Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Steiner ME, Ness PM, Assmann SF, et al. Effects of red-cell storage duration on patients undergoing cardiac surgery. N Engl J Med 2015;372:1419-29. DOI: 10.1056/NEJMoa1414219

Supplementary Appendix

Effects of red cell storage duration in cardiac surgery patients

Table of Contents

Acknowledgments

The following investigators and staff participated in the study: **Froedert Memorial Lutheran Hospital, Milwaukee, WI:** Z. Rashid (principal investigator), B. Alivo, J. McFarland, D. Nischick; **Aurora St. Luke's Medical Center, Milwaukee, WI:** K. Puca (principal investigator), C. Zywicki; **Boston Children's Hospital, Boston, MA:** S. Sloan (principal investigator), L. Lashley, J. Salvin, E. Neufeld; **Beth Israel Deaconess Medical Center, Boston, MA:** L. Uhl (principal investigator), B. Malynn, K. Brideau; **Brigham and Women's Hospital, Boston, MA:** R.M. Kaufman (principal investigator), A. Dunn-Morgan, R. Smeland-Wagman; **New York Presbyterian Hospital - Weill Cornell, New York, NY:** J. Bussel (principal investigator), M. Cushing, L. Girardi, N. Haq, S. Hill; **Duke University, Durham, NC:** T.L. Ortel (principal investigator), M. Gleim, P. Jordan, L. Talbott, E. Thames; **Emory University, Atlanta, GA:** C. Josephson (principal investigator), M. Castillejo, K. Egan; **Johns Hopkins University, Baltimore, MD:** P. Ness (principal investigator), K. Behrens, J. Boyd, R. Case, K. Condon, R. Ohkuma, G. Whitman; **Massachusetts General Hospital, Boston, MA:** C. Stowell (principal investigator), L. Bornikova, C. Brueggeman, J. McNamara , M. Sanchez-Hernandez; **Swedish Medical Center, Seattle, WA:** M. Delaney (principal investigator), L. Fitzpatrick, D. Gartman, J. Nagel, D. Tinlin, C. Truva, S. Youssef; **University of Iowa, Iowa City, IA:** T. Raife (principal investigator), D. Schrock; **University of Maryland, Baltimore, MD:** P. Rock (principal investigator), A. Anazado, R. Cooke, K. Wallace; **Fairview–University Medical Center, Minneapolis, MN:** M. Steiner (principal investigator), S. Pulkrabek; **University of North Carolina, Chapel Hill, NC:** N. Key (principal investigator), R. Kellerman , Y. Park, W. Simmons; **University of Oklahoma, Oklahoma City, OK:** J. George (principal investigator), Z. Al-Nouri, Q. Khan, J. Reese, P. Roberts, D. Terrell; **University of Pittsburgh, Pittsburgh and University of Pittsburgh-Mercy Hospital, Pittsburgh, PA:** D.J. Triulzi (principal investigator), P. D'Andrea, C. Wiskeman; **Mayo Clinic, Rochester, MN:** G. Nuttall (principal investigator), D. Haugen, W. Oliver, S. Veettil; **Vanderbilt University, Nashville, TN:** P. Young (principal investigator), N. Bratcher, S. Dixon , J. Janssen, C. Madison, L. Michalowski; **Rutgers Robert Wood Johnson, New Brunswick, NJ:** J. Carson (principal investigator), K. Dragert; **St. Luke's- Texas Heart Institute, Houston, TX:** A. Bracey (principal investigator), S. Kavazovic, S. Moore, C. Ramos , C. Wellington; **Baystate Medical Center, Springfield, MA:** R. Engelman (principal investigator), B. Burkott , N. Corriveau , J. Germain; **Indiana/Ohio Heart, Fort Wayne, IN and Indiana/Ohio**

Heart- St. Joseph, Fort Wayne, IN: V. Scavo (principal investigator), H. Downey, S. Eichman, S. James; **UT Southwestern Medical Center, Dallas, TX:** P. Greilich (principal investigator), K. Landgraf, V. Patel; **University of Florida, Gainesville, FL:** C. Klodell (principal investigator), K.Berg, C. King , N. Staples; **St. Elizabeth's Medical Center, Boston, MA:** R. Hunsaker (principal investigator), C. Keith, S. Lin, A. Weiffenbach, J. Wise; **Newark Beth Israel Medical Center, Newark, NJ:** R. Karanam (principal investigator), B. Patel; **Aspirus Heart and Vascular Institute, Wausau, WI:** R. Miles (principal investigator), J. Kaliebe, M. Johnson; K. Matsche; **Sanford Heart Center, Fargo, ND,** C. Dyke (principal investigator), D. Augustadt, J. Wurgler; **Columbia University Medical Center, New York, NY:** E. Hod (principal investigator), L. Goldsmith, J. Netterwald, S. Rizvi, J. Schwartz; **Tulane University, New Orleans, LA:** Cindy Leissinger (principal investigator); **New England Research Institutes (Data Coordinating Center), Watertown, MA:** S.F. Assmann (principal investigator), V. Butler, E. Devlin, S. Granger, M. Grenache, K. Hayes, J. Miller, L. Miller; **TMH Steering Committee Chair, McMaster University, Hamilton ON**: M. Blajchman; **Medical Monitors:** L. Katz, G. Ailawadi, D. Jobes; **Saint Louis University Core ECG Reading Facility:** B. Chaitman (director), K. Stocke, T. Bertram; **National Heart, Lung, and Blood Institute, Bethesda, MD:** T. Mondoro (project officer), S. Glynn; **Data and safety Monitoring Board**: D. Chen, V. Durkalski, R. Gorman, H. Hume, J. Lusher, B. McLeod, J. Menitove, A. Neff, A. Reitsma, P. Roberson, A. Shapiro, E. Snyder, R. Weiskopf, C. Whitsett.

Complete List of Eligibility Criteria

Enrollment Inclusion Criteria:

1) Patients ≥ 12 years old
2) Patients ≥ 40 kg
3) Scheduled complex cardiac surgery with planned use of median sternotomy. The procedure may be performed either on-pump or off-pump. Procedures that qualify as complex cardiac surgery include the following ("Repeat procedure" means that the subject had a previous cardiac surgery with median sternotomy):
    - Single Vessel Coronary Artery Bypass Graft, repeat procedure
    - Multiple Coronary Artery Bypass Grafts, first or repeat procedure
    - Single Valve Repair or Replacement, repeat procedure
    - Multiple Valve Repair or Replacement, first or repeat procedure
    - Surgery involving both Coronary Artery Bypass Graft(s) and Valve Repair(s), first or repeat procedure
    - One or more of the following procedures, with or without Coronary Bypass Graft(s):
        - left ventricular aneurysm repair,
        - ventricular and/or atrial septal defect repairs,
        - batista (surgical ventricular remodeling),
        - surgical ventricular restoration,
        - congenital defect repair, and
        - aortic root procedures
4) Patients ≥ 18 years must have a TRUST[1] probability score ≥ 3, which corresponds to a high likelihood of receiving RBC transfusions during surgery or within 96 hours post-operatively (see Appendix 1). Calculate the TRUST score using the most recent test results done within the previous 60 days. If data for some components of the TRUST score are not available, but enough data are available to know that the TRUST score is at least 3, the patient meets this eligibility criterion. For patients < 18 the TRUST score need not be calculated.

Enrollment Exclusion Criteria:

1) Refusal of blood products
2) Planned surgery is minimally invasive
3) Known transfusion reaction history
4) Requirement for washed products, volume reduced products, or products with additive solution removed
5) Expected residual cyanosis with O_2 saturation < 90
6) Left ventricular assist device (LVAD) or Extracorporeal membrane oxygenation (ECMO) support pre-operatively or planned need post-operatively
7) Cardiogenic shock requiring pre-operative placement of an Intra-aortic balloon pump (IABP) (IABP done for unstable angina or prophylactically for low ejection fraction is not excluded)
8) Planned Deep Hypothermic Circulatory Arrest (DHCA)
9) Renal dysfunction requiring pre-operative renal replacement therapies such as hemodialysis (HD) or continuous venovenous hemofiltration (CVVH)
10) Planned use of alternative to heparin, e.g. bivalirudin
11) Planned use of autologous or directed donations
12) Prior RBC transfusion during hospitalization for the study-qualifying surgery

Statistical Details of Interim Monitoring

The Data and Safety Monitoring Board reviewed primary outcome, mortality, and adverse event data quarterly.

- For the primary outcome, the pre-defined stopping boundary was 0.000002 at each interim look, with the final p-value to declare significance calculated from an alpha-spending approach.[1] This boundary was chosen to have < 3% chance of stopping early for a true difference of < 1.2 points, a difference deemed to be too small to warrant a major change in practice even if statistically significant, while having over 40% chance of stopping early for a true difference of at least 2 points.
- For mortality, stopping boundaries were calculated from an alpha-spending approach approximating O'Brien-Fleming boundaries, with the final p-value to declare significance calculated from an alpha-spending approach.
- A p-value < 0.05 was considered significant for other outcomes, which did not have formal stopping boundaries.

Table S1 Calculation of Transfusion Risk Understanding Scoring Tool (TRUST), from Alghamdi et al. The TRUST score is the sum of the patient's points for individual characteristics, with a range from 0 to 8.

Patient Characteristic	**Points**
Hemoglobin < 13.5 g/dL	1
Wediht < 77 kg	1
Female sex	1
Age > 65 years	1
Nonelective surgery	1
Serum creatinine level > 120 μmol/L	1
Previous cardiac surgery	1
Nonisolated surgery	1

Table S2. Content of additive solutions (all values in mg/100 mL), from AABB Technical Manual 17th edition.

Constituent	**AS-1**	**AS-3**	**AS-5**
Dextrose	2200	1100	900
Adenine	27	30	30
Monobasic sodium phosphate	0	276	0
Mannitol	750	0	525
Sodium chloride	900	410	877
Sodium citrate	0	588	0
Citric acid	0	42	0

Table S3. Definition of MODS components and scores [Marshall 1997]

	Score				
Organ System	**0**	**1**	**2**	**3**	**4**
Respiratory [a]					
($P0_2/Fi0_2$ ratio)	>300	226-300	151-225	76-150	≤75
Renal [b]					
(serum creatinine)	≤100	101-200	201-350	351-500	>500
Hepatic [c]					
(serum bilirubin)	≤20	21-60	61-120	121-240	>240
Cardiovascular [d]					
(PAR)	≤10.0	10.1-15.0	15.1-20	20.1-30	>30
Hematologic [e]					
(platelet count)	>120	81-120	51-80	21-50	≤20
Neurologic [f]					
(Glasgow Coma Score)	15	13-14	10-12	7-9	≤6

[a] The $P0_2/Fi0_2$ ratio is calculated without reference to the use of mode of mechanical ventilation, and without reference to the use or level of positive end-expiratory pressure

[b] the serum creatinine concentration is measured in µmol/L, without reference to the use of dialysis

[c] the serum bilirubin concentration is measured in µmol/L

[d] the pressure-adjusted heart rate (PAR) is calculated as the product of the heart rate (HR) multiplied by the ratio of the right atrial (central venous) pressure (RAP) to the mean arterial pressure (MAP): PAR= HR x RAP/mean BP

[e] the platelet count is measured in platelets/mL 10^{-3}

[f] the Glasgow Coma Score is preferably calculated by the patient's nurse and is scored conservatively (for the patient receiving sedation or muscle relaxants, normal function is assumed, unless there is evidence of intrinsically altered mentation)

Table S4. Protocol Compliance with Storage Duration of RBC Units

A. Through Post-Operative Day 7

Characteristic	RBC ≤10 Days N (%)	RBC ≥21 Days N (%)
Compliance By Transfusion	**2394 units**	**2546 units**
Storage Duration in Subject's Randomized Treatment Arm	2191 (92)	2378 (93)
Storage Duration 11-20 Days	123 (5)	121 (5)
Storage Duration in Other Treatment Arm	78 (3)	47 (2)
Unknown	2 (<1)	0 (0)
Compliance By Subject	**538 subjects**	**560 subjects**
All RBC Units Compliant with Subject's Randomized Treatment Arm	483 (90)	493 (88)
Received At Least One Unit With Storage Duration 11-20 days But None With Storage Duration in Other Treatment Arm	33 (6)	43 (8)
Received At Least One RBC Unit With Storage Duration in Other Treatment Arm	21 (4)	24 (4)
Unknown	1 (<1)	0 (0)

B. Through Post-Operative Day 28

Characteristic	RBC ≤10 Days N (%)	RBC ≥21 Days N (%)
Compliance By Transfusion	**2584 units**	**2730 units**
Storage Duration in Subject's Randomized Treatment Arm	2358 (91)	2554 (94)
Storage Duration 11-20 Days	136 (5)	126 (5)
Storage Duration in Other Treatment Arm	88 (3)	50 (2)
Unknown	2 (<1)	0 (0)
Compliance By Subject	**538 subjects**	**560 subjects**
All RBC Units Compliant with Subject's Randomized Treatment Arm	478 (89)	488 (87)
Received At Least One Unit With Storage Duration 11-20 days But None With Storage Duration in Other Treatment Arm	34 (6)	47 (8)
Received At Least One RBC Unit With Storage Duration in Other Treatment Arm	25 (5)	25 (4)
Unknown	1 (<1)	0 (0)

Table S5. Treatment group comparisons for each MODS component from baseline to the worst post-operative value through earliest of hospital discharge, death, study withdrawal, or 7-days

Component	**RBC ≤10 Days N = 538 Unadjusted Mean (±SD)**	**RBC ≥21 Days N = 560 Unadjusted Mean (±SD)**	**Difference between ≤ 10 d Arm and ≥ 21 d Arm, (95% Confidence Interval)**	P-value
ΔRespiratory	3.74 (± 0.93)	3.70 (± 0.98)	0.01 (-0.09, 0.12) [a]	0.79 [a]
ΔRenal	0.38 (± 0.77)	0.38 (± 0.71)	0.01 (-0.07, 0.10) [a]	0.76 [a]
ΔHepatic	0.53 (± 0.82)	0.74 (± 0.88)	-0.22 (-0.32, -0.11) [a]	<0.001 [a]
ΔCardiovascular	1.84 (± 1.25)	1.79 (± 1.28)	0.04 (-0.11, 0.19) [a]	0.63 [a]
ΔHematolotic	1.22 (± 1.03)	1.29 (± 1.00)	-0.07 (-0.19, 0.05) [a]	0.25 [a]
ΔNeurologic [b]	0.78 (± 1.16)	0.78 (± 1.10)	0.01 (-0.13, 0.14) [a]	0.94 [a]

[a] Adjusted for baseline value
[b] Neurologic component missing for 4 in ≤10 day group and 7 in ≥21 day group.

Table S6a. Frequency of serious adverse events and other adverse events, by subject.

	Serious Adverse Events					Other Adverse Events				
	≤10 Days N = 538		≥21 Days N = 560			≤10 Days N = 538		≥21 Days N = 560		
	N	%	N	%	P-Value	N	%	N	%	P-Value
Number Of Participants With At Least One Serious Adverse Event/Other Adverse Event Of Any Type	283	53	288	51	0.72	324	60	334	60	0.85
Number Of Participants With At Least One Serious Adverse Event/Other Adverse Event Of Specified Type										
BLOOD AND LYMPHATIC SYSTEM DISORDERS	16	3	17	3	1.0	9	2	4	1	0.17
CARDIAC DISORDERS	111	21	132	24	0.25	57	11	61	11	0.92
CONGENITAL, FAMILIAL AND GENETIC DISORDERS	2	<1	2	<1	1.0	4	1	2	<1	0.44
EAR AND LABYRINTH DISORDERS	0	0	1	<1	1.0	0	0	0	0	--
ENDOCRINE DISORDERS	1	<1	0	0	0.49	0	0	0	0	--
EYE DISORDERS	1	<1	1	<1	1.0	0	0	0	0	--
GASTROINTESTINAL DISORDERS	16	3	13	2	0.57	0	0	0	0	--
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS	22	4	17	3	0.42	4	1	3	1	0.72
HEPATOBILIARY DISORDERS	29	5	51	9	0.02	78	14	99	18	0.16
IMMUNE SYSTEM DISORDERS	1	<1	0	0	0.49	2	<1	0	0	0.24
INFECTIONS AND INFESTATIONS	42	8	49	9	0.59	22	4	22	4	1.0
INJURY, POISONING AND PROCEDURAL COMPLICATIONS	29	5	41	7	0.22	13	2	18	3	0.47

Table S6a. Frequency of serious adverse events and other adverse events, by subject. (continued)

	Serious Adverse Events					Other Adverse Events				
	≤10 Days N = 538		≥21 Days N = 560			≤10 Days N = 538		≥21 Days N = 560		
	N	%	N	%	P-Value	N	%	N	%	P-Value
INVESTIGATIONS	40	7	39	7	0.82	38	7	30	5	0.26
METABOLISM AND NUTRITION DISORDERS	72	13	67	12	0.53	209	39	210	38	0.66
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS	1	<1	1	<1	1.0	0	0	0	0	--
NERVOUS SYSTEM DISORDERS	17	3	19	3	0.87	0	0	3	1	0.25
PSYCHIATRIC DISORDERS	1	<1	2	<1	1.0	0	0	0	0	
RENAL AND URINARY DISORDERS	38	7	36	6	0.72	25	5	22	4	0.66
REPRODUCTIVE SYSTEM AND BREAST DISORDERS	0	0	2	<1	0.50	1	<1	0	0	0.49
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS	106	20	109	19	0.94	53	10	53	9	0.84
SKIN AND SUBCUTANEOUS TISSUE DISORDERS	2	<1	2	<1	1.0	2	<1	1	<1	0.62
SURGICAL AND MEDICAL PROCEDURES	10	2	9	2	0.82	1	<1	2	<1	1.0
VASCULAR DISORDERS	124	23	116	21	0.38	98	18	99	18	0.88

Table S6b. Frequency of serious adverse events and other adverse events, by event

	Serious Adverse Events				Other Adverse Events			
	≤10 Days N = 841		≥21 Days N = 902		≤10 Days N = 750		≥21 Days N = 737	
	N	%	N	%	N	%	N	%
Number Of Serious Adverse Events/Other Adverse Events Of Each Type								
BLOOD AND LYMPHATIC SYSTEM DISORDERS	17	2	18	2	9	1	4	1
CARDIAC DISORDERS	161	19	179	20	66	9	64	9
CONGENITAL, FAMILIAL AND GENETIC DISORDERS	2	<1	2	<1	4	1	2	<1
EAR AND LABYRINTH DISORDERS	0	0	1	<1	0	0	0	0
ENDOCRINE DISORDERS	1	<1	0	0	0	0	0	0
EYE DISORDERS	1	<1	1	<1	0	0	0	0
GASTROINTESTINAL DISORDERS	16	2	13	1	0	0	0	0
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS	22	3	17	2	4	1	3	<1
HEPATOBILIARY DISORDERS	31	4	59	7	89	12	108	15
IMMUNE SYSTEM DISORDERS	1	<1	0	0	2	<1	0	0
INFECTIONS AND INFESTATIONS	48	6	53	6	27	4	27	4
INJURY, POISONING AND PROCEDURAL COMPLICATIONS	32	4	47	5	13	2	19	3
INVESTIGATIONS	51	6	53	6	43	6	31	4
METABOLISM AND NUTRITION DISORDERS	86	10	93	10	282	38	268	36
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS	1	<1	1	<1	0	0	0	0
NERVOUS SYSTEM DISORDERS	20	2	22	2	0	0	3	<1
PSYCHIATRIC DISORDERS	1	<1	2	<1	0	0	0	0

Table S6b. Frequency of serious adverse events and other adverse events, by event (continued)

	Serious Adverse Events				Other Adverse Events			
	≤10 Days N = 841		≥21 Days N = 902		≤10 Days N = 750		≥21 Days N = 737	
	N	%	N	%	N	%	N	%
RENAL AND URINARY DISORDERS	41	5	38	4	29	4	25	3
REPRODUCTIVE SYSTEM AND BREAST DISORDERS	0	0	2	<1	1	<1	0	0
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS	148	18	150	17	66	9	68	9
SKIN AND SUBCUTANEOUS TISSUE DISORDERS	2	<1	2	<1	2	<1	1	<1
SURGICAL AND MEDICAL PROCEDURES	10	1	11	1	1	<1	2	<1
VASCULAR DISORDERS	149	18	138	15	112	15	112	15

Figure S1. Patient Flow Diagram

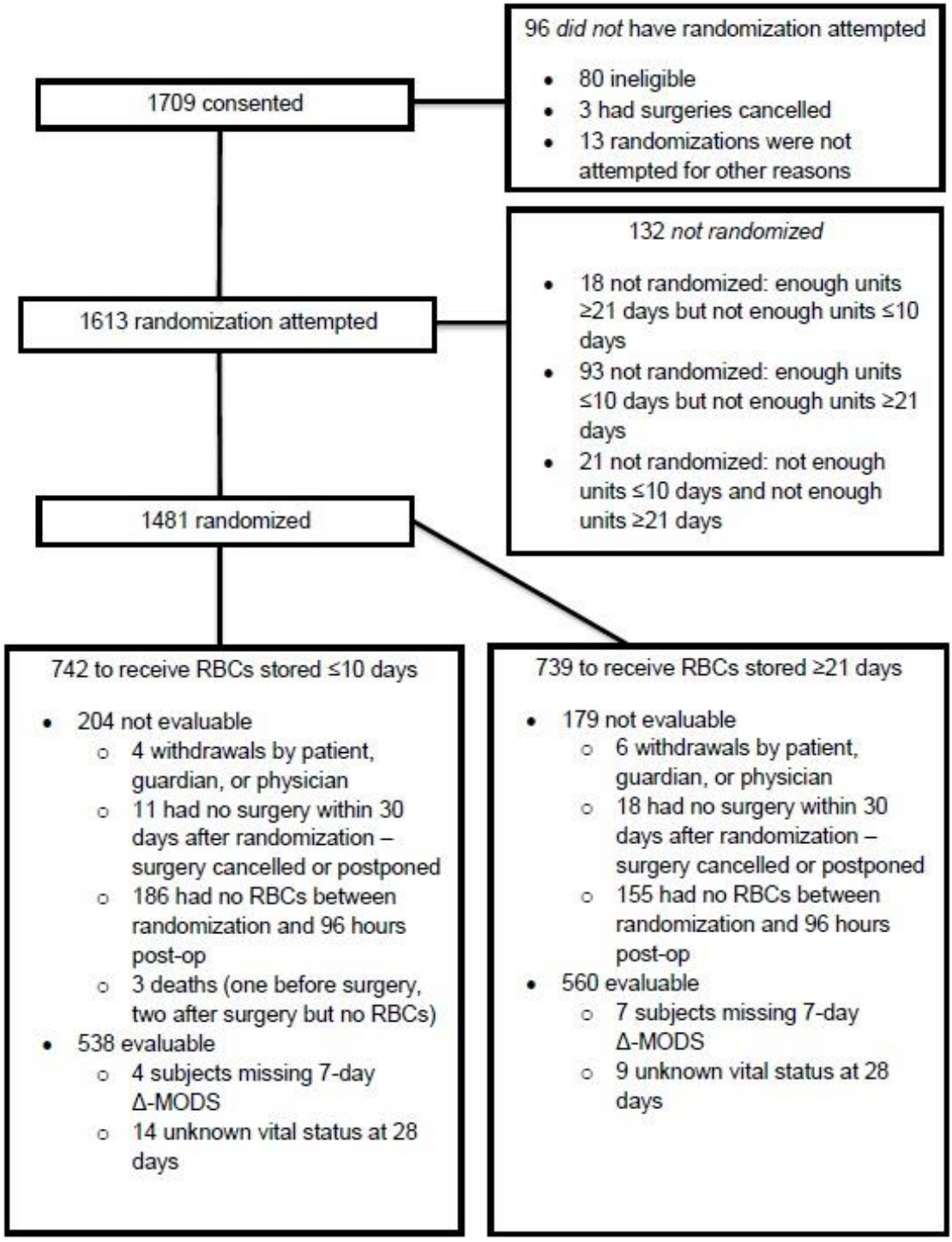
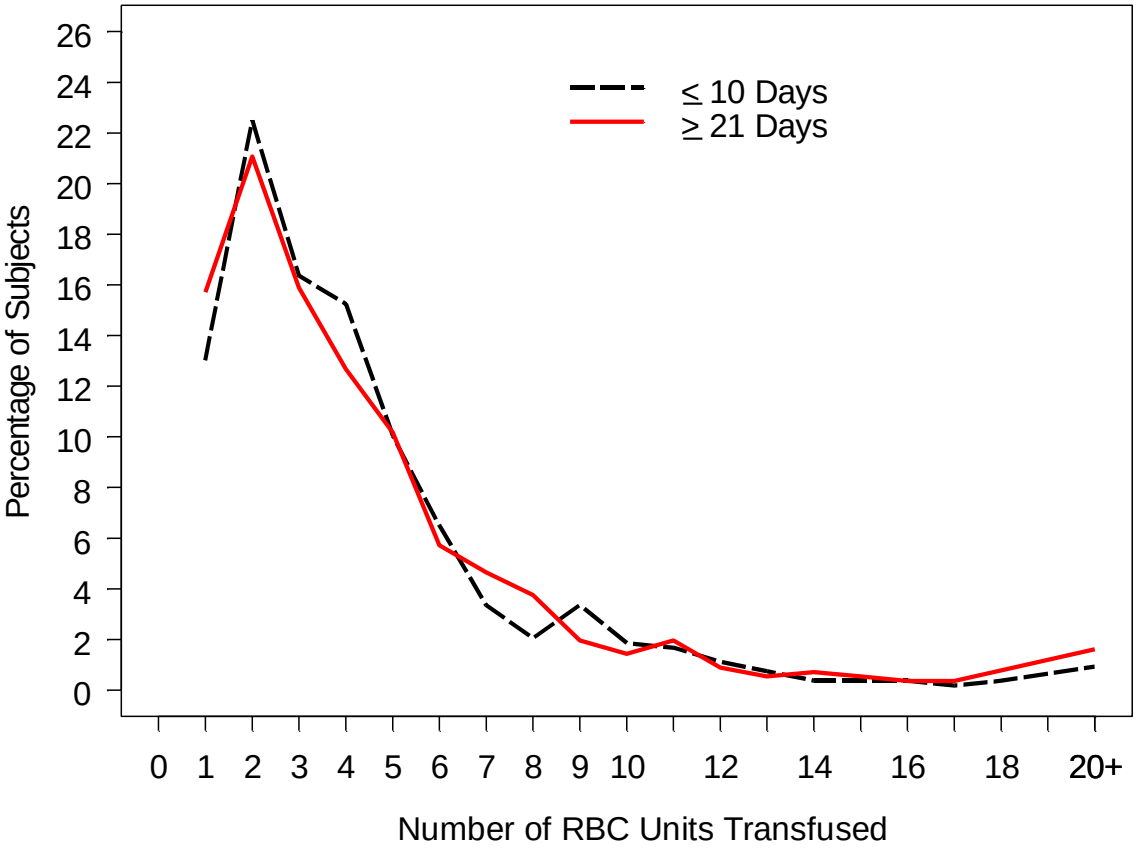


Figure S2. Number of RBC units received by subjects in each treatment group. The black line is the distribution of the number of units given to each subject in the ≤ 10 day arm and the red line is the distribution of the number of units given to each subject in the ≥ 21 day arm.

A)Through post-operative day 7, hospital discharge or death; B) Through post-operative day 28, hospital discharge or death

a.



b.

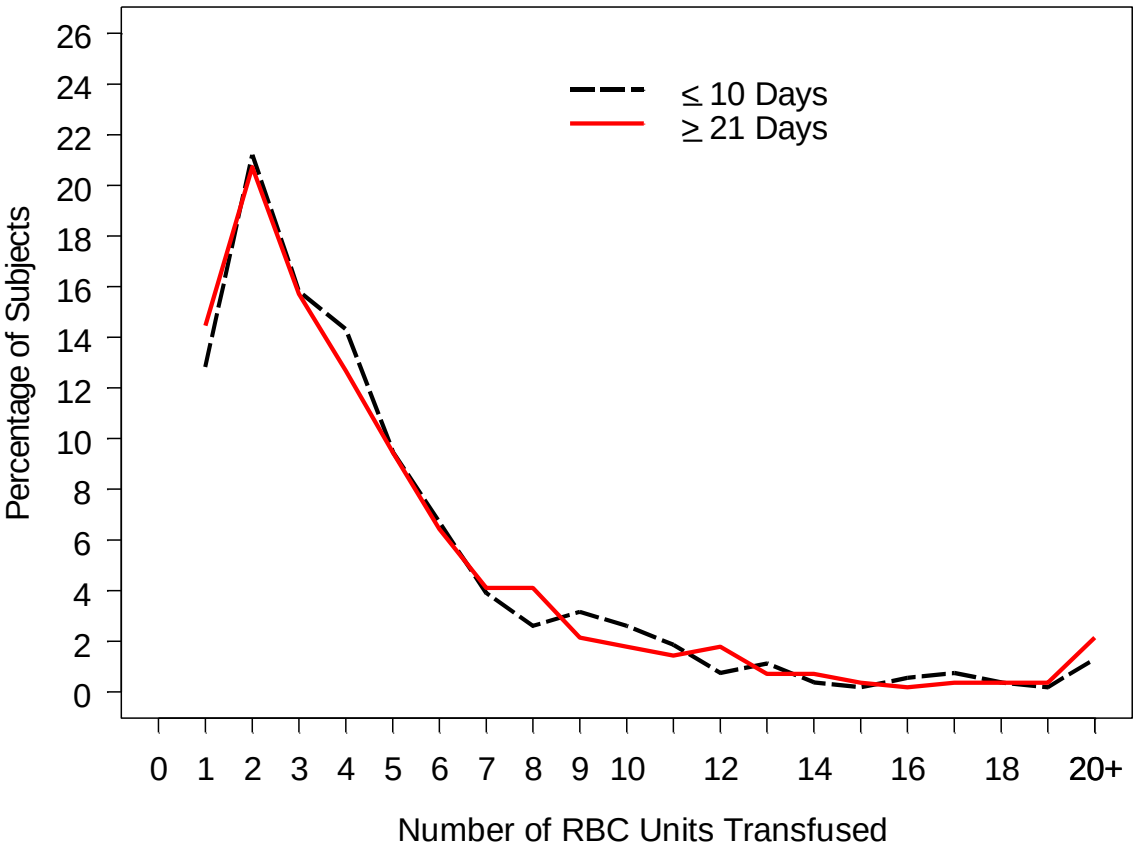
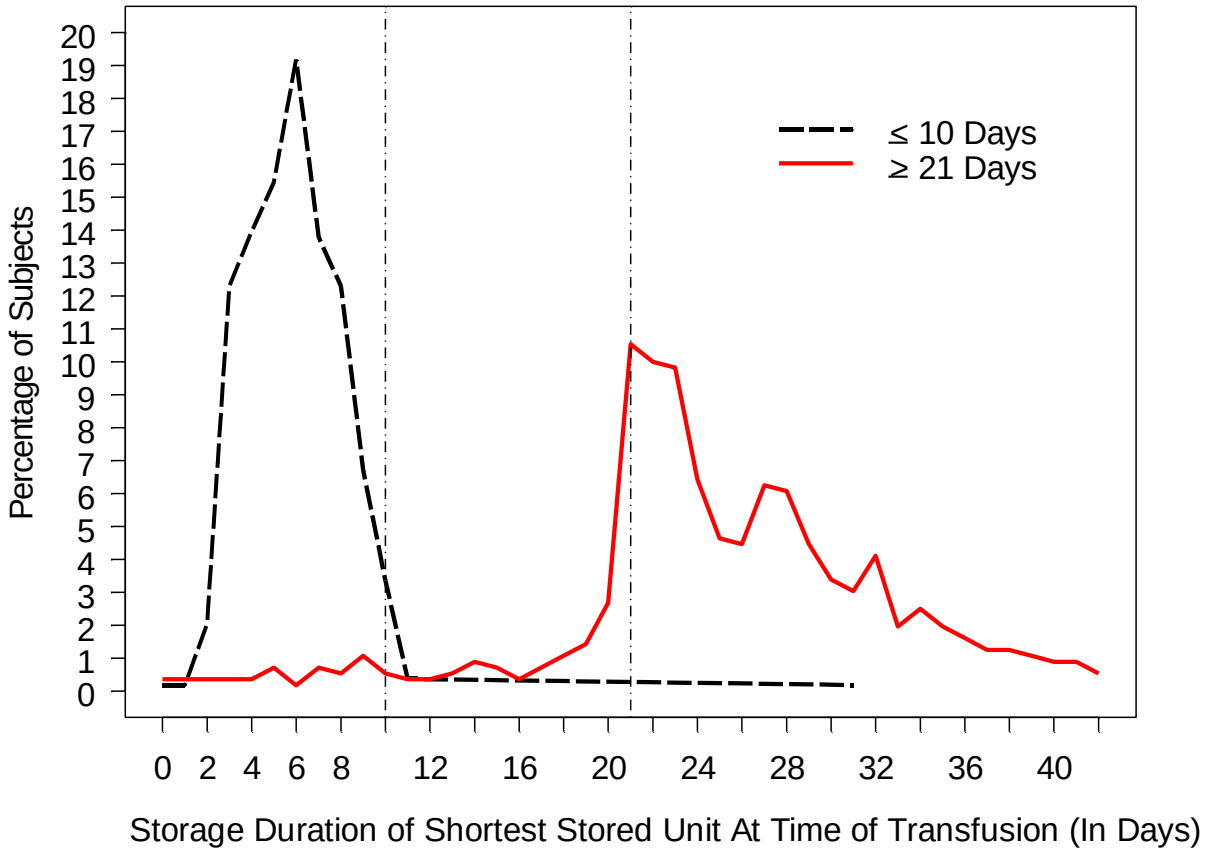

Percentage of Subjects
0
2
4
6
8
10
12
14
16
18
20
22
24
26
≤ 10 Days
≥ 21 Days
0 1 2 3 4 5 6 7 8 9 10 12 14 16 18 20+
Number of RBC Units Transfused

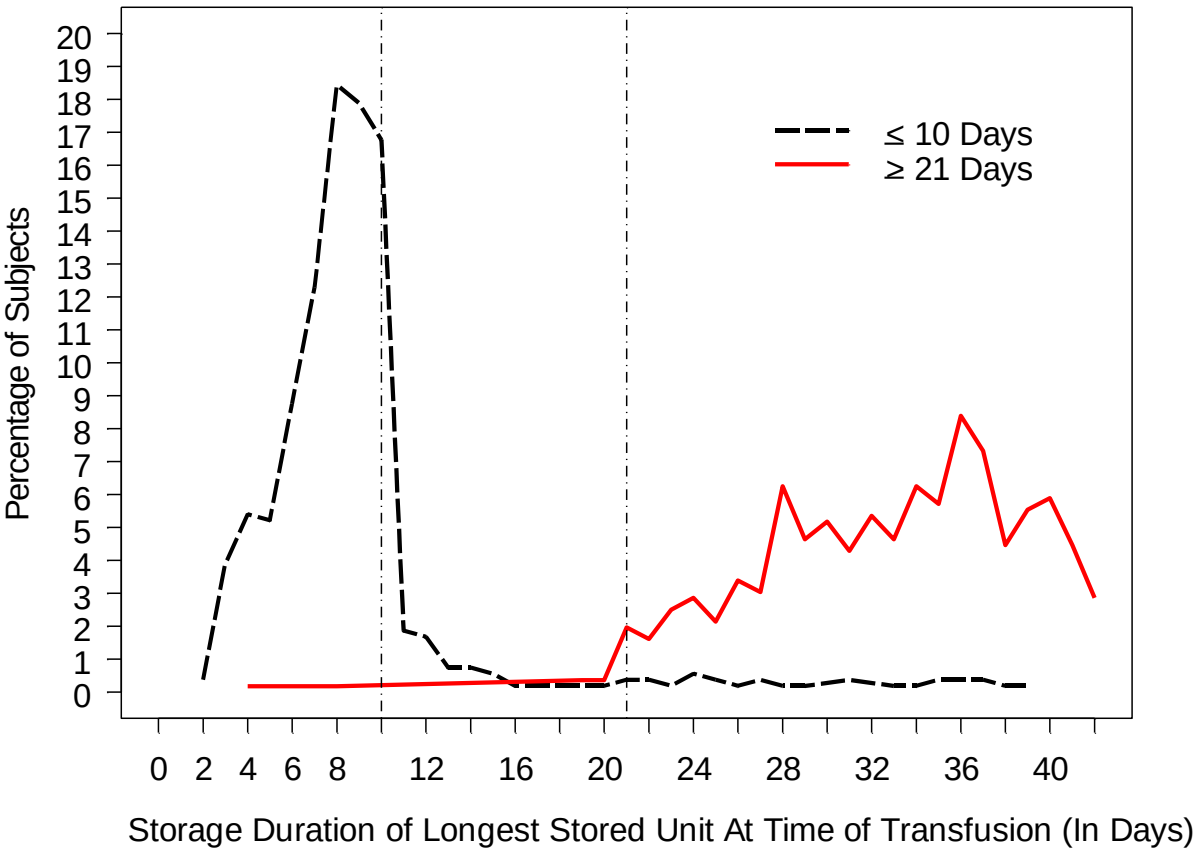
Figure S3. Distribution of RBC unit storage times on a per-patient basis. The black lines are the distributions of storage durations for units given to subjects in the ≤ 10 day arm and the red lines are the distributions of storage durations for units given to subjects in the ≥ 21 day arm. One subject is omitted from these analyses. This subject received 30 units, two of which have missing data on storage duration. Among the other 28 units, the shortest storage duration was 4 days, the longest storage duration was 37 days, and the average storage duration was 17.8 days.

a) Shortest storage duration of all RBC units received by the patient, b) Longest storage duration of all RBC units received by the patient, c) Average storage duration of all RBC units received by the patient, rounded to the nearest integer.

a)



b.


Percentage of Subjects
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
≤ 10 Days
≥ 21 Days
0
2
4
6
8
12
16
20
24
28
32
36
40
Storage Duration of Longest Stored Unit At Time of Transfusion (In Days)

c.

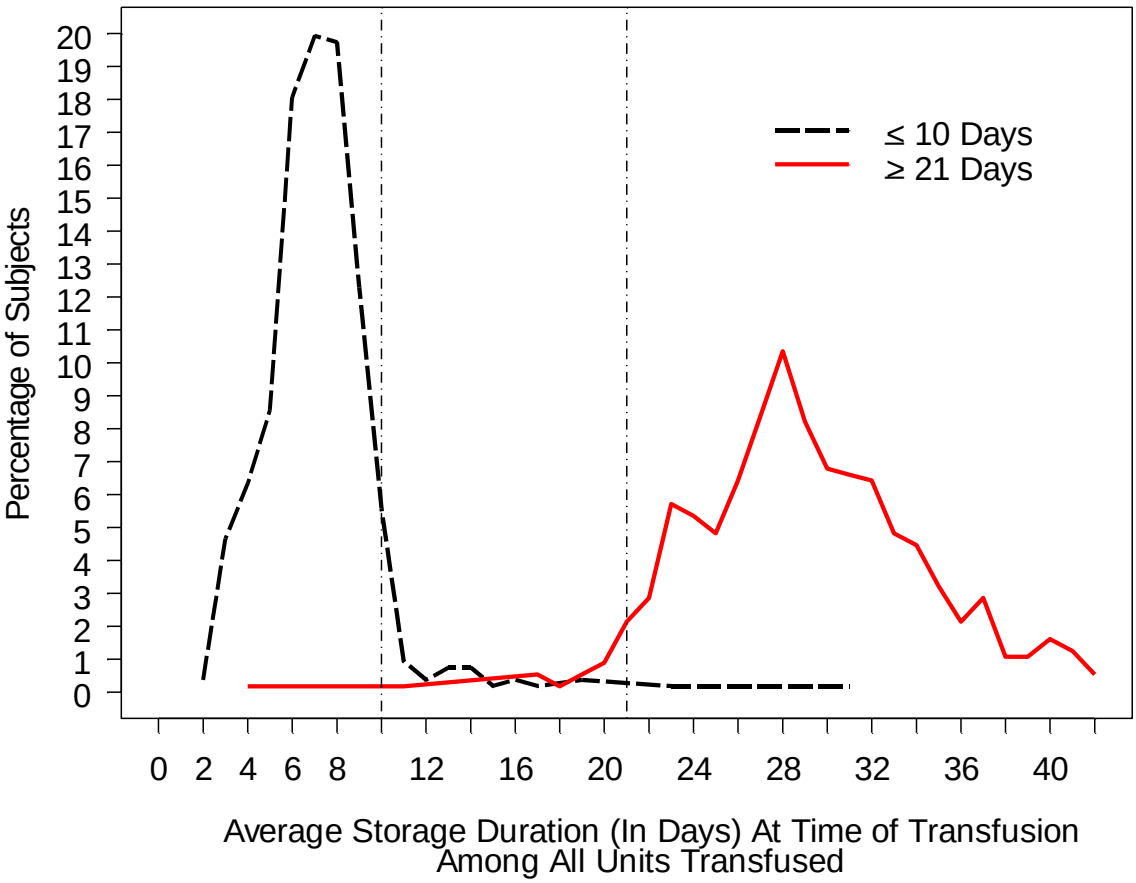
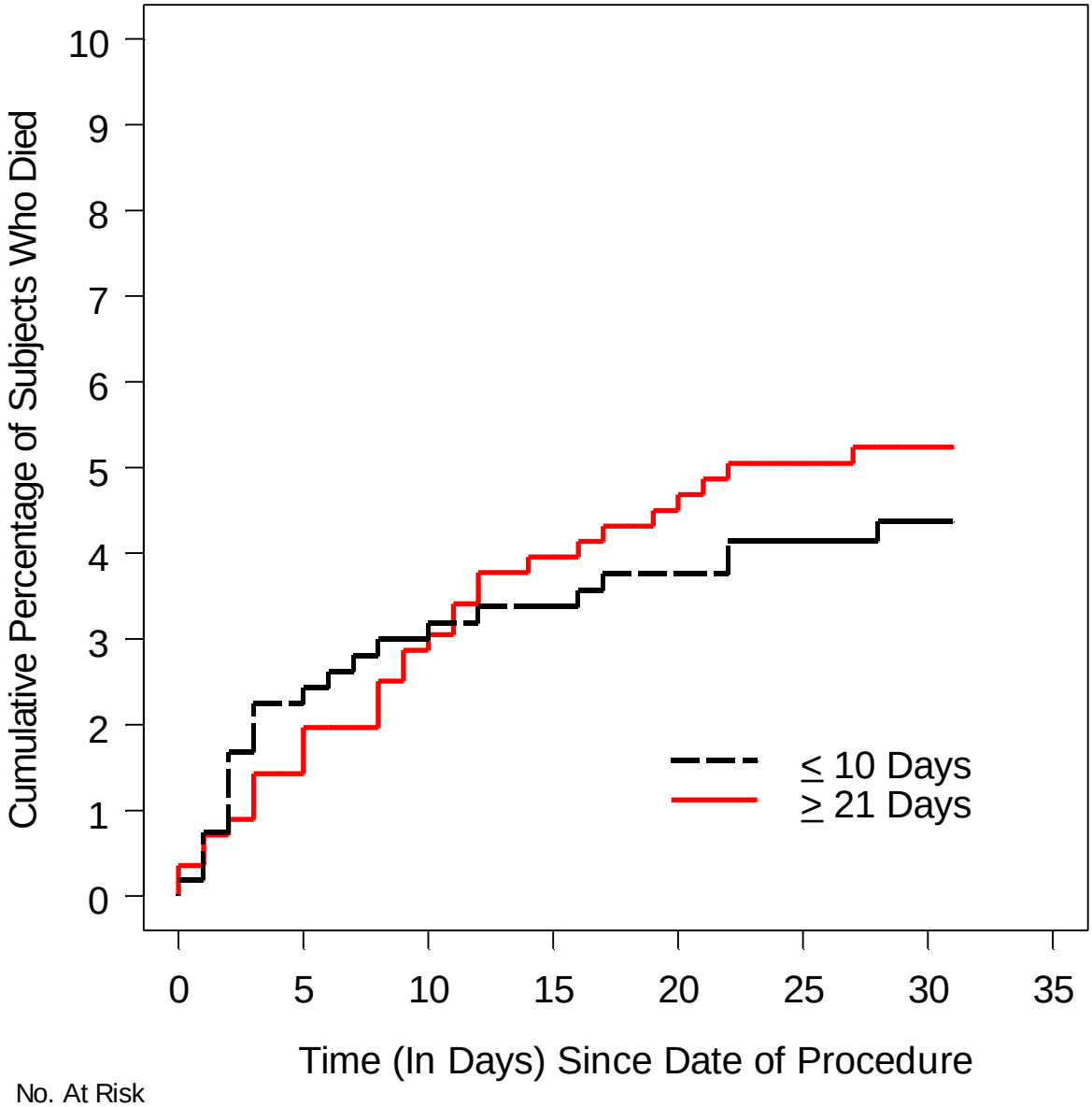

Percentage of Subjects
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
≤ 10 Days
≥ 21 Days
0
2
4
6
8
12
16
20
24
28
32
36
40
Average Storage Duration (In Days) At Time of Transfusion
Among All Units Transfused

Figure S4: Kaplan-Meier plot of time to all-cause mortality



No. At Risk								
≤ 10 Days	538	521	510	506	502	495	46	0
≥ 21 Days	560	550	537	530	524	518	48	0

References

1. Alghamdi AA, Davis A, Brister S, Corey P, Logan A. Development and validation of Transfusion Risk Understanding Scoring Tool (TRUST) to stratify cardiac surgery patients according to their blood transfusion needs. Transfusion 2006; 46:1120-1129
2. Lan K.K.G. and DeMets D.L. (1983). Discrete sequential boundaries for clinical trials. Biometrika 70;3:659-63
3. Roback JD, Grossman BJ, Harris T, Hillyer CD (editors). Technical Manual 17th edition. AABB Press, Bethesda MD, 2011, p. 192.