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Storage Age of Transfused Red Blood Cells During Liver Transplantation and Its Intraoperative and Postoperative Effects

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Abstract
Background Recent studies suggest that the storage age of red blood cells (RBCs) may be associated with morbidity and mortality in surgical patients. We studied perioperative effects of RBC storage age in patients undergoing orthotopic liver transplant (OLT).
Methods Adult patients who received ≥5 U of RBCs during OLT between January 2004 and June 2009 were studied. The subjects were divided into two groups according to the mean storage age of RBCs they received: new or old RBCs (stored ≤14 or >14 days, respectively). Effects of storage age of transfused RBCs during OLT on intraoperative potassium (K^+) concentrations, incidence of hyperkalemia (K^+ ≥5.5 mmol/L), postoperative morbidity, and patient and graft survival were studied.
Results The mean serum K^+ concentrations and the incidence of hyperkalemia during OLT were significantly associated with storage age of the RBCs. Logistic analysis showed that storage age of RBCs was an independent risk factor for intraoperative hyperkalemia (odds ratios 1.067–1.085, $p < 0.001$) in addition to baseline K^+ concentration and units of RBCs transfused. Patient and graft survival and postoperative morbidity including postoperative ventilation, reoperation, acute renal dysfunction defined by the RIFLE criteria was not associated with old RBCs.
Conclusions Transfusion of RBCs stored for a longer time was associated with intraoperative hyperkalemia but not with postoperative adverse outcomes in adult OLT. Prevention and treatment of potentially harmful hyperkalemia should be considered when old RBCs are administered.

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Introduction

Transfusion of stored red blood cells (RBCs) is often required to treat blood loss during surgical procedures. Despite being widely considered as necessary therapy, blood transfusion, particularly massive transfusion, may be harmful. Numerous studies have demonstrated that intraoperative blood transfusion is associated with increased postoperative morbidity and mortality [1–8]. RBCs undergo progressive and significant changes during storage. Recently, these well-documented storage-related changes have been implicated as a possible cause for increased postoperative morbidity and mortality. In fact, several clinical studies have indicated that transfusion of

RBCs stored for a long period of time is associated with unfavorable postoperative outcomes. These unfavorable outcomes include prolonged hospital and intensive care unit (ICU) stay, extended requirement for endotracheal intubation, multiorgan failure, sepsis, and mortality [4–7, 9–12].

Several limitations have been noted in these clinical studies. Many studies enroll only a small number of patients [9, 11]. Some studies include a large number of patients, but the number of transfused RBC units is rather small, averaging only one to two units per patient [6]. Moreover, most of the studies focus on postoperative outcomes, ignoring any potential intraoperative effect. Patients undergoing orthotopic liver transplantation (OLT) often have severe pretransplant coagulopathy and significant intraoperative blood loss, making massive intraoperative blood transfusion a routine practice [13]. Thus, OLT provides an ideal setting for studying the influence of RBC storage age on intraoperative complications and postoperative outcomes. Unfortunately, the potential effects of RBC storage in this patient population have not been extensively studied.

In this retrospective, single-center study of adult OLT patients, we sought to determine whether transfusion of RBC stored for a long period of time was associated with: (1) higher intraoperative potassium (K^+) concentration and incidence of hyperkalemia; (2) longer duration of postoperative endotracheal intubation, higher incidence of reoperation, and renal dysfunction; and (3) worse postoperative patient and graft survival.

Methods

It was a retrospective single-center study. The institutional review board of the University of California, Los Angeles approved the study. Adult patients ($\geq$18 years) who underwent OLT at our medical center between January 2004 and June 2009 were identified. To increase the sensitivity of the potential effects of RBC storage age, we included only patients who received $\geq$5 U of allogeneic packed RBCs during OLT. Patients who received <5 U of RBCs or who underwent combined liver-kidney or liver-bowel transplants were excluded. Data were collected from our transplant database and electronic medical records.

Patients were induced and maintained with intravenous and inhalational anesthetics according to the protocol of our liver transplantation team. Intraoperative fluids included crystalloids, human albumin, and packed RBCs. Other blood products included fresh frozen plasma, apheresis platelets, and cryoprecipitate. Pulmonary artery catheters and transesophageal echocardiography were used for hemodynamic monitoring during OLT. Vasopressors and fluids were used to maintain intraoperative hemodynamic stability. There was no standard protocol for blood transfusion. However, in general, transfusion of packed RBCs was guided by maintaining the hematocrit between 28 and 30 %. Blood was administered using a rapid transfusion device with a large central port. Blood salvage was not used in our center during the study period. University of Wisconsin solution was used to preserve the liver graft during the study period. Intraoperative hemodialysis was chosen by agreement among surgeons, anesthesiologists, and nephrologists for patients with intractable metabolic acidosis, hyperkalemia, or electrolyte abnormalities.

Intraoperative serum K^+ levels were monitored throughout OLT and plotted hourly in relation to the reperfusion of the liver graft. Hyperkalemia was defined as a serum K^+ $\geq$5.5 mmol/L. Prereperfusion, early postreperfusion, and late postreperfusion hyperkalemia were defined as hyperkalemia that occurred within 2 h before reperfusion of the liver graft, within 15 min after reperfusion, and from 1 h after reperfusion to the end of surgery, respectively, as described previously [14]. Any patient who developed intraoperative cardiac arrest or major ST changes on electrocardiography (ECG) was identified and a possible relation with intraoperative hyperkalemia was analyzed. Postoperative acute renal dysfunction was defined by the risk, injury, function, loss, and end-stage (RIFLE) criteria [15]. Acute renal injury and failure were defined, respectively, as a two-fold (acute) and three-fold (failure) increase in the serum creatinine (Cr) during the first week after OLT as compared to pretransplant levels. We documented the postoperative ventilation requirement and reoperation for any reason within 30 days after OLT as well as mortality and graft failure. The storage duration of each RBC unit was obtained from the blood bank. Investigators who collected the demographic and outcome variables were blinded to the duration of RBC storage.

The storage duration of each unit of RBC was obtained from blood bank. The mean storage duration of RBC units transfused for each patient was calculated. The patients were then divided into two groups based on the mean storage duration of the RBC units they received. Patients transfused with RBCs stored for $\leq$14 days were considered to have received "new" blood, and those transfused with RBCs stored for >14 days were considered to have received "old" blood. Perioperative outcomes of the two groups were compared. Investigators who collected the demographic and outcome variables were blinded to the duration of RBC storage.

Data were expressed as the mean ± SD for continuous variables and proportions for categoric data. All analyses were performed using the PASW statistics 18.0 for windows (SPSS, Chicago, IL, USA). All reported *p* values are two-sided. Student's *t* test, one-way analysis of variance

(ANOVA), and the Pearson's χ^2 test were used for group comparisons among continuous or categoric variables. Variables with a statistically significant relation to the endpoints in the univariate analysis were entered into multivariate binary logistic regression. Odds ratios (OR) and 95 % confidence intervals (CI) were reported. Patient and graft survival were evaluated using Kaplan–Meier survival analysis. Statistical significance was defined as $p < 0.05$.

Results

A total of 906 adult patients underwent OLT between January 2004 and June 2009. After excluding 103 (11.4 %) patients who received <5 U of RBCs intraoperatively, we included 803 patients for analysis in the study. Most of the studied patients were male (65.1 %), had a diagnosis of cirrhosis caused by chronic hepatitis C (55.7 %), and had a mean Model for end-stage liver disease (MELD) score of 29 prior to OLT. A total of 13,415 U of RBC were transfused during OLT for the studied patients, ranging from 5 to 145 U per OLT. The mean and median storage ages of transfused RBC per OLT were 15.8 and 15.0 days, respectively. The minimum storage age was 1 day, and the maximum age was 42 days. The rest of the patient demographics and RBC characteristics are presented in Table 1.

In general, K^+ levels increased as OLT progressed. For example, the incidence of hyperkalemia was 1.9 % at the baseline and was higher at later time points (3.7 and 7.6 % at 2 and 1 h before reperfusion, respectively). The incidence of hyperkalemia reached the highest rate of 11.7 % immediately after reperfusion. The overall incidence of hyperkalemia during the prereperfusion and late postreperfusion periods were significantly higher in patients receiving old RBCs than those receiving new RBCs (18.1 vs. 10.2 and 9.0 vs. 3.9 %, both $p < 0.005$). Risk factors for hyperkalemia during the prereperfusion and late postreperfusion periods were analyzed using multivariate logistic regression. In addition to two previously known risk factors—baseline K^+ levels and the number of units of RBC transfused [14]—the storage age of RBCs was an additional independent risk factor for both preperfusion and late postreperfusion hyperkalemia. As shown in Table 2, the odds of developing hyperkalemia were 7–9 % higher if the storage age of transfused blood increased by 1 day. Preliminary analysis showed that prereperfusion hyperkalemia may be associated with intraoperative catastrophic events (cardiac arrest, $n = 8$) or ischemic events (ST changes on ECG, $n = 5$): 4.3 % (5/112) in hyperkalemic patients versus 1.2 % (8/678) in nonhyperkalemic patients ($p = 0.014$).

Table 1 Patient demographics and RBC characteristics

Characteristic	Data
Patient age (year)	52.9 ± 10.6
Male gender (%)	65.1
Total RBC transfused (U)	16.7 ± 13.3
Total FFP transfused (U)	20.5 ± 13.7
MELD score	29.1 ±7.7
Hepatitis C (%)	55.7
Acute hepatitis (%)	8.2
Alcoholic cirrhosis (%)	21.6
Retransplantation (%)	10.3
Donor age (years)	40.0 ± 16.6
RBC storage age	
Mean ± SD	15.8 ± 7.4
1–14 days	347 (43.2 %)
>14 days	456 (56.8 %)
ABO type (%)	
A	31.6
B	6.0
AB	19.1
O	43.3

The results are the mean ± SD or the number

RBC red blood cell, *FFP* fresh frozen plasma, *MELD* model for end-stage liver disease

Because previous studies [6] suggested that adverse effects became more apparent after RBCs were stored for >14 days, we used 14 days as a cutoff point to divide the patients into two groups. Patient demographics, baseline and intraoperative characteristics, and postoperative outcomes were compared between patients receiving new and old RBCs. As shown in Table 3, there was no difference in the number of units of transfused RBCs for the patients receiving new or old blood. RBC storage solutions included mannitol-adenine-dextrose (AS) (84.1 %) and citrate–phosphate–dextrose–adenine (CPDA) (15.9 %). A total of 420 patients received RBCs that were stored only in AS, and a small number of patients ($n = 11$) received RBCs that were stored only in CPDA. The rest of the patients ($n = 372$) received RBCs stored in both AS and CPDA. Other demographics and baseline, intraoperative, and donor characteristics were not significantly different between the old and new RBC groups.

The postoperative impact of the storage age of RBCs was compared between patients who received new and old RBCs. The results are shown in Table 4. Postoperative requirement of mechanical ventilation for ≥3 days was not significantly different between the two groups (48.1 vs. 46.9 %, $p = 0.737$). OLT patients frequently underwent reoperation for a variety of reasons, but the overall reoperation rate (one-third of patients in either group) within

Table 2 Logistic regression showing risk factors for hyperkalemia during the prereperfusion and postreperfusion periods

Risk factor	Odds ratio	95 % Confidence interval		p
		Lower	Upper	
Prereperfusion				
Baseline K^{+}	8.217	5.309	12.717	<0.001
RBC transfused during the prereperfusion period (units)	1.125	1.091	1.160	<0.001
RBC storage age (days)	1.067	1.034	1.102	<0.001
Postreperfusion				
Baseline K^{+}	2.950	1.915	4.545	<0.000
RBC transfused during the postreperfusion period (units)	1.064	1.039	1.090	<0.000
RBC storage age (days)	1.085	1.043	1.127	<0.000

Table 3 Comparison of patient demographics, preoperative and intraoperative characteristics, and donor variables

Variable	New RBCs (n = 347)	Old RBCs (n = 456)	p
Age (years)	53.3 ± 10.0	52.6 ± 11.0	0.334
Male sex	64.2	65.9	0.619
MELD score	29.2 ± 7.9	29.4 ± 8.3	0.809
Hepatitis C (%)	55.9	55.6	0.933
Nonalcoholic steatotic hepatitis (%)	4.5	4.0	0.707
Preoperative intubation (%)	19.9	25.2	0.077
Variceal bleeding (%)	36.8	39.4	0.467
Baseline hematocrit (%)	28.9 ±5.0	29.3 ± 4.9	0.229
Baseline platelet (×1,000/μl)	70.1 ±56.2	69.4 ± 47.7	0.853
Baseline INR	1.8 ± 0.7	1.8 ± 1.3	0.633
Baseline creatinine (mg/dl)	1.8 ± 1.9	1.8 ± 1.2	0.826
Surgery time (min)	300.4 ± 144.0	308.4 ± 154.2	0.457
Storage age (day)	9.2 ± 3.0	20.7 ± 5.7	NA
RBCs transfused (U)	16.7 ± 13.6	16.7 ± 13.0	0.949
Fresh frozen plasma (U)	20.4 ± 13.7	20.5 ± 13.8	0.912
Platelets (U)	1.4 ± 1.2	1.4 ± 1.2	0.821
Use of insulin (%)	54.7	55.9	0.721
Total dose of insulin (units)	15.8 ± 13.0	18.1 ± 22.1	0.190
Intraoperative hemodialysis (%)	4.2	5.9	0.283
Donor variables			
Age (years)	40.0 ± 17.0	39.2 ± 16.3	0.502
Donation after cardiac death (%)	4.5	6.3	0.370
Hospital stay (day)	4.4 ± 3.0	4.4 ± 3.1	0.883
Cold ischemia time (min)	372.6 ± 180.9	378.1 ±174.8	0.660
Warm ischemia time (min)	40.4 ± 59.7	36.6 ± 13.8	0.180
Macrosteatosis (≥20%) (%)	10.2	9.1	0.616

30 days after OLT was not significantly different between the two groups. Graft survival was similar between the patients who received old and new RBCs. Patients who received new blood showed slightly better survival in the Kaplan–Meier survival analysis compared with the old blood group, but the difference was not statistically significant (Fig 1).

We also compared postoperative renal dysfunction in patients who received new and old blood during OLT. Patients who had pretransplant renal failure were excluded

Table 4 Posttransplant outcomes

Outcome	New RBCs ($n = 347$)	Old RBCs ($n = 456$)	p
Reoperation (%)	33.7	30.5	0.613
Ventilation for ≥3 days (%)	48.1	46.9	0.737
Creatinine level (mg/dl)	New RBCs ($n = 231$)	Old RBCs ($n = 294$)	p
Baseline	1.6 ± 2.1	1.4 ± 0.8	0.161
Highest 7 days after OLT	2.2 ± 1.3	2.3 ± 1.8	0.123
Highest 14 days after OLT	1.4 ± 1.0	1.4 ± 0.8	0.786
Highest 30 days after OLT	1.3 ± 1.3	1.3 ± 0.7	0.885
Acute renal injury (%)	21.6	18.4	0.288
Acute renal failure (%)	11.3	15.6	

OLT orthotopic liver transplantation

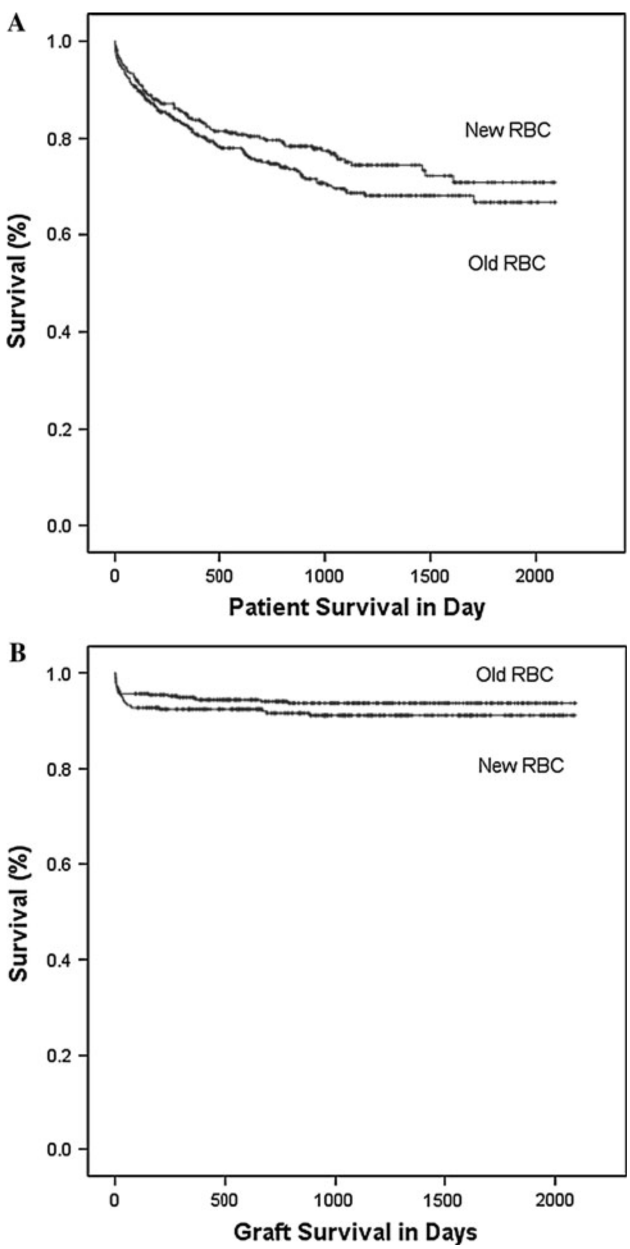

Fig. 1 Patient (**a**) and graft (**b**) survivals

from analysis of renal complications according to the RIFLE criteria. After exclusion of 278 patients who had undergone preoperative hemodialysis, 525 patients were included in the analysis. The baseline Cr concentration, the highest Cr during 7 PODs, and acute renal injury and acute renal failure according to the RIFLE criteria are showed in Table 4. Among 231 patients in the new-blood group, 21.6 % developed acute renal injury and 11.3 % developed acute renal failure within the first week following OLT; whereas in the old-blood group, 18.4 % of the patients developed acute renal injury and 15.6 % developed acute renal failure. There were no significant differences between the two groups.

Discussion

In this study of 803 adult patients receiving ≥5 U of RBCs during OLT, we demonstrated that transfusion of RBCs stored for longer periods of time was associated with significantly higher intraoperative mean K^+ concentrations and a higher incidence of hyperkalemia. The blood storage age correlated with highest K^+ concentrations during the prereperfusion and postreperfusion periods. As the storage age increased, the mean K^+ concentrations and incidence of hyperkalemia increased as well. The odds of developing hyperkalemia during OLT were 7–9 % higher if the storage age of transfused RBCs increased by 1 day. In contrast to previous studies, postoperative morbidity—requirement for intubation, reoperation within 30 days, renal dysfunction—was not associated with the duration of RBC storage. Patient and graft survival was not significantly different in patients who received old or new RBCs in our study.

Red blood cells undergo significant functional and structural changes during storage [1, 2]. Among the functional changes are depletion of 2,3-diphosphoglycerate, reduction of adenosine triphosphate (ATP), and loss of nitric oxide [16]. The structural changes include decreased deformability and increased adhesiveness, aggregability, and fragility [17–19]. The storage medium undergoes significant changes as well. A progressive decrease in pH, an increase in K^+, release of free hemoglobin, and accumulation of bioactive substances are often seen with a longer period of storage [18, 19]. The low temperature used during RBC preservation shuts down the ATP-dependent Na^+–K^+ pump. As a result, K^+ slowly leaks out of RBCs and the concentration of extracellular K^+ in the storage medium typically rises at a rate of about 1 mEq/day [2]. In general, adult patients can equilibrate the K^+ load rapidly and maintain K^+ concentration within a narrow range. Life-threatening hyperkalemia can occur if transfused blood contains a high concentration of K^+, as seen in old RBCs, and the rate of blood transfusion exceeds the rate of the equilibrium. Clinically, this has been reported only in small patients such as neonates receiving rapid transfusion via a central line [20]. In this study, we showed that the storage age of transfused RBCs affected the mean concentrations of intraoperative K^+ and the incidence of hyperkalemia during OLT.

Although it has been long suspected that transfusion of RBCs stored for a long period of time may lead to intraoperative hyperkalemia, the strong correlation shown in this study had not been reported in this clinical setting. Surprisingly, administration of insulin, a simple and effective K^+-reducing therapy, was not used more frequently in patients receiving old RBCs (Table 3) in this study. It seems that information regarding the RBC storage age was not fully appreciated and incorporated into clinical practice. In our center, each unit of RBCs had a label indicating its expiration date. To know the exact storage age, three pieces of information are required: transfusion date, collection date, and maximum storage days allowed by the particular preservation solution (RBCs can be stored in AS solution for 42 days and in CPDA for 35 days). This calculation is cumbersome, time-consuming, and difficult to perform, particularly during critical situations such as massive blood loss and rapid transfusion. Therefore, prior to a unit of RBC being issued, calculation with a clearly printed storage time instead of the sole expiration date may help clinicians better utilize this important piece of information.

Blood supply is a fundamental element of the health care system. The safety and availability of stored RBCs are equally important and need to be carefully balanced. Although storage leads to significant changes and deterioration of RBCs, it does provide flexibility to the blood supply. To ensure the safety of the stored RBCs and improve current blood banking practices and inventory management, studies identifying the storage-related changes and potential impact on patients are warranted. In this study, we confirmed that intraoperative hyperkalemia was associated with the storage age of transfused RBCs in adult OLT patients who received $\geq$5 U of RBCs.

The magnitude of RBC storage-related problems can be lessened by a joint effort between blood bank staff and the clinicians who are treating the patients. These efforts include (1) simplification of storage data to allow easy access at the site of transfusion; (2) aggressive prevention and treatment of intraoperative hyperkalemia, including the use of intraoperative hemodialysis and RBC "washing"; and (3) release of newer blood during massive transfusion if possible.

Several limitations are worth mentioning. OLT surgery is unique in its organ allocation and patient population. The organ allocation system in the United States prioritizes the sickest patient on the waiting list [21]. The sickest patients often have serious co-morbidities that may have an overwhelming impact on posttransplant mortality and morbidity [13]. This may contribute to the nonsignificance of storage age in this study. Many statistical methods can be used to control the impact of co-morbidities. We did both propensity-matching and nonmatching analyses and chose to report results by nonmatching analysis only. When a unit of RBCs was issued from the blood bank at our center, the process was blinded, although not randomized, and therefore we believed that such matching was unnecessary. The mean storage age (mixed, not exclusively new or old) was used in our study and prevented us from knowing how many units of old RBCs a patient received. This may underestimate the adverse effects of old RBCs in our study. Some investigators believe that exclusion of mixed-age RBCs may reduce the potential confounding effects. In our center, however, the average amount of RBCs transfused during OLT was between 12 and 15 U [13], and few patients received exclusively new or old blood. To augment the impact of the RBC age, we included patients who received $\geq$5 U of RBCs for analysis. Irradiation of RBCs before transfusion accelerates storage lesion, but its effect on outcomes are unknown. In this study, a small number of patients ($n = 45$) received irradiated RBCs, and most of those patients ($n = 32$, 71 %) received old blood by mean RBC age, making analyzing irradiated and unirradiated RBC not feasible. Additionally, storage-related changes may be different based on the use of either AS or CPDA as the storage solution, and the storage solution was not accounted for in our analysis. Finally, the retrospective design of this study has many known shortcomings and limitations. Despite the limitations mentioned above, this large retrospective study confirmed the long-suspected relation between the storage age of transfused RBCs and intraoperative hyperkalemia. Because hyperkalemia may be associated with catastrophic events during OLT, it should be taken seriously.

Conclusions

We compared intraoperative hyperkalemia and postoperative outcomes in 803 adult patients receiving $\geq$ 5 units of RBC during OLT. Transfusion of RBCs stored for longer periods of time was associated with a significant increase in serum K^+ concentrations and the incidence of hyperkalemia during OLT. As the storage age increased, the mean K^+ concentrations increased. Postoperative morbidity—requirement for intubation, reoperation within 30 days, incidence of renal dysfunction—was not associated with the duration of RBC storage. Graft and patient survivals were not significantly different in patients who received old or new RBCs. Therapies preventing or treating intraoperative hyperkalemia should be considered when old RBCs are transfused, particularly at a fast rate, during OLT.

Conflict of interest There are no conflicting interests or financial involvements in this study for any of the authors.

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