**Red Cell Storage Duration and Outcomes in Cardiac Surgery:**
**A Randomized Controlled Trial**

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Currently RBC units that have been stored up to 42 days are available for transfusion. Increasing RBC storage duration has been shown to alter red cells by reducing oxygen availability, creating proinflammatory and immunomodulatory effects, as well as reducing red cell deformability, and aggregability. Prior observational investigations report variable results in terms of morbid outcomes related to increasing length of RBC storage. We propose a randomized blinded clinical trial with a maximum enrollment of 2840 adult cardiac surgical patients. Consecutive consenting patients who meet the inclusion criteria will be randomized to 'age of blood' less than two weeks or greater than 20 days shelf life for all of the units allocated to the patient. The trial uses a group sequential design with three internal interim analyses that examine group sequential boundaries of both efficacy and futility. The overall type I error rate is 0.05 and power is 85%. The study we propose will provide an appropriate level of evidence for determining whether increased storage duration is associated with increased morbidity. We estimate that it will take approximately 2-3 years to complete the enrollment and randomization.

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**A. Specific Aims**

During preservation, RBC undergo functional and structural changes. What is termed 'storage lesion' is an amalgamation of reversible and irreversible rheological changes that may begin in the second week of storage, progress with storage duration [1] and lead to reduced red cell function and viability following transfusion. [2] Increasing length of storage of RBC units has been shown to reduce oxygen availability and create immunomodulatory and proinflammatory effects. [3] Depletion in 2,3-diphosphoglycerate with increased length of storage may adversely impact oxygen delivery to tissues via shifts the oxyhemoglobin dissociation curve.[4] In addition, reductions in RBC deformability and aggregability have also been noted with increasing storage time. [3] A number of observational investigations [5-10] have reported on the impact of length of storage of RBC and outcomes after transfusion of RBC. The clinical impact of storage-related changes associated with age of RBC has been inconsistent [5, 7, 9-14]. Prior investigations have been limited by small sample size [10, 15], heterogeneous patients [15] and an inability to adequately control for confounding factors related to outcomes [10]. Additionally, many of their end-points lack sensitivity to detect differences in organ function in so much as they examine broad outcomes such as survival or length of stay. [16] Although there are well-documented changes in rheologic properties with increasing length of RBC storage [1, 2, 17, 18], it is unknown whether these changes contribute to adverse patient outcome following transfusion. Our objective was to determine whether length of RBC storage duration is associated with adverse morbid outcomes in a large cohort of patients undergoing cardiac surgery. Among patients who are transfused RBC units, we will randomize units to less than two weeks and greater than 20 days storage.

- Our primary aim is to determine whether length of storage of RBC is related to postoperative morbid outcomes.

The purpose of a RBC transfusion is to increase tissue oxygenation, thereby reducing tissue oxygen debt. During storage progressive morphological and biochemical changes occur to the RBC product which may contribute to a reduction in microvascular tissue flow and contribute to tissue hypoxia.[19-23] The observed increase in complications in transfused patients could be related, in part, to older RBC units contributing to reductions in local tissue oxygenation. After RBC transfusion, an increase of hemoglobin levels is readily observed, but the effect of RBC transfusion on tissue oxygenation is rarely measured. Therefore, a quantitative evaluation of the effectiveness of stored RBC in increasing tissue oxygenation is required to assess the risk of complications related to tissue oxygenation and storage duration of the RBC units transfused.

**B. Background and Significance:**

***Effect of RBC Storage Time***

Considerable evidence suggests that transfusions increase the risk of serious complications and mortality in both cardiac surgical patients [24-31] and in critically ill patients.[32-34] But evidence also suggests that, within the transfused population, risk of complications increases when administered blood has been stored for long periods of time.[5, 6, 9, 15, 35] Blood banks, including ours, generally provide clinicians with the oldest matched units to minimize the number that expire. Under American Red Cross guidelines, packed red cells can be stored up to 42 days. It is well established that storage provokes a progressive degradation in erythrocyte shape and deformability, beginning after about two weeks.[1, 2, 17, 18] However, the clinical importance of transfusing older versus younger red cells remains unclear with some studies identifying adverse consequences [5, 6, 9, 15, 35] and others not.[12, 16, 36, 37] Raat and colleagues[19] reported that among 74,084 units of RBC transfused, more than one-third were units transfused with storage durations greater than 3 weeks. Among patients with type AB negative, up to 68.4% of units transfused were greater than 3 weeks storage duration.[19]

During preservation, RBC undergo a series of reversible and irreversible functional and structural changes. A number of investigators have demonstrated storage-induced decreases in RBC deformability that may contribute to decreased microvascular flow, local hypoxia and posttransfusion complications. [17] [1] Changes in erythrocytes with storage time include depletion of 2,3-diphosphoglycerate [4] which may result in a leftward shift of the oxyhemoglobin dissociation curve and adversely impact oxygen delivery to the tissues. Reductions in red cell deformability, adhesiveness and aggregability have also been noted with increasing storage time. [3] [22] Hemorrheological changes in stored blood may begin in the second week of storage and progress during the rest of the storage period. [1] [23] Furthermore, storage time progressively reduces levels of nitric oxide [38] and reductions in adenosine triphosphate (likely correlating with reduced post-transfusion survival). Recent work by Stamler and colleagues [21]demonstrated impaired vasodilatory response to hypoxia secondary to reductions in nitric oxide bioactivity with storage duration.[21] In an animal model, Rigamonti and colleagues [20] recently demonstrated differences in cerebral tissue oxygen delivery with fresh vs. older blood. Fresh blood demonstrated improved cerebral oxygen delivery as compared to stored blood.[20] Others [14] [1] [2] [17] have described progressive time-related alterations in erythrocyte shape and deformability beginning as early as 2 weeks duration and progressing throughout storage. Recent work by Anniss and colleagues [39] demonstrated that increased RBC storage duration was associated with an increase in the number and strength of adhesion of RBC to the vascular endothelium. Of note, the authors comment that these findings are of importance as increased adhesion of RBC to the vascular endothelium can reduce tissue oxygen delivery due to a reduction in local tissue blood flow.[39, 40]

Mynster and colleagues [6] reported transfusion of blood stored $\geq$ 21 days was associated with an increased risk for postoperative infectious complications in patients undergoing surgery for rectal cancer. Leal-Noval et al [9]examined the influence of RBC storage time on postsurgical morbidity in cardiac surgery patients. Outcomes were prolonged ICU stay, or mechanical ventilation time or perioperative MI, and sepsis. Prolonged storage was not related to an increase in morbidity but storage beyond 28 days was related to an increase in nosocomial pneumonia. [9] Purdy et al [35] reported an increased mortality associated with age of blood transfused in septic ICU patients. Positive correlation between mortality in severe sepsis and age of non-leukoreduced RBC units transfused. The median age of RBC transfused to survivors was 17 days compared with 25 days for non survivors. Others [14] [1] [2] [17] have described progressive time-related alterations in erythrocyte shape and deformability beginning as early as 2 weeks duration and progressing throughout storage. Kirkpatrick [18] and colleagues similarly reported markedly abnormal flow and biochemical properties of blood cells in stored bank blood and salvaged blood. While some of the storage related changes in RBC are reversible such as restoration of 2,3 DPG, it has been reported that that recovery of 2,3 DPG may be at 50-70% of normal at 24 hours post RBC transfusion.[41, 42]

Marik and colleagues,[13] were unable to demonstrate improved systemic oxygen uptake as measured by indirect calorimetry for up to 6 hours following RBC transfusion. In addition, they found an inverse association between the changes in gastric intramucosal pH and the age of the transfused blood for patients who received RBC stored >15 days. Furthermore, patients receiving old RBC developed evidence of splanchnic ischemia. The authors postulated that the poorly deformable transfused RBC caused microcirculatory occlusion in some organs, potentially contributing to tissue ischemia. While RBC physiology is restored with time following transfusion, the

authors could not demonstrate time-dependent changes in systemic oxygen uptake for up to 6 hours post transfusion. [13]

Zallen and colleagues reported that the age of red blood cells was an independent predictor of multiple organ failure. [5] Similarly, in a small investigation of 61 trauma patients, Offner and colleagues, [15] reported that transfusion of older blood was associated with increased infection following major traumatic injury. The authors [15] commented on the laboratory work of Silliman and colleagues [43, 44] describing the significant priming of the nicotinamide adenine dinucleotide phosphate oxidase system after 2 weeks storage, reaching a peak at the shelf life of the RBC units and thought to be primarily due to accumulation of proinflammatory lipids. [45]

There are however, investigations that do not report adverse effects of RBC storage time as reflected by similar measurements of tissue oxygenation [7, 8]. Vamvakas and colleagues [12] reported no increase in postoperative morbidity (as defined by length of intensive care unit stay, length of hospital stay and length of postoperative ventilation) with length of storage in 268 patients undergoing cardiac surgery. Walsh and colleagues [7] examined the effect of RBC storage time on regional and global indexes of tissue oxygenation in anemic critically ill patients in a randomized double-blind study in 22 patients. They reported that transfusion of RBC to euvolemic, anemic critically ill patients had no clinically significant adverse effects on gastric tonometry or global indexes of tissue oxygenation. [7] Interestingly, there were also unable to demonstrate a beneficial effect of RBC transfusion on indexes of tissue hypoxia when hemoglobin concentration was increased from 8-9 to 9-11, even when fresh RBC were transfused.[7] van de Watering and colleagues [16] did not report an impact with increasing storage time of RBC units on 30-day survival, and ICU and hospital length of stay end-points. In a small investigation, Weiskopf and colleagues [37] reported that both fresh and stored blood were similarly efficacious in reversing anemia-induced brain oxygen deficits.[37] A recent investigation by Yap and colleagues[36] reported similar clinical outcomes for RBC units of varying storage duration in 670 cardiac surgical patients. The investigation was limited due to an inability to separately analyze patients receiving exclusively fresh vs. older RBC units; the impact of patients receiving an admixture of RBC units with varying storage duration is unknown. In addition, the sample size and number of outcome events was limited.[36]

Ho et al [3] recently highlighted RBC storage duration and the relationship to the efficacy of transfusion. Their review examined the clinical evidence for benefit/harm of RBC transfusions and possible adverse effects associated with changes in RBC storage.[3] The authors concluded that clinical effects beyond post-transfusion viability are uncertain, but a growing body of evidence suggests that the storage lesions may reduce tissue oxygen availability, have proinflammatory and immunomodulatory effects and influence morbidity.

From preliminary data on approximately 6000 cardiac surgical patients in our institution, we report that increasing RBC storage duration is associated with increased risk for morbid outcomes following cardiac surgery. Hospital death, intubation morbidity, renal failure, sepsis, multisystem organ failure and composite outcome were higher in the group with increasing RBC storage duration. We are also aware of the strong and incrementally increased risk for morbid outcomes associated with each unit of RBC transfused [24-26].

### C. Preliminary Studies

Recent work[46] from our institution demonstrated a significant risk-adjusted impact of RBC transfusion on in-hospital mortality, pulmonary, cardiac, renal, infectious and neurological morbidity in over 11,963 isolated CABG patients. Transfusion was the single factor most reliably associated with increased risk for these morbid events. Figure 1 displays the unadjusted relationship between units of RBC transfused, platelet transfusion and morbidity and mortality outcomes. As the number of units of RBC transfused is increased, so is the risk for every postoperative adverse outcome. Figure 2 displays a Forrest plot of unadjusted and adjusted odds ratios and their confidence limits for the seven postoperative morbid outcomes.

**Figure 1.**

Unadjusted morbid outcomes and RBC, and platelet transfusion. (Solid lines depict patients who received platelet transfusions per unit of RBC transfused; Dashed lines represent those patients who only received RBC units).

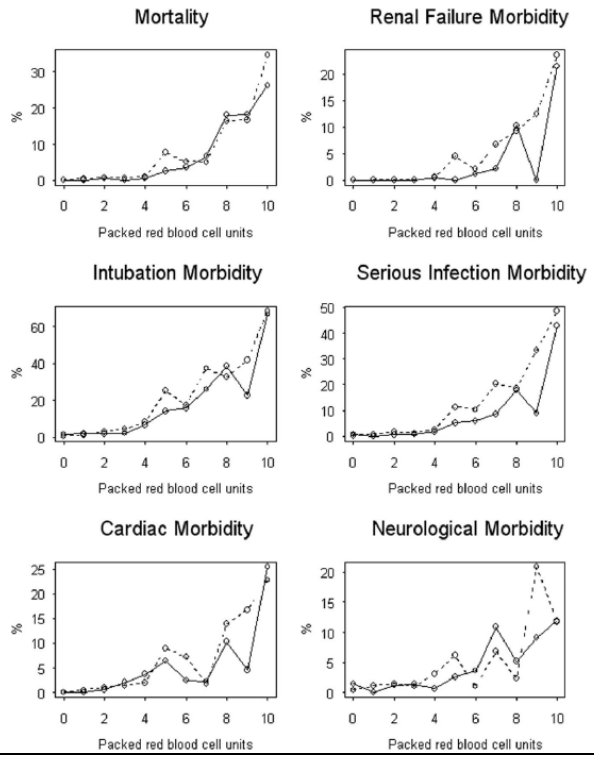
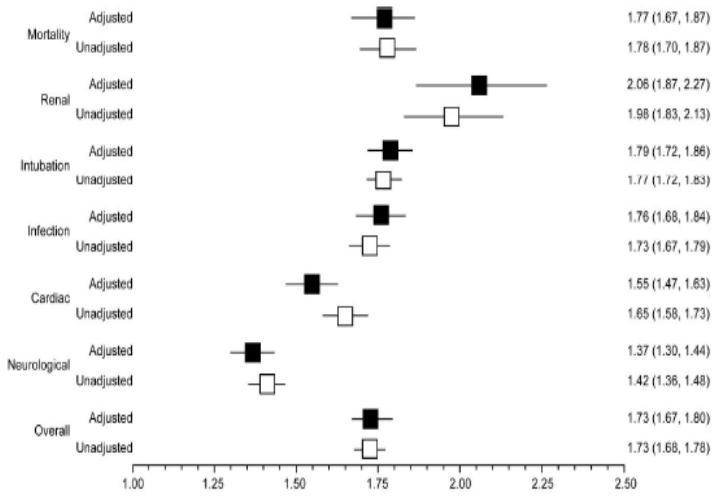



**Figure 2.**

Forrest plot displays adjusted and unadjusted odds ratios and their confidence limits for 7 postoperative morbid outcomes.

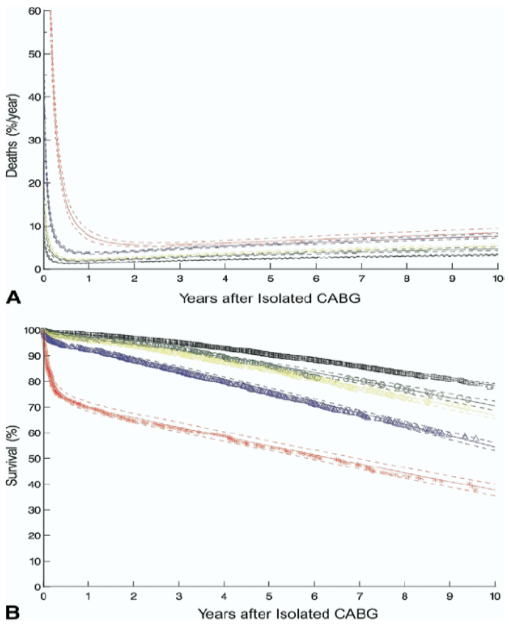



In a separate investigation [25] , we demonstrated the association between RBC transfusion and an increased early (within 6 months after surgery) and late hazard (out to 10 years) for death. We examined the incremental influence of perioperative RBC transfusion and component therapy on long-term survival after isolated coronary artery bypass grafting after controlling for the effects of demographics, comorbidity, operative factors and the early hazard for death. Approximately half of the 10,289 patients received at least 1 unit of RBC in the perioperative period. Survival among transfused patients was significantly reduced compared to those who did not receive a RBC transfusion. Figure 3. The hazard for death displayed a more influential early impact of RBC transfusion out to 6 months postoperatively. While the late hazard for death was less, the association was remained statistically significantly different from those who did not receive RBC transfusion throughout the follow-up period. Risk-adjusted reduction in survival for both early and late phases were (0.34 +/- 0.02, P <.0001) and late phases (0.074 +/- 0.016, P <0.0001).

**Figure 3.**

**(A).** Hazard function by transfusion status. The curves display the non-proportionality with an increased early risk for red blood cell (RBC) transfusion, and a reduced late risk after 6 months throughout the entire follow-up period. Note increasing units of RBC is associated with an increased hazard for death.

**(B).** Survival by transfusion for the entire follow-up period by transfusion status. Increasing units of RBC are associated with incremental decrement in survival (black, no blood transfusion; green, 1 unit RBC; yellow, 2 units RBC; blue, 3-5 units RBC and red >/= 6 units of RBC)




*Number of Patients Under Observation at Different Time Points*

| PRBC Units | 3 Months | 6 Months | 1 Year | 3 Years | 6 Years | 9 Years |
|---|---|---|---|---|---|---|
| 0 | 5,188 | 5,176 | 5,140 | 4,757 | 2,741 | 536 |
| 1 | 1,266 | 1,258 | 1,242 | 1,123 | 550 | 137 |
| 2 | 1,451 | 1,443 | 1,426 | 1,267 | 704 | 168 |
| 3–5 | 1,576 | 1,548 | 1,524 | 1,294 | 719 | 206 |
| 6+ | 498 | 471 | 449 | 377 | 233 | 88 |

Patient centered outcomes are important measures of assessing the success of an operative procedure. We examined [47] the impact of perioperative RBC transfusion in 7,321 cardiac surgical patients with the use of the Duke Activity Status Index (DASI). Functional recovery after cardiac surgery was significantly reduced in patients who received RBC transfusions perioperatively, even after adjustments for baseline DASI, preoperative clinical status, comorbidities, hematopoietic system measurements, laboratory values, operative factors, surgical procedure and postoperative morbid events. Figure 4 displays the follow-up DASI scores for those who received and did not receive perioperative RBC transfusions. Figure 5 displays the empirical probabilities of five grouped follow-up DASI groups at each RBC by platelets (yes/no) usage. As the number of RBC units increases, more patients were in the lowest DASI score group (0-34.7) and fewer achieved the highest DASI score group (58.2). The predicted probability of achieving the highest follow-up DASI by increasing age and transfusion status is shown in figure 6. The predicted probability of achieving the highest follow-up DASI category decreases with increasing age and is further decreased with the addition of a blood transfusion.

**Figure 4.**

Mirrored histogram displays nominally 6-month follow-up Duke Activity Status Index score according to whether red blood cells had been transfused. (Blue bars=no blood transfusion; red bars=red blood cell transfusion).

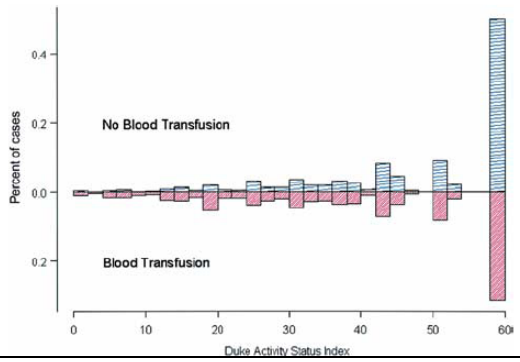


**Figure 5.**

Empirical probabilities of five grouped follow-up DASI groups at each PRBC by platelets (yes/no) usage. The solid lines denote with platelet usage; the dashed lines represent no platelet usage. Patient representation in lowest functional category (black) increases with increasing RBC units administered.

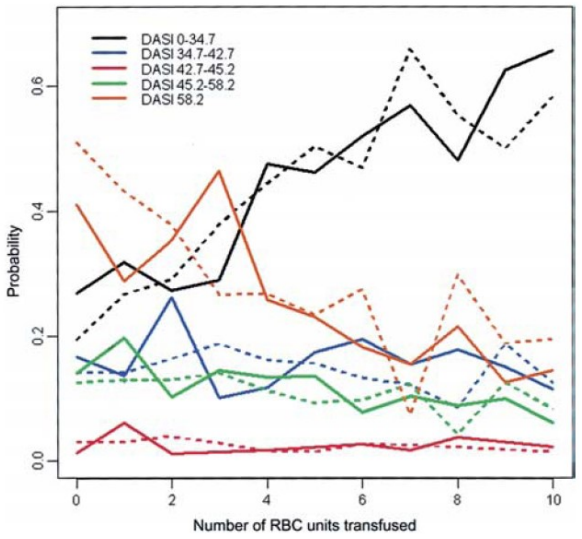
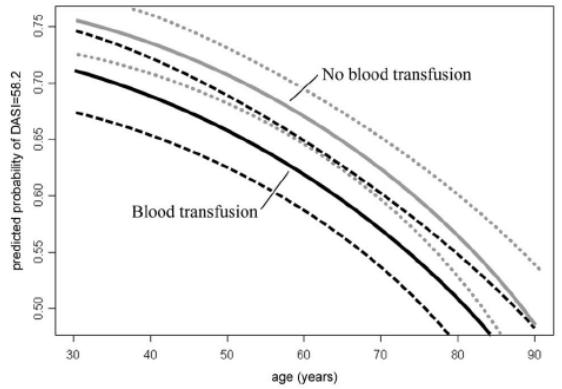


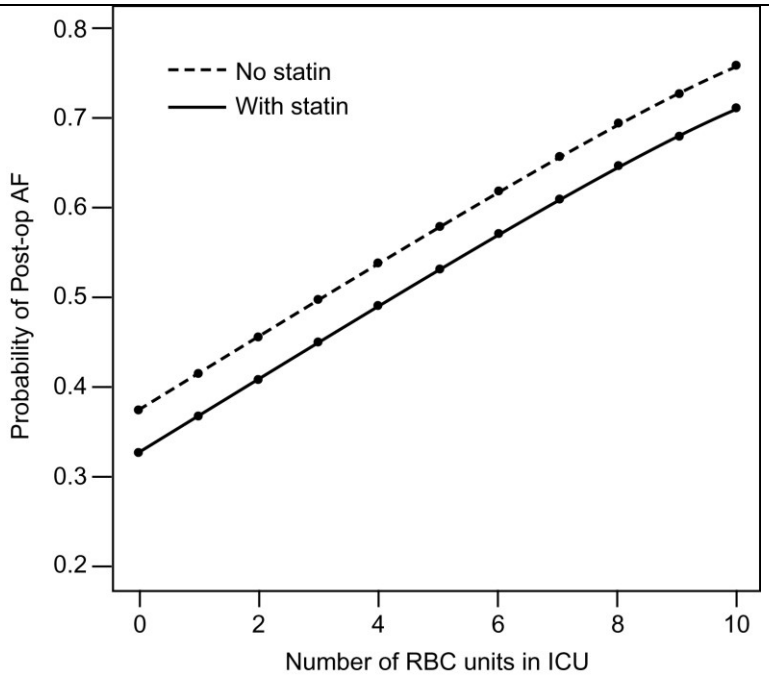

**Figure 6.**
Relationship between increasing age, recipient of RBC units (solid black line) or not (gray line) and the probability of being in the highest follow-up DASI functional category (58.2) based on a low risk patient and the equation represented in table 1 below. The dotted lines represent the 95% confidence limits.




We recently tested the hypothesis that RBC transfusion increases the risk of postoperative atrial fibrillation in cardiac surgical patients via modulating inflammation. In 5841 patients we were able to demonstrate that transfusion of RBC in the ICU was associated with a statistically significant increased risk for the development of new onset postoperative atrial fibrillation, odds ratio per unit transfused 1.18, confidence limits 1.14, 1.23, P<0.0001.[26]

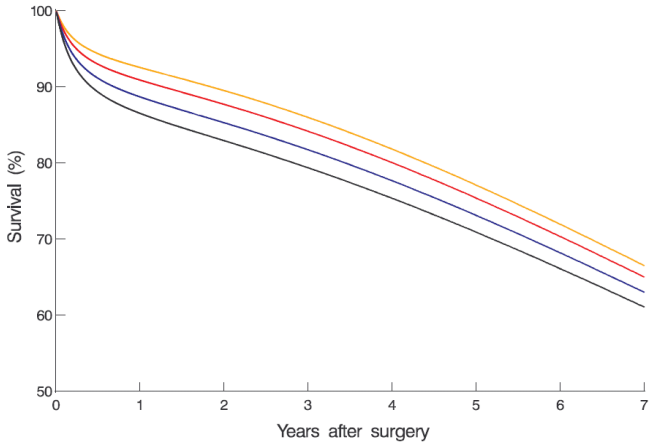
**Figure 7.**

Predicted probability for developing new onset postoperative atrial fibrillation by red blood cell units transfused and preoperative statin therapy for a low risk patient undergoing valve surgery. As the number of red cell units transfused increases, the probability of developing atrial fibrillation also increases. Preoperative statin therapy ameliorates the impact of red cell transfusion on new onset postoperative atrial fibrillation.




In addition, we have recently examined length of RBC storage duration on morbid outcomes from approximately 6000 transfused cardiac surgery patients from the Cleveland Clinic Heart Center. There were 2872 patients who received exclusively RBC units ≤14 days and 3130 patients received units that were exclusively >14 days storage duration. Morbid outcomes were prospectively collected on every patient. The blood bank was blinded to patient clinical status and specific operative procedure when assigning RBC units of particular storage duration. The allocation could be considered 'random' as is reflected by an even distribution for a majority demographics, comorbidity, and operative factors among the 2 groups. Patients whose RBC unit storage duration was >14 days verus ≤14 days had a higher incidence of in-hospital mortality: 1.7% versus 2.8%, P=0.004; intubation morbidity: 9.7% vs 5.6%, P<0.001; renal failure: 2.7% vs 1.6%, P=0.003; septicemia / sepsis: 4.0% vs 2.8%, P=0.010; multisystem organ failure: 0.73% vs 0.24%, P=0.007; and adverse composite outcome: 26% vs 22%, P=0.001, respectively. From a multivariable logistic regression model on composite adverse outcome the maximum age of RBC units was significantly related to the composite outcome as was increasing number RBC units transfused and other important demographic variables and comorbidity. Figure 8.

**Figure 8.**
Predicted survival of an intermediate-risk patient with different maximum age of blood (1 day, 15 days, 30 days, and 42 days). This nomogram displays the "dose-response" relationship between maximum age of blood and survival, adjusting for other relevant confounding factors. Maximum age of blood groups: 1 day = orange; 15 days = red; 30 days = blue and 42 days= black.



## D. Research Design and Methods:

We propose to provide an appropriate level of evidence for determining whether increasing length of storage of RBC impacts morbid outcomes following cardiac surgery. We plan to study a large and diverse population so our results will be generalizable to most adults undergoing cardiac surgery.

- Our aim is to determine whether length of red cell storage is related to adverse perioperative outcomes.

### Setting and Population

Cleveland Clinic Heart Center is a large tertiary care center and is among the busiest cardiac surgical centers in the US. Our annual volume over the last 5 years for coronary artery bypass grafting, and valve procedures has been between 3500-3790 surgeries. Transfusion of RBC currently occurs in approximately 40-50% of these patients.

#### *Inclusion Criteria*

All primary and reoperative adult cardiac surgical patients undergoing cardiopulmonary bypass for coronary artery bypass grafting, coronary artery bypass grafting with a valve procedure, isolated valve procedures and ascending aortic aneurysm or dissection repair alone or combined with CABG and valve procedures.

#### *Exclusion criteria*:

Age less than 18 years, descending thoracic aortic aneurysm repairs, left or right ventricular assist devices, and those unwilling to receive blood for religious reasons (Jehovah Witness).

#### *Protocol*

Our primary aim is to determine whether increased length of storage of RBC is related to adverse perioperative outcomes.

### Study Intervention

Consecutive consenting patients who meet the inclusion criteria will be randomized to ‘age of blood’ less than two weeks or greater than 20 days shelf life for all of the units allocated to the patient. Red blood cell and component transfusion will follow management decisions of the care team. Adherence to the treatment protocol will be required for the patients in the operating room, intensive care unit and postoperatively until discharge from the hospital. Good clinical judgment supersedes the protocol.

### Measurements

#### *Baseline Information:*

Baseline demographics, laboratory values, comorbidity, surgical procedure, operative variables, and postoperative morbid events are contained in Appendix 1. (CRF).These variables are routinely prospectively collected by the Departments of Cardiothoracic Anesthesia and Thoracic and Cardiovascular Surgery Cardiovascular Information Registries. These research registries have been approved by the institutional Internal Review Board for research purposes. Individual patient consent has been waived. Anesthesia automated record keeping system (ARKS) currently stores all hemodynamic information, intraoperative medication usage, arterial blood gas analyses, amount of crystalloid administered, time, number and identification number for RBC units transfused. Component blood product use and urine output are also automatically recorded and stored within the automated system.

**Primary Outcome**

Major and minor composite outcomes are defined by the Society of Thoracic Surgeons (STS) definitions for postoperative events www.sts.org. Major serious composite outcomes include in-hospital death, myocardial infarction, asystole, ventricular tachycardia or fibrillation, tamponade, femoral or aortic dissection, renal failure, sepsis, respiratory insufficiency, pulmonary embolism, pneumonia, cerebral vascular accident, coma, gastrointestinal morbidity, deep sternal wound infection, multisystem organ failure, acute limb ischemia, return to the operating room for any one of the following: graft occlusion, valve dysfunction, or bleeding. Two additional major outcome variables prolonged postoperative ventilation and occurrence of atrial fibrillation occur with greater frequency and will be considered separately from the other major composite outcomes. Minor composite postoperative morbid outcomes include urinary tract infection, superficial wound and leg harvest infection, complete heart block and transient stroke. These outcome measures are routinely collected as part of a data harvest for the STS database. Length of intensive care unit and hospital stay will also be examined. Appendix 1 (CRF). While considered as an expected outcome for the investigation, death and prolonged hospital length of stay will be collected as serious adverse events and reported as outlined by IRB guidelines. Other expected outcome events are not considered as SAE however will be reported at the annual renewal date.

**Statistical Analysis Plan**

This trial is a single center, two-arm parallel design with two interventions: newer blood versus older blood. Patients randomized to the newer blood group will receive blood units that have been stored less than or equal to 14 days; patients randomized to the older blood group will receive blood units that have been stored more than or equal to 20 days. The primary outcome is a binary composite outcome composed of major and minor postoperative morbid events defined by STS definitions. The secondary outcomes include prolonged postoperative ventilation and occurrence of atrial fibrillation, length of intensive care unit and length of hospital stay. These outcomes will be assessed by research assistants blinded to the treatment assignment. All the outcomes are short-term endpoints that can be ascertained within a few weeks after the operation, which makes it possible to do sequential monitoring. The randomized groups will be descriptively compared on all baseline variables using summary statistics such as mean and standard deviation, median and quartiles or frequency and percent, as appropriate. No two-sample tests on baseline variables are necessary, but those that are believed to be strongly correlated with the outcomes will be included in the covariate adjustment in the primary and secondary analyses. The level of statistical significance used in the primary analysis will be 0.05, and all tests in the primary and secondary analyses are two-tailed. The statistical analysis will be performed using SAS 9.1 (SAS Institute, Cary, NC).

If a patient meets the inclusion criteria and gives consent, he/she could potentially be randomized in the trial. Before the start of surgery, the research coordinator responsible for randomization will check the randomization list and determine that person's randomization allocation. The coordinator will then contact the blood bank and check whether there are adequate type-matched newer or older blood units available for that patient. Occasionally, there is short of supply of newer or older blood for a certain blood type. If the Blood Bank can not ensure that the patient receive only the kind of blood he/she is randomized to receive, that patient will be taken out of the trial and not randomized. We expect that this failure of randomization occurs infrequently, and it more likely occurs to patients with rare blood types. Since there is no evidence for an interaction between rare blood types and age of blood, this practice is not expected to introduce bias to the study. Another situation that may cause failure of randomization is that the patient may not need any transfusion for the entire hospital stay as determined by the surgeon. In that case the patient will also be taken out of the trial at hospital discharge. We expect that about 40-50% patients will not need any transfusion. This is a special kind of drop-out after randomization. However, since the dropping out is not related to the treatment assignment, we do not believe it will introduce bias to the study, and the two treatment arms will still be balanced.

The primary analysis is a two-sided chi-square test comparing the two treatment groups on the rate of composite outcome, based on the intent-to-treat (ITT) principal. The 95% confidence interval of the rate of

composite outcome will be calculated for the two treatment groups, and for the treatment effect which is quantified by the odds ratio.

We will test the interaction between the treatment groups and the recipients' blood type, and donors' blood type. In this way, we may identify subgroups more sensitive to the treatment. Although pre-specified, we will interpret the estimated interactions and their statistical significance with much caution due to multiplicity and they are not the primary analysis or focus. Our assessment of interactions will be viewed as exploratory and hypothesis generating.

**Sample Size Calculation and Sequential Monitoring**

The sample size calculation is based on the primary analysis, which is a Chi-squared test comparing the proportion of composite outcome between the two randomized groups. We estimate that the unadjusted odds ratio of the composite outcome between older and newer blood groups is 1.21 (25.9% vs. 22.4%). The adjusted odds ratio is 1.16 (95% confidence interval 1.01-1.33). That result was from a comparison between age of blood less than 14 days versus more than 14 days. For this trial we expect a bigger effect because we define the older group as age of blood being longer than 20 days instead of 14 days. Therefore, for this sample size calculation we assume the odds ratio is 1.3. The overall proportion of composite outcome is estimated to be roughly around 30% in an observational data set from CVIR. These two assumptions lead to the estimated composite event rate of the newer blood group being 27.3% and that of the older blood group being 32.7%. If we set the type I error to be 0.05 and power to be 0.85, the study would need to enroll 1328 transfused patients per treatment arm, for a total of 2656 patients with equal assignment. The total sample size could increase to 4810 transfused patients if the odds ratio is 1.2 (32% vs. 28%), and it becomes 1600 if the odds ratio is 1.4 (33.5% vs. 26.5%).

We plan on three interim analyses at 25%, 50%, 75% of the accrual, plus the final analysis. Our calculations of group sequential boundaries assume non-binding stopping rules and account for monitoring both the null (efficacy) and alternative hypothesis (futility). We use the gamma family spending function with gamma = -4 for efficacy and gamma = -2 for futility, which is between Pocock and O'Brien-Fleming approaches **Figure 9.** We are thus spending beta somewhat faster than the alpha during the trial, allowing for early termination if there is little treatment group difference. The stopping boundaries are shown in **Figure 10** (on the scale of Z-statistic of two-sample proportions test) and Table 1 (on the *P* value scale). The total sample size has to be modified up a bit (n=2838) in order to properly account for the interim looks. Note that this is the maximum sample size for this study. Because the group sequential design makes it possible to terminate the study early for either efficacy or futility, the expected sample size is 2004 if the null hypothesis of no difference is true and it is 2063 if the alternative hypothesis (27.3% vs. 32.7%) is true.

**Table 1. Stopping boundaries on the *P* value scale and probabilities of crossing the boundaries (H0: there is no difference in the proportions of composite outcome between the treatment groups; H1: there is a difference)**

| *P* value threshold | Interim 1 (n=708) | Interim 2 (n=1418) | Interim 3 (n=2128) | Final (n=2838) |
|---|---|---|---|---|
| To reject H0, p must be less than or equal to | 0.0016 | 0.0048 | 0.0147 | 0.044 |
| To reject H1, p must be larger than or equal to | 0.9478 | 0.7128 | 0.2424 | |
| Boundary crossing probability under H0 | 0.054 | 0.271 | 0.472 | 0.203* |
| Boundary crossing probability under H1 | 0.071 | 0.251 | 0.376 | 0.302* |

*** Probability of reaching the final analysis without early boundary crossing**

We assume stable enrollment (uniform accrual) during the trial. Based on the number of cardiac surgeries conducted at the Cleveland Clinic each year, we estimate that it may take 2-3 years to complete the enrollment. The clinical endpoint can usually be determined within 30 days after the operation. Therefore, the interim/final

analyses will be performed at six months, one year, one and half year, and two year. The probabilities of crossing (at least one) boundary at each interim looks are calculated in Table 1. The stopping rules presented here will be used for reference by the Data Safety Monitoring Board to make decisions.

In summary, we recommend that the study enroll a maximum of 2840 patients, with 1420 patients in each treatment arm in order to achieve 0.05 type I error and 0.85 power. The enrollment could take 2-3 years. The sample size and grouping sequential boundary calculations were performed using NCSS-PASS (www.NCSS.com) and East 5.0 (Cytel Inc.).

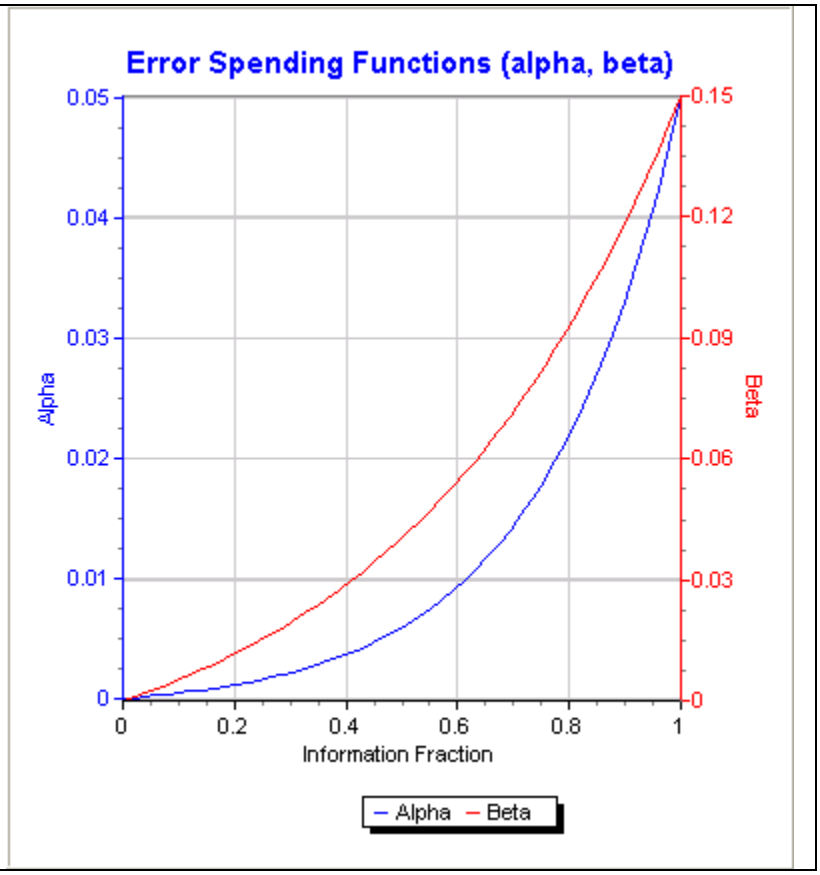
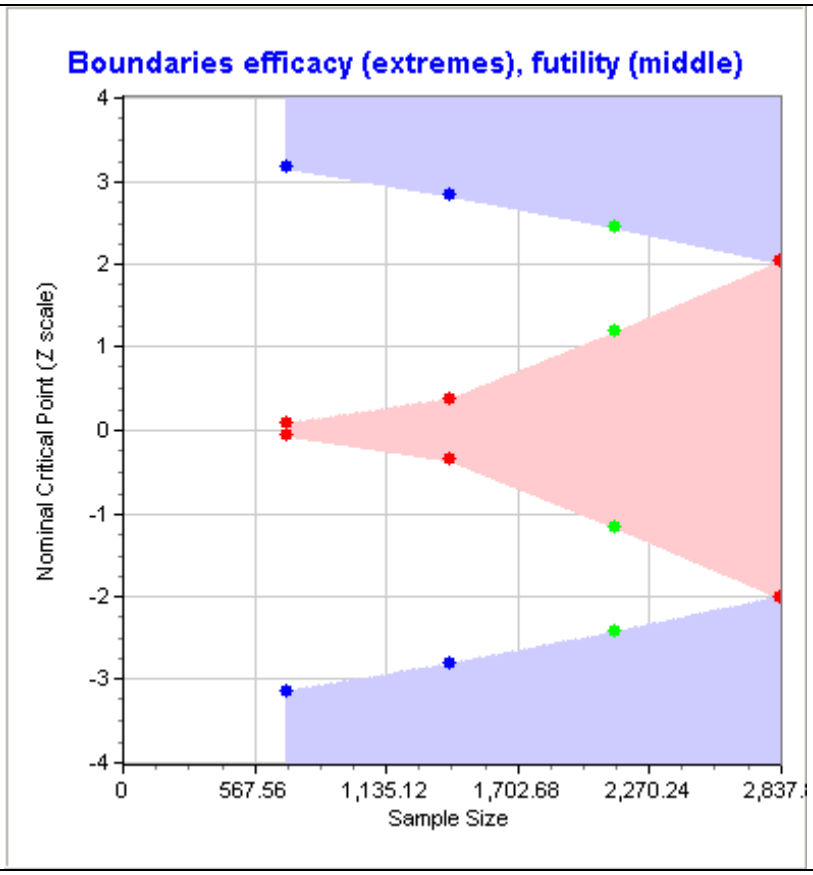



Figure 9. Alpha (blue) and beta (red) spending functions used in the calculation of group sequential boundaries. The beta (futility) is spent faster than alpha (efficacy), allowing for early termination is there is little difference between the treatment groups.

Figure 10. Grouping sequential boundaries for efficacy (extremes) and futility (middle). If the test statistic (normalized difference of two proportions) crosses the boundaries and enters into the purple or pink areas, we may terminate the trial with claim of either efficacy or futility.




***Strengths, Limitations and Significance***

**Strengths**

Cleveland Clinic Heart Center is among one of the largest cardiac surgical programs in the United States. Our procedural volume coupled with the research infrastructure at the Cleveland Clinic will allow for us to perform this study with sufficient power within a time frame of approximately 2-3 years. Our protocol will provide evidence-based medical guidelines for red cell storage duration.

**Significance**

Blood is a limited resource and should our findings indicate that older blood is detrimental, current blood inventory management strategies may need to be reconsidered. For example, 'first in, first out' (FIFO) is presently considered the responsible approach to managing a limited blood supply. The FIFO approach releases older red cell units first to prevent outdating of inventory and minimize the patient-care and financial impact of lost inventory. It may be that a 'last in, first out' (LIFO), which releases the newest red cell units first, is more feasible in terms of inventory management and patient outcome. However, to maximize use of "young" blood, mathematical modeling of blood supply, inventory, and utilization,[48] and application of modern methods of dynamic optimization of resources, so successful in the business world, may be required. [49]

***Data Management and Quality Assurance***

This investigation will be registered with **Clinical Trials.Gov** before the first patients are enrolled. An Executive Committee will be comprised of the experienced clinical trialists. The executive committee will evaluate overall results and adverse events from the proposal trial at six-month intervals, but more often if the Committee deems it necessary. It will be the responsibility of this committee to alert the IRB via letter to any

untoward toxicity in one of the study groups. This committee along with the IRB, will have authority to stop the study either because the hypotheses have been confirmed or denied, or because of adverse events are detected.

All interviewers will undergo training by our social worker, and must demonstrate a high level of proficiency before being certified to interview subjects. Procedures used to assure the integrity of data include: 1) quarterly external audits by Outcomes Research staff; 2) data entry procedures following standard operating procedures (SOPs); and, 3) data queries and resolution processes following SOPs. Frequent interaction among members of the study Executive Committee (PI, co-investigators, consultants), RAs and others as necessary, will maintain overall quality assurance. They will meet or have conference calls at least quarterly throughout the data collection period, and as necessary thereafter.

Hard-copy forms will be stored in locked cabinets within a secured area. To protect electronic records and files against loss, duplicate files will be maintained and on Division of Anesthesiology servers at the Cleveland Clinic. These servers are highly secured because they already contain much patient-related information and are backed up daily to tape which is maintained in a remote location. The system meets all applicable HIPAA Privacy and Security rules. Access to the database and backups are strictly monitored according to need.

An initial training meeting before study enrollment and frequent meetings thereafter will ensure reliability and consistency in the use of diagnostic criteria and written standard operating procedures (SOPs). All data will be independently audited to confirm consistency among patient records, study data sheets and the main database. Data will be maintained on a custom-designed Filemaker or SQL relational database. (CVIR, CTA Registry).

Upon completion of the trial and publication of the main results which will be provided to PubMed Central, the master data set will be de-identified to meet HIPAA requirements. The resulting limited data set will be published on the Outcomes Research web site (www.or.org).

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**Appendix 1. Case Report Form**

**Table 1. Baseline Continuous Variables**

| **Demographics** |
|---|
| Age at surgery (years) |
| Height (cm) |
| Weight (kg) |
| Body mass index ($kg/m^2$) |
| Body surface area ($m^2$) |
| **Preoperative Laboratory Values** |
| Hematocrit (%) |
| Bilirubin (mg/dl) |
| Albumin (g/dl) |
| Blood urea nitrogen (mg/dl) |
| Serum creatinine (mg/dl) |
| Triglycerides (mg/dl) |
| High density lipoprotein cholesterol (mg/dl) |
| Low density lipoprotein cholesterol (mg/dl) |
| Red cell mass estimate (L) |

**Table 2. Baseline Categorical Variables**

| **Preoperative Cardiac Variables** |
|---|
| Myocardial infarction |
| Cardiogenic shock |
| Intra-Aortic balloon pump |
| Emergency surgery |
| Unstable angina |
| Atrial fibrillation |
| Left ventricular ejection fraction (EF) |
| EF>60% |
| EF=50-59% |
| EF=46-49% |
| EF=41-45% |
| EF=35-40% |
| EF<35% |
| New York Heart Association functional class |
| 1 |
| 2 |
| 3 |
| 4 |
| Reoperation number 0-3 |
| 0 |
| 1 |
| 2 |
| 3 |
| ***Coronary artery disease (>70%)*** |
| Left circumflex disease |
| Left main disease |
| Left anterior descending disease |
| Right coronary artery disease |
| ***Preoperative Valve Pathology*** |
| Aortic valve regurgitation |
| Aortic valve stenosis |
| Mitral valve regurgitation |
| **Comorbidities** |
| Chronic obstructive pulmonary disease |
| Smoking |
| Hypertension |

| |
|---|
| Diabetes: insulin dependent |
| Diabetes: non-insulin dependent |
| Stroke |
| Peripheral vascular disease |
| Renal disease |
| Alcohol use |
| **Operative Factors** |
| Aortic Cross clamp time (minutes) |
| Cardiopulmonary bypass time (minutes) |
| Internal thoracic artery grafting use |
| Procedure performed |

**Table 3. Outcome Variables as defined by Cleveland Clinic CTA and CVIR**

| **Postoperative Complications** |
|---|
| Hospital death |
| **Neurological Morbidity** |
| Encephalopathy |
| Focal deficit |
| Global deficit |
| **Pulmonary Morbidity** |
| ARDS |
| Aspiration pneumonia |
| Pneumonia |
| Pulmonary edema |
| Tracheostomy |
| Pulmonary embolus |
| Plural effusion |
| Hours on ventilator in intensive care unit |
| **Renal Morbidity** |
| Renal failure |
| Anuria |
| Renal protective dopamine |
| **Infectious Morbidity** |
| Sternal wound infection |
| Mediastinitis |
| Septicemia/ sepsis |
| Endocarditis |
| **Cardiac Morbidity** |
| Low cardiac output |
| Cardiac arrest |
| Atrial fibrillation |
| Myocardial infarction |
| Intra/Postop IABP |
| Anti-arrhythmics |
| Inotropic agents |
| Vasopressors |
| **Multisystem organ failure** |
| **Reoperation for bleeding / tamponade** |

**Table 4. Outcome Variables as defined by STS data collection**

| |
|---|
| **Mortality** |
| Hospital death |
| **Neurologic Morbidity** |
| Transient stroke |
| Coma |
| Cerebral vascular accident |
| **Pulmonary Morbidity** |
| Pneumonia |
| Pulmonary embolus |
| Respiratory insufficiency |
| Prolonged ventilation |
| **Renal Morbidity** |
| Renal failure |
| **Infectious Morbidity** |
| Deep Sternal wound infection |
| Septicemia/ sepsis |
| Septicemia |
| Superficial wound infection |
| Urinary tract infection |
| Leg harvest site infection |
| **Cardiac Morbidity** |
| Atrial fibrillation |
| Myocardial infarction |
| Complete heart block |
| Ventricular tachycardia |
| Ventricular fibrillation |
| Asystole |
| **Gastrointestinal Morbidity** |
| **Multisystem organ failure** |
| Return to operating room for bleeding / tamponade |
| Reopen for graft occlusion, valve dysfunction, noncardiac reasons |
| Aortic or femoral artery dissection |
| Acute limb ischemia |