Clinical consequences of red cell storage in the critically ill

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Red cell (RBC) transfusions are a potentially life-saving therapy employed during the care of many critically ill patients to replace losses in hemoglobin to maintain oxygen delivery to vital organs. During storage, RBCs undergo a series of biochemical and biomechanical changes that reduce their survival and function. Additionally, accumulation of other biologic by-products of RBC preservation may be detrimental to recipients of blood transfusions. Laboratory studies and an increasing number of observational studies have raised the possibility that prolonged RBC storage adversely affects clinical outcomes. In this article, the laboratory and animal experiments evaluating changes to RBCs during prolonged storage are reviewed. Subsequently, the clinical studies that have evaluated the clinical consequences of prolonged RBC storage are reviewed. These data suggest a possible detrimental clinical effect associated with the transfusion of stored RBCs; randomized clinical trials further evaluating the clinical consequences of transfusing older stored RBCs are required.

Over the past 25 years, we have witnessed a dramatic "paradigm shift" whereby red blood cell (RBC) transfusions, once regarded as "one of the great advances in modern medicine," are now considered harmful in some clinical situations. This paradigm shift has focused attention on the quality of stored transfused blood. Changes accompanying the storage of red cells (RBCs) are known as the "storage lesion," which can be defined as a series of biochemical and biomechanical changes in the RBC and storage media during ex vivo preservation that reduce RBC survival and function. Although the storage lesion has been well documented for decades,[1] our understanding of the mechanisms involved in these changes and clinical consequences remains incomplete. Recent clinical trials and animal experiments have raised fundamental questions about the efficacy of stored RBCs,[2,3] which may have

ABBREVIATIONS: CO = cardiac output; DO_2 = O_2 delivery; ICU = intensive care unit; LOS = length of stay; pO_2 = partial pressures of oxygen.

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important implications for the future of transfusion research.

In critically ill patients, clinical studies have reported an association between RBC transfusions and increased morbidity and mortality, an effect that may increase with the age of the transfused RBCs. Anemia is very common in the critically ill with 95 percent of patients admitted to the intensive care unit (ICU) experiencing a hemoglobin (Hb) level below normal by the third day[4,5] and 40 percent to 45 percent of critically ill patients receive 5 units of RBCs during their ICU admission.[4,5] More recently, a seminal multicenter randomized controlled clinical trial in critically ill patients (TRICC, Transfusion Requirements in Critical Care) demonstrated a lower 30-day mortality rate in the patients randomly assigned to the restrictive transfusion strategy[6] (23.3% vs. 18.7%, p = 0.11; Fig. 1). Plausible explanations for the increased morbidity and mortality seen in TRICC may be that prolonged storage renders RBCs ineffective oxygen (O_2) carriers and/or modifies RBCs, which cause harm when transfused into vulnerable patients via either a proinflammatory effect or the direct toxic effects of by-products of RBC storage. To date, the mechanisms of action accounting for increased morbidity and mortality remain unknown. In this article, we will review the laboratory and clinical studies evaluating changes to RBCs with prolonged storage followed by a review of studies evaluating the clinical consequences of prolonged RBC storage.

RBC AND OXYGEN DELIVERY

The goal of RBC transfusions is to increase the Hb concentration, thereby improving O_2 delivery to tissues.[7,8] The amount of O_2 delivered, either to the whole body or to specific organs, is the product of blood flow and arterial O_2 content. For the whole body, O_2 delivery (DO_2) is the product of total blood flow or cardiac output (CO) and arterial O_2 content (CaO_2)[8-10] In terms of CaO_2, more than 99 percent of O_2 is transported by Hb and only a negligible amount is dissolved in the plasma fraction at ambient PaO_2 in room air. Thus, under most circumstances, DO_2 can be calculated by

$$DO_2 = CO\ (L/min) \times [Hb\ (g/L) \times Arterial\ O_2\ saturation \times 1.39 mL\ of\ O_2/g\ Hb].$$

From this equation, the causes of tissue hypoxia include decreased DO_2 from decreases in Hb concentrations (anemic hypoxia), CO (stagnant hypoxia), or Hb saturation (anoxic hypoxia).

Additionally, tissue hypoxia may be related to abnormalities in oxygen-Hb dissociation and the ability of the RBC to traverse the microcirculation. The loading and unloading of oxygen to Hb is described as a sinusoidal oxyhemoglobin dissociation curve, which enables both efficient oxygen loading at high oxygen tensions in the lungs and the efficient unloading at low partial pressures of oxygen (pO_2) in the microcirculation of the tissues. Hb O_2 binding affinity, however, may be altered by acidosis and factors such as 2,3-diphosphoglycerate (2,3-DPG), which promotes unloading of O_2 in the tissues by a right shift of the oxyhemoglobin dissociation curve. These factors may potentially have a significant beneficial or negative effect in the adaptive response to anemia.

To deliver O_2 to the tissues, the RBC must navigate the microcirculation where the capillary diameter ranges from 3 to 8 μm. For the 8-μm RBC to navigate these narrow channels, it must retain its deformability. This deformability is dependent on a number of factors including surface area-volume ratio, membrane elasticity, and intracellular viscosity.[11] To maintain these properties, the RBCs depend on the catabolism of glucose and generation of high energy adenosine triphosphate (ATP) via the Embden-Meyerhoff pathway. Loss of their normal biconcave shape and deformability impairs the ability of the RBC to deliver O_2 and remove CO_2 from the tissues via the microcirculation. These senescent RBCs and poorly deformable cells are culled from the circulation as they pass through the splenic circulation.

The healthy host has substantial physiologic reserves for DO_2, thereby enabling the human body to adapt to significant increases in O_2 requirements or decreases in DO_2 as a result of various diseases. In health, the amount of O_2 delivered to the whole body exceeds resting O_2 requirements by a factor of two- to fourfold.[8] RBC transfusion implicitly assumes that an increase in Hb with transfusion will increase the O_2 content of blood and deliver O_2 in a form that will be readily utilized by tissue. After RBC transfusions, an increase Hb concentration is

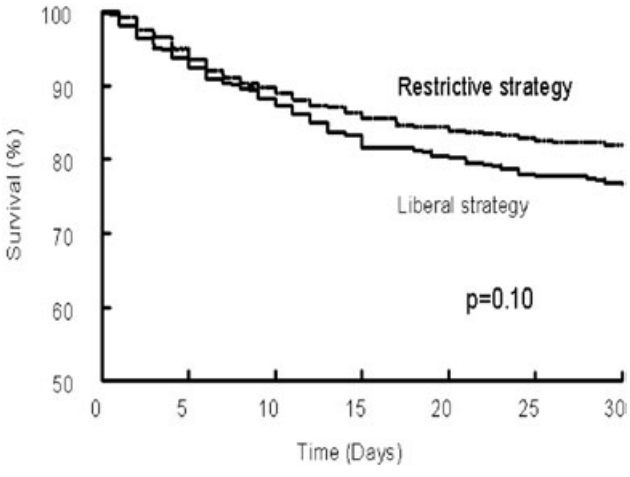

Fig. 1. Critical care patients randomly assigned to a restrictive transfusion strategy to maintain their Hb level between 7 and 9 g per dL had improved survival compared to patients randomly assigned to a liberal transfusion strategy (Hb level, 10-12 g/dL).[6]

readily measured, but the effects of RBC transfusion on utilization of O_2 in peripheral tissues is rarely measured. As a result, in clinical practice, an increase in Hb concentration still remains the common measure of RBC transfusion efficacy.

HOW WAS RBC STORAGE TIME ESTABLISHED?

Both the inability to define the optimum and minimum clinical transfusion thresholds and the inability to reliably measure tissue oxygenation have made it difficult to study and determine the efficacy of RBC transfusions. As a consequence, the determination of shelf life for RBCs has been based exclusively on the maintenance corpuscular integrity and posttransfusion 24-hour survival as surrogate markers for therapeutic benefit.[2,12,13] Determining the concentration of chromium-radiolabeled RBCs remaining in the peripheral circulation 24 hours after the administration of a fixed dose of RBCs has been the standard measurement for RBC survival. Early studies of blood stored in citrate and glucose adopted a threshold of 70 percent for the acceptable 24-hour posttransfusion survival. Below this threshold, transfused RBCs were deemed not beneficial in the treatment of anemia,[12] but this may have been more of a pragmatic threshold limited by the technology of the time. The introduction of acid-citrate-dextrose (ACD) in 1943 with its enhanced preservative properties increased the shelf life of blood to 21 days.[14] Sustained effort over the past 60 years to maintain corpuscular integrity and improve posttransfusion viability has extended the storage period for blood to 35 to 42 days by the addition of phosphate, adenine, and nutrient solutions.[15-18] Unfortunately, few studies have examined the efficacy of RBCs in the transport of oxygen when stored for such a prolonged period of time.

CHANGES IN RBCS DURING THE STORAGE PROCESS

During storage, RBCs undergo a predictable change in morphology, evolving from deformable biconcave disks to reversibly deformed echinocytes to irreversibly deformed spheroechinocytes (Fig. 2). These corpuscular changes are associated with a multitude of biochemical and biomechanical changes, which have been previously summarized and have been collectively referred to as the storage lesion.[1-3,19,20]

These biochemical and biomechanical changes associated with RBC storage include a depletion of ATP[21-23] and 2,3-DPG,[22-25] membrane phospholipid vessiculation[26-29] and loss, protein oxidation[1,30] and lipid peroxidation of RBC membrane,[31] and, ultimately, loss of deformability.[32-35] These corpuscular changes may contribute to adverse clinical consequences as a result of altered or diminished oxygen transport. RBC storage increases RBC-endothelial interactions,[36] which are further increased by endotoxins and inflammatory cytokines.[37] The loss of deformability and increased interactions with vascular endothelium compromise microvascular flow of stored RBCs[38] and critically ill patients would be expected to be particularly vulnerable.[3] Even if able to navigate the microcirculation, the unloading of oxygen to the tissues may be impaired by the well-documented depletion of 2,3-DPG in RBCs stored for more than 48 hours.

ATP depletion has been well documented,[21-23] but the consequences of this phenomenon are not clear. Rapid depletion of ATP in RBCs reproduces the morphologic changes observed during storage and restoration of ATP levels reverses these changes.[39] During storage, however, the more gradual depletion of ATP does not correlate well with observed morphologic changes, and a large number of irreversible spherocytes persist despite restoration of normal ATP levels.[21,40] The total available adenine nucleotide pool (ATP + ADP + AMP) may be of greater importance than the concentration of ATP alone[41] because this may allow transfused RBCs to regenerate the necessary ATP to perform normal metabolic functions and repair cellular damage resulting from storage.[42] Some studies have suggested that ATP levels may be important to prevent secondary mediators of the "storage lesion" including dephosphorylation of proteins[43] or changes in the phospholipid composition of the RBC membrane.

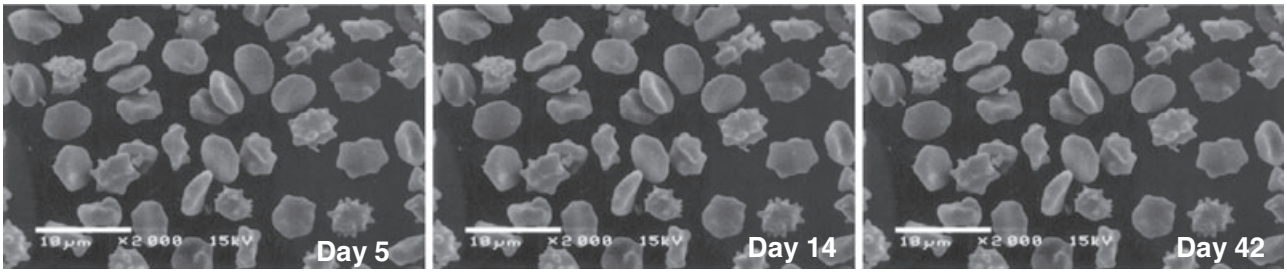

Fig. 2. Electron micrographs of RBCs over 42 days of storage demonstrating progressive changes from biconcave discs to reversibly deformed echinocytes to irreversibly deformed spheroechinocytes.[33]

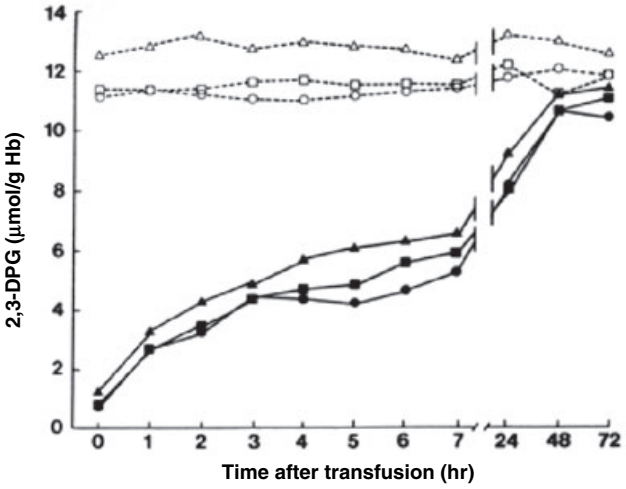

Fig. 3. Mean 2,3-DPG levels in recipient (- - -) and in transfused RBCs (—) during 3 days after transfusion for CPDA-1 (●), AS-1 (■), and AS-3 (▲) RBCs.[44]

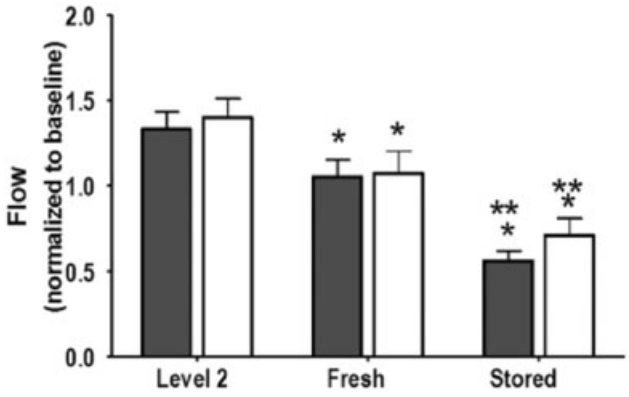

Fig. 4. Changes in arteriolar (■) and venular (□) blood flow after moderate hemodilution (Level 2) and in the two experimental groups: fresh and stored RBCs. In both experimental groups, fresh and stored RBCs, the blood flow was reduced from Level 2 (*) and was also significantly different from each other (; $p < 0.05$). Data are presented means ± SEM.[59]**

The depletion of RBC 2,3-DPG, the major allosteric modifier of oxygen affinity, is a well-characterized event that occurs early during storage.[22-24] De novo synthesis of 2,3-DPG occurs after transfusion, restoration of RBC 2,3-DPG can take up to 72 hours.[25,44-48] In man and nonhuman primates, after transfusion of 2,3-DPG–depleted RBCs, systemic DPG levels, as well as the p50 values (a measure of oxyhemoglobin affinity indicated by the O_2 tension at 50% Hb saturation), decrease significantly and then regenerate at a variable rate[25,49] (Fig. 3). Based on these observations, it has been speculated that transfusion of large amounts of stored RBCs may have an adverse clinical consequence on DO_2 in patients whose O_2 reserve is compromised.[45-48] This hypothesis has not been tested in controlled clinical trials, however.

TABLE 1. Changes in percentage of reversibly and irreversibly deformed RBCs and associated changes in deformability as measured by nuclear pore filtration[33]

	RBC change (%)		
Days	Reversibly deformed	Irreversibly deformed	Deformability index
5	14.0 ± 1.7	7.0 ± 1.6	118.9 ± 9.4
7	13.6 ± 1.7	8.4 ± 1.6	114.7 ± 7.6
14	27.9 ± 1.9*	14.7 ± 2.6*	70.9 ± 20.5*
21	30.6 ± 3.0	15.7 ± 3.3*	51.8 ± 23.3*
28	35.2 ± 1.6*	17.2 ± 4.1*	70.5 ± 13.2*
35	40.6 ± 3.4*	21.9 ± 5.0*	36.7 ± 7.9*
42	46.8 ± 6.7*	29.9 ± 4.0*	63.9 ± 14.0*

* $p < 0.05$ compared to blood stored for 5 days.

The formation of microvesicles composed of phospholipids, transmembrane proteins, and cytoskeletal during RBC storage results in a decrease in the surface-to-volume ratio and the formation of echinocytes and spheroechinocytes.[26] These shape changes are associated with decreased RBC deformability, as measured by filterability,[33] ektacytometry,[34] and increased osmotic fragility,[50-52] which have both been correlated with decreased RBC survival[35,51] (Table 1). Experiments with the additions of additive solutions (AS-1)[53] and experimental hypotonic storage solutions[54,55] have demonstrated decreased RBC membrane loss and vesicle formation during storage, which were associated with increased RBC survival.

Oxidative damage is a well-recognized mechanism contributing to the storage lesion and a plausible mechanism contributing to microvessicle formation and the loss of deformability. Oxidation of the spectrin-actin-protein 4.1 complex, which binds the phospholipid bilayer to RBC cytoskeleton, has been correlated with vessiculation of the RBC membrane.[11,30,56] Similarly, lipid peroxidation has also been associated with increased RBC deformability[57] and osmotic fragility.[50] During storage, the loss of band 3, an intrinsic RBC membrane proteins, results in a shift toward glycolysis with a resultant decrease in intracellular levels of NADPH and ATP, which may make the RBCs vulnerable to oxidative stress.[58]

Storage of RBCs also increases RBC adhesion to human vascular endothelium in vitro[36] and in vivo animal models.[38] Tsai and coworkers[59] observed a decrease of 63 percent decrease in microvascular flow after a 25 percent exchange transfusion with stored RBCs in a hamster model (Fig. 4). In vitro, RBC adhesions and microvascular occlusion appear be abrogated by prestorage leukodepletion,

suggesting that the altered RBC adhesiveness is associated with white blood cell (WBC) contaminants.[36]

There are also a number of reports suggesting that disease processes, such as sepsis, also impair RBC deformability.[60-64] and impair microcirculatory blood flow.[65-67] This combination may dramatically affect tissue DO_2 in sepsis and septic shock.[60-63] In this setting, transfusion of poorly deformable, 2,3-DPG–depleted stored RBCs with increased vascular adhesion could potentially exacerbate preexisting microcirculatory dysfunction and further impair tissue perfusion. The available evidence would suggest that the transfusion of stored RBCs may have adverse effects on microcirculatory flow and oxygen utilization, particularly in vulnerable patients.

EFFICACY OF RBC TRANSFUSIONS

In terms of establishing the efficacy of RBC transfusions, we can calculate the systemic DO_2 because it is proportional to the product of the Hb concentration and CO. There are, however, many inherent difficulties with tissue-specific indicators of cellular respiration and adequacy of oxygen transport and utilization. As a consequence, the current criteria for clinical efficacy for stored RBCs focus on their physical and biochemical characteristics while having little to do with their function. In clinical practice, we rely on increased Hb concentrations and changes in other crude markers of oxygenation such as mixed venous O_2 and lactate to determine whether a transfusion was efficacious (Fig. 5). Unfortunately, as previously described, RBCs may be ineffective transporters of oxygen, especially in compromised critically ill patients who have microcirculatory abnormalities from a number of pathologic processes.[68-70]

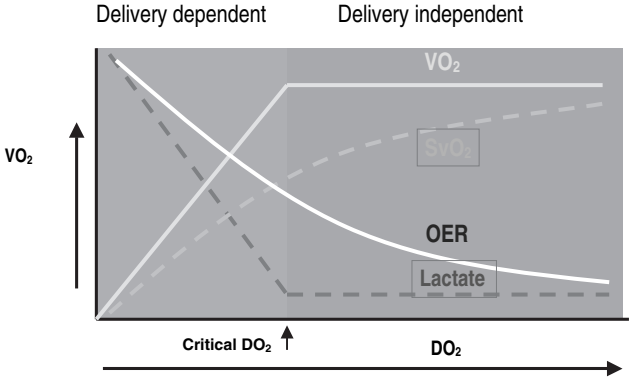

Fig. 5. As DO_2 decreases, there is a gradual increase in the oxygen extraction ratio (OER) and a corresponding decrease in the systemic venous O_2 (SVO_2). With the increased OER, initially there is no decrease in oxygen consumption (VO_2) by the tissues. The VO_2 only decreases when the DO_2 falls below a critical threshold (critical DO_2); this is associated with an increase in lactate (anaerobic metabolism).

ANIMAL STUDIES

Earlier animal studies demonstrated that stored rat blood compared to fresh RBCs did not improve tissue O_2 consumption.[71,72] The effect of RBC transfusions was assessed through isovolemic hemodilution of animals just beyond the point of O_2 supply dependency. In the supply-dependent state, the efficacy of transfusing old versus fresh RBCs and blood substitutes to DO_2 (CO, arterial O_2 saturation, and Hb concentration), to improve O_2 consumption (measured directly) and decrease arterial lactate was measured. With this model, the two studies consistently noted that transfusion of rat RBCs stored under standard conditions for 28 days when compared to fresh rat blood (less than 5 days) were not efficacious in improving tissue O_2 consumption or other measures of tissue hypoxia.[71,72] These findings were further supported by an additional study that 28-day-old rat blood stored in CPD failed to improve microvascular pO_2 in a hemorrhagic rat model compared to fresh RBCs or RBCs stored in saline-adenine-glucose-mannitol.[73] Subsequent work, however, demonstrated that the storage lesion in rat RBCs is significantly worse than the storage lesion in human RBCs after similar storage times.[74] Although 28-day rat blood is significantly "older" than 28-day human blood, these experiments still serve to highlight the possible deleterious effects of the storage effect to impair oxygen delivery to the tissues.[3] To overcome the differences between rat and human blood, Raat and associates[23] compared the transfusion of 2- to 6-day-old, 2- to 3-week-old, and 5- to 6-week-old human blood in a rat isovolemic exchange model.[23] The exchange transfusion with the old blood resulted in a decrease in microvascular pO_2 compared to the fresh and intermediate blood (Fig. 6). Several

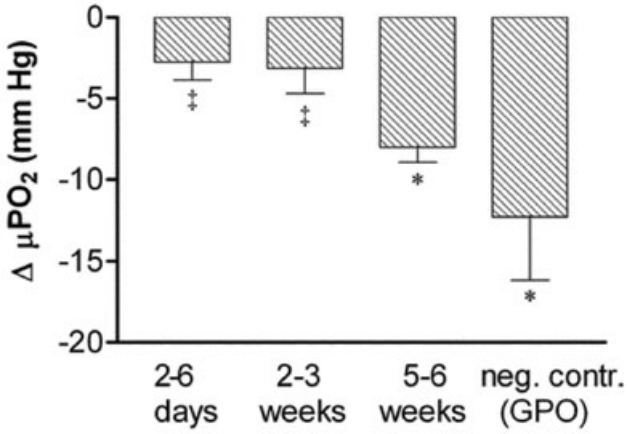

Fig. 6. Difference between intestinal microvascular oxygen concentration (μPO_2) in rat isovolemic exchange model at the start and end of exchange transfusion with washed human RBCs that had been stored up to 6 week. Values are displayed as means ± SEM, n = 8. *$p < 0.05$ versus 2- to 6-day stored RBCs; ‡$p < 0.05$ versus no change (zero).[23]

conclusions may be drawn from these in vivo experiments. First, these animal studies provide some of the only evidence confirming that RBCs effectively increase oxygen delivery and release oxygen at the cellular level. Second, the experiments in supply-dependent animals demonstrate that the many changes observed in the laboratory may have important in vivo consequences. Indeed, older transfused RBCs may have a limited ability to acutely improve O_2 availability. These experiments raise a number of questions. Specifically, it is unclear whether the observed effects of transfusing old stored blood were due to corpuscular changes within the RBC or associated with bioreactive substances in plasma supernatant of stored RBCs. Finally, the magnitude of this effect and its clinical consequences have yet to be established.

CLINICAL STUDIES OF O_2 KINETICS

Clinical studies attempting to determine the effect of RBC transfusions on O_2 kinetics have not provided definitive answers. We identified 19 clinical studies[75-93] evaluating the impact of RBC transfusions on O_2 kinetics in humans. All studies measured DO_2 and O_2 consumption before and after the transfusion of a specified number of allogeneic RBCs. DO_2 uniformly increased but O_2 consumption was observed to change in only 6 of the studies.[8] The lack of change in O_2 consumption reflects either methodologic errors[94] or patients with an elevated anaerobic threshold rather than an indication that RBCs were unnecessary, as was suggested by one of the studies.[75] The most recent study also evaluated systemic oxygen transport by measuring skeletal oxygen tension with microelectrodes.[78] Even though a number of clinical trials[91-93] have attempted to define optimal levels of DO_2, there is still no consensus as to which patients are most likely to benefit and which intervention or approach is superior (i.e., fluids, inotropic agents, or a combination of these interventions).

CLINICAL STUDIES EXAMINING CONSEQUENCES OF RBC STORAGE

A systematic literature search was conducted to identify previous systematic reviews on the topic of clinical effectiveness of stored RBCs, and none were identified. In the absence of published systematic reviews, we conducted a systematic search and synthesis of the literature with standard methods.

Numerous studies have demonstrated an association between RBC transfusions and increased mortality[4,5,95-99] and morbidity[4,5,95-97,100-113] (Table 2). Most of these studies are prospective or retrospective cohort studies and therefore despite multivariate analysis, the relationship between RBC transfusions and increased adverse events may still be a result of confounding. In the TRICC trial, however, patients randomly assigned to a more restrictive transfusion threshold received fewer units of RBCs (mean, 2.6 vs. 5.6; $p < 0.01$) and had a significant decrease in hospital mortality (22.8% vs. 28.1%; $p = 0.05$) and an increase in respiratory failure, which suggests that RBC transfusions may indeed cause harm.[6] The prolonged storage of the RBCs is a potential cause for the increase in adverse events associated with higher transfusion rates, but this study was not designed to look at the effects for prolonged RBC storage.

A number of retrospective clinical studies have examined the association between prolonged storage times and adverse clinical outcomes and they have documented an increase in mortality,[96,114,115] pneumonia,[96,103] serious infections,[96,105] multiorgan failure,[115,116] and length of stay (LOS)[96,99,115,117] in many patient populations including critically ill patients, multiple trauma victims, and patients undergoing cardiac surgical procedures (Table 3). Martin and associates[99] observed a significant association between the transfusion of aged blood (>14 days old) and increased length of ICU stay ($p = 0.003$) in 698 critically ill patients. In patients receiving a transfusion, aged RBCs was the only predictor of LOS ($p < 0.0001$). In survivors, from this analysis, only the median age of blood was predictive of LOS ($p < 0.0001$). Purdy and coworkers[114] demonstrated a negative correlation ($r = -0.73$) between the proportion of RBC units of a given age transfused to survivors in patients admitted to the ICU with a diagnosis of severe sepsis ($n = 31$). Purdy and coworkers also noted that these latter units were more likely to be older.

With a prospective database of trauma victims, Zallen and colleagues[116] and Offner and colleagues[105] have examined the influence of the age of the transfused RBCs in trauma victims who received between 6 and 20 units of RBCs in the first 12 hours after injury. The mean age of units of RBCs and the mean number of units greater than 14 and 21 days, respectively, were greater in patients ($n = 63$) with multiple organ failure.[116] The number of units greater than 14 days old (odds ratio [OR], 1.13; confidence interval [CI], 1.01-1.26) and the 21 days old (OR, 1.13; CI, 1.00-1.27) were also independent risk factors for serious infection ($n = 61$).[105] In a separate study of 86 trauma patients who received transfusions in the first 48 hours of admission and were discharged alive, Keller and coworkers[117] demonstrated a significant association between the number of RBC units older than 14 days and hospital LOS ($p = 0.02$), but not ICU stay or duration of mechanical ventilation.

In cardiac surgery patients, Basran and associates[115] demonstrated an increase in in-hospital mortality and out-of-hospital associated with increased mean age of RBCs transfused. Additionally, the age of the transfused RBCs was associated with increased ICU length of stay and acute renal dysfunction. Vamvakas and Carven[103] found an increased risk of pneumonia of 1 percent for each additional day in the mean storage time of the trans-

TABLE 2. Association of RBC transfusions with mortality and morbidity in critically ill in observational studies

Study: first author, year	Population	Design	Number	Outcomes
Ciesla, 2005[113]	Trauma	Prospective cohort	1,344	Increased multiorgan failure
Gong, 2005[106]	ICU patients	Prospective cohort	688	Increased risk of ARDS*
Lebron, 2005[109]	Liver transplant	Retrospective cohort	241	Increased early postoperative renal failure
Shorr, 2005[107]	ICU patients	Prospective cohort	3,502	Increased ICU acquired bacteremia
Silverboard, 2005,[112]	Trauma	Prospective cohort	102	Increased risk of ARDS
Smith, 2004[108]	Subarachnoid hemorrhage	Prospective cohort	441	Worse outcome with intraoperative transfusions
Vincent, 2004[5]	ICU patients	Prospective cohort	1,136	Increased ICU, hospital and 28-day mortality Increased organ dysfunction
Leal-Noval, 2003[104]	Cardiac surgery	Prospective cohort	103	Increased ICU LOS, mechanical ventilation, and pneumonia
Malone, 2003[98]	Trauma	Prospective cohort	15,534	Increased mortality
Chelemer, 2002[100]	CABG	Prospective cohort	533	Increased bacterial infections
Claridge, 2002[110]	Trauma	Prospective cohort	1,593	Increased infection
Corwin, 2002[4]	ICU	Prospective cohort	4,892	Increased ICU and hospital LOS Increased complications
Taylor, 2002[95]	ICU	Retrospective cohort	1,717	Increased nosocomial infections, ICU LOS, and mortality
Vamvakas, 2002[111]	Cardiac surgery	Retrospective cohort	416	Increased postoperative ventilation associated with volume of RBC supernatant
Leal-Noval, 2001[96]	Cardiac surgery	Prospective cohort	738	Increased ICU LOS, mechanical ventilation, and pneumonia
Chang, 2000[97]	Colorectal surgery	Retrospective cohort	282	Increased postoperative infection Increased mortality
Carson, 1999[101]	Hip fracture	Retrospective cohort	9,598	Increased risk of serious bacterial infection and pneumonia
Offner, 1999[105]	Trauma	Prospective cohort	61	Increased infection
Vamvakas, 1999[103]	Cardiac surgery	Retrospective cohort	416	Increased postoperative infection (5% /unit)
Carson, 1998[141]	Hip fracture	Retrospective cohort		No change in mortality or morbidity
Moore, 1997[102]	Trauma	Prospective cohort	513	Increased multiorgan failure
Martin, 1994[99]	ICU	Retrospective cohort	698	Increased mortality

* ARDS = acute respiratory distress syndrome.

TABLE 3. Association of RBC storage with clinical outcomes: observational studies

Study: first author, year	Population	Design	Number	Outcomes
Basran, 2006[115]	Cardiac surgery	Retrospective cohort	321	Increased mortality associated with mean age of RBC units and age of oldest RBC unit
Leal-Noval, 2003[104]	Cardiac surgery	Prospective cohort	897	Increased pneumonia associated with oldest unit
Keller, 2002[117]	Trauma	Retrospective cohort	86	Increased LOS with number of RBC units >14 days
Offner, 2002[105]	Trauma	Prospective cohort	61	Increased infections with number of units >14 and 21 days
Vamvakas, 2000[118]	Cardiac surgery	Retrospective cohort	268	No change in LOS or mechanical ventilation associated with age of RBC units
Vamvakas, 1999[103]	Cardiac surgery	Retrospective cohort	416	Increased risk of pneumonia with median age of transfused RBC units
Zallen, 1999[116]	Trauma	Prospective cohort	63	Increased multiorgan failure with number of units >14 and 21 days
Purdy, 1997[114]	Septic ICU	Retrospective cohort	31	Increased mortality associated with older median age of RBC units
Martin 1993[99]	ICU	Retrospective cohort	698	Increased LOS with number of units >14 days

fused RBCs. Additionally, Leal-Noval and coworkers[104] described an increase of 6 percent in the risk of pneumonia for each additional day of storage of the oldest unit of RBCs transfused. Duration of RBC storage and changes in postoperative pneumonia rates, however, were not associated with a prolongation of mechanical ventilation or ICU or hospital stay.[104,118]

Unfortunately, all cohort studies evaluating prolonged RBC storage will invariably be subject to the confounding influences of factors such as the number RBC units transfused, the mixture of storage times from the multiple units transfused throughout a hospital stay, and patient factors including severity of illness. Inferences related to the clinical consequences of transfusing RBCs

with a storage time of less than 8 days are also limited by a small sample size and imbalances in clinically important baseline characteristics. Additionally, all the published studies demonstrate an adverse effect associated with prolonged storage of RBCs, which may reflect a publication bias.

Recently, two small randomized controlled trials in adults examining the effects of the storage time of transfused RBCs have been reported. Walsh and colleagues[119] evaluated changes in gastric intramucosal pH (pHi), a measures of gastric perfusion, in 22 mechanically ventilated critically ill patients who required a RBC transfusion. In this study, the authors were not able to detect any adverse consequences on pHi and changes in the arterial-gastric mucosal CO_2 gap with a storage time exceeding 20 days as compared to patients receiving RBCs less than 5 days. These results contradicted earlier observations in a before and after study conducted by Marik and Sibbald[68] who documented an inverse relationship between the age of transfused RBCs and gastric intramucosal pH($r = -.71$; $p < 0.001$) in a prospective trial of 23 critically ill ICU patients (Fig. 7). The former trial differed from the latter as patients received filtered leukodepleted RBCs and were not septic and stable enough to withhold RBC transfusions for 12-18 hours while consent was obtained. We recently completed and published a second study of prolonged RBC storage.[120] The goal of the pilot study was to ensure that blood banks could comply with requests for fresh RBCs, adhere to the inventory management strategies and to ensure that our approach would result in clear separation of RBC storage times. In the 57 patients studied, the number of units transfused averaged 5.5 ± 8.43 RBC units in the experimental group as compared

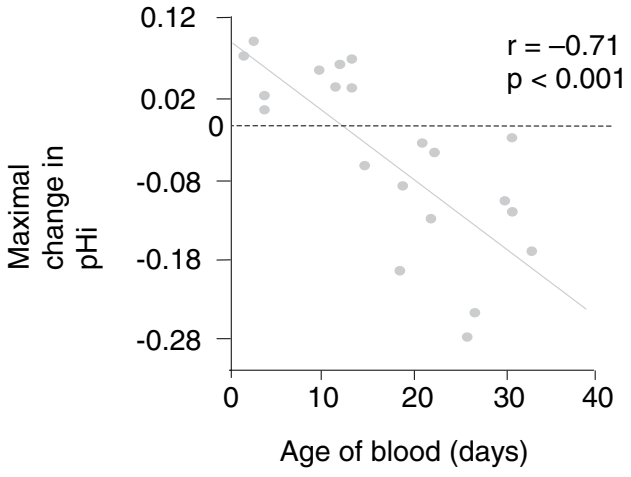

Fig. 7. In 13 septic patients who received 3 units of RBCs, there was no evidence of increased oxygen as determined by gastronomic tonometry measurements of gastric pH (pHi). Transfusion of older blood (>15 days) was associated with evidence for gastric mucosal ischemia.[68]

3.3 ± 3.27 RBC units in the standard group ($p = 0.25$). The median storage time was 4 days in the experimental group as compared to 19 days in the standard group (difference of 15 days, interquartile range of 12-16 days, $p < 0.001$). Overall, 91 percent of patients allocated to the fresh group received RBCs with storage times below 8 days. The group receiving RBCs less than 8 days of age tended to be older on average (68 ± 8.5 years vs. 63 ± 15.3 years, P = 0.13) and have more comorbid illnesses (85 percent vs. 65 percent, P = 0.09). In total, 27 percent of patients in the experimental group died or had a life-threatening complication as compared to 13 percent in the standard group ($p = 0.31$). There were no differences in prolonged respiratory, cardiovascular or renal support after randomization (all P > 0.05). This pilot trial demonstrates the feasibility of performing a large randomized clinical trial to evaluate the effect of prolonged RBC storage. The small sample does not allow for any conclusions to be reached regarding the adverse effects of RBC storage on mortality and morbidity.

Four neonatal trials conducted in critically ill premature infants have also evaluated prolonged RBC storage in the context of dedicated RBC units (multiple aliquots from a single unit are transfused to the same infant over time) programs.[121-124] These programs involve prolonged storage as a consequence of approach rather than specifically examining the hypothesis of fresh versus stored blood. A meta-analysis of these four randomized trials[121-124] showed that infants receiving fresh blood were exposed to just over 2 more donors than those receiving stored blood (weighted mean difference of 2.31 donors, 95 percent CI 1.09-3.53; p = 0.0002) (Fig. 8). Unfortunately, all studies were of small sample size and none of the four trials evaluated clinically important outcomes. Thus, conclusive evidence on outcomes can not be ascertained. Finally, a recent study published in The New England Journal of Medicine (p.e.) found that fresh whole blood for cardiopulmonary bypass pump-priming in children less than 1 year of age offered no advantage over reconstituted blood products.[125]

PROPOSED MECHANISMS LINKING PROLONGED STORAGE OF RBCS AND ADVERSE CLINICAL CONSEQUENCES

Evidence from laboratory studies that prolonged RBC storage may result in either (1) an impaired ability of stored RBCs to transport or deliver oxygen as described above; or (2) stimulation of the inflammatory cascade by the transfused blood product. Once initiated, either mechanism may exacerbate or eventually lead to organ failure and death in the critically ill. As a consequence or as a direct cause of critical illness, there are a number of potential events that result in an altered host immune function, either initiating a pro-inflammatory or anti-inflammatory response. The Systemic Inflammatory

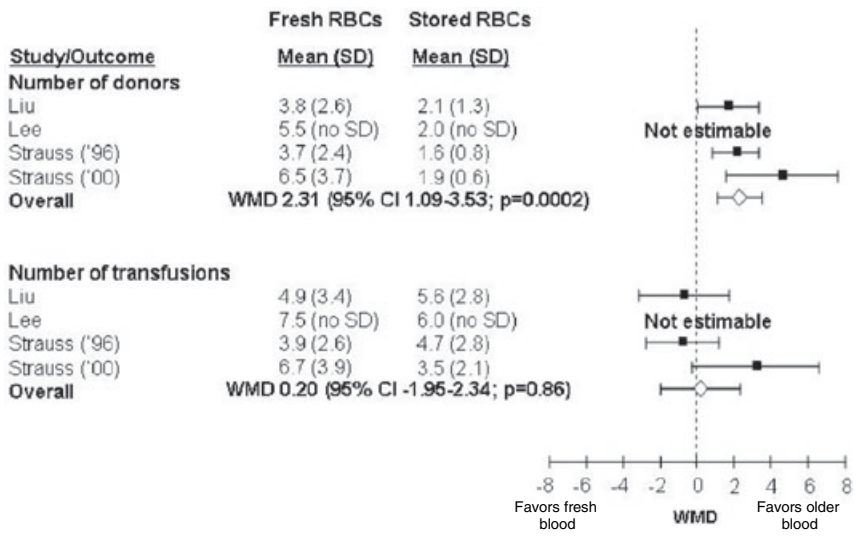

Fig. 8. A meta-analysis of four randomized trials of neonates[121-124] demonstrating that infants receiving fresh blood were exposed to just over 2 more donors compared to infants receiving stored blood.

Response Syndrome (SIRS), as an example, may result from a variety of insults to the human host including severe pancreatitis, cardiopulmonary bypass, trauma, burns and infections.[126] Transfused RBCs and platelets products may not only be a trigger but could potentially prime the body so that subsequent insults are much more significant (second hit hypothesis).[127] There is also evidence that transfused RBCs may have pro-inflammatory activities.[128-136] Many pro-inflammatory molecules are detected in RBC units, including cytokines,[128,132,134-136] histamine,[132] lyso-phosphatidyl-choline species,[129] and other bio-reactive substances, which may initiate, maintain or enhance an inflammatory process. The altered immune responses following RBC transfusions may predispose critically ill transfusion recipients to SIRS, sepsis syndrome, nosocomial infections,[60-65,129,137] and multiorgan failure,[67,70,103,114] which may ultimately result in higher mortality rates.[70] Through changes in RBC units following prolonged storage, older RBCs may result in endothelial injury and possibly activation.

Other pathophysiological mechanisms may result in adverse clinical outcomes. For example, it is known that the concentration of free Hb increases with time in RBC units. Free Hb reacts with endothelial nitric oxide, which can lead to vasoconstriction.[138] The binding and inactivation of nitric oxide may lead to increases in intravascular thrombosis, WBC adhesion and diapedesis, endothelial permeability, and smooth muscle proliferative responses after vascular injury.[139] Free Hb may also induce inappropriate vasoconstriction.[118] The interaction between free Hb and nitric oxide might explain why a significant drop in arterial O_2 tension (PaO_2 of 32 mmHg) and in forced vital capacity of 32 percent is observed in children with thalassemia major following blood transfusion.[140] Through these mechanisms (and others previously mentioned), one can also postulate that prolonged RBC storage can result in a failure to provide adequate oxygen to vital organs which will eventually lead to their failure. Other cellular by-products may also have detrimental effects. Silliman and colleagues[139] showed that the plasma fraction of packed RBCs stored for 42 days caused vasoconstriction and lung injury; this may be caused by lysophosphatidyl-choline species probably released from the cellular membrane of old RBCs.[31] In summary, a number of described storage related changes of transfused RBCs adversely affects the quality of stored blood and could potentially explain the adverse clinical consequences.

CONCLUSIONS

From our exhaustive review of the literature, we conclude that (1) there is strong laboratory evidence suggesting that prolonged RBC storage may be deleterious and (2) observational studies report a number of associations between prolonged storage and adverse clinical outcomes such as mortality and organ failure. Only two small adult trials have been published assessing clinical consequences of prolonged RBC storage. Given the importance of the question and limited evidence in humans, further clinical studies are required to address these issues. While animal and smaller clinical studies may further elucidate the RBC storage lesions and possible mechanisms for harm, they will never be able to determine if clinically important adverse events are caused by the transfusion of older RBCs. As a result, large definitive randomized controlled trials with clinically important endpoints including mortality are needed. Such trials will need to focus on the comparison of fresh RBCs versus standard issue in patient populations such as critical care patients who are most likely to be adversely affected by the transfusion of older stored RBCs. A comparison involving RBCs near outdate would also be of scientific interest, but the current knowledge of the RBC changes that occur during storage make it ethically problematic to randomize patients to "old" RBCs, which are possibly inferior to current standard therapy.

Ideally, in any future clinical trial assessing the storage lesion, the blood in the fresh arm of the trial should be as "fresh" as possible. A minimum storage window, however, is required to 1) allow sufficient time to complete infectious disease testing and ship to hospitals, 2) ensure

that there is sufficient inventory for the fresh arm of the trial, and 3) allow for a shelf life for fresh units that would be logistically possible to implement after the clinical trial. A storage limit of less than 8 days for fresh RBC units meets these practical considerations and, additionally, is based on our current knowledge of the RBC storage lesion. Both biochemical and biomechanical deterioration occurs in RBCs by the second week of storage. The levels of 2,3-DPG have fallen to near zero by the second week.[22-25] Although the levels are rapidly restored, the ability of these RBCs to deliver oxygen is impaired in the first 24 hours after transfusion. Additionally, the percentage of irreversibly deformed cells and overall deformability remains stable from Storage Day 5 to Storage Day 7[33] but significantly increases by Day 14.[33,34]

Randomized trials in potentially vulnerable patients such as premature infants or critically ill patients may have significant implications on blood procurement services. Negative trials would reassure clinicians and blood bankers regarding the effectiveness of prolonged storage. If no clinical benefits were detected (null result or harmful effect of RBCs stored less than 8 days), then blood banks would have evidence to support current inventory management strategies in adult who are critically ill. In addition, trauma surgeons and critical care practitioners would no longer be justified in requesting fresh blood. At least one positive trial would confirm that prolonged RBC storage has clinical consequences, either because prolonged RBC storage renders the product ineffective or because of direct toxic effects of prolonged storage. Documenting improved outcomes with fresh blood will provide much needed evidence to determine the most important mechanism leading to tissue injury and death. From a large study, we would expect to document the overall benefits of fresh RBCs, if present, and also better understand if fresh RBCs might benefit some patients more than others. A positive study would also result in a significant investment in research on the prolongation of shelf life such as improvements in storage media and rejuvenation solution, additional randomized controlled trials of RBC storage in different patient populations, and a reevaluation of regulatory policies on RBC storage. In conclusion, a series of clinical studies, regardless of their conclusions, will result in a major change or affirmation of clinical practice, health policy, and the management of the blood supply.

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