Editor’s key points

- Transfusion of older stored erythrocytes might be associated with increased morbidity and mortality.
- Storage can result in functional erythrocyte defects (‘storage lesion’).
- Some clinical studies suggest that transfusion of older erythrocytes is associated with worsened outcomes.
- The quality of available evidence is too poor to recommend changes in transfusion practice.

Summary. Blood transfusion saves many lives but carries significant risk of injury. Currently, red blood cell (RBC) concentrates can be stored up to 42 days. Concerns have recently been raised about the safety and efficacy of transfusing stored RBCs. Refrigerated storage results in a ‘storage lesion’ that is reflected by metabolic derangements, RBC shape modification, rheological changes, oxidative injury to lipids and proteins, alterations in oxygen affinity and delivery, increased adhesion of RBCs to endothelial cells, and accumulation of bioactive substances in storage media. In animal models, transfusion of aged, but not fresh, RBCs induces organ injury, inflammation, coagulopathy, and impaired oxygen delivery. A number of clinical studies, mostly observational or retrospective and from a single centre, have reported an association between transfusion of older RBCs and increased clinically significant outcomes, such as increased morbidity and mortality in certain patient populations, including trauma, critical care, and cardiac surgery. Others, however, have failed to indicate an influence of RBC age on outcome. The quality of evidence is currently too poor to make recommendations to change current transfusion practice; however, the transfusion community looks forward to the results of randomized trials currently addressing the long-standing question regarding the effects of RBC storage on clinically significant outcomes.

Keywords: blood erythrocytes; blood transfusion; erythrocyte storage; outcome

Red blood cell (RBC) transfusion is considered one of the most important and common medical interventions in many clinical situations. It is indicated for improvement of oxygen-carrying capacity of blood to tissues. RBC units are refrigerated and stored for increasing periods of time in order to optimize availability and minimize wastage. Storage is limited mainly by US FDA standards that require <1% haemolysis at the end of storage and at least 75% of allogeneic erythrocytes still circulating in the recipient’s blood 24 h after transfusion. The current upper limit of storage conforming to these requirements is 42 days, and optimization of storage time mandates the principle of transfusing the oldest compatible unit that is available when an order is made from the blood bank.

Stored RBC units experience biochemical and structural changes involving intracellular, membranous and extracellular medium components. These alterations, referred to as the ‘storage lesion’, probably influence erythrocyte oxygen affinity, ability to change shape, membrane stability, and other factors affecting RBC function and transfusion efficacy. The efficacy of transfusing stored, refrigerated erythrocytes in improving oxygen-carrying capacity and delivery to the tissues has not been very well studied in the past. Recent data question the efficacy, and more worrisome, the safety of this prevalent treatment, which according to several researchers should be reserved to specific extreme clinical situations of increased, unmet oxygen demand, such as major trauma and haemorrhage or to supply-dependent patients such as those with acute coronary syndrome.

This article reviews the scientific data available to date regarding the RBC ‘storage lesion’, experimental data regarding the efficacy and possible side-effects of stored RBC transfusion, and the contradictory clinical data addressing these issues. If available data and ongoing studies confirm current concerns regarding clinical risks associated with transfusion of older blood, it will entail major operational and financial impact on national blood supplies and the organization of blood banks would be anticipated.

The storage lesion

Many biochemical, morphological, and molecular changes are known to occur during storage that affect RBC function and possibly safety despite improvements in preservation methods. These are collectively referred to as the ‘storage lesion’, the significance of which is discussed below and
described in Figures 1 (as a proposed timeline) and 2 (as a process).

Concentrations of potassium and lactate in the RBC storage medium more than double within a week of storage, and reach 10 times their baseline level within 42 days. Sodium and glucose concentrations, and also pH, decrease considerably over the first 14 days of storage. Haemolysis also occurs and increases with time. Free haemoglobin levels in the supernatant and haemolysis rates are greater after 28 days of refrigeration compared with 7 days. Bioreactive substances also accumulate in the storage medium. These include various proinflammatory cytokines such as interleukin-1β (the levels of which increase already on day 1 of rat RBC storage), monocyte chemoattractant protein-1 (MCP-1) (the levels of which increase considerably from collection to 28 days of storage), and lysophosphatidylcholine, that some researchers, but not all, have implicated in transfusion-related lung injury and neutrophil activation. Additional alterations include membrane phospholipid vesiculation, protein oxidation, and lipid peroxidation of the cell membrane, all leading to structural changes.

Deformability is the ability of erythrocytes to change shape. It is crucial for flow through small capillaries and to avoid stasis, as some capillaries are narrower than the erythrocyte width. Many researchers have attributed reduced deformability for the negative effects seen after RBC transfusions. In rats, RBC deformability is reduced at 7 days of storage, inducing a...
five-fold increase in the number of rigid, undeformable cells, whereas RBC adherence to endothelial cells was not affected by the increased storage time. In humans, Frank and colleagues demonstrated that long periods of storage (>38 days) resulted in reduced RBC deformability, both in vivo (post-transfusion) and in vitro (in a sample obtained from the unit before transfusion), compared with short (<14 days) storage duration. Reiley and colleagues also demonstrated a clear alteration of the physical properties of human RBCs because of cold storage. These changes, already evident after 2 weeks of storage, include reduced deformability and increased aggregability, rigidity, and adhesion to endothelial cells. Enhanced adhesion is mediated by increased phosphatidylserine translocation to the RBC surface, probably due to growing oxidative stress in stored erythrocytes. This effect has been shown to be reversed by a ‘rejuvenation solution’ (Rejuvesol enCyte Systems, Inc.) containing sodium pyruvate, inosine, adenine, dibasic sodium phosphate, and monobasic sodium phosphate, pH 6.7–7.4) applied to stored RBCs before infusion. Others were unable to demonstrate a reduction in rat RBC deformability, while some researchers suggest that deformability and adhesion alterations are mainly seen when whole, non-leucoreduced blood is examined, and that this effect is mostly mediated by donor leucocytes.

One of the principal RBC functions is oxygen delivery. Cold storage has long been known to deplete both ATP and 2,3-diphosphoglycerate (2,3-DPG) very early in the storage period. Because of the higher affinity of haemoglobin to oxygen due to the decrease in 2,3-DPG, oxygen delivery is altered. Moreover, very early after processing, even before significant decline in 2,3-DPG occurs, oxygen delivery by stored RBCs has been reported to be deficient.

Prolonged storage reduces the capability of RBCs to vasodilate the microvasculature in response to increased metabolic demand (e.g. regional hypo-perfusion), in part through alteration of nitric oxide availability. This effect is reversible by restoring nitric oxide utilization of stored erythrocytes. Bennett-Guerrero and colleagues described the decline of S-nitrosohaemoglobin starting from the earliest hours of RBC storage, independent of preservative addition. They suggest that the reduction in haemoglobin’s ability to bind and deliver nitric oxide, thus impairing RBC-induced ‘hypoxic vasodilation’, contributes significantly to the negative effects observed with RBC transfusion. This emerging field of interest has recently been extensively reviewed elsewhere.

Preclinical studies

Numerous studies using different animal and human models have explored the impact of RBC storage on organ injury, tumour progression, and tissue oxygenation.

While reviewing these animal studies, it is important to remember that there are major biological differences between rat and human erythrocytes. d’Almeida and colleagues previously documented these storage-related differences. For example, 7 day stored rat erythrocytes are more representative (but not equivalent) to human RBCs stored for 4 weeks. Thus, extrapolation of results found in animal studies to humans must be cautious.

RBC storage and inflammation, coagulopathy, and organ injury

Several studies have attempted to identify the mechanisms behind the deleterious effects of ‘aged’ RBC transfusion. These mechanisms involve effects on both inflammation and coagulation systems. Recently, Callan and colleagues reported an exaggerated inflammatory response after transfusion of old (28 days of storage) but not fresh (7 days of storage) RBCs to healthy dogs. This inflammatory response was characterized by elevated levels of the pro-inflammatory cytokine MCP-1 and neutrophil counts, and also thrombocytopenia. To evaluate whether the stored RBC-induced inflammatory response could lead to pulmonary or systemic injury, Vlaar and colleagues examined two groups of rats—healthy and lipopolysaccharide (LPS)-induced septic rats. Healthy rats had a prominent inflammatory lung reaction after aged but not fresh erythrocyte transfusion with no coagulopathy. In LPS-pretreated rats, however, transfusion of aged but not fresh erythrocytes augmented lung injury by inducing coagulopathy but not inflammation, both in the pulmonary and systemic compartments, suggesting a ‘two hit’ mechanism of transfusion-related acute lung injury. Furthermore, coagulopathy induced by aged erythrocytes in septic animals was abrogated by washing the aged erythrocytes, indicating that the damaging effect originated from the supernatant of the stored RBCs.

A recent study by Matot and colleagues evaluated liver injury induced by haemorrhagic shock followed by resuscitation with either aged blood or packed RBCs stored for 7, 4, or 0 days. Whereas transfusion of fresh blood partially restored liver haemodynamics and function, transfusion of aged erythrocytes (7 days storage) not only did not improve liver outcome but rather exacerbated injury as reflected by significantly increased liver apoptosis and necrosis, elevations in serum liver enzymes, and worse perfusion.

The effect of transfusion of stored blood on cardiac function and tissue injury was recently evaluated in a rat model of anaemia and myocardial infarction. The study showed that the beneficial effects of transfusion were limited to fresh blood only, as only fresh blood (4 h storage), but not stored blood (7 days storage), was effective in salvaging ischaemic myocardium. This highlights the need for such studies in patients.

RBC storage and tumour progression

The effect of stored blood on tumour progression has also been evaluated in both animal and human studies. A comprehensive study examining two cancer models in rats (mammary adenocarcinoma and leukaemia) found that RBC transfusion was an independent and significant risk factor for cancer progression in both models, causing up to a four-fold increase in lung tumour retention, and doubling mortality rates. The RBC storage time was the critical determinant of these deleterious
Erythrocyte storage duration and outcome

Aged erythrocytes (9 days and older), rather than leukocytes or soluble factors, mediated the harmful effects of RBC transfusion. A recent human study retrospectively evaluated a prospectively collected database of more than 27,000 cancer patients. It sought to establish whether transfusion of RBCs after prolonged storage in cancer patients influences overall survival or cancer recurrence. Transfused RBC units were categorized as ‘new’ if stored < 14 days, ‘intermediate’ if stored 14–28 days, and ‘old’ if stored > 28 days. After excluding patients who received RBCs from more than one ‘age’ category, data of 1335 patients were analysed. Overall survival did not correlate with the duration of RBC storage. Although cancer recurrence was significantly higher in patients who received erythrocyte transfusion than those who did not, this effect was not related to the duration of RBC storage.

RBC storage and tissue oxygenation

One of the main debates regarding transfusion of stored RBCs is the relative lack of evidence to show they restore or increase tissue oxygenation, which is the main rationale behind their administration. In a rat model, old RBCs (28 days storage) failed to improve systemic oxygen supply in either control or septic animals, whereas fresh RBCs (3 days storage) acutely increased oxygen supply. In an attempt to measure organ-specific oxygenation capability of old RBCs, isovolaemic haemodilution was performed in a rat model to a mean haematocrit of 14% in order to achieve supply dependency. At this extreme level of anaemia, old RBCs (5–6 weeks old) failed to maintain baseline oxygen supply compared with fresh (2–6 days) or intermediate (2–3 weeks) RBCs. It should be noted that when haematocrit was allowed to increase with regular, non-diluted transfusion, oxygen supply increased in all groups, suggesting that old RBC transfusion disrupts oxygenation mainly during extreme demand. A subsequent experiment found similar results when renal microvascular oxygenation was measured, showing that after mild haemodilution in rats (to a haematocrit of 30%), transfusion of old RBCs (5–6 weeks old) lowered tissue oxygenation significantly more than fresh (3 days old) RBCs. Interestingly, treating old RBCs with rejuvenation solution reversed the effect and returned renal oxygenation to baseline, fresh RBC levels.

A study on human volunteers attempted to examine the influence of transfusion of a fresh (7 days storage) vs old (42 days storage) single, autologous, leucoreduced RBC unit on tissue oxygenation in the brain and thenar eminence, and also sublingual microvascular blood flow, all of which were measured non-invasively. The study failed to demonstrate any significant changes in measured parameters before and after a single unit RBC transfusion, either fresh or old. The authors concluded that transfusing a single RBC unit of old blood does not cause any measurable detrimental effect on oxygenation or microcirculation in healthy patients. It should be noted, though, that the study group was extremely small (eight participants) and consisted of healthy, non-anaemic participants, and only one blood unit was transfused. All of these characteristics might be quite different from clinical scenarios of massive transfusion in anaemic, hypovolaemic, supply-dependent patients.

Clinical outcome studies

Numerous studies have aimed to resolve the controversy around the presumed deleterious effects of stored blood on patient outcome. Most have focused on four patient populations: critical care patients, trauma patients, cardiac surgery patients, and neonates, and with the exception of the extremely ambitious study in neonates described later (the ARISPI trial), the majority of these studies have critical flaws. Most reports are small size (underpowered), single-centre, and retrospective. In many, no adjustments for confounding parameters were performed, arbitrary thresholds for fresh vs aged RBCs were used, and/or subjects were transfused with units of mixed storage times (for both control and study groups). Also, different storage media were used, there is no documentation of transfusion protocol, and various outcomes or endpoints have been evaluated.

Intensive care unit patients

Despite recent improvement in the policy of RBC transfusion to intensive care unit (ICU) patients, the prevalence of RBC administration to this patient population remains significant. For example, a Scottish prospective study found that as many as 40% of patients admitted to ICU were transfused, with a haemoglobin threshold of 7.8 g dL–1 in the absence of haemorrhage. Moreover, critical care patients are more likely to develop complications resulting from RBC transfusion as a result of their inflammatory state, impaired tissue oxygen demand–supply status, and perturbations of their microcirculation. It is, therefore, not surprising that intensive care patients were the first group to be evaluated regarding effects of RBC storage time on oxygen delivery and outcome.

One of the earliest reports suggesting a possible correlation between RBC storage age and mortality involved 31 patients admitted to a single ICU during 1992 with a diagnosis of severe sepsis. The authors retrospectively examined risk factors for increased mortality by comparing the clinical characteristics of the 12 survivors with the 19 non-survivors. The data confirmed that the mean age of transfused RBCs was the only parameter to influence the relative risk of mortality. This study, however, did not adjust for confounding factors. Another early study from that decade, which was a prospective, controlled study enrolling 23 critically ill, mechanically ventilated patients, attempted to evaluate the effect of stored RBCs on oxygen uptake, and availability to the splanchnic bed. It demonstrated that 3 units of stored RBCs failed to raise systemic oxygen uptake as measured by indirect calorimetry. Moreover, the investigators found a decrease in splanchnic oxygen availability that correlated with the age of the stored RBC units, that is, administration of older units reduced gastric intramucosal pH. The authors concluded that stored RBC administration not only failed in its main role of increasing oxygen delivery, but rather augmented end-organ
ischaemia. A study on neurosurgical intensive care patients that enrolled 102 traumatic brain injury patients reported that unlike fresh RBCs, stored RBCs stored >19 days did not increase brain oxygenation. A recent, noteworthy multicentre, prospective observational study enrolled 757 intensive care patients from 47 Australian and New Zealand ICUs. In this study, patients received a mix of RBC units of different ages, and the analysis used the age of the oldest RBC unit administered as representative of age of RBCs transfused for statistical assessment of effect. Accordingly, the data showed a significant increase in ICU length of stay and in-hospital mortality in patients receiving old RBCs (median age 17.6 days, range 12.9–24.0) compared with fresher RBCs (median age 7.5 days, range 5.7–9.0). This effect was seen after adjustment for several confounding factors, including severity of illness (APACHE III score), number of transfusions, fresh-frozen plasma and platelet transfusions, leucodepletion status, pre-ICU transfusions, pre-transfusion haemoglobin concentration, and cardiac surgery.

Several other studies were not conclusive or failed to demonstrate a significant deleterious effect of old blood on oxygenation and flow parameters. In a prospective, single-centre, observational study conducted in 35 patients with severe sepsis and septic shock, Sakk and colleagues did not find any impact of storage time on sublingual microvascular perfusion. In neurosurgical ICU patients, Smith and colleagues examined the ability of stored RBCs to increase brain tissue oxygen supply. In three-quarters of the 35 evaluated patients, there was an elevation in brain oxygen partial pressure, whereas in the others, a disturbing decrease in brain oxygenation was documented. In this small cohort, no correlation was demonstrated between RBC storage age and changes in brain oxygenation. It should be noted, however, that patients received a varying number of packed RBCs (from 1 to 6 units) for diverse indications, with the main indication being a haemoglobin <10 g dl⁻¹. Yet, another study in anaemic critically ill patients (double-blind, randomized) that compared the effect of RBC transfusion on tonometric indexes when RBC units were stored <5 vs >20 days failed to show any significant differences between the study groups.

Recently, a randomized controlled trial (RCT) enrolling 100 mechanically ventilated ICU patients failed to show any impact of storage duration of transfused RBCs (<5 days in the study group vs standard care in controls) on pulmonary gas exchange (as reflected in the PaO₂/FI₂O₂ ratio) and on immune and coagulation status. Negative results regarding the effects of storage duration on organ injury (acute lung injury, deep vein thrombosis, stroke, and myocardial ischaemia), nosocomial infection, mortality, and/or on length of stay in the ICU or hospital were also reported by several other investigators in both retrospective and prospective, double-blind, randomized, controlled, multicentre study—aimed to determine whether fresh RBC units transfused to premature neonates produce a better outcome than old RBC units. Three hundred and seventy-seven premature very-low-birth-weight (<1250 g) neonates from six Canadian neonatal ICUs that needed at least one RBC unit were randomized to either receive fresh units (<7 days old, mean 5.1 days) or older units (>7 days old, according to local blood bank standard practice, mean 14.6 days). This study failed to demonstrate any advantage of fresh erythrocytes over standard practice RBC units in premature neonates. The reasons for this are yet to be determined, but it should be noted that the ‘old’ RBCs in the AR.edu trial were not that old (mean 14.6 days of storage). Moreover, there was no predefined protocolized transfusion threshold, and each hospital adhered to its own standard practice. In general, the practice in the AR.edu study appears to follow a liberal transfusion strategy. It is, therefore, likely that the enrolled preterm infant did not reach a critical degree of anaemia necessary to unmask potential harmful effects of prolonged RBC storage on oxygen delivery. Finally, 7.5% of neonates in the fresh RBC group were actually transfused with ‘old’ blood but were analysed according to the ‘in tension to treat’ approach. All these limitations should not dismiss the results of this large-scale study, but might discourage one from generalizing these results to patients of other age groups, from other countries with different blood-bank practices, with other illnesses, different transfusion thresholds, or with larger amounts of RBC units required.

Trauma patients

Trauma patients are frequently treated with allogeneic blood transfusion, either during the resuscitation phase or the first day of management. It is, therefore, not surprising that many investigators have studied the relationship between blood storage duration and outcome in this population. An early publication in this area was a small, retrospective, case–control single-centre study from 1999 that compared trauma victims that developed multi-organ failure (MOF) with a control group that did not develop MOF. Only patients who received 6–20 units of RBCs were included. After multivariate analysis, the authors found that predicting factors for developing MOF were higher mean age of transfused blood and absolute number of transfused units older than 14 or 21 days. They concluded that trauma patients should preferably receive fresh RBCs (<14 days of storage), since the age of transfused erythrocytes was an independent risk factor for developing MOF.

Another retrospective case–control single-centre study enrolled 202 trauma patients who received at least 5 units of RBCs. The incidence of deep vein thrombosis was more than doubled in patients transfused with even 1 unit of old erythrocytes (>21 days of storage) compared with the control group. Moreover, mortality was also nearly doubled in the group that received even 1–2 RBC units older than 28 days compared with patients transfused with younger blood. This study, which was significantly larger than the previous one described,

Neonatal ICU patients

The most comprehensive trial published to date involved premature, very low-birth-weight neonates. The AR.edu trial—a
showed a significant and remarkable increase in mortality after exposure to even 1 unit of old RBCs.

In order to overcome the problem inherent in most studies, in which a mixture of old and fresh units is administered to trauma patients, Weinberg and colleagues\(^59\) compared mortality in 1637 patients receiving exclusively fresh (≤ 14 days) or old (≥ 14 days) RBC units during the first day after major trauma. Although they did not demonstrate a difference in mortality compared with patients receiving only 1 or 2 units of RBCs, in patients receiving ≥ 3 units, mortality was significantly higher in patients transfused with old RBCs.

Except for one prospective single-centre study,\(^50\) all other studies reporting harmful effects from transfusion of ‘old’ RBC units to trauma patients\(^59\) were retrospective, single-centre studies in which transfusion protocols (for RBCs and other blood components) were not standardized, and in most studies, a mixture of old and fresh units was administered. The cut-off point for the definition of old RBCs was 14 days of storage, and evaluated outcomes were organ injury (renal failure and pneumonia), length of stay, and/or mortality.

### Cardiac surgery patients

Cardiac surgery provides a good opportunity for investigation, since this is often an elective procedure with detailed databases collected for many years, for which many patients are transfused with RBCs, many remain on mechanical ventilation in the early postoperative period, and postoperative morbidity is relatively high.

The only available prospective study showing the effect of RBC storage duration on outcome was carried out by Leal-Noval and colleagues,\(^57\) who enrolled nearly 800 patients undergoing cardiac surgery (mostly CABG, valve surgery, or both) in a single centre. Patients with early (< 48 h) mortality or ICU discharge and those with early signs of infection were excluded. There was no correlation of RBC age with length of stay, prolonged ventilation, or perioperative myocardial infarction. There was an elevated risk of postoperative pneumonia for blood stored >28 days. These results correlate with two additional studies: an earlier report by Vamvakas and Carven,\(^55\) who demonstrated in a single-centre retrospective study of 416 CABG patients an increased rate of pneumonia and/or infection in patients transfused with older RBCs, and a second more recent retrospective multi-centre study that enrolled 1748 patients undergoing CABG or valve surgery in which a higher risk of severe postoperative infections was documented in patients transfused exclusively with RBCs stored >14 days.\(^59\)

Although retrospective and from a single centre, the largest trial conducted to date in surgical cardiac patients got the attention of the media, the public, and the US Department of Health and Human Services (http://www.hhs.gov/ash/bloodsafety/advisorycommittee/recommendations/resmy08.pdf), published in 2008 by Koch and colleagues.\(^60\) The authors evaluated 6002 patients who received nearly 20,000 units of RBCs during cardiac surgery (both CABG and valve surgeries). Only patients who received exclusively ‘old’ (≥ 14 days of storage) or ‘fresh’ (<14 days of storage) blood were included in the study, reducing the confounding effect of patients administered a mixture of both old and fresh blood. Patients who were transfused with exclusively older RBCs suffered higher in-hospital mortality, 1 yr mortality, renal failure, and sepsis, and had prolonged mechanical ventilation compared with those who received exclusively fresh RBCs. Moreover, transfusion of older RBCs was independently associated with an increased risk-adjusted rate of a composite of serious adverse events (25.9% vs 22.4%, \(P = 0.001\)). Despite the critical limitations of this study, the impact that this report created pushed in part the initiation of several randomized controlled studies, the results of which are yet to be published.

Several other studies\(^61–66\) have not confirmed a positive correlation between RBC age and adverse postoperative events. A group from Australia reviewed the records of 670 patients undergoing cardiac surgery (CABG, valve, or both)\(^63\) for early mortality, renal failure, pneumonia, ICU length of stay, and prolonged mechanical ventilation. None of these end-points was found to correlate with either mean or maximal age of RBCs transfused, although there was a clear association of all these end-points with the number of units transfused. These negative results have been confirmed by other single-centre retrospective studies that enrolled a larger number of patients.\(^64–66\)

### Ongoing clinical trials

The enormous amount of both experimental and clinical data suggests, but does not prove, deleterious effects of transfusion of RBCs after longer storage duration. However, the quality of the evidence, as summarized in this review, is much too poor to make recommendations to change current transfusion practice. Currently, several studies are comparing the effects of different blood storage durations on patient outcomes in randomized clinical trials.

The RECESS study\(^67\) (Red Cell Storage Duration Study) is a multi-centre, prospective, partially blinded, RCT that aims to enrol 1434 patients from more than 25 medical centres in the USA, undergoing mid-sternotomy cardiac surgery. Participants are being allocated to receive either exclusively ‘fresh’ units of RBCs (≤ 10 days of storage) or ‘older’ units (≥ 21 days of storage). Patients are enrolled into the study only if the blood service is capable of supplying units of both arms described, meaning that patients in the control (‘older’ RBCs) group are actually being treated according to common practice (i.e. they would get the oldest matching blood available at that time even if not in the study). Anticipated completion is in October 2013.

The ABLE study\(^68\) (age of blood evaluation) is another multi-centre, prospective, double-blind, RCT being conducted in Canada. It will enrol just over 2500 severely ill ICU patients from more than 20 hospitals, randomized to receive either exclusively fresh (≤ 7 days of storage) RBC units or ‘older’ RBC units of uncontrolled length of storage (control, common practice group). This trial is unique in its control group, where there is no threshold for the age of RBCs administered. This issue might minimize differences between the study and control...
groups with regard to age of transfused RBCs, but simulates ‘real-life’ situations in a better fashion.

The TRANSFUSE trial [http://www.anzicrc.monash.org/transfuse-rct.html (accessed August 2013)] (STandaRd Issue TrAnsfusion versus Fresher red blood cell Use in IntensIve care), a randomized, multi-centre controlled study from Australia, is intended to enrol 5000 non-cardiac ICU patients and, much like the ABLE study, randomize them to receive either the youngest RBC unit available (study group) or the oldest unit available (control, standard care group). This study is again unique in not pre-setting the age of the RBC unit in either group. The protocol chosen specified only that the unit transfused should either be the freshest or oldest one available, according to the study arm. This protocol best reflects real clinical life, although it might blur the difference among groups.

These three large-scale clinical trials are still in different stages of progress, and will shed light on this controversial issue. Although limited to specific patient populations, data from these studies are expected to have a major impact on the clinical practice of transfusion medicine.

**Summary**

Published retrospective and prospective studies have raised concerns that patients receiving older erythrocytes have increased morbidity and mortality compared with those receiving fresher units. Two recent meta-analyses have struggled to summarize these studies because of variability in methods of reporting data in individual studies, and reached contradictory conclusions. In Vamvakas’ meta-analysis, only observational studies that presented adjusted results and had adequate adjustment for confounding factors were assessed. The author concluded that no adverse effect could be attributed to transfusion of ‘old’ units, and pointed out that current data do not support conducting further trials with an aim to answer the equipoise in opinions. An updated meta-analysis from the same author, published a year later, strengthens these conclusions. The latest meta-analysis by Wang and colleagues, published in 2013, reported contradictory conclusions. This meta-analysis included more studies, and thus more patients in total and in each subgroup than the previous one, and used different enrollment criteria. The authors reviewed more than 400,000 patients from 21 reports, mostly cardiac surgical and ICU patients. The results showed increased mortality among patients receiving older erythrocytes. Subgroup analysis of these trials indicated that the increased risk was not restricted to a particular type of patient, size of trial, or amount of blood transfused. The authors calculated that 97 patients who receive exclusively ‘fresh’ RBCs will result in one life saved.

The ongoing discussion and debate, as reflected in these two meta-analyses, and the many reviews published in recent years, introduced RBC storage duration as a new parameter that cannot be ignored (Table 1). Some studies now indicate that in their hospital, ‘younger’ blood is used for certain patient populations.

The available scientific data suggest potential adverse effects associated with prolonged RBC storage, particularly in certain clinical settings (e.g. intensive care, cardiac surgery, and trauma). Nevertheless, as long as data regarding safety from adequately controlled clinical trials are lacking, and in the absence of satisfactory analysis of the impact of shortening storage duration on blood availability, a change in practice is premature. At present, the medical community looks forward to the results of several ongoing randomized trials. Additionally, further research addressing specifically effects of erythrocyte transfusion and storage duration on organ function is needed. More specifically, those organs that are at risk because of previous or ongoing independent injury should be further assessed. A proposed mechanism in which the first event (such as infection or hypoperfusion) predisposes to adverse outcome when a second potentially injurious event is introduced, referred to as the ‘two hit’ phenomenon, is compelling. RBC transfusion, and more so stored erythrocytes, might well serve as the second hit. This has been looked at in a paucity of experimental studies and should now be looked at in more depth in clinical trials.

**Authors’ contributions**

B.C. and I.M. actively participated in data retrieval, and the organization, writing, revision, and approval of this paper.

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**References**

1. FDA workshop on red cells stored in additive solution systems. Bethesda, MD: US Food and Drug Administration, 1985


Fitzgerald RD, Martin CM, Dietz GE, Daig G, Potter RF, Sibbald WJ. Transfusing red blood cells stored in citrate phosphate dextrose adenine-1 for 28 days fails to improve tissue oxygenation in rats. *Crit Care Med* 1997; 25: 726 – 32


Vamvakas EC, Carven JH. Transfusion and postoperative pneumonia in coronary artery bypass graft surgery: effect of the length of storage of transfused red cells. Transfusion 1999; 39: 701–10
