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2,3-Diphosphoglycerate Concentrations in Autologous Salvaged Versus Stored Red Blood Cells and in Surgical Patients After Transfusion

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Abstract

Background—Stored red blood cells (RBCs) are deficient in 2,3-diphosphoglycerate (2,3-DPG), but it is unclear how autologous salvaged blood (ASB) compares to stored blood, and how rapidly 2,3-DPG levels return to normal after transfusion. Therefore, we compared levels of 2,3-DPG in stored versus ASB RBCs, and in patients' blood following transfusion.

Methods—Twenty-four patients undergoing multilevel spine fusion surgery were enrolled. We measured 2,3-DPG and the oxyhemoglobin dissociation curve (P_{50}) in samples taken from the ASB and stored blood-bags before transfusion, and in blood samples drawn from patients before and after transfusion.

Results—The mean storage duration for stored RBCs was 24 ± 8 days. Compared with fresh RBCs, stored RBCs had decreased 2,3-DPG levels (by $\approx 90\%$; $P < 0.0001$), and a decreased P_{50}

(by $\approx 30\%$; $P<0.0001$). However, ASB RBCs did not exhibit these changes. The mean 2,3-DPG concentration decreased by $\approx 20\%$ ($P<0.05$) in postoperative blood sampled from patients who received 1 – 3 stored RBC units, and by $\approx 30\%$ ($P<0.01$) in those who received 4 stored RBC units. 2,3-DPG was unchanged in patients who received no stored blood or ASB alone. After surgery, 2,3-DPG levels recovered gradually over three postoperative days in patients who received stored RBCs.

Conclusions—Stored RBCs, but not ASB RBCs, have decreased levels of 2,3-DPG and a left shift in the oxyhemoglobin dissociation curve. Postoperatively, 2,3-DPG levels remain below preoperative baseline levels for up to three postoperative days in patients who receive stored RBCs, but are unchanged in those who receive only ASB RBCs.

Introduction

Blood transfusion has been used as a life-saving medical intervention since the 1800s. However, it did not gain widespread acceptance until World War I, when it quickly evolved to meet the needs of the masses of wounded soldiers. The ability to store blood, with anticoagulants in a cool space, allowed healthcare workers to transfuse patients with blood collected previously instead of transfusing directly from one person to another.¹ Now, preservation techniques have increased the shelf life of stored (banked) blood up to 42 days. Although controversial, evidence from retrospective studies has linked longer storage duration of red blood cells (RBCs) to increased risk of postoperative complications^{2,3} and increased mortality.^{4,5} However, three prospective, randomized clinical trials found no difference in outcomes among patients transfused with fresher vs. older blood.⁶⁻⁸ Of note however, few RBC units in these recent trials had been stored for long durations (5 or 6 weeks). Nonetheless, it is proposed that the adverse effects associated with stored blood transfusion are likely related to the RBC “storage lesion”.

Storage lesions are inimical changes to the RBC structure and biochemical properties that occur during the storage of blood.⁹ During storage, RBCs undergo morphologic changes, including echinocytosis, spherocytosis, and vesiculation.^{10,11} Biochemically, lactic acid accumulation and a decrease in RBC pH cause metabolic dysfunction. RBCs also exhibit reductions in 2,3-diphosphoglycerate (2,3-DPG), ATP, and intracellular potassium and increases in calcium and oxidative damage.^{10,12,13} The loss of 2,3-DPG is of particular importance because it results in a left-shift of the oxyhemoglobin dissociation curve (ODC). This left-shifted ODC decreases the P_{50} (partial pressure of O_2 at which 50% of hemoglobin is saturated with O_2) which, in turn, decreases peripheral oxygen unloading.¹⁴

Although it is well recognized that 2,3-DPG levels are very low after 14 days storage,¹⁵ and nearly depleted after 21 days of storage^{15,16}, it is not entirely clear how rapidly this storage lesion is reversed after transfusion. Evidence suggests that recovery of 2,3-DPG begins within a few hours^{15,16} but is not complete until between 1 - 3 days after transfusion.¹⁷⁻¹⁹ It is also controversial whether transfused RBCs that are depleted of 2,3-DPG are effective in delivering oxygen. In a volunteer study,²⁰ Weiskopf, et al. present evidence that stored RBCs, even when deficient in 2,3-DPG, deliver oxygen effectively. In a recent editorial,²¹ the same author presents multiple reasons explaining these findings.

An alternative to transfusion of stored blood is the use of autologous salvaged blood (ASB), which is a cost-effective and safe method of blood conservation.^{22,23} Since ASB RBCs are only removed from the circulation for hours, not days or weeks, it is unlikely that storage lesions would be present. In fact, 2,3-DPG levels are thought to be normal in ASB RBCs,²⁴ but it is unclear how ASB and stored RBCs compare, and whether significant changes in 2,3-DPG occur in patients after receiving either ASB or stored RBCs.

In the current study, we performed in vitro measurements to determine differences in 2,3-DPG levels and P_{50} in samples drawn from stored blood and ASB blood bags. We also performed an in vivo study to determine 2,3-DPG changes in blood drawn from postoperative patients over a 3-day period. We tested the hypotheses: 1) that 2,3-DPG levels in stored RBCs would be much lower than levels in ASB RBCs, and 2) that patients have persistently lower 2,3-DPG levels after receiving stored RBC transfusions, but not after ASB RBC transfusions.

Methods

After receiving Johns Hopkins Hospital IRB approval and written informed consent, we enrolled 24 patients undergoing multilevel spine fusion surgery at the Johns Hopkins Hospital. Patients received general anesthesia with opioids, inhaled agents, and neuromuscular blockade. The intraoperative hemoglobin transfusion trigger during active bleeding was 9 g/dL, and the postoperative transfusion trigger was 8 g/dL. During surgery, an autologous blood salvage system was used (Cell Saver Elite, Haemonetics Corp, Braintree, MA) with either a 70 or 125 mL bowl for processing of shed blood, according to the manufacturer's recommendations. When the salvaged blood was inadequate in volume, allogeneic packed RBCs (stored in AS-1 solution) were obtained from the blood bank. The duration of storage for these banked blood units was according to standard blood issue protocols at our institution.

Venous blood samples were drawn into heparinized tubes preoperatively, upon completion of surgery (postoperatively) and on postoperative days 1 (POD1), 2 (POD2), and 3 (POD3). In addition to the venous blood samples, we collected an aliquot of blood from the stored and ASB blood bags before transfusion.

Sample Processing

All blood samples were processed as follows, immediately after the blood draw. Samples were centrifuged at 5000 rpm for 5 min at 4°C, the plasma was removed, and the RBC pellet was washed twice with 10 mL of ice-cold phosphate-buffered saline (PBS, pH 7.4). After being washed, a 0.25 mL aliquot of packed RBCs was suspended in 0.5 mL of PBS. To determine and standardize the 2,3-DPG concentrations by grams of hemoglobin (Hb), a 30 μ L aliquot of the PBS-RBC solution was taken for Hb concentration determination. Next, 30 μ L of 11.6 M perchloric acid was mixed into the PBS-RBC solution to precipitate all proteins and thus arrest any metabolic activity. After centrifugation, 0.5 mL of the supernatant (containing soluble metabolites present in the RBCs) was removed, neutralized with 56 μ L of 2.5 M Na_2CO_3 solution, and stored at -85°C .

Analytic Methods

P₅₀ Determination—The ODC curve was determined with a Hemox-analyzer (TCS Scientific Corporation, New Hope, PA) according to the manufacturer's instructions. Each day before the samples were analyzed, the membrane was subjected to progressively hypoxic mixture of nitrogen gas to check the membrane function. If the P₅₀ was not reached at 2 torr, the electrode membrane was replaced. 20uL of red blood cells pellet and 10uL of anti-foam reagent were mixed in 3 ml of hemox-solution pH 7.4 (manufacturer supplied) and transferred into the instrument cuvette. The sample was equilibrated to 37 °C temperature and 100% oxygenated with air. After adjusting the pO₂ to 150 torr, the sample was deoxygenated with nitrogen and oxygen dissociation curve was recorded. The P₅₀ was determined as the partial pressure of oxygen at which the hemoglobin was 50% saturated.

2,3-DPG Determination—After sample processing as described above, 2,3 DPG was determined in the processed sample with an enzymatic colorimetric reaction kit obtained from Roche Diagnostics (Catalog. No. 10-148-334-001, Roche Diagnostics, Indianapolis, IN). This measurement was based on the consumption of NADH that is proportional to 2,3 DPG content of the sample. The decrease in NADH during the enzymatic reaction was determined spectrophotometrically using an extinction coefficient of 6.3 mM⁻¹ cm⁻¹ at 340 nm as per kit instructions. The Hb concentration for the processed sample was determined spectrophotometrically. After calculating the 2,3-DPG concentration, we used the Hb concentration to express 2,3-DPG concentration as micromoles of 2,3-DPG / gram Hb.

Statistical Methods

Differences in the mean measured values within groups and between groups were analyzed by two-way repeated measures ANOVA. The Tukey-Kramer *post-hoc* test was used to adjust for multiple comparisons of means. Differences in mean measured values over time within only one group were analyzed by one-way ANOVA. Individual means were compared with an unpaired Students t-test, and dichotomous variables were analyzed by the Fisher's exact test. Variables that were not normally distributed are reported as median and interquartile range (IQR) and were analyzed by Kruskal-Wallis. Continuous variables are reported as mean ± standard deviation (SD). $P < 0.05$ (two-tailed tests), was used to define significance. Analyses were performed using JMP version 9.0.2 (SAS Institutes, Inc., Cary, NC).

Results

The patient characteristics and transfusion requirements are summarized in Table 1. The patients were stratified into three groups based on the total number of stored RBC units they received through POD3. The three groups were: 1) no stored RBC units or ASB alone (n=7), 2) 1-3 stored RBC units and ASB (n=7), and 3) 4 stored RBC units and ASB (n=10). Age, sex, ASB volume transfused, preoperative Hb, immediate postoperative Hb, and number of stored RBC units transfused on POD1, POD2, and POD3 were not significantly different between the groups. The number of intraoperative stored RBC units transfused, as well as POD1, POD2, and POD3 Hb values, were significantly different between the three groups. It should be noted that multiple patients received ongoing low doses of postoperative stored

RBC transfusions on POD1 and POD 2, and in some patients on POD3. The mean storage duration for stored RBCs was 24 ± 8 days, and ranged from 3 to 38 days. Only 10% of RBC units were < 10 days duration of storage, and 25% of RBC units were < 21 days duration of storage.

The preoperative 2,3-DPG concentration and P_{50} values were determined in fresh patient blood from each subject and compared to the 2,3-DPG and P_{50} values of stored blood samples and ASB samples drawn from the blood bags. The mean 2,3-DPG concentration was approximately 90% lower in stored RBCs than in preoperative RBCs ($P<0.0001$), whereas the mean concentration in ASB RBCs was not different from that in preoperative RBC samples ($P=0.81$; Fig. 1).

The P_{50} data followed a pattern similar to that of 2,3-DPG (Fig. 2). The P_{50} value of stored RBCs was approximately 30% lower than that of preoperative and ASB RBCs ($P<0.0001$). We also found a small but statistically significant decrease (<5% difference) in the mean P_{50} value of ASB RBCs compared to that of preoperative RBCs. Figure 3 shows representative ODCs from fresh, stored, and ASB blood samples, and illustrates the leftward shift of the curve for the stored RBCs as compared to that of the fresh preoperative patient RBCs and ASB RBCs.

We analyzed the *in vivo* effect of stored RBC transfusion by plotting the mean 2,3-DPG concentrations measured postoperatively and on POD1, POD2, and POD3. 2,3-DPG concentrations varied considerably between patients in both the preoperative and postoperative samples. To adjust for this variability, and to use each patient as their own control, we analyzed the percent change from preoperative baseline levels, rather than the absolute measured concentration. We then compared the percent change in mean postoperative, POD1, POD2, and POD3 2,3-DPG concentrations according to the number of stored RBC units transfused over the entire three perioperative days (Fig. 4). This analysis showed that 2,3-DPG was decreased below the preoperative baseline (by $\approx 20\%$, $P<0.05$) in the patient groups that received the lower dose stored RBC transfusion (1-3 units), and by $\approx 30\%$ ($P<0.01$) in the patient group that received the higher dose stored RBC transfusion (4 units). Furthermore, these levels did not recover to baseline, even by POD3 ($P<0.05$; for within group comparisons). However, those who received no stored RBCs or ASB alone had no change from baseline, and had a higher mean 2,3-DPG level compared to those transfused any stored RBCs ($P<0.05$; for between group comparisons).

There were four patients who received no stored blood transfusions after the morning of POD1, and for these patients the percent change in 2,3-DPG concentrations from the preoperative baseline were analyzed to assess the recovery of 2,3-DPG over three perioperative days in the absence of ongoing postoperative transfusions (Fig. 5). The mean number of stored RBC units given between the start of surgery and the morning of POD1 in these patients was 3.5 ± 2.0 . After an approximate 50% decrease in 2,3-DPG (from preoperative to immediate postoperative; $P<0.03$), there was a gradual recovery over the three postoperative days with an approximate 30% decrease below baseline on POD 1 and POD2 (both $P<0.03$), and 20% decrease below baseline on POD3 ($P=0.03$).

Discussion

Our findings show that 2,3-DPG concentrations in ASB RBCs are comparable to those of fresh RBCs and significantly higher than those of stored RBCs. The P_{50} values followed the same pattern, with a lower value for stored RBCs and nearly equivalent values for ASB and fresh RBCs. During and after surgery, 2,3-DPG concentrations decreased significantly in patients who received any stored RBCs but remained unchanged in patients who received no stored RBCs or ASB alone. Since multiple patients (30-60%) received ongoing low-dose postoperative stored RBC transfusions, over the three postoperative days, we are unable to comment on the exact recovery time of 2,3-DPG after transfusion. We did, however, observe that 2,3-DPG remained below baseline levels over the three postoperative days, even in those receiving relatively small amounts of stored RBCs.

The RBC uses 2,3-DPG to facilitate oxygen release from hemoglobin. When the 2,3-DPG molecule binds to the beta chain of one tetramer of hemoglobin, the resulting conformational change reduces the affinity of the hemoglobin molecule for O_2 , thereby increasing the amount of oxygen delivered to the peripheral tissue or organ. Thus, 2,3-DPG causes a right-shift in the ODC and a higher P_{50} .^{25,26} Because 2,3-DPG is vital to the oxygen delivery system, substantial amounts of research have been done to characterize this molecule.

Soon after the function of 2,3-DPG was discovered, scientists began to characterize the effects of storing RBCs, with particular interest in determining the concentrations of 2,3-DPG throughout storage. Early on, it was realized that RBCs become depleted of 2,3-DPG within 14-21 days of storage.^{15,16,25} It was suggested that dysfunction of bisphosphoglycerate mutase, the enzyme that makes 2,3-DPG, leads to the decrease. Bisphosphoglycerate mutase is part of the glycolytic pathway, and its function is largely dependent on a physiologic pH.²⁷ A few studies have measured ASB RBC 2,3-DPG concentrations, ODC (P_{50}), and other parameters seen in the storage lesion, but it remains unclear how these ASB RBCs compare to stored RBCs.^{24,28-30} Our results showed a striking difference between 2,3-DPG concentrations in the stored RBCs and those in both preoperative (fresh) patient blood and ASB RBCs. However, we found no significant difference between 2,3-DPG concentration in ASB and fresh RBCs. The ODCs also were virtually identical in these two groups. Although the difference in ASB and fresh patient RBC P_{50} values was statistically significant, the mean values were very close (<5% difference), and both were much higher than that measured in stored RBCs.

In this study, we also aimed to determine the time course of 2,3-DPG changes following transfusion of stored RBCs by analyzing samples drawn from postoperative surgical patients. A few previous studies have examined how rapidly 2,3-DPG recovers *in vivo* after transfusion. However, most included only a small number of patients (<5) who received large amounts of transfused blood (3-5 units), used RBC preservation solutions that are not commonly used today, and/or were carried out in healthy subjects rather than in surgical patients.³¹⁻³⁵ Previous investigators have shown anywhere from a 1 to 3 day 2,3-DPG *in vivo* recovery time to reach baseline levels after stored RBC transfusion,^{31,34,36} whereas our study showed an incomplete recovery by POD3. This lack of recovery might be attributable

to the continued low-dose administration of stored RBCs on POD1, POD2, and POD3 (between 0-1 units/day), as the patients recovered from surgery. These additional transfusions may have maintained the 2,3-DPG concentrations below normal. If so, this suppression of 2,3-DPG with such ongoing low-dose stored blood transfusion is a new finding that has not been previously shown. We commonly use a restrictive intraoperative transfusion strategy that may lead to ongoing postoperative transfusions, especially after major surgical cases that are prone to a postoperative downward Hb drift.³⁷ Although there were only four patients who received no stored blood after the morning of POD1, the analysis of these patients provides additional evidence for the gradual recovery of 2,3-DPG over a 3-day time course.

Although there is little information on the subject of phosphate metabolism and recovery of 2,3-DPG, increased phosphate ion concentration is known to promote increased 2,3-DPG levels in RBCs.^{38,39} It has also been reported that as many as half of hospitalized patients have hypophosphatemia after major surgery,⁴⁰ perhaps due to poor dietary intake, which may occur in the perioperative setting,⁴¹ or due to renal wasting of phosphate.⁴¹ It is possible, therefore, that the relatively slow recovery of 2,3-DPG after stored blood transfusions in our study could be explained in part by abnormal phosphate homeostasis. Given that CPD anticoagulant has been shown to be an important source of phosphate in transfused perioperative patients,⁴⁰ and that our patients received AS-1 stored RBCs, which is devoid of phosphate, this may also have contributed to a relatively delayed recovery of 2,3-DPG.

Although we observed a large decrease in 2,3-DPG considering the low to moderate doses of stored RBCs, one possible explanation should be considered: If the hemoglobin falls from 12 g/dL preoperatively to 9 g/dL on POD2, after 3 units of stored RBCs have been given, it is likely that almost half of the native RBCs are no longer in the circulation. Until the allowable blood loss is reached, the healthy native cells are lost, and not replaced. In other words, the ratio of transfused RBCs to native RBCs is higher than it would be if the patient hadn't experienced major blood loss. Another interesting phenomenon deserves some comment. For patients who received no stored RBCs, we observed a slight upward trend in 2,3-DPG, albeit not statistically significant. We hypothesize that this may be the body's natural adaptation to blood loss (in this case surgical blood loss) and the resulting anemia. Thus, if the Hb concentration decreases, the body may compensate by increasing the 2,3-DPG concentration in order to deliver more oxygen per gram of Hb. Previously, it has been shown that 2,3-DPG concentrations increase in low O₂ environments, such as in mountain climbers, and in people with chronic anemia,^{42,43} so it is plausible that postoperative anemia might result in a similar response.

Whether or not the decreased 2,3-DPG levels actually impair oxygen unloading from the hemoglobin molecule and delivery to peripheral tissues is also controversial. In an elegant study by Weiskopf and colleagues, using an isovolemic hemodilution model, 3-week old stored transfused blood was equally effective as fresh transfused blood in reversing anemia-induced cognitive impairment.²⁰ The authors discuss several potential explanations, including the possibility that the decreased intracellular pH in the RBC shifts the ODC to the right (the Bohr effect),⁴⁴ which counteracts the effects of depleted 2,3-DPG. This rightward

shift would result in an increased “functional P₅₀”, according to this theory, which would enhance the delivery of oxygen. This hypothesis is certainly interesting and deserves further study.

Our study did have some limitations. We did not assign patients to their treatment groups, since we offered routine care, which included ASB as an option for all patients. Therefore, we could not randomly assign patients to ASB vs. stored blood treatment groups, since most often the ASB alone would not suffice to meet the transfusion requirements in these patients. Furthermore, the volume of ASB given could not be controlled for between the groups, and the overall ASB dose was relatively low. During these surgeries, a substantial amount of shed blood was not recovered by the ASB system, since the surgeons often used three suction catheters, one or two of which were connected to a waste container. We also did not control for RBC storage duration, as we transfused standard issue RBC units at our institution. The storage duration very well may affect the recovery of 2,3-DPG, but we were unable to systematically assess this effect. Finally, the time required for 2,3-DPG recovery to baseline levels after transfusion, may have been influenced by the ongoing low-dose stored blood given to patients on postoperative days 1, 2, and 3. Nonetheless, we can say that under these typical clinical circumstances, 2,3-DPG levels remained below baseline levels, even at three days following surgery. Finally, the changes in 2,3-DPG demonstrated in this study may or may not have an impact on clinical outcomes, as this has yet to be determined. Since our study was not designed or powered to assess clinical outcomes, we are unable to comment on whether lower 2,3-DPG levels are related to perioperative morbidity.

In summary, 2,3-DPG levels are substantially depleted in stored RBCs compared to ASB RBCs, and transfusion of low to moderate doses of stored RBCs result in a postoperative decrease in 2,3-DPG following surgery. This finding, along with other aspects of the storage lesion, such as decreased RBC cell membrane deformability,⁴⁵ may impair oxygen delivery to tissues after stored blood transfusion. Our findings also suggest that RBCs in ASB are of higher quality than stored RBCs in terms of 2,3-DPG and P₅₀.

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Figure 1.

Mean 2,3-DPG concentrations were significantly less in stored RBCs than in preoperative (preop, fresh) RBCs or in RBCs from autologous salvaged blood (ASB). However, concentrations did not differ significantly between fresh and ASB RBC samples. Data are shown as mean $\pm$ SD. ** $P < 0.0001$ versus preop and ASB.

Figure 2.

The P₅₀ of stored RBCs was significantly lower than that of preoperative (preop, fresh) RBCs and autologous salvaged blood (ASB) RBCs. Additionally, there was a small but statistically significant difference between the P₅₀ of preop fresh RBCs and that of ASB RBCs. Data are shown as mean ± SD. ** $P < 0.0001$ versus preop fresh and ASB; * $P = 0.016$ versus preop fresh.

Figure 3.

In this representative figure, the oxyhemoglobin dissociation curves (ODC) for preoperative (fresh) RBCs and autologous salvaged blood (ASB) RBCs overlap, with P_{50} values around 28.5 mmHg. The stored RBC ODC is left-shifted compared to those of the other two, with a P_{50} around 19 mmHg.

Figure 4.

The mean percent change in 2,3-DPG concentrations over the three postoperative days is shown in patients stratified by the total number stored RBC units transfused. Data are shown as mean $\pm$ SD. * $P < 0.05$ versus 0 units of stored RBCs or ASB alone; # $P < 0.05$ for within group comparison to preoperative baseline. POD = postoperative day.

Figure 5.

The mean percent change in 2,3-DPG concentration over the three postoperative days is shown for the four patients who did not receive any transfusions after the morning of POD1. Immediately following surgery (Postop), the mean 2,3-DPG concentration was approximately 50% below baseline, followed by a gradual recovery - 30% below baseline on POD1 and POD2, and 20% below baseline on POD3. # $P < 0.05$ vs. preoperative baseline. POD = postoperative day.

Table 1

Patient Characteristics by Total Number of Stored Red Blood Cell Transfusions

Characteristic	0 Units stored RBCs or ASB alone (n=7)	1-3 Units stored RBCs and ASB (n=7)	4 Units stored RBCs and ASB (n=10)	P Value
Age (mean $\pm$ SD)	59 $\pm$ 21	57 $\pm$ 15	65 $\pm$ 8	0.18
Sex (Male)	5	2	3	0.06
Estimated blood loss [median (IQR)]	400 (288, 575)	800 (625, 988)	1,800 (1,500, 2,650)	0.0004
Intraoperative stored RBC units (mean $\pm$ SD)	0 $\pm$ 0	1.1 $\pm$ 0.9	4.3 $\pm$ 3.1	0.0008
No. of stored RBC units (mean $\pm$ SD)				
POD 1	0 $\pm$ 0	0.4 $\pm$ 0.8	1.0 $\pm$ 1.1	0.06
POD 2	0 $\pm$ 0	0.7 $\pm$ 1.1	0.4 $\pm$ 0.7	0.23
POD 3	0 $\pm$ 0	0 $\pm$ 0	0.5 $\pm$ 0.7	0.05
No. (%) of patients transfused stored RBCs				
POD 1	0 (0%)	2/7 (29%)	6/10 (60%)	0.03
POD 2	0 (0%)	3/7 (43%)	3/10 (30%)	0.16
POD 3	0 (0%)	0/7 (0%)	4/10 (40%)	0.03
ASB volume (mean $\pm$ SD)	65 $\pm$ 116	18 $\pm$ 47	127 $\pm$ 169	0.24
Preoperative Hb, g/dL (mean $\pm$ SD)	14.3 $\pm$ 1.6	12.2 $\pm$ 2.1	12.0 $\pm$ 2.0	0.10
Postoperative Hb, g/dL (mean $\pm$ SD)	12.8 $\pm$ 1.8	10.4 $\pm$ 2.3	10.1 $\pm$ 1.9	0.07
POD 1 Hb, g/dL (mean $\pm$ SD)	11.9 $\pm$ 2.0	9.4 $\pm$ 0.9	10.0 $\pm$ 0.9	0.04
POD 2 Hb, g/dL (mean $\pm$ SD)	12.6 $\pm$ 1.2	10.7 $\pm$ 2.1	9.6 $\pm$ 1.4	0.01
POD 3 Hb, g/dL (mean $\pm$ SD)	12.7 $\pm$ 1.0	10.9 $\pm$ 1.6	9.8 $\pm$ 0.6	0.01

RBC = Red Blood Cell; ASB = autologous salvaged blood; IQR = interquartile range; POD = postoperative day; Hb = hemoglobin, IQR = interquartile range