Supplementary Material for:

Stowell CP, Whitman G, Assmann SF, Gomez H, Granger S, Massey M, Shapiro N, Steiner ME, Bennett-Guerrero E., The Impact of Red Blood Cell Storage Duration on Tissue Oxygenation in Cardiac Surgery: An Ancillary Physiologic Study to the Red Cell Storage Duration Study

MATERIALS and METHODS

Tissue Hemoglobin Oxygen Saturation: Data Analysis

Raw data were uploaded to a cloud-based server and synchronized to a secure server at the central processing facility. The data files for tissue hemoglobin oxygen saturation (S_tO_2) of the thenar eminence and cerebrum were parsed for absolute time and value data entries and converted to a common file format for analysis. Time data embedded in the S_tO_2 data streams were correlated with data that were recorded directly on Case Report Forms which indicated the time points defined in the Materials and Methods section of the main text. Using a custom software application written in Matlab (Mathworks, Natick, MA), S_tO_2 data were plotted as a function of time. Color-coded vertical marks delineated the times that the sensors were applied or removed, the four time points described above, all recorded transfusion start and stop times, and the time the patient entered the ICU. An analyst visually inspected the data plots for stability and selected a region of the plot for each measurement that was stable and within the timepoint window using a running average single spot measurement. The 150-sample average was taken over a period of 2.5 minutes before to 2.5 minutes after the spot to be measured.

For timepoint A, the spot measurement was selected during a stable period in the signal within 6 hours prior to the incision and at least 2.5 minutes after the start of data recording and at least 2.5 minutes before entering the OR. For timepoint B, the spot measurement was selected during a stable period in the signal within 2 hours prior to the index transfusion AND at least 2.5 minutes before the start of the index transfusion. For timepoint C, the spot measurement was selected during a stable period in the signal within 2 hours after the index transfusion AND at least 2.5 minutes after the end of the index transfusion. For timepoint D, the spot measurement was selected during a stable period in the signal most proximal to 24 hours post-ICU arrival AND at least 2.5 minutes after the beginning of timepoint D. The software then computed and recorded an average S_tO_2 value for that region of the plot.

Microcirculatory Blood Flow: Data Analysis

The Microscan video files were pre-processed to enhance contrast, edited to 5 sec duration, and evaluated for image quality using the method described.(1) Video files with unacceptable quality were excluded from further analysis. Video clips were sorted by quality score and up to 3 clips were selected at each of the 4 timepoints. Selected video files were assigned a random identification number before further analysis using AVA 3.1 software (Microvision Medical BV, Amsterdam, The Netherlands). Vessel centerlines and lumen boundaries were drawn using semi-automated or manual tools in AVA. All of the microcirculatory parameters reported in this study were derived from analyses of small vessels (defined as having lumen diameters below 20 μm) because capillaries and post-capillary venules of this size are the primary sites of oxygen diffusion between blood and tissue. Blood flow in vessel segments was evaluated in a semi-quantitative fashion using a scale of 0-3 (0: no flow, 1:

intermittent flow, 2: sluggish flow, 3: continuous flow). Microcirculatory parameters were then derived from the measured small vessel total length and the perfused length.

RESULTS

Table S1. Comparison of Transfused Subjects in RECESS (Parent Study) and RECAP (Ancillary Study). These subjects received at least one RBC transfusion from 6 h prior to surgery to 24±4 h after surgery.

Parameter	RECESS Subjects N=1098	RECAP Subjects N=89
Baseline Characteristics		
Age – median y (Q1,Q3)	72 (66, 79)	71 (66, 80)
Male – n (%)	475 (43%)	41 (46%)
Hemoglobin – median g/dl (Q1,Q3)	11.9 (10.7, 12.9)	11.5 (10.5, 12.4)
Creatinine – median mg/dL (Q1,Q3)	1.0 (0.8, 1.4)	1.1 (0.9, 1.4)
TRUST – mean (± SD)	3.98 (± 0.91)	3.87 (± 0.80)
MODS - mean (± SD)	0.67 (± 0.81)	0.72 (± 0.75)
Surgery Characteristics		
Repeat Sternotomy – n (%)	293 (27%)	21 (24%)
Cardiopulmonary bypass duration[a] – median min (Q1,Q3)	140 (102, 190)	151 (102, 209)
Transfusion Data (from time entering the OR until 24 h after leaving the OR)		
RBC Transfused - median units (Q1,Q3)	3.0 (2.0, 5.0)	3.0 (2.0, 5.0)
Shorter Storage Time – mean d (± SD)	16.2 (± 11.1)	15.3 (± 10.5)
Longer Storage Time - mean d (± SD)	20.0 (± 12.8)	19.8 (± 13.1)
Outcome Measures		
Δ MODS 7 d – mean (± SD)	8.58 (± 3.58)[b]	8.74 (± 3.55)[c]
ICU length of stay – median d (Q1,Q3)	3 (2 , 5)	2 (1, 4)
Mortality 7 d – n (%)	26 (2.4%)	2 (2.3%)

[a] Calculated only for patients undergoing cardiopulmonary bypass.

[b] For the change in MODS at 7 days, data were unavailable for 11 subjects.

[c] For the change in MODS at 7 days, data were unavailable for one subject.

Table S2. Patient Clinical Characteristics Prior to Post-operative RBC (index) Transfusion

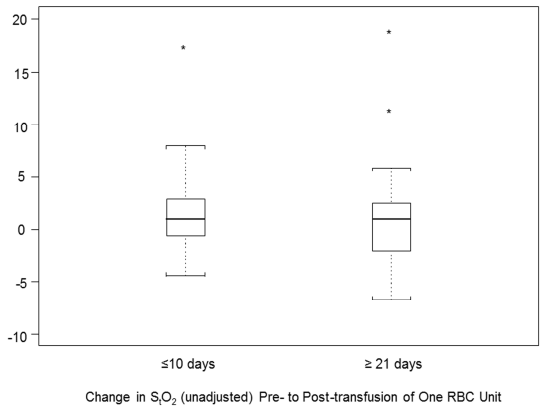
Clinical characteristics within 2 h of start of post-operative RBC transfusion	**RECAP Subjects who had an index transfusion**			
	≤ 10 d Arm (N=23)	**≥21d Arm (N=32)**	**P-Value[1]**	**Effect Size[2]**
Receiving supplemental oxygen – n (%)	20 (87%)	31 (97%)	0.30	0.19
Unknown	*0*	*0*		
Intubated – n (%)	17 (74%)	26 (81%)	0.53	0.09
Unknown	*0*	*0*		
Receiving Inotrope - n (%)	17 (77%)	21 (70%)	0.75	0.08
Unknown	*0*	*0*		
Systolic BP – mmHg mean (± SD)	104.8 (15.9)	109.4 (22.6)	0.53	0.23
Diastolic BP – mmHg mean (± SD)	50.4 (8.6)	51.9 (13.8)	0.76	0.13
Heart Rate – bpm mean (± SD)	85.3 (12.6)	88.9 (11.3)	0.46	0.30
FiO2 - mean (± SD)	48.5 (15.7)	46.1 (14.2)	0.57	0.16
Blood Oxygen Saturation				
S_aO_2 - mean (± SD)	100 (0) N=2	97 (2.9) N=6	0.09	1.13
S_pO_2 - mean (± SD)	98.5 (2.1) N=17	98.6 (3.2) N=25	0.23	0.03
Unknown	3	0		

[1] P-Values for continuous variables are from a Kruskall-Wallis non-parametric test; p-Values for categorical variables are from Fisher's exact test.

[2] Effect sizes for binary and categorical variables were calculated using Cramer's phi statistic and were calculated as the absolute difference in means divided by the pooled standard deviation for continuous variables.

References

1. Massey MJ, Larochelle E, Najarro G, Karmacharla A, Arnold A, Trzeciak S, et al. The microcirculation imaging quality score: development and primary evaluation of a proposed approach to grading quality of image acquisition for bedside videomicroscopy. *J Crit Care*. 2013;28:913-7.



Change in S_tO_2 (unadjusted) Pre- to Post-transfusion of One RBC Unit