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Effects of Storage-Aged RBC Transfusions on Endothelial Function in Hospitalized Patients:

Storage Aged Blood Units and Endothelial Function

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Abstract

Background—Clinical and animal studies indicate that transfusions of older stored RBCs impair clinical outcomes as compared to fresh RBC transfusions. It has been suggested that this effect is due to inhibition of NO-mediated vasodilation following transfusion of older RBC units. However, to date this effect has not been identified in human transfusion recipients.

Study Design and Methods—Forty-three hospitalized patients with transfusion orders were randomized to receive either fresh (< 14 days) or older stored (> 21 days) RBC units. Prior to transfusion, and at selected time points after the start of transfusion, endothelial function was assessed using non-invasive flow-mediated dilation assays.

Results—Following transfusion of older RBC units, there was a significant reduction in NO-mediated vasodilation at 24 hours after transfusion (p=0.045), while fresh RBC transfusions had no effect (p=0.231).

Conclusions—The present study suggests for the first time a significant inhibitory effect of transfused RBC units stored > 21 days on NO-mediated vasodilation in anemic hospitalized patients. This finding lends further support to the hypothesis that deranged NO signaling mediates

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adverse clinical effects of older RBC transfusions. Future investigations will be necessary to address possible confounding factors and confirm these results.

Keywords

Blood transfusion; RBC; storage lesion; endothelial function; FMD

Introduction

Blood transfusion is the most common therapeutic procedure in hospitalized patients, with approximately 15 million red blood cell (RBC) units transfused annually in the US to approximately 5 million patients (1). Despite undeniable therapeutic benefits, transfusions are also associated with serious adverse reactions (2). Even when the well-recognized hazards are excluded, transfusion of RBCs remains an independent contributor of morbidity and mortality; furthermore most (but not all) studies show that these effects increase with RBC storage time (2–20). A recent meta-analysis supports this association (21), as do studies in dogs where transfusion of older stored RBC units (storage-aged RBCs; saRBCs), but not fresh RBCs, increased morbidity and mortality in animals with experimental pneumonia (22, 23). While data from ongoing randomized controlled trials of fresh versus saRBC transfusion are eagerly anticipated (RECESS (24), ABLE (25), and the Red Cell Storage Duration and Outcomes in Cardiac Surgery study (26), the results may not be definitive, as mathematical modeling suggests that some adverse effects of RBC storage time on recipient outcomes may be beyond the ability of such trials to detect (27).

Several pathologic changes occur in RBCs during storage (the “RBC storage lesion”) that may contribute to adverse effects of saRBC transfusions. These changes include the depletion of adenosine triphosphate (ATP) and 2,3-diphosphoglycerate (2,3-DPG) (14, 15), reduced blood flow velocity and increased endothelial adhesion (28, 29), progressive membrane dysfunction leading to hemolysis, elaboration of lipid mediators, microparticle formation (30–35) and disruptive effects on nitric oxide (NO)-mediated vasodilation (36, 37). In particular, we and others proposed saRBC-mediated reductions in NO bioavailability and inhibition of NO-mediated vasodilation as potentially important mechanisms underlying adverse transfusion effects (22, 23, 31, 37–39). We demonstrated in rat aortic rings that compared to fresh RBCs, saRBCs that were stored for 28–42 days produced significant inhibition of methacholine-stimulated and NO-mediated vasodilation (36). However, the validity of the proposed mechanisms has not been directly explored in human subjects.

In this study we measured endothelial function using brachial artery flow-mediated dilation (FMD) (40) in hospitalized subjects randomized to receive either fresh (<14 days old) or saRBC units (>21 days old) to test the hypothesis that saRBCs adversely affect NO-mediated vasodilation.

Materials and Methods

Subjects

Eligible subjects were anemic inpatients at Emory University Hospital between the ages of 18 and 80 years, whose physician ordered a clinically indicated transfusion of one or more units of allogeneic packed RBCs. Patients were excluded if: they had received blood transfusions within a week of screening; they were due to receive directed donor, autologous, or whole blood units; they were on nitrates or vasoactive infusions; they were pregnant; they were unsuitable for FMD measurements due to anatomy or clinical conditions; or they were unable to give informed consent. Additionally patients requiring emergent transfusion, transfusion after working hours or requiring invasive procedures during the study period were excluded. Written informed consent was obtained and the study was approved by the Emory Institutional Review Board.

Study Design

Consented and enrolled subjects were typed, cross-matched, and randomized to receive either fresh RBCs (<14 days) or saRBCs (>21 days). Randomization was performed by the Emory University Hospital Blood Bank using sealed envelopes opened individually for each participant. Investigators performing and analyzing study measurements were blinded to the randomization. Following hospital policy, full details of each unit were available to the patient and clinical care team. Demographics and clinical characteristics were documented. Leukoreduced CPD/AS1 RBC units were purchased from the American Red Cross Blood Services, Southern Region. RBC units were irradiated prior to transfusion if requested by the clinical team. Transfusions were performed according to standard hospital protocols. Blood tests and vascular measurements were performed at the pretransfusion baseline, 30 minutes after initiation of transfusion, 1 hour and 24 hours post-completion of transfusion. The study team did not alter any clinical care for the participants, including medication administration or diet.

Endothelial function assessment

Subjects underwent ultrasonography for determination of brachial artery FMD prior to transfusion, and at the aforementioned time points to assess the primary outcome measure of changes in FMD following fresh RBC vs. saRBC transfusions. FMD is a reliable surrogate for vascular health, and reflects endothelium and NO-mediated arterial function.(41, 42) Ultrasonography of the brachial artery was performed at the bedside using a high-resolution 10 MHz ultrasound transducer (Acuson) before and after suprasystolic inflation of a blood pressure cuff for 5 minutes in the ipsilateral upper arm. (43) Briefly, on cuff deflation reactive hyperemia produces an acute increase in shear stress; imaging of the brachial artery is performed continually for the next 120 seconds. Subsequent image analysis was performed by an investigator blinded to the timing using a validated program (Brachial Analyzer, Medical Instruments, Inc.). Brachial artery FMD was calculated as (hyperemic diameter-baseline diameter)/baseline diameter*100. Values obtained were adjusted during statistical analysis for baseline diameter using allometric scaling as recently described.(44) With regard to reproducibility, the mean difference in FMD (%) between two consecutive assessments performed in 11 subjects an average of 8 days apart was 1.26% (+/−0.76%,

r=0.75) in our laboratory. The mean difference in the FMD (%) between two readings of the same 11 measurements was 0.82% (+/−0.48%, r=0.97).

Laboratory Measurements

Plasma cell-free hemoglobin quantitation—Plasma free hemoglobin was determined by spectrophotometry. Standards utilized were from Count-A-Part Cyanmethemoglobin Standard Set (Diagnostic Technology, Inc.). Drabkin's Reagent was used to make 1:2 dilutions of the hemoglobin standards (24.0 mg/dL and 77.4 mg/dL) in order to generate a standard curve. Samples were centrifuged at 3400 RPM for 10 minutes at 4°C, and the plasma supernatant was removed to another tube. The plasma was centrifuged again under the same conditions, the supernatant was removed to a clean tube, and then diluted in Drabkin's Reagent and concentrations were calculated in reference to the standard curve. The assay was performed at an absorbance of 540 nm with standards and samples being run simultaneously within the same 96 well plate. Drabkin's Reagent was used as a blank in order to correct for background and subtracted from the resultant value of each well.

2,3-Diphosphoglycerate and adenosine triphosphate assays—In order to stabilize 2,3-DPG and ATP in blood samples for subsequent quantitation, perchloric acid extraction was performed. A 1 mL blood sample was added to a tube containing 2 mL of 5% perchloric acid (PCA), which was being continuously vortexed. The solution was maintained at 4°C for 20 minutes, and then centrifuged at 4°C for 10 minutes at 3400 RPM. The clear supernatant was transferred to a clean tube and centrifuged again. 2 mL of the resulting supernatant was transferred to a clean tube and, while vortexing, 300 μL of 3M K_2CO_3 was added. The resulting precipitate was allowed to settle for 20 minutes at 4°C. The extract was then centrifuged at 3400 RPM (4°C) for 10 minutes. The resulting clear, neutralized supernatant was transferred to a clean tube; a 0.3 mL aliquot was set aside at 4°C for 2,3-DPG quantitation and the remaining volume was capped tightly and stored frozen at −80°C in aliquots. The PCA extract was tested for 2,3-DPG and ATP content utilizing commercially available kits (Roche Diagnostics GmbH) according to the manufacturer's instructions. 2,3-DPG content of the blood samples was assayed within 24 hours of extraction and the concentration calculated using correction factors provided by the manufacturer. ATP content was calculated using an internal standard curve.

Nitrite and nitrate levels—Blood samples were collected in distilled water-rinsed centrifuge tubes containing 100 μL of 100 mM N-ethylmaleimide and 5 μL of 0.5 mM ethylenediaminetetraacetic acid (EDTA). Blood samples were then centrifuged to obtain a plasma sample. Plasma samples were flash frozen and stored at −80°C until further analysis. At the time of measurement, plasma samples were thawed on ice and nitrite and nitrate concentrations were quantified by ion chromatography using an ENO20 Analyzer (Eicom USA, San Diego, CA, USA) as previously described.(45)

Measurement of additional plasma analytes—IL-6, IL-2, TNF-alpha and MCP-1 were quantified by Luminex assay, while C-reactive protein was quantified by ELISA.

Statistical Analysis

Normally distributed variables were reported as means ± standard deviation. In contrast, non-normal data were reported as median (IQR) since means would have been heavily affected by data skewness. Differences between groups were assessed using t-tests for continuous variables, and chi-square or Fischer exact tests for categorical variables, where appropriate. Statistical analysis of allometrically scaled FMD was performed using a linear mixed effects model to account for baseline diameter, and provided estimates of these parameters by time in both groups. The model-based means are unbiased with unbalanced and incomplete data, provided that the missing data are non-informative. An unstructured form in the repeated measurements was assumed. Comparison of 2,3-DPG, ATP, and absolute change in FMD were performed using repeated measures ANOVA. The remaining metabolites were not normally distributed. Differences between these were analyzed using Mann-Whitney U, Friedman's two-way ANOVA and Wilcoxon Signed Rank tests when log-transformation was not possible. Two-tailed P-values <0.05 were considered statistically significant. Analyses were performed using IBM SPSS Statistics Version 21 (Armonk, NY, USA).

Results

Subject Characteristics

Of the 142 patients screened, 98 failed screening or were excluded; 44 subjects were randomized. One subject withdrew later, and of the remaining 43 subjects, 25 were randomized to receive saRBC units and 18 to fresh RBCs (Table 1). There were no significant differences in the clinical characteristics between the groups; 57% of subjects had an underlying malignancy and 25% had received chemotherapy

All transfused units were leukoreduced CPD/AS1 packed RBC units that were crossmatch-compatible with the recipient. The majority of subjects (82%) were transfused two units of RBCs. Two subjects in the fresh group received three and four units, while the remaining six subjects received a single unit. There were no significant differences in the pre- or post-transfusion hemoglobin or the number of units transfused per patient between groups. Only two of the study participants received plasma transfusions within 2 days of the study, and those units were ABO-identical with the recipient; none of the patients were transfused with platelets in the peri-study period. No adverse transfusion reactions occurred during the study period, and no positive direct antiglobulin test results were seen. The mean storage duration of saRBC and fresh RBC units was 29.6 ± 4.9 and 9.6 ± 3.9 days, respectively ($p<0.001$).

Endothelial function

There was no significant difference in the baseline pre-transfusion FMD between subjects randomized to saRBC compared to fresh RBC transfusion (5.2 ± 4.4% versus 4.7 ± 4.4% respectively, $p=0.7$). Although the change in FMD between the two groups did not reach statistical significance ($p=0.2$), post-hoc analysis revealed that while FMD remained unchanged in subjects receiving fresh RBCs ($p=0.231$), it significantly declined in those receiving saRBCs over the 24 hours following transfusion ($p=0.045$) (Figure 1). Resting brachial artery diameter did not change significantly over time in either group ($p=0.7$ and

p=0.3 for fresh RBC and saRBC) or between the groups (p=0.9), and peak-velocity at hyperemia did not change significantly post-transfusion (p=0.08 and p=0.4 for saRBC and fresh RBC). FMD analyzed without allometric adjustment produced similar results.

2,3-Diphosphoglycerate Levels

Although there were no differences in 2,3-DPG levels at baseline in recipients of saRBC and fresh RBC (55.2 ± 35.6 and 44.3 ± 21.8 μM/gHgb respectively, p=0.3), there were small but statistically significant changes during the next 24 hours between the groups (Figure 2). Specifically, at 1 hour after transfusion 2,3-DPG levels declined in recipients of saRBCs, but increased in those transfused with fresh RBCs (p=0.024). The trend at 24 hours was similar but did not reach statistical significance (Figure 2).

Nitrite and Nitrate Levels

The changes in serum nitrite and nitrate levels were not statistically significant with either saRBC or fresh RBC transfusion, however, there was a trend towards a decrease in nitrite levels 24 hours after saRBC compared to fresh RBC transfusions (p=0.052) (Table 2).

Other Metabolites

A small increase in cell-free hemoglobin levels was noted 1 hour after transfusion of saRBC (p=0.039) but not fresh RBCs. ATP levels increased 1 hour after fresh RBC transfusion (p=0.043) and decreased 24-hours after saRBC (p=0.026). The remaining metabolites and inflammatory marker levels did not change significantly (Table 2).

Discussion

NO is a crucial vasodilator that actively modulates blood flow and oxygen delivery to local tissues (46, 47). Common disorders including cardiovascular disease and its risk factors are known to reduce NO bioavailability leading to vascular dysfunction and related morbidities (48, 49). We and others have hypothesized that older stored RBCs can also reduce NO bioavailability in transfusion recipients, contributing to endothelial dysfunction, abnormalities of vasodilator tone, and ultimately to end-organ dysfunction (14, 37–39, 46, 50). In this study, we randomized patients to fresh vs. old stored RBCs, and measured their vascular reactivity with FMD, which is a specific measure of vascular NO bioavailability. (51) While there were no significant differences in the primary outcome measure of changes in FMD following fresh RBC vs. saRBC transfusions, the results showing a decrease in FMD response in recipients of saRBC (but not fresh RBC) transfusion suggest that infusion of RBCs stored > 21 days can significantly reduce NO-mediated vasodilation in stable anemic hospitalized patients. These findings will need to be confirmed in subsequent larger studies.

Despite some of the limitations of the present study, as described below, previous investigations using *in vitro* aortic ring systems (36, 37) as well as rat (31) and dog (22, 23) transfusion models support our findings. However, there is currently limited clinical data on the vascular effects of saRBC transfusions in human recipients to either confirm or refute our results. Berra et al reported that RBC storage time (3 days versus 40 days) had no effect

on the reactive hyperemia index measured by pulsatile arterial tomography in healthy volunteers receiving a single unit of autologous blood, although older blood was associated with increased markers of hemolysis and plasma nitrite (52). Likewise, Roberson and colleagues failed to demonstrate a differential effect of 7 versus 42 day aged autologous transfusion on microcirculatory flow in healthy subjects (53). As compared to the present findings, the lack of an effect of blood storage duration in these previous clinical studies may have been due to the differing characteristics of the study populations as well as the use of pulsatile tonometry which is not a direct measure of vascular NO activity. (54) Interestingly, in a study of 93 transfused trauma patients, increased storage age of RBC units was associated with decreased perfused capillary density and thenar eminence tissue oxygen saturation (55). Furthermore, transfusion of a single unit of RBC stored over 26 days significantly decreased whole blood nitrite levels (37). However, as compared to the trauma cohort studied in the aforementioned investigations (37, 55), we studied older subjects (mean age 60±15 versus 46+20 years) with predominantly medical versus traumatic/surgical etiologies of anemia, and we used FMD which is a specific measure of conductance vessel NO-bioavailability. Our subjects also had relatively lower average baseline FMD (5.1%), which reflects their age and degree of illness.

The approximate 2 percentage-point FMD decrease in the saRBC group (from 4.7% pre-transfusion to 2.4% at 24 hours post-transfusion) is comparable to, or larger than, other FMD effects described in the literature. For example, a 12 week course of statin therapy in patients with metabolic syndrome significantly improved FMD from 5.0% to 6.1% (p=0.02) (56). In patients with known coronary artery disease (CAD), coexisting diabetes mellitus type 2 was associated with a reduction in FMD to 2.5%(57). Furthermore, for every 1% decrease in FMD, the risk of cardiovascular events increases by 13% (58). Thus, the magnitude of FMD observed in recipients of saRBC units are meaningful and may have clinical implications.

While the observed FMD changes may be due to the effects of saRBC transfusions, it is important to rule out other causes. For example, several factors such as smoking, diabetes, food, medications, hypertension and body mass index (BMI) can affect FMD. However, our study population was randomized and patient characteristics, including risk factor distribution and baseline FMD were not different between the groups. While 2 of 25 patients randomized to the saRBC arm were smokers, compared to none of the 18 recipients of fresh RBCs, the difference between groups in smoking prevalence is not statistically significant (p=0.5). Additionally, it is unlikely that the cardiovascular risk factor profile changed during the 24-hour time interval of our study.

While these data suggest that saRBC transfusions reduce NO-mediated vasodilation, the present data do not allow us to identify potential mechanisms. The vasoinhibitory effects may be due to decreased NO synthesis, increased NO scavenging, reduced smooth muscle vasodilatory response to NO, and/or other causes. Although we found elevated plasma hemoglobin levels after saRBC but not fresh RBC transfusions, which would be consistent with previous experimental data suggesting that the NO scavenging effects of free hemoglobin may be partly responsible (22, 23, 31), the effect was small and the reduction in plasma hemoglobin in recipients of fresh RBC units was unexpected. Given these

unexpected results, we cannot draw firm conclusions on the effects of plasma free hemoglobin on FMD in this study Recent studies demonstrating the protective effects of haptoglobin and hemopexin lend support to the finding that the FMD vasoinhibitory effects may potentially be due to free hemoglobin (or its metabolites, such as hemin).(59)

The changes observed in 2,3-DPG were unexpected. Heaton previously demonstrated in healthy recipients that AS-1 RBCs stored for 35 days prior to transfusion regenerated 2,3-DPG slowly over 24 hours, with 25% restoration within 1 hour.(60) However, Correra showed that some anemic patients have inappropriately low 2,3-DPG levels (when compared to Hb),(61) possibly because in their diseased states they don't support adequate RBC 2,3-DPG synthesis. Since our transfusion recipients were ill and anemic, they may not be able to support normal rates of 2,3-DPG synthesis in transfused RBCs, particularly saRBCs, leading to the observed differences in 2,3-DPG levels in recipients of saRBCs vs. fresh RBCs. This possibility will require further investigations.

Strengths of our study include the large sample size, detailed study of vascular NO responsiveness, and investigation of potential mediators of vascular dysfunction in anemic adults receiving allogeneic blood transfusions for clinical reasons, a population in whom the increased risks of blood transfusion has been observed.

There are also important limitations of our study. First, the primary outcome measure of changes in FMD following fresh RBC vs. saRBC transfusions showed no statistically significant differences between recipients of fresh vs saRBC transfusions, although a post-hoc analysis showed that the latter participants experienced a significant decrease in FMD 24 hours after older RBC transfusions. Second, due to the lead time required for our study, only patients receiving routine transfusion were included in the study, thus excluding many critically ill and actively bleeding patients requiring emergent transfusion. Third, medications and meals were not held to accommodate study procedures and neither was the time of day fixed which may have confounded our results because of circadian and food related effects. Fourth, there was significant heterogeneity in the etiology of anemia among subjects although there were no differences between the randomized groups. Fifth, we also observed changes in plasma hemoglobin, 2,3-DPG and ATP which were in some cases unexpected, and we did not observe any significant changes in inflammatory markers, although our subjects had multiple co-morbidities and thus already had elevated baseline levels of inflammatory markers that may have influenced our findings. Thus, while our data provides compelling suggestions that saRBC transfusions adversely affect vascular function in ill, hospitalized transfusion recipients, the current results should be interpreted with caution. We are now beginning a follow-up study to further investigate the vascular effects of saRBC transfusions using a cross-over trial design and other modifications to control for recipient variability and address other drawbacks of the present investigations.

Clinical Implications

This study suggests a significant negative effect of transfused RBC units stored > 21 days on NO-mediated vasodilation in anemic hospitalized patients. This finding lends additional support to the hypothesis that deranged NO signaling mediates adverse clinical effects of older RBC transfusions, and should be further investigated in larger clinical studies.

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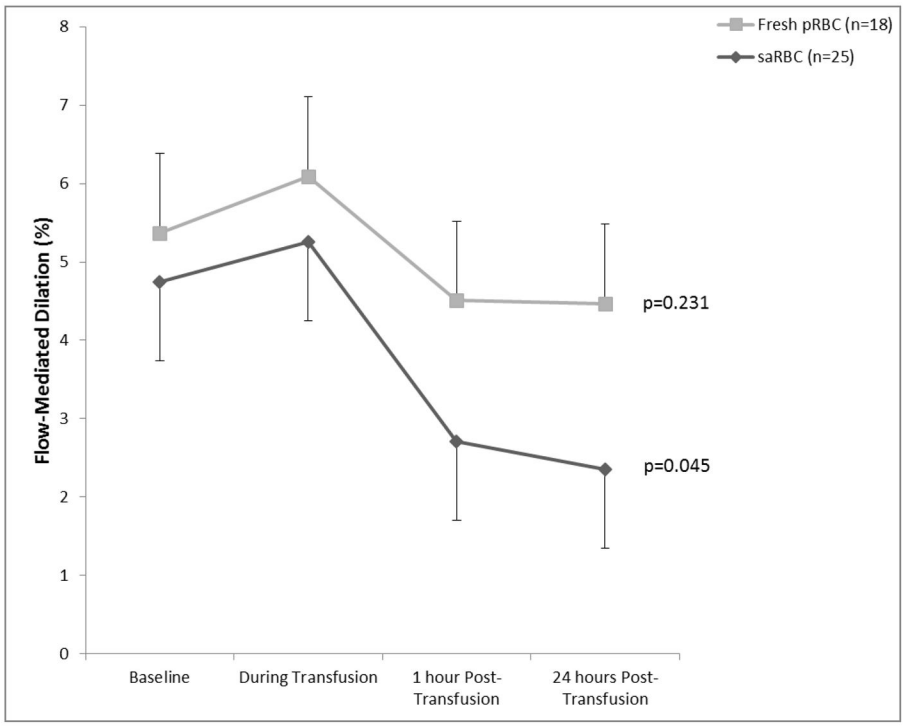

Figure 1.

Flow-Mediated Dilation during Transfusion of Storage-Aged and Fresh Blood Units. RBCs= packed red blood cells; saRBCs= storage-aged red blood cells. Values represent absolute change in FMD (%), adjusted for baseline brachial artery diameter using allometric scaling.

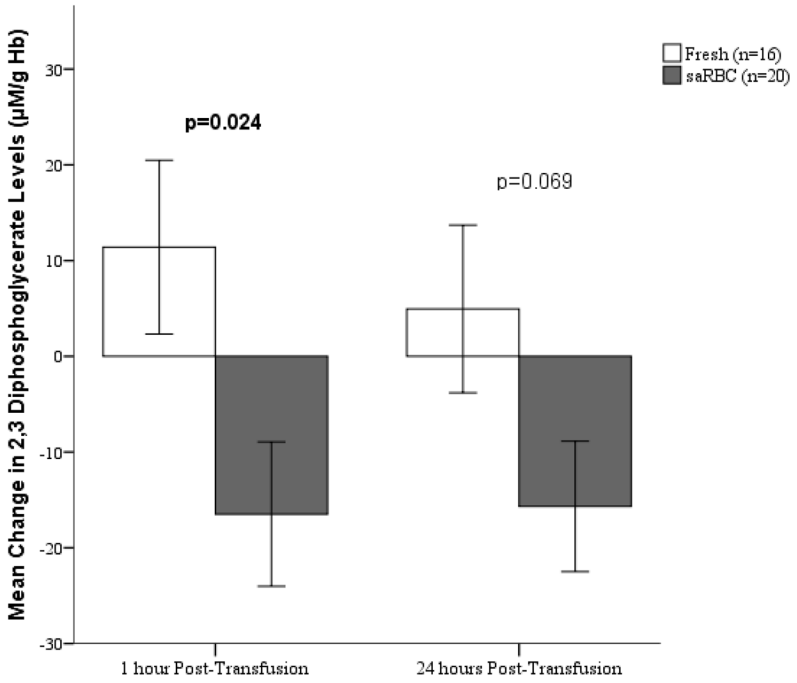

Figure 2.

Change in 2,3-diphosphoglycerate Levels after Transfusion of Storage-aged and Fresh Blood Units. RBCs= packed red blood cells; saRBCs= storage-aged red blood cells. Values represented are mean change in 2,3-diphosphoglycerate (DPG) levels compared to baseline. The interaction of time point and blood age was statistically significant (p=0.042). Note the significant difference in the change in 2,3 DPG at 1 hour between recipients of saRBC and fresh RBC.

Table 1

Demographic and Clinical Characteristics

Variables	All Patients (n=43)	Recipients of saRBCs (>21 days) (n=25)	Recipients of fresh RBCs (<14 days) (n=18)
Age, years	59 ± 13	58 ± 12	60 ± 15
Male, n (%)	24 (55)	13 (52)	11 (58)
Race			
White, n (%)	23 (54)	15 (63)	8 (42)
Black, n (%)	17 (40)	7 (29)	10 (53)
Blood Type			
A−	1 (2)	1 (4)	0
A+	12 (27)	5 (20)	7 (36)
B−	1 (2)	1 (4)	0
B+	7 (16)	5 (20)	2 (11)
O+	23 (52)	13 (54)	10 (53)
Number of Units Transfused			
1	6 (14)	5 (20)	1 (5)
2	36 (82)	20 (80)	16 (84)
3	1 (2)	0	1 (5)
4	1 (2)	0	1 (5)
Clinical Characteristics			
Baseline Hemoglobin, g/dL	7.6 ± 0.8	7.7 ± 0.7	7.4 ± 0.9
Post-Transfusion Hemoglobin, g/dL	9.6 ± 1.1	9.3 ± 1.0	9.9 ± 1.0
Systolic Blood Pressure, mmHg	126 ± 17	123 ± 17	131 ± 17
Diastolic Blood Pressure, mmHg	70 ± 10	69 ± 9	72 ± 11
Coronary Artery Disease, n (%)	11 (25)	5 (20)	6 (32)
Diabetes Mellitus, n (%)	12 (29)	5 (21)	7 (39)
Dyslipidemia, n (%)	9 (21)	3 (13)	6 (33)
Current Smoking, n (%)	2 (5)	2 (8)	0
Family History of CAD, n (%)	2 (5)	2 (9)	0
Known malignancy, n (%)	25 (57)	17 (68)	17 (90)
Undergoing chemotherapy, n (%)	11 (25)	9 (36)	2 (11)

Variables	All Patients (n=43)	Recipients of saRBCs (>21 days) (n=25)	Recipients of fresh RBCs (<14 days) (n=18)
Surgery during index admission, n (%)	19 (43)	9 (36)	10 (53)
Medications			
Statin, n (%)	3 (8)	1 (4)	2 (12)
Aspirin, n (%)	6 (15)	3 (13)	3 (18)
ACE-I/ARB, n (%)	3 (8)	1 (4)	2 (12)

Values are mean ± SD, or n (%). There were no statistically significant differences between groups. CAD; coronary artery disease, ACE-I; angiotensin-converting enzyme inhibitor, ARB; angiotensin receptor blocker.

Table 2

Metabolites and Inflammatory Markers

	saRBC			Fresh RBC		
	Baseline	**1 hour Post- Transfusion**	**24 hours Post- Transfusion**	**Baseline**	**1 hour Post- Transfusion**	**24 hours Post- Transfusion**
Free Hgb (g/dL)	0.111 (0.054)	0.114 (0.050)	0.116 (0.054)	0.142 (0.137)	0.125 (0.086)	0.109 (0.174)
NO_2 (μM)	0.97 (1.71)	0.66 (1.56)	0.64 (1.23)	1.68 (1.54)	1.90 (3.53)	1.47 (2.35)
NO_3 (μM)	99.8 (109.7)	79.7 (90.8)	77.4 (54.3)	107.0 (136.8)	96.2 (147.5)	71.6 (78.9)
ATP (μM/g Hgb)	4.90 (6.50)	5.26 (7.91)	4.70 (4.11)	4.14 (4.24)	5.82 (1.18)	3.10 (4.26)
IL-6 (pg/mL)	23.2 (29.4)	21.0 (26.3)	18.9 (30.8)	20.0 (33.4)	14.9 (33.0)	18.0 (18.6)
IL-2 (pg/mL)	0.60 (1.80)	0.40 (2.15)	0.52 (2.91)	0.01 (0.48)	0.01 (0.62)	0.01 (0.35)
TNFα (pg/mL)	6.80 (8.80)	7.32 (10.58)	6.98 (10.04)	4.76 (6.63)	4.84 (5.56)	4.84 (4.66)
MCP-1 (pg/mL)	170.1 (217.6)	161.3 (264.4)	227.7 (249.5)	170.3 (187.2)	185.8 (168.3)	191.9 (134.9)
CRP (μg/mL)	188.9 (238.7)	185.8 (330.0)	162.7 (189.0)	138.7 (100.7)	145.1 (205.0)	187.8 (230.3)

Values are median (IQR). NO2; nitrite, NO3; nitrate, ATP; adenosine triphosphate, IL; interleukin, TNF; tumor necrosis factor, MCP; monocyte chemotactic protein, CRP; C-reactive protein. The differences between saRBC and fresh RBC recipients were not statistically significant.