Clinical impact of blood storage lesions

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Recent reports suggest that transfusion of old red blood cell (RBC) units (>2 weeks) was associated with increased risks of postoperative complications and higher mortality rate caught public attention (Yap et al., Ann Thorac Surg 2008; 86:554–559 and Koch et al., 2008; 358:1229–1239). This rekindled the decades old discussion regarding the impact of RBC aging and storage lesions in patient care. The objectives of this review are to provide readers with an overview of the process of banking RBC that may have an impact on its quality, the reported clinical impact of storage lesions, the consequences of transfusing new RBC units only to the nation's blood supply and potential solutions that may improve the feasibility of blood banks to issue new blood units only. Am. J. Hematol. 85:117–122, 2010. © **2009 Wiley-Liss, Inc.**

Introduction

The ability to store blood was first started in the 1915 with the discovery of sodium citrate as blood anticoagulant [1]. Since then, much progress had been made. Currently, blood components can be stored for a prolonged time (Table I). The ability to store blood for a long time revolutionized blood transfusion practices and dramatically improved the practice of medicine and surgery. However, storing blood has pathological consequences that are collectively know as the "storage lesions."

The primary objective of red blood cell (RBC) transfusion is to improve oxygen delivery to the tissue. The trigger for transfusing RBCs varies with the cause of anemia and whether the cause is acute or chronic. In general, RBCs are transfused when hemoglobin level drops below 7 g/dl [2]. There is no clear evidence to suggest the efficiency of RBC to deliver oxygen to tissue decreases with RBC aging in vivo. However, in vitro, storage lesions significantly impair the capacity of RBC to efficiently deliver oxygen to the recipient tissue [3,4].

Storage Lesions

Decreasing ATP, decreasing pH, decreasing 2,3-Diphosphoglycerate. Blood is collected in a bag that contains preservative solution, which contains limited amounts of glucose in the form dextrose, phosphate and adenine to maintain ATP, and 2,3-DPG levels. The preservatives provide fuel for energy-requiring processes that preserve cell membrane integrity and cell functions [5]. During storage, lactic acid accumulates in the blood bag [6]. As a result, RBC pH decreases during storage [5] and this increases phosphatase 3 enzyme activity, which results in 2,3-diphosphoglycerol (DPG) degradation. 2,3-DPG binds to deoxyhemoglobin and stabilizes it [5]. This facilitates oxygen transportation from the lungs to tissues by oxyhemoglobin. A decrease in 2,3-DPG results in increased oxygen affinity of hemoglobin and therefore less oxygen delivery to tissue [5]. It is clear that the storage lesions outlined above progressively occur over the duration of storage. After 42 day storage, a unit of RBCs loses about a quarter of its ATP content and about a third of its glucose (Table II). Over 90% of 2,3-DPG is degraded after 42 day storage. However, about 50–70% of the 2,3-DPG is recovered in vivo within 24 h after transfusion [8].

Altered RBC membrane morphology and function. The overall goal of any blood bank is to issue RBCs that efficiently deliver oxygen to the tissues and survive in the recipient circulation long enough to perform its functions. Survival of transfused RBC is considered to be adequate when 70% of the transfused RBCs are present in the circulation after 24 h [3,9]. Storage conditions such as low ATP content induce morphological and physiological changes in RBC membranes [10]. This result in RBC membrane remodeling that involves loss and oxidative cross-linking of Band 3, a major integral membrane protein, and subsequent increased autologous IgG binding [11–13]. This accelerates the destruction of old RBCs after transfusion [14,15]. In addition, shedding of vesicles rich in lipid raft from spicules of cells that have undergone echinocytic transformation, were previously reported to be associated with changes in the RBC cytoskeletal organization [16]. Vesiculation takes place both during storage and after transfusion [10,17]. Vesiculation results in the loss of about 20% of cell surface and an increase in cell density [18,19]. It is thought that vesiculation protects RBC against complement lysis following transfusion [20]. Overall, stored RBCs show increased osmotic fragility and a reduced deformability [21,22].

Role of Blood Processing in the Development of Storage Lesions

Packed red blood cells (pRBC) are RBC concentrates prepared from whole blood after removal of platelet rich plasma [5]. They can also be selectively collected directly from donors using erythrocyte apheresis. However, whole blood donation is still a common practice particularly during mobile blood drives. Contrary to the practice in the US, most units in the developing countries are stored and transfused in the form of whole blood. The extent of the impact of these processing activities on RBC aging is determined by the collection technique and preparation methodologies.

Storage temperature. Packed RBC units are stored at temperatures between 1 to 6 degrees centigrade [23]. Stor-

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Conflict of interest: Nothing to report.

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Received for publication 13 November 2008; Revised 11 November 2009; Accepted 12 November 2009

Am. J. Hematol. 85:117–122, 2010.

Published online 18 November 2009 in Wiley InterScience (www.interscience.wiley.com).
DOI: 10.1002/ajh.21599

TABLE I. Shelf Life of Blood Components

Blood components	Shelf life
Red blood cells	Up to 42 days
Platelets	5 days
Fresh frozen plasma	1–7 years
Cryoprecipitate	1 years

TABLE II. Consequences of Prolonged RBC Storage

		42 day storage	
Characteristics	Function	Pre	Post
pH		6.8	6.4
ATP (mmol/g Hb)	Energy, active pumps	4.1	2.9
2,3-Diphosphoglycerate (mmol/g Hb)	Oxygen affinity	9	0.3
Potassium (mEq/L)	Maintain membrane potential	2.4	63
Glucose (mg/dl)	Energy	608	402
Free Hb (mg/dl)		39	372
Hemolysis (%)		0	0.61

Modified from Holme et al. 1998 [7].

age at this temperature range slows RBC metabolism and decrease demand for energy. This helps to extend RBC shelf life in blood banks. However, storage of RBC at 4°C impairs their ATP dependant potassium pump and as a result the intracellular and extracellular potassium ion equilibrates. Plasma potassium concentrations in stored blood increase ~1 meq/L per day due to passive leakage out of the red cell [24,25]. The potassium concentration peaks at about 90 meq/L in pRBC [25]. The resulting transfusion associated potassium load is rarely clinically significant except in the setting of hyperkalemia, renal failure or very sick neonates. In these situations, fresh or washed RBC may be transfused [26].

Leukoreduction. Leukocyte reduction of pRBC is now a common practice in the USA and Europe. Leukoreduction was introduced to minimize the risk of febrile nonhemolytic transfusion reaction [27,28], alloimmunization [29], transfusion-related immune modulation [30,31], and transfusion transmitted infections such as cytomegalovirus [32]. There is some evidence that WBC affect the quality of stored RBC [5,6,8]. Marik et al. reported one of the first studies showing the effect of stored-blood transfusion on oxygen delivery in patients with sepsis [33]. A similar study using leukoreduced units to treat anemic critically ill patients was repeated by Walsh et al. in 2004 but in contrast to Marik et al. no effect of storage duration on tissue oxygenation could be demonstrated [34]. This suggests leukoreduction may diminish the clinical impact of storage lesions. However, there is no comprehensive study that evaluates the impact of leukoreduction on RBC storage lesions.

Irradiation. Irradiation of pRBC is usually performed to minimize the risk of transfusion associated graft versus host disease [35]. Each unit is subjected to a minimum of 25Gy gamma radiation [36]. The objective is to induce enough DNA damage to prevent leukocyte proliferation upon exposure to allogeneic antigens. Since RBCs lack DNA, the main effect of radiation on RBC is increased cell membrane permeability. This increases leakage of intracellular electrolytes, particularly potassium. Irradiated RBC units have higher potassium level after irradiation [9,10]. Overall the viability of irradiated RBC is diminished [37,38]. As a result, the shelf life of irradiated pRBC units is reduced from 42 days to 28 days [23]. Therefore, it is generally believed that irradiation further accelerate RBC storage lesions.

Clinical impact. A few studies examined the impact of blood processing on clinical outcome. Two studies examined the effect of transfusing fresh whole blood (unprocessed) compared to reconstituted whole blood (processed) from stored pRBC, FFP and platelets, on clinical outcomes [39,40]. Both studies were prospective randomized, double blind studies and they involved a large number of pediatric patients who underwent cardio-thoracic surgery. The first study reported by Manno et al. suggests transfusing fresh whole blood immediately after cardiopulmonary bypass surgery is associated with significantly less post operative blood loss than reconstituted whole blood [39]. The second study was reported by Mou et al. almost 13 years after the first report by Manno's group. The study examined the use of fresh whole blood versus reconstituted blood for pump priming in open heart surgery. The study findings suggest fresh whole blood has no advantage over the use of reconstituted blood during surgery for congenital heart disease [40]. They further suggested that circuit priming with fresh whole blood is associated with increased length of stay in the intensive care unit and increased perioperative fluid overload. Even though the two studies examined different time point; pump priming and immediate postoperative period, the rationale for the observed contradictory outcomes was not apparent. One possible explanation is the difference in how blood units were processed in the 1980s and early 1990s compared to 2000s period. Prestorage leukoreduction was not a common practice before the year 2000 in the United States. Leukoreduction as highlighted above minimize storage lesion and immuno-moduator effect of blood transfusion. Even though neither Manno's nor Mou's group mentioned whether any of the blood units used in their study were leukoreduced, it is more likely that the Mou's group used leukoreduced products. In addition, the use of rejuvenation solution such adsol-1, which contains mannitol, a potent diuretic agent, was not a common practice in the 80s and early 90s. However, by early 2000s most pRBC units contain a rejuvenating solution. Mou's group clearly stated that they used AS-1 rejuvenating solution. Therefore, the lower incidence of postoperative fluid overload observed by Mou's group could be explained by the use of mannitol containing additive solution.

Impact of Blood Storage on Clinical Outcome

Association of transfusing old RBC with increased morbidity and mortality. There are reports that suggest RBC transfusions in generally are associated with increased mortality and morbidity. Several retrospective and prospective studies had evaluated the association of RBC transfusion and mortality [16,17], risk for acquiring infection [41,42], multiorgan failure [43,44] and length of stay [41,43,45].

Several studies had examined the effect of stored pRBC on patient care outcome (Table III). These studies focus mainly on limited clinical areas that include cardiac surgery, trauma and critically ill patients. Most of the studies were reported in the last ten years. The studies were made of more retrospective than prospective designs. There was no prospective randomized double blind study in adults or pediatrics that specifically addresses the effect of pRBC storage duration on patient care outcome. Only a few of the studies indicated whether they use leukoreduction or not. Even though most of the studies have different cut offs for new and old RBC units, majority used 2 weeks cut off and reported that RBC units older than 2 weeks were associated with higher mortality and morbidity. Table III summarized the study designs and outcomes of the published reports.

TABLE III. Impact of Blood Storage Lesions in Medical Practice

Study design	*N*	Outcome	References
A retrospective study that assesses the association of age of transfused RBC and clinical outcomes within 48 h post cardiac surgery. Approximately 3.8% of transfused RBC units were prestorage leukoreduction	670	RBC age was not associated with early mortality and morbidity after cardiac surgery	46
Retrospective study that evaluated duration of RBC storage and complication after cardiac surgery. Study period was 6.5 years. <50% of all cases received leukoreduced units Twice as much <14 weeks old units were leukoreduced compared to >14 weeks old units.	6,002	Transfusion of RBC stored for more than 2 weeks was associated with an increased risk of postoperative complications, reduced short-term and long-term survival.	47
Prospective study involving trauma patients at risk for developing multiorgan failure (MOF) and who received 6 to 20 units of pRBCs in the first 12 h following injury. Multiple logistic regression analysis was performed to determine if age of transfused blood is an independent risk factor.	63	The age of transfused pRBCs in the first 6 h after transfusion is an independent risk factor for postinjury MOF.	44
Prospectively studied the relationship between blood storage time and the development of disease recurrence and long-term survival after colorectal cancer surgery. Storage of buffy-coat-depleted RBCs suspended in saline for 21 days was used.	740	Transfusion of buffy-coat-depleted RBCs stored for 21 days may be an independent risk factor for development of recurrence after elective colorectal cancer surgery	48
Prospective cohort study that investigate whether transfusion of old blood increases infection risk in severely injured patients. Products were not leukocyte reduced.	61	Transfusion of old blood (14 and 21 days) is associated with increased infection after major injury.	49
Retrospective study that investigated the length of storage of transfused red cells and postoperative morbidity in patients undergoing coronary artery bypass graft surgery.	268	No corroborate with the previously reported association between transfusion of old RBCs and increased morbidity.	50
Retrospective study that evaluated the influence of erythrocyte concentrates storage time on postsurgical morbidity in cardiac surgery patients.	897	Prolonged storage of RBC does not increase morbidity in cardiac surgery. However, storage for longer than 28 days could be a risk factor for the acquisition of nosocomial pneumonia.	51
Retrospective study that evaluate the association between duration of storage of transfused red blood cells and morbidity and mortality after reoperative cardiac surgery.	321	The mean duration of storage of transfused RBCs was an independent predictor of in-hospital mortality, acute renal dysfunction, intensive care unit and hospital length of stay	43
Retrospective study that evaluated the Association of mortality with age of blood transfused in septic ICU patients.	31	There was a correlation between mortality and the age of PRBC transfused.	52
Retrospective study that evaluated RBCs transfused after prolonged storage may be associated with a higher incidence of postoperative infections than fresh RBCs in CABG pts.	416	Risk for pneumonia was associated with length of storage of transfused RBCs	42

Two large retrospective studies reported in 2008 appear to contradict each other. The relatively smaller study ($N = 670$) by Yap et al. from Melbourne Australia involved nonemergent recipients of coronary artery bypass graft or aortic valve replacement and who received at least 2 units of RBC between 2001 and 2007 [46]. Only 3.8% of the transfused blood units were leukoreduced. Thirty day cut off was used to define newer and old blood units. Age of transfused RBC was analyzed using logistic and linear regression models to determine an independent association with clinical outcome. The study findings show it was the quantity of RBC transfused and not the RBC age that independently correlated with clinical outcomes. The other relatively larger study ($N = 6,002$) reported by Koch et al. from Cleveland Clinic Ohio involved similar patient profile as the Melbourne study who were transfused between 1998 and 2006 [47]. About 33% of the newer RBC and 50% of old RBC units transfused were leukoreduced. Fourteen days was used as a cut off to define newer and old blood units. Multivariate logistic regression analysis was used to evaluate the effect of duration of RBC storage on clinical outcome. The study findings show transfusion of RBC units stored for more than 14 days was significantly associated with increased risk of postoperative complications and reduced short- and long-term survival.

There are clear differences between the studies. The geographic location of the study site could potentially influence the study outcomes. This could be attributed to slight variation in how blood products are prepared and stored in different parts of the world. The practice of universal leukoreduction is not yet a common practice in Australia while it is in the United Stated and this may have contributed to the contradictory findings. In addition, the cut off used, 14 days versus 30 days might influence the clinical outcome. Finally, the differences in transfusion practice and general patient care between the two continents may have an impact on the study outcomes.

Both reports have limitations that are commonly associated with retrospective studies. Many potential confounders were not equally represented or not considered. For example, both studies failed to account for surgical blood loss, salvaged blood transfusion, surgeon/anesthesiologist involved and intraoperative medications administered such as aprotinin, plavix, aspirin etc. It is a common practice for a patient to receive both old and new blood. It is unclear from both studies whether old blood negates the possible benefit of the newer blood in patients that received both.

Association of transfusing old blood and increased incidence of transfusion-related acute lung injury (TRALI). The incidence of transfusion-related acute lung injury (TRALI) was reported to increase with transfusion of older plasma containing blood products [53]. In addition to prolonged storage of transfused products, the presence of an underlying condition such as recent surgery, massive blood transfusion, cytokine therapy and active infection have been implicated in some, but not all, studies [59]. These reports suggest that the accumulation of bioactive products in stored blood may be important in the development of TRALI. In experimental models, lipopolysaccharide [53] and soluble CD40 ligand [60] in stored blood were shown to play an important role in the pathogenesis of TRALI. Plasma from stored (but not fresh) platelets induced lung injury in rats pretreated with endotoxin [61]. Using rat lung model, development of TRALI was demonstrated when endotoxin pretreatment, which causes increased neutrophil adherence, was followed by the infusion of plasma from stored packed red blood cells, but not with fresh plasma [61].

Association of transfusing old blood and higher incidence of bacterial contamination and sepsis. Bacterial contamination of blood products, particularly platelets, is the most common cause of transfusion transmitted infection [62,63]. In the United States, the frequency of bacterial

contamination was reported to be 1 in 5,000 for platelet units and 1 in 30,000 for pRBC [64–66]. One important risk factor is duration of blood storage. In one series that involved over 3,000 random donor platelet pools, the incidence of contamination detected by a Gram's stain was much lower in units stored for $\leq$4 days compared with units stored for 5 days. In addition, among the contaminated units, the magnitude of contamination was less in those stored for $\leq$4 days [67]. Routine bacterial detection assay for platelet units was instituted by the FDA in 2004 and the impact of this measure is yet to be ascertained. American Red Cross, the largest blood collection center in the USA and one of the earliest centers to institute bacterial detection system has reported that their bacterial detection assay had significantly reduced incidence of transfusion transmitted bacterial infection due to platelet units. However, residual risk of septic transfusion reaction due to platelet transfusion remained a significant safety concern [68,69].

Consequences of Transfusing New (<14 days) Blood Only to Blood Supply

There is a lack of consistent cut off that defines RBC storage duration among the studies that investigated the clinical impact of RBC storage lesions. As a result, the clinical importance of transfusing old RBC versus new RBC remains to be established. Some studies suggested that the clinical impact of the storage lesions become significant after 2 weeks [44,47,49]. However, there is no clear definition of what is "fresh," "new," "young," and "older" units of blood.

If we were to shorten the shelf life of RBC to 14 days as some of the above studies recommended, more blood units may potentially be wasted. The median storage duration of RBC units in the USA is about 15 days and the maximum allowed shelf life of a unit of RBC is 42 days [70]. The nation's blood supply is already under duress because of a shrinking donor pool, increased regulatory requirements and increasing processing and infectious disease screening costs and a rising demand for blood. From 1991 to 2004 the number of RBC units transfused has increased by 10%[70]. Because of more stringent criteria for donor selection and more sensitive serologic and nucleic acid testing, more donors are disqualified from blood donation. Donor deferral requirements due to travel history are also increasing. Increasing regulatory requirements that demand multiple donor screening tests are recommended or required by many voluntary and government agencies that monitor blood products and transfusion practice. All of these factors make blood components safer but shrink the donor pool. Additionally, even though the turn-around time for these tests continues to improve, it takes about 48– 72 h before the blood products are ready to be released to the general inventory. Therefore, if RBC shelf life is to be reduced to 14 days, their availability for transfusion will only be about 10–12 days. The cost of producing and screening blood components is already very high and shortening the shelve life of RBC will significantly increase the cost of a unit of blood. The impact of this may be substantial. Indeed, according to a nationwide survey conducted in 2005 by American Association of Blood Banks the number of whole blood and RBC units transfused in 2004 was 14,182,000 units [70].

Solutions

Conservative approach to blood transfusion. With the current demand for blood, it is almost impractical to shorten the shelf life of pRBC. To decrease the demand for blood to a level that we can afford to reduce the shelve life, a comprehensive blood management/conservation program will need to be set up in all healthcare centers. For a start, every healthcare institution should develop its own guidelines for RBC transfusion, indications and triggers. Moreover, institutions should develop measures to ensure the guidelines are being followed. The goal is to minimize unnecessary transfusion and to decrease the overall number of RBC units transfused. It is only when the demand for RBC is less than the supply that the shelf life of RBC can be practically reduced. There are studies that suggest a restrictive approach of RBC transfusion is at least as effective as, and potentially superior to, a liberal transfusion approach in critically ill patients with exception of patients with acute myocardial infarction or unstable angina [71]. A more recent study by Foss et al. suggests there was no statistically significant differences in postoperative rehabilitation scores and length of stay between elderly patients with hip fracture who were managed with liberal or restrictive approach to blood transfusion. In addition, they observed liberal approach to transfusing patients with hip fracture did not result in increased ambulatory scores. However, there were fewer patients in the liberal transfusion group with cardiovascular complications. Therefore, extra caution should be given when restrictive blood transfusion approach is used in elderly patients [72].

Limit transfusing new blood units to risky patients. Administration of less than 14-day-old blood can be limited to risky patients. As previously highlighted, the effectiveness of new RBC was only suggested in cardiothoracic and critically ill patients and even then it has not been proven. Detrimental effect of transfusion of old RBC units is yet to be demonstrated in patients with chronic illness. Therefore, the use of new blood can be limited to only cardiac and critically ill patients. However, those cases tend to require many units of blood all at one time. In most instances, enough new blood may not be available to meet the patients' needs. The only option then is to limit the number of units transfused. However, it is not established whether the risks of limiting blood transfusion is lower than the risks of transfusing old RBC units. A policy of issuing new blood when available cannot be instituted until studies examining the risks/benefits of issuing old blood versus limited blood transfusion are performed.

Avoid circumstances that require RBC transfusion. The best way to reduce or prevent RBC storage related ailments is to avoid circumstances that necessitate RBC transfusion. This can be achieved through aggressive management of anemia through iron supplements and erythropoietin therapy [73,74]. In addition, judicious use of cell savers whenever possible in the operating rooms will minimize RBC transfusion [75].

In summary, recent studies suggests storage lesions are associated with increased morbidity and mortality. However, there are reports with opposite results. A prospective randomized double blind study is needed to determine if the previous reports are actually true. There is also a need to identify the patient population at risk. Transfusion of new blood units is only feasible after establishment of rigorous blood inventory management, blood conservation and blood utilization programs.

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