Clinical Practice of Allogenic Red Blood Cell Transfusion and Fluid Bolus Therapy in Neonates

A thesis submitted for the degree of

DOCTOR OF PHILOSOPHY

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by

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Abstract

This thesis by publication comprises of seven chapters encompassing several aspects of allogenic red blood cell (RBC) transfusion practice, fluid bolus therapy and the use of these products utilised in both. The work aims to improve the evidence-base on which clinical and research decisions are made around both of these therapies and the products, namely RBCs and 0.9% sodium chloride, utilised within neonatal units. It includes two systematic reviews and meta-analyses to address uncertainties in neonatal transfusion practice, an in-vitro study designed to provide information to inform clinical transfusion practice and an observational, cross-sectional study to further understand the use of fluid bolus therapy in neonates. To place these studies in their broader context, an introductory chapter (*Chapter 1*) is provided.

The first study (*Chapter 2*) provides contemporary data on blood usage in neonatal units. Without recent data on the use of blood products, it is challenging to design accurate clinical studies. Prior to this study, knowledge of neonatal transfusion practices was limited to local cohort or survey-based studies. This study found blood product use remains common in neonates born before 30 weeks' gestation.

The second study (*Chapter 3*) provides, for the first time, a systematic review of the known published adverse effects and associations of neonatal allogenic RBC transfusion. The review did not find any significant differences in a range of clinical outcomes between neonates exposed to restrictive and liberal RBC transfusion practices. The predominance of non-randomised and observational studies was highlighted in this relatively highly-transfused population group.

Chapter 4 provides a systematic review of published studies examining washing RBCs prior to transfusion in neonates. It is possible that modification of RBCs prior to transfusion, through washing with 0.9% sodium chloride, may reduce adverse effects related to neonatal allogenic RBC transfusion. The review found insufficient evidence to support or refute the use of washed RBCs to prevent the development of significant neonatal morbidities or mortality. This review provided key data to support the development of a randomised study in this area.

Transfusion guidelines advise against the co-infusion of RBCs with solutions other than 0.9% sodium chloride. This study (*Chapter 5*) evaluates the impact of co-infusion with dextrose-containing fluids on markers of RBC quality in an in-vitro setting. The study found the in-vitro characteristics of RBCs co-infused with 0.9% sodium chloride or 10% dextrose were not adversely impacted, arguing against the recommendation not to co-infuse. These findings led to practice changes in several neonatal units in Australia and Canada.

Intravenous fluid bolus therapy for suspected haemodynamically compromised neonates is an apparent common intervention in neonatal units. Despite this, the volume and type of fluid used, as well as the timing and indications for this practice are not well described or understood. The NeoBolus study (*Chapter 6*) provides a contemporary description of clinical practice in relation to the types and specific indications for use of fluid therapy, including blood products, in neonates with suspected haemodynamic compromise. The data generated will provide key information to develop a randomised study in the area.

In summary, this work provides a number of insights into neonatal transfusion practice and fluid bolus therapy, key to improving the evidence-base and identifying future directions for research in these areas.

Glossary

AOP	Anaemia of prematurity
ANZNN	Australian and New Zealand Neonatal Network
CLD	Chronic lung disease
CMV	Cytomegalovirus
CNN	Canadian Neonatal Network
CPD	Citrate-phosphate-dextrose
CPDA-1	Citrate-phosphate-dextrose-adenine
ECMO	Extracorporeal membrane oxygenation
EPO	Erythropoietin
FFP	Fresh frozen plasma
GA	Gestational age
IVH	Intraventricular haemorrhage
NEC	Necrotising enterocolitis
PPHN	Persistent pulmonary hypertension of the newborn
RBC	Red blood cell
Rh	Rhesus
ROP	Retinopathy of prematurity
SAGM	Saline-adenine-glucose-mannitol
SHOT	Serious Hazards of Transfusion
TA-GvHD	Transfusion-associated graft-versus-host disease
TANEC	Transfusion-associated necrotising enterocolitis
TRIM	Transfusion-related immunomodulation

Candidate statement

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Amy Keir
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Please note:
For the manuscript "*Temporal changes in blood product usage in preterm neonates born at <30 weeks' gestation in Canada*" Dr Ruth Ware PhD provide editorial support during the manuscript preparation. Dr Warre is employed by the Maternal-Infant Care Research Centre, Mount Sinai Hospital, Toronto, Ontario, Canada. Her area of academic specialisation is in maternal-infant care research. Dr. Ware reviewed the manuscript for clarity and grammatical correctness. This is stated in the acknowledgment section of the published manuscript.

Chapter 1: Introduction

In Australia, in 2013, 309 489 babies were born with 307 277 live births and 2191 stillborn.[1] Of live births, 43 159 (14%) of neonates were admitted to neonatal units, with the majority (75%) admitted due to prematurity (born at less than 37^{+0} weeks' gestation). Many of the conditions experienced by these children, and the therapies used to manage them, have the potential to impact on their longer-term health and development. Part of improving their outcomes, includes examining the treatments provided to them and the potential immediate and longer-term, positive and negative impacts these treatments may have. This is the case with both allogenic red blood cell (RBC) transfusion and fluid bolus therapy. Both of these practices involve infusion of either 0.9% sodium chloride, most commonly in the case of fluid bolus therapy, or blood products, in the case of RBC transfusion and, on occasion in fluid bolus therapy, making them two of the commonest infused products in the neonatal unit. Both therapies and both products have the potential to improve or worsen clinical outcomes, impact on longer term outcomes and have a limited evidence-base on which the decision to use, or not use them, are made.

The overall aim of this thesis is to improve the evidence-base on which these clinical decisions are made around the use of both these therapies and products. Each of the five included papers address a specific research gap in neonatal allogenic RBC transfusion practice and fluid bolus therapy. Included are two systematic reviews and meta-analyses to address uncertainties in neonatal transfusion practice and knowledge, an in-vitro study designed to provide information to inform clinical transfusion practice, and an observational, cross-sectional study to further describe the use of fluid bolus therapy in neonates. Combined, these papers will improve the understanding of the clinical practices around neonatal allogenic RBC transfusion practice and fluid bolus therapy.

This chapter will provide a contextual statement to place the included publications within the history of neonatal medicine as well as within current knowledge and clinical practice. Throughout the statement, each specific research question, forming the basis of each subsequent chapter and included publications, will be highlighted.

Neonatal transfusion history

Over the last 100 years, whilst significant developments in neonatal transfusion practice have occurred, the effects of blood transfusion on many important neonatal outcomes remain unknown.[2] The first report of a transfusion into a neonate dates back to 1908 and describes the direct transfusion from a father's radial artery to his child's popliteal vein.[3] This first neonatal transfusion occurred on the background of continuing expansion of special care nurseries and physician involvement in neonatal care. Prior to the late 1800s, physician involvement had been minimal. However, industrialisation in the 19th century resulted in high infant mortality and falling birth rates, which led, in part, to the formation of the Infant Welfare Movement. This movement included increasing physician involvement in the care of infants, expansion of early examples of special care nurseries and a focus on the preventative health care of infants.[4] In 1857, Jean-Louis-Paul Denucé, a French surgeon, published the first report of the use of an incubator in the care of a premature infant.[5] Stephane Tarnier, a French obstetrician, refined the incubator by using it for the regular care of premature and sick infants. Madame Henry, a French midwife and colleague of Dr. Tarnier, was instrumental in developing the first special care nursery in France in 1893.[6] From the late 1890s, Martin Couney, a European-born physician, toured Europe and the United States with premature infants in incubators at fairs and other exhibitions.[7] As these special care nurseries continued to increase, so did the various aspects of neonatal transfusion practice.

In 1925, the first reported neonatal exchange transfusion was performed by Alfred Hart,[8] a Canadian physician, for Rhesus (Rh) isoimmunisation. Rh isoimmunisation was a common disease in the mid 20th century with extremely high morbidity and mortality. However, it was not until 1946 when additional reports of exchange transfusions were published[9] that exchange transfusion gained increasingly wide-spread use in the management of Rh isoimmunisation. In 1940, Karl Landsteiner, who had discovered the ABO blood group in 1900,[10] and Alexander Wiener, two American physicians, published their seminal paper "*An agglutinable factor in human blood recognised by immune sera for rhesus blood*."[11] The key linking of Rh factor and haemolytic disease of the newborn came in 1941 when Philip Levine, an American physician, published evidence that the majority of cases of haemolytic disease of the newborn were due to immunisation of a Rh-negative mother by a Rh-positive fetus.[12] In 1968, with the advent of postpartum administration of Rh immunoglobulin to Rh-negative mothers, maternal isoimmunisation decreased and, as a consequence, so did the numbers of exchange transfusions. By the early 1970s, new cases of Rh isoimmunisation were uncommon,[13] marking a key advance in perinatal medicine. Intrauterine RBC transfusions still remain a key part of management of this condition to date. However, there remains work to be done in this area with recent findings indicating altered cardiovascular development following exposure to fetal anaemia and intrauterine transfusion, with persistence of these changes into adulthood potentially indicating increased risk of cardiovascular disease.[14]

By the 1980s and early 1990s, results from North American surveys[15, 16] found variable transfusion practices throughout neonatal intensive and special care units. These surveys remain widely cited throughout the neonatal transfusion literature. Basic data around RBC transfusion numbers per neonate were gathered, however it is difficult to interpret due to

methodological limitations of the studies. Accurate, population data on neonatal transfusion practices remain scarce.[17]

In 2005, Bell and colleagues from Iowa, USA, published the BELL study, one of the two best known studies of neonatal transfusion enrolling 100 infants ≤1300 grams randomised to different transfusion thresholds.[18] A year later, Kirpalani and colleagues from Hamilton, Canada, published a second, larger study (PINT) enrolling 451 infants <1000 grams randomised to restrictive (low) compared to liberal (high) transfusion thresholds.[19] Further commentary and examination of the limitations of these two studies are provided later in this chapter.

Other developments in the 1990s and 2000s, included the use of erythropoietin (EPO) as a way to reduce the numbers of RBC transfusions. However, since the mid 2000s, EPO has not been recommended for this purpose due to a potential association with increased rates of retinopathy of prematurity.[20, 21] Minimisation of blood-sampling and smaller samples required for investigations[22] have had limited success in reducing transfusion numbers. Other methods such as the use of cord blood for initial blood tests have been suggested,[23] however, technical difficulties with sampling may occur and interfere with results.

Anaemia of prematurity - pathophysiology

Anaemia of prematurity (AOP) is a multi-factorial condition defined by early, significant anaemia in the context of phlebotomy blood losses, lower erythropoietin (EPO) production, and a limited bone marrow response.[24] Infants universally experience a decrease in haemoglobin (Hb) that results in varying degrees of anaemia after birth, regardless of gestational age. Preterm infants are particularly vulnerable to development of anaemia in the postnatal period due to a number of factors including a more pronounced postnatal drop in Hb levels compared to infants born at term and an increased likelihood of iatrogenic Hb losses due to recurrent phlebotomy tests due to intercurrent illnesses.[25] Recognition of AOP relies upon a combination of non-specific clinical symptoms of anaemia and haemoglobin or haematocrit levels.[26, 27] Anaemia becomes symptomatic when there is an imbalance between oxygen delivery and consumption. This may not occur universally at the same Hb for every preterm infant. Symptoms of anaemia (e.g. increased oxygen requirement and tachycardia) are non-specific and can be due to other aetiologies including sepsis, worsening respiratory distress syndrome or chronic lung disease).[28].

Strong evidence supports non-physiologic factors as primary contributors to the development of anaemia in prematurity and to the large numbers of RBC transfusions received by these preterm infants. Non-physiological factors such as phlebotomy losses, sepsis and limited nutrition all have a greater impact than physiologic factors such as decreased sensitivity to tissue hypoxia, shortened RBC survival, left-shifted oxygen dissociation curve, low plasma volumes, rapid growth, and cardiovascular factors. However, all these factors contribute to the low Hb levels observed in preterm infants 4-6 weeks after birth.

Estimates of laboratory phlebotomy loss among preterm infants in the neonatal unit during the first 6 weeks after birth range from 11 to 22 mL/kg per week with 70-80mL/kg being the

average circulating blood volume of a preterm infant. The similar temporal patterns in the intensity of laboratory phlebotomy loss and the administration of RBC transfusions. Approximately 50% of all RBC transfusions administered to infants with a birthweight <1000 grams are given in the first two weeks after birth, and 70% are administered within the four weeks.[25] It seems certain that AOP is, at the very least, exaggerated by significant phlebotomy losses in the first 4-6 weeks of admission to neonatal units. It is also the most likely area where interventions to minimise phlebotomy losses will have the greatest impact on AOP rates.[29]

In neonatal units today, RBC transfusions remain the key management strategies of AOP and are given to keep Hb levels above a certain threshold depending on the level of cardiorespiratory support required. In clinical practice, it is commonly thought that anaemia leads to tachycardia, hypotension, poor perfusion and decreased oxygen delivery to the tissues. The physiological adaptation to anaemia in preterm infants has been examined and no significant change in oxygen consumption, mean inspired oxygen or mean oxygen saturation following RBC transfusion were found.[30] There is no evidence that a haemoglobin or haematocrit threshold where inadequate tissue oxygenation (critical anaemic hypoxaemia) definitively occurs in infants of any gestational age. Very little remains known about the adaptive responses to anaemia in infants with a birthweight <1000 grams and the effects of RBC transfusion at various levels of anaemia.[28]

In addition to AOP, another reason for transfusion of blood products, or infusion of other intravenous fluids, is to provide volume expansion in the setting of critical illness. However, this clinical decision to provide intravenous fluid or blood products as volume expansion is made with a limited evidence base.[31]

Neonatal RBC transfusion and fluid bolus therapy – current practice

At present, the majority of published literature around usage patterns of blood products in neonatal units was published in the 1990s and is based upon data obtained from practice surveys.[15, 32] There is also limited data around the use of other blood products beyond RBCs. Consequently, there is a need for recent comprehensive data on neonatal transfusion practice and an evaluation of any temporal changes in blood product usage over recent years.

Research question 1:

What are the current usage patterns of blood products in neonatal units?

RBC transfusion practice and adverse effects in neonates

The conventional and most commonly utilised model of neonatal RBC transfusion includes a transfusion algorithm based on either haemoglobin or haematocrit, modified by chronological age and receipt of respiratory support.[33] The two previously mentioned neonatal RBC transfusion threshold studies[18, 19] provide the majority of available data to guide current neonatal transfusion practice and these, along with one other randomised study[34], are summarised in Table 1. Limited other studies examining transfusion thresholds exist,[35-37] however, they do not necessarily provide information to guide clinical decision-making in neonates from birth and throughout hospitalisation.

Cochrane review

The Cochrane review, examining liberal (high) compared to restrictive (low) RBC transfusion thresholds found no evidence that either strategy had an effect on mortality, major morbidities or on survival without major morbidity in preterm infants ≤ 1500 grams. It includes three published studies[18, 19, 37] and one unpublished.[38] Similar restrictive transfusion thresholds were used for all included studies and are shown in Table 2. Safety at haemoglobin levels below these limits in Table 2 has not been evaluated.

Table 2: Lower limits for capillary haemoglobin thresholds evaluated in the Cochrane review[38]

Postnatal week	**Hb (g/L)**	
	No respiratory support	**Respiratory support (any kind)**
1	100	115
2	85	100
≥3	75	85

Table 1: Summary of randomised studies examining differing RBC transfusion thresholds

Citation	Aim of study	Eligible study population	No. included and analyzed	Summary
Bell (the BELL study) 2005[18] USA Single centre	To determine if restrictive guidelines for RBC transfusions can reduce number of transfusions without adverse effects	216 preterm (500-1300 grams) infants and 103/216 preterm infants randomised (only those transfused).	100 included: 51 in liberal and 49 in restrictive group	No differences between groups in grade ≥3-4 IVH, ROP ≥3, CLD or mortality
Chen 2009[34] Taiwan Single centre	To examine the effect of RBC transfusion on outcomes of VLBW infants (restrictive versus liberal transfusion policies)	Infants <1500 grams Number eligible: unclear	36 randomised: 19 in restrictive and 17 in liberal group	No differences in adverse outcomes found between liberal and restrictive groups
Kirpalani (the PINT study) 2006[19] Canada/Australia/USA Multiple centres	To determine whether different transfusion thresholds affect survival or morbidity	694 infants <1000 grams and <31 weeks GA	451 randomised and analysed (223 in restrictive vs. 228 in liberal)	No differences in adverse outcomes (mortality, brain injury, CLD, ROP) were observed between restrictive and liberal transfusion groups

Abbreviations: RBC, red blood cell; IVH, intraventricular haemorrhage; ROP, retinopathy of prematurity; CLD, chronic lung disease; NEC, necrotizing enterocolitis; VLBW, very low birthweight; GA, gestational age; BW, birthweight

Preterm infants

As findings from the PINT[19] and BELL studies[18] are most commonly used to guide transfusion practice, it is worth examining them in further detail. These studies included preterm infants either 500-1300 grams[18] or <1000 grams and <31 weeks.[19] No differences in short-term clinical outcomes (Table 1) were found between the infants in low and high transfusion threshold groups in either trial, including number of donor exposures.

The BELL study, which included 100 infants, was designed to examine numbers of RBC transfusions and donor exposures per infant. The PINT study, including 451 infants, was designed to assess the composite primary outcome of death before hospital discharge or survival with any of severe retinopathy, bronchopulmonary dysplasia, or brain injury on cranial ultrasound. No differences in the composite primary outcome were found between low and high transfusion threshold groups in the PINT study. The BELL study[39] also did not find any differences in significant morbidities or mortality, however, as this study was primarily designed to examine differences in transfusion numbers between groups, it is challenging to draw any conclusions from this. Longer term follow-up of these studies[39, 40] found conflicting results in regard to neurodevelopmental outcome, however, neither study was designed to examine this outcome. The longer-term follow up to the PINT study suggested that higher haemoglobin thresholds may benefit longer-term neurodevelopmental outcomes assessed at 18-24 months of age.[39] The longer-term follow up to the BELL study found more liberal RBC transfusions were associated reduced brain volumes at 12 years of age, however, only 44 of the original 100 trial participants were followed,[40] limiting the value of interpretation of the findings.

Term infants

For term infants, there is minimal evidence to guide thresholds for RBC transfusion. There is only one study that provides some guidance, the Transfusion Strategies for Patients in Pediatric Intensive Care Units (TRIPICU) study.[41] This study showed no difference in oxygenation markers, duration of ventilation, cardiac dysfunction and length of hospital stay when critically ill infants and children were transfused at thresholds of 70g/L compared to 95g/L.

Ongoing clinical trials

Two clinical trials, the Thresholds on Neurocognitive Outcome of extremely low birth weight infants (ETTNO)[42] and the Transfusion of Prematures trial (TOP),[43] examining the short and longer term neurodevelopmental outcomes to 24 months' corrected age in extremely low birth weight infants randomised to liberal or restrictive RBC transfusion thresholds are underway. These trials may provide information to guide neonatal transfusion practice and longer-term outcomes related to transfusion.

At present, there is insufficient evidence to either accept or reject a restrictive or liberal RBC transfusion strategy in preterm or term neonates. However, there is a need to identify the potential benefits or harms that may arise from either strategy, whilst further studies are underway.

Adverse effects and associations of neonatal RBC transfusion

Associations between neonatal RBC transfusions and increased mortality[44, 45] as well as significant morbidities such as necrotising enterocolitis (NEC),[46] intraventricular haemorrhages (IVH),[47] retinopathy of prematurity (ROP),[48] chronic lung disease (CLD)[49] are

all reported in the literature. However, there is no current systematic collation of adverse effects due to, or associated with, RBC transfusion in neonates. Without this, and an accompanying meta-analysis of the data, it will be challenging to draw conclusions from this literature in an objective manner. In addition, there is emerging evidence that similar adverse effects in the adult population, such as transfusion-related acute lung injury, are under-reported and under-recognised in neonates.[50, 51] Data published from the Serious Hazards of Transfusion (SHOT) scheme from the United Kingdom highlights that transfusion reactions, usually observed in adults, do occur in neonates.[52] However, there is likely underreporting and lack of recognition of adverse transfusion effects and associations in neonates due to inter-current illness and lack of awareness of clinicians.

Adverse effects in transfusion

An adverse effect is an undesirable and unintended occurrence during or after transfusion of blood or blood component, which may be related to the administration of the blood or a component. The cause-effect relationship between receipt of the blood product and the adverse outcome is established. These may include alloimmunisation, post-transfusion purpura, transfusion-transmitted infection, e.g. hepatitis B, hepatitis C, parasites, incorrect blood component transfused and/or adverse events or reactions associated with directed donation.

Adverse associations in transfusion

An adverse association is also an undesirable and unintended occurrence during or after transfusion of blood or blood component, which may be associated with the administration of the blood or component. However, there is no cause-effect relationship definitely established. Transfusion-associated necrotising enterocolitis (TA-NEC) is an example of such an

association, which has recently gained attention in the literature. Kirpalani and Zupancic undertook a systematic review and a meta-analysis[53] of the published literature on the association between transfusions in newborns and the occurrence of NEC. They found that the direction of effect of RBC transfusions on NEC (more transfusions show lower NEC) as demonstrated in randomised trials was opposite to that seen in observational studies (transfusions are associated with NEC). A recent study by Patel *et al*[54] found that severe anaemia, not RBC transfusion, was associated with an increased risk of NEC. The authors suggest that prevention of severe anaemia may be more important than minimising RBC transfusion alone.[54]

Proposed underlying mechanisms of adverse transfusion effects and associations

The proposed mechanism or mechanisms underlying adverse transfusion effects and associations are unclear. A proposed mechanism relates to the modulation of a transfusion recipient's immune system, termed transfusion-related immunomodulation (TRIM).[55] In the clinical setting of an underlying inflammatory state priming the recipient's immune system, transfusion of allogenic RBC may trigger immune cell activation and related immunomodulation, resulting in frank inflammation. Data is available to support this hypothesis, with increases in interleukin (IL) 1β, IL-8, tumour necrosis factor (TNF) α and monocyte chemoattractant protein-1 observed after allogenic RBC transfusion in preterm infants.[56] These increases also correlated with increases in markers of endothelial activation.[56] This pro-inflammatory reaction may be a manifestation of TRIM. It may partly explain the association between RBC transfusion and the development of NEC, ROP and CLD.

Another contributory mechanism may be related to the sex of the blood donor. Recent work has bound that female donor blood was associated with an increased likelihood of recipients developing chronic lung disease, spontaneous intestinal perforation/necrotising enterocolitis and mortality.[57] Key limitations to this work include a higher number of transfusions received by those in the group receiving predominantly female donor blood. It is quite possible that those infants receiving more transfusions were less well and, thus, had more comorbidities and increased likelihood of mortality that those receiving less RBC transfusions.

To further understand the adverse effects and associations potentially related to neonatal allogenic RBC transfusion practice, a systematic collation of these reported adverse effects and associations is required and is provided in Chapter 4.

Research question 2:

What are the reported adverse effects and associations of neonatal allogenic RBC transfusions in the literature?

Providing safer blood products to neonates

Different component safety measures apply for both fetal and neonatal patients, compared to paediatric and adult populations, as they are particularly vulnerable recipients due to their small size, developmental immaturity and longest potential lifespan.[58] There are number of modifications of blood products that may or may not impact on health of neonatal recipients of these blood products.

Leukodepletion

Leukocytes, when transfused, can cause a variety of side-effects such as febrile reactions, suppression of the immune system, and transmission of viruses, such as cytomegalovirus (CMV). Leukodepletion is where the white blood cells are removed from blood prior to transfusion. This process decreases the risk of CMV infection transmission by RBC and platelet transfusion.[59] After the introduction of universal prestorage leukoreduction of RBC transfusions to neonates (<1250 grams) in 1998 in Canada, a reduction in several morbidities, including NEC, retinopathy of prematurity and chronic lung disease, were found.[60] A recent randomised controlled trial found transfusion of blood products that were both CMV-seronegative and leukoreduced prevents transmission of CMV to neonates <1000 grams. The only postnatal acquired CMV infections in this study were due to transmission from maternal breast milk.[61]

Irradiation

The British Committee for Standards in Haematology blood transfusion task force in 2009 made specific recommendations around the use of irradiated blood products.[62] They recommended the use of irradiated blood products (RBC, platelet and granulocyte infusions) to remove T-lymphocytes, which can cause a significant immune reaction in transfusion of

neonates with a history of intra-uterine transfusion (up to the age of 6 months), especially those undergoing exchange transfusion.[62] These recommendations are based on the finding that most cases of transfusion-associated graft-virus-host disease (TA-GvHD) occurred in apparently immune competent infants with a history of intra-uterine transfusion followed by exchange transfusion with non-irradiated blood products.[62] Consideration of the use of irradiated blood products is suggested for neonatal exchange transfusion without a history intra-uterine transfusion and this is recommended based on rare reports of TA-GvHD in this situation. The National Blood Authority (Australia) supports these recommendations[63] and also suggests consideration of the use of irradiated blood products in neonates with a birthweight <1300 grams (especially if <28 weeks' gestation or <900 grams). The basis for this recommendation is unclear. In contrast, the British Committee for Standards in Haematology blood transfusion task force does not recommend the use of irradiated products for this patient group as reports of TA-GvHD in preterm infants are minimal and are not reported for term infants undergoing small volume RBC transfusions.[62]

Other modifications

Current evidence, including in neonatal settings, provides moderate likelihood that use of fresher RBCs (less than 5-7 days of age) does not influence mortality and low likelihood that it does not influence adverse events.[64] Other modifications to RBCs transfused to neonates may have the potential to improve clinical outcomes. There is evidence in both adult[65] and paediatric[66] populations of benefit from washing RBCs prior to transfusion. As transfusions of blood products can alter the immune system of the recipients, it is feasible that 0.9% sodium chloride washing of RBCs prior to transfusion may reduce adverse effects and improve outcomes for all patient populations, including preterm neonates. Washed RBCs are units of whole blood or RBCs that have been washed with one to two litres of 0.9% sodium

chloride prior to transfusion. These units are depleted of 99% of plasma proteins and 85% of white blood cells.

The evidence-base for use of RBCs washed with 0.9% sodium chloride to reduces morbidities and mortality in recipient is limited but promising. The use of washed RBC transfusions in paediatric cardiac surgery was shown to reduce pro-inflammatory biomarkers and number of transfusions, and demonstrated a trend towards reduced mortality, when compared with unwashed RBCs.[66] There is further evidence in adult populations that washing RBCs prior to transfusion reduces mortality in a subset of adults with acute leukemia[65] and those undergoing coronary artery bypass surgery.[67] Further research is underway in this area in adult populations.[68] If washing RBCs for transfusion in preterm infants leads to similar benefits, this would represent a significant improvement in neonatal transfusion practice.

This topic is further explored in Chapter 4.

Research question 3:

Does washing RBCs prior to transfusion in neonates prevent morbidity and mortality?

Clinical practice of RBC administration – a key aspect

Another aspect of neonatal transfusion practice, and potential modification of RBCs prior to transfusion, is the potential safety of co-infusion of dextrose-containing fluids and RBCs.[69, 70] Current transfusion guidelines prohibit the practice of infusion both blood and dextrose-containing fluids through the same intravenous line due to the potential risk of agglutination and haemolysis.[71] The evidence for this is limited and the studies on which this recommendation is based upon are summarised in Table 3.

Table 3: Studies to determine whether co-infusion of RBCs and dextrose-containing fluids causes significant haemolysis

Citation	Study design	Key Results	Comments
Noble *et* Abott. United Kingdom 1959[72]	Laboratory-based study Whole blood in an acid-citrate-dextrose solution and isotonic dextrose-containing solutions when mixed together in a transfusion apparatus. Levels of haemolysis (as a %) were determined by a colorimeter.	Haemolysis occurred when blood and 5% dextrose was incubated in the apparatus for ≥ 12 hours at room temperature (10%); earlier at 6 hours when the solution was kept at 27 degrees (17%). Haemolysis was observed earlier (at 3 hours) when the solution was dextrose and saline combined at 27 degrees (7.5%). No haemolysis was observed when blood was incubated with 0.9% sodium chloride at either temperature.	The experimental conditions and blood products (whole blood) used in the experiments are unlikely to reflect current transfusion practice. Blood and dextrose-containing fluids, although in a transfusion apparatus, were not actually infused.
Jones *et al*. United Kingdom 1962[73]	Laboratory-based study using human subjects Red blood cells labelled with 75µg ^{51}Cr as sodium chromate were mixed with 5% dextrose and reinjected into human subjects.	Red cells aggregated by 5% dextrose solutions are destroyed within 48 hours of reinjection.	Addition of 0.9% sodium chloride to the dextrose solution may ameliorate the agglutination observed when 5% dextrose is mixed with red blood cells.
Ryden *et* Oberman. USA 1975[74]	Laboratory-based study 22-day-old RBCs stored in citrate-phosphate-dextrose (CPD) were incubated with 5% dextrose with 0.9% saline, 5% dextrose in 0.225% saline, 5% dextrose and lactated Ringer's solution. Haemolysis was determined by visual inspection of the solutions.	Gross haemolysis, as measured by visual inspection, was observed after 30 minutes of incubation of 5% dextrose and RBCs, as well as with 5% dextrose and 0.225% saline by 10 minutes. No haemolysis was observed when blood was mixed with 5% dextrose with 0.9% saline or with 0.9% sodium chloride alone.	Haemolysis was documented by visual inspection of the solutions alone. The main part of the experiment involved mixing RBCs and IV solutions in a centrifuge tube and incubating them for a period of time. This practice does not reflect current clinical practice.

Easton *et* Ternoey. Canada 1985[75]	Laboratory-based study RBCs stored in citrate-phosphate-dextrose-adenine (CPDA-1) were incubated with 3.3% dextrose and 0.3% saline at different ratios for up to 120 minutes.	Haemolysis (1+ to gross haemolysis) on visual inspection was observed in most mixtures by 20 minutes and in all mixtures by 30 minutes. 0.9% saline was used as a control measure and no haemolysis or agglutination was observed when incubated with RBCs	Only visual rating scores of haemolysis were used (0, 1+, 2+, 3+ and GH). RBCs and dextrose-containing fluids were not actually infused.
Strautz *et al*. USA 1989[76]	Laboratory-based study 6-10 day old RBCs stored in adenine-saline-dextrose (AS-1) and washed RBCs were diluted with Ringer's lactate, 5% dextrose or 0.9% sodium chloride. Samples were incubated with each IV solution in centrifuge tubes at room temperature and at 37 degrees. Mixtures were examined for agglutination, clot formation and haemolysis immediately, 1 minute and 60 minutes following mixing.	Haemolysis was not observed by visual inspection when RBCs were mixed with 5% dextrose in ratios 10:1 through to 1:40 (blood: solution) either immediately on mixing or after 30 minutes at room temperature. Haemolysis was observed by visual inspection immediately when either RBCs were mixed with 5% dextrose and when the RBCs were mixed at a ratio 1:5 or greater (blood: solution) at 37 degrees. This was not observed at lower ratios (10:1 through 1:1) until 60 minutes of incubation both at room temperature and at 37 degrees.	Haemolysis identified only if supernatant developed a grossly pink colour (visual scoring only and no grading system used). Experimental conditions do not resemble current infusion practice. RBCs and dextrose-containing fluids were not actually infused. Provides supporting data that incubation of RBCs and 0.9% saline does not lead to haemolysis.
Jankov *et* Roy. Australia 1997[77]	Laboratory-based study RBCs stored in saline-adenine-glucose-mannitol were co-infused (at 5ml/hr and 15ml/hr) with various dextrose-containing solutions (5% dextrose, 10% dextrose, 15% dextrose and amino acid).	Minimal amounts of haemolysis (as a % of fHb levels of the control co-infusate – water and RBCs) were observed with any of the solutions. The greatest % level of haemolysis observed was 0.14% with RBCs and 15% dextrose.	RBCs used for the study had a mean age of 5 days of age, which is not necessarily reflective of the age of RBCs used in neonatal transfusion practice. Actual levels of fHb not stated for the co-infused samples. Levels of haemolysis seen with 0.9% saline and water (control solutions) were not given.

	Co-infused 0.9% saline and water were used as 0% and 100% haemolysis controls.		
van den Bos *et al.* Netherlands 2003[78]	Laboratory-based study Level IV evidence 5% dextrose and 0.9% saline were co-infused with irradiated RBCs stored in saline-adenine-glucose-mannitol as the control arm of an experimental study. RBCs were both co-infused with IV solutions (dynamic model) as well as incubated for 10-30 minute time periods (static models).	Levels of fHb in the co-infused RBC concentrates in various models: Dynamic co-infused model: 0.9% saline vs. 5% dextrose = 7 vs. 8 µmol/L Static 10min model: 0.9% saline vs. 5% dextrose = 8 vs. 6 µmol/L Static 30min model: 0.9% saline vs. 5% dextrose = 8 vs. 6 µmol/L.	No significant difference in levels of fHb were found when RBCs where co-infused with 0.9% saline vs 5% dextrose. High infusion rates (100ml/hr) were used and are not reflective of the rates used in neonatal transfusion practice.

Stark *et al.* Australia 2011[69]	Laboratory-based study Level IV evidence RBCs stored in saline-adenine-glucose-mannitol were co-infused with various dextrose-containing solutions (5% dextrose, 10% dextrose and amino acid).	Levels of haemolysis observed in the co-infusate were comparable to the levels observed in the control infusate (RBCs and 0.9% saline). Co-infusion of 0.9% sodium chlroide and RBCs led to fHb 1.9-2.1 µmol/L compared to when 10% dextrose was co-infused with RBCs resulting in a fHb level 1.7-2.5 µmol/L. Agglutination (macroscopic or microscopic) was not observed during co-infusion of any type of solution.	Aspects of the experimental design did not reflect current neonatal transfusion practice. For example, RBCs used for the study had a mean age of 5 days of age, not necessarily reflective of the age of RBCs used in neonatal transfusion practice.

*This table is based upon one from an evidence-based review I published in 2013 entitled "*Is it safe to co-infuse dextrose-containing fluids with red blood cells*?"[79]

Of the seven studies identified, all were in-vitro studies comparing infusion/incubation of RBCs with various dextrose-containing fluids and later observed levels of haemolysis.[69, 72-78] Four of the studies used equipment and RBC products that no longer reflect current practice[72-74, 76] or experimental conditions not indicative of current neonatal transfusion practice in highly resourced countries.[78]

The study by Stark *et al*[69] did not demonstrate agglutination or haemolysis of RBCs with various dextrose containing fluids. The experimental conditions in this study likely reflects current neonatal transfusion practice in several countries, including Australia, Canada, Europe, New Zealand and United Kingdom. However, it is unlikely that the majority of infants in highly resourced countries transfused RBCs receive ones that are 5 days of age and it is possible that older RBCs or irradiated RBCs may be at a greater risk of haemolysis. However, there remains no in-vivo data available at present to support the findings by Stark *et al.*[69] An additional in-vitro study could address several of the concerns regarding the previous study design and would be a useful base which to develop in-vivo studies from.

Research question 4:

Is it safe to co-infuse dextrose-containing fluids and RBCs?

Fluid bolus therapy in neonates

Another aspect of neonatal transfusion practice includes the use of blood products as a volume expander or fluid bolus. Fluid bolus therapy may be used in neonates as part of management of haemodynamic compromise, for example, due to hypotension. However, the volume and type of fluid used, as well as the timing and the indications for fluid boluses are not well described. Types of fluids used to manage suspected haemodynamic compromise in neonates may include crystalloids, most commonly 0.9% sodium chloride,[80] or blood products including fresh frozen plasma (FFP), albumin and RBCs.

Fluid bolus therapy: underlying principles

Due to our current lack of ability to predict which, and whether, neonates may benefit from a fluid bolus, standard practice in neonatal medicine is to empirically administer a fluid bolus to suspected haemodynamically compromised infants. Then the effect on cardiac output or other variables, such as acid-base and/or lactate, to draw conclusions about benefit at the tissue and cellular level is evaluated. This approach is indiscriminate and suboptimal because it is likely not all neonates will respond to a fluid bolus in the desired way. In non-responders, repeated fluid boluses will increase fluid load, possibly inducing harm.[81] In a preload responsive individual whose heart is operating at the steep portion of the Frank-Starling curve, additional volume will increase stroke volume and increase cardiac output.[82] The inferred consequence is improved tissue perfusion, in turn improving cell and organ function. These are the physiologic principles on which fluid bolus therapy is based. It is supported by previous data revealing an increase in cardiac output post-fluid bolus in preterm infants.[83]

Preterm infants

There are no randomised studies primarily designed to examine fluid bolus compared to no fluid bolus in preterm infants with haemodynamic compromise.[84] Several studies[85-89] published between 1976 to 2000 comparing fluid bolus (volume expansion) to no fluid bolus in preterm infants are available; however, the majority of included infants did not have signs of haemodynamic compromise. Meta-analysis of these studies found no differences in clinical outcomes, including mortality, grade 3-4 intraventricular haemorrhage and neurodevelopmental impairment .[90]

The largest, and best-known study examining the use of fluid boluses in preterm neonates, is the Northern Neonatal Nursing Initiative (NNNI) Trial Group study (n=776).[89] The study was designed to determine whether early volume expansion, including with FFP administration, would reduce morbidity and mortality in infants < 32 weeks' gestation. Prophylactic FFP (20 ml/kg followed by 10 ml/kg after 24 hour); or a similar volume of an inert gelatin plasma substitute; or control management with a maintenance infusion of 10% dextrose were compared. The study found no effect of use of FFP as early volume expansion on cranial ultrasound abnormalities or mortality prior to initial discharge. In the two-year follow-up study,[91] no significant differences between groups in severe disability or mortality were reported. The study published in 1996 and likely does not reflect current clinical practice, limiting its relevance.[92] Critically, volume expansion was used 'prophylactically' as opposed as part of management of haemodynamic compromise, limiting the conclusions able to be drawn from its findings. As a consequence, this study does not provide information on whether or not fluid boluses are beneficial in preterm infants with haemodynamic compromise.

Late preterm and term infants

Only two studies are currently available to assess whether fluid bolus therapy in late preterm and term infants has any objective clinical benefit.[93, 94] No relevant randomised controlled trials were identified. These studies are summarised in Table 4.

Table 4: Use of intravenous fluid bolus therapy in late preterm and term infants with suspected haemodynamic compromise*

Citation	Study group	Study type (level of evidence)	Outcome	Key result	Comments
Mydam *et al*[93] 2014	Infants >34 weeks GA with persistent pulmonary hypertension of the newborn (PPHN); n=98.	Retrospective cohort study with comparator group (level 4). Study group = Infants who received inhaled nitric oxide (iNO) and mechanical ventilation only, who survived to discharge. Comparator group = Infants who received ECMO or who died.	Identification of variables, which may predict adverse outcome (ECMO and/or death) in PPHN.	Infants in the comparator group received higher amounts of fluid boluses during the first 7 days of hospitalization compared to the study group (p=0.018). However, after logistic regression analysis of statistically significant parameter, illness severity scores were the only variable that retained statistical significance between groups.	Excess fluid bolus therapy observed in the comparator group was likely a marker of illness severity only.

Wyckoff *et al*[94] 2005	Infants ≥34 weeks GA who received cardiopulmonary resuscitation (defined as >1 minutes of positive pressure ventilation and chest compressions); n= 23.	Retrospective cohort study with comparator group (level 4).	Characterisation of use of fluid bolus therapy in the delivery room.	Infants who received fluid bolus therapy (n=13) in the delivery room had lower arterial cord pH and base deficits (p=0.02) with longer periods of chest compressions and receipt of more adrenaline (p=<0.001) than infants who did not. On admission to NICU (n=13), infants who had received fluid bolus therapy did not differ in arterial pH, pCO_2, heart rate, additional fluid bolus therapy or mortality (p=NS) from those that did.	Receipt of fluid bolus therapy in the delivery room may be a marker of illness severity and, in the absence of hypovolaemia secondary to blood loss, not have any objective clinical benefit.

Abbreviations: ECMO = extracorporeal membrane oxygenation; GA = gestational age; iNo = inhaled nitric oxide; PPHN = persistent pulmonary hypertension of the newborn

*This table is based upon one from an evidence-based review I published in 2016 entitled "*Are intravenous fluid boluses beneficial in late preterm or term infants with suspected haemodynamic compromise*?"[31]

The two identified studies were retrospective studies with comparator groups.[95] These studies found that receipt of fluid bolus therapy was likely to be a marker of illness severity, rather than a cause of adverse effects in infants with persistent pulmonary hypertension of the newborn[93] and hypoxic-ischaemic encephalopathy.[96] Whilst the clinical pathophysiological need for additional fluid in infants with gastroschisis and reduced intravascular volume might appear more justified based on physiological first principles, use of fluid bolus therapy in these patient groups still remains based on expert opinion rather than robust clinical trials.

Potential benefits and harm of fluid bolus therapy

Potential adverse effects of all types of fluid boluses in neonates include volume overload, dilutional coagulopathy, hypothermia and electrolyte disturbances. In addition, particular fluids may cause specific complications, such as red cell and plasma associated transfusion reactions,[46, 97] or 0.9% sodium chloride-induced hyperchloraemic metabolic acidosis. Observational studies suggest dose-related adverse effects of volume overload; in preterm infants, multiple fluid boluses have been associated with increased mortality[98] and intraventricular haemorrhages[99] whereas lower total fluid intakes in the first week of age were correlated with decreased chronic lung disease and mortality.[100, 101] The Fluid Expansion as Supportive Therapy (FEAST) study found increased 48-hourly mortality in critically ill children randomised to receive fluid bolus therapy.[102]

It is clear the evidence base for fluid bolus therapy is limited in both preterm and term infants[31] and may be associated with potential harm. It is, therefore, timely to examine fluid bolus therapy in the neonatal unit in much greater detail.

Research question 5:

What are the types, doses, indications and short-term outcomes of fluid bolus therapy in neonates?

Research gaps and aims of included publications

A number of research gaps exists in the clinical practice of allogenic RBC transfusion and fluid bolus therapy in neonates. Specific questions that will be addressed:

Research question 1: What are the current usage patterns of blood products in neonatal units? Chapter 2 will address this question through a description of the types, patterns and trends of use of blood products in the neonatal unit.

Research question 2: What are the current known adverse effects and associations of neonatal RBC transfusions? Chapter 3 will provide a systematic collation of reported adverse effects and associations of RBC transfusion in neonates.

Research question 3: Does washing RBCs prior to transfusion in neonates prevent morbidity and mortality? Chapter 4 contains a systematic review and meta-analysis of the evidence for modification of RBCs prior to transfusion on the impact of morbidities and mortality in preterm neonates is provided.

Research question 4: Is it safe to co-infuse dextrose-containing fluids and RBCs? Chapter 4 will provide the in-vitro evidence exploring whether it is safe to co-infuse RBCs and dextrose-containing fluids.

Research question 5: What are the types, doses, indications and short-term outcomes of fluid bolus therapy in neonates? Chapter 6 consists of an international, cross-sectional, observational study examining the clinical practice of fluid bolus therapy to provide the answers and explore this practice in neonatal units around the world.

Each of these publications aims to contribute to improving the outcomes of children through improving the understanding of current neonatal knowledge and clinical practice. It will allow for identification for future directions for research in this area.

Chapter 2

Use of blood products in contemporary neonatal intensive care units

This chapter includes the published paper "*Temporal changes in blood product usage in preterm neonates born at less than 30 weeks' gestation in Canada*."

The data presented in the study is from the Canadian Neonatal Network (CNN) database. During 2012-2014, I worked as a Neonatal Fellow at the University of Toronto. This allowed me to work with researchers from the CNN and to access data from the network. The network data available through the CNN is more detailed than that is currently available from the Australian and New Zealand Neonatal Network (ANZNN) database. Transfusion data is not available from the ANZNN and I was fortunate to be able to access contemporary transfusion data from a country with a similar healthcare system and socio-demographics to Australia and New Zealand.

The correct figures are included as supplementary material at the end of Chapter 2.

It addresses the previously identified research question:

Research question 1:

What are the current usage patterns of blood products in neonatal units?

Authorship forms are provided in Appendix C

Temporal changes in blood product usage in preterm neonates born at less than 30 weeks' gestation in Canada

Amy K. Keir,[1,2] Junmin Yang,[3] Adele Harrison,[4] Ermelinda Pelausa,[5] Prakesh S. Shah,[1] on Behalf of the Canadian Neonatal Network

BACKGROUND: Knowledge of neonatal transfusion practices remains limited to local cohorts or survey-based studies. This study evaluated the pattern and temporal changes in the types and frequency of blood product use among preterm neonates born at less than 30 weeks' gestation in Canada.

STUDY DESIGN AND METHODS: A retrospective cohort study of preterm neonates born at less than 30 weeks' gestation and admitted to participating neonatal intensive care units in the Canadian Neonatal Network from 2004 to 2012 was conducted to evaluate blood product usage. The temporal change in red blood cell (RBC) use was evaluated by dividing the study period into three epochs: 2004 to 2006, 2007 to 2009, and 2010 to 2012.

RESULTS: Of 14,868 eligible neonates admitted to participating units in Canada during the overall study period, 8252 (56%) received RBCs, 2151 (15%) platelets, 1556 (11%) fresh-frozen plasma, 915 (6%) albumin, and 302 (2%) cryoprecipitate. Temporal evaluation over three epochs revealed a trend toward fewer RBC transfusions among neonates born at 26 to 29 weeks' gestation (p = <0.01-0.04) but use remained unchanged or increased for neonates born at 23 to 25 weeks' gestation (p = 0.02-0.54).

CONCLUSION: Blood product use remains at a very high frequency in preterm neonates born at less than 30 weeks' gestation. Evolutionary practice changes and relative high tolerance for anemia may be associated with a reduction in RBC usage in recent years in neonates born at at least 26 weeks' gestation. This contrasts with the ongoing higher usage of blood products observed at extremely low gestational ages.

Small-volume red blood cell (RBC) transfusions are often used to manage anemia of prematurity,[1] with the assumption that the transfusion will lead to an increase in oxygen-carrying capacity and, thus, oxygen delivery to tissues. Neonates, especially those born preterm, are probably the highest consumers of blood products compared with all other patient or age groups, with reports of up to 90% of neonates born weighing less than 1500 g receiving at least one RBC transfusion during the course of their initial hospitalization.[2] Studies from the 1990s[2,3] are often cited to support this concept and are based on data obtained from practice surveys.

In recent years, key neonatal transfusion trials examining low versus high thresholds have been conducted.[4,5]

ABBREVIATIONS: CLD = chronic lung disease; NEC = necrotizing enterocolitis; NICU(s) = neonatal intensive care unit(s).

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This, coupled with increasing awareness of the potential adverse effects of transfusion,[6-8] may have led to a reduction in the usage of blood products compared with previous reports. Conversely, with limited data on the long-term effects of neonatal transfusion practices,[9] as well as the improved survival of extremely low gestational age neonates, it is possible that the amount of blood products transfused in neonatal intensive care units (NICUs) has not changed considerably since the early 1990s. However, there is a paucity of contemporary data on the types of blood products and the frequency of their use in the NICU with the most recent data being published as part of a quality improvement initiative.[10] This information, however, was drawn from neonates of high mean gestational age (35 ± 4 to 36 ± 4 weeks), short median length of stay (8-9 days), and low mortality rate (1-6%).[10] To address the lack of recent comprehensive data, this study aimed to provide contemporary data on the pattern of blood product usage in a large nearly population-based cohort of preterm neonates born at less than 30 weeks' gestation and to evaluate any temporal changes in blood product usage over recent years.

METHODS AND PATIENTS

This retrospective cohort study of preterm neonates admitted to participating NICUs in the Canadian Neonatal Network evaluated the pattern and temporal changes in types and frequency of blood product use among preterm neonates born at less than 30 weeks' gestation in Canada. The temporal changes were evaluated by dividing the study period into three epochs: 2004 to 2006, 2007 to 2009, and 2010 to 2012.

Population

Study participants were drawn from a population cohort of 16,010 neonates born at between 23^{+0} and 29^{+6} weeks' gestation with a birthweight of more than 500 g and admitted between January 1, 2004, and December 31, 2012. In 2004, a total of 16 NICUs were participating in the network, whereas 25 of the total 28 NICUs in Canada were members by 2012. Overall, the population represented approximately 80% of all neonates admitted to NICUs in Canada. Neonates for whom an explicit decision was made to provide only comfort care at birth or those born with planned palliative care and those with major congenital anomalies were excluded.

Data collection

The network operates a national standardized database of characteristics and outcomes for all neonates admitted to Level III NICUs across Canada. Data on each neonate from birth until discharge from the NICU were collected by trained abstractors at each site and were entered directly from the patient chart into a customized computerized data entry program with built-in error checking.[11] Blood banking practices in Canada are standardized, with RBCs for transfusion supplied by either the Canadian Blood Services or Héma-Québec. In Canadian NICUs transfused RBCs are generally irradiated, cytomegalovirus negative, leukoreduced, and unwashed. Dedicated donor packs are utilized to minimize exposure to multiple blood donors. The majority of transfusions are blood type specific; however, in a few instances, such as emergent transfusion, blood group O transfusions were given. RBCs for transfusion are usually stored in saline-adenine-glucose-mannitol (SAGM) for up to 42 days. Platelets (PLTs) are generally prepared using the buffy coat method and collected either through apheresis or through whole blood donation. Transfusion guidelines vary between units, although most units follow the Canadian Pediatric Society guidelines.[12]

Ethics approval

Data collection and transfer to the network coordinating center was approved at each site by either the institutional research ethics board or quality improvement committee. The approval for the study was obtained from the research ethics board at Mount Sinai Hospital and also from the network executive committee.

Statistical analysis

Descriptive statistical methods were used to characterize the study population. The Cochran-Armitage trend test was used to analyze changes in blood product usage over three epochs in the entire population and stratified by birthweight groups and each week of gestational age.

RESULTS

Of the eligible neonates included in the study, 8566 (58%) received at least one blood product during their admission to the neonatal unit (Fig. 1). The frequency of blood product use and percentages of neonates in the study cohort transfused with RBCs, PLTs, fresh-frozen plasma (FFP), albumin, and cryoprecipitate are shown in Table 1. Among neonates transfused with RBCs, 2098 (25%) received one transfusion, 1693 (21%) received two, 1051 (13%) received three, and the remaining 3410 (41%) received four or more transfusions. Blood product use over the entire study period and for each epoch stratified by birthweight and gestational age is displayed in Tables 2 and 3.

Trends in the use of RBC transfusions over time are shown in Figs. 2 and 3. There was a trend toward fewer RBC transfusions among neonates born at 26 to 29 weeks' gestation in recent epochs but use remained static or increased for neonates born at 23 to 25^{+6} weeks' gestation. No overall changes in the use of PLTs ($p = 0.08$-0.8) or FFP ($p = 0.19$-0.89) were observed. The number of

neonates who received cryoprecipitate and albumin were too small to analyze for trend data.

Trends in major neonatal morbidities (chronic lung disease [CLD], necrotizing enterocolitis [NEC], and sepsis) were analyzed for the gestational age groups (23-25 weeks' and 26-29 weeks' gestation) over the three periods (Table 4). For infants at 23 to 25 weeks' gestation, CLD decreased ($p = <0.01$), NEC remained unchanged, and sepsis increased ($p = <0.01$) across the three time periods. For infants at 26 to 29 weeks' gestation, CLD decreased ($p = <0.01$) with NEC and sepsis remaining unchanged ($p = 0.74$ and $p = 0.33$) across the three periods.

DISCUSSION

Building an evidence base for neonatal transfusion practices needs to start with a clear understanding of blood product usage in neonatal units. Our study provides current data on the types of blood products transfused and their frequency of use in preterm neonates born at less than 30 weeks' gestation using a large, nearly population-based cohort. Not surprisingly, the smallest and most immature preterm neonates received proportionally more blood products than their larger and more mature counterparts. Despite global attempts to minimize blood product use, preterm neonates remain a heavily transfused group, especially those born at less than 26 weeks' gestation.

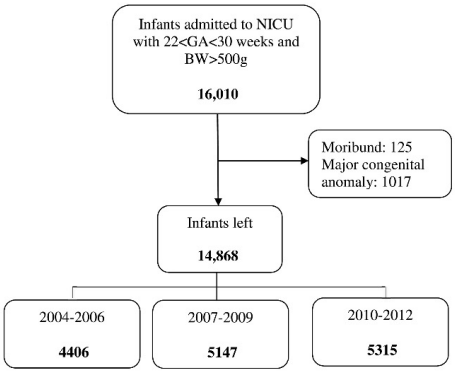

Fig. 1. Study population flow diagram.

In our study, up to 82% of neonates with a birthweight of not more than 1000 g received at least one RBC transfusion. Our results are similar to those of the study by Maier and colleagues,[13] who examined transfusion practices in the 1990s. Another study by Ringer and coworkers[14] reported that 65% to 87% of neonates with a birthweight of less than 1500 g received RBC transfusions, whereas in our study the range was 25% to 82% for a similar population. The continuing high rates of RBC transfusions in neonates born at less than 30 weeks' gestation may reflect the ongoing variations in transfusion practice reported by other research groups.[15] It is also likely that the higher transfusion requirement of such preterm neonates is a reflection of the increasingly active management and survival of neonates at the lowest gestational ages. Within our network the survival of infants born at 23 weeks' gestation has increased from 0% to 10% in early 2003 to 30% to 40% in 2012.

Our study revealed a trend toward fewer RBC transfusions in more mature preterm neonates (26-29 weeks' gestational age) with the use remaining static or increasing in the 23 to 25 weeks' gestational age group. The overall and proportionally higher use of blood products observed in the 24 weeks' gestational age group, both over the three time periods and when compared with the other gestational age groups, is likely reflective of increasing survival rates at this gestational age. These observations may also reflect changes in the prevalence of significant neonatal

TABLE 1. Blood product usage for all neonates born at less than 30 weeks' (23^{+0}-29^{+6}) gestation in 2004 to 2012 (n = 14,868)*

Transfusion frequency	RBCs	PLTs	FFP	Albumin	Cryoprecipitate
1	2098 (14)†	991 (7)	889 (6)	915 (6)	214 (1)
2	1693 (11)	393 (3)	320 (2)		88 (<1)‡
3	1051 (7)	174 (1)	154 (1)		
4	898 (6)	127 (<1)	64 (<0.5)		
5	598 (4)	84 (<1)	44 (<0.5)		
6	431 (3)	69 (<1)	25 (<0.5)		
7	344 (2)	47 (<0.5)	21 (<0.5)		
8	260 (2)	40 (<0.5)	6 (<0.1)		
9	167 (1)	40 (<0.5)	6 (<0.1)		
≥10	712 (5)	186 (1)	27 (<0.5)		
Total	8252 (56)	2151 (15)	1556 (11)	915 (6)	302 (2)

* Data are reported as number (%).
† Total percentage: 14% of all neonates in the study cohort received at one RBC transfusion only, 11% received two RBC transfusions, 7% of the entire cohort received one PLT transfusion only, and so on.
‡ Less than 1% of infants received two to 10 or more cryoprecipitate transfusions.

TABLE 2. Blood product usage over 2004 to 2012 stratified by gestational age and birthweight (n = 14,868)*

Categories	Number of admissions	RBCs	PLTs	FFP	Cryoprecipitate	Albumin	Total admissions
Birthweight (g)							
501-750	3050	2489 (82)†	963 (32)	545 (18)	101 (3)	350 (12)	2557 (84)
751-1000	4683	3269 (70)	748 (16)	579 (12)	97 (2)	329 (7)	3363 (72)
1001-1250	4163	1807 (43)	290 (7)	288 (7)	62 (2)	156 (4)	1880 (45)
1251-1500	2295	583 (25)	118 (5)	110 (5)	27 (1)	54 (2)	642 (28)
1501-1750	545	84 (15)	23 (4)	21 (4)	8 (2)	16 (3)	99 (18)
>1750	122	20 (16)	9 (7)	13 (11)	7 (6)	10 (8)	25 (20)
Gestational age (weeks)							
23	411	310 (75)	133 (32)	93 (23)	13 (3)	41 (10)	316 (77)
24	1280	1085 (85)	430 (34)	269 (21)	49 (4)	190 (15)	1113 (87)
25	1905	1610 (85)	481 (25)	337 (18)	54 (2)	225 (12)	1646 (86)
26	2219	1700 (77)	343 (16)	297 (13)	49 (2)	136 (6)	1737 (79)
27	2576	1489 (58)	292 (11)	220 (9)	35 (1)	136 (5)	1534 (60)
28	3078	1262 (41)	276 (9)	197 (6)	56 (2)	110 (4)	1333 (43)
29	3387	796 (24)	196 (6)	143 (4)	46 (1)	77 (2)	887 (26)
Total	14,868	8252 (56)	2151 (15)	156 (11)	302 (2)	915 (6)	8566 (58%)

* Data are reported as number (%).
† Total percentage of all infants in the gestational age subgroup who received one RBC transfusion only.

TABLE 3. RBC transfusions in three epochs stratified by birthweight and gestational age across 2004 to 2012 (n = 14,868)*

Categories	2004-2006 (n = 4146)	2007-2009 (n = 5147)	2010-2012 (n = 5315)	p-trend
Overall	4406	5147	5315	
Birthweight (g)				
501-750	765 (80)	827 (83)	897 (82)	0.21
751-1000	961 (70)	1154 (71)	1154 (68)	0.15
1001-1250	613 (49)	632 (43)	562 (39)	<0.01
1251-1500	200 (31)	200 (25)	183 (21)	<0.01
1501-1750	24 (16.2)	32 (15.9)	28 (14.1)	0.58
>1750	8 (27.6)	5 (9.4)	7 (17.1)	0.34
Gestational age (weeks)				
23	91 (75)	82 (73)	137 (77)	0.54
24	335 (82)	370 (85)	380 (88)	0.02
25	447 (84)	571 (84)	592 (85)	0.86
26	530 (80)	563 (78)	607 (73)	<0.01
27	462 (60)	519 (58)	508 (55)	0.04
28	457 (47)	441 (40)	364 (36)	<0.01
29	249 (26)	304 (25)	243 (20)	<0.01

* Data are reported as number (%).

morbidities across the study time periods. However, outcome trend data available for our study cohort do not support this with small variations in rates of CLD, NEC, and sepsis in the 23 to 25 weeks' gestation and 26 to 29 weeks' gestation age groups.

This is the first report describing patterns of use of RBC transfusion and the temporal trends across these extremely low gestational ages. Data on the number of donor exposures according to gestational age groups were unavailable. Most units in Canada use multipacks (division of donation into small aliquots and use in successive transfusions within a prescribed period to minimize donor exposure) and, thus, it will be important to collect such information in future studies to assess infant risk and resource utilization. Data on erythropoietin use, delayed cord clamping, and blood loss due to laboratory testing were not available for our study cohort.

PLT use in neonatal units is highly variable[16] with studies reporting up to 45% of neonates with a birthweight of not more than 1000 g being transfused with PLTs[17] and approximately 10% receiving PLTs when all birthweights and gestational ages are included.[18] In our cohort, 18% to 32% of neonates weighing not more than 1000 g at birth received at least one PLT transfusion.[17] PLT use was highest in the smallest and most immature neonates, with more than 30% of neonates with a birthweight of 501 to 750 g and 23 to 24 weeks' gestation receiving at least one transfusion. This may reflect continuing uncertainty about when to transfuse a preterm neonate with PLTs[16] or may reflect the belief of many physicians that

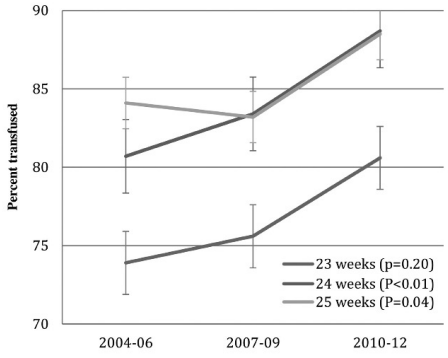

Fig. 2. Neonates born at 23 to 25 weeks' gestation transfused with RBCs: 2004 to 2012.

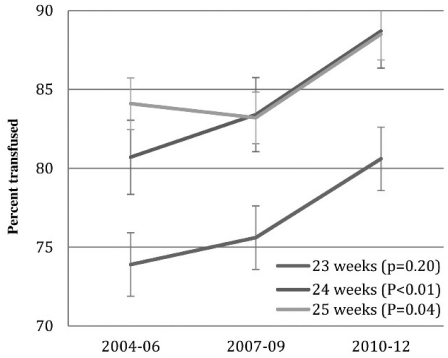

Fig. 3. Neonates born at 26 to 29 weeks' gestation transfused with RBCs: 2004 to 2012.

the smallest and most immature neonates will benefit from a PLT transfusion. An international multicenter trial is currently underway that aims to clarify prophylactic PLT transfusion thresholds.[19]

Previous studies report that up to 8% to 12% of neonates are transfused with FFP[20,21] when all birthweights and gestational ages are included. At 15%, the higher use observed in our study likely reflects the inclusion of neonates born at less than 30 weeks' gestation only. The evidence base for the use of FFP[22] in neonates is limited. A recent systematic review[23] reported no benefit of FFP in neonates with polycythemia, respiratory distress syndrome, hypotension, or sepsis, as well as no benefit in preventing intraventricular hemorrhage. With FFP carrying significant risk in the pediatric and adult populations for the development of transfusion-related acute lung injury,[23,24] a condition likely underrecognized in neonates,[25] the risk/benefit ratio of plasma transfusions should be carefully considered.

We could not compare our results for cryoprecipitate and albumin use with previous studies as such studies are lacking and the only information available is from studies that describe common usage indications.[26] The evidence base for the use of albumin[27] in neonates is also limited and controversial,[28] with no robust clinical trials to support its use.[27,29] Our study found albumin use at 6.2% overall and 11.5% in neonates with a birthweight of 501 to 750 g.

Our cohort study provides invaluable contemporary data on blood product usage in preterm neonates born at less than 30 weeks' gestation. However, our study is limited by its retrospective nature. Importantly, we do not have information regarding specific indications for transfusions; changes in institutional transfusion guidelines and adverse transfusion reactions are not part of the data collection process for the network. A prospective observational study, through an international collaboration of multiple centers or networks, is needed to gain a true understanding of blood product usage in the NICU,

TABLE 4. Outcomes trend stratified by gestational age*

Gestational age (weeks)	2004-2006	2007-2009	2010-2012	p-trend†
23-25				
Total number	1061	1227	1310	
CLD	492 (66.8)	516 (62.1)	538 (57.8)	<0.01
NEC	101 (9.9)	148 (12.4)	137 (10.9)	0.50
Sepsis	289 (27.2)	470 (38.3)	442 (33.7)	<0.01
26-29				
Total number	3345	3920	4005	
CLD	1012 (32.4)	1038 (28.4)	1003 (26.6)	<0.01
NEC	191 (5.8)	268 (6.9)	221 (5.7)	0.74
Sepsis	570 (17.0)	884 (22.6)	661 (16.5)	0.33

* Data are reported as number (%).
† Assessed by Cochran-Armitage trend test.

indications for use, potential adverse effects of blood product administration, and any associations with benefits and risks.

In conclusion, evolutionary practice changes and relatively high tolerance for anemia may be associated with an observed reduction in RBC usage in recent years in neonates born at at least 26 weeks' gestation; however, increased survival may be associated with higher usage of blood products at extremely low gestational age. Despite these observations, preterm neonates born at less than 30 weeks' gestation remain very high frequency users of blood products. Collaboration across countries and research networks is needed to further understand the benefits and risk of transfusion in this vulnerable patient group.

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CONFLICT OF INTEREST

The authors have disclosed no conflicts of interest.

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Supplementary material for Chapter 2

ERRATUM

Keir AK, Yang J, Harrison A, Pelausa E, Shah PS and on behalf of the Canadian Neonatal Network. Temporal changes in blood product usage in preterm neonates born at less than 30 weeks' gestation in Canada. Transfusion 2015;55:1340-6.

The authors regret that Figure 3 was a duplication of Figure 2. Please see the correct Figure 3 below:

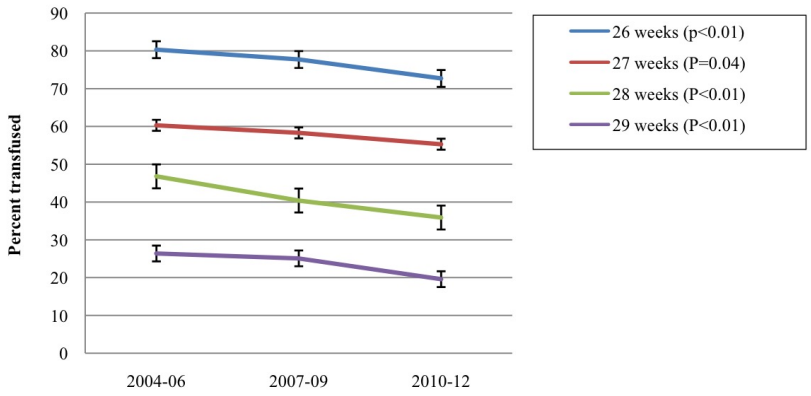

Fig. 3. Infants 26-29 weeks GA transfused with RBCs: 2004-2012

doi:10.1111/trf.13326

Chapter 3

Adverse effects and associations of neonatal red blood cell transfusions

This published protocol, systematic review and meta-analysis describes the adverse effects and associations attributed to RBC transfusions in neonates.

It addresses the previously identified research question:

Research question 2:

What are the current known adverse effects and associations of neonatal RBC transfusions?

Authorship forms are provided in Appendix C

Keir et al. Systematic Reviews 2014, 3:92
http://www.systematicreviewsjournal.com/content/3/1/92

SYSTEMATIC REVIEWS

PROTOCOL Open Access

Adverse effects of small-volume red blood cell transfusions in the neonatal population

Amy Keir[1,2*], Sanchita Pal[3], Marialena Trivella[4], Lani Lieberman[5,6], Jeannie Callum[7,8], Nadine Shehata[8,9] and Simon Stanworth[10]

Abstract

Background: Adverse transfusion reactions in the neonatal population are poorly understood and defined. The incidence and pattern of adverse effects due to red blood cell (RBC) transfusion are not well known, and there has been no systematic review of published adverse events. RBC transfusions continue to be linked to the development of morbidities unique to neonates, including chronic lung disease, retinopathy of prematurity, intraventricular haemorrhage and necrotising enterocolitis. Uncertainties about the exact nature of risks alongside benefits of RBC transfusion may contribute to evidence of widespread variation in neonatal RBC transfusion practice.
Our review aims to describe clinical adverse effects attributed to small-volume (10–20 mL/kg) RBC transfusions and, where possible, their incidence rates in the neonatal population through the systematic identification of all relevant studies.

Methods: A comprehensive search of the following bibliographic databases will be performed: MEDLINE (PubMed/OVID which includes the Cochrane Library) and EMBASE (OVID). The intervention of interest is small-volume (10–20 mL/kg) RBC transfusions in the neonatal population.
We will undertake a narrative synthesis of the evidence. If clinical similarity and data quantity and quality permit, we will also carry out meta-analyses on the listed outcomes.

Discussion: This systematic review will identify and synthesise the reported adverse effects and associations of RBC transfusions in the neonatal population. We believe that this systematic review is timely and will make a valuable contribution to highlight an existing research gap.

Trial Registration: PROSPERO, CRD42013005107
http://www.crd.york.ac.uk/PROSPERO/display_record.asp?ID=CRD42013005107

Keywords: Transfusion/adverse effects, Neonates, Systematic review, Red blood cell transfusion, Transfusion reaction

Background

Anaemia of prematurity (AOP) is a multifactorial condition with diminished plasma erythropoietin (EPO) levels in response to anaemia and hypoxia, reduced red cell life span, phlebotomy losses for laboratory testing, limited transplacental transfer of iron due to premature birth and dependence on hepatic EPO production [1]. Small-volume red blood cell (RBC) transfusions are often used to manage AOP with over 90% of preterm neonates with a birthweight at <1,000 g receiving at least one RBC transfusion [2,3]. RBC transfusions are given with the assumption that the transfusion will lead to an increase in oxygen delivery to tissues, thereby providing a rapid and effective intervention.

However, RBC transfusions are biological products, with recognised risks. Adverse effects may be classified broadly as those related to errors in the processing, storage and administration or as actual medical complications. Interpretation of the data from the UK Serious Hazards of Transfusion (SHOT) National Haemovigilance Scheme of

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a population-based epidemiological study of transfused patients has suggested that a disproportionate increased number of adverse events occur in children compared to adults, and more so in neonates [4]. A significant proportion of these reports were related to transfusion errors, including transfusion of an incorrect blood component. While SHOT has received numerous reports related to transfusion errors in the neonatal age group, there have been relatively fewer adverse reactions to transfusion reported. In the 2011 Annual SHOT report [5], there were no reports of transfusion-related lung injury (TRALI) in neonates. There were five paediatric reports classified as transfusion-associated circulatory overload (TACO) that included one neonate. It seems likely that there is under-recognition and/or under-reporting of transfusion-related adverse events in neonates [6,7] due to pre-existing critical illness, in particular around the recognition of TRALI [8] as many preterm neonates having intercurrent respiratory disease. This is compounded by the difficulties in defining adverse transfusion events in a neonatal setting.

There are several recognised potential adverse associations related to RBC transfusions unique to neonates [9]. Associations between receipt of RBC transfusions and development of necrotising enterocolitis [10], intraventricular haemorrhage [11,12] retinopathy of prematurity [13], chronic lung disease [14] as well as mortality [15,16] have all been described. The exact nature of these potential risks, alongside benefits of RBC transfusions, has likely contributed to widespread variation in neonatal RBC transfusion practice [17]. To date, there has been no systematic collation of adverse effects due to, or associated with, RBC transfusion in neonates nor assessment of the degree to which biases operate to mitigate for or against the strengths of associations with risks.

Our review aims to describe clinical adverse effects attributed to small-volume (10–20 mL/kg) RBC transfusions and, where possible, their incidence rates in the neonatal population through the systematic identification of all relevant studies. It is likely that our review will find that reporting of adverse events related to neonatal transfusion is variably described in the literature and there is a need for standardisation of definitions in this area.

Methods/design

This review will be reported in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement [18]. It has also been registered in the PROSPERO international prospective register of systematic reviews (registration number: CRD42013005107).

Study eligibility

We will include both randomised (including cluster-randomised and quasi-randomised) and non-randomised studies (including observational, cross-sectional, experimental and retrospective), with the *proviso* that any analysis will be carried out separately for randomised and non-randomised studies. Only studies examining the effects of RBC transfusion on neonates and have at least one outcome deemed relevant to our review will be included. Studies will not need to have a comparator group to be included; however, only those with a comparator group will be used in any meta-analysis. Our review will also focus its interpretation on those studies with a comparator group.

We will exclude reviews, case series with less than five neonatal participants, case reports, animal studies and laboratory (in vitro) studies. We will exclude studies that examine exchange transfusion, foetal (in utero) transfusion, large-volume transfusions and transfusions used in cardiac surgery and for extracorporeal membrane oxygenation (ECMO). These studies were excluded as we have chosen to focus on the potential adverse effects of small-volume RBC transfusions only.

Population

Neonates who received at least one RBC transfusion will be considered. Infants are defined for the purposes of this review as neonates less than 28 days of age and premature neonates (<37 weeks gestation) up to four weeks post-term corrected age.

Interventions

The intervention of interest is small-volume (10–20 mL/kg) RBC transfusions.

Comparators

For studies with a comparator group, we will include studies comparing

1. RBC transfusion with no RBC transfusion
2. Higher versus lower RBC transfusion threshold (or comparisons among RBC transfusion thresholds)
3. Higher versus lower RBC transfusion volumes
4. RBC transfusion products (e.g. leukodepletion, irradiation, age of RBC product, anticoagulant preparation versus non-modified)
5. RBC transfusion with an alternative therapy (e.g. erythropoietin-stimulating agents)

Outcomes

Depending on data availability, our outcomes will be considered separately for 'strong' (e.g. immune-mediated transfusion reactions) and 'less certain' (e.g. late-onset sepsis, NEC, BPD, severe ROP, etc.) causal pathways from transfusion to event.

Primary outcomes

1. Mortality associated with receipt of RBC transfusion
   i. Within 24–48 h of receipt of a RBC transfusion.
   ii. Before discharge from initial hospitalisation.
2. Complications during hospital stay
   Chronic lung disease (defined as requirement of supplemental oxygen at 36 weeks gestation), retinopathy of prematurity (grade 3 or above) [19], necrotising enterocolitis (stage 2 or greater using Bell's criteria) [20], intraventricular haemorrhage (grade 3 or 4) [21], adverse neurodevelopmental outcomes (at 18–24 months corrected age), cerebral palsy diagnosed following physician assessment or developmental delay (IQ or DQ > 2 standard deviations below the mean on a validated assessment tool of cognitive function), or blindness (visual acuity).

Secondary outcomes

1. Adverse transfusion events
   Immune-mediated transfusion reactions (acute haemolytic transfusion reactions, febrile non-haemolytic transfusion reactions and transfusion-related acute lung injury) within 48 h of receipt of RBC transfusion.
   Acute non-immune-mediated transfusion reactions (transfusion-related circulatory overload, metabolic complications including hypocalcaemia, hyperkalaemia, hyper/hypoglycaemia and hypothermia) within 48 h of receipt of RBC transfusion.
   Alloimmunisation, transfusion-associated graft versus host disease, post-transfusion purpura, infectious adverse effects (transfusion-transmitted infection, e.g. hepatitis B, hepatitis C, HIV, HTLV, parasites), bacterial contamination/sepsis, incorrect blood component transfused and/or adverse events or reactions associated with directed donation.
   If data availability allows, we will examine adverse transfusion events in the individual categories as outlined above.
2. Longer-term outcomes
   Long-term mortality, measured at 18–24 months, associated with previous transfusion complications/events in the neonatal period.
3. Adverse neurodevelopmental outcomes at 18–24 months, associated with previous transfusion complications/events in the neonatal period.
   Composite outcomes of relevance or additional adverse events not previously identified will also be included.

Search strategy

There will be no language restrictions, and we will attempt to translate articles in languages other than English, depending on translational services available. Literature published from 1990 onwards will be searched and studies clearly completed prior to 1990 will be excluded. These studies will be excluded as since the 1990s, increasingly restrictive RBC transfusion practices have been introduced and changes in RBC products transfused (primarily leukoreduction) have occurred. Literature and studies from 1990 onwards are more likely to reflect current neonatal transfusion practices. We will include studies available as full-text publications only as it will be difficult to apply all selection criteria and extract data for abstract-only publications. A comprehensive search of the following bibliographic databases will be performed, including MEDLINE (PubMed/OVID), EMBASE (OVID) and the CENTRAL database of the Cochrane Library. We will also undertake hand searching of reference lists and contact authors of relevant studies. We will not review other grey literature. The search strategy will include only terms relating to and describing the participants and the intervention. We will use both free-text terms and controlled vocabulary.

Selection of studies

Two reviewers will independently screen all electronically derived citations/abstracts of papers identified by the review search strategy for relevance. At this stage, screening will be based on title and abstract, and only clearly irrelevant studies will be excluded. Full text will be obtained for a selection of potentially relevant studies. The two reviewers will then formally assess the full texts for eligibility. If necessary, further information will be sought from the authors where articles contain insufficient data to make a decision about eligibility. Potential disagreements between the review authors will be resolved by consensus. If an agreement cannot be reached, a third reviewer will adjudicate. Details of excluded studies will be recorded as well as reasons for exclusion. The review authors will not be blinded to names of authors, institutions, journals or the outcomes of the trials. If any of the review group is an author on a paper identified in the search, they will be excluded from making a decision whether or not to include the study in the review, and another member of the group will make the decision.

Data extraction

Two authors will conduct data extraction independently using a data extraction form designed and piloted specifically for this systematic review. The pilot process for the data form will involve the two authors extracting data from at least one of each of the included study types for the review. The data extraction forms will then

be reviewed by the two senior members of the authorship group and revised as required. Data extracted will include information regarding study design, participants, definitions of adverse effects and associations (outcomes), RBC transfusion regimen and the control/comparison if applicable, neonatal adverse effects reported and results relevant to the review, the risk of bias assessment, including an assessment on confounding, relevance and funding sources. Specific details regarding adverse effects and associations, including grade or severity, will also be collected including were they clearly defined *a priori* and what was the period of follow-up of study participants.

If an agreement cannot be reached over any aspects of data extraction, a third reviewer will adjudicate.

Methodological quality assessment and risk of bias assessment

Studies will not be excluded based on quality of research methods. A formal risk of bias assessment will be performed. For randomised controlled trials, the Cochrane Collaboration's tool for assessing risk of bias will be used. For non-randomised studies, a modified Newcastle-Ottawa Scale (NOS) will be used to assess the quality of non-randomised studies and it will also be used to assess those without a comparator group. We are aware that the Cochrane Collaboration is developing a new risk of bias tool for non-randomised studies. If a working draft is available in time, we will also consider relevant items from this tool for inclusion into our risk of bias assessment (modified NOS). We plan to undertake sensitivity analysis by grading studies at low or high risk of bias (qualitative assessment only). We will factor in all aspects of risk of bias, for both qualitative and quantitative syntheses, when interpreting the evidence, and this will include formal risk of bias assessments, study design and quantity of data. We will separately present findings in tables for comparative and non-comparative studies. Although conclusions will be drawn from both groups, the focus of interpretation will be on studies with comparator arms, and this will apply for any quantitative analysis.

Analysis plan

Qualitative synthesis

The main analysis will be descriptive. We will provide a qualitative synthesis from the eligible studies, categorised by the type of adverse effect for primary outcomes and causal pathway for secondary outcomes. This section aims to provide a summary of adverse effects attributed to the receipt of RBC transfusion in the neonatal population.

Quantitative synthesis

If data allows a quantitative analysis of outcome data, we will analyse separately randomised and non-randomised studies. We are expecting that there will be heterogeneity among included studies, and hence, random effects models will be used to calculate separate pooled estimates for each study type. If available and according to study design, odds ratios (ORs), risk ratios (RRs), hazard ratios (HRs) and incidence ratios (IRs) will be pooled separately. If the number of studies providing data is small, and if the number of events is rather small, then it is expected that these relative measures will yield similar results. In this case, and in order to reduce heterogeneity and provide more robust estimates, we will attempt to transform ORs, RRs and HRs into a single metric [22], and we will support this strategy with a sensitivity analysis by type of measure.

We will explore clinical heterogeneity concentrating on the different RBC transfusion strategies and settings. Statistical heterogeneity (where meta-analysis is feasible) will be assessed by the I^2 test, with values above 80% classed as considerable heterogeneity. We will approach pooling cautiously, and if $I^2 > 80\%$, we will not provide pooled results, but instead we will provide information either on a table or an un-pooled forest plot. If the data permits, we will carry out subgroup analysis and sensitivity analysis based on the different types of effect measure (if they have been combined as mentioned earlier). We will also carry out sensitivity analysis based on the risk of bias assessment in terms of selection bias, and any identified confounding factors.

Discussion

This systematic review will identify and synthesise the reported adverse effects and associations of RBC transfusions in the neonatal population.

The limited reporting of adverse effects in neonatal transfusion trials, the quality of the studies identified as well as the risk of bias inherent in studies in this area are likely to be significant limitations to our review [9]. However, the identification and collation of all current known adverse effects due to, or associated with, RBC transfusion in neonates are key steps in improving the reporting of these important events. The need for standardised neonatal definitions for all relevant adverse effects is also likely to be highlighted by this review, as well as the need for consistent reporting.

By drawing together the current known adverse effects and associations of RBC transfusion in neonates, we aim to provide a clear overview of this area and clarify future research areas. This protocol may also be used in the future to examine the potential adverse effects of other blood products and intravenous fluids used in the neonatal population. We believe that this systematic review is timely and will make a valuable contribution through highlighting existing research gaps.

Abbreviations
EMBASE: Excerpta Medica Database; OR: Odds ratio; PRISMA: Preferred Reporting Items for Systematic Reviews and Meta-Analyses; PROSPERO: International Prospective Register of Systematic Reviews; RBC: Red blood cell; RR: Risk ratio.

Competing interests
The authors have no competing interests to declare.

Authors' contributions
AK, SP, MT, LL, JC, NS and SS participated in the design of the protocol and helped to draft the manuscript. All authors read and approved the final manuscript.

Authors' information
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Adverse effects of red blood cell transfusions in neonates: a systematic review and meta-analysis

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BACKGROUND: Controversy exists regarding the contribution of blood transfusions to a range of adverse clinical outcomes in neonates. The aim of our systematic review was to identify the broader literature on harmful effects and associations potentially attributable to red blood cell (RBC) transfusions.

STUDY DESIGN AND METHODS: A comprehensive search of MEDLINE (PubMed) and EMBASE was undertaken. Eligible studies included both randomized controlled trials (RCTs) and nonrandomized studies examining the effects of small volume (10-20 mL/kg) RBC transfusions on neonates. Primary outcomes of interest were mortality, chronic lung disease, retinopathy of prematurity, necrotizing enterocolitis, and intraventricular hemorrhage. Two independent authors conducted a review of abstracts and then of full-text article reviews as well as data extraction and quality assessments.

RESULTS: Sixty-one studies were eligible for inclusion, including 16 (26%) randomized studies. The majority of studies were nonrandomized (n = 45; 74%), which included 32 observational studies with and 13 studies without a comparator group. There was no evidence that rates of mortality differed between restrictive and liberal strategies for transfusion (eight RCTs: risk ratio, 1.24; 95% confidence interval, 0.89-1.672, heterogeneity = 0%) or for necrotizing enterocolitis (five RCTs: risk ratio, 1.45; 95% confidence interval, 0.91-2.33; heterogeneity = 0%). A liberal strategy also was not superior to restrictive transfusion practice in the pooled randomized studies for rates of retinopathy of prematurity, chronic lung disease, or intraventricular hemorrhage.

CONCLUSIONS: Statistically significant differences in a range of harmful outcomes between neonates exposed to restrictive and liberal RBC transfusion practice were not found. However, the risks of bias identified in many studies and the lack of consistent reporting and definitions of events limits our conclusions.

Neonates represent a group of intensive red blood cell (RBC) transfusion recipients. Over one-half of infants at less than 30 weeks' gestation (GA) and more than 80% of infants with a birthweight (BW) of less than 1000 grams receive at least one RBC transfusion during initial hospitalization.[1] These RBC transfusions are administered with the assumption that the transfusion will lead to an increase in oxygen delivery to tissues, thereby providing a rapid and effective intervention. However, RBC transfusions are biological

ABBREVIATIONS: BW = birth weight; CLD = chronic lung disease; FNHTR = febrile nonhemolytic transfusion reaction; GA = gestational age; IVH = intraventricular hemorrhage; NEC = necrotizing enterocolitis; RBC(s) = red blood cell(s); ROP = retinopathy of prematurity; RR = risk ratio.

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products with recognized risks. It has been reported that many adverse associations and outcomes are potentially related to RBC transfusions, including the development of necrotizing enterocolitis (NEC),[2] intraventricular hemorrhage (IVH),[3,4] retinopathy of prematurity (ROP),[5] and chronic lung disease (CLD).[6] One approach to evaluate the relationships between risks and the use of RBC transfusions is to report rates of adverse outcomes in patients recruited to clinical studies, comparing liberal versus restrictive use of blood. In adults, Villeneuva et al.[7] reported higher mortality in patients with gastrointestinal bleeding who received more liberal use of RBC transfusion, and a recent meta-analysis suggests that hospital-associated infections were seen more frequently in patients enrolled into liberal arms of studies.[8]

These risks and potential adverse associations with RBC transfusions are poorly described in neonates. The objective of this systematic review was to collate the clinical adverse effects and associations attributed to RBC transfusions through the identification of all relevant randomized and nonrandomized studies and conduct meta-analyses for key adverse outcomes, comparing liberal versus restrictive transfusion practices.

MATERIALS AND METHODS

This review is reported in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement.[9] A full version of the review protocol has been published[10]; therefore, only a summary of the review protocol is provided here.

Participants and intervention

Neonates who received at least one RBC transfusion were considered. Neonates were defined as infants less than 28 days of age and premature infants (<37 weeks' GA) up to 28 days post-term–corrected age. The intervention of interest was small volume (10-20 mL/kg) RBC transfusions.

Comparators

For studies with a comparator group, we included studies that compared the following:

1. RBC transfusion versus no RBC transfusion;
2. Higher versus lower RBC transfusion thresholds or comparisons among RBC transfusion thresholds;
3. Higher versus lower RBC transfusion volumes;
4. Comparisons among RBC transfusions products, e.g., leukodepletion, irradiation, age of RBC product, use of different anticoagulants; and
5. RBC transfusion compared with an alternative therapy, e.g., erythropoietin.

We classified studies in which there was a difference in transfusion numbers and/or volume between groups to compare liberal versus restrictive RBC transfusion practices. Liberal transfusion practice was defined as one group receiving a greater volume and/or number of RBC transfusions compared with the comparison group (restrictive transfusion practice).

Outcomes

Primary outcomes of interest (potential adverse associations of RBC transfusion) were:

- Mortality within 24 hours of receiving an RBC transfusion and before discharge from initial hospitalization; and
- Complications during hospital stay, including CLD (defined as requirement of supplemental oxygen at 36 weeks' gestation), ROP grade 3 or above,[11] NEC stage II or greater using Bell's criteria,[12] IVH grade 3 or 4,[13] adverse neurodevelopmental outcomes at 18 to 24 months corrected for age (defined as cerebral palsy diagnosed after physician assessment or developmental delay [intelligence quotient or development quotient > 2 standard deviations below the mean on a validated assessment tool of cognitive function]), and blindness (visual acuity).

The secondary outcomes (potential adverse effects of RBC transfusion) were:

- Adverse transfusion effects defined as immune-mediated transfusion reactions within 48 hours of receipt of RBC transfusion;
- Acute nonimmune-mediated transfusion reactions within 48 hours of receipt of RBC transfusion; and
- Alloimmunization, transfusion-associated graft versus host disease, post-transfusion purpura, infectious adverse effects, bacterial contamination/sepsis, incorrect blood component transfused, and/or adverse events or reactions associated with directed donation.

Eligibility criteria

We considered both randomized and nonrandomized studies. Only studies that examined the effects of RBC transfusion on neonates and had at least one outcome deemed relevant to the review were included. We included studies with and without a comparator group. Studies without a comparator group were included to ensure the broadest description of reported adverse transfusion effects and associations, accepting the limitations of these study designs to inform relative risks. We excluded case series that had less than five neonatal participants, case reports, animal studies, and laboratory (in vitro) studies.

Search strategy

The literature published from 1990 onward was searched, because significant changes in neonatal and blood banking practices were recognized to have occurred after this time (for example, leukodepletion). The search strategy is shown in Appendix S1 (available as supporting information in the online version of this paper).

Quality assessment and risk of bias in individual studies

We undertook a modified Newcastle-Ottawa Scale to assess the quality of randomized and nonrandomized studies with a focus on adverse effects. Part of this quality assessment for the nonrandomized studies included the identification of potential confounding variables around receipt of RBC transfusion, including birth weight, gestational age, presence/absence of sepsis, respiratory disease or need for support, and/or an illness severity score. These confounding factors were identified through an expert group consensus, which included transfusion medicine specialists, neonatologists, and hematologists. These confounding factors were selected based on the principle that, the more critically unwell the patient, the more likely an RBC transfusion will be prescribed.[14] For randomized studies, the Cochrane Collaboration's tool for assessing the risk of bias was to be used; however, bias assessments had previously been undertaken for the majority of the randomized studies identified by our review[15-17] and thus were not repeated.[16,18]

Analysis

Meta-analyses were undertaken when there were at least two studies comparing liberal versus restrictive transfusion practice for each outcome of interest; otherwise, a qualitative summary was provided. The randomized and nonrandomized studies were pooled separately for each outcome. For randomized studies, the treatment effect measures across individual studies were reported as the risk ratio (RR) with 95% confidence intervals (CIs). Heterogeneity was assessed using the chi-square test, with the significance level set at $p < 0.1$. The I^2 statistic was used to quantify the amount of possible heterogeneity, in which a threshold of $I^2 > 40\%$ indicates moderate heterogeneity, and a threshold of $I^2 > 80\%$ indicates considerable heterogeneity. For nonrandomized studies, effect estimates, where possible, were presented as RRs with 95% CIs. Only nonrandomized cohort studies with comparator groups were included in the meta-analysis for nonrandomized studies. No data from the included studies were transformed for inclusion in the meta-analyses.

RESULTS

Search results

Included and excluded studies

In total, 190 full-text publications were reviewed and, of these, 61 were deemed eligible for inclusion in the review (Appendix S2; Fig. S1, available as supporting information in the online version of this paper). Study designs and settings are summarized in Appendix S2 (Fig. S2). Summary characteristics for each study included in the review, grouped by study type, are provided in Appendix S3 (Tables S1-S3, available as supporting information in the online version of this paper).

Quality assessments across studies

Appendix S4 (Tables S4 and S5, available as supporting information in the online version of this paper) outlines the individual quality assessments for the included studies, and Appendix S5 (Figs. S3 and S4, available as supporting information in the online version of this paper) summarizes the quality assessments by study type and outcome.

Bias assessments undertaken for the majority of the randomized studies identified by this review[15-17] have been previously reported. Sources of bias identified included inability to blind caregivers to the intervention and protocol violations.[16] One review reported significant concerns around the methodological quality of many of the randomized controlled studies that also were identified by our review.[17] No significant concerns regarding the risk of bias were raised by the other systematic review.[15] The quality assessments of the nonrandomized studies found that many of these studies did not clearly identify potential confounding factors, did not adjust results to take into account potential confounding factors, and did not provide clear definitions of adverse effects related to transfusion.

Effects of interventions on primary outcomes

A summary of the RRs for randomized and nonrandomized studies for mortality, CLD, ROP, NEC, IVH, and sepsis is provided in Table 1, and detailed meta-analyses are displayed in Appendix S6 (Figs. S5-S15, available as supporting information in the online version of this paper).

Nineteen studies (31%) comparing restrictive and liberal RBC transfusion practice groups contributed to the systematic review and reported mortality during initial hospitalization as an outcome. Overall, 4036 infants were included, and the median sample size was 76 (range, 16-1077 infants). CLD was reported in 18 studies (30%) that included 4991 infants, and the median sample size was 289 (range, 22-2440 infants). Thirteen studies (21%) reported ROP (grade 3 or greater) as an outcome, with a total of 4859 infants and a median sample size of 210 (range, 20-2437 infants). IVH (grade 3 or greater) was

TABLE 1. Summary of meta-analysis of studies comparing liberal versus restrictive transfusion practice*

	Randomized studies		Nonrandomized studies	
Mortality before discharge from initial hospitalization	Eight studies; 951 infants included	RR, 1.24; 95% CI, 0.89-1.672; $I^2 = 0\%$	Five studies; 1053 infants included	RR, 0.73; 95% CI, 0.50-1.07; $I^2 = 10\%$
Chronic lung disease	Seven studies; 645 infants included	RR, 0.95; 95% CI, 0.82-1.10; $I^2 = 2\%$	Three studies; 606 infants included	RR, 0.92; 95% CI, 0.71-1.20; $I^2 = 0\%$
Retinopathy of prematurity	Seven studies; 831 infants included	RR, 0.88; 95% CI, 0.60-1.27; $I^2 = 0\%$	Two studies; 2872 infants included	RR, 0.65; 95% CI, 0.34-1.27; $I^2 = 41\%$
Necrotizing enterocolitis	Five studies; 887 infants included	RR, 1.45; 95% CI, 0.91-2.33; $I^2 = 0\%$	Meta-analysis not possible	
Intraventricular hemorrhage	Three studies; 374 infants included	RR, 0.59; 95% CI, 0.25-1.44; $I^2 = 0\%$	Two studies; 578 infants included	RR, 1.01; 95% CI, 0.53-1.93; $I^2 = 0\%$
Bacterial contamination/ sepsis	Two studies; 484 infants included	RR, 1.0; 95% CI, 0.80-1.26; $I^2 = 7\%$	Two studies; 395 infants included	RR, 1.06; 95% CI, 0.88-1.26; $I^2 = 0\%$

* RR > 1 favors liberal transfusion; RR < 1 favors restrictive transfusion.
I^2 = heterogeneity statistic.

identified in 12 studies (20%) as an outcome. The total number of infants included was 13,981, and the median sample size was 393 (range, 33-10,280 infants).

The results from meta-analyses did not identify any differences in restrictive and liberal RBC transfusion practice for any of the primary outcomes, including mortality (RR, 1.24; 95% CI, 0.89-1.672; $I^2 = 0\%$), CLD (RR, 0.95; 95% CI, 0.82-1.10; $I^2 = 2\%$), ROP (RR, 0.88; 95% CI, 0.60-1.27; $I^2 = 0\%$), NEC (RR, 1.45; 95% CI, 0.91-2.33; $I^2 = 0\%$), or IVH (RR, 0.59; 95% CI, 0.25-1.44; $I^2 = 0\%$) (Table 1). Meta-analyses of nonrandomized studies also did not demonstrate a statistically significant difference in restrictive and liberal RBC transfusion practice for mortality (RR, 0.73; 95% CI, 0.50-1.07; $I^2 = 10\%$), CLD (RR, 0.92; 95% CI, 0.71-1.20; $I^2 = 0\%$), ROP (RR, 0.65; 95% CI, 0.34-1.27; $I^2 = 41\%$), or IVH (RR, 1.01; 95% CI, 0.53-1.93; $I^2 = 0\%$) (Table 1).

NEC was reported in 24 studies (39%), and 11 of those same studies also identified transfusion-associated NEC, which was defined as the development of NEC within 24 to 72 hours of receipt of an RBC transfusion and was also included as an additional outcome for some included studies. The total number of included infants was 24,366, and the median sample size was 228 (range, 17-10,280 infants). Meta-analysis was not possible for nonrandomized studies due to a lack of studies comparing restrictive and liberal transfusion practice; however, the relative risk of NEC was not increased for infants who received liberal transfusions (Table 1).

Neurodevelopmental outcomes were the focus in two studies (3%) studies that included a total of 493 participants. Whyte et al.[19] defined adverse neurodevelopmental outcomes as cerebral palsy, cognitive delay (Mental Developmental Index <70), and severe visual or hearing impairment at 18 to 21 months of follow-up; and von Lindern et al.[20] defined an adverse neurodevelopmental outcome as a composite outcome of post-discharge mortality, severe hearing or visual impairment, and neuromotor development delay (<1 standard deviation below the mean) at 24 months. One study was a long-term follow-up of a previous randomized study, and the other was a retrospective study that included a comparator group.[19,20] Longer-term neurodevelopmental outcomes did not differ between groups for either study. Follow-up was complete (>80%) for both studies. The definition of adverse neurodevelopmental outcome was the same for both studies and was consistent with the outcome definition of this review. Meta-analysis was not possible for this outcome, because only one randomized study and one nonrandomized study were identified.

Secondary outcomes

Meta-analysis was not possible for the assessment of secondary outcomes (excluding sepsis) due to lack of comparable studies.

Transfusion-related infections

Two studies, both of which were case series, reported transfusion-related babesiosis as a study outcome and included six or seven infants in each, all with babesiosis related to the receipt of RBC transfusion. The definition of transfusion-related babesiosis was consistent across both case series. One study reported transfusion-related malaria as an outcome in 51 of 57 infants (90%) who received transfusion and was undertaken in a developing country. Another prospective observational study reported two cases of cellulitis (n = 157; 1%) relating to equipment used for transfusion and also was undertaken in a developing country.

Adverse transfusion events

Immune-mediated transfusion reactions within 48 hours of an RBC transfusion

Two studies (3%) identified a febrile nonhemolytic transfusion reaction (FNHTR) as an outcome with samples of

100 and 110 transfusion events examined.[21,22] Both studies were prospective observational studies performed in developing countries. The definition of FNHTR was the same for both studies. Eight separate FNHTR events were reported from a total of 210 transfusion events (3.8%).

Alloimmunization

Alloimmunization was an outcome in two studies (3%) that had sample sizes of 47 and 26 neonates. One study was randomized,[23] and the other was a prospective observational study.[24] In the randomized study, no RBC antibodies were observed in the first 6 months of life when tested for in 17 of 23 infants (74%) versus 18 of 24 infants (75%), and the remaining infants were not tested.[23] In the other study, four of 26 infants (15%) tested produced white blood cell antibodies.[24] The definition of alloimmunization was the same for both studies. For one of the two studies, there was incomplete follow-up of infants (<80%),[23] and follow-up was more complete (>80%) in the other study.[24]

Bacterial contamination/sepsis

Six studies (10%) that included 1771 infants reported sepsis as an outcome. The median sample size was 274 (range, 33-515 infants). None of the six included studies reported any difference in sepsis between comparator groups. No study that reported sepsis as an outcome was able to attribute the RBC transfusion directly to the development of infection. Results from meta-analyses of randomized and nonrandomized studies are shown in Table 1.

Lead exposure

Lead exposure secondary to RBC transfusion was reported in two prospective observational studies. The definition of excess intravenous lead exposure was extrapolated from World Health Organization data on excess oral lead exposure.[25] One prospective observational study[26] undertaken in the United States from 1991 to 1992 reported pretransfusion and post-transfusion lead levels. Seventy-nine transfusion events were reported, and 71 transfusions (90%) exceeded daily acceptable lead exposure. The second study,[27] which also was undertaken in the United States from 2008 to 2009, reported elevated lead levels in the RBC units received by infants as transfusions.

Glucose and electrolyte disturbances

Changes in storage media and other alterations in RBC transfusion practice have been implicated in glucose and electrolyte disturbances. Three studies observed for hyperglycemia as an outcome, with a median sample size of 61 (range, 21-87 infants) and a total of 169 infants. One study was a randomized study, and two were nonrandomized, including a prospective cohort study without a comparator group and a retrospective study without a comparator group. No episodes of hyperglycemia were reported by any of the studies. One retrospective study reported hypoglycemia as a study outcome, in which one of 61 infants experienced an episode of hypoglycemia post-transfusion. Nine studies (16%) reported hyperkalemia as an outcome, with variation in reporting as either individual infants or transfusion events. Two studies reported hyperkalemic events: a randomized study reported that two of 12 transfusion events (17%) in both groups were associated with hyperkalemia,[28] and the other retrospective study without a comparator group reported that three of 61 transfusion events (5%) were associated with hyperkalemia.[29] There were no reported adverse clinical effects secondary to hyperkalemia in either of those studies. There were no differences in hyperkalemic episodes between comparator groups from any of the included studies. Two studies identified hypocalcemia as an outcome, with sample sizes of 61 and 52 neonates: one study was randomized and reported no hypocalcemic events,[30] and the other, a prospective observational study, reported one hypocalcemic event in one of 61 (2%) infants.[29] The definitions of hyperglycemia, hypoglycemia, hyperkalemia, and hypocalcemia were not clearly described, nor were normal ranges provided, although pretransfusion and post-transfusion values were provided by all three studies. The timing of pretransfusion and post-transfusion measurements was not standardized.

Outcomes with no data available

No data were available for the following outcomes: mortality within 24 to 48 hours of receipt of an RBC transfusion, acute hemolytic transfusion reactions, transfusion-related acute lung injury, transfusion-related circulatory overload, transfusion-associated graft versus host disease, post-transfusion purpura, incorrect blood component transfused, or adverse events/reactions associated with direct donation.

DISCUSSION

In this systematic review, we identified 61 studies that reported risks of neonatal small-volume RBC transfusion practice. The aim of this review was to provide a broad synopsis of all reported risks to better understand the clinical adverse effects and associations attributed to RBC transfusions. We did not find a statistically significant association between a range of harmful outcomes and neonates exposed to restrictive or liberal RBC transfusion practices. Meta-analyses of studies that included a comparator group did not identify any consistent differences in the main outcomes, including mortality during initial hospitalization, CLD, NEC, IVH, and bacterial contamination/sepsis between neonates exposed to higher or lower volumes of RBC transfusions, in either randomized or nonrandomized studies. Our findings appear to be

contrary to current opinion about the risks of transfusion in the neonatal population.[2,18,31,32] Other studies have reported a range of risks, including associations with increased rates of NEC,[2] IVH,[3] CLD,[6] retinopathy of prematurity,[5] and mortality[33]; however, the results from our review do not support these findings when looking across the broader literature.

Although studies of a diverse nature with a variety of specific aims and primary outcomes were identified in this review, measures of statistical heterogeneity in the majority of analyses appeared to be low. Despite the number of identified studies, the quality of studies that reported many of the primary and secondary outcomes was limited. In addition, although approximately one-third of identified studies were randomized, the sample sizes in many studies would be considered inadequate to thoroughly address harm. The lack of controlling for potential confounding factors in the nonrandomized studies was a recurrent finding. Many of the studies were likely to be affected by confounding from indication bias, with the more critically ill infants more likely to receive RBC transfusion. Another potential limitation of our review included the potential relationship between the receipt of an RBC transfusion and the development of ROP. Although no differences in the outcome of severe ROP was observed between liberal and restrictive transfusion groups in the included studies, it is possible the use of erythropoietin may have affected outcomes. A detailed analysis of this was beyond the scope of the review.

The substantial methodological heterogeneity of the reports included, particularly among nonrandomized studies, further complicates the interpretation of our review. We specifically considered our findings for studies that were considered at low risk of bias. For studies at lower risk of bias (namely, randomized controlled studies and some of the better designed nonrandomized studies), our interpretation of the evidence remained unchanged, and liberal versus restrictive RBC transfusion practice in neonates was not associated with significant differences in morbidities or mortality.

Of note, very few studies included in our review provided clear definitions of the different potential adverse effects related to RBC transfusion. There is ongoing interest in understanding the risks of transfusion-associated circulatory overload and transfusion-related acute lung injury in hemovigilance systems for critically ill populations; however, it is not known how these definitions for adults relate to neonatal populations or how they may require modification for neonatal use. The standardization of definitions of adverse effects and associations of RBC transfusion in neonates, through an international consensus, is required.

In summary, this review did not demonstrate statistically significant differences in outcomes for infants who were exposed to different dose strategies for RBC transfusion practice. However, the limitations of much of the primary study evidence need to be acknowledged. The current findings do not advocate for the safety of either liberal or restrictive transfusion triggers but reiterate the importance of further research. RBC transfusion remains common practice in neonatal units,[1] and the findings from this review specifically highlight the 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 characterized by prematurity and relative immunologic immaturity. A continued focus on retrospective studies that report potential associations between RBC transfusion and the development of NEC, a devastating but rare disease, may have diverted attention from higher quality study designs to establish the real risks of neonatal transfusion. International multicenter research collaborations will be required to definitively determine the risk of RBC transfusion in neonates.

CONFLICT OF INTEREST

The authors have disclosed no conflicts of interest.

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SUPPORTING INFORMATION

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Appendix 6S

Fig. S5. Meta-analysis for mortality during initial hospitalization (randomized studies)

Fig. S6. Meta-analysis for mortality during initial hospitalization (nonrandomized studies)

Fig. S7. Meta-analysis for chronic lung disease (randomized studies)

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Supplementary material for Chapter 3

Appendices

Appendix 1S.
Medline search strategy
Run on 25 June 2013

1 Intensive Care Units, Neonatal/
2 Intensive Care, Neonatal/
3 Neonatal Nursing/
4 exp Infant, Newborn/
5 (neonat* or newborn* or prematur* or premmie* or prem or prems or "pre-term" or "pre term" or preterm or vlbw or elbw or lbw or sga or nicu or nicus).tw.
6 or/1-5
7 Erythrocyte Transfusion/
8 *Blood Transfusion/ or Blood Transfusion/ae
9 *Blood Component Transfusion/ or Blood Component Transfusion/ae
10 ((erythrocyte* or red cell* or red blood cell* or RBC*) adj5 (transfus* or hypertransfus* or retransfus* or unit*)).tw.
11 (hemotransfus* or haemotransfus* or hemotherap* or haemotherap*).tw.
12 ((blood or erythrocyte* or red cell* or red blood cell* or RBC*) adj3 (exchang* or replac*)).tw.
13 (red cell* or red blood cell* or whole blood or RBC* or transfus*).ti.
14 or/7-13
15 6 and 14
16 limit 15 to humans
17 limit 16 to yr="1990 -Current"

Medline via PubMed search updated on 15 November 2014

1. Infant, newborn
2. Transfusion
3. 1 and 2
4. limit 3 to humans
5. limit 3 to 26 June 2013 to current (15 November 2014)

EMBASE search strategy Via OVID
Run 24 July 2013

1. blood transfusion/ or blood autotransfusion/ or blood component therapy/ or erythrocyte transfusion/
2. blood/ or blood cell/ or blood component/ or venous blood/
3. blood cell/ or blood buffy coat/ or erythrocyte/
4. blood bank/
5. bloodborne bacterium/
6. blood group typing/
7. blood group incompatibility/ or blood group abo incompatibility/ or rhesus incompatibility/ or rhesus isoimmunization/
8. blood safety/ or (blood adj2 safety).ti,ab.
9. or/1-8 [****blood terms****]
10. prematurity/ or low birth weight/ or extremely low birth weight/ or small for date infant/ or very low birth weight/ or postmaturity/ or newborn/
11. exp newborn disease/
12. (neonat* or newborn* or prematur* or premmie* or prem or prems or "pre-term" or "pre term" or preterm or vlbw or elbw or lbw or sga or nicu or nicus).mp.
13. or/10-12 [****infant terms****]
14. 9 and 13 [****all infant results base set 1****]
15. ae.fs. and 14 [****blood terms with ae****]
16. randomized controlled trial.pt. or randomized controlled trial/
17. controlled clinical trial.pt. or controlled clinical trial/
18. randomized.ab.
19. placebo.ab.
20. dt.fs.
21. randomly.ab.
22. trial.ab.
23. groups.ab.
24. or/16-23
25. exp animal/ not human/
26. 24 not 25
27. meta analysis/ or "meta analysis (topic)"/ or (metaanalys* or "meta-analys*" or (meta adj2 analys*)).mp.
28. practice guiVine/ or clinical pathway/ or clinical protocol/ or consensus development/ or good clinical practice/ or nursing care plan/ or nursing protocol/ or (guideline* or (standard adj2 care) or consensus).mp.
29. "systematic review"/ or "systematic review (topic)"/ or (cochrane or medline or cinahl or embase or CCTR or scopus or "web of science" or lilacs or (systematic* adj2 review*)).mp.
30. or/27-29 [****Guideline methodology filtering terms****]
31. cohort analysis/ or longitudinal study/ or prospective study/
32. case control study/ or hospital based case control study/ or population based case control study/ or retrospective study/
33. risk/ or attributable risk/ or cardiovascular risk/ or fall risk/ or fall risk assessment/ or genetic risk/ or high risk behavior/ or high risk infant/ or high risk patient/ or high risk population/ or

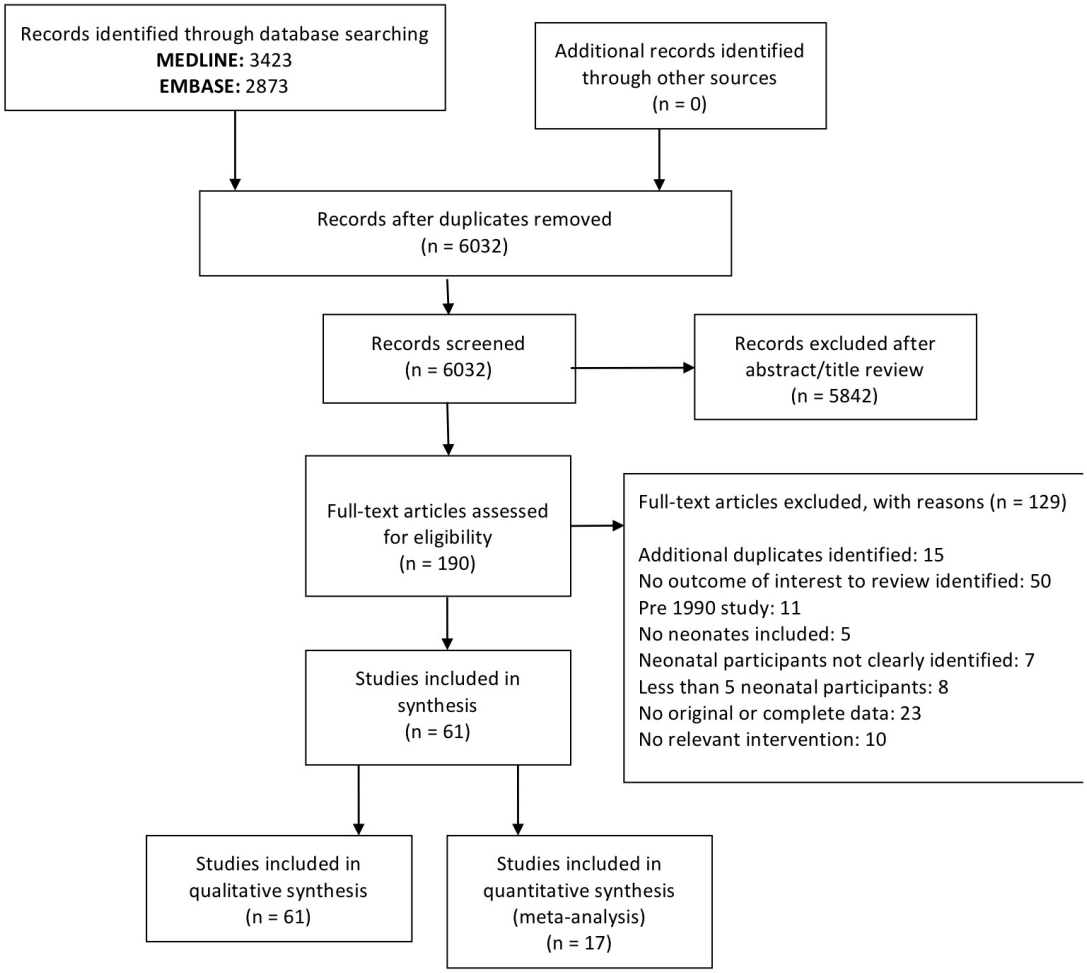
high risk pregnancy/ or infection risk/ or population risk/ or recurrence risk/ or risk factor/ or risk management/ or risk reduction/
34. (odds adj2 ratio:).ti,ab.
35. (risk: or cause or causal or causation or (relative adj2 risk:)).mp.
36. or/31-35 [***COHORT STUDY DESIGNS METHODOLOGIES OR RISK FACTORS***]
37. ct.fs. or phase 1 clinical trial/ or clinical trial/ or phase 2 clinical trial/ or phase 3 clinical trial/ or phase 4 clinical trial/ or controlled clinical trial/ or multicenter study/ or meta analysis/ or randomized controlled trial/ or crossover procedure/ or double blind procedure/ or single-blind procedure/ or triple blind procedure/ [***SENSITIVE ALL TRIAL METHODOLOGIES***]
38. cancer recurrence/ or cancer regression/ or cancer relapse/ or disease duration/ or disease exacerbation/ or prognosis/ or recurrent disease/ or reinfection/ or relapse/ or remission/ or tumor recurrence/ or tumor regression/ or survival/ or cancer survival/ or disease free survival/ or overall survival/ or survival rate/ or survival time/ or prognosis/ or incidence/ or cancer incidence/ or familial incidence/ or morbidity/ or maternal morbidity/ or perinatal morbidity/ or newborn morbidity/ or mortality/ or cancer mortality/ or childhood mortality/ or embryo mortality/ or fetus mortality/ or infant mortality/ or maternal mortality/ or prenatal mortality/ or surgical mortality/ or perinatal mortality/ or newborn mortality/ or death/ or "cause of death"/ or dying/ or heart death/ or sudden death/ or child death/ or newborn death/ or prevalence/ or treatment outcome/ or disease free interval/ or treatment failure/ or drug treatment failure/ or (natural adj2 history).mp. [***COHORT STUDY DESIGNS METHODOLOGIES OR PROGNOSIS OUTCOMES***]
39. or/26,30,36-38 [****study design terms***]
40. 39 and 14 [****base set 2****]
41. 40 and ae.fs.
42. 41 or 15 [***final base set***]
43. limit 42 to human
44. limit 43 to yr="1990 -Current"

EMBASE Search updated on 15 November 2014

1. blood transfusion or blood autotransfusion or blood component therapy or erythrocyte transfusion
2. prematurity or low birth weight or extremely low birth weight or small for date infant or very low birth weight or postmaturity or newborn
3. 1 and 2
4. limit 4 to human
5. limit 5 to 2013-06-25 to 2014-11-15

Appendix 2S. Figure S1

Figure S1: Summary of search and screening process



Appendix 2S.

Figure 2S: Aggregate data synthesis for 61 included studies

Characteristic	Number of studies (%)*
Intervention	
Type of study	
Randomized controlled trial	16 (26)
Prospective study with comparator	4 (7)
Prospective study without comparator	8 (13)
Retrospective study with comparator	28 (46)
Retrospective study without comparator	2 (3)
Case series	3 (5)
Outcomes^	
Necrotising enterocolitis	24 (39)
Retinopathy of prematurity	13 (21)
Chronic lung disease	18 (30)
Intraventricular hemorrhage	12 (20)
Sepsis	6 (10)
Mortality	19 (31)
Hyperkalaemia	8 (13)
Hyperglycemia	3 (5)
Adverse neurodevelopmental outcome	2 (3)
Other	13 (21)
Population	
Location of study	
Asia	1 (2)
Africa	2 (3)
Europe	13 (21)
USA	29 (48)
Canada	5 (8)
South America	6 (10)
Middle East	3 (5)
Other	2 (3)
Number of centers	
Single center study	41 (67)

Multiple center study	20 (33)

*Study may have examined for more than one outcome
†Two or less studies included stated outcome

Appendix 3S. Tables S1-3

Table 1S: Summary of study characteristics – randomized studies

Author Year Country MC or SC	Aim of study	Eligible study population	No. included and analyzed (included non-survivors?)	Included infants: all transfused?	RBC product and guidelines	Adverse effects /associations	Summary
Bell 2005 USA SC	To determine if restrictive guidelines for RBC transfusions can reduce no. of transfusions without adverse effects	216 eligible preterm infants and 103/216 preterm infants randomized	100 included: 51 in liberal and 49 in restrictive group Non-survivors: included	No Yes Not transfused: 6/51 (12%) liberal and 5/49 (10%) restrictive *Difference in transfusion rates/amounts between groups*	RBC product: single-donor when available Guidelines: described	**Grade ≥3-4 IVH** (p=0.55) Liberal: 8/51 (16%) Restrictive: 5/49 (10%) **ROP ≥3** (p=1.0) Liberal: 2/51 (4%) Restrictive: 2/49 (4%) **CLD** (p=0.27) Liberal: 20/50 (40%) Restrictive: 13/45 (29%) **Mortality (**p=0.61) Liberal: 1/50 (2%) Restrictive: 2/49 (4%) **NEC** (reported post publication of initial study): Liberal: 1/53 (2%) Restrictive: 1/50 (2%)	No statistically significant differences between groups in grade ≥3-4 IVH, ROP ≥3, CLD or mortality

Brooks 1999 USA SC	To determine the influence of a RBC transfusion protocol on ROP incidence and severity	165 infants <1251 grams and 50 infants randomized **Group 1**: 24 **Group 2**: 26	16/24 (67%) and 18/26 (69%) completed the study **Group 1**: transfused if deemed symptomatic **Group 2**: transfused to maintain HCT >0.40 Non-survivors: Unclear	No **Group 1**: mean (SD, range) 2.8 (3.1, 0-9) **Group 2**: mean (SD, range) 5.7 (3.1, 2-13) *Difference in transfusion rates/amounts between groups*	RBC product: no details Guidelines: described	**NEC (stage ≥2)**: Group 1 - 6/24 (25%) Group 2 - 7/26 (27%) *No difference* **CLD**: Group 1 – 16/24 (67%) Group 2 – 21/26 (81%) *No difference* **ROP**: Group 1 – 5/16 (31%) Group 2 – 3/18 (17%) *No difference*	Transfusion policy aimed at limiting RBC transfusions in neonates found no differences in adverse outcomes (IVH, CLD, NEC, ROP) between groups
Carnielli 1992 Italy SC	To determine whether prophylactic treatment with EPO and iron would reduce the need for blood transfusions	No. eligible: unclear Infants ≤1750 grams and ≤32 wks	22 randomized and analyzed: 11 control and 11 study group Non-survivors: N/A	No Transfusions: **Study** - 0.8±1.5 (0-5) **Control** - 3.1±2.1 (0-6) *Difference in transfusion rates/amounts between groups*	RBC product: not described RBC guidelines: described	**CLD**: Control – 2/11 (18%) Study – 3/11 (27%) *No difference* **Mortality**: Control – 0/11 (0%) Study – 0/11 (0%) *No difference*	No differences in outcomes (CLD, mortality) were found between groups
Chen 2009 Taiwan SC	To examine the effect of RBC transfusion on outcomes of VLBW infants (restrictive	VLBW infants No. eligible: unclear	36 randomized: 19 in restrictive and 17 in liberal group	Yes Transfusions: **Restrictive** – 2.5±1.9	RBC product: not described RBC guidelines:	**ROP grade ≥3** Restrictive - 0/17 (0%) Liberal - 2/16 (13%) (p=0.133)	No difference in adverse outcomes found between liberal and restrictive groups

	vs. liberal transfusion policies)		Non-survivors: No (excluded from analysis - 2/19 and 1/17)	**Liberal** – 3.8±2.5 *Difference in transfusion rates/amounts between groups*	described	**CLD** Restrictive - 5/17 (29%) Liberal - 3/16 (19%) (p=0.475) **IVH grade ≥3** Restrictive - 1/17 (6%) Liberal - 2/16 (13%) (p=0.59) **Sepsis** Restrictive - 9/17 (53%) Liberal - 11/16 (69%) (p=0.353) **Mortality** Restrictive - 2/19 (11%) Liberal - 1/17 (6%) *No difference*	
Fernandes da Cunha 2005 **Brazil** **SC**	To determine safety and efficacy of transfusing CPDA-1 RBCs stored up to 28 days to reduce donor exposure	108 infants <1500 grams	52 randomized and analyzed Group 1: RBCs stored up to 28 days Group 2: RBCs stored up to 3 days	Yes	RBC product: CPDA-I, irradiated, leukocyte depleted RBC guidelines: described	**Mortality**: Group 1 – 10/26 (39%) Group 2 – 9/26 (35%) **CLD** (survivors only) Group 1 – 11/18 (61%) Group 2 – 15/19 (79%) (p=0.235) **NEC**	No differences in adverse outcomes between groups

						Group 1 – 4/26 (15%) Group 2 – 6/26 (23%) (p=0.482) **Hypocalcaemia**: Group 1 – 0/26 (0%) Group 2 – 0/26 (0%) *No difference* **Hyperkalaemia**: Group 1 – 0/26 (0%) Group 2 – 0/26 (0%) *No difference*	
Fergusson 2012 Canada MC	To determine if RBCs stored for ≤ 7 days compared with standard practice (2-42 days) improved neonatal outcomes	1752 preterm infants <1250 grams	377 randomized and included Non-survivors: included	Yes	RBC product: aside from age, no specific details given Guidelines: unit dependent (not described)	**Fresh vs. standard RBCs:** **Mortality** – 30/188 (16%) vs. 31/189 (16%) RR 0.97 (0.61-1.54) **ROP** – 23/188 (12%) vs. 26 /189 (14%) RR 0.89 (0.53-1.50) **CLD** – 60/188 (32%) vs. 63/189 (33%) RR 0.96 (0.72-1.28) **NEC** – 15/188 (8%) vs. 15/189 (8%) RR 1.00 (0.48-2.12)	Use of fresh (5.1±2.0) vs. old 14.6±8.3) RBCs for transfusion made no difference to adverse outcomes

						IVH – 18/188 (10%) vs. 11/189 (6%) RR 1.65 (0.80-3.39) **Sepsis** – 127/188 (68%) vs. 121/189 (64%)	
Griffiths 1997 United Kingdom MC	To evaluate role of EPO in reducing iron infusions, which may exacerbate free radical damage, leading to CLD	VLBW infants	43 randomized and 42 analyzed: 21 infants (EPO) vs. 21 infants (no EPO) Non-survivors: included	No EPO group: 2 (0-5) transfusions No EPO group: 2 (1-8) transfusions EPO group received fewer blood transfusions (difference in medians -2, 95% CI -4, 0) (data not supplied) *Difference in transfusion rates/amounts between groups*	RBC product: not described Guidelines: described	**Mortality** EPO group: 6/21 (29%) No EPO group: 3/21 (14%) **CLD** EPO group: 7/21 (33%) No EPO group: 12/21 (57%) (Difference in proportion -0.24, 95% CI -53, 5.4)	No differences in adverse outcomes between groups
Haiden 2006 Austria SC	To investigate whether combined administration of vitamin B12, folic acid, iron and EPO reduces transfusion requirements	47 infants <32 weeks GA	40 randomized and analyzed: 21 infants (EPO) vs. 19 infants (no EPO) Non-survivors:	No EPO group: 13/21 (62%) transfused No EPO group: 18/19 (95%) (p=0.031)	RBC product: not described Guidelines: described	**Mortality** (infants excluded from further analysis) EPO group: 3/21 (14%) No EPO group: 4/19 (21%)	No differences in adverse outcomes between groups despite decreased no. of transfusion in the study group

			not included	*Difference in transfusion rates/amounts between groups*		**CLD** EPO group: 6/21 (29%) No EPO group: 8/19 (42%) p=NS **Severe ROP** EPO group: 1/21 (5%) No EPO group: 0/19 (0%) p=NS	
Kirpalani 2006 Canada/Australia/USA MC	To determine whether different transfusion thresholds affect survival or morbidity	694 infants <1000 grams and <31 wks	451 randomized and analyzed (223 in restrictive vs. 228 in liberal) Non-survivors: included	No (89% transfused in restrictive vs. 95% in liberal; p=0.037) *Difference in transfusion rates/amounts between groups*	RBC product: washed, packed RBCs, autologous or direct donation Guidelines: liberal vs. restrictive	**Mortality** Restrictive: 48/223 (22%) Liberal: 40/228 (18%) p=0.25 **NEC** (reported post publication of initial study): Liberal: 12/228 (5%) Restrictive: 19/223 (9%) **Survivors only:** **ROP ≥3** Restrictive: 33/175 (19%) Liberal: 33/188 (18%) p=0.42 **CLD** Restrictive: 101/175 (58%) Liberal: 103/188 (55%)	No differences in adverse outcomes (mortality, brain injury, CLD, ROP, sepsis) were observed between restrictive and liberal transfusion groups

						Sepsis Restrictive: 96/223 (43%) Liberal: 93/228 (41%)	
Follow-up to Kirpalani 2006 study: **Whyte 2009 Canada/Australia/USA MC**	To determine whether a restrictive vs. liberal transfusion threshold affects neurodevelopmental outcomes or mortality	Original study: 451 infants <1000 grams and <31 wks	Follow-up study: 430/451 (95%) with the primary outcome being available from 421/451 (93%) Non-survivors: included	Unclear	RBC product: washed, packed RBCs, autologous or direct donation Guidelines: liberal vs. restrictive	**Cerebral palsy, cognitive delay (MDI <70), severe visual or hearing impairment** Liberal group: 37/213 (17%) Restrictive group: 46/208 (22%) p=NS	No differences in neurodevelopmental outcomes or mortality between groups
Lee 1995 USA SC	To determine whether the use of dedicated donor packs would reduce donor exposure	32 infants <1500 grams	23 infants included and 21 analyzed 8 in control (washed) group vs. 13 in study group - (unwashed) dedicated donor packs Non-survivors: included	Yes No. of transfusions between groups was similar: study 4.0 (2.6) vs. 3.6 (1.5)	RBC product: irradiated, CPDA/AS-1, CMV negative Guidelines: not stated.	**Mortality** Control: 2/8 (25%) Study: 2/8 (25%) **Hyperkalaemia (>6.5mmol/L)** (post-transfusion): 0 in each group	No differences in adverse events/outcomes between groups

Liu 1994 USA SC	To determine the safety and efficacy of using a dedicated donor pack	Unclear no. of infants < 1500 grams	25 infants – 12 control group (pRBCs <5 days old) vs. 13 infants - study group (dedicated donor packs)	No	RBC product: CMV negative, CPDA-1, O negative, irradiated Guidelines: described	**Hyperkalaemia (>6.5mmol/L)** Control: 2/12 (17%) Study: 2/12 (17%)	No differences observed in short-term adverse effects between each group
Maier 1994 Europe MC	To determine whether early treatment with EPO reduces transfusion requirements	598 infants <1500 grams	244 randomized, included and 241 analyzed (177 infants completed the study) 120 infants in EPO group vs. 121 infants in the no EPO group Non-survivors: included in analysis	No Not transfused - EPO group: 53/120 (44%) No EPO group: 34/121 (28%) *Difference in transfusion rates/amounts between groups*	RBC product: not described RBC guidelines: described	**ROP ≥3** EPO group: 1/121 (<1%) No EPO group: 1/120 (<1%) **IVH ≥3** EPO group: 1/121 (<1%) No EPO group: 2/120 (2%) **Mortality** EPO group: 3/121 (3%) No EPO group: 3/120 (3%)	No differences in adverse outcomes between groups
Strauss 2000 USA SC	To determine the safety and feasibility of AS-3 RBCs for transfusion	Unclear no. of infants 600 to 1300 grams	Study group (11 infants): AS-3 stored and leukoreduced by filtration Control group (10 infants):	Yes	RBC product: irradiated, type O and Rh compatible RBC guidelines: not described	No statistically significant differences (p=NS) in glucose 0/11 (0%) vs. 0/10 (0%) or potassium 0/11 (0%) vs. 0/10 (0%) levels were found	No adverse clinical events were found when AS-3 stored RBCs were used for transfusion

			fresh RBCs (up to 7 days) non-leukoreduced in CPDA Non-survivors: unclear			when AS-3 RBCs vs. fresh CPDA were transfused	
Strauss 1999 USA SC	To compare occurrence of RBC, plt and WBC antibodies post-transfusion	Unclear no. of infants ≤1300 grams	47 infants included (24 in study group and 23 in control group) 28 infants completed the study and were analyzed (14 in each group) Non-survivors: unclear	No 12 infants across both groups were never transfused	RBC product: irradiated, type O and Rh compatible Study group: AS-1 or AS-3, leukoreduced by filtration vs. control group: fresh RBCs (up to 7 days) non-leukoreduced RBC guidelines: described	No RBC antibodies were detected in any infants transfused: study group 18/24 (75%) vs. control group 17/23 (74%) in the study during the 1st 6 months of life	No incidents of alloimmunisation observed in either group
Wong 2005 Canada MC	To compare different transfusion volumes	48 infants <1500 grams	20 infants included and analyzed 10 infants in the 20mL/kg study group vs. 15mL/kg in the control group Non-survivors: included	Yes High volume group: 3.4 (2.1) vs. standard volume group: 2.8 (2.1) *Difference in transfusion amounts (but not rates) between groups*	RBC product: not described RBC guidelines: described	**Treatment level ROP** Study group: 2/10 (20%) Control group: 2/10 (20%) **Mortality** Study group: 1/10 (10%) Control group: 1/10 (10%)	No statistically significant differences in outcomes noted between the higher vs. lower transfusion volume groups

Table 2S: Summary of study characteristics – non-randomized studies

Author Year Country MC or SC	Aim of study	Eligible study population	No. included/analyzed Non-survivors: Yes/No	Included infants: all transfused? Yes/No	RBC product and guidelines	Adverse effects /associations	Summary
Prospective – with comparator							
Bednarek 1998 USA MC	To compare use of RBC transfusions and outcomes between 6 NICUs (combined into 2 low/2 medium/2 high transfusing units)	825 preterm infants <1500 grams	789 included/analyzed Non-survivors: not included	Yes	RBC product: no details provided Guidelines: not described	**Grade ≥3-4 IVH** High: 18/233 (8%) AOR 1.2 (0.5-2.5) Medium: 20/311 (6%) Reference Low: 15/282 (5%) AOR 0.78 (0.4-1.6) **NEC stage ≥2** High: 15/232 (7%) AOR 1.1 (0.5-2.2) Medium: 19/305 (6%) Reference Low: 15/282 (5%) AOR 0.3 (0.1-0.8)	No difference in adverse outcomes between lower and higher transfusing NICUs except for a reduced AOR for NEC in the lower transfusing NICUs.
Miyashiro 2004 Brazil MC	To verify if strict RBC transfusion guidelines reduces the need for transfusion in the 4 weeks of life	Eligible no. = Phase 1: 173 Phase 2: 229 VLBW infants	Included and analyzed = Phase 1: 149 Phase 2: 196 Non-survivors: included if survived >24 hours	No Phase 1: 102/149 (69%) transfused Phase 2: 117/196 (60%) transfused *Difference in transfusion rates/amounts between groups*	RBC product: not described Guidelines: described	**CLD** Phase 1:34/118 (29%) Phase 2: 37/156 (24%) p=0.63 **Sepsis** Phase 1: 85/148 (57%) Phase 2: 119/195 (61%) p=0.58 **Mortality up to 28 days** Phase 1: 39/149 (26%) Phase 2: 47/196 (24%) p=0.73	No differences in outcomes (CLD, sepsis, IVH – any grade, mortality) between groups

Mimica 2008 Brazil SC	To verify if a strict transfusion guidelines reduce RBC transfusion without adverse outcomes	Phase 1 - 69 infants and phase 2 - 80 infants <37 wks and <1500 grams	Phase 1: 69 infants Phase 2: 78 infants Non-survivors: included if survived >24 hours	No Phase 1 (restrictive): 54/69 (78%) Phase 2 (more restrictive): 49/78 (63%) *Difference in transfusion rates/amounts between groups*	RBC product: irradiated, autologous, CPDA-1, leukodepleted Guidelines: described	**CLD** Phase 1: 21/55 (38%) Phase 2: 23/70 (33%) p=0.54 **IVH grade ≥3** Phase 1: 9/66 (14%) Phase 2: 10/77 (13%) p=0.91) **Mortality** Phase 1: 20/69 (29%) Phase 2: 9/78 (12%) p=0.009	No differences in CLD or IVH ≥3 between groups Increased mortality was observed in the time period with the more liberal transfusion guidelines
Silvers 1998 UK SC	To evaluate the relationship between biomarkers of antioxidant status and GA in the care of premature infants	Unclear eligible no. of infants between 22-39 weeks GA	144 were included and 105 analyzed (75%) Non-survivors: not included in CLD and transfusion analyses	No	RBC product: not described Guidelines: not described	**CLD** Outcome data (median (range)) for non-CLD survivors vs. survivors with CLD: Day 2 - 0.48 (0-2) vs. 1 (0-1) (data for 78/105) Day 3 - 0.54 (0-2) vs. 1.44 (0-3) (data for 74/105) Day 8 – 0.90 (0-4) vs. 2.28 (0-5) (data for 50/105)	For each transfusion an infant received on day 2, day 3 and day 8 they were more likely to develop CLD (1.6 times more likely on days 2 and 3; 1.5 times more likely on day 8)
Prospective – no comparator							
Ayede 2011 Nigeria SC	To evaluate blood product usage patterns	324 admitted to SCBU and 100 included in study	100 Non-survivors: unclear whether included	No 46/100 (46%) infants received at least 1 RBC transfusion	RBC product: non-irradiated Guidelines: not described	**FNHTR:** 5/100 (5%) post-transfusion (unclear whether post RBCs, plts or FFP)	FNHTR occurred in 5% of all transfused infants

Bearer 2000 USA SC	To determine the exposure of premature infants to lead from blood transfusions	19 preterm infants	19 Non-survivors: unclear whether included	Yes	RBC product: non-related donors Guidelines: not described	**Lead exposure:** No. of transfusions per infant was 4.2 ± 2.8 (mean ± SD) with 15.7 ± 1.9mL/kg RBCs for a lead dose of 1.56±1.77µg/dL	Lead exposure exceeded the acceptable daily intake for lead (World Health Organisation) in 71/79 (90%) RBC transfusions
Boo 1998 USA SC	To determine the rate of blood transfusion and the incidence of transfusion reactions in newborn infants	1 928 infants preterm and term infants	117 included and 110 analyzed Non-survivors: included	Yes	RBC product: non-irradiated and not filtered Guidelines: not described	**FNHTR**: 3/110 (3%) Febrile reactions (temp ≥38 degrees) occurred during transfusion in 3 (2.7%) infants (2 term and 1 preterm)	Incidence of FNHTR was 2.7% (3/110) of all transfused infants or 1.3% of all RBC transfusions
Elabiad 2013 USA SC	To evaluate levels of lead in RBC transfusion for ELBW infants	37 ELBW infants	37 included and analyzed Non-survivors: included	Yes	RBC product: O negative, irradiated and CMV negative stored in CPDA-1.	**Lead exposure** Average lead level per RBC unit = 18.3Ug/L 37 infants received 322 transfusions with 139 (42%) exceeding the exposure reference	All infants received at least one RBC transfusion with a lead volume greater than the exposure reference.
Jain 2001 USA SC	To determine the safety of AS-1 storage media in preterm infants	197 admissions with 87 transfused infants <1500 grams	87 infants included and analyzed Non-survivors: unclear whether included	Yes	RBC product: unwashed, CMV negative, AS-1 stored PRBCs Guidelines: described	**Hyperkalaemia** (within 6hrs post-transfusion): 0/87 (0%) **Hyperglycaemia** (within 6hrs post-transfusion: 0/87 (0%)	No biochemical adverse effects observed at a single time point 6hrs post-transfusion using AS-1 stored PRBCs
Mangel 2001 Canada SC	To determine the safety and efficacy of using AS-3 split packs for transfusion	56 infants transfused with AS-3 RBCs	56 infants were included and	Yes	RBC product: AS-3, unrelated donors only	No reported cases of **hyperkalaemia** (108/263 transfusion events).	No adverse effects related to transfusion reported

			analyzed Non-survivors: unclear whether included		Guidelines: not described		
Strauss 2000 USA SC	To determine whether preterm infants are at risk of alloimmunisation when receiving RBCs from a dedicated donor	Unclear no. of infants 600-1300 grams	31 infants were included and 30 analyzed (84 blood samples from the 30 transfused infants)	No	RBC product: Prestorage leukoreduced, dedicated donor, AS- Guidelines: not described	4/26 (15%) infants (data available) produce WBC antibodies with no clinical adverse effects	15% of infants transfused produced WBC antibodies
Ugwu 2006 Nigeria SC	To determine the rate and indications for RBC transfusion	405 infants were transfused with RBCs	405 included and analyzed 157/405 (39%) infants received small volume RBC transfusions Non-survivors: included	Yes	RBC product: not described Guidelines: not described	**Transfusion-related malaria** 51/157 (32%) **Cellulitis** 2/157 (1%)	High rates of transfusion-related malaria occurred in this Nigerian study
Retrospective – with comparator							
Allegaert 2004 Belgium MC **Case-control study**	To evaluate effect of different risk factors for ROP ≥3 stage	525 had data collected 303 admitted to NICU	175 included: 35 cases vs. 140 controls Non-survivors: not included	Unclear Transfusions (no.): 6.4±4.8 controls vs. 10.8±5.3 cases	Neither described	**ROP stage ≥3** Cases: 35/35 (100%) Controls: 140/140 (0%) No. of transfusions as a risk factor for ROP stage ≥3: OR 95% CI: 1.1189 (1.0309-1.2145) p=0.0072	A higher number of transfusions were associated with an increased likelihood of developing severe ROP

Baer 2011 USA MC **Case-control study**	To determine whether RBC transfusions are an independent risk factor for subsequent IVH	1478 admitted to NICUs 184 preterms had ≥3-4 IVH 54/184 (29%) had a normal HUS prior to IVH	155 total: 54 cases vs. 101 controls Non-survivors: included	Yes	RBC product not described Guidelines: existed but non-compliance occurred	**Grade ≥3-4 IVH** Cases: 47/47 (100%) Controls: No IVH (any grade) in 101/101 (100%)	Each RBC transfusion during 1st week was associated with an increased RR (95% CI) of developing grade ≥3-4 IVH by 2.02 (1.54-3.33)
Baer 2011 USA MC **Case-control study**	To determine whether RBC transfusions are associated with an extension of grade 1 to 3-4 IVHs	417 preterm infants with grade 1 IVH: 362 resolved and 46 extended into grade 3-4 IVHs	408 in total: 46 cases vs. 362 controls Non-survivors: unclear whether included	Unclear % of those in each group receiving RBC transfusion(s) not given	RBC product: irradiated, leukoreduced, storage in CPDA-1, unwashed, dedicated donor Guidelines: existed but non-compliance occurred	**Grade ≥3-4 IVH:** Cases: Grade ≥3-4 46/46 (100%) extension from prior grade I IVH Controls: Grade 1 IVH in 362/362 (100%) with no extension	Cases were more likely to have received RBC transfusion OR (95% CI) 3.72 (2.779-5.270, FFP 1.842 91.494-2.313) and platelets 5.415 (3.056-10.044) prior to extension of an IVH
Baer 2011 USA MC	To determine pre-post analysis of compliance with transfusion guidelines and transfusion usage	**Years** **2007**: 3303 **2008**: 3533 **2009**: 3444 Preterm/term infants	**2007**: 622/3303 (19%) **2008**: 594/3533 (17%) **2009**: 449/3444 (13%) Non-survivors: included	No	RBC product: not described Transfusion guidelines: described	**NEC:** 2007: 70/3303 (2%) 2008: 65/3533 (2%) 2009: 77/3444 (2%) **IVH:** 2007: 139/3303 (4%) 2008: 130/3533 (4%) 2009: 118/3444 (3%)	No differences in outcomes (NEC or IVH) across all 3 time periods despite increased compliance with transfusion guidelines

Birenbaum 2006 USA SC	To compare the effects of a restricted transfusion schedule with EPO therapy versus a restricted transfusion schedule alone	50 infants <1500 grams	50 infants Non-survivors: included	No 8/30 EPO group received 27 transfusions 8/20 no EPO group received 13 transfusions *Difference in transfusion rates/amounts between groups*	RBC product: not described Guidelines: yes	**Mortality** EPO group - 2/30 (7%) No EPO group - 3/20 (15%) p=NS	More transfusions received in EPO group with no differences in outcomes identified between groups
Blau 2011 USA SC	To determine whether there is an association of necrotizing enterocolitis (NEC) <48 hours of a packed red blood cells (PRBC) transfusion	256 VLBW infants	36 infants Non-survivors: included	No TA-NEC (n=9) Non-transfusion related NEC (n=15) Never-transfused NEC (n=12)	RBC product: irradiated, leukoreduced, stored in AS-3 Guidelines: yes	**NEC:** TA-NEC – 9/36 (25%) Transfused >48hr prior to NEC – 15/36 (42%) Never transfused NEC – 12/36 (33%) Similar proportions in each subgroup	25% of cases of NEC were temporarily related to RBC transfusion
Carter 2012 USA SC	To examine the relationship between treatments received prior to development of	549 <31 wks GA	549 infants Non-survivors: included	Medical NEC (n=34) Surgical NEC (n=31) No NEC (n=484)	RBC product: not described Guidelines: no	**NEC**: Medical NEC – 34/549 (6%) infants with 11.09±12.91 (mean±SD) transfusions Surgical NEC – 31/549 (6%) infants with 13.42±11.53	Infants who received more transfusions were more likely to develop NEC

	NEC					transfusions No NEC – 484/549 (88%) infants with 4.82±8.54 transfusions In infants (23-26 and 27-30 wks) a greater no. of transfusions was associated with an increased OR of developing NEC (1.1 and 1.4 respectively; CI 95%)	
Christensen 2010 USA MC **Part B: Case-control study**	To determine whether there is an association of necrotizing enterocolitis (NEC) <48 hours of a packed red blood cells (PRBC) transfusion	112 preterm and term infants with NEC requiring surgical intervention	112 included in the study with full transfusion histories for only 62/112 (55%) **Part A:** 72 infants with NEC unrelated to transfusion vs. 40 NEC within <48 hours of transfusion **Part B:** 62 infants with surgical NEC compared to 248 matched (GA, BW, sex, maternal race, day transfused) controls Non-survivors: included	No	RBC product: irradiated, leukoreduced, stored in CPDA-1 and unwashed Guidelines: yes	**Part A:** 40/112 (36%) – NEC following transfusion 72/112 (64%) – NEC unrelated to transfusion 40/112 (36%) who developed surgical NEC received a RBC transfusion in the preceding 48 hours **Part B:** Cases – 62 infants with 65% transfused DOL 1-7 and 92% after DOL 7 Controls – 248 with 46% transfused DOL 1-7 and 49% after DOL 7 Infants who developed NEC were more likely to have received a RBC transfusion at any time (OR 2.17-11.77; CI 95%) prior to NEC compared to those who never developed NEC	One-third of NEC cases were temporally associated with RBC transfusion

Cooke 1997 UK SC	To determine the relationship between oxygen-derived free-radical generation, oxidative injury and chronic lung disease	Eligible no. = unclear Infants <34 wks GA	98 included 73 infants analyzed (25 excluded as died or discharged from unit by 28 days) Non-survivors: not included	Unclear	RBC product: not described Guidelines: no	**CLD** Cases (CLD: n=30) received 8.5±3.7 transfusions Controls (no CLD: n=43) received 3.3±2.3 transfusions No. of transfusions given during the 1st month was significantly greater in the CLD group (p<0.0001)	Infants who developed CLD received more RBC transfusions than those without CLD
Couselo 2011 Spain SC **Case-control study**	To determine whether infants who received RBC transfusion prior to NEC develop a more severe grade	55 infants ≤34 wks with NEC (stage ≥2)	46 infants included (8 excluded due to CHD) 28 infants (case): transfused prior to NEC (6/28 transfused 48 prior to NEC) vs 18 infants (control): not transfused prior to NEC Non-survivors: included	Cases: yes Controls: no	RBC product: not described Guidelines: no	**Transfused prior to NEC** 20/28 (71%) – Bell stage 2 8/28 (29%)– Bell stage 3 7/28 (25%) – did not survive No relationship between RBC transfusion and NEC stage (p=0.39) **No transfusion prior to NEC:** 14/18 (78%) – Bell stage 2 4/18 (22%) – Bell stage 3 1/18 (6%) – did not survive	No relationship found between RBC transfusions, NEC stage, need for surgery or mortality
Del Vecchio 2013 Italy SC	To determine whether reducing the RBC transfusion rate through introduction of RBC transfusion guidelines is associated with decreased	3785 infants (GA and BW not specified or given) Non-survivors: excluded if died at <7	3785 (all admissions) infants included Transfusion rate 2005/2008 vs. 2009-2012: 14.8% vs. 6.3% of all admissions	Pre-transfusion guidelines (2005-2008): 1205 infants Post-transfusion guidelines (2009-2012): 1235 infants	RBC product: CPDA-1, leukoreduced, irradiated Guidelines: described	**2005-2008 vs. 2009-2012** **CLD**: 39/1205 (3.2%) vs. 11/1235 (0.9%) OR: 3.722 [1.897-7.302] **NEC**: 9/1205 (0.7%) vs. 3/1232 (0.2%) OR: 1.958 [1.247-3.073]	Introduction of restrictive RBC transfusion guidelines was associated with reductions in CLD and ROP

	neonatal morbidities	days		*Difference in transfusion rates/amounts between groups*		**ROP:** 56/1205 (4.6%) vs. 30/1232 (2.4%) OR: 3.090 [0.835-11.443]	
Demirel 2012 Turkey SC	To determine the relationship between RBC transfusion and NEC	700 VLBW infants	647 infants included and divided into 5 groups: NEC within 48hr post transfusion (n=15), NEC >48 post transfusion (n=31), never transfused NEC (n=50), transfused/no NEC (n=250), never transfused/no NEC (n=301) Non-survivors included: unclear	2 main groups (NEC < and > 48 hours post-transfusion): all transfused	RBC product: leukoreduced, irradiated Guidelines: described	**Total NEC**: 96/647 (15%) **NEC**: <48hrs post transfusion – 15/96 (16%) >48hr post transfusion – 31/96 (32%) Never transfused – 50/96 (52%) The mean interval from the last PRBC transfusion to the onset of NEC was 16 +/-8.8 h (median, 16 h; range, 6–32 h) in group 1 and 240+/-50 h (median, 157; range, 80–880 h) in group 2 (p <0.05)	Most infants developed NEC temporarily unrelated to RBC transfusion with no statistically significant differences in NEC sub-groups
Demirel 2009 Turkey SC **Case-control study**	To determine risk factors for development of CLD	312 infants <1501 grams	106 included Cases (CLD): 56 Controls (no CLD): 50 Non-survivors: included if survived long enough for CLD criteria to be applied	No	RBC product: not described Guidelines: not described	**Infants with >2 RBC transfusions** **Cases**: 13/56 (23%) **Controls**: 4/50 (8%) Increased odds 3.47 (1.05-11.49) infants with CLD received >2 RBC transfusions than those without CLD (p=0.033)	Receipt of RBC transfusion was associated with increased likelihood of being diagnosed with CLD

dos Santos 2011 Brazil MC	To assess whether RBC transfusions are associated with increased intra-hospital mortality	1077 infants <1500 grams	574 infants included Non-survivors: included	Yes	RBC product: not described RBC guidelines: 8 different ones described	**Total mortality**: 299/1077 (28%) Infants who died received 2.2±3.3 RBC transfusions vs. infants who survived at 1.6±0.3 (p=0.003) More infants who died received >2 RBC transfusions (26.8%) vs. infants who survived (21.6%) (p=NS)	Greater than 2 RBC transfusions during the entire hospital stay were associated with a significantly increased OR of mortality after 28 days but reduced OR of death prior to 28 days
Elabiad 2013 USA SC **Case-control study**	To evaluate the association between RBC transfusion and development of NEC	3462 VLBW infants	3060 infants included Cases (NEC): 174 Controls (no NEC): 2886 Non-survivors: excluded if died before day 7	No 67% (cases) transfused vs. 60% (controls)	RBC product: O negative, irradiated, leukodepleted, CMV negative Guidelines: not described	**NEC**: 174/3060 (5.7%) infants 116/174 (67%) developed NEC within 48 hours RBC transfusion	Infants with BW ≤750, 751-1000, 1001-1250 and 1251-1500 g (n=52, 51, 46 and 25) had a RR of 0.14, 0.46, 1.83 and 1.78 (p<0.01, 0.02, 0.07 and 0.17), to develop NEC after a RBC transfusion
El-Dib 2011 USA SC **Case-control study**	To determine whether preterm infants were more likely to be transfused <48-72hrs prior to NEC	747 infants <32 wks and <2500 grams 25 developed NEC	25 cases (NEC) vs. 25 matched (BW, GA, gender) controls (no NEC) Non-survivors: included	Yes	RBC product: O negative, CPDA-1, leukoreduced and CMV negative RBC guidelines: described	**NEC**: 25/747 (3%) No. of transfusions prior to NEC; for controls – no. prior to matched day for occurrence of NEC in cases (p=0.52): **Cases** - 3 (1-6) transfusions **Controls** - 3 (2-6) transfusions 65% of infants with NEC received a RBC transfusion 72 hours prior to NEC	Infants with NEC where more likely to be transfused in the preceding 48-72 hours

						compared to 20% controls transfused but did not develop NEC in matched time period	
Fergusson 2003 Canada MC	To evaluate outcomes following the introduction of prestorage RBC leukoreduction	<1250 grams	516 included and 515 analyzed (268 pre-intervention vs. 247 post-intervention) Non-survivors: included if survived >48hrs	Yes	RBC product: CPD-2, AS-3 (washed in 1 unit) vs. prestorage leukodepletion, CPD-2, AS-3 (washed in 1 unit) RBC guidelines: variable	**Pre- vs. post-implementation** **Mortality**: 45/268 (17%) vs. 44/247 (18%) AOR= 1.22 (0.59-2.50) **NEC**: 32/268 (12%) vs. 16/247 (7%) AOR= 0.39(0.17-0.90) **IVH ≥3-4:** 45/268 (17%) vs. 33/247 (13%) AOR= 0.65 (0.35-1.19) **Sepsis**: 79/268 (30%) vs. 63/247 (26%) AOR= 0.59 (0.34-1.01)	Post-implementation of prestorage leukodepletion: no changes in mortality or sepsis (primary outcomes) and decreases in CLD, NEC, IVH (secondary outcomes) were found
Giannantonio 2012 Italy SC **Case-control study**	To identify postnatal risk factors for development of stage ≥3 ROP	723 VLBW infants assessed for eligibility, 102 eligible and 93 included	93 infants included and analyzed (≥3 ROP: 44 requiring treatment vs. 49 not requiring treatment) Non-survivors: not included	No	RBC product: not described Guidelines: described	**Treatment requiring ROP (group A: 44 infants):** 0.5±0.6 (0-2) transfusions **ROP not requiring treatment (group B: 49 infants):** 0.9±1.1 (0-5) transfusions OR 1.82 (1.07-3.01)	RBC transfusion was identified as a risk factor for developing treatment requiring ROP

Josephson 2010 USA MC **Case-control study**	To test the hypothesis that RBC transfusions increase the risk of NEC in premature infants	93 infants diagnosed ≤34 wks with NEC, 2 excluded from analysis as controls not able to be found	91 infants included and analyzed plus an additional 91 control infants Non-survivors: included	No	RBC product: leukoreduced, irradiated, CPDA-1 preserved RBCs stored for ≤14 days. Guidelines: described.	**NEC** Transfused within preceding 48hrs of NEC: 18/91 (20%) NEC with no hx of transfusion within 48hrs: 75/91 (80%) Infants who developed NEC within 48hrs of transfusion had lower BW (median 735 vs. 1160grams; p=0.0003), GA (25.9 vs. 28.4 wks; p=0.001) and were receiving more respiratory support (p=<0.0001).	RBC transfusions were temporally unrelated to most cases of NEC
Khodabux 2009 Netherlands MC	To compare how different volumes of RBC transfusion affects the total no. of transfusions and neonatal outcomes	Unit A: 221 Unit B: 248 Infants <32 weeks GA	218 infants in unit A (15mL/kg) vs. 241 infants in unit B (20mL/kg) Non-survivors: included	No A: 128 (59%) B: 186 (77%) *Difference in transfusion rates/amounts between groups*	RBC product: SAGM, irradiated Guidelines: same for both units	**Mortality** A: 11/218 (5%) B: 13/241 (5%) (p=1.0) **Outcomes for survivors only:** **CLD** A: 20/207 (10%) B: 28/228 (12%) (p=0.44) **ROP ≥3** A: 7/207 (3%) B: 5/228 (2%) **IVH ≥3** A: 7/207 (3%) B: 7/228 (3%) (p=0.41)	No difference in adverse outcomes observed between units

Follow-up of subgroup from previous study of Khodabux 2009 **Von Lindern 2011 Netherlands MC**	To determine the effect of transfusion volume on long-term outcomes	Infants <28 weeks GA included in prior study by Khodabux 2009	44 infants in unit A (15mL/kg) vs. 43 infants in unit B (20mL/kg) Non-survivors: 4 excluded who died within 24 hours of birth (never transfused) and another 12 infants who died in the neonatal period (all transfused)	Unclear Unit A (n=31): 5.5±2.7 Unit B (n=36): 5.55±3.2 (p=NS)	RBC product: SAGM, irradiated Guidelines: same for both units	**Composite of post discharge mortality, severe hearing or visual impairment, or neuromotor developmental delay (<1SD below mean) at 24 months CA** A: 9/31 (29%) B: 12/36 (33%)	No differences in outcomes between units in a follow-up sub-group of a previous study
Mally 2006 USA SC **Case-control study**	To determine whether an association between RBC transfusions and NEC	17 cases of NEC of which 6/17 developed post-transfusion	6/17 infants with NEC associated with transfusion vs. 11/17 NEC not associated with transfusion Non-survivors: included	No	RBC product: irradiated, leukoreduced, AS-1 or CPD storage media Guidelines: described	**NEC (17 infants)** 6/17 (35%) developed NEC within 22hrs of being transfused 11/17 (65%) developed NEC not temporally related to transfusion	Temporal association between receipt of RBC transfusion and development of NEC reported
Martin 2013 Spain SC **Case-control study**	To determine risk or protective factors for the development of necrotizing enterocolitis in VLBW infants	576 VLBW infants	60 infants: 30 cases of NEC matched for GA with 30 controls Non-survivors: included	No Cases 19/30 (63%) vs. controls 23/30 (77%) were transfused (p=0.26)	RBC product: irradiated, leukoreduced,, CPDA-1 storage media Guidelines: described	**Number of RBC transfusions prior to NEC diagnosis:** **Cases** 3.58±3.11 RBC transfusions **Controls** 1.83±1.15 (p=0.04)	Infants with NEC received a greater number of RBC transfusion (prior to NEC) but were just as likely to be transfused as infants without NEC

Paul **2011** **USA** **SC**	To determine whether RBC transfusions are associated with increased odds of developing NEC	122 infants <1500grams	122 included and analyzed	No	RBC product: CMV negative, irradiated, sickle-cell negative, type specific or O, Rh compatible, Adsol preserved Guidelines: described	**NEC** NEC with no transfusion: 30/122 (25%) NEC within 48 hours after transfusion: 33/122 (27%) NEC >48 hours after transfusion: 59/122 (48%)	Odds of NEC in infants transfused with RBC (prior to NEC) compared to those not transfused was increased at 2.1 (1.1-4.3) OR (95% CI)
Singh **2011** **USA** **MC** **Case-control study**	To determine whether there is an association between anaemia, RBC transfusion and NEC	111 infants ≤32 weeks GA with NEC	111 study infants matched with 2 (222 infants) controls each (GA ±1 wk and BW ±2 wks) Non-survivors: included	No	RBC product: not described Guidelines: described	**NEC cases** transfused Within 24 hrs: 36/111 (32%) Within 48 hrs: 44/111 (40%) Within 96 hrs: 49/111 (44%) **"NEC" controls** (matched with index case for their day of diagnosis of NEC) transfused Within 24 hrs: 15/202 (7%) p=0.001 Within 48 hrs: 23/202 (10%) p=0.001 Within 96 hrs: 46/202 (21%) p=0.001	Infants who developed NEC, when compared to controls at same chronological age at that same day, were more likely to have received a RBC transfusion with the preceding 96 hours
Stritzke **2013** **Canada** **MC** **Case-control study**	To evaluate the association between RBC transfusion within the preceding 2 days prior to development of NEC	1026 infants with NEC (study) and 57 887 infant without NEC (control)	927 study infants and 2731 1:3 GA matched control infants Non-survivors: included	No	RBC product: CMV negative, irradiated, leukoreduced, type specific or type O Guidelines: described as variable	**NEC** Transfusion in preceding 2 days were more likely in NEC cases than in controls: 143/927 (16%) vs. 210/2731 (8%)	Infants who developed NEC, when compared to controls, were more likely to have received a RBC transfusion in the preceding 2 days

Valieva 2009 USA SC	To determine risks and benefits associated with RBC transfusions	60 infants 500-1000 grams and ≤28 weeks GA	52 infants were included and analyzed Non-survivors: if survived beyond immediate postnatal period	No 47 were transfused and 5 were not *Difference in transfusion rates/amounts between groups*	RBC product: AS-5, CMV negative or leukoreduced, Hg S negative and irradiated Guidelines: described	**CLD** Transfused group: 36/43 (84%) Non-transfused: 3/3 (100%) p=NS **NEC** Transfused group: 14/47 (30%) Non-transfused: 0/5 (0%) p=<0.05 **Mortality** Transfused group: 8/47 (17%) Non-transfused: 0/5 (0%)	Infants who were transfused were more likely to develop NEC or not survive
Weintraub 2011 Israel SC **Case-control study**	To evaluate the association between stage 3 or higher ROP and other disorders and treatments of prematurity	55 infants with ROP≥3 and 2:1 controls (<1500 grams and <32 wks)	55 study infants and 110 control infants included Non-survivors: unclear	No	RBC product: not described Guidelines: not described	RBC transfusion increased odds of the diagnosis of **ROP grade ≥3** by an OR 12.376 (1.57-127.7 95% CI)	RBC transfusions were associated with an increased odds of being diagnosed with ROP grade ≥3
Retrospective – no comparator							
Beeram 2001 USA MC	To evaluate the practice of RBC transfusions in VLBW infants in the 1990s	527 preterm infants	289/476 received at least 1 RBC transfusion		RBC product: stored in dextrose-adenine-monobasic sodium phosphate-sodium chloride, single	**CLD at 36 wks:** 83/476 (17%) **Grade ≥3-4 IVH:** 47/476 (10%) **ROP grade ≥3:** 60/476 (13%)	No adverse events found related to transfusion

					donor where available Transfusion guidelines: described		
Dani 2008 Italy SC	To assess if RBC transfusions induce significant changes in acid-base, electrolyte and glucose status	No. eligible unclear <31 wks GA	61 Non-survivors: Included	Yes	RBC product: SAGM, <7 days of age, irradiated	**Hypocalcaemia**: 1/61 (2%) **Hyperkalaemia**: 3/61 (5%) **Hyperglycaemia**: 0/61 (0%) **Hypoglycaemia**: 1/61 (2%)	No clinically significant changes in electrolyte or glucose status were observed

Table 3: Summary of study characteristics – case series

Author (Year) Country Single or multi-centre (SC/MC)	Aim of study (primary outcome)	Participants (Total number)	Intervention (exposure)	RBC product and guidelines	Primary outcome/s as per review	Secondary outcome/s as per review	Notes
Dobroszycki 1999 USA SC	To investigate a cluster of blood transfusion-associated babesiosis	6 infants affected and infected with *Babesia microti*-infected donor blood	RBC transfusion with retrospectively identified parasitemia	RBC product: not described Guidelines: not described	Babesiosis infection	Nil	
Martin 2003 Spain SC	To determine effects of transfusion of irradiated/stored RBCs to neonates	10 infants	Irradiated and stored (>10 days) RBCs	RBC product: SAGM, irradiated, stored for >10 days	Changes in K+ post-transfusion of irradiated and stored RBCs	Nil	No statistical difference in pre (median 3.7mmol/L) and post levels (median 3.55mmol/L) of K+ in any case (p=0.218)
Simonsen 2011 USA MC	To review a series of neonatal transfusion-associated babesiosis cases	7 infants ≤ 2500 grams	RBC transfusion with retrospectively identified parasitemia	RBC product: not described	Babesiosis infection	Nil	Cases were from an endemic area with an estimated 1 in 21 000 RBC units are infected with *Babesia*

Appendix 4S: Tables S4-56
Table S4: Quality assessment – randomized studies

Author Year Country	Clear definitions of adverse effects*	Clear definitions of adverse associations*	Adverse outcome data reported in >80% of studied infants	Additional quality assessment comments
Bell 2005 USA SC	No	Yes	Yes	
Brooks 1999 USA SC	No	Yes	No Missing data for 8/24 (33%) and 8/18 (44%) infants for ROP outcome only	
Carnielli 1992 Italy SC	No	No	Yes	No *a priori* sample size calculation
Chen 2009 Taiwan SC	No	No	Yes	Restrictive vs. liberal policies were only undertaken for first 30 days of age

da Cunha 2005 Brazil SC	No	No	Yes	Sample size not determined *a priori*
Fergusson 2012 Canada MC	No	Yes	Yes	
Griffiths 1997 United Kingdom MC	No	Yes	Yes	*A priori* sample size not reached as trial stopped early due to recruitment challenges
Haiden 2006 Austria SC	No	No	Yes	*A priori* sample size calculated and reached
Kirpalani 2006 Canada/Australia/USA MC	No	Yes	Yes	A priori sample size calculation reached No. of transfusions per infant was not statistically significantly different between groups: restrictive 4.9 (4.2) vs. liberal 5.7 (5.0) p=0.07
Follow-up to Kirpalani 2005 study: **Whyte 2009 Canada/Australia/USA MC**	N/A	Yes	Yes	

Lee **1995** **USA** **SC**	Yes	No	Yes	A priori sample size not reached due to trial being stopped early as significant difference between groups (in primary outcome) demonstrated
Liu **1994** **USA** **SC**	Yes	No	Yes	
Maier **1994** **Europe** **MC**	No	Yes	Yes	Infants were withdrawn from the study (post-randomisation) if they required ventilation or CPAP with a FiO2 >0.4 after day 6, major surgery, death, venous hematocrit >0.59, discharge before 42 days or a vertically transmitted infection
Strauss **2000** **USA** **SC**	Yes	No	Yes	Study did not meet *a priori* calculated sample size
Sub-study of Strauss 2000 study: **Strauss** **1999** **USA** **SC**	Yes	No	Yes	Post-hoc sample size: study was a sub-study of a large trial and alloimmunisation was not an initial study outcome

Wong 2005 Canada MC	No	Yes	Yes	

*As per definition outlined in the methods section of our review

Table S5: Quality assessment – non-randomized studies						
Author Year Country SC or MC	**Clear definitions of identified adverse effects**	**Clear definitions of identified adverse associations**	**Were key confounding factors identified?**	**Analysis adjusted for key confounding factors*?**	**Complete (>80%) adverse outcome data**	**Overall comment**
Prospective cohort – comparator						
Bednarek 1998 USA MC	No	Yes	2/5	0/5	Yes	
Miyashiro 2004 Brazil MC	No	No	5/5	5/5	Yes	
Mimica 2008 Brazil SC	No	Yes	5/5	2/5	Yes	Baseline clinical characteristics between infants in the different phases were statistically different and may account for the differences in outcomes observed, rather than change in transfusion practice Other clinical differences present in infants between different phases may account for the differences in outcomes (mortality and any grade ROP) rather than due to transfusion
Silvers 1997 UK SC	No	Yes	3/5	2/5	No Complete	Infants with CLD are more likely to be transfused (higher level of respiratory support vs. no support/minimal) and is likely to led to higher transfusion rates

					data available for 50-78/105 (48- 74%) for the various outcome point	
Prospective cohort – no comparator						
Ayede 2011 Nigeria SC	No	No	2/5	N/A	Unclear	
Bearer 2000 USA SC	Yes	N/A	0/5	N/A	Yes	Limited generalizability as study carried out in 1990s and environmental lead exposure is likely to have changed and lead levels in donated blood may be less
Boo 1998 Kuala Lumpur SC	Yes	No	2/5	N/A	Yes	
Elabiad 2013 USA SC	Yes	N/A	2/5	N/A	Yes	
Jain 2001 USA SC	No	N/A	0/5	N/A	Yes	Single time point measurement of biochemical markers makes drawing widespread safety conclusions challenging

Mangel 2001 Canada SC	Yes	No	2/5	N/A	No Missing data (59% of events) for pre/post K+ levels	Safety study with limited reported short-term adverse effects measured
Strauss 2000 USA	Yes	N/A	N/A	N/A	Yes	
Ugwu 2006 Nigeria SC	Yes	No	1/5	N/A	Yes	Observational study of Nigerian transfusion practices
Retrospective – comparator						
Allegaert 2004 Belgium MC	No	Yes	4/5	4/5	Yes	128 infants were not included in study due to death (total infants included in study = 175)
Baer 2011 USA MC	No	Yes	4/5	2/5	Yes	Outcome of grade ≥3-4 IVH was diagnosed on HUS (proxy and no standardisation of timing of scan) and may have occurred at an earlier stage; therefore, timing of exposure and outcome cannot be clearly established. Mortality in cases was higher than in controls (33% vs. 8%)

Baer 2011 USA MC	No	Yes	5/5	2/5	Yes	Outcome of extension to grade ≥3-4 IVH was diagnosed on HUS (proxy and no standardisation of timing of scan) and may have occurred at an earlier stage; therefore, timing of exposure and outcome cannot be clearly established Variation in gestational age between cases (25 wks) and controls (30 wks)
Baer 2011 USA MC	No	Yes	1/5	0/5	Unclear	Findings may not be generalizable to many NICUs as study was made up of a mature cohort of infants (mean GA 35±4 wks in perinatal centres and 36±4 wks in the surgical centre) with low mortality at 1-6%
Birenbaum 2006 USA SC	No	No	3/5	0/5	Yes	Subgroup analysis showed the lower birthweight infants received more transfusions
Blau 2011 USA SC	No	Yes	4/5	0/5	Yes	Infants with NEC temporally related to RBC transfusion potentially received transfusion as a "marker of developing illness" vs. as a contributory/causative factor Transfused infants who developed NEC (within 48 hours or longer from RBC transfusion) were more likely to be receiving some form of respiratory support compared to non-transfused NEC (87-89% vs. 42%)

Carter 2012 USA SC	No	Yes	2/5	0/5	Yes	RBC transfusion may have been a marker of illness alone
Christensen 2010 USA MC	No	Yes	2/5	2/5	No 62/112 (55%) had complete transfusion histories	Part A: Infants with NEC related to transfusion were significantly smaller and more immature than those with NEC deemed unrelated to transfusion Part B: Matching for some demographic features (GA±2wks, BW±500grams) had a wide margin, as did the matching for same day of age transfused (±7 days)
Cooke 1997 UK SC	No	Yes	3/5	3/5	No 73/98 (74%) had full data sets	
Couselo 2011 Spain SC	No	Yes	3/5	Unclear	Yes	
Del Vecchio 2013 USA SC	No	Yes	0/5	0/5	Yes	No details provided for the gestational age or birthweight of any of the included infants
Demirel 2012 Turkey SC	No	Yes	4/5	4/5	Yes	

Demirel 2009 Turkey SC	No	Yes	4/5	4/5	Yes	206/312 (66%) infants excluded from initial eligible study population as they did not survive long enough for CLD criteria to be assessed
dos Santos 2011 Brazil MC	No	Yes	5/5	3/5	Yes	Challenging study to draw conclusions from due to conflicting data regarding mortality rates associated with receipt of RBC transfusion
Elabiad 2013 USA SC	No	Yes	3/5	1/5	Yes	Baseline differences (no controlled for) in infants with NEC compared to controls may account for the differences in transfusion observed between groups
El-Dib 2011 USA SC	No	Yes	4/5	2/5	Yes	
Fergusson 2003 Canada MC	No	Yes	5/5	4/5	Yes	Retrospective before and after study over total 3-year period; other changes in neonatal care (beyond prestorage leukoreduction) may have influenced observed outcome improvements
Giannantonio 2012 Italy SC	No	Yes	4/5	2/5	Yes	

Josephson 2010 USA MC	No	Yes	5/5	0/5	Yes	Very detailed sub-group analysis leading to limited numbers in the groups
Khodabux 2009 Netherlands MC	No	Yes	4/5	0/5	Yes	Despite standard transfusion guidelines and similar clinical characteristics of infants between the two units, more infants were transfused in higher transfusion volume unit
Follow-up study of Khodabux 2009 **Von Lindern 2011 Netherlands MC**	No	Yes	Unclear	Unclear	Yes	
Mally 2006 USA SC	No	Yes	4/5	0/5	Yes	Temporal association between receipt of RBC transfusion and development of NEC could indicate either a cause-effect relationship or recent of transfusion to manage early (yet unrecognized) signs of NEC
Martin 2013 Spain SC	No	Yes	4/5	4/5	Yes	No difference between number of infants transfused with RBCs prior to development of NEC but an increase in frequency of transfusion found

Paul 2011 USA SC	No	Yes	5/5	4/5	Yes	Temporal association between receipt of RBC transfusion and development of NEC could indicate either a cause-effect relationship or recent of transfusion to manage early (yet unrecognised) signs of NEC
Singh 2011 USA MC	No	Yes	2/5	0/5	Yes	
Stritzke 2013 Canada MC	No	Yes	3/5	2/5	Yes	Large database study consistent with findings of other smaller retrospective studies, however, it remains difficult to identify whether infants were transfused because they were becoming unwell or if a cause-effect relationship exists
Valieva 2009 USA SC	No	Yes	4/5	0/5	No 24/52 (46%) infants had outcome data missing	
Weintraub 2011 Israel SC	No	Yes	2/5	2/5	Yes	Study designed to highlight characteristics of infants likely to be diagnosed with severe ROP rather than examine cause-effect relationship

Retrospective – no comparator						
Beeram 2001 USA SC	No	No	2/5	N/A	Yes	
Dani 2001 Italy SC	No	Yes	3/5	N/A	Yes	Blood sampling methods (capillary heel sticks) may have led to falsely elevated potassium levels (study outcome)

Abbreviations: abx: antibiotics; BW: birth weight; CRIB: clinical risk index for babies; FNHTR: febrile non-haemolytic transfusion reaction; FFP: fresh frozen plasma; GA: gestational age; HUS: head ultrasound; iNO: inhaled nitric oxide; IVH: intraventricular haemorrhage; min: minute; PNA: postnatal age; RBC: red blood cell; ROP: retinopathy of prematurity; wks: weeks
*no. of days

Appendix 5S. Figures S3-S4

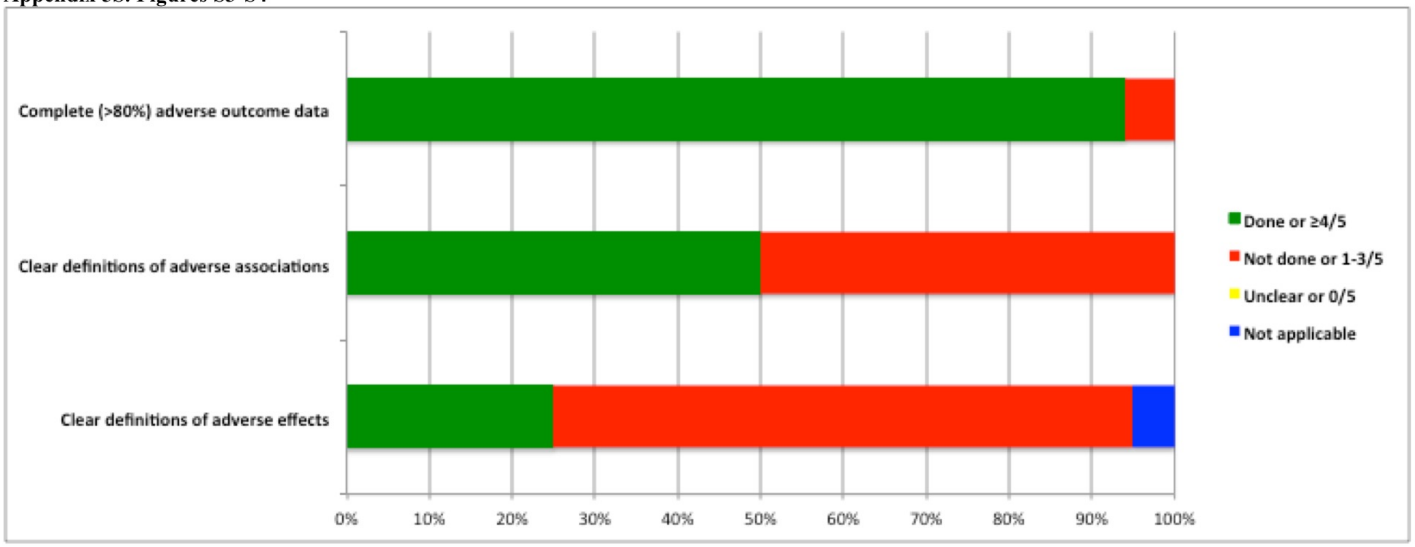


Figure S3A: Quality assessment of randomized studies (n=16)

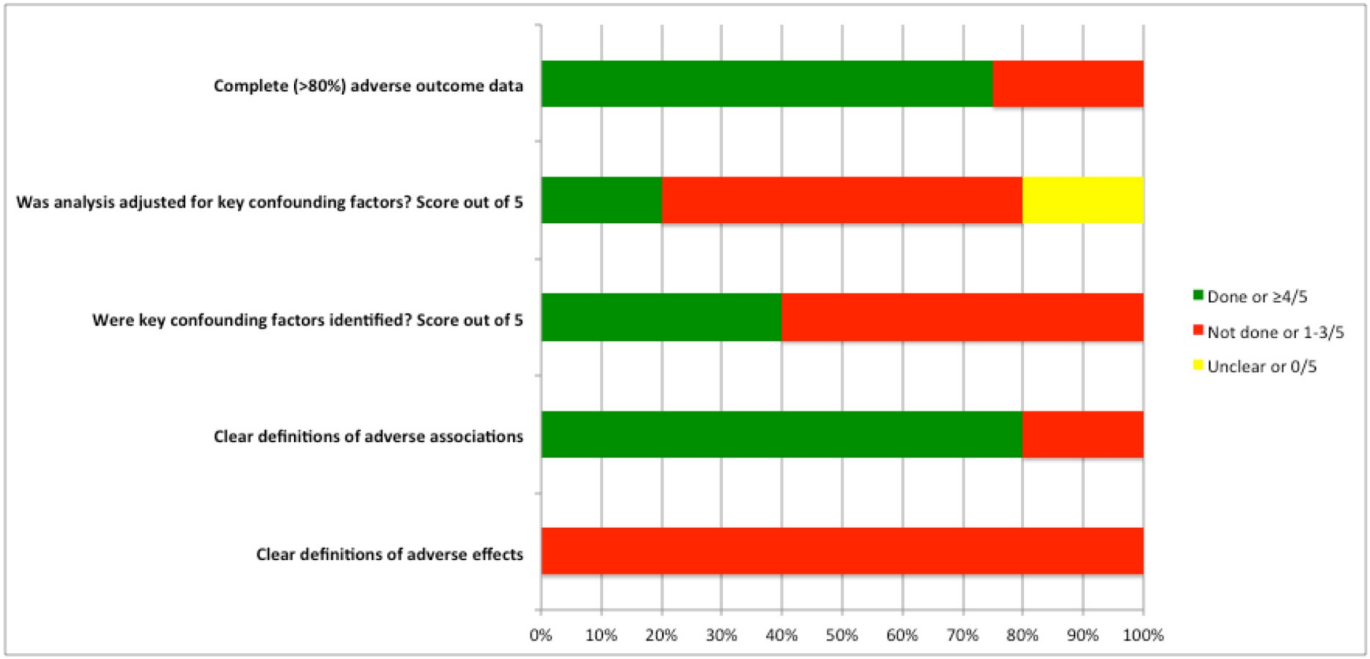


Figure S3B: Quality assessment of prospective non-randomized studies with a comparator group (n=4)

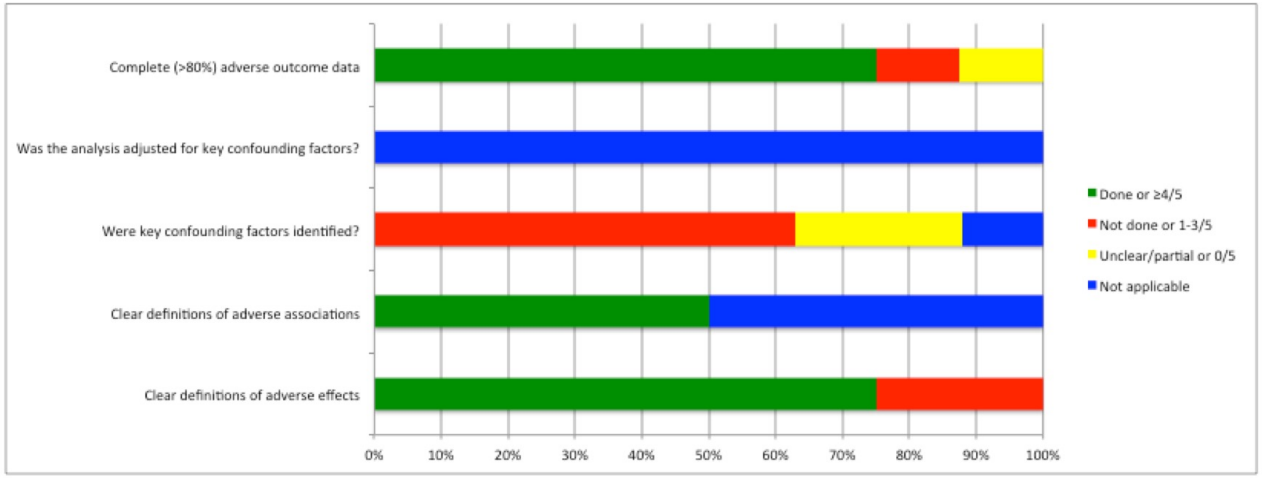


Figure S3C: Quality assessment of prospective non-randomized studies without a comparator group (n=8)

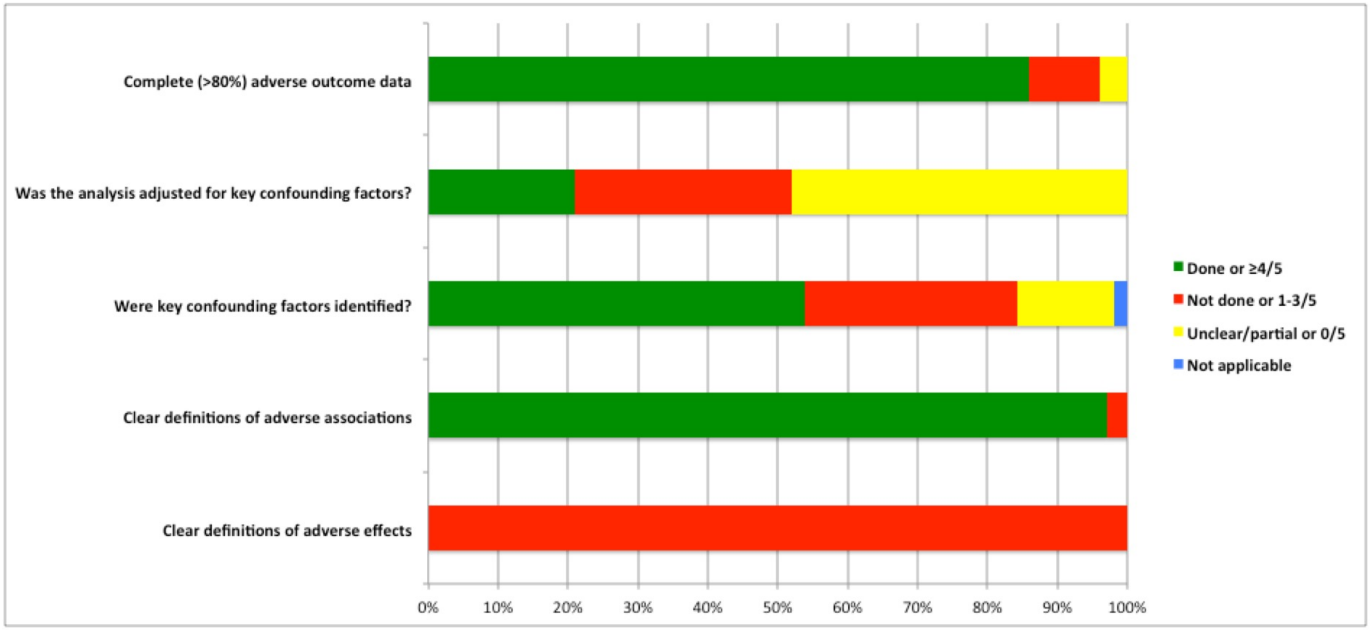


Figure S3D: Quality assessment of retrospective studies with a comparator group (n=25)

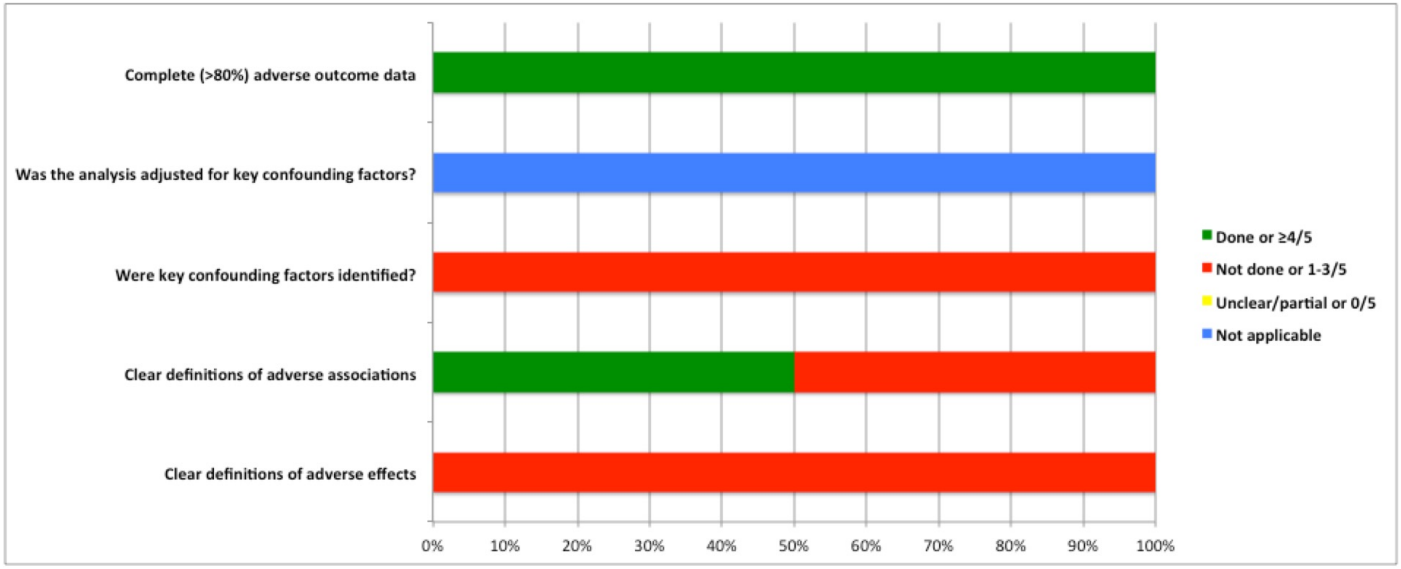


Figure S3E: Quality assessment of retrospective studies without a comparator group

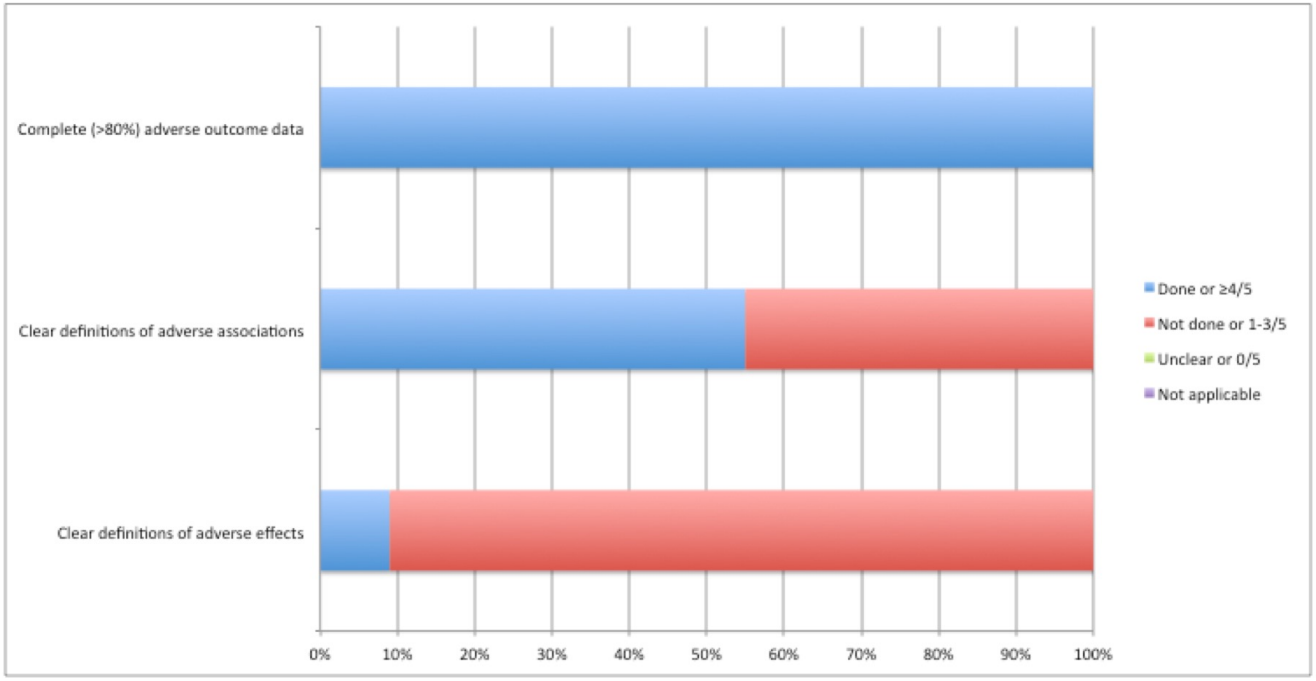


Figure S4A: Quality assessment by outcome - mortality during initial hospitalization (randomized studies; n=11)

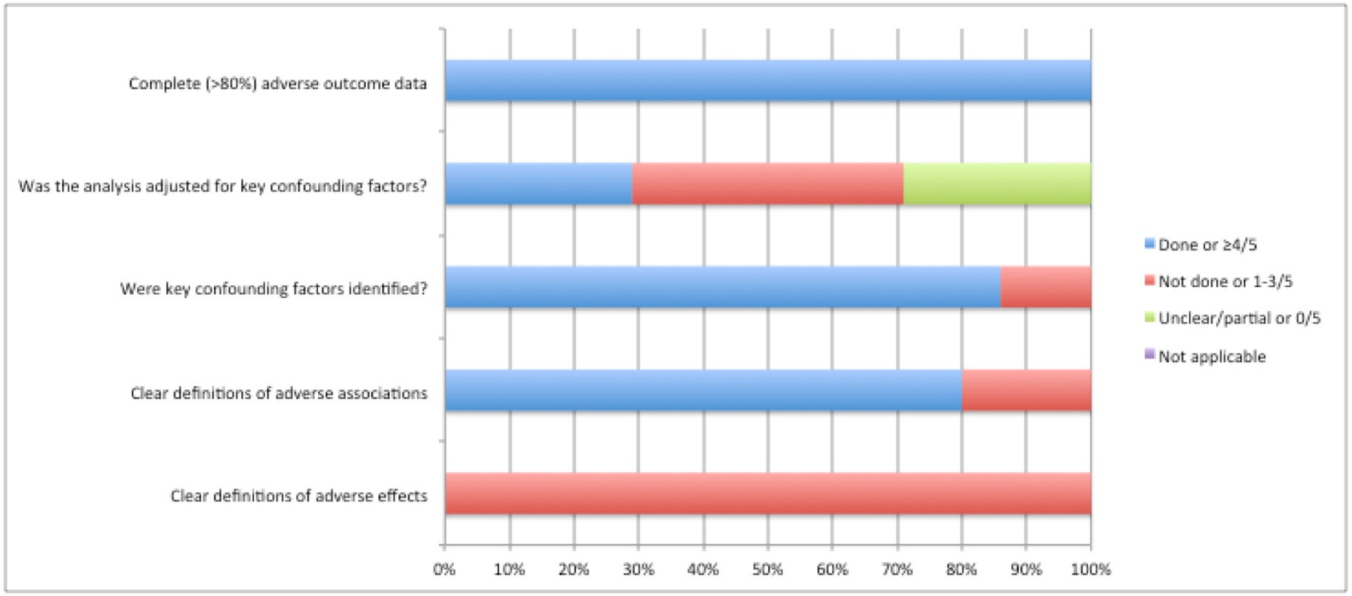


Figure S4B: Quality assessment by outcome - mortality during initial hospitalization (non-randomized studies; n=7)

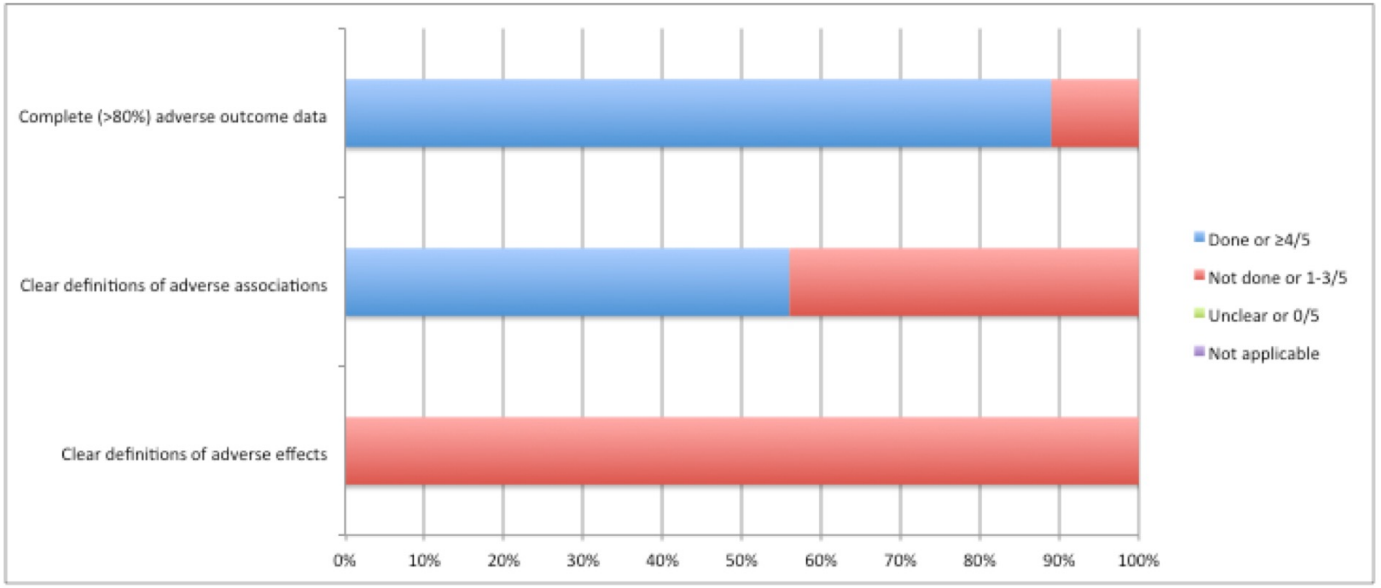


Figure S4C: Quality assessment by outcome - chronic lung disease (randomized studies; n=9)

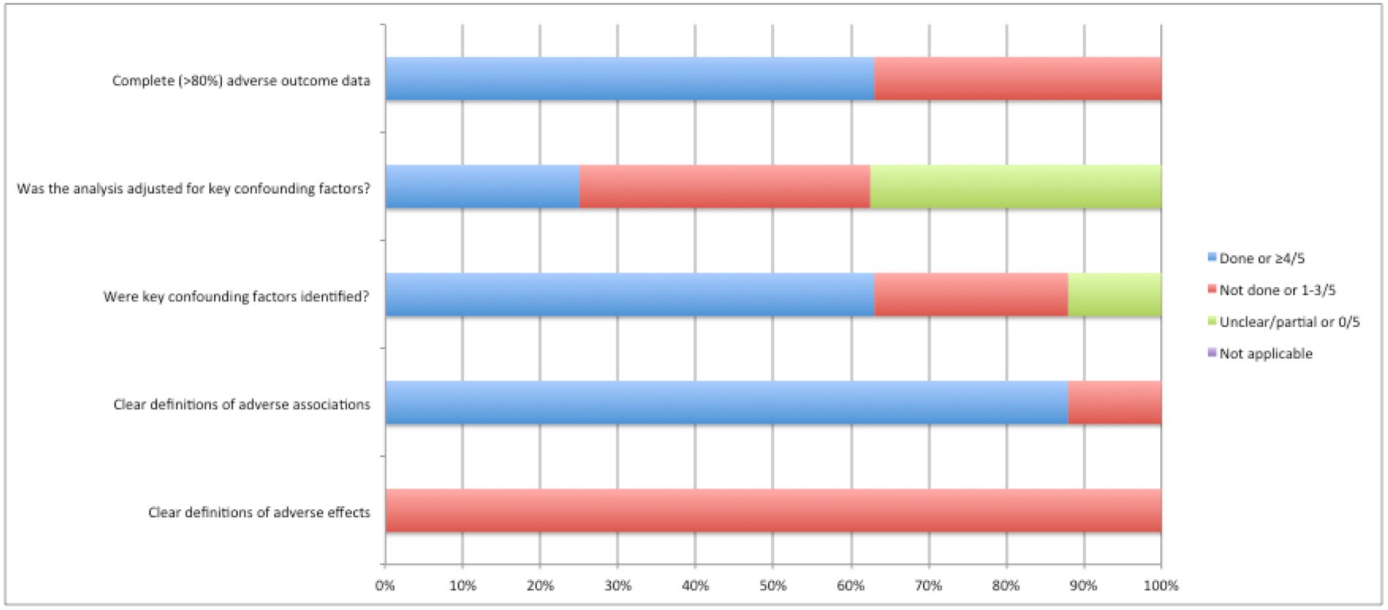


Figure S4D: Quality assessment by outcome - chronic lung disease (non-randomized studies; n=8)

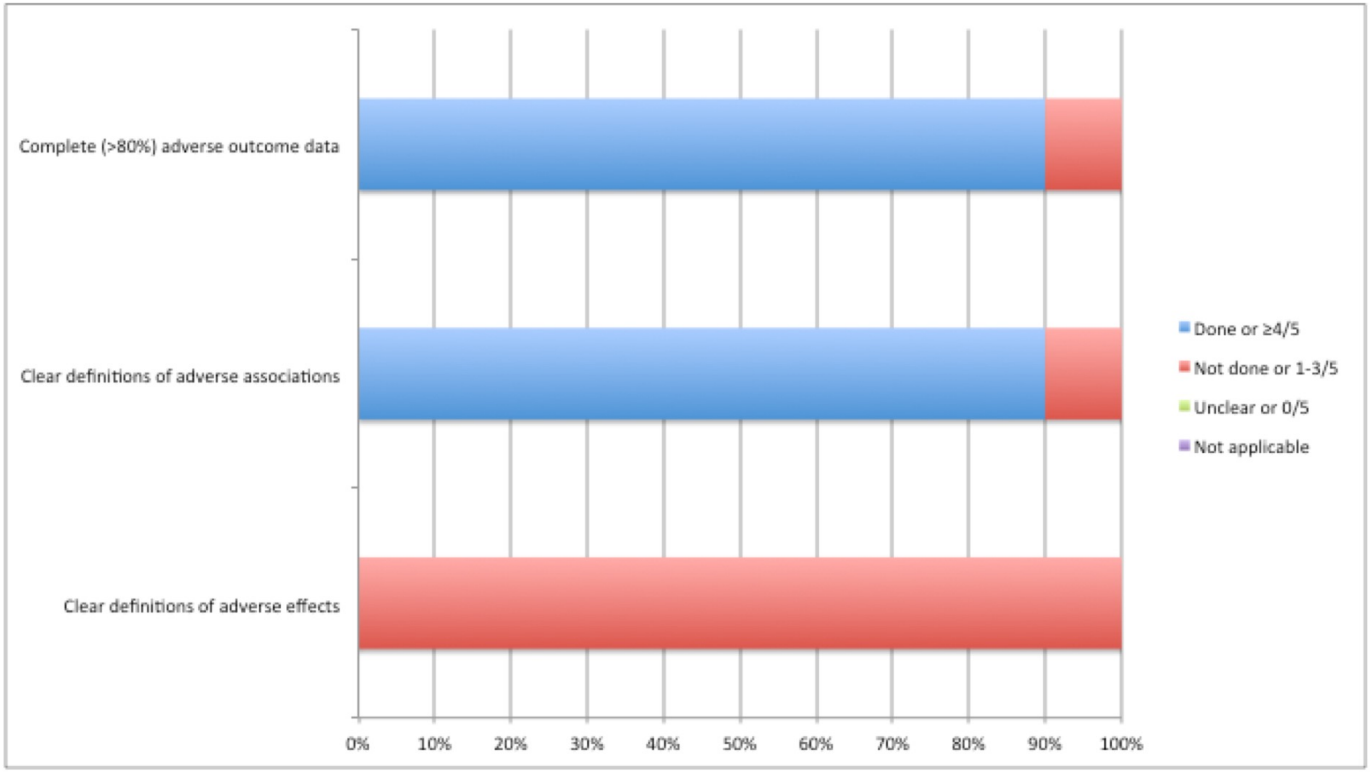


Figure S4E: Quality assessment by outcome - necrotizing entercolitis (randomized studies; n=5)

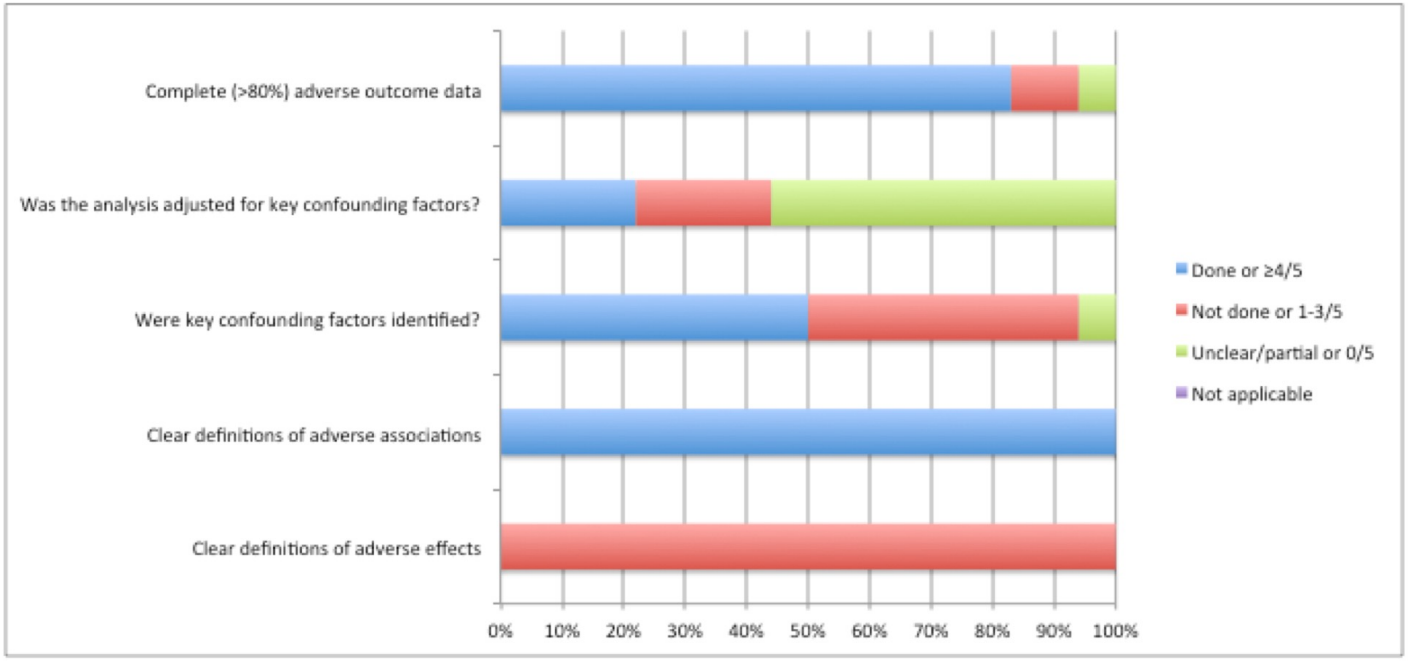


Figure S4F: Quality assessment by outcome - necrotizing entercolitis (non-randomized studies; n=18)

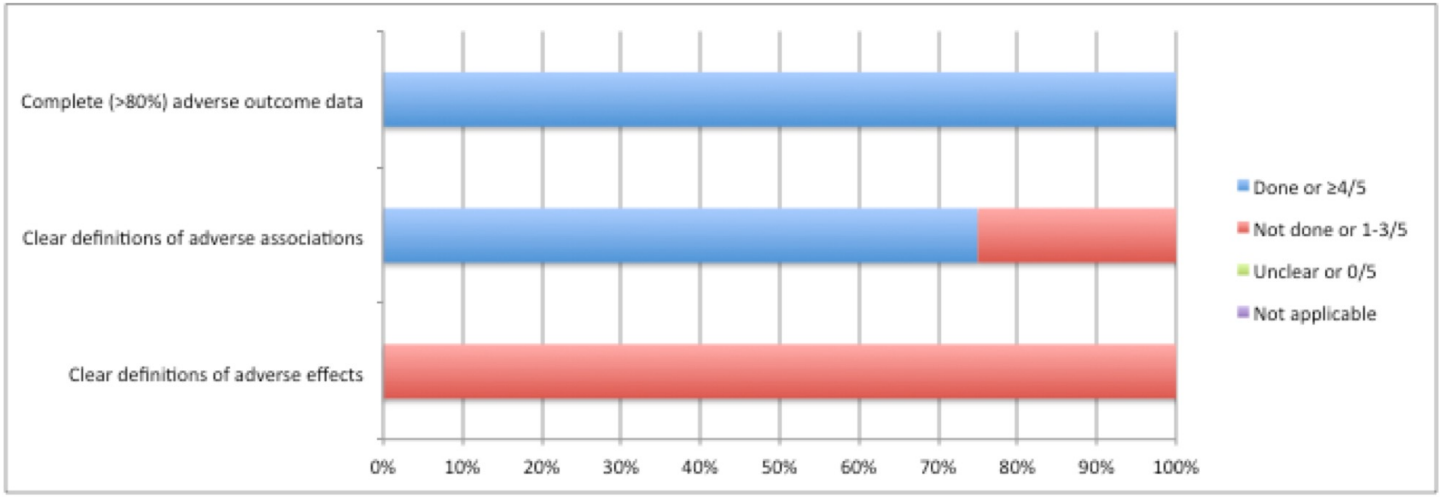


Figure S4G: Quality assessment by outcome - intraventricular hemorrhage (randomized studies; n=4)

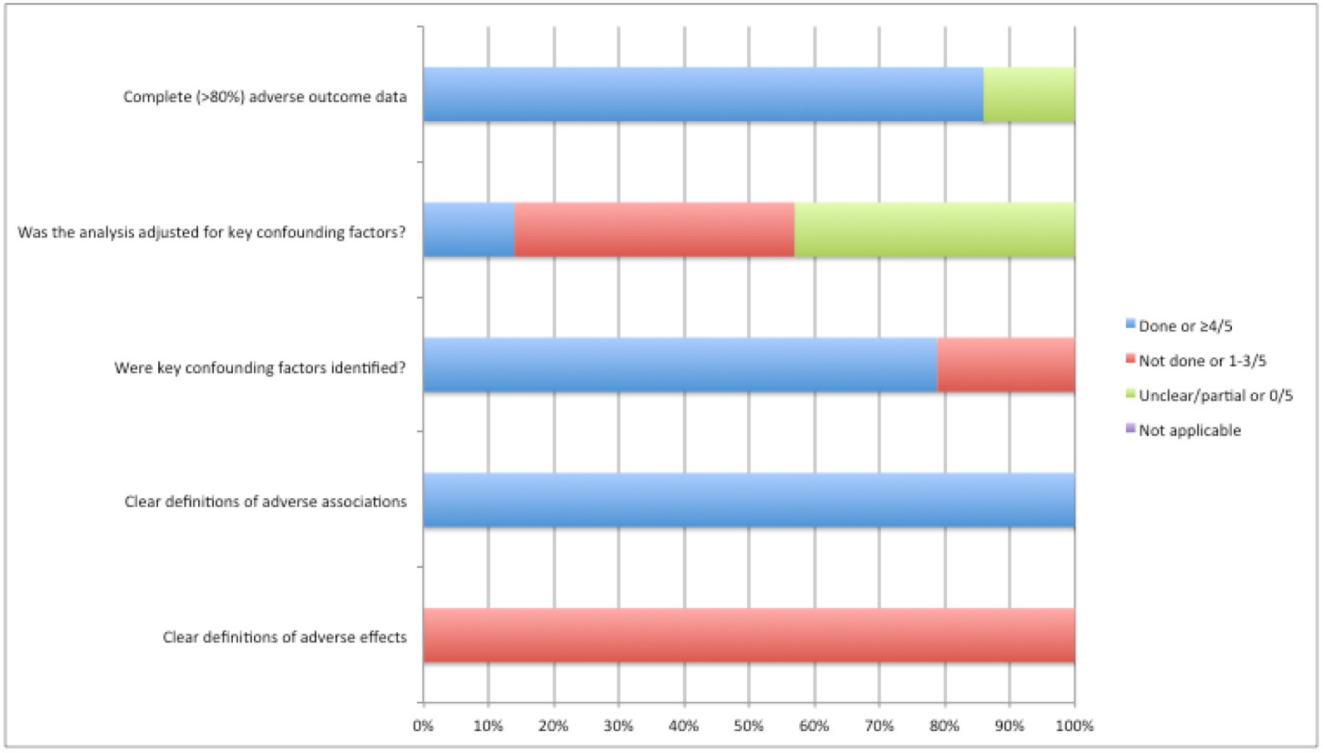


Figure S4H: Quality assessment by outcome - intraventricular hemorrhage (non-randomized studies; n=7)

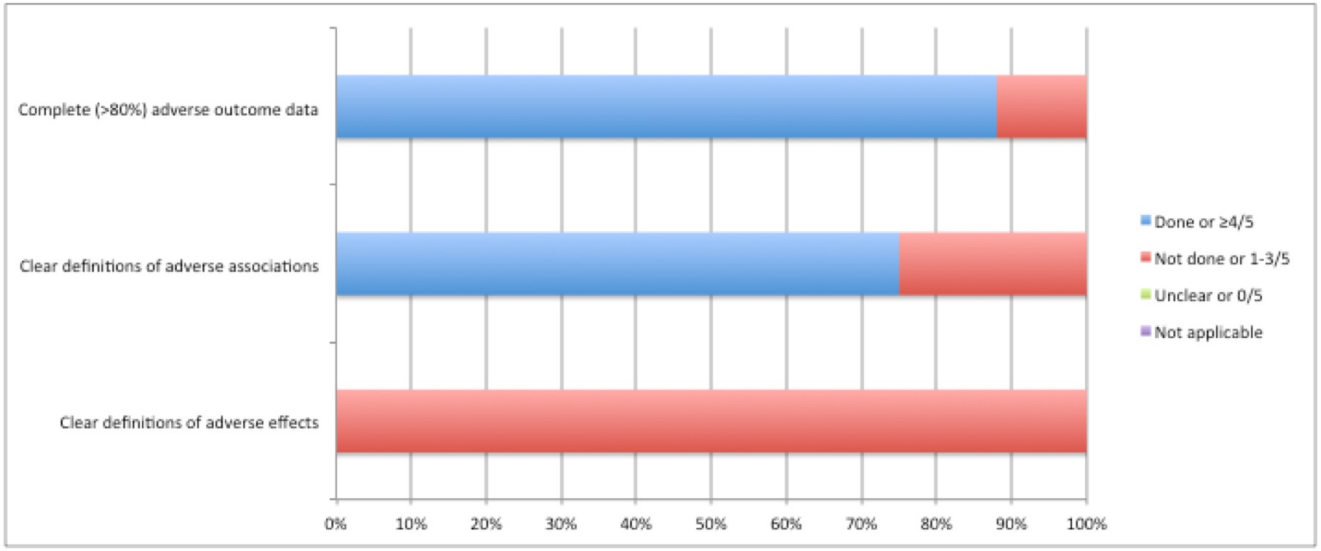


Figure S3I: Quality assessment by outcome - retinopathy of prematurity (randomized studies; n=8)

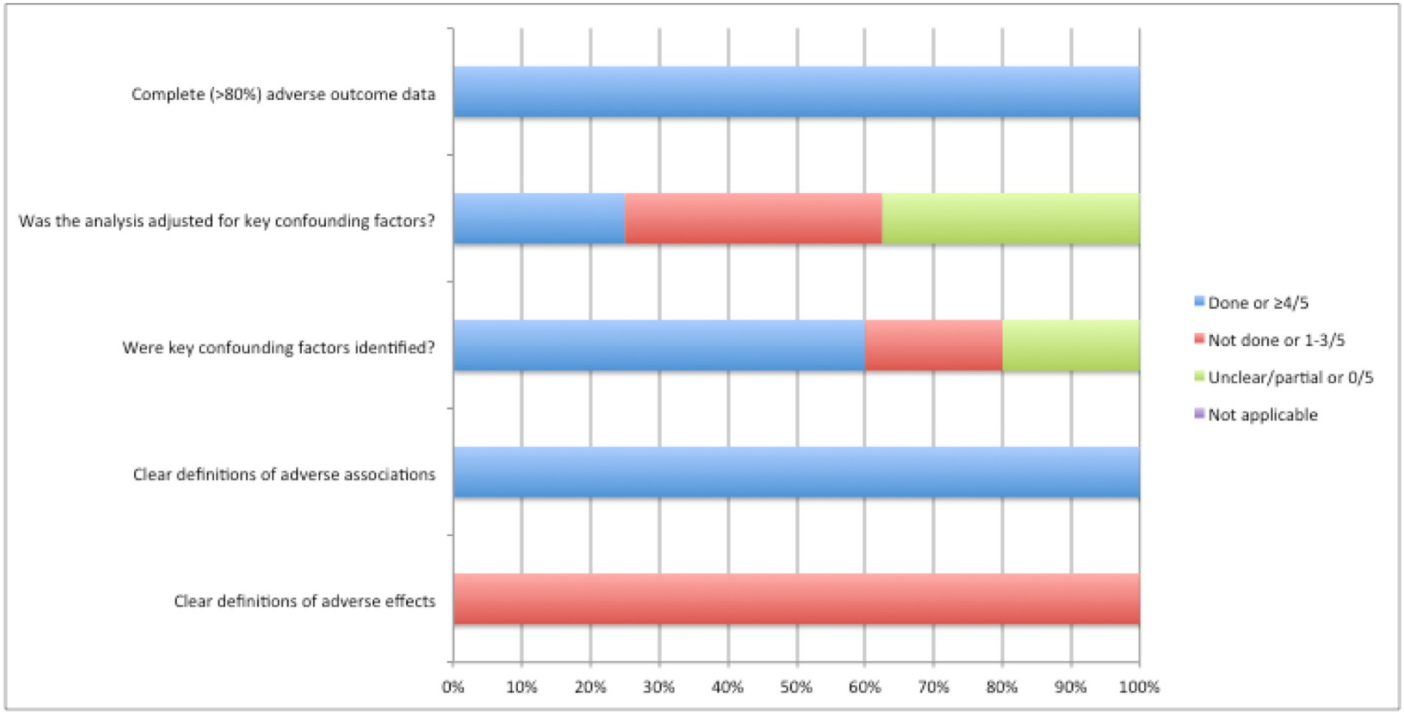


Figure S4J: Quality assessment by outcome – retinopathy of prematurity (non-randomized studies; n=4)

Appendix 6S. Figures S5-S15

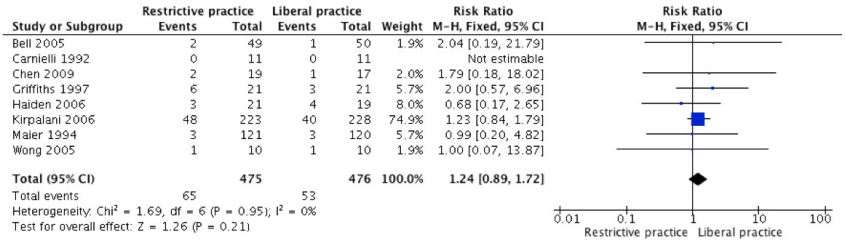


Figure S5: Meta-analysis for mortality during initial hospitalization (randomized studies)

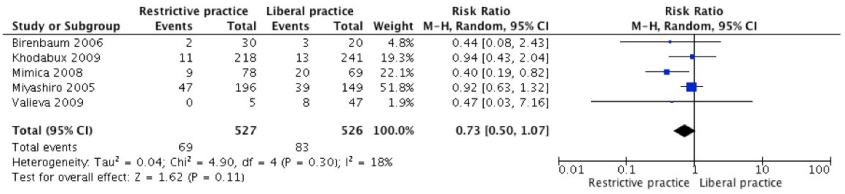


Figure S6: Meta-analysis for mortality during initial hospitalization (non-randomized studies)

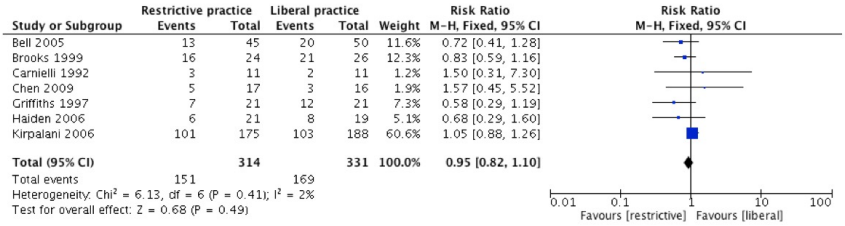


Figure S7: Meta-analysis for chronic lung disease (randomized studies)

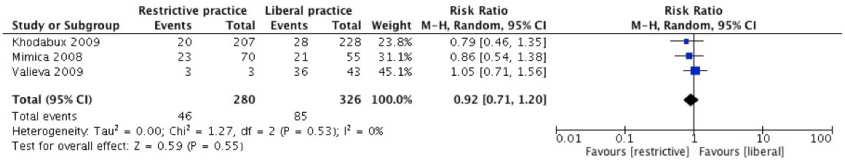


Figure S8: Meta-analysis for chronic lung disease (non-randomized studies)

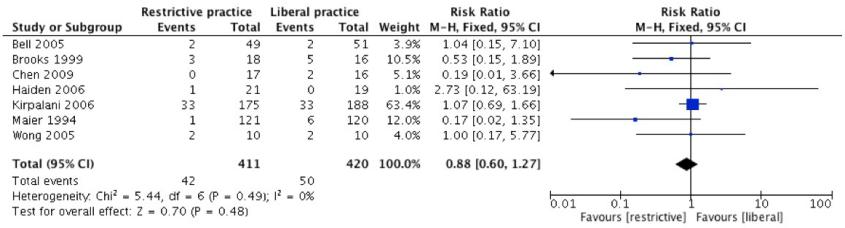


Figure S9: Meta-analysis for retinopathy of prematurity (randomized studies)

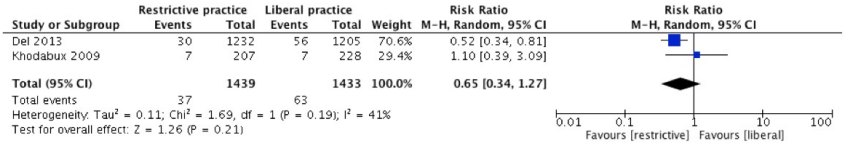


Figure S10: Meta-analysis for retinopathy of prematurity (non-randomized studies)

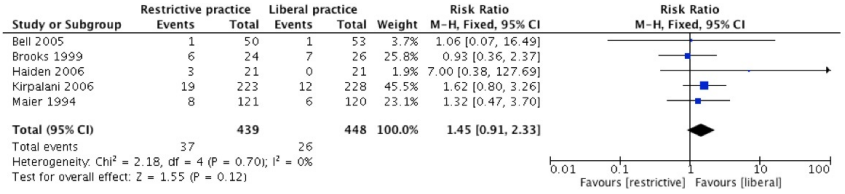


Figure S11: Meta-analysis for necrotizing enterocolitis (randomized studies)

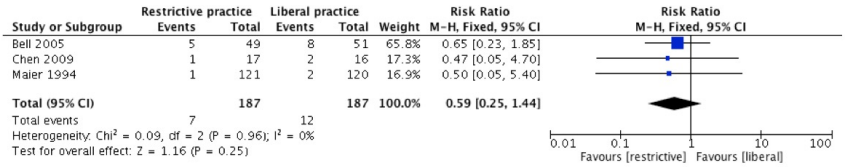


Figure S12: Meta-analysis for intraventricular hemorrhage (randomized studies)

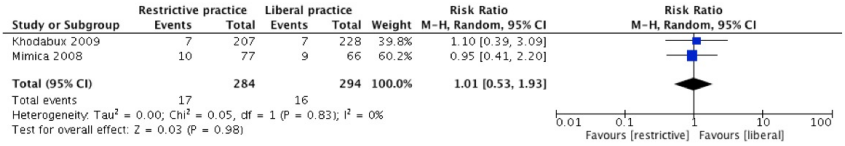


Figure S13: Meta-analysis for intraventricular hemorrhage (non-randomized studies)

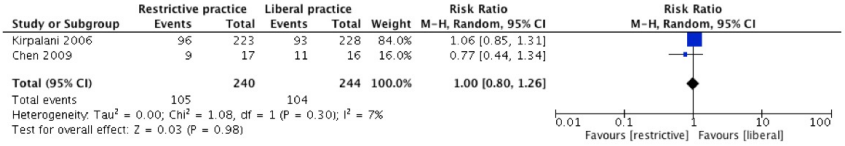


Figure S14: Meta-analysis for sepsis (randomized studies)

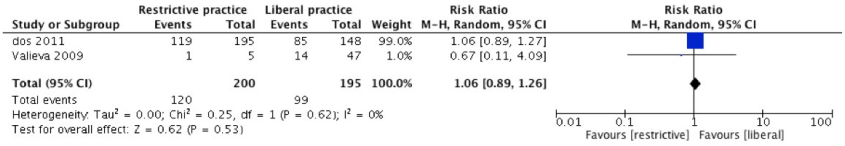


Figure S15: Meta-analysis for sepsis (non-randomized studies)

Chapter 4

Washed versus unwashed red blood cells for neonatal transfusion and impact on morbidities and mortality

This published Cochrane systematic review examines the current evidence for pre-transfusion washing of RBCs in reducing important morbidities and mortality in preterm neonates.

It addresses the previously identified research question:

Research question 3:

Does washing RBCs prior to transfusion in neonates prevent morbidity and mortality?

Authorship forms are provided in Appendix C

Cochrane Library

Cochrane Database of Systematic Reviews

Washed versus unwashed red blood cells for transfusion for the prevention of morbidity and mortality in preterm infants (Review)

Keir AK, Wilkinson D, Andersen C, Stark MJ

Keir AK, Wilkinson D, Andersen C, Stark MJ.
Washed versus unwashed red blood cells for transfusion for the prevention of morbidity and mortality in preterm infants.
Cochrane Database of Systematic Reviews 2016, Issue 1. Art. No.: CD011484.
DOI: 10.1002/14651858.CD011484.pub2.

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TABLE OF CONTENTS

[Intervention Review]

Washed versus unwashed red blood cells for transfusion for the prevention of morbidity and mortality in preterm infants

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Editorial group: Cochrane Neonatal Group.
Publication status and date: New, published in Issue 1, 2016.
Review content assessed as up-to-date: 31 July 2015.

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ABSTRACT

Background

Infants born very preterm often receive multiple red blood cell (RBC) transfusions during their initial hospitalisation. However, there is an increasing awareness of potential adverse effects of RBC transfusions in this vulnerable patient population. Modification of RBCs prior to transfusion, through washing with 0.9% saline, may reduce these adverse effects and reduce the rate of significant morbidity and mortality for preterm infants and improve outcomes for this high-risk group.

Objectives

To determine whether pre-transfusion washing of RBCs prevents morbidity and mortality in preterm infants.

Search methods

We used the standard search strategy of the Cochrane Neonatal Review Group to search the Cochrane Central Register of Controlled Trials (CENTRAL 2015, Issue 7), MEDLINE via PubMed (31 July 2015), EMBASE (31 July 2015), and CINAHL (31 July 2015). We also searched clinical trials databases, conference proceedings, and the reference lists of retrieved articles for randomised controlled trials and quasi-randomised trials.

Selection criteria

Randomised, cluster randomised, and quasi-randomised controlled trials including preterm infants (less than 32 weeks gestation) or very low birth weight infants (less than 1500 g), or both, who received one or more washed packed RBC transfusions.

Data collection and analysis

Two review authors independently assessed the eligibility of the trials. We identified four studies from the initial search. After further review of the full-text studies, we found one study meeting the selection criteria.

Main results

We included a single study enrolling a total of 21 infants for analysis in this review and reported on all-cause mortality during hospital stay, length of initial neonatal intensive care unit (NICU) stay (days), and duration of mechanical ventilation (days). There was no significant difference in mortality between the washed versus the unwashed RBCs for transfusion groups (risk ratio 1.63, 95% confidence interval (CI) 0.28 to 9.36; risk difference 0.10, 95% CI -0.26 to 0.45). There was no significant difference in the length of initial NICU stay between the washed versus the unwashed RBCs for transfusion groups (mean difference (MD) 25 days, 95% CI -21.15 to 71.15) or the duration of mechanical ventilation between the washed versus the unwashed RBCs for transfusion groups (MD 9.60 days, 95% CI -1.90 to 21.10).

Authors' conclusions

We identified a single small study. The results from this study show a high level of uncertainty, as the confidence intervals are consistent with both a large improvement or a serious harm caused by the intervention. Consequently, there is insufficient evidence to support or refute the use of washed RBCs to prevent the development of significant neonatal morbidities or mortality. Further clinical trials are required to assess the potential effects of pre-transfusion washing of RBCs for preterm or very low birth weight infants, or both, on short- and long-term outcomes.

PLAIN LANGUAGE SUMMARY

Does pre-transfusion washing of red blood cells for preterm babies improve their health outcomes?

Background: Babies born preterm or with a low birth weight may be given blood transfusions for a number of reasons. For example, they are sometimes unable to make their own blood well yet; they may need several blood tests to monitor their condition; or they may need extra blood if they become critically unwell.

Studies in older children and in adults have found that a process of 'washing' blood cells before transfusion improved short- and longer-term outcomes. Washing blood removes almost all plasma proteins and most white blood cells, which may help reduce the side effects of a blood transfusion. We wanted to learn if preterm babies might experience these same positive effects.

Review question: We wanted to learn whether washing blood cells before transfusion reduces the chance of illnesses that tend to occur in preterm babies. Some of the outcomes that we looked at were illnesses affecting eyes (retinopathy of prematurity), lungs (chronic lung disease), brain (intraventricular haemorrhages or cysts), and long-term developmental problems. We also wanted to look at other outcomes like length of hospital stay and acute transfusion reactions.

Key results: This review found only one study that evaluated the effects of washing blood cells before transfusion in preterm babies. This study included small numbers of babies. The outcomes the study reported that were relevant to our review were mortality, duration of mechanical ventilation, and length of initial hospitalisation. The results for all these outcomes were very uncertain. Washing blood cells might be helpful or harmful, but we cannot make a determination.

Quality of the evidence: It was hard from the available evidence to draw any conclusions about whether washing blood would be helpful or not for preterm babies. As of now, there is no strong evidence showing that washing blood makes any difference to the outcomes of preterm babies.

BACKGROUND

Anaemia of prematurity (AOP) is a common multifactorial complication of preterm birth. Contributing causes include reduced levels of plasma erythropoietin (EPO) in response to anaemia and hypoxia, diminished red blood cell (RBC) life span, phlebotomy losses for laboratory testing, limited transplacental transfer of iron due to premature birth, and dependence on hepatic EPO production (Venkatesh 2012). Small-volume RBC transfusions are used

to manage AOP, with over 90% of preterm neonates with a birth weight at less than 1000 g receiving at least one RBC transfusion during their initial hospitalisation (Baer 2011; Mohamed 2012). While it is assumed that these transfusions are beneficial in preterm infants, the evidence available to support this is limited (Venkatesh 2012).

There is an increasing awareness of potential adverse effects related to RBC transfusions in the neonatal population. Transfusion may be associated with necrotising enterocolitis (NEC) (Mohamed 2012), intraventricular haemorrhage (IVH) (Baer 2011), retinopathy of prematurity (ROP) (Giannantonio 2012), chronic lung disease (CLD) (Cooke 1997), and mortality (dos Santos 2011; Valieva 2009). Furthermore, there is emerging evidence that other transfusion-specific morbidities, such as transfusion-related acute lung injury, may be under-reported and under-recognised in preterm infants (Gauvin 2012; Rashid 2013).

Several biologically plausible mechanisms to explain these associations have been proposed, including a 'two-hit' model of post-transfusion injury (Aiboshi 2001). This model hypothesises that an underlying inflammatory state primes the recipient's immune system with subsequent RBC transfusion triggering immune cell activation and related immunomodulation, resulting in frank inflammation (Tinmouth 2006). Transfusion-related immunomodulation is proposed to underlie much of the increased transfusion-associated morbidity and mortality observed in adult populations (Tinmouth 2006). A similar mechanism may exist in the vulnerable preterm population and could explain many of the associations observed between RBC transfusion and significant neonatal morbidities such as NEC, ROP, and CLD.

Research in paediatric and adult populations suggest that modifications to blood-product processing prior to transfusion may improve both inflammatory responses and important clinical outcomes, including mortality in the recipient (Bilgin 2004; Blumberg 2004; Blumberg 2010; Fergusson 2003). In vivo studies in preterm infants suggest that allogeneic leukodepleted RBCs, used in many parts of the world as the standard blood product for preterm infants, are biologically active and result in endothelial activation, inflammation, and oxidative stress in preterm infants (Keir 2013; Stark 2013). Modification of RBCs prior to transfusion, through washing with 0.9% saline, may reduce these deleterious effects and improve outcomes for all populations, including preterm infants.

Description of the condition

Significant morbidities, including NEC, IVH, ROP, CLD, as well as increased mortality, have been associated with receipt of RBC transfusion in preterm infants. No direct causal relationship has been established, but transfusion-related immunomodulation may underlie this increased transfusion-associated morbidity and mortality. Modifications of the RBC product prior to transfusion may ameliorate some of these potential effects and lead to better outcomes for preterm infants.

Description of the intervention

Saline-washed RBCs are units of whole blood or RBCs that have been washed with 1 to 2 L of saline prior to transfusion. Pre-transfusion washing of RBCs occurs through a manual 'open' system technique (Grabmer 2006), an automated cell washer (O'Leary 2011), or via an auto-transfusion device (de Vroege 2007). Washed units contain 10% to 20% fewer RBCs than the original units. These units are depleted of 99% of plasma proteins and 85% of white blood cells. It is important to consider the clinical implications of this time- and resource-intensive processing step.

How the intervention might work

Transfusion-related inflammation and poor clinical outcomes may be caused by RBCs themselves, time-dependent accumulation of bioactive substances in the supernatant (storage lesion), or both (Lannan 2013). Transfusions can alter the immune system of recipients, and it is possible that saline washing of RBCs prior to transfusion may reduce these deleterious effects and improve outcomes for all patient populations, including preterm infants. Animal models using washed red cells have demonstrated blunting of the pro-inflammatory response posthaemorrhage when compared with unwashed RBCs (Belizaire 2012). The use of washed RBC transfusions in paediatric cardiac surgery reduced pro-inflammatory biomarkers and number of transfusions, and demonstrated a trend towards reduced mortality, when compared with unwashed RBCs (Cholette 2012). There is additional evidence in both adult and paediatric populations that washing RBCs prior to transfusion significantly reduces both mortality and morbidity (Blumberg 2004; Blumberg 2010; Cholette 2012). If a similar beneficial effect of equivalent magnitude exists in transfused preterm infants, this would represent a major advantage for this vulnerable patient population.

Why it is important to do this review

RBC transfusions are almost unavoidable in infants less than 1000 g birth weight, despite increasingly restrictive transfusion practice. These infants carry the highest mortality risk and heaviest burden of outcome-changing morbidities compared with late preterm and term infants. Consequently, there is increasing interest in methods to reduce any adverse effects attributable to RBC transfusion. This can be accomplished by minimising the number of RBC transfusions, using alternatives to RBC transfusions such as EPO, and by making the transfused products potentially safer through pre-transfusion modifications to the product itself. In this review, we

focused on one method to make transfused blood products potentially safer, that is pre-transfusion washing of RBCs with saline.

OBJECTIVES

To determine whether pre-transfusion washing of RBCs prevents morbidity and mortality in preterm infants.

METHODS

Criteria for considering studies for this review

Types of studies

Randomised, cluster randomised, and quasi-randomised controlled trials.

Types of participants

Preterm infants (less than 32 weeks' gestation) or very low birth weight infants (less than 1500 g birth weight), or both, who received one or more packed RBC transfusions during their initial hospitalisation.

Types of interventions

Transfusions of washed (through a manual 'open' system technique, in an automated cell washer, or via an auto-transfusion device) packed RBCs versus unwashed packed RBCs in emergent and non-emergent situations, excluding exchange transfusion, massive transfusion, or placental-infant (delayed cord clamping) transfusion.

Types of outcome measures

Primary outcomes

1. Mortality: before discharge from initial hospital or before a defined period of follow-up (28 days, 12 months, or 18 months postnatal age, or a combination).
2. ROP, grade 3 or more prior to discharge home (ICCRP 2005).
3. Severe adverse findings at ultrasound (grades 3 to 4 IVH (Papile 1983), hydrocephalus, cortical atrophy, or periventricular leukomalacia) during first hospitalisation (Pinto-Martin 1995).
4. CLD requiring additional oxygen at 36 weeks' postmenstrual age or prior to discharge home (Shennan 1988).
5. NEC, stage 2 or greater (Bell 1978).
6. Cerebral palsy by physician assessment.
7. Developmental delay (developmental quotient more than two standard deviations below the mean on validated assessment tool of cognitive function (e.g. Bayley Score of Infant Development).
8. Blindness (visual acuity less than 20/200 in best eye).
9. Deafness (hearing loss requiring amplification or cochlear implantation).

Secondary outcomes

1. Composite outcome of death or severe adverse outcomes:
   i) mortality or severe morbidity (or its complement, survival without severe morbidity) at initial hospital discharge, where significant morbidity is defined as:
      a) ROP, grade 3 or more prior to discharge home (ICCRP 2005);
      b) severe adverse findings at ultrasound (grades 3 to 4 IVH (Papile 1983), hydrocephalus, cortical atrophy, or periventricular leukomalacia) during first hospitalisation (Pinto-Martin 1995);
      c) CLD requiring additional oxygen at 36 weeks' postmenstrual age or prior to discharge home (Shennan 1988); or
      d) NEC, stage 2 or greater (Bell 1978).
2. Composite outcome of mortality or severe adverse neurosensory outcome (or its complement, survival without serious adverse neurosensory outcome) at a defined period of follow-up at age 18 to 24 months' adjusted gestational age or older, where adverse neurosensory outcome is defined as:
   i) cerebral palsy by physician assessment;
   ii) developmental quotient (more than two standard deviations below the mean on validated assessment tool of cognitive function (e.g. Bayley Score of Infant Development);
   iii) blindness (visual acuity less than 20/200 in best eye); or
   iv) deafness (hearing loss requiring amplification or cochlear implantation).
3. Other outcomes:
   i) late-onset sepsis (sepsis diagnosed more than 72 hours after birth)
   ii) length of mechanical ventilation (days)
   iii) donor exposure
   iv) numbers of RBC transfusions
   v) length of initial neonatal intensive care unit stay (days)
   vi) markers of inflammation or oxidative stress (if available), or both, including tumour necrosis factor (TNF)-α, monocyte chemoattractant protein-1, interleukin (IL)-1, IL-6, IL-8, and total oxidant load
4. Transfusion reactions as defined by the Serious Hazards of Transfusion (SHOT) scheme (Stainsby 2008):
   i) acute transfusion reaction;

ii) delayed transfusion reaction;
iii) transfusion-related acute lung injury;
iv) transfusion-associated graft-versus-host disease;
v) post-transfusion purpura; or
vi) transfusion-transmitted infection.

Search methods for identification of studies

We used the standard search method of the Cochrane Neonatal Review Group.

Electronic searches

We searched the following databases:

- Cochrane Central Register of Controlled Trials (CENTRAL 2015, Issue 7) (Appendix 1);
- MEDLINE (January 1996 to 31 July 2015) (Appendix 2);
- EMBASE (January 1980 to 31 July 2015) (Appendix 3);
- CINAHL (1982 to 31 July 2015) (Appendix 4).

We searched for completed or ongoing clinical trials through major clinical trial registration websites: ClinicalTrials.gov (clinicaltrials.gov), Australian New Zealand Clinical Trials Registry (anzctr.org.au), Current Controlled Trials (controlled-trials.com), European Union Clinical Trials Register (clinicaltrialsregister.eu), ISRCTN registry (isrctn.org), and National Institute of Public Health Clinical Trials Search (rctportal.niph.go.jp/en/index) (Appendix 5). We applied no language restrictions.

Searching other resources

We searched the reference lists of existing reviews and studies included in the review. We contacted experts in the field for suggestions of relevant unidentified studies (published and unpublished). We searched abstracts and conference proceedings (Pediatric Academic Societies at www.abstracts2view.com/pas/, European Society for Paediatric Research (1990 to current); American Society of Hematology Annual Meeting).

Data collection and analysis

Selection of studies

Two review authors initially screened all electronically derived citations and abstracts of papers identified by the review search strategy for relevance. A second review author initially screened the same citations and abstracts for relevance. We excluded clearly irrelevant studies at this stage.
Two review authors then formally assessed the full texts of all potentially relevant trials for eligibility. If necessary, we requested further information from the authors where articles contained insufficient data to make a decision about eligibility. Two review authors assessed the papers and recorded reasons for exclusion in the Characteristics of excluded studies table. Disagreements between the review authors were resolved by consensus.

Data extraction and management

Two review authors independently conducted data extraction using a data extraction form designed (and piloted) specifically for use in this systematic review. Disagreements between the review authors were resolved by consensus. The review authors were not blinded to names of authors, institutions, journals, or outcomes of the trials.

Assessment of risk of bias in included studies

We employed the standard methods of the Cochrane Neonatal Group.
We assessed risk of bias using the tool described in the *Cochrane Handbook for Systematic Reviews of Inventions* (Higgins 2011). We reported the following domains: selection bias (random sequence generation and allocation concealment), performance bias, detection bias, attrition bias, and other bias. We assessed these domains and entered them into the 'Risk of bias' table.

Selection bias (random sequence generation and allocation concealment)

Random sequence generation

For each included study, we categorised the risk of random sequence generation as:

- low risk - adequate (any truly random process, e.g. random number table, computer random number generator);
- high risk - inadequate (any non-random process, e.g. odd or even date of birth, hospital or clinic record number);
- unclear risk - no or unclear information provided.

Allocation concealment

For each included study, we categorised the risk of bias regarding allocation concealment as:

- low risk - adequate (e.g. telephone or central randomisation, consecutively numbered, sealed, opaque envelopes);
- high risk - inadequate (open random allocation; unsealed or non-opaque envelopes, alternation; date of birth);
- unclear risk - no or unclear information provided.

Performance bias

For each included study, we categorised the methods used to blind study personnel from the knowledge of which intervention a participant received as:

- low risk - adequate for personnel (a placebo that could not be distinguished from the active drug was used in the control group);
- high risk - inadequate (personnel aware of group assignment);
- unclear risk - no or unclear information provided.

Detection bias (blinding)

For each included study, we categorised the methods used to blind outcome assessors from knowledge of which intervention a participant received. We categorised the methods used for detection bias as:

- low risk - adequate (follow-up was performed with assessors blinded to group assignment);
- high risk - inadequate (assessors at follow-up were aware of group assignment);
- unclear risk - no or unclear information provided.

Attrition bias (outcome data)

For each included study and for each outcome, we described the completeness of data including attrition and exclusions from the analysis. We noted whether attrition and exclusions were reported, the numbers included in the analysis at each stage (compared with the total randomised participants), reasons for attrition or exclusion where reported, and whether missing data were balanced across groups or were related to outcomes. Where sufficient information was reported or supplied by the trial authors, we included the missing data in the analyses. We categorised the methods with respect to the risk attrition bias as:

- low risk - adequate;
- high risk - inadequate;
- unclear risk - no or unclear information provided.

Reporting bias (selective outcome reporting)

For each included study, we described how we investigated the risk of selective outcome reporting bias and what we found. We assessed the methods as:

- low risk - adequate (where it was clear that all of the study's prespecified outcomes and all expected outcomes of interest to the review were reported);
- high risk - inadequate (where not all of the study's prespecified outcomes were reported; one or more reported primary outcomes were not prespecified; outcomes of interest were reported incompletely and so could not be used; study did not include results of a key outcome that would have been expected to have been reported);
- unclear risk - no or unclear information provided (the study protocol was not available).

Other bias

For each included study, we described any important concerns that we had about other possible sources of bias. We assessed whether each study was free of other issues that could put it at risk of bias as:

- low risk - no concerns of other bias raised;
- high risk - concerns raised after multiple looks at the data with the results made known to the investigators; difference in number of participants enrolled in abstract and final publications of the paper;
- unclear - concerns raised about potential sources of bias that could not be verified by contacting the authors.

Two review authors independently made judgements about risk of bias. We resolved discrepancies through consensus.

Measures of treatment effect

Dichotomous data

For dichotomous data, we presented results as risk ratio, risk difference, and mean difference where appropriate using Review Manager 5 software (RevMan 2014). We calculated 95% confidence intervals.

Continuous data

For continuous data, we used the mean difference if outcomes were measured in the same way between trials. We used the standardised mean difference to combine trials that measured the same outcome but used different methods.

Unit of analysis issues

We included studies with two, or more than two, treatment groups and dealt with analyses as recommended by the *Cochrane Handbook for Systematic Reviews of Interventions* (Higgins 2011). When a multi-arm study contributes more than one comparison to a particular meta-analysis, we planned to either combine treatment groups or divide the control group, to avoid inclusion of data from the same infant more than once in the same analysis. If we had identified any cluster trials and deemed the data were not appropriately analysed, we would have adjusted for correlation using an effective sample size based on the design effect for each study (Higgins 2011).

Dealing with missing data

For all outcomes, we carried out analyses, as far as possible, on an intention-to-treat basis. The denominator for each outcome in each trial was the number of participants randomised minus any participants whose outcomes were known to be missing.

Assessment of heterogeneity

As we identified and analysed only one study, assessments of heterogeneity were not appropriate and were therefore not performed.

Assessment of reporting biases

If there were 10 or more studies in the meta-analysis, we would have investigated reporting biases using funnel plots. We would have assessed funnel plot asymmetry visually. If a visual assessment suggested asymmetry, we would have performed exploratory analyses to investigate it.

Data synthesis

We carried out statistical analysis using Review Manager 5 software (RevMan 2014). We reported the mean difference where appropriate using Review Manager 5 software and calculated 95% confidence intervals (RevMan 2014). We did not carry out further analysis as we identified and analysed one study only.

Subgroup analysis and investigation of heterogeneity

Where possible, we planned to undertake predefined subgroup analyses for the different washing techniques (manual 'open' system technique, an automated cell washer, or an auto-transfusion device). We planned to examine additional subgroups depending on whether the RBCs were irradiated or not prior to washing.

Sensitivity analysis

We planned sensitivity analysis on primary outcomes to determine what effect the exclusion of studies with high risk of bias (for allocation concealment and incomplete outcome data) might have on the overall result of the meta-analysis.

RESULTS

Description of studies

See: Characteristics of included studies; Characteristics of excluded studies

Results of the search

The preliminary electronic database search (Cochrane Central Register of Controlled Trials (CENTRAL) (Issue 10, 2014), MEDLINE (1966 to November 2014), CINAHL (1982 to November 2014), and EMBASE (1980 to November 2014) yielded 546 results. After review of the titles, we selected four records for detailed abstract review and then full-text review. One of these studies met the selection criteria and one study was an abstract of a full-text record already identified (Cholette 2012). A search of online registers of clinical trials revealed one additional study meeting the inclusion criteria; we have included this study in the table Characteristics of ongoing studies. We undertook handsearching of conference abstracts, which did not yield any relevant studies. We reviewed a previous Cochrane review in a relevant area (Wilkinson 2014), which yielded one study not previously identified through prior searches; after full-text review, we excluded this study. We have detailed the characteristics of the single included study and the three excluded studies in the tables Characteristics of included studies and Characteristics of excluded studies, respectively.

We updated the above search in July 2015 and identified no new eligible studies.

Included studies

Lee 1995 was the single study that met the inclusion criteria. It was a single-centre randomised control trial conducted in the United States of America of dedicated donor (unwashed) RBC packs versus split donor (washed) RBC packs. The population consisted of inborn and outborn infants with a birth weight of less than 1500 g admitted to a neonatal intensive care unit in San Francisco (California Pacific Medical Center) and their families. Twenty-three infants were randomised, with two infants withdrawn before undertaking the study intervention at parental request, within seven days of birth (see Characteristics of included studies). The inclusion criteria were: birth weight less than 1500 g and having an initial RBC transfusion ordered during the first week of age. The only stated exclusion criterion was no previous RBC transfusion prior to enrolment in the study.

One group of infants (unwashed RBC group) were randomised to receive type-specific packed RBCs from dedicated donor (either community or directed donation) units equipped with seven satellite bags. The other group of infants (washed RBC group) received packed RBCs from units divided into three split packs shared with other infants receiving transfusions. The packed RBCs used in the washed RBC group were washed with an automated cell washer (IBM-COBE Blood Processor 2991; COBE Laboratories, Inc., Lakewood, Colorado) and re-suspended in a saline solution to a hematocrit value of 80% to 85%. All packed RBCs used in the study were cytomegalovirus antibody negative and were irradiated prior to use. Infants in both groups could receive RBCs from individuals nominated by their families donating blood specifically

for the infant (directed donation). These directed donations were collected in citrate-phosphate-dextrose anticoagulant preservative and stored in adenine in saline anticoagulant preservation and had an expiration date of 42 days. Community donations were collected in citrate-phosphate-dextrose-adenine anticoagulant preservation and had an expiration date of 35 days.

The stated primary outcome of the study was number of donor exposures per infant. Infants were monitored until one hour post-transfusion for acute transfusion reactions. Data regarding demographics, length of hospital stay, days of mechanical ventilation, days of supplemental oxygen use, and RBC transfusion details were collected. Infants received 86 RBC transfusions in total across both groups (6.0 versus 7.5 per infant; median, control versus study). Infants were able to receive directed donations from family members; these were either unwashed or washed depending on the group to which the infant was assigned. Fewer donor exposures occurred in the unwashed group (2.0 versus 5.5 per infant; median, control versus study).

The study was stopped prematurely after enrolment of 21 infants (planned sample size 34 infants) as there was a significant difference in the primary outcome (number of donor exposures) between the two groups.

Excluded studies

We excluded Cholette 2012, Hosking 1990, and Swindell 2007 after full-text review, as they did not include infants less than 32 weeks' gestation or infants with a birth weight of less than 2500 g, or both.

Risk of bias in included studies

We assessed the overall risk of bias for the included study as low. We have included a detailed 'Risk of bias' table under Characteristics of included studies.

Allocation

The sequence generation was unclear based on the published methods in Lee 1995, but allocation was concealed from the bedside healthcare team and families.

Blinding

There was no description of how or by whom the outcomes were collected; however, the outcomes assessed (for example mortality) were at low risk of being affected by lack of blinding.

Incomplete outcome data

Two infants withdrew from the study after randomisation, but this level of missing data was unlikely to affect observed results.

Selective reporting

No published study protocol was available.

Other potential sources of bias

We identified no other potential sources of bias.

Effects of interventions

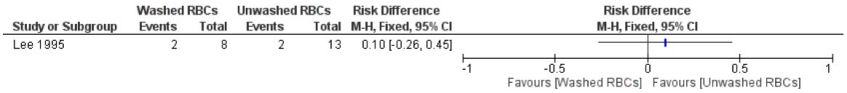
Washed versus unwashed RBCs for transfusion (Comparison I)

Primary outcomes

Mortality

One study (n = 21 infants) reported on all-cause mortality during hospital stay. There was no significant difference in mortality between the washed versus the unwashed RBCs for transfusion groups (risk ratio 1.63, 95% confidence interval (CI) 0.28 to 9.36; risk difference 0.10, 95% CI -0.26 to 0.45) (Analysis 1.1; Figure 1). Tests for heterogeneity were not applicable.

Figure 1. Forest plot of comparison: 1 Washed versus unwashed RBCs for transfusion, outcome: 1.1 Mortality.

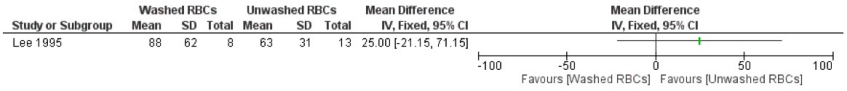


Secondary outcomes

Length of initial neonatal intensive care unit stay (days)

One study (n = 21 infants) reported on length of initial neonatal intensive care unit (NICU) stay (days). There was no significant difference in the length of initial NICU stay between the washed versus the unwashed RBCs for transfusion groups; mean difference 25 days (95% CI -21.15 to 71.15) (Analysis 1.2; Figure 2). Tests for heterogeneity were not applicable.

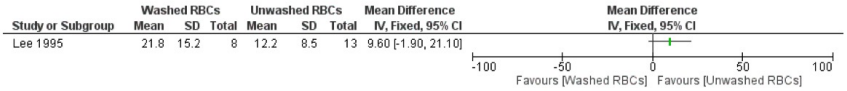
Figure 2. Forest plot of comparison: 1 Washed versus unwashed RBCs for transfusion, outcome: 1.2 Length of initial NICU stay (days).



Duration of mechanical ventilation (days)

One study (n = 21 infants) reported on duration of mechanical ventilation. There was no significant difference in duration of mechanical ventilation between the washed versus the unwashed RBCs for transfusion groups; mean difference 9.60 days (95% CI -1.90 to 21.10) (Analysis 1.3; Figure 3). Tests for heterogeneity were not applicable.

Figure 3. Forest plot of comparison: 1 Washed versus unwashed RBCs for transfusion, outcome: 1.3 Duration of mechanical ventilation (days).



DISCUSSION

Summary of main results

The single included study only assessed one method of washing, by an automated cell washer, and was a dual intervention study, with the unwashed RBC group receiving blood from a dedicated donor split into eight packs.

Due to the small sample size, 21 included infants, estimates for the three reported outcomes relevant to our review (mortality during initial hospitalisation, duration of mechanical ventilation, and length of initial NICU hospitalisation) had very wide confidence

intervals.

No outcome data was available for our other primary outcomes including retinopathy of prematurity (stage 3 or greater), necrotising enterocolitis (stage 2 or greater), chronic lung disease, adverse findings on head ultrasound screening, or adverse neurodevelopmental outcome (cerebral palsy by physician assessment, development delay, blindness or deafness). No data was available for most of our secondary outcomes, including previously discussed composite outcomes, late onset sepsis or markers of inflammation and/or oxidative stress.

Although we assessed the study as at low risk of bias, the imprecision of the estimates it provides made this study unhelpful in answering the review questions.

Overall completeness and applicability of evidence

We performed an extensive search of published and unpublished literature, including searches of trial registries for ongoing studies. We have no reason believe that there are any additional studies relevant to our review at this time. The one study we did identify examined only one method of RBC washing. The blood product processing and storage, including routine irradiation, choice of storage media and anticoagulant, that occurred in this study may not be applicable to all healthcare settings that care for preterm infants.

Quality of the evidence

The quality of the evidence provided by this single study was reasonable, however the study included only a very small number of infants.

Potential biases in the review process

It is possible that the exclusion of studies including more mature infants (more than 32 weeks' gestation) may have resulted in potentially relevant studies being missed. However, infants of this gestational age are not usually at risk of developing the primary outcomes identified by our review. The results reported by this review for the included study were straightforward, and no re-analysis or selective reporting occurred.

Agreements and disagreements with other studies or reviews

Based on the current evidence, it is unclear whether there is a benefit or risk to washing RBCs prior to transfusion for preterm infants. Studies undertaken in older infants and children (Cholette 2012), as well as in the adult population (Blumberg 2004), suggest there may be a benefit in outcomes addressed within this review. However, no such evidence is available for preterm infants, and so further clinical trials are needed.

AUTHORS' CONCLUSIONS

Implications for practice

There is insufficient evidence to support or refute the use of washed RBCs for transfusion in preterm infants to prevent morbidity or mortality.

Implications for research

As have been conducted in adult medicine, Blumberg 2004, and in paediatrics, Cholette 2012, randomised controlled trials are needed in neonatology to assess the potential benefits and the effects on short-term outcomes, neonatal morbidities, and mortality of pre-washing RBCs for preterm or low birth weight infants, or both. When designing a future study to determine whether washing RBCs prior to transfusion benefits preterm infants or not, a randomised, multicentre, controlled trial design is recommended. Included infants would be those born with a gestational age up to and including 28 weeks and 6 days, who receive a packed RBC transfusion as per a standardised clinical guideline, for example the restrictive transfusion thresholds used in the Premature Infants in Need of Transfusion (PINT) (Kirpalani 2006). Eligible infants would be randomly allocated to receive either washed or non-washed standard non-irradiated, leukodepleted allogeneic packed RBCs. At least 448 infants would be required to detect a decrease in the composite outcome from 51% to 36% (two-sided alpha 0.05, 90% power). Infants in the washed (study) group would receive 15 ml/kg non-irradiated, leukodepleted washed packed RBCs. Infants in the standard-therapy (control) group would receive 15 ml/kg non-irradiated, leukodepleted packed RBCs. All subsequent transfusions would comply with the initial randomisation. An additional component of this trial would be to assess the cost-effectiveness, safety in terms of acute adverse transfusion effects, and the practicalities of providing washed packed RBCs for routine neonatal RBC transfusions.

ACKNOWLEDGEMENTS

We would like to acknowledge the Australasian Cochrane Group for their significant support provided at the Review Completion Workshop in Melbourne, Australia, in November 2014.

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References to studies excluded from this review

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* *Indicates the major publication for the study*

CHARACTERISTICS OF STUDIES

Characteristics of included studies *[ordered by study ID]*

Lee 1995

Methods	Single-centre randomised control trial
Participants	Inborn and outborn infants with a birth weight < 1500 g
Interventions	One group of infants (unwashed RBC group) were randomised to receive type-specific packed RBCs from dedicated donor (either community or directed donation) units equipped with 7 satellite bags. The other group of infants (washed RBC group) received packed RBCs from units divided into 3 split packs shared with other infants receiving transfusions
Outcomes	The primary outcome of the study was number of donor exposures per infant
Notes	Infants were monitored until 1-hour post-transfusion for acute transfusion reactions. Data regarding demographics, length of hospital stay, days of mechanical ventilation, days of supplemental oxygen use, and RBC transfusion details were collected

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Unclear risk	No information provided
Allocation concealment (selection bias)	Low risk	Study infants were randomly assigned by hospital blood bank personnel into 1 of 2 groups
Blinding of participants and personnel (performance bias) All outcomes	Low risk	Transfusions were ordered by primary caretakers who were masked to study group assignments. RBCs were processed by hospital blood bank personnel and sent to the neonatal intensive care unit in syringes unmarked as to study group assignment
Blinding of outcome assessment (detection bias) All outcomes	Unclear risk	Low risk for mortality; unclear risk for other outcomes
Incomplete outcome data (attrition bias) All outcomes	Low risk	Two infants withdrew from the study after randomisation, but this level of missing data is unlikely to affect observed results
Selective reporting (reporting bias)	Unclear risk	No published study protocol was available to review

Lee 1995 (*Continued*)

Other bias	Low risk	No other risks of bias were identified

RBC: red blood cell

Characteristics of excluded studies *[ordered by study ID]*

Study	Reason for exclusion
Cholette 2012	Infants not in gestational age or birth weight range
Hosking 1990	Infants not in gestational age or birth weight range
Swindell 2007	Infants not in gestational age or birth weight range

Characteristics of ongoing studies *[ordered by study ID]*

ACTRN12613000237785

Trial name or title	Effect of transfusion of washed red blood cells on neonatal outcome: a randomised controlled trial
Methods	Randomised, multicentre, controlled trial
Participants	Infants born with a gestational age up to and including 28 weeks and 6 days
Interventions	Eligible infants will be randomly allocated to receive either washed or non-washed standard non-irradiated, leukodepleted allogeneic packed red blood cells
Outcomes	The primary outcome is a composite of mortality (defined as death of a live born infant > 48 hours of age) and/or major neonatal morbidities associated with organ dysfunction or failure following transfusion, until discharge from neonatal intensive care unit. Major neonatal morbidity is defined as one or more of bronchopulmonary dysplasia (oxygen and/or respiratory support - intubation/continuous positive airway pressure/high-flow nasal cannula oxygen $\geq$ 2 L/min) for any portion of the day at 36 weeks and 0 days corrected gestational age), brain injury defined as intraventricular haemorrhage (grades 3 and 4), retinopathy of prematurity (> stage 2), and necrotising enterocolitis (based on a grading of stage 2 or greater). Secondary outcomes include nosocomial infection (blood culture positive sepsis diagnosed > 48 hours after birth), length of mechanical ventilation, and length of primary admission
Starting date	Not yet recruiting
Contact information	michael.stark@adelaide.edu.au
Notes	Trial ID: ACTRN12614000419662

DATA AND ANALYSES

Comparison 1. Washed versus unwashed RBCs for transfusion

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1 Mortality	1		Risk Difference (M-H, Fixed, 95% CI)	Totals not selected
2 Length of initial NICU stay (days)	1		Mean Difference (IV, Fixed, 95% CI)	Totals not selected
3 Duration of mechanical ventilation (days)	1		Mean Difference (IV, Fixed, 95% CI)	Totals not selected

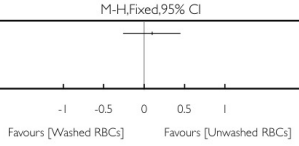
Analysis 1.1. Comparison 1 Washed versus unwashed RBCs for transfusion, Outcome 1 Mortality.

Review: Washed versus unwashed red blood cells for transfusion for the prevention of morbidity and mortality in preterm infants

Comparison: 1 Washed versus unwashed RBCs for transfusion

Outcome: 1 Mortality

Study or subgroup	Washed RBCs n/N	Unwashed RBCs n/N	Risk Difference M-H,Fixed,95% CI	Risk Difference M-H,Fixed,95% CI
Lee 1995	2/8	2/13		0.10 [-0.26, 0.45]

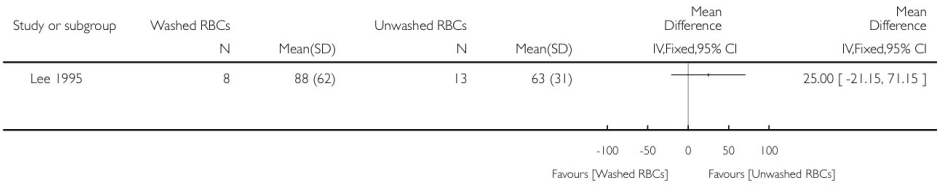


Analysis 1.2. Comparison 1 Washed versus unwashed RBCs for transfusion, Outcome 2 Length of initial NICU stay (days).

Review: Washed versus unwashed red blood cells for transfusion for the prevention of morbidity and mortality in preterm infants

Comparison: 1 Washed versus unwashed RBCs for transfusion

Outcome: 2 Length of initial NICU stay (days)

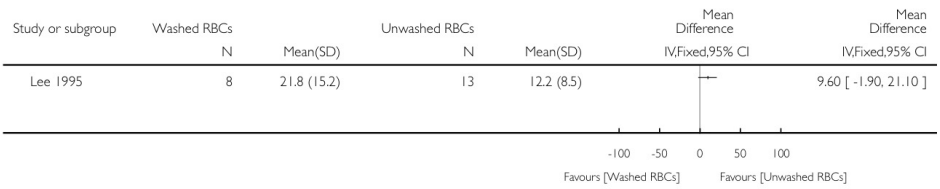


Analysis 1.3. Comparison 1 Washed versus unwashed RBCs for transfusion, Outcome 3 Duration of mechanical ventilation (days).

Review: Washed versus unwashed red blood cells for transfusion for the prevention of morbidity and mortality in preterm infants

Comparison: 1 Washed versus unwashed RBCs for transfusion

Outcome: 3 Duration of mechanical ventilation (days)



APPENDICES

Appendix 1. Cochrane Central Register of Controlled Trials (CENTRAL) search strategy

Search terms: (infant or newborn or neonate or neonatal or premature or very low birth weight or low birth weight or VLBW or LBW) AND (transfusion OR hemotransfus* OR haemotransfus* OR hemotherap*) AND (erythrocyte OR red blood cell OR RBC)

Appendix 2. MEDLINE search strategy

((infant, newborn[MeSH] OR newborn OR neonate OR neonatal OR premature OR low birth weight OR VLBW OR LBW) AND (randomized controlled trial [pt] OR controlled clinical trial [pt] OR Clinical Trial[ptyp] OR randomized [tiab] OR placebo [tiab] OR clinical trials as topic [mesh: noexp] OR randomly [tiab] OR trial [ti]) NOT (animals [mh] NOT humans [mh])) AND (transfusion OR hemotransfus* OR haemotransfus* OR hemotherap*) AND (erythrocyte OR red blood cell OR RBC) AND (Humans[Mesh] AND infant[MeSH])

Appendix 3. EMBASE search strategy

1. ((infant, newborn or newborn or neonate or neonatal or premature or very low birth weight or low birth weight or VLBW or LBW) and (human not animal) and (randomized controlled trial or controlled clinical trial or randomized or placebo or clinical trials as topic or randomly or trial or clinical trial) and (transfusion or hemotransfus* or haemotransfus* or hemotherap*) and (erythrocyte or red blood cell or RBC)).mp. [mp=title, abstract, subject headings, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword]
2. limit 1 to human
3. 1 and 2
4. limit 3 to infant <to one year>

Appendix 4. CINAHL search strategy

(infant, newborn OR newborn OR neonate OR neonatal OR premature OR low birth weight OR VLBW OR LBW) AND (randomized controlled trial OR controlled clinical trial OR randomized OR placebo OR clinical trials as topic OR randomly OR trial OR PT clinical trial) AND (transfusion OR hemotransfus* OR haemotransfus* OR hemotherap*) AND (erythrocyte OR red blood cell OR RBC)

Appendix 5. Online clinical trial registries search strategy

(transfusion OR hemotransfus* OR haemotransfus* OR hemotherap*) AND (erythrocyte OR red blood cell OR RBC) AND infant

CONTRIBUTIONS OF AUTHORS

Amy Keir (AK) screened the titles and abstracts of all studies identified by the search strategy. AK and Dominic Wilkinson (DW) screened the full text of each study identified as of potential relevance. AK and DW extracted the data separately, compared data, and resolved any differences by consensus. AK, DW, Chad Andersen and Michael Stark completed the final review.

DECLARATIONS OF INTEREST

Associate Professor Michael Stark and Dr. Chad Andersen are undertaking a clinical trial to examine the effect of pre-transfusion washing of red blood cells on neonatal outcomes.

Amy Keir and Dominic Wilkinson have no conflicts of interest to declare.

SOURCES OF SUPPORT

Internal sources

- No sources of support supplied

External sources

- Eunice Kennedy Shriver National Institute of Child Health and Human Development National Institutes of Health, Department of Health and Human Services, USA.

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DIFFERENCES BETWEEN PROTOCOL AND REVIEW

We included one additional online clinical register in our search strategy, the World Health Organization (WHO) International Clinical Trials Registry Platform.

Chapter 5

Co-infusion of dextrose-containing fluids with red blood cells

The chapter contains the published paper "*Co-infusion of dextrose-containing fluids and red blood cells does not adversely affect in vitro red blood cell quality*."

It addresses the previously identified research question:

Research question 4: Is it safe to co-infuse dextrose-containing fluids and RBCs?

Authorship forms are provided in Appendix C

Coinfusion of dextrose-containing fluids and red blood cells does not adversely affect in vitro red blood cell quality

Amy K. Keir,[1,2] Adele L. Hansen,[3] Jeannie Callum,[4,5] Robert P. Jankov,[1,6,7] and Jason P. Acker[3,8]

BACKGROUND: Transfusion guidelines advise against coinfusing red blood cells (RBCs) with solutions other than 0.9% saline. We evaluated the impact of coinfusion with dextrose-containing fluids (DW) on markers of RBC quality.

STUDY DESIGN AND METHODS: A pool-and-split design was used to allow conditions to be tested on each pool within 2 hours of irradiation. Three pools at each storage age (5, 14, and 21 days) were created for each phase. In Phase 1, samples were infused through a neonatal transfusion apparatus alone or with treatment solutions: D5W, D10W, D5W/0.2% saline, and 0.9% saline. In Phase 2, samples were incubated alone or in a 1:1 ratio with treatment solutions and tested after 5, 30, and 180 minutes. Hemolysis, supernatant potassium, RBC indices, morphology, and deformability were measured on all samples.

RESULTS: In Phase 1, RBCs transfused alone through the apparatus had higher ($p < 0.01$) hematocrit, total hemoglobin, and supernatant potassium compared to all other groups. No statistical differences were identified between groups for other measured variables. In Phase 2, mean corpuscular volume of all samples containing DW increased with incubation length and were higher ($p < 0.01$) than RBCs incubated alone or with 0.9% saline after 30 and 180 minutes. RBCs incubated with D5W and D5W/0.2% saline had greater ($p < 0.05$) hemolysis than RBCs alone after 180 minutes.

CONCLUSION: In vitro characteristics of RBCs coinfused with 0.9% saline or D10W were not adversely impacted. When developing clinical studies in neonates, we recommend use of D10W and a transfusion apparatus that minimizes the contact volume of the coinfusate with the RBC.

The practice of coinfusing red blood cells (RBCs) with dextrose-containing fluids (DW) is advised against due to the potential risk of hemolysis.[1] The evidence transfusion guidelines base this recommendation on is limited[2] and largely reflects the risk of hemolysis or crenation secondary to coinfusion of RBCs with hypotonic or hypertonic solutions, respectively.

Despite this current lack of evidence to either support or refute the practice of coinfusion of stored RBCs with DW, there is increasing interest in the potential safety of this practice in the neonatal population.[3,4] This is due, in part, to the increased awareness of the association

ABBREVIATIONS: DW = dextrose-containing fluids; NICU = neonatal intensive care unit.

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TRANSFUSION 2014;54:2068-2076.

between the development of necrotizing enterocolitis, a potentially devastating disease both with high morbidity and mortality,[5] and the receipt of a RBC transfusion within the previous 48 hours.[6,7] As a consequence, many neonatal intensive care units (NICUs) routinely withhold enteral feeds from preterm infants before, during, and after transfusion, with the aim of reducing the risk of transfusion-associated necrotizing enterocolitis.[8] Although the evidence to support this practice is limited,[6,9] it is becoming increasingly common.[9] Fasting during RBC transfusion can place infants at risk of hypoglycemia and an additional intravenous (IV) point may be needed to infuse DW to avoid this high-risk situation.[10] In some infants, gaining vascular access can be difficult and lead to iatrogenic injury. There is also an emerging association between greater number of skin breaks and poorer cognitive and motor outcomes in preterm infants.[11,12]

Previous studies examining coinfusion in in vitro settings reflecting neonatal transfusion practice[3,13] have suggested that the practice does not lead to hemolysis but significant drawbacks in methodology have led to a general lack of acceptance of these conclusions.[4] Our study aims to address these methodologic limitations and provide comprehensive in vitro evidence to determine effects of coinfusion of RBCs and DW on markers of RBC integrity.

MATERIALS AND METHODS

Blood product collection and preparation

Ethics approval was granted by the Canadian Blood Service's Research Ethics Board before initiation of the study. Whole blood (n = 45) was collected into CPD from healthy donors (CGR6494B, CPD/SAGM Quad OptiPure RC 9SBT 500 mL, Fenwal, Lake Zurich, IL), rapidly cooled to room temperature (18-24°C), and processed with semiautomated separation of components within 24 hours of the stop bleed time.[14] After separation, SAGM was added to the RBCs and leukoreduction occurred by filtration. Once processed, all units were stored at 1 to 6°C for 5, 14, or 21 days. The ages of RBCs were chosen to reflect the most commonly used age of RBCs transfused to infants in Canadian NICUs (14.6 ± 8.3 days).[15] Before use in the experiments, the RBCs were irradiated (Gammacell 3000 Elan, MDS Nordion, Ottawa, Ontario, Canada) at a dose of 25 Gy. To be able to test all conditions on the same units within 2 hours of irradiation, a pool-and-split design was used. For Phase 1 of the study, 6 RBC units at each storage age (n = 18) were pooled in pairs and divided equally into the original storage bags to make three sets of 2 pooled RBC units at 5, 14, and 21 days of storage. For Phase 2, 9 RBC units at each of the stated ages (n = 27) were pooled in sets of three and then divided equally into the original storage bags to make three sets of 3 pooled RBC units at 5, 14, and 21 days of storage. For each phase of the study, the previously outlined experimental conditions were tested on each pool.

Sampling

Ten milliliters of RBCs for each experimental condition in Phase 1 and 5 mL of RBCs for Phase 2 conditions were sampled from the pooled units with a 60-mL syringe (Becton, Dickinson and Company, Franklin Lakes, NJ) and an 18-gauge needle (Becton, Dickinson and Company) via a sampling-site coupler (Baxter Healthcare Corporation, Deerfield, IL) having passed through a 170- to 260-µm blood filter (Baxter).

Study design

Phase 1: determining the impact of the transfusion apparatus on the RBCs

Samples from each of the pools (5 days, n = 3; 14 days, n = 3; 21 days, n = 3) were passed through a neonatal transfusion apparatus either alone (control) or in a 1-to-1 ratio with D5W or D10W solution, D5W and 0.2% saline solution or 0.9% saline solution (isotonic saline control; Baxter). A 5-mL sample was collected in a tube at the end of the transfusion apparatus for testing. The syringe pumps (MedFusion 3500, Medex, Inc., Carlsbad, CA) for the RBCs and coinfusion solutions were both programmed at a rate of 15 mL/hr (3000-g infant, a 120 mL/kg/day infusion rate for the DW and a 15 mL/kg total volume over 3-hr infusion rate for the RBCs). These conditions were selected as they reflected the fastest rates within the clinical range considered by this study.

Standard neonatal transfusion apparatus. A standard neonatal transfusion apparatus was used for the experiments in Phase 1. The apparatus consisted of a 24-gauge IV cannula (Becton, Dickinson and Company) connected to a small-bore ("T-piece") extension set (ICU Medical, Inc., San Clemente, CA) with two needleless connectors (ICU Medical, Inc.) with extension tubing (Microbore tubing, MED-RX, Oakville, Ontario, Canada) attached. Connected to each extension tube was a 60-mL syringe, one containing the RBCs and the other DW or 0.9% saline (excluding when RBCs were transfused alone, when only one syringe and pump were used). The extension tubing, connected to the syringe with the RBCs, was attached to the needleless connecter closest to the cannula. The small-bore extension set held 0.38 mL in volume and this was the only area in which the RBCs and the treatment solution were in contact with each other. However, when coinfusing it took 10 minutes to collect the volume required for testing in the tube at the end of the apparatus; therefore, the samples were in contact with the DW or 0.9% saline for varying times outside the apparatus that may not be representative of a clinical setting.

Phase 2: determining the impact of rate and suspension of infusion

Phase 2 was used to investigate the effect of incubation time in the apparatus in a controlled setting. The slowest infusion rate considered in our study was 2.5 mL/hr (500-g infant, a 120 mL/kg/day infusion rate for the DW and a 15 mL/kg total volume over 3-hr infusion rate for the RBCs). The DW and RBCs would therefore be in contact in the tubing line for approximately 4 minutes, using the previously described apparatus in which tubing containing the coinfusion solution and RBC mixture holds 0.38 mL. Samples from each of the pools (5 days, n = 3; 14 days, n = 3; 21 days, n = 3) were stored in a tube for 5 minutes to mimic the slowest infusion rates being considered, 30 minutes to simulate the situation of a temporarily suspended transfusion, or 3 hours to simulate a situation where the transfusion was suspended for its entire duration, either as RBCs independently (control) or with RBCs in a 1:1 ratio with D5W or D10W solution, D5W with 0.2% saline solution, or 0.9% saline (isotonic saline control).

RBC in vitro assays

All samples were assessed for hemolysis, supernatant potassium levels, morphology, hematologic indices, and deformability. Supernatant and total hemoglobin (Hb), used to calculate percentage hemolysis, were measured using a Drabkin's-based method,[16] and spun hematocrit (Hct) was measured using a Hct centrifuge (Haematokrit, Andreas Hettich GmbH & Co., Tuttlingen, Germany). The Hct of the 0.9% saline control of the same age was used in the hemolysis calculation for all conditions involving DW to limit the impact of RBC swelling on the percent hemolysis calculation. Extracellular K^+ concentration was determined using supernatants, obtained by centrifuging a sample at $2200 \times g$ at 4°C for 10 minutes and indirect potentiometry using ion-selective electrode methodology on a cellular analysis system (DXC 800, Beckman Coulter, Inc., Fullerton, CA). The corrected K^+ values were calculated by dividing the concentration (mmol/L) by the total Hb concentration g/L measured using the Drabkin's method to obtain the potassium mmol/g Hb. This was undertaken due to the dilution of the samples that occurred in the coinfused treatment groups. RBC hematologic indices, including mean corpuscular volume (MCV), mean corpuscular Hb, mean corpuscular Hb concentration, Hb, and Hct were determined using an automated cell counter (Coulter AcT, Beckman Coulter, New York, NY). RBC gross morphology was evaluated by microscopic examination (Eclipse TE2000-U, Nikon, Tokyo, Japan) of both fixed and Wright-stained blood smears. Approximately 10 fields of view were visualized by blinded trained reviewers for each smear and 100 cells were graded based on their morphology, as previously described.[17] RBC deformability was calculated using a laser-assisted optical rotational cell analyzer (Mechatronics, Zwaage, The Netherlands) as previously described.[18]

Statistical analysis

Mean and standard deviation were calculated by treatment groups (coinfusion solution) to describe data distribution for each RBC characteristic. Linear mixed model analyses were performed to test the effects of the solution and storage age on the product quality. In the mixed model, the random effects were estimated for each of the product units to control for the clustering problem. Post-hoc tests were implemented to identify groups, which were significantly different from others. Tukey's method was used to obtain the adjusted p values in the post-hoc tests. The effect of the storage age on the quality was examined within each treatment. For the Phase 2 data, we also explored the effect of incubation time on the product quality. A p value of <0.05 was considered as statistically significant. The data analysis was performed using the statistical analysis system (SAS).

RESULTS

Phase 1: impact of the transfusion apparatus on the RBCs

The mean and standard deviation for all quality variables measured in Phase 1 of the study are summarized in Table 1. The age of the RBCs had a statistical impact on the spun Hct of samples from RBCs coinfused with D5W ($p = 0.027$) and on hemolysis for RBCs infused alone ($p = 0.001$). Extracellular potassium levels were statistically different between RBC ages for all groups ($p < 0.001$ for all). As these differences in age were observed in all groups and can be explained by RBC storage lesion, the data in Table 1 are displayed as a combined mean of all ages. Due to the dilution of samples with the coinfusion solutions samples collected from the transfusion apparatuses set up to coinfuse the RBCs with any of the DW or the 0.9% saline control had statistically lower spun Hct levels, Coulter total Hb, and extracellular potassium compared to the RBC control ($p < 0.001$ for all). Statistical differences in spun Hct were also identified between all samples collected from RBCs coinfused with any DW or 0.9% saline ($p \leq 0.001$ for all comparisons). No statistical differences were identified between any treatment groups for hemolysis, MCV, deformability, and morphology.

Phase 2: impact of incubation with DW on the RBC membrane (Fig. 1)

As incubation time increased there was a statistical increase in the hemolysis for RBCs incubated with D5W or D5W with 0.2% saline and in the potassium for RBCs incubated with any of the DW, when grouped by age, as shown by the p values in Fig. 1 (all p values ≤ 0.020). Extracellular potassium also increased as the age of the RBCs increased

TABLE 1. Impact of the transfusion apparatus on RBCs

RBC in vitro assays	D5W	D10W	D5W/0.2% saline	0.9% saline	RBCs	Overall p value
Spun Hct (%)	35.78 (1.39)*†‡§	26.78 (1.30)*†‡	33.22 (1.09)*†‡	31.22 (1.20)*	59.56 (1.67)†	<0.0001
Hemolysis (%)	0.20 (0.03)	0.18 (0.03)	0.19 (0.03)	0.22 (0.12)	0.19 (0.10)§	0.7233
Coulter Hb (g/L)	88.67 (3.08)*	89.56 (4.45)*	89.11 (2.47)*	89.11 (3.76)*	180.78 (6.57)	<0.0001
Extracellular K+ (mmol/g Hb)	0.08 (0.03)*§	0.07 (0.03)*§	0.07 (0.03)*§	0.07 (0.03)*§	0.12 (0.05)§	<0.0001
MCV (fL)	94.22 (2.04)	94.33 (2.14)	94.57 (1.83)	94.40 (2.09)	94.49 (1.81)	0.3719
Deformability						
EI_{max}	0.58 (0.02)	0.59 (0.02)	0.58 (0.02)	0.59 (0.03)	0.59 (0.02)	0.4177
K_{EI}	2.29 (0.43)	2.30 (0.39)	2.34 (0.85)	2.28 (0.38)	2.24 (0.36)	0.9916
Morphology	66 (11)	65 (4)	69(7)	64 (7)	60 (6)	0.1300

* $p < 0.05$ when compared to RBCs.
† $p < 0.05$ when compared to 0.9% saline.
‡ $p < 0.05$ when compared to any of the other dextrose-containing solutions.
§ $p < 0.05$ differences between 5-, 14-, and 21-day RBCs identified.

for all DW and controls, except for RBCs incubated for 180 minutes with D5W (groups shown on Fig. 1 denoted by a, b, and c). For 14-day RBCs there was a statistical decrease in the EI_{max} as incubation time increased with any of the DW (all p values ≤ 0.038).

Incubation of RBCs for 180 minutes with D5W (5-day p = 0.019, 14-day p = 0.017, 21-day p = 0.008) or D5W with 0.2% saline (14-day p = 0.039, 21-day p = 0.044) led to significantly higher levels of hemolysis compared to RBC control. Extracellular potassium after 30 and 180 minutes (30 min—5-day p = 0.038, 14-day p = 0.006, 21-day p = 0.019; 180 min—5-day p = 0.019, 21-day p = 0.035) and hemolysis after 180 minutes (5-day p = 0.020, 14-day p = 0.020, 21-day p = 0.013) were also significantly different compared to 0.9% saline control for RBCs incubated with D5W. The EI_{max} for 14-day RBCs was statistically lower compared to the 0.9% saline control when incubated for 30 or 180 minutes in D5W (p = 0.009, 0.027), D10W (p = 0.003, 0.025), or D5W with 0.2% saline (p = 0.033, 0.034).

At 180 minutes, the hemolysis of RBCs incubated with D5W was statistically higher when compared to D10W (5-day p = 0.006, 14-day p = 0.002, 21-day p = 0.002) and D5W with 0.2% saline (5-day p = 0.016, 14-day p = 0.009, 21-day p = 0.036) regardless of age. At 180 minutes, the hemolysis of 21-day RBCs incubated in D5W with 0.2% saline was statistically higher when compared to D10W (p = 0.012). There are statistical differences in the potassium between DW solutions after 5 minutes (5-day p = 0.003, 14-day p = 0.005, 21-day p = 0.015), 30 minutes (5-day p < 0.001, 14-day p < 0.001, 21-day p = 0.001), and 180 minutes (5-day p = 0.002, 14-day p = 0.044, 21-day p = 0.005) for all RBC ages.

Phase 2: impact of incubation with DW on the RBC characteristics (Fig. 2)

As incubation time increased there was a statistical increase in the spun Hct, Coulter Hct, and MCV for RBCs incubated with any DW as shown by the p values in Fig. 2 (all p values ≤ 0.007). The Coulter was unable to measure the Hct and MCV of 5-day RBCs incubated with D10W for the 180 minute time period.

Due to the dilution of samples with the coinfusion solutions, RBCs incubated with any of the DW or the 0.9% saline control had statistically lower spun Hct and Coulter Hct levels compared to the RBC control (p < 0.035 for all). Incubation of RBCs with D5W or D5W with 0.2% saline resulted in statistically higher spun Hct compared to the 0.9% saline control after 5 minutes (5-day p = 0.001, 0.069; 14-day p = 0.006, 0.035; 21-day p = 0.013, 0.013), 30 minutes (5-day p = 0.002, 0.004; 14-day p = 0.007, 0.012; 21-day p = 0.002, 0.028), and 180 minutes (5-day p < 0.001, p = 0.001; 14-day p < 0.001, p = 0.009; 21-day p = 0.014, 0.011) for all RBC ages (except 5-min incubation of 5 days RBC in D5W with 0.2% saline). Incubation of RBCs with D10W resulted in statistical lower spun Hct after 5 minutes (5-day p = 0.007, 14-day p = 0.002, 21-day p = 0.007) and statistically higher spun Hct after 180 minutes (5-day p = 0.001, 14-day p = 0.024, 21-day p = 0.034) for all RBC ages. After 180 minutes, 21-day RBCs incubated with D5W (p = 0.016), D10W (p = 0.005), or D5W with 0.2% saline (p = 0.001) had statistically higher Coulter Hct levels than the 0.9% saline control. Differences compared to the 0.9% saline control were also seen in Coulter Hct after 30 minutes for 21-day RBCs incubated with D5W (p = 0.034) or D5W with 0.2% saline (p = 0.015). With 5-day RBCs, differences in Coulter Hct were observed compared to the 0.9% saline control after 5 minutes with D5W (p = 0.001) and after 180 minutes with D5W (p = 0.008) and D5W with 0.2% saline (p = 0.001). MCV of RBCs incubated for 180 minutes with D5W (5-day p = 0.004, 0.004; 21-day p = 0.005, 0.004), D10W (21-day p = 0.001, 0.001), or D5W with 0.2% saline (5-day p < 0.001, 0.001; 21-day p = 0.002, 0.001) were statistically higher compared to RBC and 0.9% saline controls.

There were statistical differences in the spun Hct between DWs after 5, 30, and 180 minutes for all RBC ages

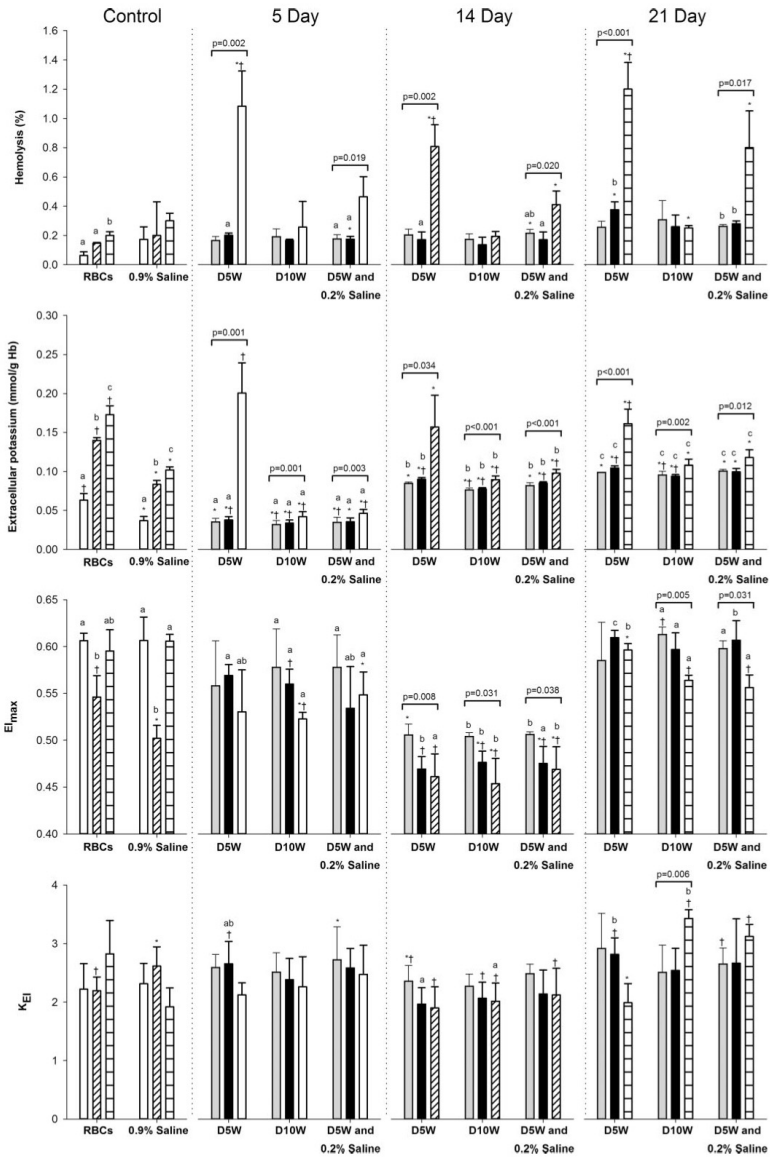

Fig. 1. Impact of incubation with DW on the RBC membrane. Results are sorted by RBC age, incubation time (▭ 5 min, ■ 30 min, ▭ 180 min/5 day, ▭ 180 min/14 day, ▨ 180 min/21 day) and coinfusion solution showing the hemolysis, extracellular K^+ levels, and deformability (EI_{max} and K_{EI}). Hemolysis and extracellular potassium levels increased with incubation time and were dependent on the coinfusion solution used. *Significant difference (at $p < 0.05$) between the indicated point and the RBC control of the same age. †Significant difference (at $p < 0.05$) between the indicated point and the 0.9% saline control of the same age. [a,b,c]Signficant difference (at $p < 0.05$) between RBC age for the indicated solution and incubation time. A bar (⁀) with a specified p value indicates a significant difference (at $p < 0.05$) between incubation times for the indicated solution and RBC age.

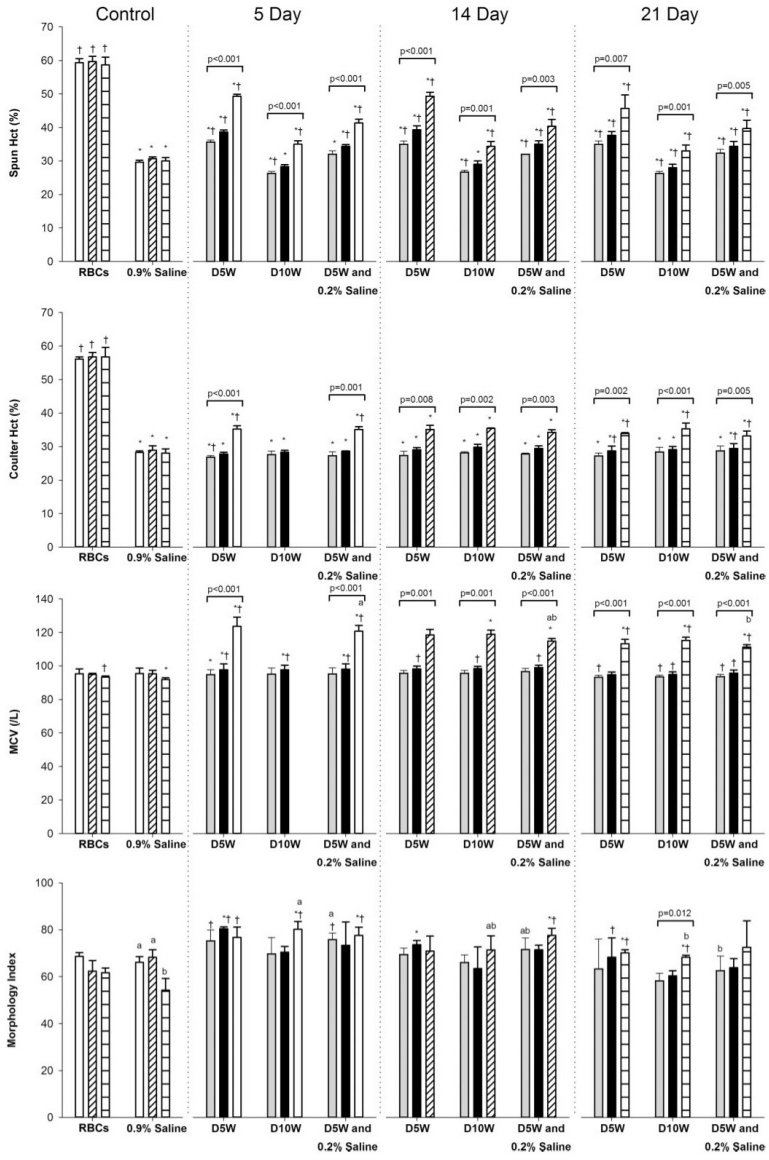

Fig. 2. Impact of incubation with DW on RBC characteristics. Results are sorted by RBC age, incubation time (▭ 5 min, ■ 30 min, ▭ 180 min/5 day, ▭ 180 min/14 day, ▨ 180 min/21 day), and coinfusion solution showing the morphology index, MCV, and Hct (spun Coulter). Hct and MCV increased with incubation time and were dependent on the coinfusion solution used. *Significant difference (at $p < 0.05$) between the indicated point and the RBC control of the same age. †Significant difference (at $p < 0.05$) between the indicated point and the 0.9% saline control of the same age. [a,b,c]Signficant difference (at $p < 0.05$) between RBC age for the indicated solution and incubation time. A bar (⌐¬) with a specified p value indicates a significant difference (at $p < 0.05$) between incubation times for the indicated solution and RBC age.

($p \leq 0.001$ for all). Regardless of age, RBCs incubated with D5W had statistically higher spun Hct levels than the RBC incubated with D10W or D5W with 0.2% saline at all incubation times ($p \leq 0.018$ for all). RBCs incubated in D5W with 0.2% saline had statistically higher spun Hct levels than the RBCs incubated with D10W at all incubation times ($p \leq 0.008$ for all ages).

DISCUSSION

Current transfusion guidelines recommended against coinfusion of RBCs with IV solutions except for 0.9% saline.[1,19] Our study has found that it may be permissible to coinfuse certain DW and RBCs in the NICU. By utilizing RBCs that reflect common transfusion practice throughout NICUs worldwide, including use of the most common age of RBC transfused[15] and irradiation before use, our study has addressed many of the methodologic concerns with prior studies.[4] Phase 2 of the study addressed one of the slowest infusion rates used for RBC transfusions in the NICU, as well as the situation that might occur if the transfusion was suspended for a period of time, further addressing criticism of earlier studies.[4]

Hemolytic damage is dependent on infusion rate, contact time as well as the composition of the solutions tested.[20] In Phase 1, the contact time of the DW with RBCs was longer than would occur in clinical practice in the NICU. In the NICU, RBCs would only be in contact with the DW for a maximum of 5 minutes in the smallest infants with the slowest infusion rates. Larger infants, with consequent of higher infusion rates, would receive RBCs that had been in contact with DW for even shorter time periods.

Water has an osmolality of 0 mOsm/L and when RBCs are placed in water, there is an increase in osmotic pressure due to water flowing into the cells leading to cell lysis. Any solution with an osmolality less than that of the RBCs at 288 mOsm/L, such as D5W at 253 mOsm/L, will have a similar effect with water flowing into the cells, causing them to lyse. As 0.9% saline is an isotonic solution, with an osmolality similar to that of blood at 310 mOsm/L, it is considered acceptable to coinfuse with RBCs. Other hypertonic solutions, such as D10W with an osmolality at 505 mOsm/L, are not considered appropriate to coinfuse with RBCs as they may cause water to shift from the cells, leading to RBC crenation. The contact period during which these changes are likely to occur is not clear. For the contact times used in Phase 1 of our experiments, we did not observe any adverse effects on RBC integrity. In Phase 2, RBCs incubated with D5W and D5W with 0.2% saline (321 mOsm/L) at 180 minutes had the highest hemolysis levels observed for all groups. The elevated Hct levels also observed in Phase 2 were likely due to cell swelling of the RBCs in contact with the DW.

In Phase 1 of the experiments, increasing age of the RBCs was associated with increased levels of corrected supernatant K^+ as well as hemolysis in 21-day-old RBCs transfused alone. However, the values observed are unlikely to be clinically significant as none of the values exceeded those of 21-day-old RBCs transfused alone or with 0.9% saline, a coinfusate permitted by current transfusion guidelines. In Phase 2 of the experiments, increasing age of the RBCs and increasing incubation time were associated with adverse effects on markers of RBC integrity. However, it is likely that some of these changes, including the effects on Hct level, may reverse on infusion into an infant. In addition, many of the statistical differences observed in Phase 2 of the study may not translate into significant effects in a clinical setting.

The US Food and Drug Administration (FDA) accepted level for licensure of RBC storage solutions recommends levels below 1.0% hemolysis and the Council of Europe Guidelines for RBC products recommends levels below 0.8% hemolysis.[21] With the US FDA introducing more stringent requirements that 95% of RBC units must meet the standard with a 95% statistical certainty, this effectively means a maximum value of 0.35% hemolysis if these standards are to be consistently met.[22] When RBCs were coinfused in Phase 1 of the study with D5W, D10W, and D5W with 0.2% saline, regardless of age, the highest hemolysis levels observed were $0.20 \pm 0.03\%$, below the maximum recommended value of 0.35%.

Our study findings support a potential practice change in the NICU allowing for coinfusion with DW. An in vitro study by Birch and colleagues[23] found that the addition of medications mixed with 0.9% saline to RBC infusions did not adversely affect RBC integrity, leading to a change in Australian transfusion guidelines.[19] The evidence that current transfusion guidelines use to support the recommendation that RBCs should not be coinfused with DW is limited and based on older studies, often using blood products and experimental conditions not resembling current clinical practice.[2] At a minimum, our findings suggest an in vivo trial of coinfusion of DW and RBCs is feasible in the NICU.

Our study is limited by its in vitro nature and small sample size. No microscopic examination for RBC aggregation was performed and all possible infusion rates for infants in the NICU setting were not examined. In addition, the varying production methods for RBCs, including use of differing storage media, mean our findings may not be generalizable to other jurisdictions, such as the United States, where there is a larger use of apheresis and nonleukoreduced RBCs. Differences in neonatal transfusion apparatus set-up were also not evaluated in this report.

Previous studies in this area have indicated that coinfusion of DW and RBCs may not adversely affect RBC integrity, consistent with our findings.[3,13] However, aspects

of these prior studies make drawing broad conclusions challenging, including the use of 5-day-old nonirradiated RBCs only, small sample sizes, and limited use of markers of RBC integrity. Several older studies have examined the effects of incubation of DW and RBCs using visual markers of hemolysis only.[24,25] These studies were performed with whole blood utilizing experimental conditions that do not resemble current clinical practice, making comparisons difficult. A more recent study,[26] again using visual markers of hemolysis, found that when RBCs were incubated with D5W in ratios 1:1 (blood:solution) hemolysis developed after 30 to 60 minutes, consistent with our findings in Phase 2.

Clinical studies are needed to ultimately determine the safest route for infusion of both RBCs and DW in the NICU setting, whether by two separate IV access sites or by coinfusion. Our study findings suggest D10W may be an acceptable DW to test as a coinfusate in the in vivo setting as it appeared to minimally effect the RBCs in Part 2 of the study. Use of an apparatus that minimizes the space the coinfusate and RBCs are in contact with each other is also important. We would recommend the use of D10W as the coinfusate in a transfusion apparatus similar to the one described in our study, where the contact area for the coinfusate and RBCs was only 0.38 mL if coinfusion is being considered.

We conclude that RBCs coinfused with 0.9% saline or D10W were not adversely impacted. Caution is warranted, however, when developing clinical studies or guidelines as the coinfusion conditions, including flow rate, incubation time, and type of DW, may affect the quality of the transfused RBCs. Coinfusion of DW with RBCs may be permissible under circumstances clinically relevant to the NICU with further in vitro and in vivo studies warranted.

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CONFLICT OF INTEREST

The authors report no conflicts of interest.

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Chapter 6

Fluid bolus therapy in neonates

This chapter includes the paper, currently submitted for publication "*An international, multi-centre, cross-sectional study of fluid bolus therapy in neonates*."

It addresses the previously identified research question:

Research question 5:

What are the types, doses, indications and short-term outcomes of fluid bolus therapy in neonates?

The published paper is now provided at the end of Chapter 6

Authorship forms are provided in Appendix C

An international, multi-centre, cross-sectional study of fluid bolus therapy in neonates

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Abstract and keywords

Aims: To assess the prevalence, types, and indications for fluid bolus therapy in neonates with haemodynamic compromise.

Methods: A pragmatic international multi-centre cross-sectional study in neonatal units across Australasia, Europe, and North America. A pre-defined study period of 10-15 study days per participating neonatal unit between December 2015-March 2017. Infants ≤28 days of age who received a fluid bolus for the management of haemodynamic compromise (≥10ml/kg given at ≤6 hours) were included.

Results: 163 neonates received a bolus over 8479 eligible patient days in 41 neonatal units. Prevalence of fluid bolus therapy varied between centres from 0% to 28.6% of admitted neonates per day, with a pooled prevalence rate of 1.5% (95% confidence interval 1.1-1.9%). The most commonly fluid used was 0.9% sodium chloride (129/163; 79%), the volume of fluid administered was most commonly 10mL/kg (115/163; 71%) over a median of 30 (interquartile range 20-60) minutes. The most frequent indications were hypotension (n=56; 34%), poor perfusion (n=20; 12%) and metabolic acidosis (n=20; 12%). Minimal or no clinical improvement was reported by clinicians in 66/163 (40%).

Conclusions: Wide international variations in types, indications and effects of fluid bolus administration in haemodynamically compromised neonates suggest uncertainty in the risk-benefit profile. This is likely to reflect the lack of robust evidence to support the efficacy of different fluid types, doses and appropriate indications. Together, these highlight a need for further clinically relevant studies.

Keywords: infant, newborn; therapy, fluid; blood pressure.

Introduction

Intravenous fluid bolus therapy for suspected haemodynamic compromise in neonates, with a variety of underlying conditions, is a common intervention in neonatal units. Fluid boluses may include crystalloids such as 0.9% sodium chloride, or colloids such as albumin or blood products including plasma, which have different biochemical properties. While this therapy represents an established component of the management of haemodynamic compromise in neonates, the volume, type of fluid, timing and indications for this practice are not well described or understood.[31, 90, 103] A Cochrane review found no benefit from the use of early fluid bolus therapy in infants ≤ 32 weeks' gestation without haemodynamic compromise.[90] This review identified no available evidence to determine whether those with clear haemodynamic compromise might benefit from volume expansion compared to no volume expansion.[90] Another review, including two more recent studies not included in the previous meta-analysis, was again unable to establish any benefit from fluid bolus therapy in late preterm and term infants with signs of haemodynamic compromise.[31] There are well documented concerns about the consequences of fluid bolus therapy in older children, but comparable data do not exist in neonates. It is possible that some fluid boluses provide no clinical benefit, and may even cause harm.[99, 104]

As a first step in evaluating and improving the use of fluid bolus therapy in clinical practice, we conducted a pragmatic, international, multi-centre, cross-sectional study to explore existing practices of fluid bolus therapy. Our primary objective was to describe the prevalence, types, indications for and doses of fluid bolus therapy administered to neonates with suspected haemodynamic compromise. Secondary objectives were to determine variations in practice of fluid bolus therapy and evaluate the degree of perceived improvement post fluid boluses.

Methods

Study design and setting

This study was an international, pragmatic, multi-centre, cross-sectional study undertaken at 41 neonatal units in Australasia (n=12), North America (n=16) and Europe (n=13). Units were recruited through neonatal research networks and specialty societies, as well as through personal communications directed by the main study investigators. Participating neonatal units collected data in blocks of 5 continuous days in 2-3 blocks for a minimum of 10 days and up to a maximum of 15 days per unit. This was a pragmatic decision, given limited study

funding, to allow units to support a period of study data collection, based (for example) on availability of local research staff or individuals. Data collection occurred between December 2015 and March 2017.

Participants

Newborn infants of any gestation at birth who were ≤28 days of age who received a fluid bolus for suspected haemodynamic compromise were included. Participants were identified by the individual study site co-investigators.

Exposure

The exposure of interest was a fluid bolus given for the purposes of intravascular volume expansion for suspected haemodynamic compromise. Fluids included were 0.9% sodium chloride, 0.45% sodium chloride, Ringer's lactate solution, albumin, frozen plasma, whole blood or RBCs. The fluid bolus had to be 10mL/kg or greater volume given over ≤6 hours. Neonates who received bolus fluids for hypoglycaemia or RBC transfusions to manage anaemia of prematurity alone were excluded.

Variables

Demographic and clinical characteristics of included neonates and participating units were collected. We collected information on type, volume and duration of bolus fluids administered. Information on indications for fluid boluses assigned according to pre-defined categories, including an "other" category where site investigator was asked to define the indication were collected; see *Supplementary material 1* for the data collection sheet. The effects of fluid boluses on short-term perceived clinical outcomes at 4-6 hours after administration were categorized according to a numerical score. These scores were based on clinician report 4-6 hours post-bolus in 4 areas: (1) the reported degree of improvement in the primary indication for fluid (no change=0, some improvement=1, large improvement=2), the need for escalation of therapy to inotrope use (new agent started=0, some up, some down =1, or no agent started, agent decreased or stopped=2), additional fluids bolus(es) within six hours of the first (more than 2=0, one additional bolus=1, no additional bolus=2) and whether another treatment was commenced for the primary indication (yes=0, no=2). These scores were summed and classified as no or minor improvement (score 0-2), mild improvement (score 3-5) or major improvement (6-8). The scoring sheet is provided in *Supplementary*

material 1 and was developed by expert consensus and a formal piloting process, including neonatologists, paediatric critical care and haematologists within the study group.

Data management

Study data were collected and managed using the Research Electronic Data Capture (REDCap) tools hosted at the University of Adelaide, Australia.[105] REDCap is a secure, web-based application designed to support data capture for research studies, providing 1) an interface for validated data entry; 2) audit trails for tracking data manipulation and export procedures; 3) automated export procedures for data downloads to common statistical packages; and 4) procedures for importing data from external sources.

Data sources

Descriptive data on unit characteristics were collected by individual study site coordinators, including type of unit, country, number of neonates admitted per year, availability of unit guidelines for fluid bolus and/or RBC transfusion.

Sample size

All infants in each participating institution who received at least one fluid bolus during the site collection period were included in the study. A sample of 41 units agreed to participate. Each patient was enrolled only once for the first bolus received during the study interval even if he/she received further boluses on a subsequent study day.

Statistical analysis

Normally distributed data were described by the mean and standard deviation (SD) and non-normally distributed data using the median and interquartile range (IQR). Analyses were carried out using R statistical software package (R version 3.1.0 (2014-04-10)[106] unless otherwise specified. Prevalence rate for receipt of bolus was calculated by dividing number of neonates who received a bolus by the number of neonates who were present in the unit during the study interval who were ≤28 days of age. Each neonate on a given day was considered to be eligible to receive a bolus until the study period ended. Pooled prevalence rate and 95% confidence interval were calculated using Der-simonian random-effects model with open access Meta-analyst software.[107]

Ethics approval

Site specific ethics approval was obtained for all sites. Two centres in Canada required individual written consent prior to collection of clinical data. French and Swiss sites had an opt-out strategy with information provided for families in the units' waiting rooms. All other ethics committees waived the requirement for individual consent given that all data were routinely collected for clinical purposes and no individual identifying data would be recorded and sent to the lead site (Women's and Children's Hospital, Adelaide, Australia).

Results

Participating centres

Forty-one units participated in the study. Ten (24%) were in Australia, eight (20%) in Canada, four (10%) in France, one (2%) in Italy, two (5%) in New Zealand, one (2%) in Portugal, four (10%) in Sweden, two (5%) in Switzerland, one (2%) in the United Kingdom and eight (20%) in the USA. Median numbers of admissions per unit per year were 650 (IQR 420 -1836). Twenty-two (55%) units were classified as general perinatal centres, 16 (39%) were surgical units including cardiac and three (7.5%) were mixed (NICU/PICU) units.

Patient characteristics

A total of 163 neonates received a bolus over 8479 eligible patient days. Pooled prevalence rate of receipt of fluid bolus was 1.5% (95% confidence interval 1.1-1.9%;) across all participating units. Prevalence of bolus administration in participating units varied from 0% to 28.6% of admitted neonates (≤28 days of age) per day. Data for individual units, grouped by geographical regional area, are provided in Figure 1.

For included infants, the birth gestation of included infants reflected a bimodal distribution with peaks at 27 and 39 weeks (Figure 2) as did birthweight with peaks at 650-850 grams and 2850-3050 grams. The majority of neonates received their first fluid bolus on the day of birth (87/163; 53%), and there was diminishing likelihood of a first fluid bolus on subsequent days; day 2 (24/163; 15%), days 3-7 (25/163; 15%) and >7 days (27/163; 17%). The reported primary indications for fluid bolus therapy are provided in Table 1.

Clinical guideline availability

Local clinical practice guidelines which referenced fluid bolus therapy were available in only 10 (24%) of the participating units.

Fluid bolus characteristics

Types of fluid used for fluid bolus therapy included 0.9% sodium chloride (n=129; 79%), RBCs (n=15; 9%), 5 or 20% albumin (n=5; 3%), Ringer's lactate (n=9; 5%), frozen plasma (n=4; 3%) and 0.45% sodium chloride (n=1; <1%). The commonest volume administered was 10mL/kg (n=115; 67%) with a median duration of administration of 30 (IQR 20-60) minutes. Table 2 shows a breakdown of the indication for each fluid bolus and type of fluid used.

Short-term outcomes

Mortality

At the end of the data collection period 151/163 (93%) of infants were alive. None of the infants died during the receipt of the fluid bolus or within 6 hours post-bolus.

Clinician-perceived improvement

Clinicians perceived no or minor improvement (score 0-2) in 25/163 (15%), a mild improvement (score 3-5) in 41/163 (25%) and a major improvement in 97/163 (60%) in response to bolus therapy. Improvement according to primary indication is reported in Table 1. Table 3 (*Supplementary material 2*) provides further breakdown of indication for fluid bolus, type of fluid used and clinical improvement scores.

Laboratory indices

The following changes in laboratory parameters were observed following fluid bolus: pH 0.03 units (IQR; -0.03 to 0.12 units; n=140) (Figure 3); lactate -0.59 mmol/L (-2.15 to 0.02 mmol/L; n=100) (Figure 4); bicarbonate 0 mmol/L (-1.35 to 2.00 mmol/L; n=139); chloride 0.5 mmol/L (-1.00 to 3.00 mmol/L; n=80); base deficit -1.10 mmol/L (-3.93 to 1.00 mmol/L; n=128) and haemoglobin -5.00 g/L (-16.00 to 9.25 g/L; n=88).

Variations in prevalence of fluid bolus therapy

Regions

The pooled prevalence for fluid bolus therapy for Australian and New Zealand units (n=12) was 1.2% (95% CI 0.6-1.7%;), in Canadian units (n=8) it was 1.5% (95% CI 0.8-2.1%), in USA-based units (n=9) it was 1.8% (95% CI 0.8-2.8%) and in European units (n=12) it was 2.7% (95% CI 1.1-4.4%) (Figure 1).

Types of centre

The pooled prevalence for fluid bolus therapy within general perinatal centres (n=22) was 1.3% (95% CI 0.9-1.8%) and within the remaining centres (surgical and mixed units) (n=19) it was 1.9% (95% CI 1.2-2.6%). The centre with the highest prevalence rate was a non-perinatal unit caring primarily for paediatric patients.

Discussion

This international study explored the prevalence, types and indications of fluid bolus therapy in neonates with haemodynamic compromise. This was a pragmatic study aimed at trying to better define the current practices of fluid bolus therapy and, as such, was developed with the need to be very restrictive on the amount of data collection. While the pooled prevalence rate was low, the prevalence of this therapy varied (0–28.6%). We identified variations in the nominated indications for and frequency of use of fluid boluses between participating units. Overall, perceived improvement following fluid bolus therapy was reported in 85% of cases. Together, these results highlight a clear lack of consistent clinical approach and perceptions of variable effects.

The interpretation of our pragmatic study needs to recognise both its strengths and limitations. Our study was supported by a large number of units across many different countries. It describes practices in units that were selected by personal approaches by the investigators. but we cannot assume they are representative of non-participating neonatal units and other countries. Participation was voluntary and units selected the most convenient time to support data collection. The calculation of prevalence was based on the assumption that the prevalence of fluid boluses over the short study intervals was constant and representative of standard practice in each unit. This assumption may not be true. However, variations from the reported rate could be on either side of estimated rate and thus overall, the averaged results could be considered representative. In attempt to maximise unit participation, data collection was kept to a minimum, and therefore a number of outcomes of potential interest were not requested, for example BP. In addition, it was not possible to collect detailed information on potential adverse effects related to fluid bolus beyond the six hours, such as volume overload, dilutional coagulopathy, hypothermia and electrolyte disturbances.[98, 99] This lack of data also extends to other specific fluid related complications, including transfusion reactions,[46, 97] or 0.9% sodium chloride-induced hypochloremic metabolic acidosis, although we did not observe any significant increase in chloride level post-fluid bolus. Reported outcomes post-bolus were made by the treating clinicians, and as the prescriber of the treatment, they may have preferred to perceive an improvement.

Published studies evaluating fluid bolus therapy in neonates are heterogeneous, and have not always included neonates with signs of haemodynamic compromise.[85-89] There are no randomised studies primarily designed to examine fluid bolus compared to no fluid bolus in

preterm infants with haemodynamic compromise.[84] Studies in late preterm and term infants with haemodynamic compromise are limited to non-randomised observational studies and do not report clinical benefit.[94, 108] A survey in Canada reported that while attitudes to the use of inotropes varied, neonatologists routinely treated suspected haemodynamic compromise in infants with a birthweight <1500 grams with a fluid bolus (97%) and most commonly used 0.9% sodium chloride (95%).[80] Our results are consistent with this, with the majority of fluid boluses (47/56; 84%) given to an infant to manage low blood pressure being 0.9% sodium chloride.

Only 10 of the units participating in this study had local clinical guidelines available to guide fluid bolus use. Use of clinical guidelines, even in areas with a limited evidence base, may reduce variation in practice.[109] Nevertheless, consensus is only helpful to patients if it is the right consensus, and the lack of intervention studies defining optimal fluid bolus therapy, such as indication, type, volume and rate, in preterm and term infants makes this not possible at this time. Clinicians are left to either extrapolate data from other patient groups, some now showing potential harmful effects from fluid bolus therapy in children (e.g. Fluid Expansion as Supporting Therapy (FEAST) study[102]), or rely on limited and potentially misleading physiological data to guide decisions.

With ongoing trials examining the use of inotropes in this group of infants (http://www.neocirculation.eu and http://www.hip-trial.com), our study also suggests the need to evaluate fluid bolus therapy. This extends to the choice of fluid as well as dose and timing. the most common fluid bolus type in our study was 0.9% sodium chloride, but this fluid is not physiological, and concerns continue to be raised about use of this fluid in other settings of critical illness, which may be more important in preterm infants with less mature renal function. With the assessment of haemodynamic compromise currently relying on a variation of clinical signs, echocardiographic findings or abnormal laboratory results,[110] additional research should consider the development of consensus definitions in this area. including a core outcome set[111].

While only a small proportion of newborn infants receive fluid bolus therapy in the neonatal period, our study highlights variations in incidence and reasons for fluid administration in different units, and uncertainties in outcomes. Further studies need to be conducted in patient populations meeting clear consensus definitions of haemodynamic compromise. As the

FEAST trial demonstrated in children,[102] our assumptions around the potential of benefits of fluid bolus therapy in neonates may need careful reconsideration.

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Tables

Table 1: Primary indication for fluid bolus therapy (n=163) and clinical-perceived scores post-fluid bolus administration

Main indication	Frequency (%)	No to minor improvement Score 0-2	Mild improvement Score 3-5	Major improvement Score 6-8
Low blood pressure	56 (34%)	14 (25%)	17 (30%)	25 (45%)
Decreased perfusion on clinical assessment	20 (12%)	1 (5%)	7 (35%)	12 (60%)
Metabolic acidosis	20 (12%)	1 (5%)	4 (20%)	15 (75%)
Elevated lactate	13 (8%)	2 (15%)	0 (0%)	11 (85%)
Decreased urinary output	9 (6%)	0 (0%)	1 (11%)	8 (89%)
Blood loss/hemorrhage	9 (6%)	1 (11%)	4 (44%)	4 (44%)
Hypovolemic shock	6 (4%)	2 (33%)	0 (0%)	4 (67%)
Echocardiography findings (decreased cardiac output)	6 (4%)	2 (33%)	0 (0%)	4 (67%)
Part of acute resuscitation in an arrested (or peri-arrest) infant	6 (4%)	2 (33%)	3 (50%)	1 (17%)
Tachycardia	4 (3%)	0 (0%)	1 (25%)	3 (75%)
Septic shock	4 (3%)	0 (0%)	2 (50%)	2 (50%)
Other[0]	10 (6%)	0 (0%)	2 (20%)	8 (80%)

[0]Other: volume replacement for gastric aspirate or urinary losses, polycythemia, dehydration and renal impairment, hypovolemia, anemia, hyperbilirubinemia, gastroschisis (unclear whether this was routine for this unit for this diagnosis, or was for another reason, such as replacement of losses or to improve perfusion).

Table 2: Indication for fluid bolus (n=163) and type of fluid used

	0.9% sodium chloride	Packed red blood cells	Frozen plasma	Ringers lactate	Other[0]
Low blood pressure	47	3	1	1	4
Decreased perfusion on clinical assessment	18	1	-	1	-
Metabolic acidosis	17	-	-	3	-
Elevated lactate	10	2	-	1	-
Decreased urinary output	8	1	-	-	-
Blood loss/hemorrhage	5	3	1	-	-
Hypovolemic shock	2	2	-	1	1
Echocardiography findings (decreased cardiac output)	5	-	1	-	-
Part of acute resuscitation in an arrested (or peri-arrest) infant	3	2	-	1	-
Tachycardia	3	-	-	-	1
Septic shock	3	-	-	1	-
Other[1]	8	1	1	-	-

[0]Other: 5% albumin, 20% albumin, 0.45% sodium chloride

[1]Other: volume replacement for gastric aspirate or urinary losses, polycythemia, dehydration and renal impairment, hypovolemia, anemia, hyperbilirubinemia, gastroschisis (unclear whether this was routine for this unit for this diagnosis, or was for another reason, such as replacement of losses or to improve perfusion).

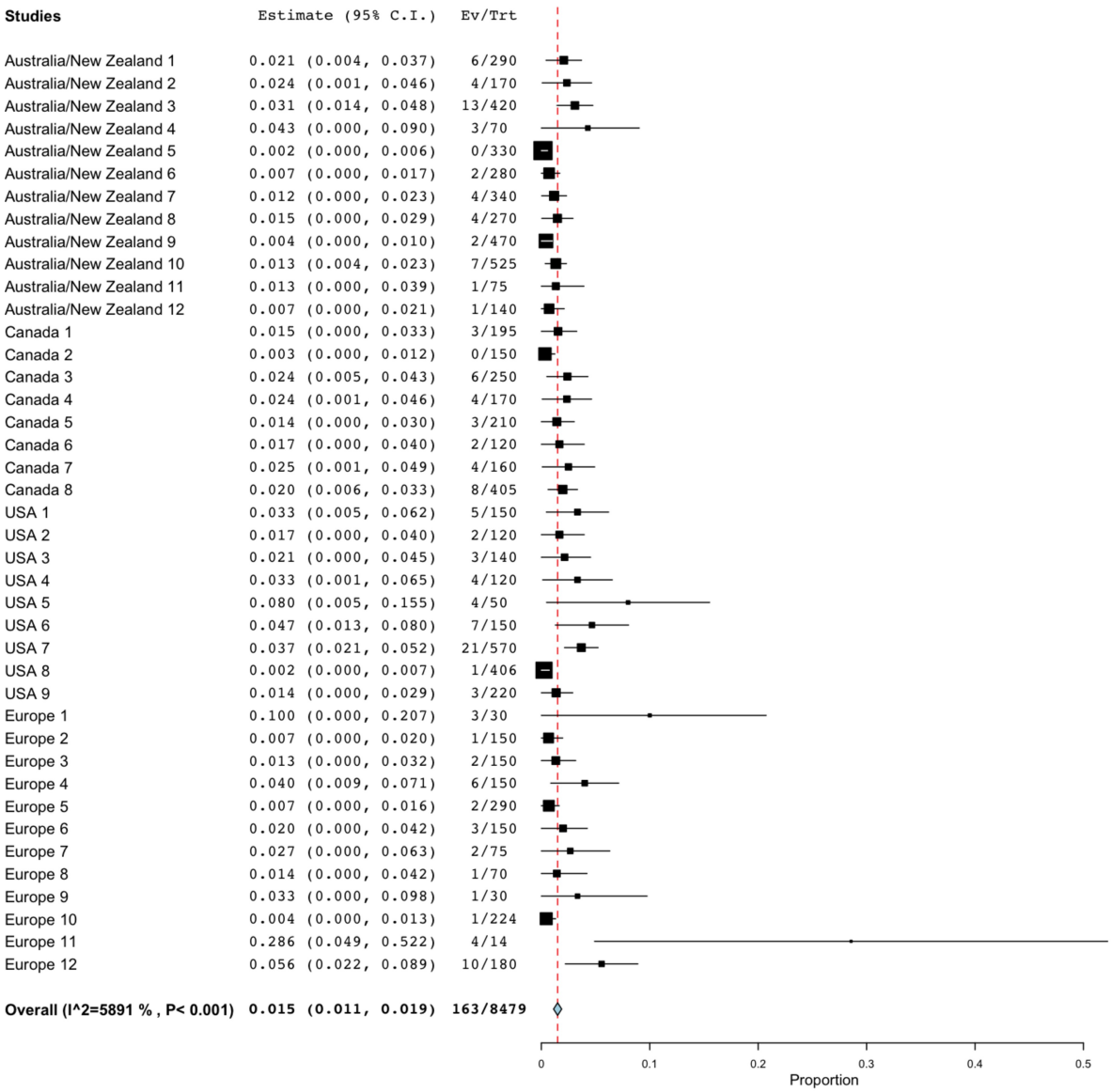

Figure 1: Prevalence of fluid bolus therapy per admitted infant per study day.

Horizontal lines represent the proportion of infants who received a fluid bolus divided by number of potentially eligible infants during the study period. For example, for site 1: 0.021 (CI 95% 0.004 to 0.037) or 2.1% of potentially eligible infants received a fluid bolus during study.

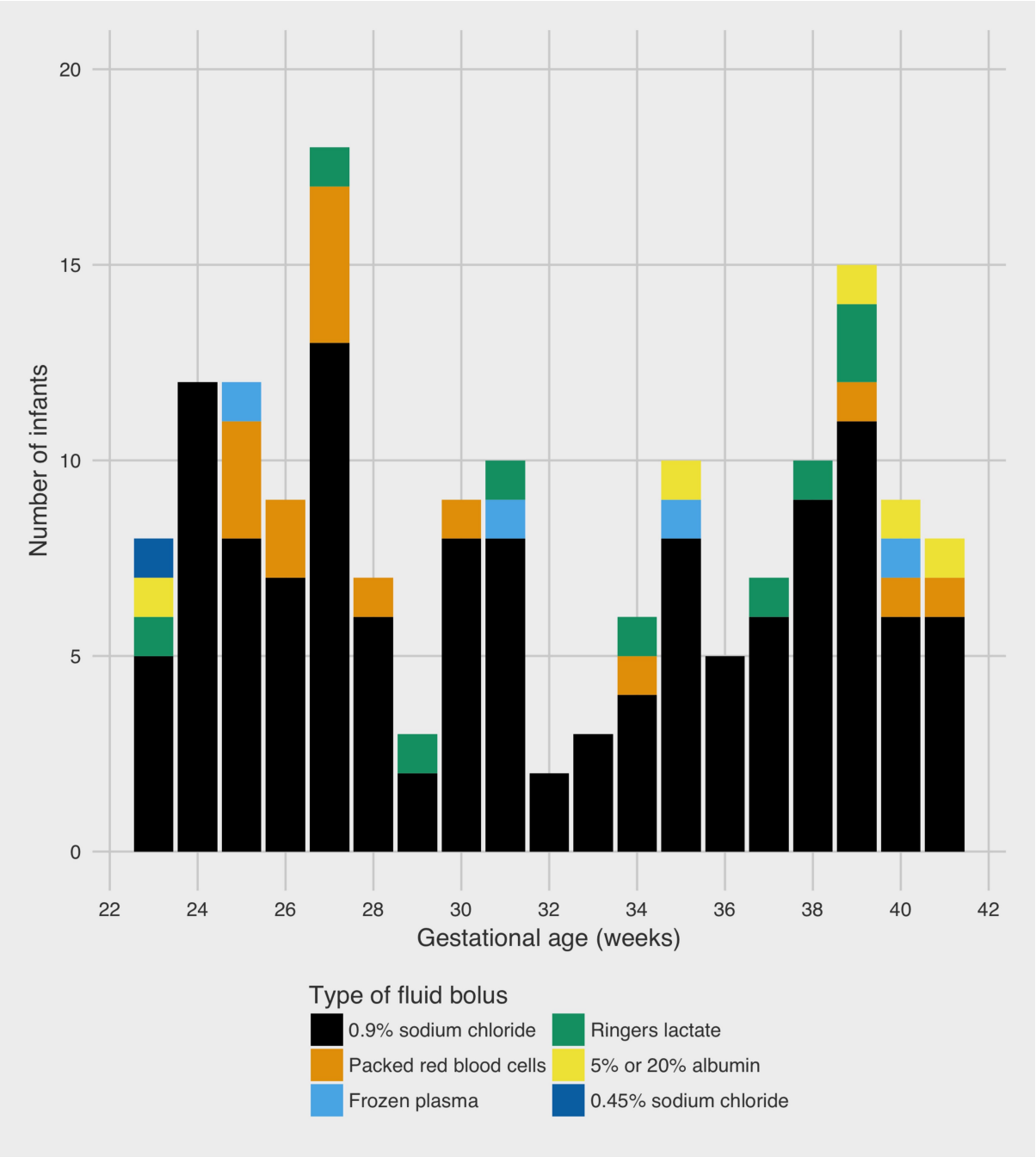

Figure 2: Distribution of gestational age at birth of included infants.

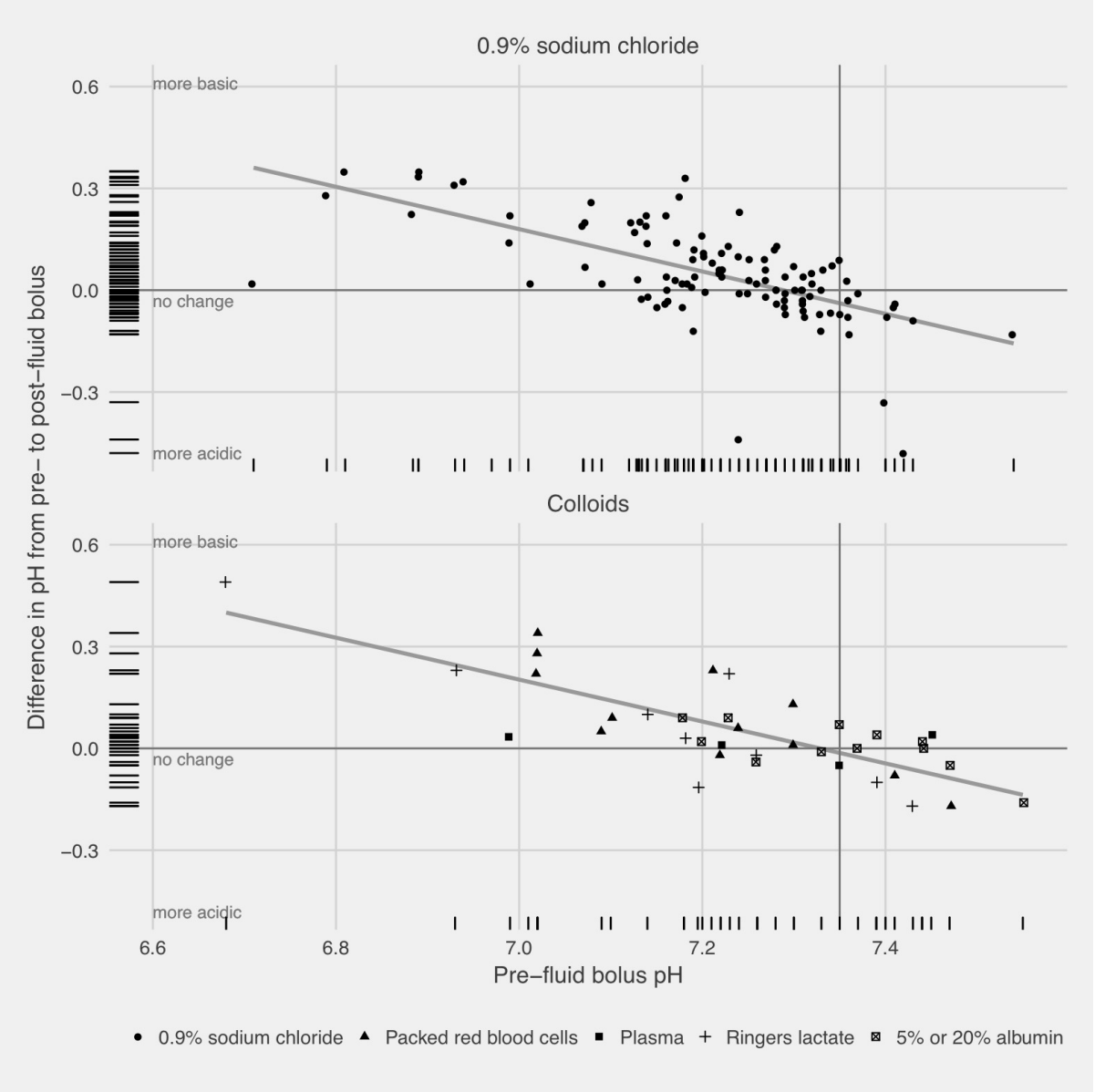

Figure 3: Differences in acid-base (pH) from pre- to post- fluid bolus.

Solid grey lines represent the linear regression change in pH compared to initial pH level.

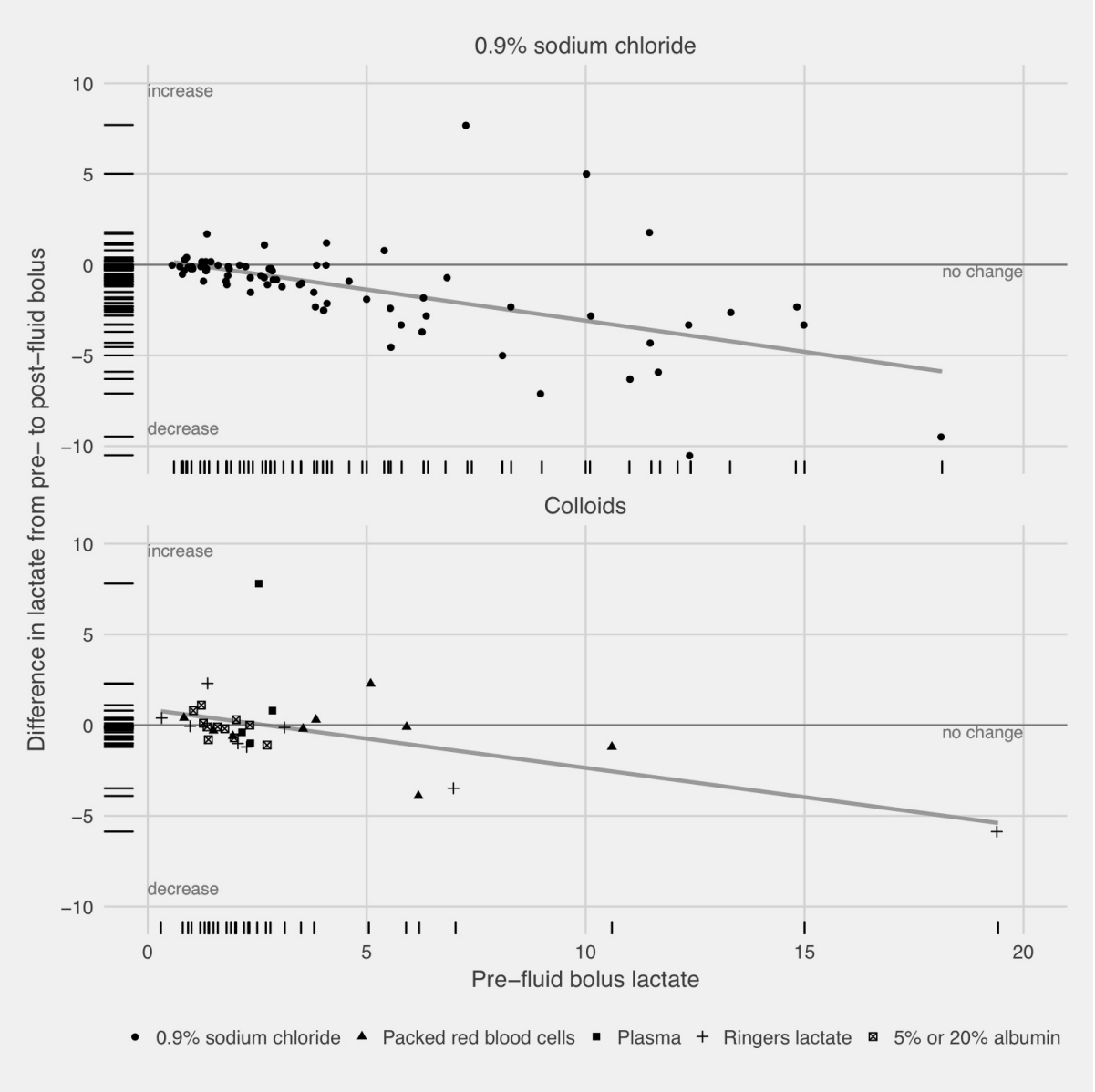

Figure 4: Difference in lactate level from pre- to post- fluid bolus.

Solid grey lines represent the linear regression change in lactate compared to initial lactate level.

Supplementary material for Chapter 6

CENTRE ID: ___¦___¦___¦ **PATIENT CODE: ___¦___¦___¦___**

CASE REPORT FORM (CRF) – PART A

Part A1 – Organisational information – do not complete if already done so for this organisation - submit this form ONCE only		
Centre ID	___¦___¦___¦	
Patient code	___¦___¦___¦___	
Country		
Number of neonates admitted to the unit on DAY 1 of the study period **(ALL neonates <28 days of age and currently on the unit on DAY 1)**		
Average number of neonates admitted per year		
Type of unit	Perinatal centre: ☐ Cardiac/surgical unit: ☐ Medical/surgical: ☐ Other (please specify): _____	
Are organisational guidelines available for fluid bolus therapy?	☐ Yes (if yes, would you provide please)	☐ No
Are organisational guidelines available for transfusion, including for 'placental' (delayed cord clamping) transfusion?	☐ Yes (if yes, would you provide please)	☐ No

CENTRE ID: ___¦___¦___¦ **PATIENT CODE: ___¦___¦___¦___**

CASE REPORT FORM (CRF) – PART B

Part B1 – Assessment of each patient receiving a 1st fluid bolus – COMPLETE ONLY ONCE FOR A SINGLE PATIENT			
Centre ID	___¦___¦___¦		
Patient code	___¦___¦___¦___		
Gestational age at birth	_____ weeks _____days		
Postnatal age (chronological) on this study day	_____days		
Birth weight	_____ grams		
Current weight on this study day	_____ grams		
Gender	☐ Male	☐ Female	
Status	☐ Inborn	☐ Outborn	
Delayed cord clamping and/or cord milking performed at delivery	☐ Yes	☐ No	☐ Unknown

Reason for admission (choose ALL THAT APPLY)		
☐ Extreme prematurity <28^{+0} weeks	☐ Prematurity 28^{+0}- 32^{+0} weeks	☐ Late prematurity (>32^{+0}- 36^{+6} weeks)
☐ Sepsis (including septic shock)	☐ Surgical condition (non-cardiac)	☐ Cardiac condition (specify _____)
☐ Metabolic (including hypoglycaemia)	☐ Hypoxic-ischaemic encephalopathy	☐ Respiratory disorder (specify _____)
☐ Neurological (excluding HIE; specify): _____	☐ Other (specify): _____	☐ Other (specify): _____

Part B2 – Assessment of the 1st fluid bolus event ONLY		
Total duration of fluid bolus (time)	**_____ : _____ Hours : Minutes**	
Total volume of fluid infused (actually received)	_____ mL	
Type and amount (mL/kg) of fluid bolus		
☐ 0.9% saline: _____mL/kg	☐ Packed red blood cells: _____ mL/kg	☐ Frozen plasma: _____ mL/kg
☐ 4.5% Albumin: _____mL/kg	☐ Ringer's lactate: _____ mL/kg	☐ Other (please specify): _____ _____ mL/kg
<u>Main</u> indication for fluid bolus (choose ONLY ONE)		
☐ Hypovolaemic shock	☐ Metabolic acidosis	☐ Elevated lactate
☐ Septic shock	☐ Decreased urinary output	☐ Low blood pressure
☐ Decreased perfusion on clinical assessment	☐ Blood loss/haemorrhage	☐ Part of acute resuscitation in an arrested (or peri-arrest) infant
☐ Echocardiography findings (Specify:_____ _____)	☐ Tachycardia	☐ Other _____

Secondary indications for fluid bolus - choose AS MANY as relevant		
☐ Hypovolaemic shock	☐ Metabolic acidosis	☐ Elevated lactate
☐ Septic shock	☐ Decreased urinary output	☐ Low blood pressure
☐ Decreased perfusion on clinical assessment	☐ Blood loss/haemorrhage	☐ Part of acute resuscitation in an arrested (or peri-arrest) infant
☐ Echocardiography findings (Specify:_____ _____)	☐ Other _____	☐ NIL _____

CENTRE ID: ___¦___¦___¦ **PATIENT CODE:** ___¦___¦___¦___

Most recent tests <u>prior</u> to commencement of 1st fluid bolus		
Timing of most recent blood gas prior to 1st fluid bolus	How long **prior** to the start of the 1st fluid bolus was a blood gas done (*up to 24 hours prior*)? _____ : _____ Hours: minutes ☐ Not done/not available	
Type	Arterial: ☐ Venous: ☐ Capillary: ☐	
pH	_____	☐ Unknown
pCO_2	_____	☐ Unknown
pO_2	_____	☐ Unknown
Bicarbonate	_____	☐ Unknown
Base deficit	_____	☐ Unknown
Chloride (if available)	_____	☐ Unknown
Lactate	_____	☐ Unknown
Results for most recent haemoglobin (Hb), PT/INR and platelet count	How long **prior** to the start of the 1st fluid bolus were the following tests done (*up to 24 hours prior*)? Hb _____ : _____; Hrs: mins; PT/INR _____ : _____ Hrs: mins; Platelets _____ : _____ Hrs: mins Not done ☐ Not done ☐ Not done ☐	
Haemoglobin level	_____	
INR/PT	_____	
Platelet count	_____	

Version 6: FINAL (December 2015)

CASE REPORT FORM (CRF) – PART C

Tests post 1st fluid bolus (*post 1 hour of completion of bolus AND within 4-6 hours if available*)		
Timing of most recent blood gas post completion of 1st fluid bolus	To the nearest hour (up to 24 hours), how long **after** to 1st fluid bolus was the gas done? _____ : _____ Hours: minutes ☐ Not done/not available	
Type	Arterial: ☐ Venous: ☐ Capillary: ☐	
pH	_____	☐ Unknown
pCO_2	_____	☐ Unknown
pO_2	_____	☐ Unknown
Bicarbonate	_____	☐ Unknown
Base deficit	_____	☐ Unknown
Chloride (if available)	_____	☐ Unknown
Lactate	_____	☐ Unknown
Timing of most recent haemoglobin (Hb), PT/INR level and platelet level	How long **AFTER** completion of the 1st fluid bolus were the following tests done (*up to 24 hours post*)? Hb _____ : _____; Hrs: mins; PT/INR _____ : _____ Hrs: mins; Platelets _____ : _____ Hrs: mins Not done ☐ Not done ☐ Not done ☐	
Haemoglobin level	_____	☐ Unknown
INR/PT level	_____	☐ Unknown
Platelet level	_____	☐ Unknown

Please complete the following <u>at 6 hours post completion or as close as feasible to 6 hours post</u> the 1st fluid bolus:
N.B: TICK ONE BOX PER COLUMN only

Indication(s) for fluid bolus (see section B2 of this form)	Inotropes*	Further fluid bolus within 6 hours of 1st bolus*	Other treatment started for main indication(s)+	Score
All or most improved (> 50%) ☐	No agent started, or all prior agents reduced or stopped ☐	None ☐	No ☐	2
Some (50% or less) ☐	Some up, some down ☐	One ☐		1
None improved, or worse ☐	New agent(s) started ☐	More than one ☐	Yes ☐	0

***Include additional fluid bolus/es and/or commencement of inotrope/s even if they were NOT started for the main indication.**
If inotropes and/or additional fluid boluses were given for ANOTHER indication aside from the main one, please state reason/s:
Indication/s:_____
Type/s of fluid and/or inotrope/s given (regardless of indication):_____
_____
+Please specify other treatment (excluding fluid bolus/es and/or inotropes started for main/other indication(s):

TOTAL SCORE (OUT OF MAXIMUM OF 8) =
<u>Please complete the following question ONLY if this it the last study day and this is the final enrolled infant:</u>
How many enrolled infants were alive at the end of the study period?
Total enrolled = _____
Alive = _____
Dead = _____

	Main indication	Type of fluid used	Total no. per indication	Nos per indication per type of fluid	Major Improvement	Mild Improvement	No Improvement
1	Hypovolaemic shock	0.9% saline	**6**	2	1	-	1
2	Hypovolaemic shock	Packed red blood cells		2	1	-	1
3	Hypovolaemic shock	Ringers lactate		1	1	-	-
4	Hypovolaemic shock	Other (please specify)		1	1	-	-
5	Metabolic acidosis	0.9% saline	**20**	17	12	4	1
6	Metabolic acidosis	Ringers lactate		3	3	-	-
7	Elevated lactate	0.9% saline	**13**	10	10	-	-
8	Elevated lactate	Packed red blood cells		2	-	-	2
9	Elevated lactate	Ringers lactate		1	1	-	-
10	Septic shock	0.9% saline	**4**	3	2	1	-
11	Septic shock	Ringers lactate		1	-	1	-
12	Decreased urinary output	0.9% saline	**9**	8	7	1	-
13	Decreased urinary output	Packed red blood cells		1	1	-	-
14	Low blood pressure	0.9% saline	**56**	47	19	16	12
15	Low blood pressure	Packed red blood cells		3	2	-	1
16	Low blood pressure	Frozen plasma		1	1	-	-
17	Low blood pressure	Ringers lactate		1	1	-	-
18	Low blood pressure	Other (please specify)		4	2	1	1
19	Decreased perfusion	0.9% saline	**20**	18	11	6	1
20	Decreased perfusion	Packed red blood cells		1	1	-	-
21	Decreased perfusion	Ringers lactate		1	-	1	-
22	Blood loss/haemorrhage	0.9% saline	**9**	5	2	3	-
23	Blood loss/haemorrhage	Packed red blood cells		3	2	1	-
24	Blood loss/haemorrhage	Frozen plasma		1	-	-	1
25	Part of acute resuscitation	0.9% saline	**6**	3	-	2	1
26	Part of acute resuscitation	Packed red blood cells		2	1	-	1
27	Part of acute resuscitation	Ringers lactate		1	-	1	-
28	Echocardiography findings	0.9% saline	**6**	5	4	-	1
29	Echocardiography findings	Frozen plasma		1	-	-	1
30	Tachycardia	0.9% saline	**4**	3	3	-	-
31	Tachycardia	Other (please specify)		1	-	1	-
32	Other (please specify)	0.9% saline	**10**	8	7	1	-
33	Other (please specify)	Packed red blood cells		1	1	-	-
34	Other (please specify)	Frozen plasma		1	-	1	-

Published paper:

International, multicentre, observational study of fluid bolus therapy in neonates

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ORIGINAL ARTICLE

International, multicentre, observational study of fluid bolus therapy in neonates

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Aim: To assess the prevalence, types and indications for fluid bolus therapy in neonates with haemodynamic compromise.

Methods: This was a pragmatic, international, multicentre observational study in neonatal units across Australasia, Europe and North America with a predefined study period of 10–15 study days per participating neonatal unit between December 2015 and March 2017. Infants ≤28 days of age who received a fluid bolus for the management of haemodynamic compromise (≥10 mL/kg given at ≤6 h) were included.

Results: A total of 163 neonates received a bolus over 8479 eligible patient days in 41 neonatal units. Prevalence of fluid bolus therapy varied between centres from 0 to 28.6% of admitted neonates per day, with a pooled prevalence rate of 1.5% (95% confidence interval 1.1–1.9%). The most common fluid used was 0.9% sodium chloride (129/163; 79%), and the volume of fluid administered was most commonly 10 mL/kg (115/163; 71%) over a median of 30 min (interquartile range 20–60). The most frequent indications were hypotension (n = 56; 34%), poor perfusion (n = 20; 12%) and metabolic acidosis (n = 20; 12%). Minimal or no clinical improvement was reported by clinicians in 66 of 163 cases (40%).

Conclusions: Wide international variations in types, indications and effects of fluid bolus administration in haemodynamically compromised neonates suggest uncertainty in the risk–benefit profile. This is likely to reflect the lack of robust evidence to support the efficacy of different fluid types, doses and appropriate indications. Together, these highlight a need for further clinically relevant studies.

Key words: blood pressure; fluid; infant; newborn; therapy.

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Conflict of interest: None declared.

†Individual study site investigators/collaborators: Australia: Flinders Medical Centre: Scott Morris; Grace Centre for Newborn Care, Children's Hospital at Westmead: Kathryn Browning Carmo; John Hunter Children's Hospital: Koert De Waal, Michelle Stubbs; Mater Mothers' Hospital Brisbane: Helen Liley, Angela Pearson, Holly Campbell; The Royal Children's Hospital, Melbourne: Rodney W Hunt, Elizabeth Perkins; The Royal Hobart Hospital: Sanoj KM Ali; The Royal North Shore Hospital: Jennifer Bowen, Claire Jacobs, Mary Paradisis; The Royal Prince Alfred Hospital: David Osborn, Mark Greenhalgh; The Royal Women's Hospital: Carl Kuschel; Women's and Children's Hospital: Nicolette Hodyl, Michael Stark, Amy Keir. Canada: British Columbia Women's Hospital: Joseph Ting; Centre Hospitalier Universitaire Sainte-Justine: Keith Barrington, Anie Lapointe; Kingston General Hospital: Kimberly Dow, Helen Coo; McMaster Children's Hospital: Amit Mukerji; Mount Sinai Hospital: Adel Mohamed; Royal Columbian Hospital: Miroslav Stavel; St John's Newfoundland: Akhil Deshpandey, Nicole Tucker; Sunnybrook Health Sciences Centre: Eugene Ng, Carly Diamond. France: Hôpital de la Femme, de l'Enfant et de l'Adolescent (neonatal and paediatric intensive care units): Pierre Bourgoin, Lutz Bindl; Réanimation Pédiatrique Spécialisée Hospital Enfants: Matthieu Maria; South Paris University Hospitals, APHP and South Paris-Saclay University (Division of Pediatrics and Neonatal Critical Care, Medical Center "A.Beclere") (Prais, France): Daniele De Luca, Valentina Dell'Orto. Italy: Fondazione IRCCS Cà Granda Ospedale Maggiore Policlinico, Università degli Studi di Milano (Neonatal Intensive Care Unit, Department of Clinical Sciences and Community Health): Stefano Ghirardello. New Zealand: Christchurch Women's Hospital: Kiran More; Dunedin Hospital (South District Health Board): Liza Edmonds, Lauren Weaver. Sweden: Karolinska University Hospital in Danderyd, Karolinska University Hospital in Huddinge and Astrid Lindgren Children´s Hospital, Karolinska University Hospital: Emöke Deschmann and Mikael Norman; SUS Lund University Hospital: Owain Thomas, Jonathan Karlsson. Switzerland: Hôpitaux universitaires de Genève: Roberta De Luca; Ostschweizer Kinderspital: Bjarte Rogdo. Portugal: Hospital Pediátrico de Coimbra: Rita Moinho, Alexandra Dinis. United Kingdom: John Radcliffe Hospital: Dominic Wilkinson, Anshuman Paria. United States of America: Boston Children's Hospital: Martha Sola-Visner, Vanessa Young; Emory University Midtown, Grady Memorial Hospital and Center for Transfusion and Cellular Therapy, Pathology Department, Emory University: Cassandra D Josephson, Jane Skvarich; Levine Children's Hospital (Carolinas Medical Center): Matthew Saxonhouse, Rebecca Poliquin; University of Arkansas for Medical Sciences and Arkansas Children's Hospital: Sherry Courtney, Dalton Janssen; University of Vermont Medical Center: Sarah K Harm, Allison Bartlett; University of Washington Medical Center and Seattle Children's Hospital: Dennis Mayock, Gina Lee.

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What is already known on this topic

1 Fluid bolus therapy is used for the management of haemodynamic compromise in neonates.
2 The indications for, the type of fluids used and the short-term clinical outcomes of fluid bolus therapy are poorly understood.
3 There is increasing evidence in paediatric medicine that fluid bolus therapy may be either ineffective or harmful.

What this paper adds

1 Our study suggests that fluid bolus therapy remains a practice in preterm and term infants in neonatal units in highly resourced countries; the most common type of fluid used is 0.9% sodium chloride at 10 mL/kg over 30 min.
2 The most common indications for fluid bolus therapy in neonates are low blood pressure, decreased perfusion on clinical assessment and metabolic acidosis.
3 Current clinical trials in this area are focusing on the use of inotropes; however, fluid bolus therapy also warrants closer examination, and this study provides key data to develop interventional trials.

Intravenous fluid bolus therapy for suspected haemodynamic compromise in neonates with a variety of underlying conditions is a common intervention in neonatal units. Fluid boluses may include crystalloids such as 0.9% sodium chloride or colloids such as albumin or blood products, including plasma, which have different biochemical properties. While this therapy represents an established component of the management of haemodynamic compromise in neonates, the volume, type of fluid, timing and indications for this practice are not well described or understood.[1–3] A Cochrane review found no benefit from the use of early fluid bolus therapy in infants ≤32 weeks' gestation without haemodynamic compromise.[1] This review identified no available evidence to determine whether those with clear haemodynamic compromise might benefit from volume expansion compared to no volume expansion.[1] Another review, including two more recent studies not included in the previous meta-analysis, was again unable to establish any benefit from fluid bolus therapy in late preterm and term infants with signs of haemodynamic compromise.[3] There are well-documented concerns about the consequences of fluid bolus therapy in older children, but comparable data do not exist for neonates. It is possible that some fluid boluses provide no clinical benefit and may even cause harm.[4,5]

As a first step in evaluating and improving the use of fluid bolus therapy in clinical practice, we conducted a pragmatic, international, multicentre, observational study to explore existing practices of fluid bolus therapy. Our primary objective was to describe the prevalence, types, indications for and doses of fluid bolus therapy administered to neonates with suspected haemodynamic compromise. Secondary objectives were to determine variations in practice of fluid bolus therapy and evaluate the degree of perceived improvement post-fluid boluses.

Methods

Study design and setting

This study was an international, pragmatic, multicentre, observational study undertaken at 41 neonatal units in Australasia (n = 12), North America (n = 16) and Europe (n = 13). Units were recruited through neonatal research networks and specialty societies, as well as through personal communications from the main study investigators. Participating neonatal units collected data in blocks of five continuous days in two to three blocks for a minimum of 10 days and up to a maximum of 15 days per unit. This was a pragmatic decision, given limited study funding, to allow units to support a period of study data collection based, for example, on the availability of local research staff or individuals. Data collection occurred between December 2015 and March 2017.

Participants

Newborn infants of any gestation at birth who were ≤28 days of age and who received a fluid bolus for suspected haemodynamic compromise were included. Participants were identified by the individual study site co-investigators.

Exposure

The exposure of interest was a fluid bolus given for the purposes of intravascular volume expansion for suspected haemodynamic compromise. Fluids included were 0.9% sodium chloride, 0.45% sodium chloride, Ringer's lactate solution, albumin, frozen plasma and whole blood or red blood cells (RBCs). The fluid bolus had to be 10 mL/kg or a greater volume given over ≤6 h. Neonates who received bolus fluids for hypoglycaemia or RBC transfusions to manage anaemia of prematurity alone were excluded.

Variables

Demographic and clinical characteristics of included neonates and participating units were collected. We collected information on the type, volume and duration of bolus fluids administered. Information on indications for fluid boluses assigned according to predefined categories, including an 'other' category where site investigator was asked to define the indication, was collected. (See Appendix S1 (Supporting Information) for the data collection sheet.) The effects of fluid boluses on short-term perceived clinical outcomes at 4–6 h after administration were categorised according to a numerical score. These scores were based on clinician report 4–6 h post-bolus in four areas: (i) the reported degree

of improvement in the primary indication for fluid (no change = 0, some improvement = 1, large improvement = 2); (ii) the need for escalation of therapy to inotrope use (new agent started = 0, one or more agents increased *and* one or more agents decreased = 1 or no agent started, agent decreased or stopped = 2); (iii) additional fluids bolus(es) within 6 h of the first (more than 2 = 0, one additional bolus = 1, no additional bolus = 2); and (iv) whether another treatment, for example, sodium bicarbonate infusion or blood products, was received for the primary indication (yes = 0, no = 2). These scores were summed and classified as no or minor improvement (score 0–2), mild improvement (score 3–5) or major improvement (6–8). The scoring sheet is provided in Appendix S1 (Supporting Information) and was developed by expert consensus and a formal piloting process, including neonatologists, paediatric critical care and haematologists within the study group.

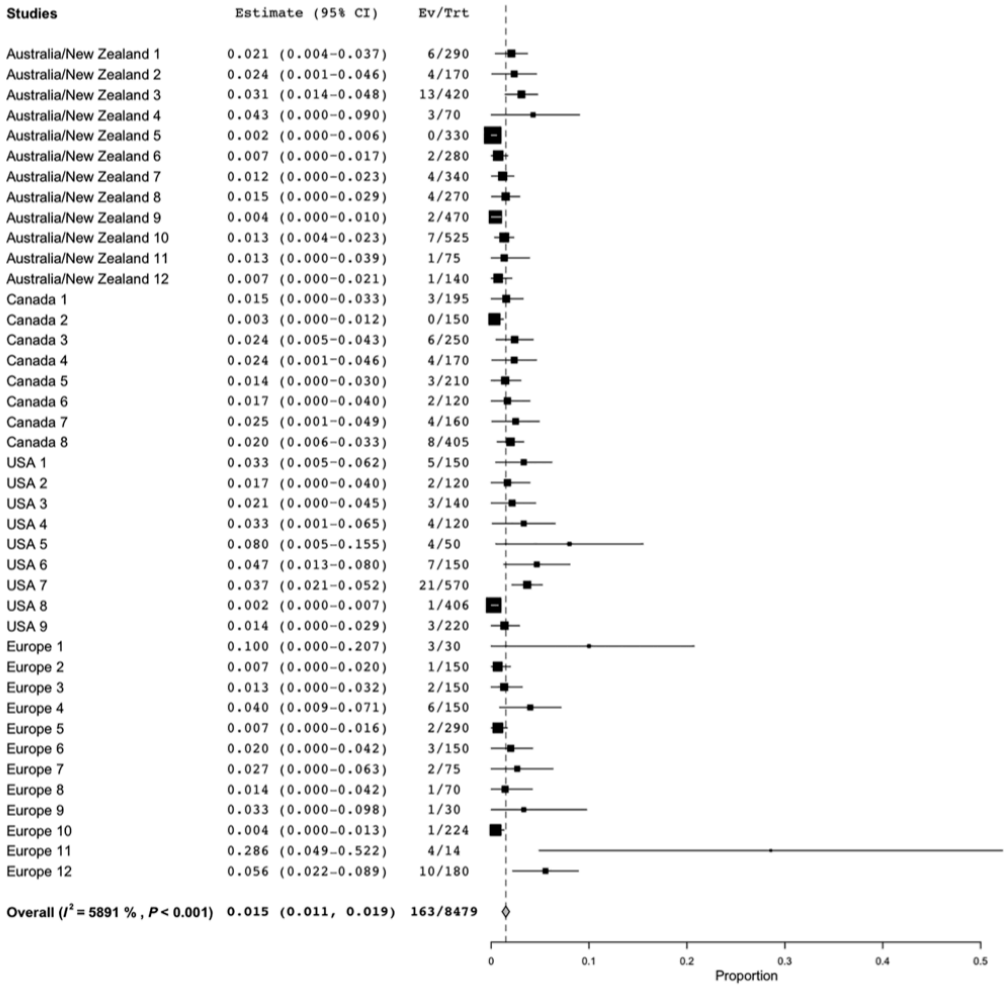

Fig. 1 Prevalence of fluid bolus therapy per admitted infant per study day. Horizontal lines represent the proportion of infants who received a fluid bolus divided by the number of potentially eligible infants during the study period. For example, for site 1:0.021 (95% CI 0.004–0.037) or 2.1% of potentially eligible infants received a fluid bolus during study.

Data management

Study data were collected and managed using the Research Electronic Data Capture (REDCap) tools hosted at the University of Adelaide, Australia.[6] REDCap is a secure, web-based application designed to support data capture for research studies, providing (i) an interface for validated data entry; (ii) audit trails for tracking data manipulation and export procedures; (iii) automated export procedures for data downloads to common statistical packages; and (iv) procedures for importing data from external sources.

Data sources

Descriptive data on unit characteristics were collected by individual study site co-ordinators, including type of unit, country, number of neonates admitted per year, availability of unit guidelines for fluid bolus and/or RBC transfusion.

Sample size

All infants in each participating institution who received at least one fluid bolus during the site collection period were included in the study. A sample of 41 units agreed to participate. Each patient was enrolled only once for the first bolus received during the study interval even if he or she received further boluses on a subsequent study day.

Statistical analysis

Normally distributed data were described by the mean and standard deviation and non-normally distributed data using the median and interquartile range (IQR). Analyses were carried out using R statistical software package (R, version 3.1.0; R Foundation for Statistical Computing, Vienna, Austria) unless otherwise specified. The prevalence rate for the receipt of bolus was calculated by dividing the number of neonates who received a bolus by the number of neonates who were present in the unit during the study interval who were ≤28 days of age. Each neonate on a given day was considered to be eligible to receive a bolus until the study period ended. Pooled prevalence rates and 95% confidence intervals (CI) were calculated using Der-simonian random-effects model with open-access Meta-analyst software.[7]

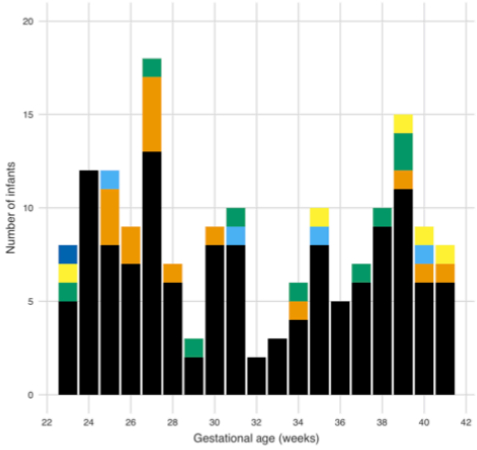

Fig. 2 Distribution of gestational age at birth of included infants. Type of fluid bolus: (■), 0.9% sodium chloride; (■), packed red blood cells; (■), frozen plasma; (■), Ringer's lactate; (■), 5 or 20% albumin; (■), 0.45% sodium chloride.

Table 1 Primary indication for fluid bolus therapy (n = 163) and clinically perceived scores post-fluid bolus administration

Main indication	Frequency, *n* (%)	No to minor improvement (score 0–2), *n* (%)	Mild improvement (score 3–5), *n* (%)	Major improvement (score 6–8), *n* (%)
Low blood pressure	56 (34)	14 (25)	17 (30)	25 (45)
Decreased perfusion on clinical assessment	20 (12)	1 (5)	7 (35)	12 (60)
Metabolic acidosis	20 (12)	1 (5)	4 (20)	15 (75)
Elevated lactate	13 (8)	2 (15)	0 (0)	11 (85)
Decreased urinary output	9 (6)	0 (0)	1 (11)	8 (89)
Blood loss/Haemorrhage	9 (6)	1 (11)	4 (44)	4 (44)
Hypovolemic shock	6 (4)	2 (33)	0 (0)	4 (67)
Echocardiography findings (decreased cardiac output)	6 (4)	2 (33)	0 (0)	4 (67)
Part of acute resuscitation in an arrested (or peri-arrest) infant	6 (4)	2 (33)	3 (50)	1 (17)
Tachycardia	4 (3)	0 (0)	1 (25)	3 (75)
Septic shock	4 (3)	0 (0)	2 (50)	2 (50)
Other†	10 (6)	0 (0)	2 (20)	8 (80)

†Other: Volume replacement for gastric aspirate or urinary losses, polycythaemia, dehydration and renal impairment, hypovolemia, anaemia, hyperbilirubinaemia, gastroschisis (unclear whether this was routine for this unit for this diagnosis or was for another reason, such as replacement of losses or to improve perfusion).

Table 2 Indication for fluid bolus (n = 163) and type of fluid used

	0.9% sodium chloride, *n*	Packed red blood cells, *n*	Frozen plasma, *n*	Ringer's lactate, *n*	Other, *n*†
Low blood pressure	47	3	1	1	4
Decreased perfusion on clinical assessment	18	1	—	1	—
Metabolic acidosis	17	—	—	3	—
Elevated lactate	10	2	—	1	—
Decreased urinary output	8	1	—	—	—
Blood loss/Haemorrhage	5	3	1	—	—
Hypovolemic shock	2	2	—	1	1
Echocardiography findings (decreased cardiac output)	5	—	1	—	—
Part of acute resuscitation in an arrested (or peri-arrest) infant	3	2	—	1	—
Tachycardia	3	—	—	—	1
Septic shock	3	—	—	1	—
Other‡	8	1	1	—	—

†Other: 4% albumin, 5% albumin, 0.45% sodium chloride. ‡Other: Volume replacement for gastric aspirate or urinary losses, polycythaemia, dehydration and renal impairment, hypovolemia, anaemia, hyperbilirubinaemia, gastroschisis (unclear whether this was routine for this unit for this diagnosis or was for another reason, such as replacement of losses or to improve perfusion).

Ethics approval

Site-specific ethics approval was obtained for all sites. Two centres in Canada required individual written consent prior to collection of clinical data. French and Swiss sites had an opt-out strategy, with information provided to families in the units' waiting rooms. All other ethics committees waived the requirement for individual consent given that all data were routinely collected for clinical purposes, and no individual identifying data would be recorded and sent to the lead site.

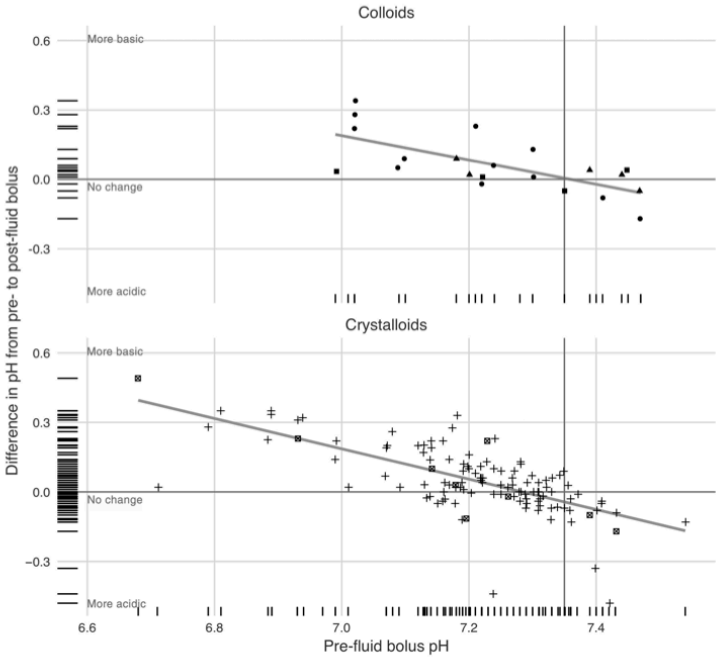

Fig. 3 Differences in acid–base (pH) from pre- to post-fluid bolus. Solid grey lines represent the linear regression change in pH compared to initial pH level. The horizontal axis is the starting value, and the vertical axis is the change over time. The trend line (solid grey line) indicates that the further away from 'normal' the value starts, the bigger the change (closer to normal). This may due to the fluid bolus (or other factors) or other factors such as sampling and a regression to the mean. (●), Packed red blood cells; (▲), 4 or 5% albumin; (■), frozen plasma; (+) 0.9% saline; (┼), Ringer's lactate.

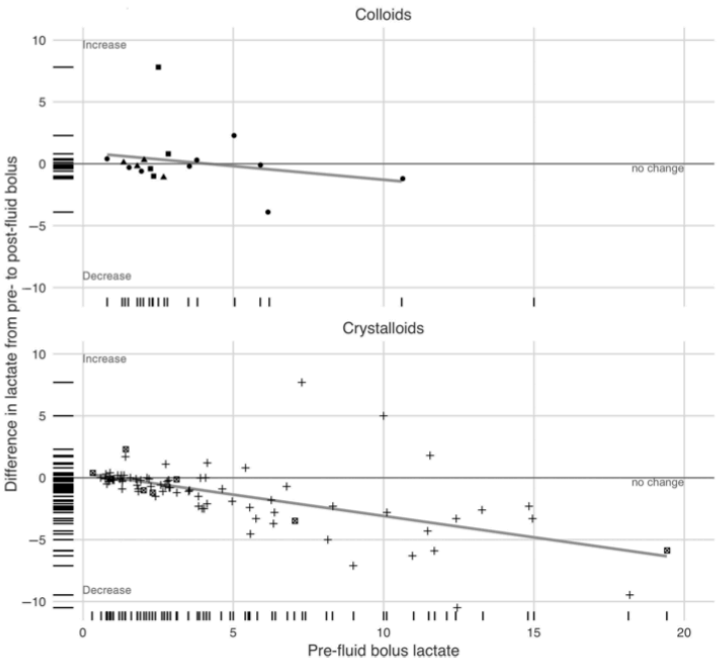

Fig. 4 Difference in lactate level from pre- to post-fluid bolus. Solid grey lines represent the linear regression change in lactate compared to initial lactate level. The horizontal axis is the starting value, and the vertical axis is the change over time. The trend line (solid grey line) indicates that the further away from 'normal' the value starts, the bigger the change (closer to normal). This may due to the fluid bolus (or other factors) or other factors such as sampling and a regression to the mean. (●), Packed red blood cells; (▲), 4 or 5% albumin; (■), frozen plasma; (+) 0.9% saline; (⊠), Ringer's lactate.

Results

Participating centres

Forty-one units participated in the study. Ten (24%) were in Australia, eight (20%) in Canada, four (10%) in France, one (2%) in Italy, two (5%) in New Zealand, one (2%) in Portugal, four (10%) in Sweden, two (5%) in Switzerland, one (2%) in the UK and eight (20%) in the USA. Median numbers of admissions per unit per year were 650 (IQR 420–1836). Twenty-two (55%) units were classified as general perinatal centres, 16 (39%) were surgical units including cardiac and three (7.5%) were mixed (neonatal and paediatric intensive care) units.

Patient characteristics

A total of 163 neonates received a bolus over 8479 eligible patient days. The pooled prevalence rate of the receipt of fluid bolus was 1.5% (95% CI 1.1–1.9%) across all participating units. The prevalence of bolus administration in participating units varied from 0 to 28.6% of admitted neonates (≤28 days of age) per day. Data for individual units, grouped by geographical regional area, are provided in Figure 1.

For included infants, the birth gestation of included infants reflected a bimodal distribution, with peaks at 27 and 39 weeks, as did birthweight, with peaks at 650–850 g and 2850–3050 g (Fig. 2). The majority of neonates received their first fluid bolus on the day of birth (87/163; 53%), and there was diminishing likelihood of a first fluid bolus on subsequent days; day 2 (24/163; 15%), days 3–7 (25/163; 15%) and >7 days (27/163; 17%). The reported primary indications for fluid bolus therapy are provided in Table 1.

Clinical guideline availability

Local clinical practice guidelines, which referenced fluid bolus therapy, were available in only 10 (24%) of the participating units.

Fluid bolus characteristics

Types of fluid used for fluid bolus therapy included 0.9% sodium chloride (n = 129; 79%), RBCs (n = 15; 9%), 4 or 5% albumin (n = 5; 3%), Ringer's lactate (n = 9; 5%), frozen plasma (n = 4; 3%) and 0.45% sodium chloride (n = 1; <1%). The most common volume administered was 10 mL/kg (n = 115; 67%), with a median duration of administration of 30 (IQR 20–60) min. Table 2 shows a breakdown of the indication for each fluid bolus and type of fluid used.

Short-term outcomes

Mortality

At the end of the data collection period, 151 of 163 (93%) infants were alive. None of the infants died during the receipt of the fluid bolus or within 6 h post-bolus.

Clinician-perceived improvement

Clinicians perceived no or minor improvement (score 0–2) in 25 of 163 (15%), a mild improvement (score 3–5) in 41 of 163 (25%) and a major improvement in 97 of 163 (60%) in response to bolus therapy. Improvement according to primary indication is reported in Table 1. Table S1 (Supporting Information) provides a further breakdown of indication for fluid bolus, type of fluid used and clinical improvement scores.

Laboratory indices

The following changes in laboratory parameters were observed following fluid bolus: pH 0.03 units (IQR −0.03 to 0.12 units; $n = 140$) (Fig. 3); lactate −0.59 mmol/L (−2.15 to 0.02 mmol/L; $n = 100$) (Fig. 4); bicarbonate 0 mmol/L (−1.35 to 2.00 mmol/L; $n = 139$); chloride 0.5 mmol/L (−1.00 to 3.00 mmol/L; $n = 80$); base deficit −1.10 mmol/L (−3.93 to 1.00 mmol/L; $n = 128$); and haemoglobin −5.00 g/L (−16.00 to 9.25 g/L; $n = 88$).

Variations in prevalence of fluid bolus therapy

Regions

The pooled prevalence rates for fluid bolus therapy in Australian and New Zealand units ($n = 12$) was 1.2% (95% CI 0.6–1.7%); in Canadian units ($n = 8$), it was 1.5% (95% CI 0.8–2.1%); in US-based units ($n = 9$), it was 1.8% (95% CI 0.8–2.8%); and in European units ($n = 12$), it was 2.7% (95% CI 1.1–4.4%) (Fig. 1).

Types of centre

The pooled prevalence for fluid bolus therapy within general perinatal centres ($n = 22$) was 1.3% (95% CI 0.9–1.8%), and within the remaining centres (surgical and mixed units) ($n = 19$), it was 1.9% (95% CI 1.2–2.6%). The centre with the highest prevalence rate was a non-perinatal unit caring primarily for paediatric patients.

Discussion

This international study explored the prevalence, types and indications of fluid bolus therapy in neonates with haemodynamic compromise. This was a pragmatic study aimed at trying to better define the current practices of fluid bolus therapy and, as such, was developed with the need to be very restrictive on the amount of data collection. While the pooled prevalence rate was low, the prevalence of this therapy varied (0–28.6%). We identified variations in the nominated indications for and frequency of use of fluid boluses between participating units. Overall, perceived improvement following fluid bolus therapy was reported in 85% of cases. Together, these results highlight a clear lack of consistent clinical approach and perceptions of variable effects.

The interpretation of our pragmatic study needs to recognise strengths and limitations. Our study was supported by a large number of units across many different countries. It describes practices in units that were selected by personal approaches by the investigators, but we cannot assume that they are representative of non-participating neonatal units and other countries. Participation was voluntary, and units selected the most convenient time to support data collection. The calculation of incidence was based on the assumption that the prevalence of fluid boluses over the short study intervals was constant and representative of standard practice in each unit. This assumption may not be true. However, variations from the reported rate could be on either side of the estimated rate, and thus, overall, the averaged results could be considered representative. In an attempt to maximise unit participation, data collection was kept to a minimum, and therefore, several outcomes of potential interest were not requested, for example, blood pressure. In addition, it was not possible to collect detailed information on potential adverse effects related to fluid bolus beyond 6 h, such as volume overload, dilutional coagulopathy, hypothermia and electrolyte disturbances.[4,5] This lack of data extends to other specific fluid-related complications, including transfusion reactions[8,9] or 0.9% sodium chloride-induced hypochloremic metabolic acidosis, although we did not observe any significant increase in chloride-level post-fluid bolus. We did not observe any significant changes in measured laboratory indices post-bolus. Reported outcomes post-bolus were described by the treating clinicians, and as the prescriber of the treatment, they may have preferred to perceive an improvement. One further point is that, as the majority of fluid boluses were administered at day one of age, factors such as post-natal age may also have a significant effect on the parameters of physiological responses to fluids.

Published studies evaluating fluid bolus therapy in neonates are heterogeneous and have not always included neonates with signs of haemodynamic compromise.[10–14] There are no randomised studies primarily designed to examine fluid bolus compared to no fluid bolus in preterm infants with haemodynamic compromise.[15] Studies in late preterm and term infants with haemodynamic compromise are limited to non-randomised observational studies and do not report clinical benefit.[16,17] A survey in Canada reported that, while attitudes to the use of inotropes varied, neonatologists routinely treated suspected haemodynamic compromise in infants with a birthweight <1500 g with a fluid bolus (97%) and most commonly used 0.9% sodium chloride (95%).[18] Our results are consistent with this, with the majority of fluid boluses (47/56; 84%) given to an infant to manage low blood pressure being 0.9% sodium chloride.

Only 10 of the units participating in this study had local clinical guidelines available to guide fluid bolus use. Use of clinical guidelines, even in areas with a limited evidence base, may reduce variation in practice.[19] Nevertheless, consensus is only helpful to patients if it is the right consensus, and the lack of intervention studies defining optimal fluid bolus therapy, such as indication, type, volume and rate, in preterm and term infants makes this not possible at this time. Clinicians are left to either extrapolate data from other patient groups, some now showing potential harmful effects from fluid bolus therapy in children (e.g. Fluid Expansion as Supporting Therapy (FEAST) study[20]), or rely on limited and potentially misleading physiological data to guide decisions. Interestingly, there was little regional variation observed in the use of fluid bolus therapy.

Conclusions

With ongoing trials examining the use of inotropes in this group of infants (http://www.neocirculation.eu and http://www.hip-trial.com), our study suggests the need for research to evaluate fluid bolus therapy. Further studies may need to explore whether

infants who are more acidotic, or with higher lactate, benefit from fluid bolus therapy as might be suggested by our findings. Research needs to consider the choice of fluid as well as dose and timing. The most common fluid bolus type in our study was 0.9% sodium chloride, although this fluid is non-physiological, and concerns have been raised about the chloride load,[21] which may be more important in preterm infants with less mature renal function. New studies should apply clear consensus outcomes of haemodynamic compromise and optimal monitoring,[22] for example, development of a core outcome set.[23] As the FEAST trial demonstrated in children,[20] our assumptions around the potential benefits of fluid bolus therapy in neonates may need careful reconsideration.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Appendix S1. Data collection sheet.
Table S1. Fluid bolus, type of fluid used and clinical improvement scores.

Chapter 7

Final conclusions and future directions

This thesis included a review of contemporary neonatal transfusion practice, identification of adverse effects related to neonatal RBC transfusion through systematic identification of relevant published studies and a review of whether changes in blood processing prior to neonatal RBC transfusion, through washing with 0.9% sodium chloride, improves clinical outcomes. It also included an in-vitro study to determine whether it is potentially safe to co-infuse dextrose-containing fluids with RBCs and lastly, it provided a contemporary description of clinical practice in relation to the types and specific indications for use of fluid therapy, including blood products, in neonates with suspected haemodynamic compromise. All these studies are key to improving the understanding of and improving clinical practice of transfusion and fluid bolus therapy in neonates.

Research question 1:

What are the current usage patterns of blood products in neonatal units?

In Chapter 2, a retrospective cohort study of preterm neonates born at less than 30 weeks' gestation and admitted to participating neonatal intensive care units in the Canadian Neonatal Network from 2004 to 2012 was conducted to evaluate blood product usage. It found blood product use remains at a very high frequency in preterm neonates born at less than 30 weeks' gestation. Three time epochs were examined and compared, revealing a trend toward fewer RBC transfusions among neonates born at 26-29 weeks' gestation. Use remained unchanged or increased for neonates born at 23-25 weeks' gestation.

Significance and contribution to knowledge

This study provided contemporary data on usage patterns of blood products in neonatal units. It is the first report describing patterns of use of RBC transfusion and the temporal trends across extremely low gestational ages. It may be that evolutionary practice changes and a relative high tolerance for anaemia may be associated with a reduction in RBC usage in recent years in neonates born at least 26 weeks' gestation. This contrasts with the ongoing higher usage of blood products observed at extremely low gestational ages.

Up to 82% of neonates with a birth-weight less than 1000 grams received at least one RBC transfusion in this study. The results are similar to those of the study by Maier *et al*[112], who

examined transfusion practices in the 1990s. Ringer *et al*[113] reported that 65% to 87% of neonates with a birthweight of less than 1500 g received RBC transfusions, whereas in this study the range was 25% to 82%.

A UK national audit of RBC use in neonates and children[114] found that for the first transfusion episode for neonates on neonatal units, the median (IQR) gestational age at birth was 27 (26-30) weeks, n=1194, and the majority (81%; 971) of the transfusions were given to infants born at <32 weeks' gestational age. Most first RBC transfusions were given for anaemia, with (60%) or without (21%) symptoms. The majority of infants (75%) were either mechanically ventilated or on continuous positive airway pressure (CPAP) at the time of the transfusion.

The ongoing high rates of RBC transfusions in infants born at less than 30 weeks' gestation likely reflects the ongoing variations in transfusion practice reported by other research groups.[113] It is also likely that the higher transfusion requirement of such preterm neonates is a reflection of the increasingly active management and survival of neonates at the lowest gestational ages. Within the Canadian Neonatal Network the survival of infants born at 23 weeks' gestation has increased from 0% to 10% in early 2003 to 30% to 40% in 2012. As this study is based on Canadian Neonatal Network data, which includes >90% of all infants admitted to neonatal intensive care units in Canada, it is likely highly reflective of true Canadian neonatal transfusion practice. Unfortunately, similar data is not available at a bi-national level in Australia and New Zealand to allow further comparisons.

Challenges and insights

This study was not able to provide information regarding specific indications for transfusions; changes in institutional transfusion guidelines and adverse transfusion reactions are not part of the data collection process for the network. A prospective observational study, through an international collaboration of multiple centres or networks, is needed to gain a true understanding of blood product usage in the NICU, indications for use, potential adverse effects of blood product administration, and any associations with benefits and risks. Collaboration across countries and research networks is needed to further understand the benefits and risk of transfusion in this vulnerable patient group. Another approach, and perhaps a necessary one, due under-reporting that may occur in database studies[115, 116] may be to develop minimal data sets for blood banks to collect at the time of blood product release.

Building an evidence base for neonatal transfusion practices needs to start with a clear understanding use of blood products in neonatal units and the included study is one step towards this. The study highlighted the need for up to date information regarding neonatal transfusion practices.

Conclusions and future directions

Without accurate knowledge blood product usage in neonatal units around the world, it will be challenging to design relevant clinical studies. Development of larger epidemiological data sets of blood product use in neonates are needed to support the ongoing development of quality improvement activities and clinical studies. Multi-network international research collaborations would be an ideal approach to allow for gathering of these large data sets to guide research in this area.

Research question 2:

What are the current known adverse effects and associations of neonatal RBC transfusions?

Controversy exists regarding the contribution of RBC transfusions to a range of adverse clinical outcomes in neonates. Neonates are particularly vulnerable transfusion recipients with concerns over infective and toxic risks as well as the potential for acute side-effects due to their small blood volume.[117] Neonatal adverse reactions, in particular, may be non-specific and appear to be a worsening of their current clinical state, for example, worsening hypoxia in an extremely preterm infant with chronic lung disease receiving a red cell transfusion for anaemia of prematurity.

The aim of this Chapter was to provide a broad synopsis of all reported risks to better understand the clinical adverse effects and associations attributed to neonatal RBC transfusions. This was done through a systematic review and meta-analysis of the literature. Studies were classified into two groups, in which there was a difference in transfusion numbers and/or volume between groups to compare liberal versus restrictive RBC transfusion practices. Liberal transfusion practice was defined as one group receiving a greater volume and/or number of RBC transfusions compared with the comparison group (restrictive transfusion practice). This allowed a comparison between the outcomes of infants who were exposed to restrictive or liberal RBC transfusion practice.

Significance and contribution to knowledge

The review did not find any differences in clinical outcomes between restrictive and liberal neonatal RBC transfusion practices. Meta-analyses of studies that included a comparator group did not identify any consistent differences in mortality during initial hospitalisation, CLD, NEC, IVH, and bacterial contamination/ sepsis between neonates exposed to higher or lower volumes of RBC transfusions, in either randomised or nonrandomised studies. These findings are contrary to current opinion about the risks of transfusion in the neonatal population.[118, 119] This was achieved by looking across the broader literature, through a systematic review and meta-analysis of the available literature. Without this process, it would have been challenging to draw these conclusions.

Challenges and insights

The limitations of much of the primary study evidence, which the review is based on, needs to be acknowledged. The current findings do not advocate for the safety of either liberal or restrictive transfusion triggers but reiterate the importance of further research. The findings from this review specifically highlight the 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. A continued focus on retrospective studies that report potential associations between RBC transfusion and the development of NEC, a devastating but rare disease, may have diverted attention from higher quality study designs to establish the real risks of neonatal transfusion. In addition, very few studies included in the review provided clear definitions of the different potential adverse effects related to RBC transfusion. The standardisation of definitions of adverse effects and associations of RBC transfusion in neonates, through an international consensus, is required.

Another key limitation to this work is the challenges establishing a cause-effect relationship in observed data. According to the Bradford-Hill criteria[120] to establish whether an observed association is likely to be casual a change in disease rates should follow from corresponding changes in exposure (dose-response). Therefore, if infants receive more RBC transfusions, then if a cause-effect relationship exists between receipt of RBC transfusion and neonatal morbidities/mortalities, then they should be more likely to develop these

morbidities/mortality. The most important limitation to this argument is that the sicker the infant is, the more likely he or she is to receive RBC transfusions and the more likely he or she is to develop neonatal morbidities/mortality. Importantly, our meta-analyses of studies that included a comparator group did not identify any consistent differences in mortality during initial hospitalisation, CLD, NEC, IVH, and bacterial contamination/ sepsis between neonates exposed to higher or lower volumes of RBC transfusions, in either randomised or nonrandomised studies. The studies included in our review did all report a difference in number and/or volume of transfused RBCs between study arms (if a comparator was available). It is acknowledged that for some studies, such as PINT study, that the difference was not necessarily great but it was present in regards to differences in overall volumes/or number of transfusions between study groups.

The Serious Hazards of Transfusion (SHOT) haemovigilance scheme has reviewed reports of adverse reactions and errors associated with transfusions in the UK since 1996 (www.shotuk.org). SHOT analyses reports according to pre-defined categories of errors in selection, handling and administration of blood, and of adverse reactions including rare reports of transfusion-associated graft vs host disease (TAGvHD) and transfusion-transmitted infections. The first nine years of paediatric SHOT data were analysed by Stainsby *et al*[52] showing a disproportionate number of adverse outcomes of transfusion in children, in particular infants, compared with adults, largely due to transfusion of the 'incorrect blood component.' The majority of paediatric SHOT adverse event reports are 'errors' with 68% in 2017.[121] This particularly is the case for the neonatal/infant group with 89% 'errors' in 2017, possibly to due to under-recognition or more subtle signs of reactions in the neonatal group or fewer reactions due to immunological immaturity. Other cases of adverse reactions following paediatric transfusion are reported, including 'confirmed' paediatric reports of transfusion-related acute lung injury (TRALI) and transfusion-associated circulatory overload (TACO) and transfusion-associated necrotising enterocolitis (TA-NEC) have been reported. Transfusion-related acute lung injury and TACO continue to be occasionally described as case reports in the broader literature in the neonatal and paediatric populations.[122-124] Transfusion-transmitted infections are uncommon, although in the neonatal and paediatric literature, some of these infectious complications include parasites.[125, 126] Haemolytic transfusion reactions and alloimmunisation as a consequence of RBC transfusion are less common in neonates, which may reflect relative immunological immaturity.[127] Finally, reports of paediatric adverse events to SHOT have also highlighted specific areas of risk in

complex specialised situations including fatal TAGvHD following intrauterine transfusion with maternal blood, morbidity and delays to neonatal exchange transfusions, and unusually high supernatant potassium levels in RBCs from donors with a mutation which increases potassium leakage during cold storage.[128] In 2015, key messages from SHOT related to neonates and children included noting of an increased reporting of TA-NEC and use of adult emergency O D-negative RBCs despite availability of neonatal emergency packs.[129]

Neonatal adverse transfusion reactions remain particularly poorly characterised. They may be difficult to distinguish from non-specific changes or a worsening of concurrent clinical morbidities such as hypoxia, apnoeic episodes, requirement for increased respiratory support, rash or fever. Due to their relatively small blood volumes and the tendency to transfuse top-up transfusions of up to 20mL/kg, a transfusion can comprise a significant fluid and electrolyte shift. Neonates are at risk of metabolic complications such as hypocalcaemia, hyperkalaemia, hypothermia and overload conditions if large volume transfusions are given rapidly and there is insufficient monitoring. Oxygen requirements and the degree of respiratory support are important indicators that guide RBC transfusions in neonates. Yet worsening hypoxia, apnoea or increased respiratory requirements in an extremely preterm infant with CLD receiving a RBC transfusion for AOP, may be the earliest indicators of an adverse transfusion reaction and be unrecognised and unreported as an transfusion adverse event.

Conclusions and future directions

There is a need for further larger studies with clear definitions of adverse events to be conducted prospectively, so that uncertainty about the safety of neonatal transfusion can be addressed. The review highlighted a continued focus on non-randomised and observational studies that report potential associations between RBC transfusion and adverse neonatal clinical outcomes, such as NEC. This, in turn, may have diverted attention from higher quality study designs to establish the real risks of neonatal transfusion. Multi-centre international research collaborations are required to definitively determine the risk of RBC transfusion in neonates. Before this occurs, standardisation of definitions of adverse effects and associations of RBC transfusion in neonates, through an international consensus, is required. This work is currently underway through the International Society of Blood Transfusion (ISBT) Clinical Transfusion working party group, of which I am a member and am leading this project. Further work may also be possible as practices such as deferred cord

clamping are likely to become more common,[132] making it possible to compare groups of infants that may avoid RBC transfusion, or are transfused less, than in the past would have been transfused.

Research question 3:

Does washing RBCs prior to transfusion in neonates prevent morbidity and mortality?

In Chapter 4, a systematic review and meta-analysis of a method to make RBC transfusions potentially safer through pre-transfusion washing of RBCs with 0.9% sodium chloride. The review found only one study that evaluated the effects of washing blood cells before transfusion in preterm infants. The outcomes the study reported that were relevant to the review were mortality, duration of mechanical ventilation, and length of initial hospitalisation. The results for all these outcomes were very uncertain. Washing RBCs with 0.9% sodium chloride prior to transfusion might be helpful or harmful, but the review was unable to make a clear determination.

Significance and contribution to knowledge

No evidence was found that either support or refute washing RBCs with 0.9% sodium chloride prior to transfusion to prevent morbidity or mortality in preterm infants. The review highlighted the gap in research knowledge in this area.

Challenges and insights

The primary challenge for this review was the lack of primary studies to inform its conclusions. This stresses the need for additional primary studies in this area. When designing a future study to determine whether washing RBCs prior to transfusion benefits preterm infants or not, a randomised, multicentre, controlled trial design would be ideal.

Conclusions and future directions

The findings of this review support the development of a randomised study examining whether or not pre-transfusion washing of RBCs prior to neonatal transfusion improves clinical outcomes.[133]

Research question 4:

Is it safe to co-infuse dextrose-containing fluids and RBCs?

Current transfusion guidelines recommended against co-infusion of RBCs with intravenous solutions except for 0.9% sodium chloride. This study found that it may be permissible to co-infuse particular dextrose-containing RBCs in the neonatal unit. By utilising RBCs that reflect common transfusion practice throughout neonatal units worldwide, including use of the most common age of RBC transfused and irradiation before use, this study addressed many of the methodologic concerns with prior studies.[69] Phase 2 of the study addressed one of the slowest infusion rates used for RBC transfusions in the neonatal unit further addressing criticism of earlier studies.

Significance and contribution to knowledge

This study found that certain in-vitro characteristics of RBC co-infused with 0.9% sodium chloride or 10% dextrose were not adversely effected. The study suggested that it may be permissible to allow for co-infusion of RBC and 10% dextrose infusions. However, a change in practice, at this point, would be based on in-vitro data alone, which has occurred, omitting an randomised controlled trial.

Challenges and insights

Based on the in-vitro data provided by the study, a decision was made at both the Hospital for Sick Children and Sunnybrook Health Centre in Toronto, Canada, to allow for co-infusion of RBC and 10% dextrose intravenous fluid. The practice also occurs at the Women's and Children's Hospital, Adelaide, Australia. Since its introduction at these three sites, no adverse effects have been observed attributable to this change in practice.

Conclusions and future directions

Ideally, a randomised controlled trial in this area ideally would have been undertaken to determine the safest route for infusion of both RBCs and dextrose-containing, through either two separate IV access sites or by co-infusion. The study findings suggest 10% dextrose may be an acceptable fluid to test as a co-infusate in the in-vivo setting as it appeared to minimally effect the RBCs in Part 2 of the study. If this is not feasible, then formal audit, including monitoring for adverse effects, should occur prior to ongoing and wider dissemination of this practice change.

Research question 5:

What are the types, doses, indications and short-term outcomes of fluid bolus therapy in neonates?

This study represents the first multi-centre international cross sectional observation of fluid bolus therapy for the management of suspected haemodynamic compromise in neonatal units. The study found fluid boluses are administered to 1-2% of neonates in highly resourced countries. The most common type of fluid used was 0.9% sodium chloride, the most common dose 10mL/kg and most common infusion time was over 30 minutes. The most frequent indication was low blood pressure, followed by decreased perfusion on clinical assessment, then metabolic acidosis, and an elevated lactate level.

This study was an international, multi-centre, cross-sectional study undertaken at 41 neonatal units in Australasia, North America and Europe. Units were recruited through neonatal research networks and specialty societies, as well as through personal communications directed by the main study investigators. Participating neonatal units collected data in blocks of 5 continuous days in 2-3 blocks for a minimum of 10 days and up to a maximum of 15 days per unit.

Significance and contribution to knowledge

The NeoBolus study demonstrated it is feasible for neonatal units around the world to collaborate successfully and provide data around a common clinical practice. By collecting data in the blocks of 10-15 days across a large number of units, it allowed for a greater representation of clinical practice. The information provided by this study characterised a level of uncertainty about this clinical practice, which will be helpful to refer to when preparing for further studies in this area.

Challenges and insights

While only a small proportion of newborn infants receive fluid bolus therapy in the neonatal period, this study highlights variations in incidence and reasons for fluid administration in different units, and uncertainties in outcomes. There were a number of identified challenges with this study. It described practices in units that were approached by the investigators and agreed to participate. These units were derived from a widespread geographical area, allowing the examination of practice from an international perspective. However, since they were not randomly selected and it cannot be assumed that they are representative of non-participating neonatal units and other countries. The calculation of incidence was based on

the assumption that the prevalence of fluid boluses over the short study intervals was constant and representative of standard practice in each unit. Detailed information on potential adverse effects related to fluid bolus beyond the six hours of data collection were not gathered. In addition, outcomes post-bolus was made by the treating clinicians, and as the prescriber of the treatment, they may have preferred to perceive an improvement. The assessment tool used to report clinical outcomes was not rigorously piloted and may have also biased the results towards a perception of improved outcomes.

With the assessment of haemodynamic compromise currently relying on a variation of clinical signs, echocardiographic findings or abnormal laboratory results,[110] it is also time for the development of consensus definitions in this area. Although this will be challenging, even the development of basic consensus definitions and a core outcome set[111] will be an invaluable step towards improving the evidence-base and study design in this area.

Conclusions and future directions

Further research is required to establish whether different types of fluid and dose leads to clinical benefit for different indications. These studies need to be conducted in patient populations meeting clear consensus definitions of hemodynamic compromise.

The ongoing use of fluid bolus therapy in neonates, as described by this study, implies that many healthcare professionals assume fluid bolus therapy is beneficial to both preterm and term infants. There were wide variations in rate and reasons for fluid administration in the different units throughout the study period. Given the lack of evidence of effectiveness and additional concerns about harm, these variations are concerning. Our study reported that 85% of cases where a fluid bolus was given, it was perceived by the prescribing clinicians to have had some clinical benefit. This is in contrast to the Cochrane review that found no evidence from randomised trials to support the routine use of early volume expansion in preterm infants without cardiovascular compromise. It also found insufficient evidence to determine whether infants with cardiovascular compromise benefit from volume expansion.[90] This review, however, only included studies with infants less than or equal to 32 weeks' gestational age and/or less than or equal to 1500 grams.

Our study supports the need for multi-centre international research collaborations. The first steps towards this are the development on international consensus of definitions of

haemodynamic compromise and a core outcome set. Through the network formed through this study, a consensus definition will be formed using established consensus methods (Delphi approach) as well as a core outcome set.

Overall significance of the work and contribution to knowledge

This thesis has contributed to improving the evidence-base in neonatal transfusion practice and fluid bolus therapy. This occurred through the provision of epidemiological data on blood product use, collation of reported adverse effects and associations of neonatal RBC transfusions, examination of whether pre-transfusion washing of RBCs impacts on neonatal morbidity and mortality, determination of the potential safety of co-infusion of RBC and dextrose-containing fluids, and finally, provision of novel data on fluid bolus use, including blood products, in neonatal units. The individual contributions of each study are outlined in the previous section of this Chapter.

Future directions

Based on the research findings presented in this thesis, the following are proposed:

1. **Expansion of the Australian and New Zealand Neonatal Network (ANZNN) data, formation of a neonatal research network and establishing links with blood services data and other research networks**

Well-designed clinical studies are essential to resolve clinical uncertainty, such as that reflected in the NeoBolus study findings. Such trials would require effective recruitment and data collection beyond what was gathered during the NeoBolus study. Point-of-care study designs using routinely collected data, short patient information sheets and opt-out consent for comparative effectiveness research are likely to be beneficial.[134] These approaches could be applied more widely to facilitate large, simple trials, reduce research waste and speed reductions in uncertainties in neonatal care.[134]

In Australia, the Australian and New Zealand Neonatal Network (ANZNN) is a collaborative network that collects a minimum data set to monitor the mortality and morbidity of infants admitted to neonatal intensive care units across the region. Formally developing a research network based within the ANZNN may be helpful as would expansion of data set collected. The primary limitations around this will be funding sources. However, the ANZNN already collaborates with a number of other national neonatal networks in the area of quality

improvement indicating it is feasible.[135] Another approach using ANZNN data would be periodic additional data collection periods, for example, 1-2 months blocks per year, where a particular aspect of neonatal care is focused on and relevant data collected.

Linking ANZNN data with the Australian Red Cross and New Zealand Blood Services data would facilitate future directions for research in this area. This would also allow for further exploration of effects of different degrees and timing of blood product exposure and neonatal morbidity, as well as donor-recipient interactions.

Further collaborations between existing research and data networks would also be beneficial. Establishing more formal links between ANZNN and the Interdisciplinary Maternal Perinatal Australasian Collaborative Trials (IMPACT) Network would be a positive future direction for neonatal research in Australia and New Zealand.

2. International consensus definitions of adverse effects and associations of neonatal transfusion

The standardisation of definitions of adverse effects and associations of transfusion in neonates and children, through an international consensus, is required to better report, understand and prevent them. Many countries now support haemovigilance systems[136] to promote and monitor safety and other issues, but there is little understanding of how these are adapted for children and neonates. In addition to working towards an international consensus on definitions of adverse transfusion events in neonates and children, other areas for development in haemovigilance include collection of key dominator data and engagement of patients and families in haemovigilance systems. This work is currently underway through the International Society of Blood Transfusion (ISBT) and I am undertaking leadership role with this work with the ISBT.

3. Assessment of current practices of co-infusion of dextrose-containing fluids and RBCs for transfusion in neonates

This practice is now routine in a number of neonatal units, including the Hospital for Sick Children and Sunnybrook Health Sciences Centre in Toronto, Canada as well as at Women's and Children's Hospital in Adelaide, Australia. Whilst no adverse effects have been reported due to this change in practice, a formal assessment of the practice ideally will be carried out.

This could be done through the use of a simple data sheet to prospectively evaluate haematological and biochemical effects of co-infused versus non co-infused transfusions.

4. International consensus definitions of haemodynamic compromise and core outcome set in neonates

As part of the work towards developing a clinical study (NeoBolus II) derived from the findings of the NeoBolus study, international consensus definition of haemodynamic compromise in neonates is required. With the network developed from the NeoBolus study, an international consensus is feasible. In addition, expansion of a core outcome set into this specific area is needed. Core outcome sets are an agreed, standardised group of outcomes to be reported by all studies within a research field. This broad initiative is underway through a United Kingdom based group.[111] The expansion of specific outcome sets for different areas of neonatal research are needed. This will be undertaken as part of the work towards the NeoBolus II study and will also include a modified Delphi process around definitions of haemodynamic compromise in neonates.

5. Randomised controlled study of fluid bolus therapy in neonates

Once the previously described work is undertaken, development of a randomised controlled study (NeoBolus II) will occur. It will evaluate the feasibility of fluid bolus therapy compared to no fluid bolus therapy for the management of suspected haemodynamic compromise in preterm infants (<30 weeks' gestation) within the first 24-48 hours of age. The detailed development of the study will rely on the work to be carried out as described in the previous section, in particular, around definitions and outcomes. Validation of the clinical questionnaire used in the initial NeoBolus trial would also need to occur. Initially, the study will examine the feasibility and acceptability of such a trial and then move towards a larger randomised controlled study.

Overall summary

This thesis provided a number of insights into neonatal transfusion practice and fluid bolus therapy. The research findings generated from this have allowed for identification of future directions for research in these areas, including enhanced data collection and collaborations through neonatal networks, development of international consensus definitions of transfusion adverse effects and associations, as well as haemodynamic compromise in neonates and a randomised controlled trial, the NeoBolus II study.

Appendices

APPENDIX A: List of publications included in the thesis

Keir AK, Yang J, Harrison A, Pelausa E, Shah PS on behalf of the Canadian Neonatal Network

Temporal changes in blood product usage in preterm neonates born at <30 weeks' gestation in Canada *Transfusion* 2015; 55(6): 1340-6. doi: 10.1111/trf.12998

Keir A, Pal Sanchita, Trivella M, Lieberman L, Callum J, Shehata N, Stanworth S

***Systematic review protocol*: Adverse effects of small volume red blood cell transfusions in the neonatal population** *Systematic Reviews* 2014; 3:92. doi:10.1186/2046-4053-3-92

Keir AK, Pal S, Trivella M, Lieberman L, Callum J, Shehata N, Stanworth S

Adverse effects of RBC transfusions in neonates: a systematic review and meta-analysis *Transfusion* 2016; 56(11): 2773-2780. doi: 10.1111/trf.13785.

Keir AK, Wilkinson D, Andersen C, Stark M

Washed versus unwashed red blood cells for transfusion for the prevention of morbidity and mortality in preterm infants (Protocol). Cochrane Database of Systematic Reviews 2015, Issue 1. Art. No.: CD011484. doi: 10.1002/14651858.CD011484.

Keir AK, Wilkinson D, Andersen C, Stark M

***Cochrane review*: Washed versus unwashed red blood cells for transfusion for the prevention of morbidity and mortality in preterm infants**

Cochrane Database Syst Rev. 2016; doi: 10.1002/14651858.CD011484

Keir A, Hansen A, Jankov R, Callum J, Acker J

Co-infusion of dextrose-containing fluids and red cell concentrates does not adversely affect in-vitro red blood cell quality *Transfusion* 2014; 54(8): 2068-2076. doi: 10.1111/trf.12618.

Keir A, Karam O, Hodyl N, Stark M, Liley H, Shah PS, Stanworth SJ on behalf of the NeoBolus study group

An international, multicenter, cross sectional study of fluid bolus therapy in neonates

Currently under peer-review – March 2018

APPENDIX B: Grants used to support the work included in the thesis

Women's and Children's Hospital Foundation Research Project Grant **2016**

Adelaide, Australia

For: Fluid bolus therapy in infants in the neonatal unit

Project team: Dr. Amy Keir, Dr. Nicolette Hodyl and A/Professor Michael Stark

Value: $68 740

The Eric Burnard Fellowship **2015**

The Royal Australasian College of Physicians (RACP) Foundation

The Fellowship commemorates the life and work of Dr Eric Burnard who died in 1991

Purpose: To assist a Fellow or trainee travel overseas to undertake a research project in neonatology

Value: $5 000

Division of Neonatology Trainee Grant Program **2013 - 2014**

The University of Toronto

Funding for up to $20 000 to undertake a research program as part of the University of Toronto's Neonatal-Perinatal Medicine Fellowship Program

Hospital for Sick Children Trainee Start-Up Fund (TSUF) **2013**

Toronto, Canada

Funding for up to $3 000 to undertake a research project

APPENDIX C: Abstract presentations from the work included in the thesis

Invited presentations – national and international

2018

International Society of Blood Transfusion Regional Congress (Toronto, Canada)
Invited Speaker: "Transfusion practice and adverse effects in neonates"

2015

Annual Scientific Meetings of the HAA (Adelaide, Australia)
The Haematology Society of Australia and New Zealand, the Australian & New Zealand Society of Blood Transfusion and the Australasian Society of Thrombosis and Haemostasis
Invited Speaker: "Transfusion practice in preterm infants

Abstract presentations – national and international

2018

Pediatric Academic Societies (PAS) Annual Scientific Meeting (Toronto, Canada)
An international, multi-centre, observational study of fluid bolus therapy in neonates
Keir A, Karam O, Hodyl N, Stark M, Liley H, Shah PS, Stanworth on behalf of the NeoBolus study group

Perinatal Society of Australia and New Zealand (PSANZ) Annual Congress (Auckland, New Zealand)
An international, multi-centre, observational study of fluid bolus therapy in neonates
Keir A, Karam O, Hodyl N, Stark M, Liley H, Shah PS, Stanworth on behalf of the NeoBolus study group

2015

International Society for Blood Transfusion (ISBT) (London, UK)
Transfusion practice does not appear to impact on neonatal morbidities or mortality: a systematic review
Keir AK, Pal S, Trivella M, Lieberman L, Callum J, Sheheta N, Stanworth S

Perinatal Society of Australia and New Zealand (PSANZ) Annual Congress (Melbourne, Australia)
Cochrane review: Washed versus unwashed red blood cells for transfusion for the prevention of morbidity and mortality in preterm infant
Keir AK, Wilkinson D, Andersen C, Stark M

Paediatric Academic Societies (PAS) Annual Scientific Meeting (Vancouver, Canada)
Use of blood products in preterm infants <30 weeks' gestation in the Canadian Neonatal Network
Keir A, Harrison A, Lee SK, Shah PS and the Canadian Neonatal Network

Late RBC transfusions and outcomes of preterm infants <30 weeks gestational age: a nested cohort study
Keir A, Aziz K, McMillan DD, Monterrosa L, Ojah CR, Lee SK, Shah PS and the Canadian Neonatal Network

2014
Perinatal Society of Australia and New Zealand (PSANZ) Annual Congress (Perth, Australia)
Late RBC transfusions and outcomes of preterm infants <30 weeks gestational age: a nested cohort study
Keir A, Aziz K, McMillan DD, Monterrosa L, Ojah CR, Lee SK, Shah PS and the Canadian Neonatal Network

Use of blood products in preterm infants <30 weeks' gestation in the Canadian Neonatal Network
Keir A, Harrison A, Nwaesei C, Lee SK, Shah PS and the Canadian Neonatal Network

2013
AABB Annual Meeting (Denver, Colorado)
Co-infusion of dextrose-containing fluids and packed red blood cells does not adversely affect markers of red cell integrity
Keir A, Hansen A, Callum J, Jankov R, Acker J

APPENDIX D: Authorship forms

Authorship form for:

Keir AK, Yang J, Harrison A, Pelausa E, Shah PS on behalf of the Canadian Neonatal Network

Temporal changes in blood product usage in preterm neonates born at <30 weeks' gestation in Canada

Transfusion 2015; 55(6): 1340-6. doi: 10.1111/trf.12998

Statement of Authorship

Title of Paper	Temporal changes in blood product usage in preterm neonates born at <30 weeks' gestation in Canada
Publication Status	○ Published, ◉ Accepted for Publication, ○ Submitted for Publication, ○ Publication style
Publication Details	Accepted for publication in the journal Transfusion on 1 December 2014.

Author Contributions

By signing the Statement of Authorship, each author certifies that their stated contribution to the publication is accurate and that permission is granted for the publication to be included in the candidate's thesis.

Name of Principal Author (Candidate)	Amy Keir		
Contribution to the Paper	1. Conception and design of the project; 2. Analysis and interpretation of research data; 3. Writing of the first draft of the paper and responsibility for ongoing revisions as directed by the authorship group.		
Signature		Date	

Name of Co-Author	Adele Harrison		
Contribution to the Paper	1. Collection, analysis and interpretation of research data; 2. Critically revising the paper so as to contribute to the interpretation.		
Signature		Date	2 Dec, 2014

Name of Co-Author	Ermelinda Pelausa		
Contribution to the Paper	1. Collection, analysis and interpretation of research data; 2. Critically revising the paper so as to contribute to the interpretation.		
Signature		Date	

Name of Co-Author	Please see page two for additional author/senior author		
Contribution to the Paper			
Signature		Date	

Statement of Authorship

Title of Paper	Temporal changes in blood product usage in preterm neonates born at <30 weeks' gestation in Canada
Publication Status	○ Published, ◉ Accepted for Publication, ○ Submitted for Publication, ○ Publication style
Publication Details	Accepted for publication in the journal Transfusion on 1 December 2014.

Author Contributions

By signing the Statement of Authorship, each author certifies that their stated contribution to the publication is accurate and that permission is granted for the publication to be included in the candidate's thesis.

Name of Principal Author (Candidate)	Please see page one.		
Contribution to the Paper			
Signature		Date	

Name of Co-Author	Junmin Yang		
Contribution to the Paper	1. Analysis and interpretation of research data; 2. Critically revising the paper so as to contribute to the interpretation.		
Signature		Date	12/02/2014

Name of Co-Author	Prakesh Shah		
Contribution to the Paper	1. Conception and design of the project; 2. Analysis and interpretation of research data; 3. Responsibility for ongoing revisions and critically revising the paper so as to contribute to the interpretation.		
Signature		Date	July 14, 2016

Name of Co-Author			
Contribution to the Paper			
Signature		Date	

Statement of Authorship

Title of Paper	Temporal changes in blood product usage in preterm neonates born at <30 weeks' gestation in Canada
Publication Status	○ Published, ◉ Accepted for Publication, ○ Submitted for Publication, ○ Publication style
Publication Details	Accepted for publication in the journal Transfusion on 1 December 2014.

Author Contributions

By signing the Statement of Authorship, each author certifies that their stated contribution to the publication is accurate and that permission is granted for the publication to be included in the candidate's thesis.

Name of Principal Author (Candidate)	Amy Keir		
Contribution to the Paper	1. Conception and design of the project; 2. Analysis and interpretation of research data; 3. Writing of the first draft of the paper and responsibility for ongoing revisions as directed by the authorship group.		
Signature	[redacted]	Date	14 July 2016

Name of Co-Author	Adele Harrison		
Contribution to the Paper	1. Collection, analysis and interpretation of research data; 2. Critically revising the paper so as to contribute to the interpretation.		
Signature		Date	

Name of Co-Author	Ermelinda Pelausa		
Contribution to the Paper	1. Collection, analysis and interpretation of research data; 2. Critically revising the paper so as to contribute to the interpretation.		
Signature	[redacted]	Date	December 15, 2014

Name of Co-Author	Please see page two for additional author/senior author		
Contribution to the Paper			
Signature		Date	

Authorship forms for:

Keir A, Pal Sanchita, Trivella M, Lieberman L, Callum J, Shehata N, Stanworth S

***Systematic review protocol*: Adverse effects of small volume red blood cell transfusions in the neonatal population**

Systematic Reviews 2014; 3:92. doi:10.1186/2046-4053-3-92

Keir AK, Pal S, Trivella M, Lieberman L, Callum J, Shehata N, Stanworth S

Adverse effects of RBC transfusions in neonates: a systematic review and meta-analysis

Transfusion 2016; 56(11): 2773-2780. doi: 10.1111/trf.13785.

Statement of Authorship

Title of Paper (PROTOCOL ONLY)	Adverse effects of small-volume red blood cell transfusions in the neonatal population
Publication Status	**Published**
Publication Details	Keir A, Pal S, Trivella M, Lieberman L, Callum J, Shehata N, Stanworth S. Syst Rev. 2014 Aug 20;3:92. doi: 10.1186/2046-4053-3-92. PMID: 25143009

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Name of Principal Author (Candidate)	Amy Keir		
Contribution to the Paper	1. Substantial contribution to conception and design, acquisition of data, analysis and interpretation of data 2. Drafting the article and revising it for important intellectual content 3. Final approval of the version to be published.		
Overall percentage (%)	70%		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	13 July 2016

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Sanchita Pal		
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Contribution to the Paper	1. Substantial contribuation to conception and design, analysis and interpretation of data 2. Drafting the article and revising it for important intellectual content 3. Final approval of the version to be published		
Signature		Date	13/7/2016

Name of Co-Author	Lani Lieberman

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Signature		Date	Aug 31/2017.

Name of Co-Author	Jeannie Callum		
Contribution to the Paper	1. Contribuation to acquisition of data 2. Revising the article for important intellectual content 3. Final approval of the version to be published		
Signature		Date	2017/8/29

Name of Co-Author	Nadine Shohata		
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Name of Co-Author	Simón Stanworth		
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Signature		Date	23 August 2017

Contribution to the Paper	1. Contribuation to acquisition of data 2. Revising the article for important intellectual content 3. Final approval of the version to be published		
Signature		Date	

Name of Co-Author	Jeannie Callum		
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Signature		Date	

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Contribution to the Paper	1. Substantial contribuation to conception and design, acquisition of data, analysis and interpretation of data 2. Drafting the article and revising it for important intellectual content 3. Final approval of the version to be published		
Signature		Date	July 13/16

Name of Co-Author	Simon Stanworth		
Contribution to the Paper	1. Substantial contribuation to conception and design, acquisition of data, analysis and interpretation of data 2. Drafting the article and revising it for important intellectual content 3. Final approval of the version to be published		
Signature		Date	

Statement of Authorship

Title of Paper (PROTOCOL ONLY)	Adverse effects of small-volume red blood cell transfusions in the neonatal population
Publication Status	**Published**
Publication Details	Keir A, Pal S, Trivella M, Lieberman L, Callum J, Shehata N, Stanworth S. Syst Rev. 2014 Aug 20;3:92. doi: 10.1186/2046-4053-3-92. PMID: 25143009

Principal Author

Name of Principal Author (Candidate)	Amy Keir		
Contribution to the Paper	1. Substantial contribuation to conception and design, acquisition of data, analysis and interpretation of data 2. Drafting the article and revising it for important intellectual content 3. Final approval of the version to be published.		
Overall percentage (%)	70%		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	13 July 2016

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Sanchita Pal		
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Name of Co-Author	Lani Lieberman

Statement of Authorship

Title of Paper	Adverse effects of RBC transfusions in neonates: a systematic review and meta-analysis
Publication Status	**Accepted for publication**
Publication Details	Keir AK, Pal S, Trivella M, Lieberman L, Callum J, Shehata N, Stanworth S **Adverse effects of RBC transfusions in neonates: a systematic review and meta-analysis** *Transfusion*; accepted for publication 27 June 2016

Principal Author

Name of Principal Author (Candidate)	Amy Keir		
Contribution to the Paper	1. Substantial contribution to conception and design, acquisition of data, analysis and interpretation of data 2. Drafting the article and revising it for important intellectual content 3. Final approval of the version to be published.		
Overall percentage (%)	70%		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	13 July 2016

Co-Author Contributions

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iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Sanchita Pal		
Contribution to the Paper	1. Contribuation to acquisition of data 2. Revising the article for important intellectual content 3. Final approval of the version to be published		
Signature		Date	

Name of Co-Author	Marialena Trivella		
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Signature		Date	13/7/2016

Name of Co-Author	Lani Lieberman

Contribution to the Paper	1. Contribuation to acquisition of data 2. Revising the article for important intellectual content 3. Final approval of the version to be published		
Signature		Date	Aug 31/2017.

Name of Co-Author	Jeannie Callum		
Contribution to the Paper	1. Contribuation to acquisition of data 2. Revising the article for important intellectual content 3. Final approval of the version to be published		
Signature		Date	2017/8/29

Name of Co-Author	Nadine Shehata		
Contribution to the Paper	1. Substantial contribuation to conception and design, acquisition of data, analysis and interpretation of data 2. Drafting the article and revising it for important intellectual content 3. Final approval of the version to be published		
Signature		Date	

Name of Co-Author	Simon Stanworth		
Contribution to the Paper	1. Substantial contribuation to conception and design, acquisition of data, analysis and interpretation of data 2. Drafting the article and revising it for important intellectual content 3. Final approval of the version to be published		
Signature		Date	23 August 2017

Statement of Authorship

Title of Paper	Adverse effects of RBC transfusions in neonates: a systematic review and meta-analysis
Publication Status	**Accepted for publication**
Publication Details	Keir AK, Pal S, Trivella M, Lieberman L, Callum J, Shehata N, Stanworth S **Adverse effects of RBC transfusions in neonates: a systematic review and meta-analysis** *Transfusion*; accepted for publication 27 June 2016

Principal Author

Name of Principal Author (Candidate)	Amy Keir		
Contribution to the Paper	1. Substantial contribuation to conception and design, acquisition of data, analysis and interpretation of data 2. Drafting the article and revising it for important intellectual content 3. Final approval of the version to be published.		
Overall percentage (%)	70%		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	

Co-Author Contributions

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i. the candidate's stated contribution to the publication is accurate (as detailed above);
ii. permission is granted for the candidate in include the publication in the thesis; and
iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Sanchita Pal		
Contribution to the Paper	1. Contribuation to acquisition of data 2. Revising the article for important intellectual content 3. Final approval of the version to be published		
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Name of Co-Author	Marialena Trivella		
Contribution to the Paper	1. Substantial contribuation to conception and design, analysis and interpretation of data 2. Drafting the article and revising it for important intellectual content 3. Final approval of the version to be published		
Signature		Date	13/7/2016

Name of Co-Author	Lani Lieberman

Contribution to the Paper	1. Contribuation to acquisition of data 2. Revising the article for important intellectual content 3. Final approval of the version to be published		
Signature		Date	

Name of Co-Author	Jeannie Callum		
Contribution to the Paper	1. Contribuation to acquisition of data 2. Revising the article for important intellectual content 3. Final approval of the version to be published		
Signature		Date	

Name of Co-Author	Nadine Shehata		
Contribution to the Paper	1. Substantial contribuation to conception and design, acquisition of data, analysis and interpretation of data 2. Drafting the article and revising it for important intellectual content 3. Final approval of the version to be published		
Signature		Date	July 13/16

Name of Co-Author	Simon Stanworth		
Contribution to the Paper	1. Substantial contribuation to conception and design, acquisition of data, analysis and interpretation of data 2. Drafting the article and revising it for important intellectual content 3. Final approval of the version to be published		
Signature		Date	

Authorship form for:

Keir AK, Wilkinson D, Andersen C, Stark M

***Cochrane review*: Washed versus unwashed red blood cells for transfusion for the prevention of morbidity and mortality in preterm infants**

Cochrane Database Syst Rev. 2016; doi: 10.1002/14651858.CD011484

Statement of Authorship

Title of Paper	Washed versus unwashed red blood cells for transfusion for the prevention of morbidity andmortality in preterm infants (Review)
Publication Status	[X] Published ☐ Accepted for Publication ☐ Submitted for Publication ☐ Unpublished and Unsubmitted w ork w ritten in manuscript style
Publication Details	Keir AK, Wilkinson D, Andersen C, Stark MJ. Cochrane Database Syst Rev. 2016 Jan 20;1:CD011484. doi: 10.1002/14651858.CD011484.pub2. Review. PMID: 26788664

Principal Author

Name of Principal Author (Candidate)	Amy Keir
Contribution to the Paper	1. Substantial contribuation to conception and design, acquisition of data, analysis and interpretation of data 2. Drafting the article and revising it for important intellectual content 3. Final approval of the version to be published. Published authorship statement (applies to all authors): "Amy Keir (AK) screened the titles and abstracts of all studies identified by the search strategy. AK and Dominic Wilkinson (DW) screened the full text of each study identified as of potential relevance. AK and DW extracted the data separately, compared data, and resolved any differences by consensus. AK, DW, Chad Andersen and Michael Stark completed the final review."
Overall percentage (%)	70%
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.
Signature	Date: 16 May 2016

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Text

Name of Co-Author	Dominic Wilkinson
Contribution to the Paper	1. Substantial contribuation to conception and design, acquisition of data, analysis and interpretation of data 2. Drafting the article and revising it for important intellectual content 3. Final approval of the version to be published
Signature	Date: 17 May 2016

Name of Co-Author	Chad Andersen		
Contribution to the Paper	1. Contribution to conception and design 2. Revising the article for important intellectual content 3. Final approval of the version to be published		
Signature		Date	17/5/16
Name of Co-Author	Michael Stark		
Contribution to the Paper	1. Contribution to conception and design 2. Revising the article for important intellectual content 3. Final approval of the version to be published		
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Authorship form for:

Keir A, Hansen A, Jankov R, Callum J, Acker J

Co-infusion of dextrose-containing fluids and red cell concentrates does not adversely affect in-vitro red blood cell quality

Transfusion 2014; 54(8): 2068-2076. doi: 10.1111/trf.12618.

Statement of Authorship

Title of Paper	Co-Infusion of Dextrose-Containing Fluids and Red Cell Concentrates Does Not Adversely Affect In-Vitro Red Blood Cell Quality
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Publication Details	Accepted for publication in Transfusion on 15 January 2014 and is currently 'in-press'.

Author Contributions

By signing the Statement of Authorship, each author certifies that their stated contribution to the publication is accurate and that permission is granted for the publication to be included in the candidate's thesis.

Name of Principal Author (Candidate)	Amy Keir
Contribution to the Paper	1) Substantial contributions to conception and design, acquisition of data and analysis and interpretation of data 2) Drafting the article and revising it critically for important intellectual content 3) Final approval of the version to be published
Signature	Date: 2014-02-09

Name of Co-Author	Adele Hansen
Contribution to the Paper	1) Substantial contributions to conception and design, acquisition of data and analysis and interpretation of data 2) Drafting the article and revising it critically for important intellectual content 3) Final approval of the version to be published
Signature	Date: 2014-02-07

Name of Co-Author	Jeannie Callum
Contribution to the Paper	1) Contributions to conception and design 2) Revising it critically for important intellectual content 3) Final approval of the version to be published
Signature	Date: 2014/02/13

Name of Co-Author	Jason Acker
Contribution to the Paper	1) Substantial contributions to conception and design, acquisition of data and analysis and interpretation of data 2) Drafting the article and revising it critically for important intellectual content 3) Final approval of the version to be published
Signature	Date: 2014-02-07

Statement of Authorship

Title of Paper	Co-Infusion of Dextrose-Containing Fluids and Red Cell Concentrates Does Not Adversely Affect In-Vitro Red Blood Cell Quality
Publication Status	○ Published, ◉ Accepted for Publication, ○ Submitted for Publication, ○ Publication style
Publication Details	Accepted for publication in Transfusion on 15 January 2014 and is currently 'in-press'. *PAGE 2 OF AUTHORSHIP FORM*

Author Contributions

By signing the Statement of Authorship, each author certifies that their stated contribution to the publication is accurate and that permission is granted for the publication to be included in the candidate's thesis.

Name of Principal Author (Candidate)	Amy Keir		
Contribution to the Paper	1) Substantial contributions to conception and design, acquisition of data and analysis and interpretation of data 2) Drafting the article and revising it critically for important intellectual content 3) Final approval of the version to be published		
Signature		Date	2014-02-09

Name of Co-Author	Robert Jankov		
Contribution to the Paper	1) Substantial contributions to conception and design 2) Drafting the article and revising it critically for important intellectual content 3) Final approval of the version to be published		
Signature		Date	Feb 14, 2014

Name of Co-Author			
Contribution to the Paper			
Signature		Date	

Name of Co-Author			
Contribution to the Paper			
Signature		Date	

Authorship form for:

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An international, multicenter, cross sectional study of fluid bolus therapy in neonates

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Statement of Authorship

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Contribution to the Paper	Amy Keir significantly contributed to the concept and design of the study, led the acquisition of the data and co-ordination/recruitment of the study sites and undertook the analysis and interpretation of the data with support of her co-authors. She wrote the initial draft of the manuscript and critically revised it with the input and assistance of her co-authors.		
Overall percentage (%)	75		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
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Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

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