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REVIEW

Current issues relating to the transfusion of stored red blood cells

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The development of blood storage systems allowed donation and transfusion to be separated in time and space. This separation has permitted the regionalization of donor services with subsequent economies of scale and improvements in the quality and availability of blood products. However, the availability of storage raises the question of how long blood products can and should be stored and how long they are safe and effective. The efficacy of red blood cells was originally measured as the increment in haematocrit and safety began with typing and the effort to reduce the risk of bacterial contamination. Appreciation of a growing list of storage lesions of red blood cells has developed with our increasing understanding of red blood cell physiology and our experience with red blood cell transfusion. However, other than frank haemolysis, rare episodes of bacterial contamination and overgrowth, the reduction of oxygen-carrying capacity associated with the failure of some transfused cells to circulate, and the toxicity of lysophospholipids released from membrane breakdown, storage-induced lesions have not had obvious correlations with safety or efficacy. The safety of red blood cell storage has also been approached in retrospective epidemiologic studies of transfused patients, but the results are frequently biased by the fact that sicker patients are transfused more often and blood banks do not issue blood products in a random order. Several large prospective studies of the safety of stored red blood cells are planned.

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Introduction

Peyton Rous was the first person to store red blood cells. He had learned from Roger Lee that citrate was an anticoagulant [1]. He kept rabbit red blood cells in a mixture of citrate and glucose for 4 weeks in a refrigerator and observed that they did not haemolyse [2]. When these stored red blood cells were infused back into the donor rabbits, they raised the haematocrit and did not cause haemoglobinuria or bilirubinuria [3]. Two years later, in military hospitals adjacent to World War I battlefields, Rous's post-doctoral fellow, Oswald Robertson, used this solution to store human red blood cells for up to 26 days and used this 'banked' blood to resuscitate soldiers in shock [4,5]. However, Robertson's US Army colleagues became concerned about the possibility of bacterial contamination of the stored blood, and the commission that approved stored blood transfusion for general use approved it only for storage in citrate without glucose and only for 5 days of storage [6]. Robertson, in his private writings, noted that this restriction both limited the utility of blood banking and reduced the quality of stored blood, because some units ran out of glucose in less than 5 days.

The controversy between those who seek longer red blood cell storage for logistical reasons and those who have concerns about the safety and efficacy of stored blood continues. Storing red blood cells for longer times does have advantages. It allows the accumulation of inventory, takes advantage of

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economies of scale in collection, processing and testing, and allows the development of quality controls. Longer cold storage reduces potential transmission of syphilis and reduces transfusion-associated graft-versus-host disease. However, stored red blood cells lose functional capacity during storage: their 2,3-diphosphoglycerate (DPG) concentrations decrease, they lose membrane, and they eventually become non-viable [7]. Stored red blood cells can be frankly dangerous with high potassium concentrations, bacterial overgrowth, and the lytic elaboration of toxic lipids. Determining the optimal duration of red blood cell storage that maximizes both safety and availability requires the careful weighing of different kinds of information and values, the interpolation of frequently missing critical evidence, the judgement of motive, and the respect for different points of view.

In this article, we will review the history of standards for red blood cell storage, the state of knowledge about the storage lesion, the concerns that have been raised about prolonged storage, and prospect for obtaining critical evidence.

Standards for red blood cell storage: recovery, survival and haemolysis

The first standards for red blood cell storage were that the cells did not haemolyse in the bottle and that they appeared to circulate when reinjected into the donor or were transfused into a recipient [3]. In a sense, these remain the only standards. They are now formalized in the US licensure requirements that at the end of the approved storage period, an average of at least 75% of the cells remain in the circulation 24 h after infusion and that haemolysis be less than 1%.

For 50 years, labelling red blood cells with chromium-51 has been the accepted way to measure their recovery and survival [8]. The recovery is the fraction of the injected cells that circulate after infusion and their survival is the length of time that either the average cell or the longest surviving cell circulates. With the recognition of the high frequency of post-transfusion hepatitis, autologous recovery and survival measures, where a volunteer donor's own red blood cells are returned after storage, became the standard method for evaluating blood storage systems. Such studies have the added advantage that as the infused red blood cells are the donor's own, antibody-mediated clearance of the cells does not typically interfere with the observations, and documented reductions in recovery or survival can be presumed to be the result of damage to the cells inflicted by the storage system or the passage of time. Several groups have defined objective ways of performing these studies [9]. The standard measure is now the 24-h post-infusion *in vivo* recovery, with the survival measured as the half-life of the radioactive label. One laboratory has developed and used a system for measuring recovery of allogenic red blood cells by measuring the fraction carrying alloantigens by flow cytometry [10].

The establishment of 75% as the recovery standard in the USA came out of historical experience [11]. Whole blood stored for 3 weeks in acid–citrate–dextrose solution had an approximately 75% autologous *in vivo* recovery. With 3-week storage of whole blood in CPD solution, this improved to 79% [12]. When adenine was added to CPD solution to make CPDA-1, the licensure study showed 81% autologous *in vivo* recovery after 5-week storage as whole blood, but only a 72% autologous *in vivo* recovery after 5-week storage as packed red blood cells [13]. The solution was licenced, because it met the 1970s standard of 70% autologous *in vivo* recovery, but because of the sense that performance had actually gotten worse, the Food and Drug Administration raised the standard to 75% in 1985. All of the red blood cell additive solution storage systems licenced subsequently in the USA, AS-1, AS-3, and AS-5, have met this higher standard. Furthermore, in a large review of licensure trials by Dumont and AuBuchon, all appeared to be equivalent, with approximately 82% 24-h *in vivo* recovery when stored as red blood cells in additive solution for 6 weeks [8]. When the red blood cells are leucoreduced at the time of initial processing, the red blood cell recovery is about 2% higher [14]. The survival of red blood cells that circulate for 24 h has been normal with a half-life of about 60 days in all systems where it has been measured.

The problem with 24-h *in vivo* recovery as a red blood cell storage quality standard is that its measurement is time-consuming, expensive to conduct, requires exposing volunteers to modest amounts of radiation, and gives quite different results from one volunteer to another [15]. Examination of the results of a large number of such studies shows that the population distribution has a large standard deviation and negative skew with a long lower tail [8]. Examinations of cross-over studies where individual volunteers are measured several times show that some volunteers' red blood cells consistently store better than others [15].

Measures of haemolysis are easier to perform, and several very large series are available from national blood service quality-assessment programmes. Typically, red blood cells in additive solution have 0·2–0·4% haemolysis after 5–6 weeks of storage, and 1–4% of such cells typically exceed standards. Leucoreduction tends to reduce storage haemolysis by about 50% [16].

Other changes occurring during storage: the red blood cell storage lesion

There are many other changes that occur during red blood cell storage that have not served as conditions of storage system licensure in the past [7]. These changes include shape change, slowed metabolism with decreased concentrations of adenosine 5′-triphosphate (ATP), acidosis with resulting decreased concentrations of DPG, loss of cation pumping

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with loss of intracellular potassium, oxidative injury with changes in band 3 structure and lipid peroxidation, and apoptotic changes with membrane phospholipid racemization and membrane loss [16].

Storing living red blood cells in a closed plastic bag means that the products of ongoing glycolytic metabolism, lactic acid and protons accumulate over time [16]. Other metabolic processes, such as the breakdown of adenosine by adenosine deaminase, mean that other breakdown products accumulate as well, but the generally small amounts of ammonia and inosine formed do not seem to be clinically important for themselves. The protons, however, decrease the pH in the blood bag and alter glycolysis, first leading to a rapid drop in DPG concentrations with a concomitant burst in ATP production, followed by an increased slowing of glycolysis and falling ATP production as acid accumulates. DPG is typically gone by the 10th day of red blood cell storage, whereas ATP concentrations initially increase or are stable during the first 2 to 4 weeks of storage with generally declining concentrations thereafter. New experimental solutions may be able to extend high concentrations of ATP longer [17].

Acidification and decreasing ATP concentrations both affect red blood cell shape [16]. Acidosis causes the initial manifestations of red blood cell shape change during storage, the development of bumps that grow to become the typical surface protrusions of echinocytes. Most of the early aspects of echinocytic shape change appear to be reversible with red blood cell warming and certainly disappear when stored red blood cells are incubated in a neutral pH solution of nutrients, a process called rejuvenation. However, as red blood cell ATP concentrations fall, irreversible changes associated with increased red blood cell calcium concentrations develop. These include the loss of phospholipid asymmetry, the development of negatively charged phospholipid rafts on the cell surface, and their shedding as microvesicles. Membrane loss during red blood cell storage would appear to be permanent. As storage progresses, red blood cells become more rigid and more adherent to endothelium [18,19].

Red blood cell concentrates are not a pure product, being derived from whole blood by simple centrifugation techniques. Many red blood cell concentrates are still made this way with the white blood cells left behind as a buffy-coat when the platelet-rich plasma is removed. When these white blood cells are exposed to the acidic conditions of storage and refrigerated, they respond with activation and cytokine production before they die [20]. After they die, the white blood cells break down and release constituents including enzymes such as phospholipase-A2. Phospholipase-A2 in turn attacks and breaks down phospholipids released by red blood cells, creating lysophospholipids such as the dialkylglycerol platelet-activating factor. The longer the red blood cells are stored, the more of these biologically active lipids are produced. Leucoreduction of red blood cell concentrates shortly after collection markedly reduces the concentrations of lysophospholipids. Leucoreduction also decreases the changes that cause stored red blood cells to stick to endothelial cells in culture and probably to post-capillary venules in the circulation [21].

Oxidative damage also occurs to red blood cells during storage [22]. The haemoglobin in venous blood is partially saturated with oxygen, so the oxygen is constantly leaving one haemoglobin molecule and binding to another. This reaction is not perfectly reversible, and occasionally, the leaving oxygen takes an electron with it, forming ferric methemoglobin and superoxide (O_2^-). Normally, methemoglobin is reduced and superoxide is desmuted without consequences, but occasionally superoxide interacts with iron and water in the Fenton reaction to form hydroxyl radical, which can attack and damage proteins and lipids. Damage to spectrin and glycoprotein band 3 can occur, and interaction with triacylglycerols can lead to deacylation and the formation of lysophospholipids. While damage to glycoprotein band 3 appears to have consequences as a determinant in the natural 120-day lifespan of red blood cells in the circulation, its much slower rate during cold storage probably reduces its importance as part of the storage lesion. On the other hand, the slow accumulation of lysophospholipids in the blood bag during storage, without an opportunity for their continuous removal and detoxification, remains as a safety concern.

Are stored red blood cells safe?

There are several circumstances in which transfusion or even reinfusion of stored red blood cells are associated with bad outcomes. Deaths have been associated with the overgrowth of red blood cell units by cold-growing bacteria, with the rapid central infusion of older units with high concentrations of extracellular potassium, with haemolysed units of various causes, and from transfusion-related acute lung injury (TRALI) from oxidation-induced lysophospholipids. There may also be hypercoagulation associated with the infusion of microvesicles exposing negatively charged phospholipids.

One in 2000 units of blood is contaminated from skin or blood at the time that it is drawn [23,24]. Despite leucoreduction and cold storage, about 1 in 30 000 stored red blood cell units can be demonstrated at some point to be bacterially contaminated. Infections related to bacterial contamination could be demonstrated in 1 in 5 million red blood cell units, and in a typical year, about one of the five annual deaths from bacterially contaminated blood products is reported to be associated with a unit of red blood cells [25]. Most bacterial organisms do not survive in the cold, but a few such as *Serratia marcesans*, *Yersinia enterocolitica*, and *Aeromonas* species can grow at refrigerator temperatures [26]. They tend to grow slowly in cold blood, dividing about once a day and

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so to take approximately 27 days for a single organism to grow to 10^8 organisms and present with an overwhelming infection or endotoxic shock. Examination of units of red blood cells for evidence of haemolysis or a dark colour indicative of bacterial consumption of oxygen is a routine blood bank procedure.

The activity of the sodium potassium-dependent ATPase 'pump' on the red blood cell surface is highly temperature dependent [7]. In the cold, it does not have the activity to overcome diffusive cation loss. Red blood cells therefore leak potassium, and, in additive solutions, the extracellular potassium concentration of stored units increases at a rate of about 1 mEq/l each day. The rate is greatest early on when the intra- to extracellular concentration gradient is highest, then slows as an equilibrium is reached. As the equilibrium point is about 60 mEq/l, most units never achieve this concentration in 42 days of storage, so the approximately 1 mEq/l/day rule is useful. Deaths have been reported when such units were infused through central lines into infants or used to prime cardiopulmonary bypass or other high-flow devices [27]. As the red blood cells will reabsorb the potassium as soon as they warm and equilibrate to body pH and osmolality, the problem is not the total potassium load, but its local extracellular concentration in the older stored units and its delivery to the central circulation where it can be associated with cardiac arrhythmias. Rules to provide young units of red blood cells to small infants and for bypass priming or to use washed red blood cells when young units are not available largely prevent these incidents when the rules are followed.

Infusion of haemolysed red blood cell units can cause reactions that look like immune haemolytic transfusion reactions. Typically, they are less severe, because they do not cause the complement activation associated with antibody-mediated haemolysis, but they can be associated with acute renal failure or hyperkalemic sudden death. Such reactions are more frequently associated with older units, because such units have had more time for mishaps of storage to occur.

As noted above, lytic and oxidative damage to red blood cell membrane phospholipids and the elaboration of lysophospholipids occurs continuously during red blood cell storage. Silliman and his colleagues have shown that this can be a mechanism of acute lung injury, and Gajic and his colleagues have shown that concentrations of lysophospholipids in stored red blood cell units are associated with increased rates of lung injury in intensive care patients [28,29]. However, rates of TRALI are markedly reduced when plasma from women donors is removed from the blood supply, so the role of lysophospholipids in causing clinically important lung injury is not clear [30].

Finally, microvesicles from stored red blood cells are shed in relatively greater numbers toward the end of storage when ATP concentrations are low [31]. These vesicles expose negatively charged phospholipids on their surfaces that are potentially proinflammatory and procoagulant. Although there are suggestions that transfusion is associated with increased inflammation in studies of transfusion and multiple organ failure and with thrombosis in critically ill patients, these are deeply confounded studies of very sick patients receiving many kinds of therapy.

Are stored red blood cells effective?

The suggestion that stored red blood cells lose efficacy is generally based on claims that they do not flow or they do not deliver oxygen [32]. Suggestions that they do not flow are based on direct observation of the microvasculature or reologic studies in various instruments and are associated with membrane stiffness, membrane loss, and the loss of secretion of local vasodilators such as ATP and nitric oxide. Suggestions that stored red blood cells do not deliver oxygen are usually based on their low concentrations of DPG.

Red blood cell flow is reduced after prolonged storage in supravital studies of the microvasculature and in artificial capillary systems [33]. Their deformability is reduced in ectocytometers [34]. The artificial capillary systems tend to be exquisitely sensitive to membrane loss and the ectocytometers to membrane rigidity. Both would be expected to reduce flow in the living capillary systems. The problem is that the same cells, stored in solutions of nutrients that maintain ATP concentrations, tend to have normal flow despite the membrane loss, suggesting that the reduced flow is a function of the red blood cells' interaction with its environment [35]. Since rejuvenating solutions rapidly restore red blood cell ATP concentrations and ATP is important for membrane fluidity, by facilitating cytoskeletal rearrangement, and for vascular flow, by the secretion of ATP in response to shear effects resulting in local vasodilation, it seems plausible that ATP is involved. Considerable work on the development of the next generation of red blood cell storage solutions is aimed at improving red blood cell ATP concentrations at the end of storage to prevent these kinds of problems.

Nitric oxide bound to the sulfur of β-93 cysteine (SNO-Hb) is also rapidly lost during red blood cell storage, is not regenerated in rejuvenating solutions, and not involved in the artificial capillary systems. It takes several hours to regenerate SNO-Hb after returning cells the body, so the prompt restoration of flow associated with better-stored red blood cells suggests that it is not critical to flow regulation. Moreover, genetically engineered mice having the β-93 cysteine replaced with alanine still have red blood cell-dependent hypoxic vasodilation, suggesting an entirely different source for red blood cell-derived nitric oxide such as nitrate reduction by deoxyhemoglobin that is not altered by storage [36].

2,3-Diphosphoglycerate intercalates between the β globin chains of deoxyhemoglobin, stabilizing the deoxy form and

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moving the base of the oxygen equilibrium curve to the right. This makes the curve steeper and moves the mid-point of the curve, the P50, to the right. In the absence of DPG, oxygen loading in the lung is about the same, because the upper end of the equilibrium curve is little affected, but the unloading of oxygen in the tissues takes place at a lower PO_2, so the driving force for tissue oxygenation is lower. This can result in reduced oxygen delivery to tissue, especially when it is combined with reduced capillary flow and reduced functional capillary density. Attempts to measure the effect of prolonged red blood cell storage on oxygen delivery, such as that of d'Almeida and his colleagues, suggest that the effect is small, reducing oxygen delivery by about 15%, and is only important near the critical point where low haematocrit limits oxygen delivery so as to restrict oxygen consumption [37]. However, an attempt to reproduce this work in larger numbers of animals was not able to demonstrate any difference in critical oxygen delivery between fresh and stored red blood cells [38].

Data from clinical studies

In 1993, Marik and Sibbald reported that the transfusion of longer-stored units was associated with a decrease in gastric pH in 23 patients in septic shock, each transfused with 3 units of red blood cells as a goal-directed therapy to increase their oxygen delivery in an attempt to increase their oxygen consumption [39] (Table 1). This was an incidental finding in a larger study in which many factors were analysed. Gastric tonometry was a widely used intensive care monitoring system at the time, but it has since been discredited and is no longer widely used. The authors speculated that poor red blood cell deformability associated with longer storage led to microvascular occlusion. None of the red blood cell units used in this study would have been leucoreduced.

The next year, Martin and his colleagues reported in an abstract that transfusion of red blood cells older than 14 days was associated in multiple logistic regression analysis with increased length of intensive care unit (ICU) stay in a cohort of 698 ICU patients [40]. However, in the initial analysis, only the total number of units was associated with mortality. No full publication of the work was found.

In 1997, Purdy and his colleagues described a cohort of 31 transfused septic intensive care patients [41]. In this group, the 19 patients who died received more red blood cells (15 vs. 13 units) and proportionately more red blood cells stored longer (52 vs. 74% stored longer than 16 days).

Two years later, Zallen and his colleagues reported on a cohort retrospectively identified from a trauma centre registry on the basis of having received 6–20 units of red blood cells in the first 12 h after admission and consisting of 63 individuals, 23 of whom developed multiple organ failure [42]. The patients who developed multiple organ failure were older, 46 ± 4·7 vs. 33 ± 2·3 years, but also received red blood cells that had been stored longer, 30·5 ± 1·5 vs. 24 ± 0·5 days on average. In a multiple logistic regression analysis, age of the units of red blood cells administered in the first 12 h was an independent risk factor for multiple organ failure as were the total number of units stored longer than 14 and 21 days. At the time that this study was performed, the red blood cell units would not have been leucoreduced.

In 1999 and 2000, Vamvakas and Carven published two reports on the incidence of pneumonia in coronary artery bypass grafting (CABG) patients following transfusion. In the first article, they described a logistic regression model of the occurrence of postoperative pneumonia in a series of 416 consecutive CABG patients [43]. In their model, they observed an association between the length of storage of non-leucoreduced red blood cell units administered and the occurrence of pneumonia. The next year, they published a similar analysis of a further 268 consecutive CABG patients [44]. Using several regression models they could not confirm a relationship between length of incubation, length of ICU stay, or length of hospitalization and the length of storage of the longest stored red blood cell unit transfused, the mean length of storage of the longest two stored units, or the mean length of storage of all red blood cell units administered. The authors noted that while they were unable to confirm the previously noted relationship, there was a paucity of research on the effects of the storage of red blood cells.

In 2002, Offner and his colleagues reported on a second trauma cohort of 61 patients again identified on the basis of having received 6–20 units of red blood cells in the first 12 h after admission [45]. In this second cohort, patients who received older red blood cells had an increased number of major infections that led to increased length of ICU stay. The patients with infections were also older and had higher injury severity scores. On average, they received 12 as opposed to 9 units of red blood cells stored longer than 14 days. The group concluded that leucoreduced red blood cells or red blood cell substitutes might be more appropriate products for the initial resuscitation of trauma patients.

That same year, Keller and her colleagues issued a preliminary report describing an increased hospital length of stay in patients who received older red blood cell products [46]. However, there was no correlation ICU length of stay or ventilator days. There has been no follow-up.

Also that year, Schulman and his colleagues at the Ryder Trauma Center in Miami reported in an attempt to perform a prospective randomized clinical trial of shorter- vs. longer-stored red blood cells in a trauma population [47]. In the course of 8000 admission, they were able to enroll only 17 patients of whom four short storage patients and two long storage patients died.

Because of the importance of the original Marik and Sibbold study in the debate over the quality of stored red blood

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Table 1 A table describing 20 clinical studies that have looked at the effects of long-stored red blood cells in critically ill patients presented in order of publication

References	Type of study	Number of patients	Major finding	Caveats
[39]	Interventional trial of RBC transfusion in septic shock	23	Older RBC units associated with lower gastric pH	Not primary end-point of trial
[40]	Regression analysis of 1-year ICU patients	69	Older RBC units associated with ICU length of stay	Older RBC units not associated with mortality
[41]	Review of transfused septic patients in an ICU	31	Patients who died received older RBC units	Total numbers of RBC associated with mortality. Abstract, full report promised, never came
[42]	Review of trauma database for patients receiving 6–20 units of RBC in first 12 h	23	Patients developing multiple organ failure received older RBCs (30 vs. 24 days)	Patients developing multiple organ failure were older (46 vs. 33 years), a known risk factor
[43]	Review of coronary artery bypass database	416	Longer-stored non-leucoreduced RBCs associated with pneumonia	Patients who died received more RBC units
[44]	Review of coronary artery bypass database	268	Older RBCs not associated with any specific or all cause morbidity	Repeat of above study failed to confirm findings
[45]	Review of trauma database for patients receiving 6–20 units of RBC in first 12 h	61	Older RBCs associated with increased ICU stay, but not multiple organ failure	No association with multiple organ failure or mortality. Patients receiving older RBCs were older and had higher injury severity scores. Repeat of Zallen study from same institution
[46]	Review of 18 months of trauma database for urban medical centre	86	Increased hospital length of stay associated with older non-leucoreduced RBC units	No association with ICU length of stay or ventilator days. Titled 'A preliminary study'. No follow-up
[47]	Randomized prospective trial of < 11 or > 20-day-old RBCs	17	No significant effects. Able to enroll 17 patients out of 8000 trauma admissions	Short storage group received 9.3 U and had four deaths, long storage group received 10.6 U and had two deaths
[48]	Randomized clinical trial of effect of transfusion on gastric pH	22	No difference for RBCs < 5 or > 20 days	Formal repeat of the original Marik and Sibbald study found no effect
[49]	Trial of effect of transfusion on brain tissue PO_2	35	Transfusion raised haemoglobin by 17% and increased $BtPO_2$ 15%	No effect of age of RBCs
[50]	Review of a trauma database of urban public hospital for 2001–2003	275	Examined mortality and length of ICU stay. No effect on mortality, ICU length of stay longer in those receiving older RBCs	Patients receiving older blood had longer ICU stay but did not have a higher need for ICU care
[51]	Randomized trial of RBC < 10 or > 10 days in surgical and critically ill patients	66	Life-threatening complication or death in 27% of fresh vs. 13% of old ($P = 0.31$)	Pilot for a planned larger randomized trial
[52]	Review of coronary artery bypass grafting (CABG) database for a large Dutch hospital for 1993–1999	2732	No untoward effects of long-stored RBCs were seen	This largest well-conducted retrospective review found no association
[53]	Review of registry of all repeat midline sternotomy patients for CABG, valve, or both	321	Age of RBCs, associated in a regression model with in-hospital and out-of-hospital mortality	Age of red blood cells, associated in a regression model with in-hospital and out-of-hospital mortality

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Table 1 *Continued*

References	Type of study	Number of patients	Major finding	Caveats
[54]	Prospective cohort of ICU patients transfused with presampled blood products	901	Women donors, HLA class I & II antibodies and lisophospholipids associated with increased risk of acute lung injury	ALI patients more likely to have sepsis and alcoholism. Age of RBC was 21.1 days in alanine aminotransferase (ALI) and non-ALI groups
[55]	Retrospective review of a CABG database at Cleveland Clinic for patients who received only all < 2-week or > 14-day RBCs	5902	Patients who received older RBCs had higher in and out of hospital mortality	Enough more patients in the old RBC group received more units to account for most of the excess mortality
[56]	Trial of effect of transfusion on brain tissue PO_2	66	RBCs greater than 19 days old did not improve $BtPO_2$ of male patients	Study heavily manipulated including dropping female patients and ignoring group effects
[57]	Review for patients who received 1 unit of RBCs in first 24 h at University of Alabama Hospital	1813	For patients receiving 6 or more units of RBCs including 3 or stored more > 13 days, there was increased risk of death by a regression model	No significant indifference in the confidence limit analysis

cells, Walsh and his colleagues at the Edinburgh Royal Infirmary reported a randomized, prospective double blind trial in 2004 [48]. Twelve patient volunteers in septic shock were randomized to receive transfusions with red blood cells stored for greater than 20 days, and 10 more were given red blood cells stored for less than 5 days. There were no changes in gastric pH observed and no other significant differences between the groups. The researchers concluded, 'Our data do not support the hypothesis that transfusing stored red cells adversely effects tissue oxygenation in anaemic, euvolemic, critically ill patients with no evidence of bleeding.'

In 2005, Smith and her colleagues described the effects of transfusion on the brain tissue oxygen partial pressure ($BtPO_2$) in brain injured patients in a neurologic ICU who had brain oxygen electrodes implanted in normal brain contralateral to the site of injury [49]. In these patients, a 17% increase in haemoglobin concentration resulted in a 15% increase in $BtPO_2$. The length of storage of the red blood cells had no effect on the size of the increment in $BtPO_2$.

Also that year, Murrell and his colleagues described a retrospective cohort study of 275 patients in a trauma centre where the receipt of longer-stored red blood cells was associated with increased ICU length of stay [50]. However, the in-hospital mortality was not different between the patients receiving younger and older stored blood units. Again, in this study the study period of 2001–2003 spanned a period when increasing amounts of leucoreduced red blood cells were being used.

At about the same time, Hebert and his colleagues in the Canadian Critical Care Trials Group published the results of a multicentre randomized pilot study of 66 cardiac surgery and critical care patients randomized to receive either red blood cells stored less than 8 days or standard blood bank issue red blood cells that would usually have longer storage because of first-in-first-out practices [51]. Nine patients did not require any red blood cells, but among the 57 patients who did, the mean durations of storage were 4 and 19 days. Twenty-seven per cent of the patients receiving the short storage red blood cells and 13% of those receiving the longer stored cells sustained life-threatening complications or death. This pilot study has now been continued as the Age of Blood Experiment (ABLE) described below.

Basran and her colleagues published a review of a database of cardiac surgery patients where they identified 321 patients who underwent repeat midline sternotomy and used at least 1 unit of red blood cells in the first 24 h after surgery [52]. They found an association with receiving longer-stored units measured both as the age of the longest-stored unit and the median time of storage with both in-hospital and out-of-hospital mortality. However, they also found that patients who received more units generally received more longer-stored units. Multivariate analysis usually cannot deconstruct this kind of bias.

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In 2006, van der Watering and his colleagues published a review of the experience of 2732 patients who underwent CABG in a single hospital between 1993 and 1999 and who had received transfusions of buffy-coat-reduced but not leucocyte-filtred red blood cells [53]. They looked for an association between red blood cell length of storage measured as (i) mean storage time of all perioperative red blood cell transfusions; (ii) storage time of the youngest red blood cell transfusion; and (iii) storage time of the oldest red blood cell transfusion; and (iv) in patients receiving red blood cells with a storage time below the median storage of 18 days vs. patients receiving red blood cell with a storage time above the median and outcome measures of 30-day mortality, hospital length of stay, and ICU length of stay. They observed univariate correlations between storage time and the end-points survival and ICU stay, but also correlations with established risk factors such as the number of transfusions. The multivariate analyses showed no independent effect of storage time on survival or ICU stay.

The next year, Gajic and his colleagues at the Mayo Clinic reported the results of a prospective nested case–control study of the relation of transfusion to acute lung injury in the ICU [54]. They found evidence of lung injury in 74 of 901 transfused patients and an association with elevated concentrations of lysophosphotidyl choline in the blood products, but no association with the length of storage of the red blood cell units transfused.

In March of 2008, Koch and her colleagues published a retrospective review of the large cardiac surgery database of the Cleveland Clinic and compared large cohorts who received either all red blood cells stored for less than 15 days (2872 patients) or all longer than 14 days (3130 patients) [55]. The authors claimed that the older red blood cells were associated with excess in-hospital mortality, but more than half of these deaths could be explained by greater numbers of patients receiving more than 6 units of red blood cells among the patients who received longer-stored blood, which in a prior publication from the same database the author's group had shown was associated with very high mortality. The residual risk to be associated with receiving old red blood cells that remains after correcting for the sampling bias is no longer significant.

Three months later, Leal-Noval and his colleagues reported on a study of transfusion in brain-injured patients with brain tissue oxygen electrodes implanted in the side contralateral to the injury [56]. They reported that $BtPO_2$ did not improve in male patients after transfusion of red blood cell units stored longer than 19 days. This result can only be justified through a highly selective reading of their data after the female patients were excluded. A more straightforward analysis, such as comparing the mean increment in $BtPO_2$ at all reported time-points in the first 24 h after transfusion, showed that $BtPO_2$ increased by 1·7 Torr in the group receiving the shortest stored red blood cells compared to 2·5 Torr in the group receiving the longest stored cells.

In September 2008, Weinberg and his colleagues reported on a review of 1813 trauma patients transfused at least 1 unit of red blood cells over a 7·5-year period [57]. The group performed by both contingency table and regression analysis of the relationship of numbers of units of long-stored red blood cells and mortality and found an association by regression but not in the contingency table analysis. Again, patients who received more blood received more longer-stored red blood cells.

In aggregate, the clinical papers on the effects of transfusion of red blood cells stored for prolonged periods do not show a significant adverse effect. All five prospective studies are negative. The large retrospective series with attention to epidemiologic rigor are also negative. The positive studies all show direct or suggestive evidence of database dredging, the searching for a positive result among many possible associations and then reporting it with conventional probability as if it had been the goal all along. As noted above, Koch and her colleagues ignored the strong association of mortality with the number of red blood cell units administered that they had reported previously, Leal-Noval and his colleagues removed the females from the analysis when their data did not support the desired conclusion, and the Denver Health groups of Offner and of Zallen chose to analyse cohorts of patients receiving 6–20 units of red blood cells in the first 12 h of care and report as positive two different end-points that were negative in the other review. As van der Watering concluded, 'no justification could be found for use of a particular maximum storage time for RBC transfusions in patients undergoing CABG surgery.'

Planned large studies

Two large studies of the effects of the duration of red blood cell storage are planned. These trials are well within the technical capabilities of the groups planning to undertake them but are resource-constrained and have been designed with only a limited sense of what the actual frequency of the sought outcomes might be. Lacroix and his colleagues in the Canadian Clinical Care Trials Group have received funding to start the ABLE study, and Steiner and her colleagues in the US National Heart, Lung, and Blood Institute's Transfusion Medicine/Hemostasis Clinical Trials Network have designed a trial called the Red Cell Storage Age Study. Although both studies are large by conventional standards, they may not be large enough to detect an effect of the size suggested by analysis of prior experience.

The ABLE study was planned as a large simple randomized trial in which trauma or intensive care patients with anticipated massive transfusion would be randomized to receive either all red blood cells stored for 8 days or less or all stored for longer than 8 days and followed to a mortality outcome at the end of the hospital stay [58]. The initial plan was to try to randomize

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4000 patients in 100 ICUs in trauma centres and academic medical centres in Canada, the USA, Europe, and Australia. Present funding is only to start the trial in Canada where the total number of patients is probably too small to achieve the enrolment goals in any reasonable period of time. However, once the problems involved in running the trial are determined, additional high quality sites and sources of funding will be sought. While the planned trial would be smaller than the retrospective review by Koch described above, the inclusion of many more massively transfused patients may provide additional power.

The Red Cell Storage Age Study trial would involve recruitment and randomization of 1606 cardiac surgery patients selected on the basis a risk factor scoring system that predicts an 80% likelihood of requiring perioperative transfusion. The study would follow the patients until hospital discharge or 30 days for changes in a multiple organ dysfunction score or mortality and would also include a smaller physiologic substudy of thenar eminence perfusion spectral imaging and sublingual supravital microscopy to look for changes at the time of transfusion.

What do we do now?

Red blood cells clearly degrade during storage. They change shape, become acidotic, lose DPG, ATP and membrane. Some break down, and others fail to circulate. While the body has evolved mechanisms for clearing normal numbers of aged cells, the bulk loads of acid, abnormal cells, and cell breakdown products associated with transfusion, especially massive transfusion, may have pathologic consequences. Bacterial growth and opportunities for other mishaps increase with storage time.

On the other hand, blood storage allows the ability to build and maintain blood inventory to support large institutions and major surgical procedures. Very large medical centres may use hundreds of units of red blood cells a day, and occasionally individual patients require massive amounts of blood in the course of major procedures. Storage maximizes red blood cell utilization so that now most major medical centres find a recipient for more than 99% of their red blood cell inventory. When red blood cells could only be stored for 3 weeks, the wastage rates approached 30%.

Review of the evidence suggests that there is no need for immediate change in transfusion practice, but that in planning for the future, we should work both for greater knowledge and better products. Greater knowledge will come from both basic and clinical research. The development of better products is ongoing but needs guidance and support. Choosing the best ways to take advantage of better products will depend on the information available at the time the products become available. In the mean time, we need to work to inform the best choices and to have the best options.

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