

Keith Collard, PhD, Associate Professor, Series Editor

Transfusion related morbidity in premature babies: Possible mechanisms and implications for practice

Keith James Collard

Keith James Collard, Peninsula Allied Health Centre, School of Health Professions, Faculty of Health and Human Sciences, University of Plymouth, Plymouth, PL6 8BH, United Kingdom

Author contributions: Collard KJ solely contributed to this paper. Supported by The Northcott Devon Medical Foundation; The NHS SW Research; and Development Fund

Correspondence to: Keith Collard, PhD, Associate Professor, Peninsula Allied Health Centre, School of Health Professions, Faculty of Health and Human Sciences, University of Plymouth, Derriford Road, Plymouth, PL6 8BH, United Kingdom. keith.collard@plymouth.ac.uk

Telephone: +44-1752-587585 Fax: +44-1752-588874

Received: November 27, 2013 Revised: March 19, 2014

Accepted: May 31, 2014

Published online: August 8, 2014

Abstract

Many premature babies, especially those with a low birth weight are given multiple transfusions during their first few weeks of life. The major serious complications of prematurity include bronchopulmonary dysplasia, with lesser incidences of retinopathy of prematurity, intraventricular haemorrhage, and necrotising enterocolitis. Many studies have shown correlations between the receipt of blood transfusions and the development of these conditions, but little is known of the underlying pathophysiology of this relationship. Recent studies are beginning to provide some answers. This review examines recent findings with regard to the influence of preparation and storage of paediatric packed red blood cell units on heme, iron, and oxidative status of the units and relates these to the ability of the premature baby to deal with these changes following the receipt of blood transfusions. Paediatric packed red blood cell units are a potential source of heme, redox active iron and free radicals, and this increases with storage age. Haemolysis of transfused red blood cells may add further iron and cell free haemoglobin to the recipient baby. Premature babies, particularly those with low birth weight and gestational age appear to have little

reserve to cope with any additional iron, heme and/or oxidative load. The consequences of these events are discussed with regard to their contribution to the major complications of prematurity and a novel hypothesis regarding transfusion-related morbidity in premature babies is presented. The review concludes with a discussion of potential means of limiting transfusion related iron/heme and oxidative load through the preparation and storage of packed red blood cell units and through modifications in clinical practice.

© 2014 Baishideng Publishing Group Inc. All rights reserved.

Key words: Blood transfusions; Premature babies; Iron; Heme; Oxidative stress; Storage lesion; Complications of prematurity

Core tip: Many premature babies, especially those with a low birth weight are given multiple transfusions during their first few weeks of life. Studies have shown correlations between the receipt of blood transfusions and the development of the major complications of prematurity. Little is known of the underlying pathophysiology of this relationship. This review examines novel potential mechanisms which are related to the changes that occur in iron, heme and oxidative status in paediatric packed cell units during preparation and storage, and in the ability of the premature baby to deal with these changes following receipt of blood transfusion.

Collard KJ. Transfusion related morbidity in premature babies: Possible mechanisms and implications for practice. *World J Clin Pediatr* 2014; 3(3): 19-29 Available from: URL: <http://www.wjgnet.com/2219-2808/full/v3/i3/19.htm> DOI: <http://dx.doi.org/10.5409/wjcp.v3.i3.19>

INTRODUCTION

Many premature babies, especially those with a birth

weight of less than 1000 g are given multiple transfusions during their first few weeks of life^[1,2]. The major serious complications of prematurity include bronchopulmonary dysplasia (BPD), with lesser incidences of retinopathy of prematurity (ROP), intraventricular haemorrhage (IVH), and necrotising enterocolitis (NEC). Many studies have shown correlations between the receipt of blood transfusions and the development of these conditions^[1,3-16]. Few have been able to provide any strong evidence that the receipt of blood transfusions is an independent risk factor in the development of these conditions^[3,4,6,9,15,17], although some more recent studies have provided better evidence of transfusion being an independent risk factor for NEC^[13] and IVH^[9]. This lack of consistency is probably related to the multifactorial nature of these conditions, the multifactorial nature of the consequences of the receipt of packed cell transfusions^[18], and the fact that smallest and sickest babies are those most likely to receive blood transfusions^[19]. This makes it difficult to tease out the relative risks of the many factors involved^[20] even with sophisticated multiple regression analysis^[2,4,19]. Despite this, some explanations have been proposed to account for the relationship between the receipt of blood transfusions and some of the consequences of prematurity. Many of these involve transfusion mediated iron induced factors such as infection and oxidative stress^[19], changes in immune function^[21] and also other factors such as changes in nitric oxide (NO) mediated vasodilation and responsiveness^[22,23].

This paper attempts to determine more about transfusion-related morbidity in premature babies by relating some of the recently observed changes which occur in paediatric packed cell units during preparation and storage to the particular physiology of the premature baby. The result has provided strong evidence to suggest that a major contributor to transfusion-related morbidity in these babies is the enhanced level of non-protein-bound iron, heme and oxidative stress in the paediatric packs and the limited ability of the premature baby to deal with a transfusion-mediated iron, heme and oxidative load. It is a relatively new concept that does not preclude other aspects of the "storage lesion" also contributing^[24,25], but at present it appears to be a good marriage between the known physiology of the premature baby and the effects of packed red blood cell transfusions.

BLOOD TRANSFUSION AND CLINICAL OUTCOME IN PREMATURE BABIES

The relationship between blood transfusion and NEC has been linked to possible adverse immunological consequences of the receipt of blood and the timing of this with feeding^[11,21,26], although this should be less of a problem when using leukoreduced blood preparations^[27]. Transfusion related gut morbidity is however a multifactorial condition related to a dynamic balance of immune, infectious, vascular, angiogenic and mechanical mediators of brush border integrity^[28]. It also appears to be related to particular changes occurring at 31-32 wk postconcep-

tional age, irrespective of postconceptional age at birth. There is evidence that the older the storage age of the blood transfused the more likely NEC is to develop^[17], and oxidative stress has been mentioned as a potential factor^[28]. However, the complexity of the condition has not permitted a clear understanding of the relationship between the receipt of blood and the development of NEC. Also, many babies who receive blood transfusions do not develop NEC^[15], indicating that those in which the relationship is seen may have other underlying factors that predispose towards developing NEC following transfusions^[29,30]. Certainly the smaller the birth weight the more likely the baby is to develop transfusion associated NEC^[17]. Thus the receipt of blood may be one factor in a complex multifactorial condition.

The link between IVH and the receipt of blood may be related to volutrauma and damage to the weak blood vessels in the germinal matrix^[10]. This may be further exacerbated by the loss of NO from erythrocytes during storage^[22,31] which would impair capillary vasodilation to accommodate the donated erythrocytes^[9]. It should be noted however, that not all studies support this model of erythrocyte mediated vasodilation^[9,32]. However, it has been shown that blood which had been stored for up to 42 d has a progressive vaso-inhibitory effect which is mediated not by scavenging NO or loss of NO, but by inhibiting endothelial NO production in the recipient^[23]. Thus there is evidence of disruption of NO vasodilatory mechanisms in the recipient following the receipt of stored red blood cells. Again, it should be noted that not all babies who develop IVH receive blood transfusions, and many babies who receive blood transfusions do not develop IVH^[9]. Yet again there is a subset of vulnerable babies in which the receipt of blood becomes a risk factor. Current views on the link between transfusion and IVH does not include iron-induced oxidative stress. However, it could play a major role in events subsequent to the haemorrhage as blood (potentially rich in redox active iron) entering the extracellular compartment is likely to contribute to iron-induced oxidative damage to the cells of the developing brain^[33,34].

While volutrauma may also contribute to ROP, most studies suggest that iron overload and associated oxidative stress may be a major player in ROP^[12,35]. Increased iron load due to post-transfusional red blood cell (RBC) breakdown and associated oxidative stress has been suggested^[12,35]. In addition, enhanced O₂ delivery to the developing retinal vasculature following transfusion with adult RBC's may impair the function of the growth factors which regulate vascularisation of the retina^[12]. Again low birth weight and respiratory distress also appeared to be independent risk factors for the development of ROP^[35], highlighting the multifactorial nature of the condition.

Though not so well investigated, transfusion-related iron overload and resulting oxidative stress has been suggested as a potential mechanism linking transfusion to the development of BPD^[19,36-38]. The relationship between blood transfusion and BPD may be related to the finding that babies with BPD were usually smaller, required more

ventilator support and required more blood sampling leading to iatrogenic anaemia. Consequently more blood transfusions would be required to replace that removed by sampling^[38]. This suggests a potential consequence of very low birth weight rather than a direct cause of BPD. However, the receipt of blood and associated complications caused by it may exacerbate a condition developing from other causes. A major factor in the development of BPD is endotracheal infection^[4]. A recent study in critically ill adults has shown that transfusion with blood stored for more than 14 d is associated with increased bacterial infection^[39]. Since iron availability is essential for bacterial colonisation^[40], and the level of non-transferrin bound iron in paediatric packs rises significantly throughout storage (around 6 $\mu\text{mol/L}$ on day 14, around 15 $\mu\text{mol/L}$ on day 35, compared with plasma levels of around 0.3 $\mu\text{mol/L}$ in healthy adults^[41]), transfusion mediated iron promoted bacterial infection may be involved in the development of BPD.

Thus, in summary, there is a reasonable amount of evidence to support of increased risk of developing the major complications of prematurity following the receipt of blood transfusions in some premature babies. As indicated above, potential mechanisms which have been proposed to account for this relationship include disruption of NO mediated vasodilation^[22-24], immune dysfunction^[11,21,42], and transfusion mediated iron and oxidative load^[19,41,43]. These are not necessarily mutually exclusive and have not been established as independent risk factors in all cases. Interaction between these factors probably occurs. For example, structural changes in the stored red blood cells which influence the deformability and survival of red blood cells post transfusion, lead to the build-up of extracellular haemoglobin which can have major effects on NO availability^[44-47], and also provides a potential source of heme and iron^[48] for iron mediated pathology and immune modulation^[42].

Particularly interesting are the findings that the link between blood transfusions and the complications of prematurity are more prevalent in the smaller birth weight babies, and that one complication may be associated with the presence of another in this group^[49]. This may indicate that this subset of premature babies may require specific attention with regard to transfusion practice.

The awareness of the potential risks of receiving blood transfusions has led to a number of studies and changes in clinical practice to try to limit the use of blood transfusions in the neonatal intensive care unit as a means of improving clinical outcome^[50-58]. These procedures are beyond the scope of this review.

Recent findings have strengthened the idea that transfusion-mediated iron and oxidative load may play a major role in some of the complications of prematurity. These findings include factors involved in the preparation, storage and use of packed red blood cell units in premature babies and the ability of the baby to deal with potential adverse consequences of the receipt of blood. This review will investigate the possible link between transfusion-mediated iron overload and oxidative stress and the ability of the premature baby to deal with such a

situation, and the implications with regard to the development of the complications of prematurity.

PREPARATION OF PAEDIATRIC PACKED CELL UNITS FOR TRANSFUSION

In order to try to understand the potential mechanisms of any relationship between the receipt of blood transfusions and clinical outcome some knowledge of the procedures involved in the preparation of packed cell units is required. Paediatric packed red blood cell units are prepared from adult blood. One unit of adult blood usually anticoagulated with citrate is spun down to yield the red blood cells. Blood for paediatric use is usually filtered to remove the majority of leucocytes. The majority of plasma is removed and replaced by additive solution to provide a haematocrit of 55%-60%. Various different additive solutions are used but they all tend to contain various amounts of dextrose, adenine, phosphate, mannitol and occasionally citrate, either residual from the original anticoagulation or added^[59]. The additive solutions are designed to provide anticoagulant and buffering capacity and a source of metabolic energy for RBCs. In addition, mannitol and adenine act as preservatives to allow the storage of RBCs up to 35 d for paediatric use and 42 d for adult use^[60] in the United Kingdom. These latter substances stabilise the RBC membrane and ensure adequate 2,3-diphosphoglycerate and ATP availability within the RBCs. Each adult unit is then divided to provide 6-8 paediatric packs of 40-50 mL each. While these additives have been designed to help preserve RBC integrity and shelf life, the removal of plasma has significant implications with regard to iron and iron-induced oxidative stress. The replacement of plasma with additive removes the major iron binding proteins and extracellular antioxidants from the final preparation^[41]. This provides the opportunity for the build up of redox active iron in the extracellular medium and the potential to drive iron mediated oxidative damage to the RBCs, and to induce iron mediated oxidative damage to the baby post transfusion^[61]. The purpose of this review is to evaluate the possibility that the contribution of the receipt of blood transfusions to the development of the complications of prematurity may reside, to some extent, in poor iron status in the paediatric packs and the consequences alluded to above. This will require an understanding of changes in iron and oxidative status of paediatric packs during storage, and the extent to which the baby might cope with increased iron and oxidative load post transfusion.

THE INFLUENCE OF STORAGE ON THE IRON AND OXIDATIVE STATUS OF PACKED CELL UNITS AND THE RELATIONSHIP BETWEEN STORAGE AND CLINICAL OUTCOME

Traditionally, only blood stored for less than 7 d had

been deemed acceptable for neonatal transfusions^[62]. Because some babies require frequent transfusions, the number of different donors that an individual baby may be exposed to could be high. This was considered as potentially detrimental to the baby and wasteful with regard to resources^[63]. For these reasons, the use of small volume paediatric packs prepared from a single donor (as described above) was adopted as standard use^[63]. This allowed the packs to be stored up to 35 d and ensured that the baby should only receive blood from a single donor. The move from the use of fresh blood to stored blood required some understanding of the consequences of RBC storage on the status of the blood and the influence of older stored blood on clinical outcome. A number of studies have shown adverse relationships between the storage age of the blood used in transfusion and clinical outcome^[64,65]. This relationship holds whether the blood is transfused to critically ill infants^[64,65] or adults^[66]. However it should be noted that not all studies support this contention. To some extent this may be related to the failure to address all the potential confounding variables^[20,67-70]. The potential adverse effects of storage on the biochemistry and validity of stored erythrocytes has been given the term “the storage lesion”^[52,71]. The controversy surrounding this contention, and the need to improve our understanding of the influence of storage on clinical outcome is illustrated by the development of two current large scale studies looking at the influence of storage age on clinical outcome in critically ill adults^[72], and in premature babies^[73]. Both these studies are prospective studies using clearly defined storage ages and outcome measures. The initial results of the latter study showed that babies who received blood of an average storage age of 5.1 d did not have an improved outcome compared to babies who received blood stored under the current standard procedure which averaged out in this study at 14.6 d^[74]. However, as discussed later, it is more likely to observe adverse outcomes in babies who have received blood stored for greater than 14 d. This is difficult to study prospectively because of the ethics of randomly assigning blood stored for more than 14 d to a group of babies knowing it might compromise outcome.

The involvement of iron and oxidative status in the storage lesion has received little attention, despite the potential adverse influence of the procedures involved in the preparation of packed cell units on the iron and oxidative status of the units. This can have consequences on the viability of erythrocytes, and the behaviour of haemoglobin within the erythrocytes and on iron bioavailability^[75,76].

A number of studies have indicated that transfusion mediated iron overload may contribute to morbidity and mortality in some situations^[78], and that this may be increased as a function of the storage age of the blood transfused. Other studies have indicated that this may be mediated by iron released from RBCs during storage as a result of oxidative damage to RBC membranes and haemoglobin^[76]. The first study to show that iron is indeed lost from packed RBCs during storage was that

of Marwah *et al*^[79]. In this study little non-transferrin-bound iron (NTBI) was seen in the extracellular medium surrounding the stored RBCs over the first 10 d of storage. Thereafter it rose steadily up to 28 d storage. In our laboratory using leucoreduced paediatric packed cell units iron was already detectable in the extracellular medium on arrival from the blood transfusion centre (3 d post donation) and then rose linearly to a level of 34 $\mu\text{mol/L}$ after 35 d storage^[41,43]. Moreover a high percentage was in the potentially damaging NTBI form. This suggests that some release of iron was occurring as a result of damage occurring during preparation^[80], and that some changes occur very rapidly in the first few days of storage^[81]. It is not believed that the filtration process used to remove the leucocytes is the cause of the initial haemolysis, but other factors such as shear stress, exposure to anticoagulants, exposure to additive solutions and contact with the plastic material in the bags^[80]. Leukoreduction may improve the storage of RBCs^[82]. Packed cell units used in neonatal intensive care units are routinely leucoreduced. In our studies the rise in extracellular iron and NTBI with storage is associated with a gradual increase in malondialdehyde (MDA) over the first 21 d of storage with a steeper rise from 21 d to 35 d. Similar findings have recently been reported by Stark *et al*^[83], and the findings reflect changes in cellular MDA during storage^[84]. The appearance of Hb in the extracellular medium parallels the rise in MDA. This indicates that lipid peroxidation in the RBC membrane may contribute to the loss of iron and Hb. In addition to the rise in iron with storage, there is also a large increase in heme in the extracellular milieu^[48,85] with the pattern of increase running parallel to that of MDA. MDA is marker of lipid peroxidation. It is well known that oxidative stress, normal ageing and aerobic incubation lead to the release of free chelatable iron from Hb within erythrocytes^[77,86]. There is evidence that iron released within erythrocytes can mediate oxidative damage to the cell membrane, leading to haemolysis and the release of Hb^[76]. This may be further oxidised to produce superoxide, methemoglobin and free iron^[86,87]. Methemoglobin is relatively unstable and will readily release the heme moiety from the heme pocket^[77]. Further oxidation of the heme molecule leads to the release of free iron.

The small amount of residual ascorbate present in the extracellular medium was mostly in the oxidised form and fell dramatically from 21 to 35 d^[41]. Thus the lack of antioxidant protection contributes to oxidative damage to the cell membrane and iron binding proteins such as Hb^[76,88,89]. The findings from our laboratory support the previous findings of Karon *et al*^[90] who noted similar findings with regard to the development of adverse effects in the membranes of stored RBCs including enhanced lipid peroxidation and build-up of extracellular Hb. Thus the evidence suggests that oxidatively mediated haemolytic changes to RBC membranes and damage to iron binding proteins leads to release of iron from the RBCs into the extracellular medium during storage. The

finding that these changes may occur early in storage, coupled with the lack of antioxidant protection suggests that the released iron can generate more free radical species and potentially initiate a vicious cycle of oxidative damage and iron release. This scenario is supported by the pattern of changes in heme and MDA, with the rise in both parameters initially being gradual over the first 14-21 d of storage and then more rapidly during the latter stages^[48,85]. An alternative, but related hypothesis, to account for iron-induced adverse effects has been presented by Hod *et al*^[91,92]. Their work using a murine model of transfusion with stored blood has suggested that extravascular haemolysis of transfused RBC's by macrophage-mediated phagocytosis leads to a pro-inflammatory response which is associated with increased circulating NTBI. Furthermore, reactive oxygen species induced by NTBI may mediate cytokine production and promote the pro-inflammatory response. These studies were followed up by investigating the situation in healthy human adults^[93] and premature babies^[83]. Both studies reported transfusion mediated increases in NTBI, and this was associated with increased oxidative stress in the premature babies^[83]. However, neither study was able to demonstrate the increased appearance of pro-inflammatory cytokines. Thus the involvement of NTBI in transfusion related immunomodulation in premature babies has yet to be established^[83], although immune modulation mediated by other components that increase during storage (such as heme) may occur^[42].

The enhanced NTBI levels seen post-transfusion may also promote bacterial infection, a factor of relevance to conditions such as chronic lung disease of prematurity^[4]. It is also suggested that heme present in transfused blood may promote nosocomial infection through its effect on the innate immune system^[42].

To develop these ideas further, some understanding of how well the premature baby is able to handle an enhanced transfusion-mediated iron load is required.

THE ABILITY OF THE PREMATURE BABY TO DEAL WITH TRANSFUSION MEDIATED IRON AND OXIDATIVE LOAD

Because of the physiological importance of iron and the potential toxic effects of free iron the body is equipped with a very precise homeostatic mechanism to regulate iron bioavailability^[61]. The function of the major components of this system in premature babies has been investigated in a number of studies. Studies into iron status and iron binding and transporting proteins in premature babies are complicated because the levels of these proteins may be influenced by oxidative stress and free iron levels^[94,95]. Although results are not always conclusive, the results suggest that both the levels and binding capacity of transferrin in the plasma of premature babies is low^[96-99]. The most recent study^[100] showed that premature babies had elevated iron and percentage iron binding

levels compared to normal reference values. This was particularly so in male babies, who tend to show a greater degree of morbidity than their female counterparts^[101,102]. In addition to the specific iron binding to transferrin, albumin may also play a role as an antioxidant by binding free iron and limiting the ability of iron to generate free radicals^[103]. The ability of albumin to bind iron seems to be particularly important as a defence mechanism against iron induced oxidative damage^[60]. Studies have reported significantly lower serum albumin levels in premature infants compared with term infants^[104]. Serum albumin in premature infants is particularly susceptible to oxidative damage^[105] which would further limit its ability to bind iron. Caeruloplasmin, which converts iron to the form necessary to bind to transferrin may also be low in prematures. Serum hepcidin concentrations were lower in preterm infants than full term babies^[106]. This was believed to reflect the lower total iron stores of premature babies. There was a good correlation between hepcidin levels and the levels of ferritin and erythropoietic activity. However, it was not possible to detect a significant correlation between hepcidin and transferrin levels or transferrin saturation, despite this population of babies having low transferrin levels and high transferrin saturation^[96,100]. Post-transfusional changes in serum hepcidin have not been studied in premature babies, but oral iron supplementation in low birth weight infants led to increases in circulating hepcidin^[107] as did blood transfusions in adults with thalassemia^[108]. Should this also occur in premature babies it may enhance iron sequestration in macrophages and limit iron-induced toxicity. However, more studies are required to explore this further. Thus it appears that some aspects of the regulation of hepcidin activity by iron status appear to be functional in premature babies, but how well hepcidin is able to be upregulated in response to a transfusionally mediated enhanced iron load has yet to be elucidated. In addition to potential iron overload, the large rise in heme provides a second potentially toxic mediator in stored packed red blood cells. Free heme has both pro-oxidant and pro-inflammatory activity^[109,110] and many other potentially toxic activities^[111]. The potential toxicity of heme is limited by the presence of the heme binding protein hemopexin^[110]. Thus an adequate availability of hemopexin is necessary to prevent the toxic effects of heme. Premature babies have very low levels of hemopexin^[112] which makes them vulnerable to the effects of transfusion mediated heme overload.

The results of studies on the antioxidant status of premature babies are largely in agreement. It appears that they have limited antioxidant defences to protect against circulating free radicals^[5,4,113-116]. With regard to the low molecular weight antioxidants ascorbate, urate and possibly glutathione in serum and bronchoalveolar lavage fluid in premature babies, studies have shown that the levels of these antioxidants fall during the first week of life and recover over the next few weeks^[5,4,113,117]. Premature babies who require blood transfusions will receive their first transfusion, and possibly the majority of their treatments within

the first week of life. Thus the receipt of blood, with the possibility of generating excessive free radicals, coincides with a period when antioxidant protection through the low molecular weight antioxidants is falling. Studies on the major enzymic antioxidants in premature babies have shown reduced levels of glutathione peroxidase and superoxide dismutase^[116]. Furthermore, the ability to upregulate pulmonary superoxide dismutase in response to inspired O₂ and free radicals is impaired in premature babies^[118] and animal models^[119,120]. Similarly, peroxiredoxin does not appear to upregulate in preterm baboons in response to high inspired O₂ concentrations^[121].

In summary, the premature baby appears to be poorly equipped to deal with any form of heme and iron overload and subsequent iron induced oxidative stress. Consequently the premature baby is likely to be at risk of transfusion-related heme, iron and oxidative overload.

POSTTRANSFUSIONAL CHANGES IN IRON AND OXIDATIVE STATUS IN PREMATURE BABIES

A limited number of studies have examined iron status in premature babies following the receipt of blood transfusions. Assessment of premature babies at 35 wk post menstrual age indicated that 50% of babies who received more than 3 erythrocyte infusions were iron overloaded at that stage of their care irrespective of when they received the transfusions^[122]. Earlier studies^[123] showed no difference in plasma bleomycin-detectable iron (NTBI) between babies who did or did not receive blood transfusions. However, the total number of samples containing bleomycin-detectable iron was significantly greater in babies who developed BPD compared to those who did not. Later studies by Hirano *et al*^[124] showed that bleomycin-detectable iron was present in 30% of premature babies before transfusion and rose to 80% after transfusion. Measurement of total iron in premature babies (post mortem) showed that those who received more than 100 mL of blood had a higher total serum iron level than those who received less than 100 mL^[125]. More recently, Dani *et al*^[126] found that the plasma level of NTBI increased significantly following blood transfusion, but that this was not associated with any evidence of increased oxidative stress in plasma up to 3 h after transfusion. In contrast to this, studies in our laboratory showed that pulmonary oxidative stress increased following blood transfusion^[2], and in babies that received more than one transfusion oxidative stress increased after each transfusion. The most recent study^[83] supports our findings showing increases in blood MDA following blood transfusion in premature babies. NTBI also increased and was correlated to the storage age of the packed cells transfused. Positive correlations between NTBI and MDA were also reported. The effect of transfusion on NTBI was transient, as was the increase in pulmonary MDA seen in our studies following transfusion^[2]. The

major difference between the studies of Collard *et al*^[61] and Stark *et al*^[83] and those of Dani *et al*^[126] was the age of the babies studied. In the study by Dani *et al*^[126] the gestational age of the babies studied was almost 10 wk greater than those studied in our study, and also older than those studied by Stark *et al*^[83]. The antioxidant capacity, which increases with gestational age and birth weight^[127], may have been sufficiently well developed to deal with the pro-oxidant effect of iron in the babies studied by Dani *et al*^[126] but not in those in the other two studies. This interpretation is supported by the findings of Minghetti *et al*^[128] who showed that the antioxidant capacity of weight disparate twin babies was lower in the smaller babies and associated with enhanced lipid peroxidation. This adds to the previous data that indicated that there may be a subset of smaller lower gestational age babies which are particularly vulnerable to transfusion related morbidities. Little is known about post-transfusional changes in free heme in premature babies. This lack of knowledge needs rectifying urgently.

INTERIM SUMMARY, IMPLICATIONS FOR FUTURE STUDIES AND FOR BLOOD TRANSFUSION PRACTICE IN THE NEONATAL INTENSIVE CARE UNIT

Premature babies, particularly those with low birth weight and gestational age appear to have little reserve to cope with any additional iron, heme and/or oxidative load.

Paediatric packed red blood cell units are a potential source of heme, redox active iron and free radicals, and this increases with storage age. Haemolysis of transfused red blood cells may add further iron and cell free haemoglobin to the recipient baby.

The link between the receipt of packed cell transfusions and the complications of prematurity may be due to some extent to the additional heme, iron and oxidative load caused by transfusion. This relationship may be particularly significant in low birth weight and gestational age babies.

In order to develop this idea further or to refute these suggestions there is an urgent need to conduct appropriate clinical studies. Clinical studies have shown that blood stored for an average of 14 d does not cause any additional complications when compared with fresh (5 d storage) blood^[72]. This fits well with the findings of the biochemical studies which indicate that changes in the parameters discussed above progress slowly over the first 14-21 d and then change more rapidly. The effect of blood stored for longer periods has yet to be established in this group of babies. This would be difficult to study by means of a prospective randomised trial because it would be unethical to randomly subject babies to receive blood stored for more than 14-21 d. An alternative strategy would be to conduct a study in which all babies in a neonatal intensive care unit receive blood stored for

less than 14 d for an appropriate period (say 2 years) and compare outcomes with data from the previous 2 years in which blood stored for up to 35 d was routinely used.

Perhaps the best way of obtaining data in a shorter time span would be to conduct a retrospective study using clinical records from neonatal intensive care units in which the storage age of the blood used was recorded. This would allow the categorisation of data into groups in which blood beyond 14 d storage could be compared with those receiving blood less than 14 d old. This approach has recently been suggested by Flegel^[129]. Because of the multifactorial nature of the clinical conditions under investigation, all confounding variables will need to be recorded and a detailed multifactorial analysis conducted in order to tease out the relative risk factors of all the variables^[4] including storage age of blood used.

There are also some modifications to the preparation of the paediatric packed red cell units which might be investigated in an attempt to limit the availability of redox active iron and free radicals in the units. This could include the addition of haptoglobin, hemopexin, apotransferrin and/or antioxidants to the additive fluid. The effects could be easily evaluated *in vitro* and in animal models, but transferring the findings to clinical applications would be difficult in premature babies without detailed studies on the safety and efficacy of such preparations in appropriate human subjects.

In the short term, until the outcome of further clinical studies is known, it might make sense to limit the storage age of blood given to premature babies to 14 d. This is supported by studies which indicated that, from a biochemical and molecular standpoint, the parameters defining the integrity of saline, adenine, glucose mannitol stored leucoreduced red blood cells may be acceptable up to 14 d of storage, but then decline^[84], and clinical studies have shown no additional adverse effects of blood stored for a mean age of 14 d compared to fresh blood^[74]. A cut-off point of 14 d is supported by many other studies. Blood stored for more than 14 d was associated with multiple organ dysfunction and prolonged stay in the paediatric intensive care unit^[65]. In adults, the incidence of bacterial infection increased following transfusion with blood stored for more than 14 d^[39], and the incidence of mortality almost doubled in those patients receiving blood stored for more than 14 d compared to those receiving blood stored for 7 d^[130]. The most detailed investigation conducted on adults undergoing cardiac surgery^[131] showed conclusively that blood that had been stored for more than 14 d was associated with significantly increased risks of post operative complications, in-hospital mortality and poorer long term outcome compared to patients receiving fresher blood. The study investigated large numbers of patients and provided strong statistical power. Thus the biochemical and clinical data support the view that to reduce the incidence of morbidity and mortality in patients requiring transfusions with packed red blood cells, the blood should be stored for no more than 14 d. Limiting storage age to 14 d would have clear logistic and cost implications. Limiting the maximum

shelf life from 35 d to 7 d is predicted to result in a 50% decrease in the number of available units and a fourfold increase in the number of units outdated each year^[132]. An expiration date of 14 d would have a significant impact on hospital reserves, and would require a substantial increase in collections to preserve hospital stocks^[132]. These figures are based on blood group matched adult units. The situation regarding O negative paediatric packs for use in premature babies would be more disruptive. It may make sense to conduct a small pilot study to fully evaluate the feasibility of such a change in practice.

In addition as there appears to be a subset of premature babies which are particularly vulnerable to the adverse effects of transfusion, we should already be giving the freshest blood available to the smallest and youngest babies. The need to re-evaluate transfusion practice with regard to the storage age of the blood has recently been suggested for blood transfusion in adults^[129]. There is perhaps a more urgent need to do the same in premature babies who are probably at a greater risk of transfusion mediated morbidity than adults.

REFERENCES

- 1 **Valieva OA**, Strandjord TP, Mayock DE, Juul SE. Effects of transfusions in extremely low birth weight infants: a retrospective study. *J Pediatr* 2009; **155**: 331-337.e1 [PMID: 19732577 DOI: 10.1016/j.peds.2009.02.26]
- 2 **Collard KJ**, Godeck S, Holley JE. Blood transfusion and pulmonary lipid peroxidation in ventilated premature babies. *Pediatr Pulmonol* 2005; **39**: 257-261 [PMID: 15668937]
- 3 **Silvers KM**, Gibson AT, Russell JM, Powers HJ. Antioxidant activity, packed cell transfusions, and outcome in premature infants. *Arch Dis Child Fetal Neonatal Ed* 1998; **78**: F214-F219 [PMID: 9713035]
- 4 **Collard KJ**, Godeck S, Holley JE, Quinn MW. Pulmonary antioxidant concentrations and oxidative damage in ventilated premature babies. *Arch Dis Child Fetal Neonatal Ed* 2004; **89**: F412-F416 [PMID: 15321959]
- 5 **Shohat M**, Reisner SH, Krikler R, Nissenkorn I, Yassar Y, Ben-Sira I. Retinopathy of prematurity: incidence and risk factors. *Pediatrics* 1983; **72**: 159-163 [PMID: 6408596]
- 6 **Dani C**, Reali MF, Bertini G, Martelli E, Pezzati M, Rubaltelli FF. The role of blood transfusions and iron intake on retinopathy of prematurity. *Early Hum Dev* 2001; **62**: 57-63 [PMID: 11245995]
- 7 **Binkhathlan AA**, Almahmoud LA, Saleh MJ, Srungeri S. Retinopathy of prematurity in Saudi Arabia: incidence, risk factors, and the applicability of current screening criteria. *Br J Ophthalmol* 2008; **92**: 167-169 [PMID: 18227198 DOI: 10.1136/bjo]
- 8 **Karkhaneh R**, Mousavi SZ, Riazi-Esfahani M, Ebrahimzadeh SA, Roohipoor R, Kadivar M, Ghalichi L, Mohammadi SF, Mansouri MR. Incidence and risk factors of retinopathy of prematurity in a tertiary eye hospital in Tehran. *Br J Ophthalmol* 2008; **92**: 1446-1449 [PMID: 18728050 DOI: 10.1136/bjo.2008.145136]
- 9 **Baer VL**, Lambert DK, Henry E, Snow GL, Butler A, Christensen RD. Among very-low-birth-weight neonates is red blood cell transfusion an independent risk factor for subsequently developing a severe intraventricular hemorrhage? *Transfusion* 2011; **51**: 1170-1178 [PMID: 21166684]
- 10 **Lee JY**, Kim HS, Jung E, Kim ES, Shim GH, Lee HJ, Lee JA, Choi CW, Kim EK, Kim BI, Choi JH. Risk factors for periventricular-intraventricular hemorrhage in premature infants. *J Korean Med Sci* 2010; **25**: 418-424 [PMID: 20191041 DOI:

- 10.3346/jkms.2010.25.3.418]
- 11 **El-Dib M**, Narang S, Lee E, Massaro AN, Aly H. Red blood cell transfusion, feeding and necrotizing enterocolitis in preterm infants. *J Perinatol* 2011; **31**: 183-187 [PMID: 21252964 DOI: 10.1038/jp.2010.157]
 - 12 **Romagnoli C**. Risk factors and growth factors in ROP. *Early Hum Dev* 2009; **85**: S79-S82 [PMID: 19828269 DOI: 10.1016/j.earlhumdev.2009.08.026]
 - 13 **Wan-Huen P**, Bateman D, Shapiro DM, Parravicini E. Packed red blood cell transfusion is an independent risk factor for necrotizing enterocolitis in premature infants. *J Perinatol* 2013; **33**: 786-790 [PMID: 23702619 DOI: 10.1038/jp.3013.60]
 - 14 **Bak SY**, Lee S, Park JH, Park KH, Jeon JH. Analysis of the association between necrotizing enterocolitis and transfusion of red blood cell in very low birth weight preterm infants. *Korean J Pediatr* 2013; **56**: 112-115 [PMID: 23559972]
 - 15 **Marin T**, Moore J, Kosmetatos N, Roback JD, Weiss P, Higgins M, McCauley L, Strickland OL, Josephson CD. Red blood cell transfusion-related necrotizing enterocolitis in very-low-birthweight infants: a near-infrared spectroscopy investigation. *Transfusion* 2013; **53**: 2650-2658 [PMID: 23480548 DOI: 10.1111/trf.12158]
 - 16 **Christensen RD**, Baer VL, Lambert DK, Ilstrup SJ, Eggert LD, Henry E. Association, among very-low-birthweight neonates, between red blood cell transfusions in the week after birth and severe intraventricular hemorrhage. *Transfusion* 2014; **54**: 104-108 [PMID: 23672455]
 - 17 **Josephson CD**, Wesolowski A, Bao G, Sola-Visner MC, Dudell G, Castillejo MI, Shaz BH, Easley KA, Hillyer CD, Maheshwari A. Do red cell transfusions increase the risk of necrotizing enterocolitis in premature infants? *J Pediatr* 2010; **157**: 972-978.e1-3 [PMID: 20650470 DOI: 10.1016/j.jpeds.2010.05.046]
 - 18 **Eder AF**, Chambers LA. Noninfectious complications of blood transfusion. *Arch Pathol Lab Med* 2007; **131**: 708-718 [PMID: 17488156]
 - 19 **Collard KJ**. Is there a causal relationship between the receipt of blood transfusions and the development of chronic lung disease of prematurity? *Med Hypotheses* 2006; **66**: 355-364 [PMID: 16236459]
 - 20 **Venkatesh V**, Khan R, Curley A, Hopewell S, Doree C, Stanworth S. The safety and efficacy of red cell transfusions in neonates: a systematic review of randomized controlled trials. *Br J Haematol* 2012; **158**: 370-385 [PMID: 22639894 DOI: 10.1111/j.1365-2141.2012.09180.x]
 - 21 **Blau J**, Calo JM, Dozor D, Sutton M, Alpan G, La Gamma EF. Transfusion-related acute gut injury: necrotizing enterocolitis in very low birth weight neonates after packed red blood cell transfusion. *J Pediatr* 2011; **158**: 403-409 [PMID: 21067771 DOI: 10.1016/j.jpeds]
 - 22 **Reynolds JD**, Hess DT, Stampler JS. The transfusion problem: role of aberrant S-nitrosylation. *Transfusion* 2011; **51**: 852-858 [PMID: 21496046 DOI: 10.1111/j.1537-2995.2011.03097.x]
 - 23 **Alexander JT**, El-Ali AM, Newman JL, Karatela S, Predmore BL, Lefer DJ, Sutliff RL, Roback JD. Red blood cells stored for increasing periods produce progressive impairments in nitric oxide-mediated vasodilation. *Transfusion* 2013; **53**: 2619-2628 [PMID: 23480490 DOI: 10.1111/trf.12111]
 - 24 **Gladwin MT**, Kim-Shapiro DB. Storage lesion in banked blood due to hemolysis-dependent disruption of nitric oxide homeostasis. *Curr Opin Hematol* 2009; **16**: 515-523 [PMID: 19701085]
 - 25 **Kanias T**, Gladwin MT. Nitric oxide, hemolysis, and the red blood cell storage lesion: interactions between transfusion, donor, and recipient. *Transfusion* 2012; **52**: 1388-1392 [PMID: 22780890 DOI: 10.1111/j.1537-2995.2012.03748.x]
 - 26 **Stritzke AI**, Smyth J, Synnes A, Lee SK, Shah PS. Transfusion-associated necrotizing enterocolitis in neonates. *Arch Dis Child Fetal Neonatal Ed* 2013; **98**: F10-F14 [PMID: 22447991 DOI: 10.1136/fetalneonatal-2011-30128]
 - 27 **Locke R**, Paul D, Touch S, Mackley A, Maduskuie V, Fawcett P. Cytokine load in prestorage leukoreduced PRBC transfusions in premature infants. *J Perinatol* 2005; **25**: 526-530 [PMID: 15908985]
 - 28 **La Gamma EF**, Blau J. Transfusion-related acute gut injury: feeding, flora, flow, and barrier defense. *Semin Perinatol* 2012; **36**: 294-305 [PMID: 22818551 DOI: 10.1053/j.semperi]
 - 29 **Singh R**, Visintainer PF, Frantz ID, Shah BL, Meyer KM, Favila SA, Thomas MS, Kent DM. Association of necrotizing enterocolitis with anemia and packed red blood cell transfusions in preterm infants. *J Perinatol* 2011; **31**: 176-182 [PMID: 21273983 DOI: 10.1038/jp.2010.145]
 - 30 **Demirel G**, Celik IH, Aksoy HT, Erdevi O, Oguz SS, Uras N, Dilmen U. Transfusion-associated necrotizing enterocolitis in very low birth weight premature infants. *Transfus Med* 2012; **22**: 332-337 [PMID: 22738152]
 - 31 **Reynolds JD**, Ahearn GS, Angelo M, Zhang J, Cobb F, Stampler JS. S-nitrosohemoglobin deficiency: a mechanism for loss of physiological activity in banked blood. *Proc Natl Acad Sci USA* 2007; **104**: 17058-17062 [PMID: 17940022]
 - 32 **Hess JR**. Red cell changes during storage. *Transfus Apher Sci* 2010; **43**: 51-59 [PMID: 20558107 DOI: 10.2450/2010.003s]
 - 33 **Chen-Roetling J**, Chen L, Regan RF. Apotransferrin protects cortical neurons from hemoglobin toxicity. *Neuropharmacology* 2011; **60**: 423-431 [PMID: 21034753 DOI: 10.1016/j.neuropharm]
 - 34 **Caliaperumal J**, Ma Y, Colbourne F. Intra-parenchymal ferrous iron infusion causes neuronal atrophy, cell death and progressive tissue loss: implications for intracerebral hemorrhage. *Exp Neurol* 2012; **237**: 363-369 [PMID: 22809564 DOI: 10.1016/j.expneurol.2012.07.001]
 - 35 **Akkoyun I**, Oto S, Yilmaz G, Gurakan B, Tarcan A, Anuk D, Akgun S, Akova YA. Risk factors in the development of mild and severe retinopathy of prematurity. *J AAPOS* 2006; **10**: 449-453 [PMID: 17070481]
 - 36 **Korhonen P**, Tammela O, Koivisto AM, Laippala P, Ikonen S. Frequency and risk factors in bronchopulmonary dysplasia in a cohort of very low birth weight infants. *Early Hum Dev* 1999; **54**: 245-258 [PMID: 10321791]
 - 37 **Hernández-Ronquillo L**, Téllez-Zenteno JF, Weder-Cisneros N, Salinas-Ramírez V, Zapata-Pallagi JA, da Silva O. Risk factors for the development of bronchopulmonary dysplasia: a case-control study. *Arch Med Res* 2004; **35**: 549-553 [PMID: 15631883]
 - 38 **Zhang H**, Fang J, Su H, Chen M. Risk factors for bronchopulmonary dysplasia in neonates born at ≤ 1500 g (1999-2009). *Pediatr Int* 2011; **53**: 915-920 [PMID: 21605281 DOI: 10.1111/j.1442-200x.2011.05399.x]
 - 39 **Juffermans NP**, Vlaar AP, Prins DJ, Goslings JC, Binnekade JM. The age of red blood cells is associated with bacterial infections in critically ill trauma patients. *Blood Transfus* 2012; **10**: 290-295 [PMID: 22395349 DOI: 10.2450/2012.0068-11]
 - 40 **Schaible UE**, Kaufmann SH. Iron and microbial infection. *Nat Rev Microbiol* 2004; **2**: 946-953 [PMID: 15550940]
 - 41 **Collard K**, White D, Copplestone A. The effect of maximum storage on iron status, oxidative stress and antioxidant protection in paediatric packed cell units. *Blood Transfus* 2013; **11**: 419-425 [PMID: 23058860 DOI: 10.2450/2012.0046-12]
 - 42 **Neal MD**, Raval JS, Triulzi DJ, Simmons RL. Innate immune activation after transfusion of stored red blood cells. *Transfus Med Rev* 2013; **27**: 113-118 [PMID: 23434246 DOI: 10.1016/j.jtmrv.2012.01.001]
 - 43 **Collard K**, White D, Copplestone A. The influence of storage age on iron status, oxidative stress and antioxidant protection in paediatric packed cell units. *Blood Transfus* 2014; **12**: 210-219 [PMID: 24333084 DOI: 10.2450/2013.0142-13]
 - 44 **Yalcin O**, Ortiz D, Tsai AG, Johnson PC, Cabrales P. Microhemodynamic aberrations created by transfusion of stored blood. *Transfusion* 2014; **54**: 1045-1027 [DOI: 10.1111/trf.12361]
 - 45 **Liu C**, Zhao W, Christ GJ, Gladwin MT, Kim-Shapiro DB.

- Nitric oxide scavenging by red cell microparticles. *Free Radic Biol Med* 2013; **65**: 1164-1173 [PMID: 24051181 DOI: 10.1016/j.freeradbiomed.2013.09.002]
- 46 **Vermeulen Windsant IC**, de Wit NC, Sertorio JT, Beckers EA, Tanus-Santos JE, Jacobs MJ, Buurman WA. Blood transfusions increase circulating plasma free hemoglobin levels and plasma nitric oxide consumption: a prospective observational pilot study. *Crit Care* 2012; **16**: R95 [PMID: 22624585 DOI: 10.1186/cc.11359]
- 47 **Minneci PC**, Deans KJ, Zhi H, Yuen PS, Star RA, Banks SM, Schechter AN, Natanson C, Gladwin MT, Solomon SB. Hemolysis-associated endothelial dysfunction mediated by accelerated NO inactivation by decompartmentalized oxyhemoglobin. *J Clin Invest* 2005; **115**: 3409-3417 [PMID: 16294219 DOI: 10.1172/JCI25040]
- 48 **Collard KJ**, White DL. On the source of the non-transferrin-bound iron which accumulates in packed red blood cell units during storage. *Blood Transfusion* 2014; Epub ahead of print [PMID: 24960650 DOI: 10.2450/2014.0271-13]
- 49 **Weintraub Z**, Carmi N, Elouti H, Rumelt S. The association between stage 3 or higher retinopathy of prematurity and other disorders of prematurity. *Can J Ophthalmol* 2011; **46**: 419-424 [PMID: 21995985 DOI: 10.1016/j.cjco.2011.07.04]
- 50 **Mercer JS**, Vohr BR, McGrath MM, Padbury JF, Wallach M, Oh W. Delayed cord clamping in very preterm infants reduces the incidence of intraventricular hemorrhage and late-onset sepsis: a randomized, controlled trial. *Pediatrics* 2006; **117**: 1235-1242 [PMID: 16585320]
- 51 **Rabe H**, Reynolds G, Diaz-Rossello J. Early versus delayed umbilical cord clamping in preterm infants. *Cochrane Database Syst Rev* 2004; (4): CD003248 [PMID: 15495045]
- 52 **Rabe H**, Diaz-Rossello JL, Duley L, Dowswell T. Effect of timing of umbilical cord clamping and other strategies to influence placental transfusion at preterm birth on maternal and infant outcomes. *Cochrane Database Syst Rev* 2012; **8**: CD003248 [PMID: 22895933 DOI: 10.1002/14651858.CD003248]
- 53 **Kirpalani H**, Whyte RK, Andersen C, Asztalos EV, Heddle N, Blajchman MA, Peliowski A, Rios A, LaCorte M, Connelly R, Barrington K, Roberts RS. The Premature Infants in Need of Transfusion (PINT) study: a randomized, controlled trial of a restrictive (low) versus liberal (high) transfusion threshold for extremely low birth weight infants. *J Pediatr* 2006; **149**: 301-307 [PMID: 16939737]
- 54 **Bell EF**. Transfusion thresholds for preterm infants: how low should we go? *J Pediatr* 2006; **149**: 287-289 [PMID: 16939732]
- 55 **Von Kohorn I**, Ehrenkranz RA. Anemia in the preterm infant: erythropoietin versus erythrocyte transfusion--it's not that simple. *Clin Perinatol* 2009; **36**: 111-123 [PMID: 19161869 DOI: 10.1016/j.clp.09.009]
- 56 **Takami T**, Suganami Y, Sunohara D, Kondo A, Mizukaki N, Fujioka T, Hoshika A, Akutagawa O, Isaka K. Umbilical cord milking stabilizes cerebral oxygenation and perfusion in infants born before 29 weeks of gestation. *J Pediatr* 2012; **161**: 742-747 [PMID: 22578578 DOI: 10.1016/j.jpeds.2012.03.053]
- 57 **Christensen RD**. Associations between "early" red blood cell transfusion and severe intraventricular hemorrhage, and between "late" red blood cell transfusion and necrotizing enterocolitis. *Semin Perinatol* 2012; **36**: 283-289 [PMID: 22818549]
- 58 **Carroll PD**, Widness JA. Nonpharmacological, blood conservation techniques for preventing neonatal anemia--effective and promising strategies for reducing transfusion. *Semin Perinatol* 2012; **36**: 232-243 [PMID: 22818543]
- 59 **Sloan SR**. Neonatal transfusion review. *Paediatr Anaesth* 2011; **21**: 25-30 [PMID: 21155924 DOI: 10.1111/j.1460-9592.2010.03458.x]
- 60 **Loban A**, Kime R, Powers H. Iron-binding antioxidant potential of plasma albumin. *Clin Sci (Lond)* 1997; **93**: 445-451 [PMID: 9486090]
- 61 **Collard KJ**. Iron homeostasis in the neonate. *Pediatrics* 2009; **123**: 1208-1216 [PMID: 19336381]
- 62 **Levy GJ**, Strauss RG, Hume H, Schloz L, Albanese MA, Blazina J, Werner A, Sotelo-Avila C, Barrasso C, Blanchette V. National survey of neonatal transfusion practices: I. Red blood cell therapy. *Pediatrics* 1993; **91**: 523-529 [PMID: 8441554]
- 63 **Mangel J**, Goldman M, Garcia C, Spurl G. Reduction of donor exposures in premature infants by the use of designated adenine-saline preserved split red blood cell packs. *J Perinatol* 2001; **21**: 363-367 [PMID: 11593369]
- 64 **Gauvin F**, Spinella PC, Lacroix J, Choker G, Ducruet T, Karam O, Hébert PC, Hutchison JS, Hume HA, Tucci M. Association between length of storage of transfused red blood cells and multiple organ dysfunction syndrome in pediatric intensive care patients. *Transfusion* 2010; **50**: 1902-1913 [PMID: 20456697 DOI: 10.1111/j.1537-2995.2010.02661.x]
- 65 **Karam O**, Tucci M, Bateman ST, Ducruet T, Spinella PC, Randolph AG, Lacroix J. Association between length of storage of red blood cell units and outcome of critically ill children: a prospective observational study. *Crit Care* 2010; **14**: R57 [PMID: 20377853 DOI: 10.1186/cc.8953]
- 66 **Eikelboom JW**, Cook RJ, Liu Y, Heddle NM. Duration of red cell storage before transfusion and in-hospital mortality. *Am Heart J* 2010; **159**: 737-743.e1 [PMID: 20435180 DOI: 10.1016/j.ahj]
- 67 **van de Watering L**. Pitfalls in the current published observational literature on the effects of red blood cell storage. *Transfusion* 2011; **51**: 1847-1854 [PMID: 21831185 DOI: 10.1111/j.1537-2995.2010.03015.x]
- 68 **Isbister JP**, Shander A, Spahn DR, Erhard J, Farmer SL, Hofmann A. Adverse blood transfusion outcomes: establishing causation. *Transfus Med Rev* 2011; **25**: 89-101 [PMID: 21345639 DOI: 10.1016/j.tmr.2010.11.001]
- 69 **Lelubre C**, Piagnerelli M, Vincent JL. Association between duration of storage of transfused red blood cells and morbidity and mortality in adult patients: myth or reality? *Transfusion* 2009; **49**: 1384-1394 [PMID: 19453985 DOI: 10.1111/j.1537-2995.2009.02211.x]
- 70 **Steiner ME**, Stowell C. Does red blood cell storage affect clinical outcome? When in doubt, do the experiment. *Transfusion* 2009; **49**: 1286-1290 [PMID: 19602209 DOI: 10.1111/j.1537-2995.2009.02265.x]
- 71 **Triulzi DJ**, Yazer MH. Clinical studies of the effect of blood storage on patient outcomes. *Transfus Apher Sci* 2010; **43**: 95-106 [PMID: 20656558 DOI: 10.1016/j.transci]
- 72 **Steiner ME**, Assmann SF, Levy JH, Marshall J, Pulkrabek S, Sloan SR, Triulzi D, Stowell CP. Addressing the question of the effect of RBC storage on clinical outcomes: the Red Cell Storage Duration Study (RECESS) (Section 7). *Transfus Apher Sci* 2010; **43**: 107-116 [PMID: 20655807 DOI: 10.1016/j.transci.2010.05.014]
- 73 **Fergusson D**, Hutton B, Hogan DL, LeBel L, Blajchman MA, Ford JC, Hebert P, Kakadekar A, Kovacs L, Lee S, Sankaran K, Shapiro S, Smyth JA, Ramesh K, Bouali NR, Tinmouth A, Walker R. The age of red blood cells in premature infants (ARIPI) randomized controlled trial: study design. *Transfus Med Rev* 2009; **23**: 55-61 [PMID: 19056034 DOI: 10.1016/j.tmr]
- 74 **Fergusson DA**, Hébert P, Hogan DL, LeBel L, Rouvinez-Bouali N, Smyth JA, Sankaran K, Tinmouth A, Blajchman MA, Kovacs L, Lachance C, Lee S, Walker CR, Hutton B, Ducharme R, Balchin K, Ramsay T, Ford JC, Kakadekar A, Ramesh K, Shapiro S. Effect of fresh red blood cell transfusions on clinical outcomes in premature, very low-birth-weight infants: the ARIPI randomized trial. *JAMA* 2012; **308**: 1443-1451 [PMID: 23045213 DOI: 10.1001/2012.jama.11953]
- 75 **Ferrali M**, Signorini C, Ciccoli L, Comporti M. Iron release and membrane damage in erythrocytes exposed to oxidizing agents, phenylhydrazine, divicine and isouramil. *Biochem J* 1992; **285** (Pt 1): 295-301 [PMID: 1637315]

- 76 **Comporti M**, Signorini C, Buonocore G, Ciccoli L. Iron release, oxidative stress and erythrocyte ageing. *Free Radic Biol Med* 2002; **32**: 568-576 [PMID: 11909691]
- 77 **Kanias T**, Acker JP. Biopreservation of red blood cells--the struggle with hemoglobin oxidation. *FEBS J* 2010; **277**: 343-356 [PMID: 19968714 DOI: 10.1111/j.1742-4658.07472.x]
- 78 **Ozment CP**, Turi JL. Iron overload following red blood cell transfusion and its impact on disease severity. *Biochim Biophys Acta* 2009; **1790**: 694-701 [PMID: 18992790 DOI: 10.1016/j.bbagen]
- 79 **Marwah SS**, Blann A, Harrison P, Lumley MA, Wright J, McDowell J, Phillips JD, Rea C, Bareford D. Increased non-transferrin bound iron in plasma-depleted SAG-M red blood cell units. *Vox Sang* 2002; **82**: 122-126 [PMID: 11952985]
- 80 **Sowemimo-Coker SO**. Red blood cell hemolysis during processing. *Transfus Med Rev* 2002; **16**: 46-60 [PMID: 11788929]
- 81 **Bennett-Guerrero E**, Veldman TH, Doctor A, Telen MJ, Ortel TL, Reid TS, Mulherin MA, Zhu H, Buck RD, Califf RM, McMahon TJ. Evolution of adverse changes in stored RBCs. *Proc Natl Acad Sci USA* 2007; **104**: 17063-17068 [PMID: 17940021]
- 82 **Phelan HA**, Gonzalez RP, Patel HD, Caudill JB, Traylor RK, Yancey LR, Sperry JL, Friese RS, Nakonezny PA. Prestorage leukoreduction ameliorates the effects of aging on banked blood. *J Trauma* 2010; **69**: 330-337 [PMID: 20699741 DOI: 10.1097/TA.0b013e3181e0b253]
- 83 **Stark MJ**, Keir AK, Andersen CC. Does non-transferrin bound iron contribute to transfusion related immunomodulation in preterms? *Arch Dis Child Fetal Neonatal Ed* 2013; **98**: F424-F429 [PMID: 23475935 DOI: 10.1136/archdischild-2012-203353]
- 84 **D'Alessandro A**, D'Amici GM, Vaglio S, Zolla L. Time-course investigation of SAGM-stored leukocyte-filtered red blood cell concentrates: from metabolism to proteomics. *Hematologica* 2012; **97**: 107-115 [PMID: 21993682]
- 85 **Ozment CP**, Mamo LB, Campbell ML, Lokhnygina Y, Ghio AJ, Turi JL. Transfusion-related biologic effects and free hemoglobin, heme, and iron. *Transfusion* 2013; **53**: 732-740 [PMID: 22882431 DOI: 10.1111/j.1537-2995]
- 86 **Signorini C**, Ferrali M, Ciccoli L, Sugherini L, Magnani A, Comporti M. Iron release, membrane protein oxidation and erythrocyte ageing. *FEBS Lett* 1995; **362**: 165-170 [PMID: 7720865]
- 87 **Nagababu E**, Rifkind JM. Heme degradation by reactive oxygen species. *Antioxid Redox Signal* 2004; **6**: 967-978 [PMID: 15548894]
- 88 **Dumaswala UJ**, Wilson MJ, Wu YL, Wykle J, Zhuo L, Douglass LM, Daleke DL. Glutathione loading prevents free radical injury in red blood cells after storage. *Free Radic Res* 2000; **33**: 517-529 [PMID: 11200085]
- 89 **Chaudhary R**, Katharia R. Oxidative injury as contributory factor for red cells storage lesion during twenty eight days of storage. *Blood Transfus* 2012; **10**: 59-62 [PMID: 22044961 DOI: 10.2450/2011.0107-10]
- 90 **Karon BS**, van Buskirk CM, Jaben EA, Hoyer JD, Thomas DD. Temporal sequence of major biochemical events during blood bank storage of packed red blood cells. *Blood Transfus* 2012; **10**: 453-461 [PMID: 22507860 DOI: 10.2450/2012.0099-11]
- 91 **Hod EA**, Zhang N, Sokol SA, Wojczyk BS, Francis RO, Ansaldi D, Francis KP, Della-Latta P, Whittier S, Sheth S, Hendrickson JE, Zimring JC, Brittenham GM, Spitalnik SL. Transfusion of red blood cells after prolonged storage produces harmful effects that are mediated by iron and inflammation. *Blood* 2010; **115**: 4284-4292 [PMID: 20299509 DOI: 10.1182/blood-2009-10-245001]
- 92 **Hod EA**, Spitalnik SL. Stored red blood cell transfusions: Iron, inflammation, immunity, and infection. *Transfus Clin Biol* 2012; **19**: 84-89 [PMID: 22682673 DOI: 10.1016/j.trcli.2012.04.001]
- 93 **Hod EA**, Brittenham GM, Billote GB, Francis RO, Ginzburg YZ, Hendrickson JE, Jhang J, Schwartz J, Sharma S, Sheth S, Sireci AN, Stephens HL, Stotler BA, Wojczyk BS, Zimring JC, Spitalnik SL. Transfusion of human volunteers with older, stored red blood cells produces extravascular hemolysis and circulating non-transferrin-bound iron. *Blood* 2011; **118**: 6675-6682 [PMID: 22021369 DOI: 10.1182/blood-2011-08-371849]
- 94 **Cairo G**, Pietrangelo A. Iron regulatory proteins in pathobiology. *Biochem J* 2000; **352** Pt 2: 241-250 [PMID: 11085915]
- 95 **Nappi AJ**, Vass E. Interactions of iron with reactive intermediates of oxygen and nitrogen. *Dev Neurosci* 2002; **24**: 134-142 [PMID: 12401951]
- 96 **Evans PJ**, Evans R, Kovar IZ, Holton AF, Halliwell B. Bleomycin-detectable iron in the plasma of premature and full-term neonates. *FEBS Lett* 1992; **303**: 210-212 [PMID: 1376697]
- 97 **Lindeman JH**, Lentjes EG, van Zoeren-Grobbe D, Berger HM. Postnatal changes in plasma ceruloplasmin and transferrin antioxidant activities in preterm babies. *Biol Neonate* 2000; **78**: 73-76 [PMID: 10970997]
- 98 **Moison RM**, Haasnoot AA, Van Zoeren-Grobbe D, Berger HM. Plasma proteins in acute and chronic lung disease of the newborn. *Free Radic Biol Med* 1998; **25**: 321-328 [PMID: 9680178]
- 99 **Lackmann GM**, Hesse L, Töllner U. Reduced iron-associated antioxidants in premature newborns suffering intracerebral hemorrhage. *Free Radic Biol Med* 1996; **20**: 407-409 [PMID: 8720912]
- 100 **Molloy EJ**, El-Khuffash A, Bieda A, Jelinek MM, Baley J. Elevated iron indices in preterm infants: association with male gender. *Am J Perinatol* 2009; **26**: 7-11 [PMID: 19023849 DOI: 10.1055/s-0028-1090588]
- 101 **Zisk JL**, Genen LH, Kirkby S, Webb D, Greenspan J, Dysart K. Do premature female infants really do better than their male counterparts? *Am J Perinatol* 2011; **28**: 241-246 [PMID: 21046537 DOI: 10.1055/j-0030-1268239]
- 102 **Binet ME**, Bujold E, Lefebvre F, Tremblay Y, Piedboeuf B. Role of gender in morbidity and mortality of extremely premature neonates. *Am J Perinatol* 2012; **29**: 159-166 [PMID: 21818733 DOI: 10.1055/s-0031-1284225]
- 103 **Fukuzawa K**, Saitoh Y, Akai K, Kogure K, Ueno S, Tokumura A, Otagiri M, Shibata A. Antioxidant effect of bovine serum albumin on membrane lipid peroxidation induced by iron chelate and superoxide. *Biochim Biophys Acta* 2005; **1668**: 145-155 [PMID: 15670740]
- 104 **Galiniere A**, Périquet B, Lambert W, Garcia J, Assouline C, Rolland M, Thouvenot JP. Reference range for micronutrients and nutritional marker proteins in cord blood of neonates appropriated for gestational ages. *Early Hum Dev* 2005; **81**: 583-593 [PMID: 16009283]
- 105 **Marzocchi B**, Perrone S, Paffetti P, Magi B, Bini L, Tani C, Longini M, Buonocore G. Nonprotein-bound iron and plasma protein oxidative stress at birth. *Pediatr Res* 2005; **58**: 1295-1299 [PMID: 16306211]
- 106 **Müller KF**, Lorenz L, Poets CF, Westerman M, Franz AR. Hepcidin concentrations in serum and urine correlate with iron homeostasis in preterm infants. *J Pediatr* 2012; **160**: 949-953.e2 [PMID: 22284565 DOI: 10.1016/j.jpeds.2011.12.030]
- 107 **Berglund S**, Lönnerdal B, Westrup B, Domellöf M. Effects of iron supplementation on serum hepcidin and serum erythropoietin in low-birth-weight infants. *Am J Clin Nutr* 2011; **94**: 1553-1561 [PMID: 22071701]
- 108 **Pasricha SR**, Frazer DM, Bowden DK, Anderson GJ. Transfusion suppresses erythropoiesis and increases hepcidin in adult patients with β -thalassemia major: a longitudinal study. *Blood* 2013; **122**: 124-133 [PMID: 23656728 DOI: 10.1182/blood-2012-12-471441]
- 109 **Jeney V**, Balla J, Yachie A, Varga Z, Vercellotti GM, Eaton JW, Balla G. Pro-oxidant and cytotoxic effects of circulating heme. *Blood* 2002; **100**: 879-887 [PMID: 12130498]
- 110 **Kumar S**, Bandyopadhyay U. Free heme toxicity and its detoxification systems in human. *Toxicol Lett* 2005; **157**: 175-188

- [PMID: 15917143]
- 111 **Tracz MJ**, Alam J, Nath KA. Physiology and pathophysiology of heme: implications for kidney disease. *J Am Soc Nephrol* 2007; **18**: 414-420 [PMID: 17229906]
 - 112 **Kanakoudi F**, Drossou V, Tzimouli V, Diamanti E, Konstantinidis T, Germeris A, Kremenopoulos G. Serum concentrations of 10 acute-phase proteins in healthy term and preterm infants from birth to age 6 months. *Clin Chem* 1995; **41**: 605-608 [PMID: 7536645]
 - 113 **Vyas JR**, Currie A, Dunster C, Kelly FJ, Kotecha S. Ascorbate acid concentration in airways lining fluid from infants who develop chronic lung disease of prematurity. *Eur J Pediatr* 2001; **160**: 177-184 [PMID: 11277380]
 - 114 **Turgut M**, Başaran O, Cekmen M, Karataş F, Kurt A, Aygün AD. Oxidant and antioxidant levels in preterm newborns with idiopathic hyperbilirubinaemia. *J Paediatr Child Health* 2004; **40**: 633-637 [PMID: 15469533]
 - 115 **Davis JM**, Auten RL. Maturation of the antioxidant system and the effects on preterm birth. *Semin Fetal Neonatal Med* 2010; **15**: 191-195 [PMID: 20452845]
 - 116 **Nassi N**, Ponziani V, Becatti M, Galvan P, Donzelli G. Antioxidant enzymes and related elements in term and preterm newborns. *Pediatr Int* 2009; **51**: 183-187 [PMID: 19405912 DOI: 10.1111/j.1442-200x.2008.02662.x]
 - 117 **Rook D**, Te Braake FW, Schierbeek H, Longini M, Buonocore G, Van Goudoever JB. Glutathione synthesis rates in early postnatal life. *Pediatr Res* 2010; **67**: 407-411 [PMID: 20057337 DOI: 10.1203/PDR.0b013e3181d22cf6]
 - 118 **Asikainen TM**, Heikkilä P, Kaarteenaho-Wiik R, Kinnula VL, Raivio KO. Cell-specific expression of manganese superoxide dismutase protein in the lungs of patients with respiratory distress syndrome, chronic lung disease, or persistent pulmonary hypertension. *Pediatr Pulmonol* 2001; **32**: 193-200 [PMID: 11536448]
 - 119 **Morita-Fujimura Y**, Fujimura M, Gasche Y, Copin JC, Chan PH. Overexpression of copper and zinc superoxide dismutase in transgenic mice prevents the induction and activation of matrix metalloproteinases after cold injury-induced brain trauma. *J Cereb Blood Flow Metab* 2000; **20**: 130-138 [PMID: 10616801]
 - 120 **Morton RL**, Das KC, Guo XL, Iklé DN, White CW. Effect of oxygen on lung superoxide dismutase activities in premature baboons with bronchopulmonary dysplasia. *Am J Physiol* 1999; **276**: L64-L74 [PMID: 9887057]
 - 121 **Das KC**. Thioredoxin and its role in premature newborn biology. *Antioxid Redox Signal* 2004; **7**: 1740-1743 [PMID: 16356134]
 - 122 **Amin SB**, Scholer L, Srivastava M. Pre-discharge iron status and its determinants in premature infants. *J Matern Fetal Neonatal Med* 2012; **25**: 2265-2269 [PMID: 22734563]
 - 123 **Cooke RW**, Drury JA, Yoxall CW, James C. Blood transfusion and chronic lung disease in preterm infants. *Eur J Pediatr* 1997; **156**: 47-50 [PMID: 9007491]
 - 124 **Hirano K**, Morinobu T, Kim H, Hiroi M, Ban R, Ogawa S, Ogihara H, Tamai H, Ogihara T. Blood transfusion increases radical promoting non-transferrin bound iron in preterm infants. *Arch Dis Child Fetal Neonatal Ed* 2001; **84**: F188-F193 [PMID: 11320046]
 - 125 **Ng PC**, Lam CW, Lee CH, To KF, Fok TF, Chan IH, Wong E. Hepatic iron storage in very low birthweight infants after multiple blood transfusions. *Arch Dis Child Fetal Neonatal Ed* 2001; **84**: F101-F105 [PMID: 11207225]
 - 126 **Dani C**, Martelli E, Bertini G, Pezzati M, Rossetti M, Buonocore G, Paffetti P, Rubaltelli FF. Effect of blood transfusions on oxidative stress in preterm infants. *Arch Dis Child Fetal Neonatal Ed* 2004; **89**: F408-F411 [PMID: 15321958]
 - 127 **Rogers S**, Witz G, Anwar M, Hiatt M, Hegyi T. Antioxidant capacity and oxygen radical diseases in the preterm newborn. *Arch Pediatr Adolesc Med* 2000; **154**: 544-548 [PMID: 10850499]
 - 128 **Minghetti L**, Suppiej A, Greco A, Franzoi M, Pascoli I, Zannardo V. Oxidative stress in twin neonates is influenced by birth weight and weight discordance. *Clin Biochem* 2011; **44**: 654-658 [PMID: 21349257 DOI: 10.1016/j.clinbiochem.2011.02.005]
 - 129 **Flegel WA**. Fresh blood for transfusion: how old is too old for red blood cell units? *Blood Transfus* 2012; **10**: 247-251 [PMID: 22889813]
 - 130 **Pettilä V**, Westbrook AJ, Nichol AD, Bailey MJ, Wood EM, Syres G, Phillips LE, Street A, French C, Murray L, Orford N, Santamaria JD, Bellomo R, Cooper DJ. Age of red blood cells and mortality in the critically ill. *Crit Care* 2011; **15**: R116 [PMID: 21496231 DOI: 10.1186/cc10142]
 - 131 **Koch CG**, Li L, Sessler DI, Figueroa P, Hoeltge GA, Mihaljevic T, Blackstone EH. Duration of red-cell storage and complications after cardiac surgery. *N Engl J Med* 2008; **358**: 1229-1239 [PMID: 18354101 DOI: 10.1056/NEJMoa1229-1239]
 - 132 **Dzik WH**, Beckman N, Murphy MF, Delaney M, Flanagan P, Fung M, Germain M, Haspel RL, Lozano M, Sacher R, Szczepiorkowski Z, Wendel S. Factors affecting red blood cell storage age at the time of transfusion. *Transfusion* 2013; **53**: 3110-3119 [PMID: 23550702 DOI: 10.1111/trf.12171]

P- Reviewer: Allegaert K, Dal Monte M, Zapol WM
S- Editor: Wen LL **L- Editor:** A **E- Editor:** Liu SQ





Published by **Baishideng Publishing Group Inc**

8226 Regency Drive, Pleasanton, CA 94588, USA

Telephone: +1-925-223-8242

Fax: +1-925-223-8243

E-mail: bpgoffice@wjgnet.com

Help Desk: <http://www.wjgnet.com/esps/helpdesk.aspx>

<http://www.wjgnet.com>

