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Point-of-care washing of allogeneic red blood cells for the prevention of transfusion-related respiratory complications (WAR-PRC): a protocol for a multicenter randomized clinical trial in patients undergoing cardiac surgery

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ABSTRACT

Introduction: The transfusion-related respiratory complications, transfusion-related acute lung injury (TRALI) and transfusion-associated circulatory overload (TACO), are leading causes of transfusion-related morbidity and mortality. At present, there are no effective preventive strategies with red blood cell (RBC) transfusion. Although mechanisms remain incompletely defined, soluble biological response modifiers (BRMs) within the RBC storage solution may play an important role. Point-of-care (POC) washing of allogeneic RBCs may remove these BRMs, thereby mitigating their impact on post-transfusion respiratory complications.

Methods and analysis: This is a multicenter randomized clinical trial of standard allogeneic versus washed allogeneic RBC transfusion for adult patients undergoing cardiac surgery testing the hypothesis that POC RBC washing is feasible, safe, and efficacious and will reduce recipient immune and physiologic responses associated with transfusion-related respiratory complications. Relevant clinical outcomes will also be assessed. This investigation will enroll 170 patients at 2 hospitals in the USA. Simon's two-stage design will be used to assess the feasibility of POC RBC washing. The primary safety outcomes will be assessed using Wilcoxon Rank-Sum tests for continuous variables and Pearson chi-square test for categorical variables. Standard mixed modeling practices will be employed to test for changes in biomarkers of lung injury following transfusion. Linear regression will assess relationships between randomized group and post-transfusion physiologic measures.

Ethics and dissemination: Safety oversight will be conducted under the direction of an independent Data and Safety Monitoring Board (DSMB). Approval of the protocol was obtained by the DSMB as well as the institutional review boards at each institution prior to enrolling the

first study participant. This study aims to provide important information regarding the feasibility of POC washing of allogeneic RBCs and its potential impact on ameliorating post-transfusion respiratory complications. Additionally, it will inform the feasibility and scientific merit of pursuing a more definitive phase II/III clinical trial.

Strengths:

- Significant knowledge gap, specifically understanding whether point-of-care washing of allogeneic red blood cells (RBCs) is safe, feasible, and efficacious in ameliorating recipient immune and physiologic responses to transfusion that are associated with transfusion-related respiratory complications
- In addition to exploring immune and physiologic response, the trial is also designed to explore clinical outcomes in order to inform the merit and feasibility of future phase II/III clinical trials
- Large and accessible at-risk population
- Established multicenter clinical trial infrastructure
- Detailed and measured statistical approach
- Multidisciplinary expertise in translational, patient-centered transfusion research
- Potential for substantial clinical impact should the intervention prove safe and effective

Limitations:

- Unproven feasibility of point-of-care washing in a time-sensitive environment
- Candidate biomarkers for transfusion-related lung injury may not fully represent or capture true causal pathways
- The inflammatory response accompanying cardiac surgery may mask between-group differences in the immune and physiologic responses to transfusion therapies
- Inconsistent timing and dose of red blood cell transfusion
- Study will test the impact of modifying the RBC storage solution with POC washing, but will not clarify the impact of storage on the RBCs themselves
- Unclear effects of RBC storage duration
- Study not adequately powered for clinical outcomes

INTRODUCTION

Transfusion-related pulmonary complications, including transfusion-related acute lung injury (TRALI) and transfusion-associated circulatory overload (TACO), are the leading cause of serious transfusion-related adverse events. TRALI is the primary cause of transfusion-related death and, although seemingly less appreciated, TACO has been the second leading cause of transfusion-related death in recent years. In addition to their associated mortality, both syndromes result in substantial resource utilization and associated healthcare cost. A large proportion of patients who develop TRALI will require intensive care unit (ICU) admission and ventilator support.[1,2] Similarly, up to 21% of TACO cases have been reported as life-threatening and associated with increased lengths of ICU and hospital stays.[3-6] Although specific preventative strategies have dramatically reduced the incidence of plasma-associated TRALI (e.g., male-only plasma donation), no prevention strategies exist for red blood cell (RBC)-associated TRALI or TACO. Indeed, the lack of safe and feasible strategies that can mitigate risk of RBC-associated TRALI and TACO represent critical knowledge gaps in transfusion medicine.

While TRALI and TACO share a similar clinical phenotype of pulmonary edema and hypoxemic respiratory insufficiency, each is believed to result from distinct pathologic processes.[3,5,7-9] TRALI is believed the result of a two-hit process beginning with pulmonary endothelial activation resulting in leukocyte priming, sequestration, and activation followed by endothelial injury with inflammatory lung edema. The first insult typically relates to recipient factors (e.g., surgery, trauma, infection) and the second "hit" from the infusion of mediators in the blood component. For high-plasma volume components including plasma or apheresis platelets, this is believed most often the result of donor anti-leukocyte antibodies reacting with

recipient cognate antigens. In contrast, multiple lines of evidence suggest alternate mechanisms are at play with RBC-associated TRALI.[7,10-12] Here, the second insult is generally attributed to the infusion of soluble biological response modifiers (BRMs) residing in the RBC supernatant.

Conversely, TACO has classically been attributed to fluid overload in the setting of transfusion. However, a large proportion of reported TACO cases present after a single blood unit exposure without overt signs of systemic volume overload.[13,14] Moreover, TACO is characteristically accompanied by a marked hypertensive response that exceeds what would be expected from a volume challenge alone, suggesting the potential presence of vasoactive substances in the transfused product that may increase systemic vascular resistance.[15-17] An abrupt increase in systemic vascular resistance may result in increased cardiac filling pressures, thereby increasing risk for hydrostatic pulmonary edema. Hence, it is possible that additional and potentially synergistic pathophysiologic processes are at play in TACO. Indeed, a growing body of evidence suggests that BRMs contained within the supernatant of stored RBC (e.g., free hemoglobin, RBC microparticles) may act on vascular smooth muscle tone and contribute to TACO.[18-23]

Washing of allogeneic RBCs can remove soluble contaminants in the RBC supernatant including chemokines, biologically active lipids, cellular debris, microaggregates, and other BRMs.[24-27] Interestingly, a large investigation noted a complete absence of reported TRALI and TACO cases following transfusion of more than 28,000 units of washed allogeneic RBCs.[28] Additionally, RBC washing has been associated with decreased adverse immunologic effects in transfused trauma patients and improved survival in transfusion recipients with acute leukemia.[29,30] Although promising, washing stored RBCs has been largely discounted due to concerns related to cost and feasibility.[31] However, as there are no effective prevention strategies

for RBC-associated TRALI or TACO, and there is clear biologic plausibility for cause-effect relationships between the infusion of soluble BRMs and the development of life-threatening transfusion-related respiratory complications, further investigation is clearly warranted.

To enhance our understanding regarding the role of point-of-care allogeneic RBC-washing as a means to mitigate transfusion-related respiratory complications, the Washing of Allogeneic Red blood cells for the Prevention of Respiratory Complications (WAR-PRC) Study was developed. This is a multicenter randomized clinical trial, supported by the National Institutes of Health-National Heart, Lung and Blood Institute (NIH grant number: R01 HL121232, PIs: Drs. Kor, Welsby). The trial aims to test the feasibility, safety, and efficacy of point-of-care RBC washing using an FDA-approved autotransfusion device known as the Continuous Autotransfusion System (CATS) in adult cardiac surgery patients receiving allogeneic RBC transfusion. Cardiac surgical patients were selected as the target population given the frequency of large-volume RBC transfusion in this practice location,[32] the well-described risks of postoperative respiratory complications in this patient population,[33-36] and the presence and routine use of the cell washing strategies (auto-transfusion) in this environment. This paper describes the study procedures and planned analyses for this clinical trial.

METHODS AND ANALYSIS

Study design

To test the hypothesis that point of care (POC) allogeneic RBC-washing will be safe, feasible, and associated with amelioration of intermediate markers of TRALI and TACO, a multi-center, single-blinded (outcome assessor), parallel group, phase I/II randomized clinical trial has been designed. The ClinicalTrials.gov registration number is NCT02094118. An outline of the study design, procedures, and aims is displayed in Figure 1.

Study population

Adult patients aged 18 years and older undergoing cardiac surgery with heightened risk for large volume RBC transfusion, defined as a predicted RBC transfusion requirement of greater than or equal to 4 units, will be enrolled. To facilitate the identification of patients at high risk for RBC transfusion, a validated cardiac surgery prediction model will be utilized.[37] A cut off of 4 predicted units of RBC administration was chosen because this transfusion volume is associated with increased risk of pulmonary complications following cardiac surgery.[38] Additionally, it has been identified as a common "RBC dose" implicated in patients with TRALI and/or TACO.[8,39] This threshold will also still identify a sizable cardiac surgery population, ensuring study feasibility. A complete list of exclusion criteria including the justification for each is shown in table 1.

Table 1. Study exclusion criteria.

Exclusion criteria	Justification
Emergency surgery	Inability to randomize/perform study procedures
IgA deficiency	Not ethical to randomize to standard issue RBCs
History of severe recurrent transfusion	Not ethical to randomize to standard issue RBCs

reaction	
Refusal to receive allogeneic RBCs	Inability to administer intervention of interest
Refusal to provide informed consent	Not ethical to enroll into trial
Prevalent acute lung injury prior to randomization	Inability to adequately assess outcome
Prevalent hydrostatic pulmonary edema prior to randomization	Inability to adequately assess outcome
Expected hospital stay < 48 hours	Incomplete study procedures and outcome data
Not anticipated to survive > 48 hours	Incomplete study procedures and outcome data
Previously enrolled in this trial	Violation of the independence assumption
Pulmonary artery catheter placement not planned for the surgical procedure	Inability to assess key physiologic parameters outlined in the study protocol
Use of home oxygen therapy	Inability to assess oxygen use outcome
Complex RBC antibody profiles	Washing not feasible due to testing delays
Need for the use of irradiated RBCs	Intervention contraindicated

Patients will be recruited and enrolled at 2 academic medical centers in the USA (Mayo Clinic, Rochester, MN; Duke University Medical Center, Raleigh, NC) with substantial experience in RBC-washing and transfusion management for cardiac surgery. With regards to type of cardiac surgery, study coordinators will screen all adult patients scheduled to undergo coronary artery bypass grafting (CABG) surgery, complex cardiac valve surgery, pericardial resection, and/or ascending aortic surgery in one of the two participating institutions. Eligible patients will be approached before their elective surgical procedure by a member of the study team for informed consent. A study identification (ID) number will be assigned to each study participant and randomization will occur after receipt of informed consent, but before entry to the operating room for the scheduled procedure. Screening logs will be maintained at each site to allow generation of a CONSORT diagram.

Interventions

Study intervention: The intervention in this investigation will be implemented for all allogeneic

RBCs administered on the day of sugery, including intraoperative and postoperative transfusions. The decision to administer an allogeneic RBC transfusion will be left to the responsible clinical service and will not be prespecified in the study protocol. There are several reasons for this lack of prespecified indications for RBC transfusion. First, the target population frequently experiences major acute blood loss. During these circumstances, typical measures assessing the need for RBC transfusion, such as threshold hemoglobin or hematocrit values, do not reflect true RBC cell mass nor the need for RBC transfusion. Moreover, the process of obtaining these laboratory results may be associated with unacceptable time delays when bleeding is severe. Additionally, this design facilitates a more meaningul understanding of the feasibility of RBC washing in clinical practice.

When a clinical decision to proceed with allogeneic RBC transfusion has been made, the RBC product will be immediately prepared in the operating room (or in the ICU room if administered postoperatively) according to the allocated treatment assignment (washed versus standard issue). For patients randomized to the control group arm, all RBCs administered on the day of surgery will be standard-issue allogeneic RBCs. For patients randomized to the intervention arm of this trial, all allogeneic RBCs administered on the day of surgery will be washed with the CATS device prior to transfusion. The CATS device was chosen over more traditional cell washing machines (e.g. the Cobe 2991 Cell Processor) due to the reduced time needed for cell washing with CATS as well as the reduced risk for hemolysis with the CATS device.[40] As previously described and confirmed in our preliminary data, pre-dilution of stored, allogeneic RBCs results in the most effective elimination of supernatant.[26] Therefore, a 4:1 dilution consisting of 1200 ml saline to 300 ml RBCs will be added to the reservoir of the CATS by gravity drainage. The “high quality” wash mode option will be selected for processing.[29]

Washed RBCs will then be drained from the reinfusion reservoir into sterile transfer bags (Fenwal Inc, Lake Zurich, IL) for transfusion. A full description of the standard operating procedures for RBC washing may be found as supplemental material. Of note, all allogeneic RBC units at the two participating institutions undergo pre-storage leukocyte reduction.

Off-protocol transfusions. In the setting of cardiac surgery, it is occasionally necessary to provide allogeneic RBCs in an emergent fashion (e.g. acute, life-threatening bleeding). In this circumstance, time-delays due to study-related activities may prove unsafe. To address this potential scenario, our study protocol will allow the administration of emergency "off-protocol" allogeneic RBCs. "Off-protocol" RBC transfusions will be administered as per standard institutional practice. These RBC transfusion episodes will be specifically noted as "off-protocol" and will be summarized and analyzed to assist in assessing the feasibility of point-of-care RBC washing in patients undergoing cardiac surgery (see statistical description below). In addition, autotransfusion ("cell-saver") is frequently used in this patient population. Cell-salvage will be implemented at the discretion, and under the direction, of the clinical team. If cell-salvage is employed, the device used for this procedure will be distinct and separate from the intervention CATS device.

Co-interventions. Intraoperative care that is not directly related to this study protocol will be at the discretion of the responsible clinical team(s) (e.g., this protocol will not standardize intraoperative anesthetic care or surgical procedures). However, clinical care decisions that may affect the development of respiratory dysfunction and associated outcomes will be standardized to the greatest extent possible. To this end, the protocol specifies optimal ventilator strategies for both the operating room (OR) and ICU environments, including tidal volumes less than or equal to 8 mL/kg predicted body weight, peak inspiratory pressures less than 35 cm H_2O, and positive

end-expiratory pressures (PEEP) equal to or greater than 5 cm H_2O. Similarly, although RBC transfusion thresholds are not pre-specified in this protocol, restrictive transfusion practices will be advised in the postoperative period with a hemoglobin target greater than 8 g/dL in the absence of acute bleeding and/or ischemia. This transfusion threshold was chosen as it is the current standard of care at the two participating institutions. Standardization of best practices in at-risk patients will decrease the heterogeneity of the risk modifiers that may otherwise confound our associations of interest. Additionally, each center has adopted protocols on daily spontaneous awakening and spontaneous breathing trials to facilitate standardized weaning from ventilators following cardiac surgery. Non-intubated patients will undergo standard titration of oxygen twice daily (at 0700 and 1900, ± 2 hours). Patients saturating ≥92% on room air will not receive supplemental oxygen, unless specifically requested by the primary service. If the primary care service requests oxygen supplementation for a patient saturating ≥92% on room air, the reason for the deviation from oxygen weaning will be documented. Patients will continue to undergo evaluation for oxygen titration until liberation from oxygen therapy for 24 hours, hospital day 28, or hospital discharge, whichever comes first.

Related conditions and variables of interest: Pertinent baseline demographics and clinical characteristics such as age, sex, race, and comorbidities will be recorded. Additional variables of note will include vital signs and laboratory values that are obtained during the course of routine care, APACHE IV scores, administration of statins, ace-inhibitors, angiotensin-receptor blockers, beta-blockers, diuretics, antiplatelet agents, non-steroidal anti-inflammatory drugs, insulin, amiodarone, or steroids, blood product administration up to day 28 or hospital discharge, whichever comes first, daily fluid status, and vasopressor requirements.

Outcomes

Feasibility, Safety, and Efficacy Outcomes (Study aim 1): The primary feasibility outcome will be the number and proportion of off-protocol allogeneic RBC transfusions administered during the study intervention period (i.e. day of surgery). A secondary feasibility outcome will be the time required for the RBC washing procedures defined as the time from determination of allogeneic RBC need by the clinical team to time of delivery of the RBC unit to the clinical team. This time will be computed for all patients and all transfusions during the study intervention period in both the intervention and control cohorts.

The primary safety outcomes include the change in the RBC recipient's hemoglobin concentration from pre- to post-transfusion as well as the concentration of cell-free hemoglobin (CFH) and haptoglobin following RBC transfusion. To assess the primary safety outcomes, samples for total hemoglobin, CFH, and haptoglobin will be obtained prior to transfusion, within 30 minutes following the first RBC transfusion, as well as 6 hours (± 30 minutes) and 18 hours (±30 minutes) after the end of the first RBC transfusion for all study participants. Evidence for acute kidney injury defined according to Acute Kidney Injury Network (AKIN) criteria will be assessed throughout hospitalization as a secondary safety outcome measure.[41] If the patient remains in the hospital, safety labs will also be drawn on study day number 5.

The primary outcome evaluating the efficacy of the washing procedures will be the change in the concentration of BRMs including neutral lipids, soluble CD40 ligand (sCD40L), chemokine ligand 5/regulated on activation, normal T-cell expressed and secreted (CCL5/RANTES), RBC microparticles (RBC-MPs), and CFH in the washed RBC component from the pre- to the post-wash phase. These data will allow for calculation of CATS-related elimination rates of BRMs. Blood sampling and biomarker handling procedures have been

previously described,[42,43] and a brief overview of laboratory handling is provided as supplemental material.

Mechanistic Outcomes (Study aim 2): The concentration of multiple, validated biomarkers representing the primary pathways leading to development of lung injury will be assessed in the study participants. These pre-specified plasma biomarkers are displayed in Table 2.

Table 2. Lung injury biomarkers and exploratory potentially pathogenic biologic response modifiers.

Validated lung injury biomarkers	**Primary process evaluated**	**Supporting Evidence**
Interleukin-6	Inflammation	44-46
Interleukin-8	Inflammation	44-48
Plasma activator inhibitor-1	Dysregulated coagulation	46,47,49-51
von Willebrand Factor	Endothelial injury	52-57
sICAM-1	Endothelial injury	44,47,48,58-60
Surfactant protein D	Epithelial injury	44,48,61,62
Receptor of advanced glycation end products	Epithelial injury	48,63,64
Exploratory pathogenic BRMs	**Primary process evaluated**	**Supporting Evidence**
Neutral lipids	Lung inflammation	10,65
sCD40L	Lung inflammation	12
CCL5/RANTES	Lung inflammation	15,66,67
RBC-derived microparticles	NO scavenging	21,23,68
Cell-free hemoglobin	NO scavenging	18,22,68
N-terminal brain natriuretic peptide	Ventricular stretch/volume-overload	69-71

ICAM-1, intercellular adhesion molecule-1; BRMs, biologic response modifiers; sCD40L, soluble CD40 ligand; CCL5/RANTES, chemokine ligand 5/regulated on activation, normal T-cell expressed and secreted; NO, nitric oxide.

As study participants are expected to receive variable numbers of RBC transfusions at inconsistent times, four discrete time points have been chosen for assessment of these lung injury biomarkers. The first and second samples will be obtained from the recipient prior to transfusion and within 30 minutes following the first intervention or control RBC unit administered. For the third and fourth assessments, samples will be obtained 6 hours (± 30 minutes) and 18 hours (± 30 minutes) from the end of the first study RBC transfusion.

Secondary analyses will include detailed assessment of cardiopulmonary responses to RBC transfusions, with specific variables to be assessed shown in Table 3.

Table 3. Physiologic assessments during the study intervention period.

Respiratory Variables	**Hemodynamic Variables**
Arterial partial pressure of oxygen	Mean arterial pressure
Arterial oxygen saturation	Heart rate
Fraction of inspired oxygen	Cardiac output
Tidal volume	Right atrial pressure
Peak airway pressure	Pulmonary artery wedge pressure
Plateau airway pressure	Systemic vascular resistance*
Positive end-expiratory pressure	

*Systemic vascular resistance (SVR) will be calculated using the following equation: SVR (dyns/cm^5) = [(Mean arterial pressure – right arterial pressure)/cardiac output] x 80.

Each of these physiologic variables will be assessed and recorded immediately prior to the study RBC transfusion and again immediately after the transfusion (within 30 minutes). Standard operating procedures for these cardiopulmonary assessments will be defined prior to study onset

according to previously established recommendations.[72] These secondary outcomes will allow a more detailed assessment of the cardiopulmonary response to RBC transfusion and will be expected to provide important insight into the pathophysiology of TACO. Of note, pulmonary artery catheter placement is standard of care for this patient population at both enrolling institutions.

In an attempt to evaluate specific potential mechanistic pathways for TRALI and TACO, exploratory putative BRMs have also been selected (Study aim 2; Table 2). In the recipient, samples for the putative BRM assessments will be obtained at the same time points outlined above for the lung injury biomarker samples. To better elucidate the relationship between the dose of these soluble BRMs in the RBC components, their subsequent concentration in the recipient, and their ultimate relationship to the recipient's cardiopulmonary response to transfusion, levels of these potential putative agents will be determined in the RBC components prior to transfusion in both the washed and standard issue cohorts as well as in the transfusion recipient. As enrolled patients are expected to receive 4 or more units of allogeneic RBCs, samples will be obtained from the RBC component for all RBC units administered up to and including the fourth unit for each study participant. For those in the intervention arm, this will be a post-wash sample. Of note, there exists the potential for incomplete capture of relevant information in those who receive larger volumes of RBC transfusion. However, a four-unit cutoff represents a compromise between study feasibility and scientific validity.

Clinical Outcomes (Study aim 3): To facilitate the design and conduct of future clinical trials, we will also pursue a number of exploratory clinical outcomes, with study coordinators collecting data daily until hospital discharge or death. The primary clinical outcome will be the duration of postoperative mechanical ventilation for each study participant, determined by

subtracting the time of ICU admission from the time of endotracheal extubation. If the study participant is extubated prior to ICU admission, the duration of mechanical ventilation will be assigned as 0 hours. Recognizing the potential for early death (intraoperative or early postoperative) biasing the primary clinical outcome, the number of ventilator-free days (VFD) at postoperative day 28 will also be determined, with those who die prior to day 28 being assigned zero VFD. Participants discharged from the hospital alive prior to day 28 will be assumed to have had no additional days of mechanical ventilation following hospital discharge. Additional secondary clinical outcomes will include evaluations of hypoxemia including oxygen saturation measured by pulse oximetry (SpO_2) and the ratio of partial pressure of oxygen (PaO_2) to fraction of inspired oxygen (FiO_2), duration of oxygen supplementation, clinical diagnoses of TRALI, possible TRALI, and/or TACO, sequential organ failure assessment (SOFA) scores, and durations of ICU and hospital stay.

Sample size estimation

The sample size for this clinical trial is based on the aforementioned mechanistic outcomes (study aim 2), with estimates of the range of effect sizes for biomarkers considered in this study derived from a previous investigation.[48] Using an approximation of the standard deviation (SD) derived from the interquartile range (IQR; i.e. SD ≈ IQR/1.35), the median effect size was found to be 0.4, a magnitude of change that is considered relevant and appropriate to power this study. With equal allocation between groups, the sample size is estimated to be 78 participants per group. This assumes a type 1 error rate (alpha) = 0.10 (two-sided) and a power of 80%. Actual power is expected to be higher due to the repeated measures design. To allow for drop out and non-feasible cases, 170 total participants will be randomized with approximately 85 per

treatment arm.

Randomization and blinding

Randomization in a 1:1 fashion will occur following the acquisition of signed informed consent. Randomization to the RBC washing or control group will be conducted by the study's electronic data management system's Balance (Medidata) algorithm. This software uses dynamic minimization stratified by clinical center. The software will return a confirmation of the randomization indicating the study participant's treatment allocation status. A note will be placed into the electronic health record (EHR) identifying the patient as a study participant.

In light of the time-sensitive, point-of-care nature of the intervention, the patient, clinical team, and study team will not be blinded to the patient's treatment allocation status. Additionally, the transfusion medicine service will have unblinded, electronic access to the treatment assignment. Blinding, however, will be ensured for the physicians and laboratory personnel involved in biomarker analyses.

Statistical methods

Aim 1 of the protocol is centered on the feasibility, safety, and efficacy of POC allogeneic RBC washing. Feasibility is defined as the administration of protocol RBCs instead of off-protocol standard-issue RBCs. At the patient level, a washed arm patient is considered feasible if at least 50% of administered RBCs are washed per-protocol. Simon's optimal two-stage design will be used to determine if the protocol needs to be modified to prepare RBCs prior to the surgical procedure. The null hypothesis feasibility rate, p_0=0.75, will be tested against a one-sided alternative that feasibility is higher. In the first stage, 16 patients will be accrued in the RBC

washing arm with at least one RBC unit transfused on the operative day. If 12 or fewer patients were deemed feasible, the protocol will be modified to pre-wash RBCs (see limitations section of discussion for more details). If 13 or more are feasible, 32 additional patients in the RBC washing arm will be evaluated. The null hypothesis will be rejected if 40 or more of the 48 studied patients are considered feasible and the study will continue as originally planned. Else if 39 or fewer patients are deemed feasible, the protocol will be modified to pre-wash. This design yields a type I error rate of 0.10 and at least 90% power when the true patient feasibility rate is 0.90 or higher. The change in hemoglobin after the first transfused unit will be used as the primary safety measure. Additional safety endpoints including CFH and haptoglobin levels will be collected and analyzed at multiple time points as described previously. Wilcoxon rank sum tests will be used to compare changes in these continuous outcome measures between randomized groups. These analyses will be conducted utilizing 'as-treated' principles. Specifically, a participant who has received one or more units of CATS washed allogeneic RBC transfusion(s) on the day of surgery will be assigned and analyzed as a member of the washed cohort. Those who received allogeneic RBC transfusions on the day of surgery, none of which were washed, will be assigned to the standard-issue cohort. Categorical variables (e.g., development of acute kidney injury) will be assessed with chi-square tests. The change in concentrations of soluble BRMs pre- to post-wash (washed arm, washed RBC units only) will be assessed using paired t-tests or Wilcoxon signed-rank tests. To balance assay costs while ensuring scientific success, pre- and post-wash samples will be obtained and analyzed for the first 75 washed RBC units. If efficacy is not clearly established, pre- and post-wash samples will be obtained for an additional 75 washed units.

Aim 2 examines the changes in RBC recipient's intermediate markers of respiratory

injury or dysfunction (biomarkers) over four time points relative to the patient's first transfusion as described above. Mixed models will be fit to model the linear trajectory of these biomarkers. A model with a random slope and intercept will be considered initially, and the primary parameter of interest will be the treatment group by time interaction. For these analyses, an as-treated principle will be considered. Patients who receive at least one unit of washed cells will be included in the RBC washing group. Those not receiving RBC washed cells will be in the control group, as this aim is focused on mechanistic action and demonstrating biologic plausibility prior to formal evaluation of clinical outcomes, which would be analyzed using traditional intention to treat (ITT) considerations. Since the functional form of the changes in biomarkers over time is not known, a discrete (3 D.F. test) representation of time will also be used to gauge the linearity assumption, as well as provide a sensitivity analysis to the primary regression model. Standard mixed modeling practices will be utilized (e.g., assessment of residuals, verification of variance components, nested modeling to simply variance components and covariance patterns). This modeling scenario will be conducted for each biomarker of interest. Since prior research has noted that these outcomes are clustered, the previously described methodology by Shi et al. will be used to adjust for multiple comparisons.[73] We will also compute O'Brien's nonparametric global test statistic to provide an overall measure of treatment effect between the two treatment groups.

Due to our desire to evaluate the impact of RBC washing in a pragmatic and clinically relevant setting, effect modification by RBC storage duration will be assessed. For each patient, separately mean and maximum RBC storage duration (among transfused RBC units) will be considered as effect modifiers using interaction terms in the above models. Similarly, total number of transfused RBC units will be considered as a potential effect modifier.

Cardiopulmonary response values are measured pre- and post-transfusion for each transfused unit. Linear regression with generalized estimating equations (GEE) will assess the relationship between randomized group and change in cardiopulmonary response, accounting for the correlation of observations within individuals receiving multiple transfusions.

As a final component of aim 2, we will test the hypothesis that lower levels of putative BRMs (neutral lipids, sCD40L, CCL5, RBC-MPs, CFH) in transfused RBC components (and in the RBC recipient) will be associated reduced levels of lung injury biomarkers and an attenuated cardiopulmonary response to RBC transfusion. We will specifically quantify the relationships of the putative BRMs as measured in the post-wash bag or unwashed bag (as well as in the recipient) with measures of lung injury and cardiopulmonary response. Multiple linear regression models will test for the joint effect of randomized group and the randomized group by BRM interaction term in order to determine if the relationships of BRMs with markers of lung injury and cardiopulmonary response are co-incident (similar relationship) between study groups. Validated lung-injury associated biomarkers levels are measured at 4 time points relative to a patient's first RBC transfusion [pre-transfusion (but after the decision to transfuse is made), within 30 minutes post-transfusion, 6 hours post, and 18-hours post (all relative to first transfusion)]. Mixed models will be fit to model the linear trajectory of these biomarkers. Cardiopulmonary response is measured before and immediately after each RBC Unit transfused; linear regression using GEE will assess this relationship.

Aim 3 will utilize standard analytical measures for comparing randomized treatment groups under the ITT paradigm. Continuous outcomes will be analyzed using t-tests, or, for skewed data such as duration of mechanical ventilation, Wilcoxon rank sum tests will be used to compare groups. Binary outcomes will be analyzed using Pearson chi-square or exact tests.

Serial measurements (e.g. arterial oxygen saturation) will be analyzed using longitudinal summary statistics. Of note, this study is not powered for these intermediate clinical outcomes. Estimates of precision with confidence intervals along with the range of responses will be used to guide subsequent trial designs, including a larger phase II/III trial with clinical outcomes as the primary outcome of interest.

Consistent with early phase clinical trials, a higher level of significance than 0.05 is selected and we consider p-values less than 0.10 to be significant. This will facilitate advancement of the technique should it prove feasible with potential efficacy. Multiple testing may also increase the overall family wise error rate, so further research, particularly with clinical events, may be needed to quantify clinical efficacy of the approach. Missing data is expected to be minimal given the close surveillance provided in the surgical and ICU environments. However, missing specimens may occur in the event of patient discharge, death or administrative issues. Initial analyses will be conducted with the assumption of missing completely at random. Sensitivity analyses using multiple imputation and pattern mixture models will be used to assess the robustness of the model assumptions.

Data quality and management

Data quality and safety will be monitored by each site's principal investigator (PI). In addition, strategies to achieve a high level of protocol adherence will include: (1) refresher education sessions for study coordinators, (2) weekly checks of protocol compliance by the Mayo Clinic research coordinators, and (3) computerized identification of protocol violations in the database. Mayo Clinic has implemented an enterprise-wide Clinical Trials Management System (CTMS). CTMS is a data management infrastructure that operates in compliance with 21 Code of Federal

Regulations (CFR) Part 11 to support multicenter clinical trials and participant registries. The core of the CTMS project is the Medidata RAVE product, which will serve as the electronic data capture and randomization system for the study. The system has comprehensive audit trails, user authentication, security and disaster plans, and standardized training for users. The system provides real-time data integrity checks, maximizing data integrity while lessening the need for on-site source document verification. Protocol amendments will be fully vetted by the site's principal investigators (Mayo Clinic: Daryl J. Kor, MD; Duke University Medical Center: Ian Welsby, MBBS) prior to submission for approval by each site's IRB. The investigation's final trial dataset will be available to both sites principal investigators (Mayo Clinic: Daryl J. Kor, MD; Duke University Medical Center: Ian Welsby, MBBS). Study data will be available for secondary use by contacting the Mayo Clinic principal investigator, Dr. Daryl J. Kor, MD. Access to study data will be made available only for the subset of trial participants who have consented to the use of their study data for this purpose.

ETHICS AND DISSEMINATION

Adverse outcomes

Safety data including adverse events such as the development of TRALI, TACO, organ failure (including acute kidney injury), prolonged hospitalization, ICU admission, and mortality will be recorded. Other adverse events will be monitored by the site PI and research specialist in real time from the start of randomization to hospital discharge or death. Adverse events will be defined as "unexpected," "expected" and "serious." As our patient population is by definition "critically ill" due to their high-risk surgical procedure, it is expected that they will have a number of unrelated adverse health events during the course of their hospital stay. Therefore, we will limit the scope of our adverse event monitoring and recording to the following:

1. Serious adverse events (SAEs) will be defined as:
    - Death, believed to be related to the study procedures or a death that is unexpected considering the acuity of a patient.
    - A life-threatening experience believed to be related to the study procedures.
    - Persistent or significant disability or incapacity that is of greater frequency or severity than what would be normally expected in the perioperative course.
    - An event that jeopardizes the human subject and may require medical or surgical treatment to prevent one of the preceding outcomes and is not expected in the perioperative course.
2. Adverse events possibly related to the study procedures will be defined as:
    - Profound anemia (hemoglobin < 7 g/dL).
    - Renal failure requiring renal replacement therapy.
    - Myocardial infarction.

- Non-hemorrhagic stroke.
- Mesenteric ischemia requiring laparotomy (ischemic events secondary to anemia).
- Bloodstream infections.

Role of the data safety and monitoring board

All serious adverse events will be reported to the site institutional review board (IRB) within 24 hours of discovery followed by a more detailed written report to the IRB. The following information about adverse events will be collected: (1) the onset and resolution of the event, (2) an assessment of the severity or intensity of the event, (3) an assessment of the relationship of the event to the intervention, and (4) any action taken because of event. Reporting of SAEs to the respective IRBs will be conducted by the PI at each site. All potentially related SAEs will be reported to the data safety monitoring board (DSMB) and to NHLBI within 7 days of discovery. Additionally, a summary report will be provided to the DSMB prior to each DSMB meeting, at least every 6 months. Safety oversight will be performed by a DSMB, whose members will be independent from the study investigators. Safety endpoints consisting of expected clinical events, including death, will be assessed for all participants who are enrolled in the study on an intent-to-treat basis. Safety endpoints, as well as all serious and unexpected adverse events, will be summarized by treatment group. Trial conduct will be audited by the DSMB at least every 6 months.

Ethics approval

Prior to enrollment of the first study participant, protocol approval was obtained from the DSMB, each participating institutional IRB, and the NHLBI. Compliance of informed consent

forms with NHLBI requirements and the CFRs Title 21 Part 50 Section 50.25 was ensured. Documentation of all IRB approvals, including all finalized consent forms, have been collected and stored by the study team.

Considerations for continuation to a phase II/III clinical trial

This phase I/II clinical trial is not powered to detect subtle differences in clinical outcomes, which would be more adequately addressed in a much larger phase II/III clinical trial. Nonetheless, the clinical evaluations outlined in this protocol will provide essential preliminary data that can inform the merit and feasibility of a future phase II/III clinical trial. Moreover, if POC RBC-washing is determined not to be feasible, safe, or efficacious (aim 1), then this would provide evidence against pursuit of a larger clinical trial. Additionally, if no substantial impact is seen in the intermediate markers of respiratory injury/dysfunction (aim 2), there would be limited benefit in pursuing a larger trial.

Protocol amendments

Protocol amendments will be fully vetted by the site's principal investigators (Mayo Clinic: Daryl J. Kor, MD; Duke University Medical Center: Ian Welsby, MBBS) prior to submission for approval by each site's IRB.

Access to Data

The investigation's final trial dataset will be available to both sites principal investigators (Mayo Clinic: Daryl J. Kor, MD; Duke University Medical Center: Ian Welsby, MBBS). Study data will be available for secondary use by contacting the Mayo Clinic principal investigator, Dr.

Daryl J. Kor, MD. Access to study data will be made available only for the subset of trial participants who have consented to the use of their study data for this purpose.

Dissemination Policy

Study findings, including those of associated ancillary studies, will be disseminated to the scientific community in abstract and oral presentation formats at major national and international medical specialty meetings. All published manuscripts will be submitted to Pub Med Central in accordance with the National Institute of Health Public Access Policy.

Ancillary studies

Ancillary study proposals that complement or advance the specific proposals of this study protocol will be encouraged. Proposals will be reviewed by the Co-PIs of this protocol (Drs. Daryl Kor and Ian Welsby), both to ensure scientific merit and validity as well as ensuring consistency with the goals and conduct of the main study. Such ancillary studies may utilize data and/or samples accrued during the clinical trial or, when feasible, additional data may be collected. All statistical plans will be reviewed a priori and approved before data analysis is initiated. All presentations and manuscripts will require explicit review and approval by this investigation's Co-PIs.

Protocol funding

This study is supported by the NIH-NHLBI (Grant Number: R01 HL121232), the Mayo Clinic Critical Care and Anesthesiology and Perioperative Medicine Research Committees, as well as the Duke Clinical Anesthesia Research Endeavors (CARE). Funding and time allotment has

been provided by each of these entities to support study personnel, protocol development and data management (Medidata Rave), sample acquisition, transfusion procedures, sample and data processing and storage, and statistical support. There is no influence exerted by funding sources on the scientific conduct of the study protocol including data collection, analyses, or interpretation. Additionally, funding sources will play no role in the preparation of study results for presentation or publication.

DISCUSSION

Strategies that may decrease the incidence of RBC-associated pulmonary complications, particularly those that can be rapidly disseminated to clinical practice, remain undefined. We have presented the study protocol and data analysis plans for a phase I/II, multicenter, randomized clinical trial that seeks to test the feasibility, safety, and efficacy of POC washing of allogeneic RBCs in cardiac surgery with the goal of attenuating transfusion-related pulmonary complications. Specifically, we hypothesize that POC washing of allogeneic RBCs in cardiac surgery patients will be feasible, safe, and efficacious for the removal of soluble BRMs. Additionally, we hope to gain important mechanistic information regarding the relationship between these potentially pathogenic BRMs and intermediate markers of both TRALI (lung injury biomarkers) and TACO (cardiopulmonary physiologic indices) in transfused patients undergoing cardiovascular surgery. Finally, important clinical outcomes will also be assessed in order to provide essential information in determining the value and feasibility of a larger phase II/III clinical trial of RBC-washing for the reduction of transfusion-related pulmonary complications.

Limitations

Despite notable strengths of this study protocol including a large and accessible at-risk population, an established clinical trial infrastructure, and multidisciplinary experience and expertise in translational, patient-centered transfusion research, there are also limitations. The first relates to the feasibility of point-of-care RBC washing in a time-sensitive environment such as cardiac surgery. Though experience regarding the feasibility of washing allogeneic RBC units in this patient environment is limited, both centers have substantial experience with the

successful implementation of autotransfusion practices. This will be invaluable in ensuring the feasibility of the study protocol. In the unlikely circumstance that feasibility becomes a barrier to progress, we will perform pre-washing of two units of allogeneic RBCs at the time of OR entry for those adjudicated to the washing arm of the trial. These pre-washed RBC units would be stored in appropriate blood coolers until the time of RBC need is determined by the clinical team. All subsequent units could then be washed as described above. Of note, storage of allogeneic RBCs in blood coolers in the OR for the duration of the surgical procedure is standard of care at the two participating institutions for patients who are predicted at high-risk for RBC transfusion. It should be noted the proposed design to evaluate feasibility of real-time washing preserves the blood product supply and minimizes waste in the event RBCs are not required by the patient.

As a second limitation, our candidate biomarkers may not represent or capture true causal pathways. If promising alternative biomarkers and mechanistic pathways are identified, our stored blood samples from this investigation will be available for future analyses for all study participants providing consent for the use of their specimens for this purpose. Importantly, it is also possible that the putative agents are the RBCs themselves rather than contaminants of the RBC supernatant. Indeed, if washed RBC transfusions show no impact on recipient responses, this may in fact support a key role for the RBC itself rather than BRMs in the RBC storage supernatant. Although an unexpected finding, this would provide essential insight guiding future research on mitigating RBC-associated TRALI and TACO.

A third concern is that the inflammatory response seen in cardiac surgery may mask between-group differences in our analyses. Previous evaluations of patients undergoing cardiac surgery have identified a significant increase in IL-6 concentrations in those who receive RBC

transfusion versus those who do not.[74] However, IL-6 concentrations following cardiac surgery have been shown to remain under 200 pg/ml.[75,76] This concentration falls well below levels typically encountered with lung injury, which are frequently greater than 500 pg/mL.[46,48] Additionally, we expect an even greater separation of biomarkers evaluating specific lung-injury pathways rather than markers of general inflammation.

Another area of concern relates to RBC storage duration. Although equipoise remains, clinical data suggests the potential importance of RBC storage duration on patient-important outcomes.[77-80] Previous work has also shown clear temporal changes in the biochemical profile of stored RBC supernatant.[19,21,22,65,81,82] Recent evidence suggests that RBC storage age beyond 6 weeks results in increased extravascular hemolysis but storage age of 5 weeks or less does not.[83] Therefore, it is possible that variability in RBC storage duration may impact our results. However, we have outlined a statistical plan to address this potential concern (see statistical considerations). In addition, we hypothesize that the washing protocol will attenuate the effects of storage duration.

Finally, the study protocol is not adequately powered to fully evaluate clinical outcomes (Aim 3). Such hypotheses would more adequately be addressed in a larger phase II/III clinical trial. Nonetheless, we believe the clinical evaluations outlined in this protocol will provide essential preliminary data that can inform the merit and feasibility of a future more definitive phase II/III clinical trial.

CONCLUSION

This manuscript describes the study protocol and associated data analysis plans for a phase I/II randomized clinical trial of POC washing of allogeneic RBCs in cardiac surgery patients with the ultimate goal of attenuating transfusion-associated pulmonary complications. Inherent in this protocol is the novel repurposing of a cell-salvage device, which is widely available in surgical environments. If feasibility, safety, and efficacy are established, this could represent an innovative and cost-effective approach to improving transfusion safety with rapid clinical translation. In addition to assessing the efficacy of RBC washing for the removal of BRMs, this trial will also evaluate novel mechanisms underlying TRALI and TACO, while also assessing important clinical outcomes that may inform the development of future clinical trials.

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Contributorship Statement.

Matthew A. Warner, MD. Contributed to the study design and conduct, and writing of the manuscript.

Ian J. Welsby, MBBS. Contributed to the conception of the work, study design and conduct, acquisition and interpretation of data, and writing of the manuscript.

Philip J. Norris, MD. Contributed to the study design and conduct, acquisition and interpretation of data, and writing of the manuscript.

Christopher C. Silliman, MD, PhD. Contributed to the study design and conduct, and writing of the manuscript.

Sarah Armour, MD. Contributed to the study design and conduct, and writing of the manuscript.

Erica D. Wittwer, MD, PhD. Contributed to the study design and conduct, and writing of the manuscript.

Paula J. Santrach, MD. Contributed to the study design and conduct, and writing of the manuscript.

Laurie A. Meade, RN. Contributed to the study design and conduct, acquisition and interpretation of data, and writing of the manuscript.

Lavonne M. Liedl, RRT. Contributed to the study design and conduct, acquisition and interpretation of data, and writing of the manuscript.

Chelsea M. Nieuwenkamp, MLS(ASCP). Contributed to the study design and conduct, acquisition and interpretation of data, and writing of the manuscript.

Brian Douthit, RN. Contributed to the study design and conduct, acquisition and interpretation of data, and writing of the manuscript.

Camille M. van Buskirk, MD. Contributed to the study design and conduct, and writing of the manuscript.

Phillip J. Schulte, PhD. Contributed to the study design and conduct, acquisition and interpretation of data, and writing of the manuscript.

Rickey E. Carter, PhD. Contributed to the conception of the work, study design and conduct, acquisition and interpretation of data, and writing of the manuscript.

Daryl J. Kor, MD, MSc. Contributed to the conception of the work, study design and conduct, acquisition and interpretation of data, and writing of the manuscript.

All co-authors have provided final approval of the current manuscript version and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Competing Interests. The authors declare that they have no conflicts of interest.

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Data Sharing Statement. Study data will be available for secondary use by contacting the Mayo Clinic principal investigator, Dr. Daryl J. Kor, MD. Access to study data will be made available only for the subset of trial participants who have consented to the use of their study data for this purpose.

References

1. Vlaar AP, Binnekade JM, Prins D, et al. Risk factors and outcome of transfusion-related acute lung injury in the critically ill: a nested case-control study. Crit Care Med 2010;38:771-8.

2. Gajic O, Rana R, Winters JL, et al. Transfusion-related acute lung injury in the critically ill: prospective nested case-control study. Am J Respir Crit Care Med 2007;176:886-91.

3. Silliman CC, Fung YL, Ball JB, Khan SY. Transfusion-related acute lung injury (TRALI): current concepts and misconceptions. Blood Rev 2009;23:245-55.

4. Popovsky MA. The Emily Cooley Lecture 2009 To breathe or not to breathe-that is the question. Transfusion 2010.

5. Popovsky MA. Transfusion and the lung: circulatory overload and acute lung injury. Vox Sang 2004;87 Suppl 2:62-5.

6. Li G, Kojicic M, Reriani MK, et al. Long-term survival and quality of life after transfusion-associated pulmonary edema in critically ill medical patients. Chest 2010;137:783-9.

7. Bux J, Sachs UJ. The pathogenesis of transfusion-related acute lung injury (TRALI). Br J Haematol 2007;136:788-99.

8. Rana R, Fernandez-Perez ER, Khan SA, et al. Transfusion-related acute lung injury and pulmonary edema in critically ill patients: a retrospective study. Transfusion 2006;46:1478-83.

9. Kopko PM, Popovsky MA. Pulmonary injury from transfusion-related acute lung injury. Clin Chest Med 2004;25:105-11.

10. Silliman CC, Moore EE, Kelher MR, Khan SY, Gellar L, Elzi DJ. Identification of lipids that accumulate during the routine storage of prestorage leukoreduced red blood cells and cause acute lung injury. Transfusion 2011;51:2549-54.

11. Silliman CC. The two-event model of transfusion-related acute lung injury. Crit Care Med 2006;34:S124-31.

12. Khan SY, Kelher MR, Heal JM, et al. Soluble CD40 ligand accumulates in stored blood components, primes neutrophils through CD40, and is a potential cofactor in the development of transfusion-related acute lung injury. Blood 2006;108:2455-62.

13. Bierbaum BE, Callaghan JJ, Galante JO, Rubash HE, Tooms RE, Welch RB. An analysis of blood management in patients having a total hip or knee arthroplasty. J Bone Joint Surg Am 1999;81:2-10.

14. Popovsky MA, Audet AM, Andrzejewski C, Jr. Transfusion-associated circulatory overload in orthopedic surgery patients: a multi-institutional study. Immunohematology 1996;12:87-9.

15. Bless NM, Huber-Lang M, Guo RF, et al. Role of CC chemokines (macrophage inflammatory protein-1 beta, monocyte chemoattractant protein-1, RANTES) in acute lung injury in rats. J Immunol 2000;164:2650-9.

16. Andrzejewski C, Jr., Popovsky MA, Stec TC, et al. Hemotherapy bedside biovigilance involving vital sign values and characteristics of patients with suspected transfusion reactions associated with fluid challenges: can some cases of transfusion-associated circulatory overload have proinflammatory aspects? Transfusion 2012;52:2310-20.

17. Klein HG, Anstee DJ, eds. Mollison's Blood Transfusion in Clinical Medicine. 11 ed. Malden, MA: Oxford: Blackwell Publishing; 2006.

18. Vermeulen Windsant IC, de Wit NC, Sertorio JT, et al. Blood transfusions increase circulating plasma free hemoglobin levels and plasma nitric oxide consumption: a prospective observational pilot study. Crit Care 2012;16:R95.

19. Stapley R, Owusu BY, Brandon A, et al. Erythrocyte storage increases rates of NO and nitrite scavenging: implications for transfusion-related toxicity. Biochem J 2012;446:499-508.

20. Singel DJ, Stamler JS. Chemical physiology of blood flow regulation by red blood cells: the role of nitric oxide and S-nitrosohemoglobin. Annu Rev Physiol 2005;67:99-145.

21. Jy W, Ricci M, Shariatmadar S, Gomez-Marin O, Horstman LH, Ahn YS. Microparticles in stored red blood cells as potential mediators of transfusion complications. Transfusion 2011;51:886-93.

22. Gladwin MT, Kim-Shapiro DB. Storage lesion in banked blood due to hemolysis-dependent disruption of nitric oxide homeostasis. Curr Opin Hematol 2009;16:515-23.

23. Burnier L, Fontana P, Kwak BR, Angelillo-Scherrer A. Cell-derived microparticles in haemostasis and vascular medicine. Thromb Haemost 2009;101:439-51.

24. Westphal-Varghese B, Erren M, Westphal M, et al. Processing of stored packed red blood cells using autotransfusion devices decreases potassium and microaggregates: a prospective, randomized, single-blinded in vitro study. Transfus Med 2007;17:89-95.

25. Rosolski T, Matthey T, Frick U, Hachenberg T. Blood separation with two different autotransfusion devices: effects on blood cell quality and coagulation variables. Int J Artif Organs 1998;21:820-4.

26. Gruber M, Breu A, Frauendorf M, Seyfried T, Hansen E. Washing of banked blood by three different blood salvage devices. Transfusion 2013;53:1001-9.

27. Booke M, Fobker M, Fingerhut D, Storm M, Mortlemans Y, Van Aken H. Fat elimination during intraoperative autotransfusion: an in vitro investigation. Anesth Analg 1997;85:959-62.

28. Blumberg N, Heal JM, Gettings KF, et al. An association between decreased cardiopulmonary complications (transfusion-related acute lung injury and transfusion-associated circulatory overload) and implementation of universal leukoreduction of blood transfusions. Transfusion 2010;50:2738-44.

29. Blumberg N, Heal JM, Rowe JM. A randomized trial of washed red blood cell and platelet transfusions in adult acute leukemia [ISRCTN76536440]. BMC Blood Disord 2004;4:6.

30. Biffl WL, Moore EE, Offner PJ, Ciesla DJ, Gonzalez RJ, Silliman CC. Plasma from aged stored red blood cells delays neutrophil apoptosis and primes for cytotoxicity: abrogation by poststorage washing but not prestorage leukoreduction. J Trauma 2001;50:426-31; discussion 32.

31. Silliman CC, Moore EE, Johnson JL, Gonzalez RJ, Biffl WL. Transfusion of the injured patient: proceed with caution. Shock 2004;21:291-9.

32. The 2011 National Blood Collection and Utilization Survey. Washington, DC: Department of Health and Human Services, 2011.

33. Reeves BC, Murphy GJ. Increased mortality, morbidity, and cost associated with red blood cell transfusion after cardiac surgery. Curr Opin Cardiol 2008;23:607-12.

34. Koch CG, Li L, Sessler DI, et al. Duration of red-cell storage and complications after cardiac surgery. N Engl J Med 2008;358:1229-39.

35. Koch CG, Li L, Duncan AI, et al. Morbidity and mortality risk associated with red blood cell and blood-component transfusion in isolated coronary artery bypass grafting. Crit Care Med 2006;34:1608-16.

36. Koch C, Li L, Figueroa P, Mihaljevic T, Svensson L, Blackstone EH. Transfusion and pulmonary morbidity after cardiac surgery. Ann Thorac Surg 2009;88:1410-8.

37. Goudie R, Sterne JA, Verheyden V, Bhabra M, Ranucci M, Murphy GJ. Risk scores to facilitate preoperative prediction of transfusion and large volume blood transfusion associated with adult cardiac surgery. Br J Anaesth 2015;114:757-66.

38. Murphy GJ, Reeves BC, Rogers CA, Rizvi SI, Culliford L, Angelini GD. Increased mortality, postoperative morbidity, and cost after red blood cell transfusion in patients having cardiac surgery. Circulation 2007;116:2544-52.

39. Li G, Rachmale S, Kojicic M, et al. Incidence and transfusion risk factors for transfusion-associated circulatory overload among medical intensive care unit patients. Transfusion 2011;51:338-43.

40. O'Leary MF, Szklarski P, Klein TM, Young PP. Hemolysis of red blood cells after cell washing with different automated technologies: clinical implications in a neonatal cardiac surgery population. Transfusion 2011;51:955-60.

41. Mehta RL, Kellum JA, Shah SV, et al. Acute Kidney Injury Network: report of an initiative to improve outcomes in acute kidney injury. Critical care 2007;11:R31.

42. Bakkour S, Acker JP, Chafets DM, et al. Manufacturing method affects mitochondrial DNA release and extracellular vesicle composition in stored red blood cells. Vox Sang 2016;111:22-32.

43. Danesh A, Inglis HC, Jackman RP, et al. Exosomes from red blood cell units bind to monocytes and induce proinflammatory cytokines, boosting T-cell responses in vitro. Blood 2014;123:687-96.

44. Ware LB, Koyama T, Billheimer DD, et al. Prognostic and pathogenetic value of combining clinical and biochemical indices in patients with acute lung injury. Chest 2010;137:288-96.

45. Parsons PE, Eisner MD, Thompson BT, et al. Lower tidal volume ventilation and plasma cytokine markers of inflammation in patients with acute lung injury. Crit Care Med 2005;33:1-6; discussion 230-2.

46. Meduri GU, Headley S, Kohler G, et al. Persistent elevation of inflammatory cytokines predicts a poor outcome in ARDS. Plasma IL-1 beta and IL-6 levels are consistent and efficient predictors of outcome over time. Chest 1995;107:1062-73.

47. McClintock D, Zhuo H, Wickersham N, Matthay MA, Ware LB. Biomarkers of inflammation, coagulation and fibrinolysis predict mortality in acute lung injury. Crit Care 2008;12:R41.

48. Fremont RD, Koyama T, Calfee CS, et al. Acute lung injury in patients with traumatic injuries: utility of a panel of biomarkers for diagnosis and pathogenesis. J Trauma 2010;68:1121-7.

49. Ware LB, Matthay MA, Parsons PE, et al. Pathogenetic and prognostic significance of altered coagulation and fibrinolysis in acute lung injury/acute respiratory distress syndrome. Crit Care Med 2007;35:1821-8.

50. Prabhakaran P, Ware LB, White KE, Cross MT, Matthay MA, Olman MA. Elevated levels of plasminogen activator inhibitor-1 in pulmonary edema fluid are associated with mortality in acute lung injury. Am J Physiol Lung Cell Mol Physiol 2003;285:L20-8.

51. Christie JD, Robinson N, Ware LB, et al. Association of protein C and type 1 plasminogen activator inhibitor with primary graft dysfunction. Am J Respir Crit Care Med 2007;175:69-74.

52. Ware LB, Koyama T, Billheimer DD, et al. Prognostic and pathogenetic value of combining clinical and biochemical indices in patients with acute lung injury. Chest 2010;137:288-96.

53. Fremont RD, Koyama T, Calfee CS, et al. Acute lung injury in patients with traumatic injuries: utility of a panel of biomarkers for diagnosis and pathogenesis. J Trauma 2010;68:1121-7.

54. Ware LB, Eisner MD, Thompson BT, Parsons PE, Matthay MA. Significance of von Willebrand factor in septic and nonseptic patients with acute lung injury. American journal of respiratory and critical care medicine 2004;170:766-72.

55. Ware LB, Conner ER, Matthay MA. von Willebrand factor antigen is an independent marker of poor outcome in patients with early acute lung injury. Crit Care Med 2001;29:2325-31.

56. Rubin DB, Wiener-Kronish JP, Murray JF, et al. Elevated von Willebrand factor antigen is an early plasma predictor of acute lung injury in nonpulmonary sepsis syndrome. J Clin Invest 1990;86:474-80.

57. Moss M, Ackerson L, Gillespie MK, Moore FA, Moore EE, Parsons PE. von Willebrand factor antigen levels are not predictive for the adult respiratory distress syndrome. American journal of respiratory and critical care medicine 1995;151:15-20.

58. Calfee CS, Eisner MD, Parsons PE, et al. Soluble intercellular adhesion molecule-1 and clinical outcomes in patients with acute lung injury. Intensive Care Med 2009;35:248-57.

59. Covarrubias M, Ware LB, Kawut SM, et al. Plasma intercellular adhesion molecule-1 and von Willebrand factor in primary graft dysfunction after lung transplantation. Am J Transplant 2007;7:2573-8.

60. Agouridakis P, Kyriakou D, Alexandrakis MG, et al. The predictive role of serum and bronchoalveolar lavage cytokines and adhesion molecules for acute respiratory distress syndrome development and outcome. Respir Res 2002;3:25.

61. Eisner MD, Parsons P, Matthay MA, Ware L, Greene K. Plasma surfactant protein levels and clinical outcomes in patients with acute lung injury. Thorax 2003;58:983-8.

62. Greene KE, Wright JR, Steinberg KP, et al. Serial changes in surfactant-associated proteins in lung and serum before and after onset of ARDS. American journal of respiratory and critical care medicine 1999;160:1843-50.

63. Christie JD, Shah CV, Kawut SM, et al. Plasma levels of receptor for advanced glycation end products, blood transfusion, and risk of primary graft dysfunction. Am J Respir Crit Care Med 2009;180:1010-5.

64. Calfee CS, Ware LB, Eisner MD, et al. Plasma receptor for advanced glycation end products and clinical outcomes in acute lung injury. Thorax 2008;63:1083-9.

65. Silliman CC, Clay KL, Thurman GW, Johnson CA, Ambruso DR. Partial characterization of lipids that develop during the routine storage of blood and prime the neutrophil NADPH oxidase. J Lab Clin Med 1994;124:684-94.

66. Grommes J, Alard JE, Drechsler M, et al. Disruption of platelet-derived chemokine heteromers prevents neutrophil extravasation in acute lung injury. Am J Respir Crit Care Med 2012;185:628-36.

67. Conti P, DiGioacchino M. MCP-1 and RANTES are mediators of acute and chronic inflammation. Allergy Asthma Proc 2001;22:133-7.

68. Donadee C, Raat NJ, Kanias T, et al. Nitric oxide scavenging by red blood cell microparticles and cell-free hemoglobin as a mechanism for the red cell storage lesion. Circulation 2011;124:465-76.

69. Li G, Daniels CE, Kojicic M, et al. The accuracy of natriuretic peptides (brain natriuretic peptide and N-terminal pro-brain natriuretic) in the differentiation between transfusion-related acute lung injury and transfusion-related circulatory overload in the critically ill. Transfusion 2009;49:13-20.

70. Tobian AA, Sokoll LJ, Tisch DJ, Ness PM, Shan H. N-terminal pro-brain natriuretic peptide is a useful diagnostic marker for transfusion-associated circulatory overload. Transfusion 2008;48:1143-50.

71. Zhou L, Giacherio D, Cooling L, Davenport RD. Use of B-natriuretic peptide as a diagnostic marker in the differential diagnosis of transfusion-associated circulatory overload. Transfusion 2005;45:1056-63.

72. Bridges EJ, Woods SL. Pulmonary artery pressure measurement: state of the art. Heart Lung 1993;22:99-111.

73. Shi Q, Pavey ES, Carter RE. Bonferroni-based correction factor for multiple, correlated endpoints. Pharmaceutical statistics;11:300-9.

74. Fransen E, Maessen J, Dentener M, Senden N, Buurman W. Impact of blood transfusions on inflammatory mediator release in patients undergoing cardiac surgery. Chest 1999;116:1233-9.

75. Hirai S. Systemic inflammatory response syndrome after cardiac surgery under cardiopulmonary bypass. Ann Thorac Cardiovasc Surg 2003;9:365-70.

76. Allan CK, Newburger JW, McGrath E, et al. The relationship between inflammatory activation and clinical outcome after infant cardiopulmonary bypass. Anesth Analg 2010;111:1244-51.

77. Koch CG, Li L, Sessler DI, et al. Duration of red-cell storage and complications after cardiac surgery. The New England journal of medicine 2008;358:1229-39.

78. Eikelboom JW, Cook RJ, Liu Y, Heddle NM. Duration of red cell storage before transfusion and in-hospital mortality. American heart journal 2010;159:737-43.e1.

79. Manlhiot C, McCrindle BW, Menjak IB, et al. Longer blood storage is associated with suboptimal outcomes in high-risk pediatric cardiac surgery. The Annals of thoracic surgery 2012;93:1563-9.

80. Basran S, Frumento RJ, Cohen A, et al. The association between duration of storage of transfused red blood cells and morbidity and mortality after reoperative cardiac surgery. Anesthesia and analgesia 2006;103:15-20, table of contents.

81. Kristiansson M, Soop M, Saraste L, Sundqvist KG. Cytokines in stored red blood cell concentrates: promoters of systemic inflammation and simulators of acute transfusion reactions? Acta Anaesthesiol Scand 1996;40:496-501.

82. Nielsen HJ, Reimert CM, Pedersen AN, et al. Time-dependent, spontaneous release of white cell- and platelet-derived bioactive substances from stored human blood. Transfusion 1996;36:960-5.

83. Rapido F, Brittenham GM, Bandyopadhyay S, et al. Prolonged red cell storage before transfusion increases extravascular hemolysis. J Clin Invest 2017;127:375-82.

Figure Legends

Figure 1. Schematic of the planned study procedures.

ALI – acute lung injury; CATS - Continuous Autotransfusion System; CCL5, chemokine ligand 5; CFH – cell free hemoglobin; CHF – congestive heart failure; FiO2 – fraction of inspired oxygen; Hb – hemoglobin; MAP – mean arterial pressure; PAI-1 – plasminogen activator inhibitor 1; PaO2 – arterial partial pressure of oxygen; PCWP – pulmonary capillary wedge pressure; PEEP – positive end expiratory pressure; PO – postoperative; POD – postoperative day; RAGE – receptor of advanced glycation end-products; RBC – red blood cell; RBC-MP – red blood cell microparticle; Rxs – reactions; sCD40L – soluble CD40 ligand; SOFA – sequential organ failure assessment; SpO2 – oxygen saturation by pulse oximetry; SVR – systemic vascular resistance; TACO – transfusion-associated circulatory overload; TRALI – transfusion-related acute lung injury; Trx – transfusion.

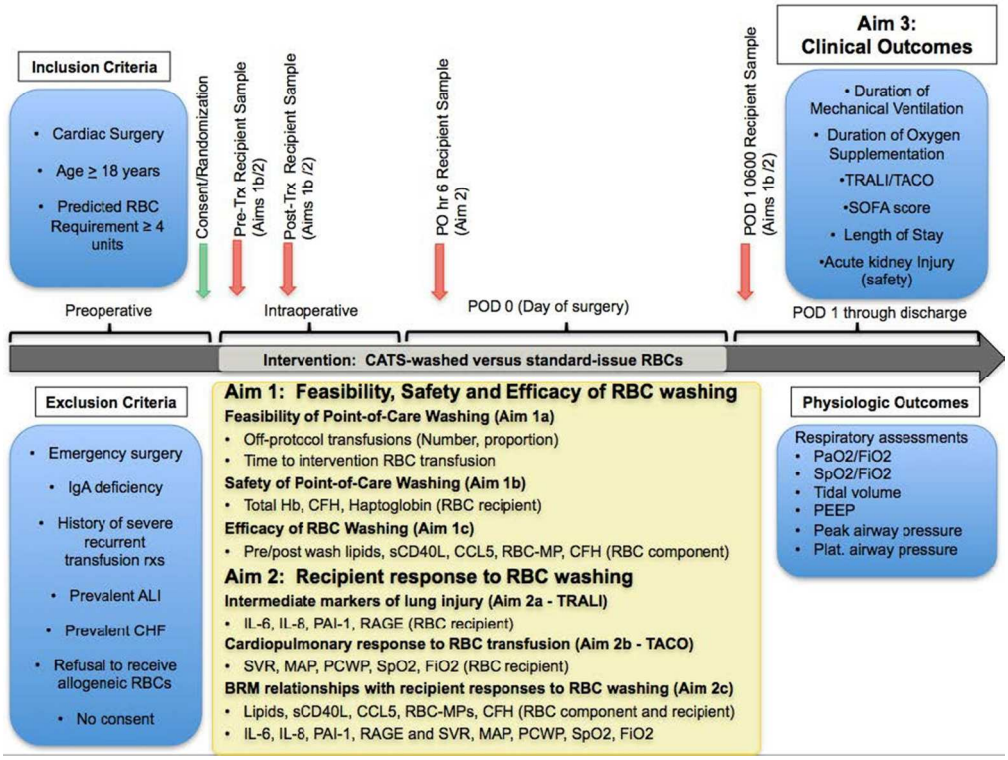

Figure 1: Schematic of the planned study procedures.

172x130mm (141 x 141 DPI)

Supplemental Materials:

Laboratory sample and biomarker handling.

Aim 1b: 8.5 ml of blood will be drawn from each study participant prior to transfusion, within 30 minutes following the first RBC transfusion, as well as 6 hours (± 30 minutes) and 18 hours (±30 minutes) after the end of the first RBC transfusion for all study participants. If the patient remains in the hospital, safety labs will also be drawn on study day number 5. These safety laboratory assessments (total hemoglobin, CFH, haptoglobin) will be analyzed locally at the enrolling sites using standard clinical assays.

Aim 1c: A 6 ml sample will be taken pre- and post-wash from the already anticoagulated intervention RBC units. An additional aliquot of the RBC unit will be sealed in a capillary tube, centrifuged at 2000 g, and expressed as a decimal fraction using a micro-hematocrit reader. A single 6 ml sample will also be drawn from the standard-issue RBC units prior to administration.

Aim 2: 10 ml of blood will be drawn from each study participant at baseline prior to transfusion, within 30 minutes following the first RBC transfusion, as well as 6 hours (± 30 minutes) and 18 hours (±30 minutes) after the end of the first RBC transfusion. At each time point, blood will be placed in a 10-ml EDTA tube. All samples will be centrifuged at 2500 g for 20 minutes at 20°C within 4 hours of blood draw. The platelet-poor plasma will then be stored in 1.8 ml cryotubes at -80°C. Samples will be batch shipped and analyzed in at Blood Systems Research Institute (San Francisco, CA, USA).

Biomarkers: 240 µl of thawed plasma will be diluted with assay buffers and measured on the Milliplex multi- and singleplex assay platforms (a**ims 1c/2c**: sCD40L, CCL5/RANTES; **aims 2a/2c**: IL-6, IL-8, PAI-1; Millipore, Billerica, MA). Washed, incubated, and labeled samples will be acquired on a Labscan 200 analyzer (Luminex, Austin, TX) and analyzed using Bio-Plex

manager 6.1 software (Bio-Rad). A further 150 µl of plasma will used to perform ELISA-based measurements of RAGE (a**im 2a/2c**; R&D Systems, Minneapolis, MN). NT-proBNP (a**im 2c**) will be measured using a clinical diagnostic system (Novus Biologicals, Littleton, CO). RBC-derived microparticles **(aims 1c/2c):** Thawed platelet-poor plasma will be spun at 13,000 g for 10 minutes at 20°C, then labeled in preparation for flow cytometric measurement (BD LSR II flow cytometer, San Jose, CA). Vesicles will be lysed with NP-40 detergent and samples re-run to confirm results and allow setting of gates. Free hemoglobin **(aims 1c/2c):** The Human Hemoglobin ELISA Kit will be utilized for the detection of free hemoglobin in plasma (Bethyl Laboratories, Inc., Montgomery, TX). Neutral lipids **(aims 1c/2c):** Following the addition of ice-cold methanol, proteins will be precipitated, and non-polar lipids will be extracted/analyzed using high-pressure liquid chromatography (LC) interfaced into the electrospray source of a triple quadrupole mass spectrometer (MS) (liquid chromatography coupled to electrospray ionization mass spectrometry [LC/MS/MS]). Lipid concentrations will be estimated using ratios to an internal standard ($^{2}H^{8}$-5-HETE), as previously described.[1-3]

References:

1. Gijón MA, Zarini S, Murphy RC. Biosynthesis of eicosanoids and transcellular metabolism of leukotrienes in murine bone marrow cells. Journal of lipid research 2007;48:716-25.
2. Jordan JR, Moore EE, Sarin EL, et al. Arachidonic acid in postshock mesenteric lymph induces pulmonary synthesis of leukotriene B4. Journal of applied physiology (Bethesda, Md : 1985) 2008;104:1161-6.
3. Zarini S, Gijón MA, Ransome AE, Murphy RC, Sala A. Transcellular biosynthesis of cysteinyl leukotrienes in vivo during mouse peritoneal inflammation. Proceedings of the National Academy of Sciences of the United States of America 2009;106:8296-301.

Supplemental materials:

RBC washing protocol with the CATS device.

Responsible	Step	Action
OPERATING ROOM/PATIENT ROOM SET-UP		
Autotransfusion Personnel	1.	Enter OR or patient room with CATS (designated for study) and perform patient ID per Patient Identification.
	2.	Press the I (power on) key until the screen turns on.
	3.	Press the Select Program key to choose the desired wash program. a. Use the ↓ key to find the High Quality Wash program. b. Once the High Quality Wash program is selected, press the Enter key.
	4.	Continue with disposable set-up per CATS.
	5.	Press the Prime key. NOTE: The CATS device recognizes High Quality Wash as an adult prime and will prime with approximately 283 mL of saline.
	6.	Record patient/surgery information on yellow AT worksheet.
	7.	Record lot numbers of disposables on reverse side of yellow AT worksheet.
	8.	Record the AT tech pager number that is responsible for case on RBC Study Sheet for In-Room Provider (see attachment)
PRE – SAMPLE COLLECTION		
	9.	Anesthesia will hand over one unit of RBCs to Autotransfusion personnel.
	10.	Record time of RBC unit request on RBC Study Sheet for In-Room Provider
	11.	Place one unit number label on RBC Study Sheet for In-room Provider and one unit number label on RBC BLOOD BAG PLASMA COLLECTION FORM
	12.	Spike one port of the RBC unit with a sterile plasma transfer set device.
	13.	Attach a sterile 10 mL syringe to plasma transfer set device.
	14.	Draw 6mL of RBCs into syringe.

	15.	Dispense contents of syringe into 10 mL EDTA tube provided by research coordinator.
	16.	Place one patient ID label on EDTA tube and place in biohazard bag.
	17.	Fill out required sections of RBC BLOOD BAG PLASMA COLLECTION FORM: a. Site ID = 001 b. Subject ID = 4 digit # located on In-Room provider sheet c. Check the box next to Plasma #1 Pre-wash d. Date/time of sample collection
	18.	Fill out pink study card with the following information: a. Place one patient ID label in upper left corner b. Record Subject ID c. Record Date/time sample was collected
	19.	Place the RBC BLOOD BAG PLASMA COLLECTION FORM and pink study card in biohazard bag with EDTA sample.
	20.	Hang RBC unit on CATS pole.
PRE-DILUTION OF RBCS BEFORE WASHING		
	21.	Open one 1000 mL bag of saline and hang on CATS pole
	22.	Open Y-type Blood Set with Pump and close both roller clamps.
	23.	Attach female end of Y-type blood set to the male port on the side of the blood collection reservoir.
	24.	Spike 1000 mL bag of saline and open roller clamp.
	25.	Drain entire volume of saline bag into blood collection reservoir.
	26.	Disconnect empty bag and attach another 1000 mL bag of saline.
	27.	Drain 200 mL of saline into blood collection reservoir and close clamp.
	28.	Spike one unit of RBCs with remaining spike.
	29.	Drain contents of RBCs bag into blood collection reservoir. NOTE: The pre-dilution of the RBCs/saline mixture is 4:1.
	30.	Swirl the contents of the reservoir to ensure proper mixing.
	31.	Track the total amount of saline bags used on Comments section of AT worksheet.

WASHING (PROCESSING)		
	32.	Press the start key to begin processing.
	33.	Record the following information on the yellow AT worksheet: a. Unit number (take label from original bag) b. Processed by c. Processing time (Time processing began until processing finished. E.g. 0915-0930)
	34.	Once blood reservoir is empty, press Save Final PRC key.
	35.	Press Save Final PRC key on next screen.
	36.	Record the remaining information in the processing section of the yellow AT worksheet: a. RBC volume recovered b. Transfer pack volume c. Comments section: Track number of saline bags used.
POST - SAMPLE COLLECTION		
	37.	Attach a sterile plasma transfer set device to one port on the reinfusion bag and close clamp.
	38.	Attach a sterile 10 mL syringe to plasma transfer set device and open clamp.
	39.	Draw 6mL of RBCs into syringe.
	40.	Dispense contents of syringe into 10 mL EDTA tube provided by research coordinator
	41.	Place one patient ID label on EDTA tube and place in biohazard bag.
	42.	Fill out required sections of RBC BLOOD BAG PLASMA COLLECTION FORM: e. Site ID = 001 f. Subject ID = 4 digit # located on In-Room provider sheet g. Check the box next to Plasma #2 Post-wash h. Date/time of sample collection
	43.	Fill out pink study card with the following information: d. Place one patient ID label in upper left corner e. Record Subject ID f. Record Date/time sample was collected
	44.	Place the RBC BLOOD BAG PLASMA COLLECTION FORM and pink study card in biohazard bag with EDTA sample.

	45.	Open clamp and drain contents of reinfusion bag into transfer pack.
	46.	Expel air from transfer pack.
	47.	Seal transfer pack using the hand sealer and two hand sealer clips. Cut between the clips.
LABELING		
	48.	Complete Research Only - Washed Allogenic Blood label in the following manner a. Retrieve unit number label from original RBC unit and place in the upper left corner of blood label. b. Place a patient ID label on the lower left corner. c. Record the volume of the washed unit. d. Record the time (hh:mm) that the CATS began washing the RBC unit. e. Record the expiration date/time (4 hours from beginning of wash).
	49.	Affix label to transfer pack.
ADMINISTRATION		
	50.	Perform visual inspection of unit and release unit to Anesthesia/nursing/Perfusion personnel. a. Record initials in the "Inspected and Release by" box on the AT worksheet. b. Record Time transfused/volume transfused.
SENDING SAMPLES		
	51.	OPERATING ROOM: a. Place samples in OR window for lab personnel. b. Press the LAB button the communication panel. PATIENT ROOM: a. Using the nearest small tube station, send all collected samples to 4th tower.
ADDITIONAL WASHED RBCS DURING STUDY TIME PERIOD		
	52.	If additional units are requested to be washed in the current OR or patient room, repeat the following sections in this procedure: a. Pre-sample collection b. Pre-dilution of RBCs before washing c. Processing d. Post-Sample Collection e. Labeling

		f. Administration
	53.	After surgery is complete, perform tear-down/cleaning of CATS device per procedure.
	54.	Transport CATS device and yellow AT worksheet with patient information to patient room.
	55.	Once an order for the first RBC unit is received, go to patient room and perform steps in OPERATING ROOM/PATIENT ROOM SET-UP section.
	56.	Complete all steps in the following sections in this procedure for all subsequent RBC orders: a. Pre-sample collection b. Pre-dilution of RBCs before washing c. Processing d. Post-Sample Collection e. Labeling f. Administration
	57.	After the last order for RBCs and the washing process has been completed, perform tear-down/cleaning of CATS device per procedure.
	58.	Transport CATS device to Autotransfusion office.
	59.	Place yellow AT worksheet on Quality Specialist desk.

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Name and Clinic Number

Approval Date: **January 27, 2017**
Not to be used after: **September 15, 2017**

RESEARCH PARTICIPANT CONSENT AND PRIVACY AUTHORIZATION FORM

Study Title: Point-of-Care RBC Washing to Prevent Transfusion-Related Pulmonary Complications

IRB#: 13-005965

Principal Investigator: Dr. D. Kor and Colleagues

Please read this information carefully. It tells you important things about this research study. A member of our research team will talk to you about taking part in this research study. If you have questions at any time, please ask us.

Take your time to decide. Feel free to discuss the study with your family, friends, and healthcare provider before you make your decision.

To help you decide if you want to take part in this study, you should know:

- Taking part in this study is completely voluntary.
- You can choose not to participate.
- You are free to change your mind at any time if you choose to participate.
- Your decision won't cause any penalties or loss of benefits to which you're otherwise entitled.
- Your decision won't change the access to medical care you get at Mayo Clinic now or in the future if you choose not to participate or discontinue your participation.

For purposes of this form, Mayo Clinic refers to Mayo Clinic in Arizona, Florida and Rochester, Minnesota; Mayo Clinic Health System; and all owned and affiliated clinics, hospitals, and entities.

If you decide to take part in this research study, you will sign this consent form to show that you want to take part. We will give you a copy of this form to keep. A copy of this form will be put in your medical record.

MAYO CLINIC

Name and Clinic Number

Approval Date: **January 27, 2017**
Not to be used after: **September 15, 2017**

CONTACT INFORMATION

You can contact …	At …	If you have questions about …
Principal Investigator: Dr. Daryl Kor **Study Team Contact:** Laurie Meade, RN	**Phone:** (507) 255-6051 **Phone:** (507) 255-1829 **Address:** 200 First Street SW Rochester, MN 55905	▪ Study tests and procedures ▪ Research-related injuries or emergencies ▪ Any research-related concerns or complaints ▪ Withdrawing from the research study ▪ Materials you receive ▪ Research-related appointments
Mayo Clinic Institutional Review Board (IRB)	**Phone:** (507) 266-4000 **Toll-Free:** (866) 273-4681	▪ Rights of a research participant
Research Subject Advocate **(The RSA is independent of the Study Team)**	**Phone:** (507) 266-9372 **Toll-Free:** (866) 273-4681 **E-mail:** researchsubjectadvocate@mayo.edu	▪ Rights of a research participant ▪ Any research-related concerns or complaints ▪ Use of your Protected Health Information ▪ Stopping your authorization to use your Protected Health Information
Research Billing	**Rochester, MN:** (507) 266-5670	▪ Billing or insurance related to this research study

MAYO CLINIC

Name and Clinic Number

Approval Date: **January 27, 2017**
Not to be used after: **September 15, 2017**

A description of this clinical trial will be available on http://www.ClinicalTrials.gov, as required by U.S. Law. This Website will not include information that can identify you. At most, the Website will include a summary of the results. You can search this Website at any time.

1. Why are you being asked to take part in this research study?

You are being asked to take part in this research study because you are having cardiovascular surgery at Mayo Clinic. About 170 people will take part in this research study. The plan is to have about 85 people take part in this study at Mayo Clinic.

2. Why is this research study being done?

The purpose of this study is to determine if washing red blood cells just before blood transfusion prevents pulmonary complications in patients undergoing cardiovascular surgery.

3. Information you should know

Who is Funding the Study?

The National Heart, Lung, and Blood Institute is funding the study. National Heart, Lung, and Blood Institute will pay the Principal Investigator or the institution to cover costs related to running the study.

4. How long will you be in this research study?

You will be in the study until you are discharged from the hospital, or day 28, whichever comes first.

MAYO CLINIC

Name and Clinic Number

Approval Date: **January 27, 2017**
Not to be used after: **September 15, 2017**

5. What will happen to you while you are in this research study?

If you agree to be in the study, you will be asked to participate in the following: If you are eligible for the study, we will assign you by chance (like a coin toss) to the *standard red blood cell (RBC)* group or a *washed RBC* group. You and the Principal Investigator can't choose your study group. You will have *an equal chance* of being assigned to the *washed RBC* group. *The decision to transfuse with red blood cells will be left up to your surgical team.* A total of about 4 tablespoons of blood will be drawn from you for the study. Blood will be drawn at four different time points: during your surgery, six hours after your first blood transfusion, 18 hours after your first blood transfusion, and on study day 5, if you are still in the hospital. This blood will be used to look for markers in blood that are associated with lung injury. Your care team will check twice daily to ensure that you are receiving an appropriate level of oxygen supplementation up to day 28 or hospital discharge, whichever comes first.

6. What are the possible risks or discomforts from being in this research study?

The risks of drawing blood include pain, bruising, lightheadedness, and/or fainting, or rarely, infection at the site of the needle stick.

Your doctor will discuss the risks of blood transfusions with you as these procedures are part of your standard clinical care. The purpose of this study is to determine if washed red blood cells are safer than unwashed red blood cells. Although unlikely, it is possible that the washing procedures in this study could damage the red blood cells that are planned to be transfused. If this were to happen, it may make the transfusion less effective.

Many side effects that occur with red blood cell transfusions go away shortly after a transfusion is stopped. However, in some cases side effects can be serious, long lasting, or may never go away. Some side effects may not be known. Side effects may range from mild to life-threatening. Other drugs may be given to make side effects less serious and less uncomfortable. Talk to the researcher and/or your healthcare provider about side effects and ask any other questions.

MAYO CLINIC

Name and Clinic Number

Approval Date: **January 27, 2017**
Not to be used after: **September 15, 2017**

7. Are there reasons you might leave this research study early?

You may decide to stop at any time. You should tell the Principal Investigator if you decide to stop and you will be advised whether any additional tests may need to be done for your safety.

In addition, the Principal Investigator, the NIH or Mayo Clinic may stop you from taking part in this study at any time:

- if it is in your best interest,
- if the study is stopped.

If you leave this research study early, or are withdrawn from the study, no more information about you will be collected; however, information already collected about you in the study may continue to be used.

We will tell you about any new information that may affect your willingness to stay in the study.

8. What if you are injured from your participation in this research study?

Where to get help:

If you think you have suffered a research-related injury, you should promptly notify the Principal Investigator listed in the Contact Information at the beginning of this form. Mayo Clinic will offer care for research-related injuries, including first aid, emergency treatment and follow-up care as needed.

Who will pay for the treatment of research related injuries:

Care for such research-related injuries will be billed in the ordinary manner, to you or your insurance. You will be responsible for all treatment costs not covered by your insurance, including deductibles, co-payments and coinsurance.

MAYO CLINIC

Name and Clinic Number

Approval Date: **January 27, 2017**
Not to be used after: **September 15, 2017**

9. What are the possible benefits from being in this research study?

This study may or may not make your health better. However, it may provide important information on how to best manage blood transfusions of patients undergoing cardiovascular surgery in the future.

10. What alternative do you have if you choose not to participate in this research study?

You don't have to be in this study to receive treatment for your condition. Your other choices may include receiving the standard blood transfusion. Talk to the Principal Investigator or your doctor if you have any questions about any of these treatments or procedures.

11. What tests or procedures will you need to pay for if you take part in this research study?

You won't need to pay for tests and procedures which are done just for this research study. These tests and procedures are:

- Washing of the RBCs
- Study labs and processing

However, you and/or your insurance will need to pay for all other tests and procedures that you would have as part of your clinical care, including co-payments and deductibles.

If you have billing or insurance questions call Research Billing at the telephone number provided in the Contact Information section of this form.

MAYO CLINIC

Name and Clinic Number

Approval Date: **January 27, 2017**
Not to be used after: **September 15, 2017**

12. Will you be paid for taking part in this research study?

You won't be paid for taking part in this study.

13. What will happen to your samples?

For this study, your de-identified blood samples will be sent to two different external laboratories to look for markers that may indicate pulmonary complications.

We would like to keep your sample for future research. You can still take part in this current study even if you don't want your sample used for future research. If you agree to give your sample, it will be the property of Mayo Clinic.

Other researchers at Mayo Clinic who aren't involved with this study may ask to use your sample for future research. Researchers at other institutions may also ask for a part of your sample for future studies. Your sample will be sent to researchers in a coded format, which protects your identity.

Please read the following statements and mark your choices:

1. I permit my sample to be stored and used in future research of critical illness and lung injury at Mayo Clinic:

☐ Yes ☐ No Please initial here: _____Date: _____

2. I permit my sample to be stored and used in future research at Mayo Clinic to learn about, prevent, or treat any other health problems:

☐ Yes ☐ No Please initial here: _____Date: _____

3. I permit Mayo Clinic to give my sample to researchers at other institutions:

☐ Yes ☐ No Please initial here: _____Date: _____

There is a very small chance that some commercial value may result from the use of your donated sample. If that happens, you won't be offered a share in any profits.

MAYO CLINIC

Name and Clinic Number

Approval Date: **January 27, 2017**
Not to be used after: **September 15, 2017**

You may request to have your sample destroyed by writing to the Principal Investigator. The address is found in the "Contact Information" section of this consent form.

Because we cannot predict how your sample will be used in the future, we cannot promise that samples can be retrieved and destroyed.

14. How will your privacy and the confidentiality of your records be protected?

Mayo Clinic is committed to protecting the confidentiality of information obtained about you in connection with this research study. We will not publish personal identifying information and we use a code to help protect your identity.

During this research, information about your health will be collected. Under Federal law called the Privacy Rule, health information is private. However, there are exceptions to this rule, and you should know who may be able to see, use and share your health information for research and why they may need to do so. Information about you and your health cannot be used in this research study without your written permission. If you sign this form, it will provide that permission.

Health information may be collected about you from:

- Past, present and future medical records.
- Research procedures, including research office visits, tests, interviews and questionnaires.

Why will this information be used and/or given to others?

- To do the research.
- To report the results.
- To see if the research was done correctly.

If the results of this study are made public, information that identifies you will not be used.

Who may use or share your health information?

- Mayo Clinic research staff involved in this study.
- National Institutes of Health (NIH).

MAYO CLINIC

Name and Clinic Number

Approval Date: **January 27, 2017**
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With whom may your health information be shared?

- The Mayo Clinic Institutional Review Board that oversees the research.
- Researchers involved in this study at other institutions.
- Federal and State agencies (such as the Food and Drug Administration, the Department of Health and Human Services, the National Institutes of Health and other United States agencies) or government agencies in other countries that oversee or review research.
- The sponsor(s) of this study and the people or groups it hires to help perform this research.
- A group that oversees the data (study information) and safety of this research.

Is your health information protected after it has been shared with others?

Mayo Clinic asks anyone who receives your health information from us to protect your privacy; however, once your information is shared outside Mayo Clinic, we cannot promise that it will remain private and it may no longer be protected by the Privacy Rule.

Your Privacy Rights

You do not have to sign this form, but if you do not, you cannot take part in this research study.

If you cancel your permission to use or share your health information, your participation in this study will end and no more information about you will be collected; however, information already collected about you in the study may continue to be used.

If you choose not to take part or if you withdraw from this study, it will not harm your relationship with your own doctors or with Mayo Clinic.

You can cancel your permission to use or share your health information at any time by sending a letter to the address below:

Mayo Clinic
Office for Human Research Protection
ATTN: Notice of Revocation of Authorization
200 1st Street SW
Rochester, MN 55905

Alternatively, you may cancel your permission by emailing the Mayo Clinic Research Subject Advocate at: researchsubjectadvocate@mayo.edu

MAYO CLINIC

Name and Clinic Number

Approval Date: **January 27, 2017**
Not to be used after: **September 15, 2017**

Please be sure to include in your letter or email:

- The name of the Principal Investigator,
- The study IRB number and /or study name, and
- Your contact information.

Your permission lasts forever, unless you cancel it.

ENROLLMENT AND PERMISSION SIGNATURES:

Your signature documents your permission to take part in this research.

	/ /	: AM/PM
Printed Name	Date	Time

Signature

Person Obtaining Consent

- I have explained the research study to the participant.
- I have answered all questions about this research study to the best of my ability.

	/ /	: AM/PM
Printed Name	Date	Time

Signature

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description
Administrative information		
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym (Page 1)
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry (Page 2)
	2b	All items from the World Health Organization Trial Registration Data Set (Complete, throughout manuscript)
XProtocol version	3	Date and version identifier (Page 2)
XFunding	4	Sources and types of financial, material, and other support (Page 2, 8)
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors (Page 2, 35)
	5b	Name and contact information for the trial sponsor (Page 2)
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities (Page 36)
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee) (Page 10, 23, 24, 26)
Introduction		
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention (Page 6-8)
	6b	Explanation for choice of comparators (Page 8, 11)
Objectives	7	Specific objectives or hypotheses (Page 8, 14-18)

Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory) (Page 9, 19)
Methods: Participants, interventions, and outcomes		
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained (Page 10)
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists) (Page 9-10)
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered (Page 10-13)
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease) (Page 12)
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests) (Page 14, 19-20)
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial (Page 12-13)
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended (Page 14-18)
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure) (Figure 1, Page 10-12)
Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations (Page 18)
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size (Page 9-10)
Methods: Assignment of interventions (for controlled trials)		
Allocation:		

Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions (Page 19)
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned (Page 19)
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions (Page 10)
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how (Page 9, 19)
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial (N/A)
Methods: Data collection, management, and analysis		
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol (Page 14-18, 23-24; Supplemental materials)
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols (Page 14-18, 23-24; Supplemental materials)
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol (Page 14-18, 23-24; Supplemental materials)
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol (Page 19-23)
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses) (Page 19-23)

	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation) (Page 19-24)
Methods: Monitoring		
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed (Page 26)
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial (Page 26)
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct (Page 25-26)
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor (Page 26)
Ethics and dissemination		
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval (Page 26-27)
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators) (Page 24, 27)
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32) (Page 10, 26, supplemental consent form)
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable (Page 28)
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial (Page 23-24)
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site (Page 2, 36)
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators (Page 27-28, 36)

Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation (N/A)
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions (Page 28)
	31b	Authorship eligibility guidelines and any intended use of professional writers (N/A)
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code (Page 27-28)
Appendices		
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates (Supplemental materials)
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable (Supplemental materials)

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.

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Point-of-care washing of allogeneic red blood cells for the prevention of transfusion-related respiratory complications (WAR-PRC): a protocol for a multicenter randomized clinical trial in patients undergoing cardiac surgery

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Point-of-care washing of allogeneic red blood cells for the prevention of transfusion-related respiratory complications (WAR-PRC): a protocol for a multicenter randomized clinical trial in patients undergoing cardiac surgery

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Abbreviated Title: Allogeneic Red Blood Cell Washing to Prevent Pulmonary Complications

ABSTRACT

Introduction: The transfusion-related respiratory complications, transfusion-related acute lung injury (TRALI) and transfusion-associated circulatory overload (TACO), are leading causes of transfusion-related morbidity and mortality. At present, there are no effective preventive strategies with red blood cell (RBC) transfusion. Although mechanisms remain incompletely defined, soluble biological response modifiers (BRMs) within the RBC storage solution may play an important role. Point-of-care (POC) washing of allogeneic RBCs may remove these BRMs, thereby mitigating their impact on post-transfusion respiratory complications.

Methods and analysis: This is a multicenter randomized clinical trial of standard allogeneic versus washed allogeneic RBC transfusion for adult patients undergoing cardiac surgery testing the hypothesis that POC RBC washing is feasible, safe, and efficacious and will reduce recipient immune and physiologic responses associated with transfusion-related respiratory complications. Relevant clinical outcomes will also be assessed. This investigation will enroll 170 patients at 2 hospitals in the USA. Simon's two-stage design will be used to assess the feasibility of POC RBC washing. The primary safety outcomes will be assessed using Wilcoxon Rank-Sum tests for continuous variables and Pearson chi-square test for categorical variables. Standard mixed modeling practices will be employed to test for changes in biomarkers of lung injury following transfusion. Linear regression will assess relationships between randomized group and post-transfusion physiologic measures.

Ethics and dissemination: Safety oversight will be conducted under the direction of an independent Data and Safety Monitoring Board (DSMB). Approval of the protocol was obtained by the DSMB as well as the institutional review boards at each institution prior to enrolling the

first study participant. This study aims to provide important information regarding the feasibility of POC washing of allogeneic RBCs and its potential impact on ameliorating post-transfusion respiratory complications. Additionally, it will inform the feasibility and scientific merit of pursuing a more definitive phase II/III clinical trial.

Strengths:

- Significant knowledge gap, specifically understanding whether point-of-care washing of allogeneic red blood cells (RBCs) is safe, feasible, and efficacious in ameliorating recipient immune and physiologic responses to transfusion that are associated with transfusion-related respiratory complications
- In addition to exploring immune and physiologic response, the trial is also designed to explore clinical outcomes in order to inform the merit and feasibility of future phase II/III clinical trials
- Large and accessible at-risk population
- Established multicenter clinical trial infrastructure
- Detailed and measured statistical approach
- Multidisciplinary expertise in translational, patient-centered transfusion research
- Potential for substantial clinical impact should the intervention prove safe and effective

Limitations:

- Unproven feasibility of point-of-care washing in a time-sensitive environment
- Candidate biomarkers for transfusion-related lung injury may not fully represent or capture true causal pathways
- The inflammatory response accompanying cardiac surgery may mask between-group differences in the immune and physiologic responses to transfusion therapies
- Inconsistent timing and dose of red blood cell transfusion
- Study will test the impact of modifying the RBC storage solution with POC washing, but will not clarify the impact of storage on the RBCs themselves
- Unclear effects of RBC storage duration
- Study not adequately powered for clinical outcomes

INTRODUCTION

Transfusion-related pulmonary complications, including transfusion-related acute lung injury (TRALI) and transfusion-associated circulatory overload (TACO), are the leading cause of serious transfusion-related adverse events. TRALI is the primary cause of transfusion-related death and, although seemingly less appreciated, TACO has been the second leading cause of transfusion-related death in recent years. In addition to their associated mortality, both syndromes result in substantial resource utilization and associated healthcare cost. A large proportion of patients who develop TRALI will require intensive care unit (ICU) admission and ventilator support.[1,2] Similarly, up to 21% of TACO cases have been reported as life-threatening and associated with increased lengths of ICU and hospital stays.[3-6] Although specific preventative strategies have dramatically reduced the incidence of plasma-associated TRALI (e.g., male-only plasma donation), no prevention strategies exist for red blood cell (RBC)-associated TRALI or TACO. Indeed, the lack of safe and feasible strategies that can mitigate risk of RBC-associated TRALI and TACO represent critical knowledge gaps in transfusion medicine.

While TRALI and TACO share a similar clinical phenotype of pulmonary edema and hypoxemic respiratory insufficiency, each is believed to result from distinct pathologic processes.[3,5,7-9] TRALI is believed the result of a two-hit process beginning with pulmonary endothelial activation resulting in leukocyte priming, sequestration, and activation followed by endothelial injury with inflammatory lung edema. The first insult typically relates to recipient factors (e.g., surgery, trauma, infection) and the second "hit" from the infusion of mediators in the blood component. For high-plasma volume components including plasma or apheresis platelets, this is believed most often the result of donor anti-leukocyte antibodies reacting with

recipient cognate antigens. In contrast, multiple lines of evidence suggest alternate mechanisms are at play with RBC-associated TRALI.[7,10-12] Here, the second insult is generally attributed to the infusion of soluble biological response modifiers (BRMs) residing in the RBC supernatant.

Conversely, TACO has classically been attributed to fluid overload in the setting of transfusion. However, a large proportion of reported TACO cases present after a single blood unit exposure without overt signs of systemic volume overload.[13,14] Moreover, TACO is characteristically accompanied by a marked hypertensive response that exceeds what would be expected from a volume challenge alone, suggesting the potential presence of vasoactive substances in the transfused product that may increase systemic vascular resistance.[15-17] An abrupt increase in systemic vascular resistance may result in increased cardiac filling pressures, thereby increasing risk for hydrostatic pulmonary edema. Hence, it is possible that additional and potentially synergistic pathophysiologic processes are at play in TACO. Indeed, a growing body of evidence suggests that BRMs contained within the supernatant of stored RBC (e.g., free hemoglobin, RBC microparticles) may act on vascular smooth muscle tone and contribute to TACO.[18-23]

Washing of allogeneic RBCs can remove soluble contaminants in the RBC supernatant including chemokines, biologically active lipids, cellular debris, microaggregates, and other BRMs.[24-27] Interestingly, a large investigation noted a complete absence of reported TRALI and TACO cases following transfusion of more than 28,000 units of washed allogeneic RBCs.[28] Additionally, RBC washing has been associated with decreased adverse immunologic effects in transfused trauma patients and improved survival in transfusion recipients with acute leukemia.[29,30] Although promising, washing stored RBCs has been largely discounted due to concerns related to cost and feasibility.[31] However, as there are no effective prevention strategies

for RBC-associated TRALI or TACO, and there is clear biologic plausibility for cause-effect relationships between the infusion of soluble BRMs and the development of life-threatening transfusion-related respiratory complications, further investigation is clearly warranted.

To enhance our understanding regarding the role of point-of-care allogeneic RBC-washing as a means to mitigate transfusion-related respiratory complications, the Washing of Allogeneic Red blood cells for the Prevention of Respiratory Complications (WAR-PRC) Study was developed. This is a multicenter randomized clinical trial, supported by the National Institutes of Health-National Heart, Lung and Blood Institute (NIH grant number: R01 HL121232, PIs: Drs. Kor, Welsby). The trial aims to test the feasibility, safety, and efficacy of point-of-care RBC washing using an FDA-approved autotransfusion device known as the Continuous Autotransfusion System (CATS) in adult cardiac surgery patients receiving allogeneic RBC transfusion. Cardiac surgical patients were selected as the target population given the frequency of large-volume RBC transfusion in this practice location,[32] the well-described risks of postoperative respiratory complications in this patient population,[33-36] and the presence and routine use of the cell washing strategies (auto-transfusion) in this environment. This paper describes the study procedures and planned analyses for this clinical trial.

METHODS AND ANALYSIS

Study design

To test the hypothesis that point of care (POC) allogeneic RBC-washing will be safe, feasible, and associated with amelioration of intermediate markers of TRALI and TACO, a multi-center, single-blinded (outcome assessor), parallel group, phase I/II randomized clinical trial has been designed. The ClinicalTrials.gov registration number is NCT02094118. An outline of the study design, procedures, and aims is displayed in Figure 1.

Study population

Adult patients aged 18 years and older undergoing cardiac surgery with heightened risk for large volume RBC transfusion, defined as a predicted RBC transfusion requirement of greater than or equal to 4 units, will be enrolled. To facilitate the identification of patients at high risk for RBC transfusion, a validated cardiac surgery prediction model will be utilized.[37] A cut off of 4 predicted units of RBC administration was chosen because this transfusion volume is associated with increased risk of pulmonary complications following cardiac surgery.[38] Additionally, it has been identified as a common "RBC dose" implicated in patients with TRALI and/or TACO.[8,39] This threshold will also still identify a sizable cardiac surgery population, ensuring study feasibility. A complete list of exclusion criteria including the justification for each is shown in table 1.

Table 1. Study exclusion criteria.

Exclusion criteria	Justification
Emergency surgery	Inability to randomize/perform study procedures
IgA deficiency	Not ethical to randomize to standard issue RBCs
History of severe recurrent transfusion	Not ethical to randomize to standard issue RBCs

reaction	
Refusal to receive allogeneic RBCs	Inability to administer intervention of interest
Refusal to provide informed consent	Not ethical to enroll into trial
Prevalent acute lung injury prior to randomization	Inability to adequately assess outcome
Prevalent hydrostatic pulmonary edema prior to randomization	Inability to adequately assess outcome
Expected hospital stay < 48 hours	Incomplete study procedures and outcome data
Not anticipated to survive > 48 hours	Incomplete study procedures and outcome data
Previously enrolled in this trial	Violation of the independence assumption
Pulmonary artery catheter placement not planned for the surgical procedure	Inability to assess key physiologic parameters outlined in the study protocol
Use of home oxygen therapy	Inability to assess oxygen use outcome
Complex RBC antibody profiles	Washing not feasible due to testing delays
Need for the use of irradiated RBCs	Intervention contraindicated

Patients will be recruited and enrolled at 2 academic medical centers in the USA (Mayo Clinic, Rochester, MN; Duke University Medical Center, Raleigh, NC) with substantial experience in RBC-washing and transfusion management for cardiac surgery. With regards to type of cardiac surgery, study coordinators will screen all adult patients scheduled to undergo coronary artery bypass grafting (CABG) surgery, complex cardiac valve surgery, pericardial resection, and/or ascending aortic surgery in one of the two participating institutions. Eligible patients will be approached before their elective surgical procedure by a member of the study team for informed consent. A study identification (ID) number will be assigned to each study participant and randomization will occur after receipt of informed consent, but before entry to the operating room for the scheduled procedure. Screening logs will be maintained at each site to allow generation of a CONSORT diagram.

Interventions

Study intervention: The intervention in this investigation will be implemented for all allogeneic

RBCs administered on the day of sugery, including intraoperative and postoperative transfusions. The decision to administer an allogeneic RBC transfusion will be left to the responsible clinical service and will not be prespecified in the study protocol. There are several reasons for this lack of prespecified indications for RBC transfusion. First, the target population frequently experiences major acute blood loss. During these circumstances, typical measures assessing the need for RBC transfusion, such as threshold hemoglobin or hematocrit values, do not reflect true RBC cell mass nor the need for RBC transfusion. Moreover, the process of obtaining these laboratory results may be associated with unacceptable time delays when bleeding is severe. Additionally, this design facilitates a more meaningful understanding of the feasibility of RBC washing in clinical practice.

When a clinical decision to proceed with allogeneic RBC transfusion has been made, the RBC product will be immediately prepared in the operating room (or in the ICU room if administered postoperatively) according to the allocated treatment assignment (washed versus standard issue). For patients randomized to the control group arm, all RBCs administered on the day of surgery will be standard-issue allogeneic RBCs. Detailed RBC unit characteristics including the type of RBC product (i.e. whole-blood derived versus apheresis), processing, and additive characteristics for each clinical site are provided as supplemental material. For patients randomized to the intervention arm of this trial, all allogeneic RBCs administered on the day of surgery will be washed with the CATS device prior to transfusion. RBC washing may occur on allogenic RBCs of any storage duration until the time of expiration, and washed units may be stored for up to 24 hours if not immediately administered. The CATS device was chosen over more traditional cell washing machines (e.g. the Cobe 2991 Cell Processor) due to the reduced time needed for cell washing with CATS as well as the reduced risk for hemolysis with the

CATS device.[40] As previously described and confirmed in our preliminary data, pre-dilution of stored, allogeneic RBCs results in the most effective elimination of supernatant.[26] Therefore, a 4:1 dilution consisting of 1200 ml saline to 300 ml RBCs will be added to the reservoir of the CATS by gravity drainage. The "high quality" wash mode option will be selected for processing.[29] Washed RBCs will then be drained from the reinfusion reservoir into sterile transfer bags (Fenwal Inc, Lake Zurich, IL) for transfusion. A full description of the standard operating procedures for RBC washing may be found as supplemental material. Of note, all allogeneic RBC units at the two participating institutions undergo pre-storage leukocyte reduction, although differences in the exact timing of this intervention exist between the two sites for whole-blood derived RBCs (supplemental materials).

Off-protocol transfusions. In the setting of cardiac surgery, it is occasionally necessary to provide allogeneic RBCs in an emergent fashion (e.g. acute, life-threatening bleeding). In this circumstance, time-delays due to study-related activities may prove unsafe. To address this potential scenario, our study protocol will allow the administration of emergency "off-protocol" allogeneic RBCs. "Off-protocol" RBC transfusions will be administered as per standard institutional practice. These RBC transfusion episodes will be specifically noted as "off-protocol" and will be summarized and analyzed to assist in assessing the feasibility of point-of-care RBC washing in patients undergoing cardiac surgery (see statistical description below). In addition, autotransfusion ("cell-saver") is frequently used in this patient population. Cell-salvage will be implemented at the discretion, and under the direction, of the clinical team. If cell-salvage is employed, the device used for this procedure will be distinct and separate from the intervention CATS device.

Co-interventions. Intraoperative care that is not directly related to this study protocol will be at the discretion of the responsible clinical team(s) (e.g., this protocol will not standardize intraoperative anesthetic care or surgical procedures). However, clinical care decisions that may affect the development of respiratory dysfunction and associated outcomes will be standardized to the greatest extent possible. To this end, the protocol specifies optimal ventilator strategies for both the operating room (OR) and ICU environments, including tidal volumes less than or equal to 8 mL/kg predicted body weight, peak inspiratory pressures less than 35 cm H_2O, and positive end-expiratory pressures (PEEP) equal to or greater than 5 cm H_2O. Similarly, although RBC transfusion thresholds are not pre-specified in this protocol, restrictive transfusion practices will be advised in the postoperative period with a hemoglobin target greater than 8 g/dL in the absence of acute bleeding and/or ischemia. This transfusion threshold was chosen as it is the current standard of care at the two participating institutions. Standardization of best practices in at-risk patients will decrease the heterogeneity of the risk modifiers that may otherwise confound our associations of interest. Additionally, each center has adopted protocols on daily spontaneous awakening and spontaneous breathing trials to facilitate standardized weaning from ventilators following cardiac surgery. Non-intubated patients will undergo standard titration of oxygen twice daily (at 0700 and 1900, ± 2 hours). Patients saturating ≥92% on room air will not receive supplemental oxygen, unless specifically requested by the primary service. If the primary care service requests oxygen supplementation for a patient saturating ≥92% on room air, the reason for the deviation from oxygen weaning will be documented. Patients will continue to undergo evaluation for oxygen titration until liberation from oxygen therapy for 24 hours, hospital day 28, or hospital discharge, whichever comes first.

Related conditions and variables of interest: Pertinent baseline demographics and clinical

characteristics such as age, sex, race, preoperative cardiac function, and comorbidities will be recorded. Additional variables of note will include vital signs and laboratory values that are obtained during the course of routine care, APACHE IV scores, administration of statins, ace-inhibitors, angiotensin-receptor blockers, beta-blockers, diuretics, antiplatelet agents, non-steroidal anti-inflammatory drugs, insulin, amiodarone, or steroids, blood product administration up to day 28 or hospital discharge, whichever comes first, daily fluid status, estimated blood loss, and vasopressor requirements.

Outcomes

Feasibility, Safety, and Efficacy Outcomes (Study aim 1): The primary feasibility outcome will be the number and proportion of off-protocol allogeneic RBC transfusions administered during the study intervention period (i.e. day of surgery). A secondary feasibility outcome will be the time required for the RBC washing procedures defined as the time from determination of allogeneic RBC need by the clinical team to time of delivery of the RBC unit to the clinical team. This time will be computed for all patients and all transfusions during the study intervention period in both the intervention and control cohorts.

The primary safety outcomes include the change in the RBC recipient's hemoglobin concentration from pre- to post-transfusion as well as the concentration of cell-free hemoglobin (CFH) and haptoglobin following RBC transfusion. To assess the primary safety outcomes, samples for total hemoglobin, CFH, and haptoglobin will be obtained prior to transfusion, within 30 minutes following the first RBC transfusion, as well as 6 hours (± 30 minutes) and 18 hours (±30 minutes) after the end of the first RBC transfusion for all study participants. Additionally, the number of units and corresponding volume of RBC transfusion will be recorded and

compared between groups to evaluate the impact of washing on RBC mass in individual units. Evidence for acute kidney injury defined according to Acute Kidney Injury Network (AKIN) criteria will be assessed throughout hospitalization as a secondary safety outcome measure.[41] If the patient remains in the hospital, safety labs will also be drawn on study day number 5.

The primary outcome evaluating the efficacy of the washing procedures will be the change in the concentration of BRMs including neutral lipids, soluble CD40 ligand (sCD40L), chemokine ligand 5/regulated on activation, normal T-cell expressed and secreted (CCL5/RANTES), RBC microparticles (RBC-MPs), and CFH in the washed RBC component from the pre- to the post-wash phase. These data will allow for calculation of CATS-related elimination rates of BRMs. Blood sampling and biomarker handling procedures have been previously described,[42,43] and a brief overview of laboratory handling is provided as supplemental material.

Mechanistic Outcomes (Study aim 2): The concentration of multiple, validated biomarkers representing the primary pathways leading to development of lung injury will be assessed in the study participants. These pre-specified plasma biomarkers are displayed in Table 2.

Table 2. Lung injury biomarkers and exploratory potentially pathogenic biologic response modifiers.

Validated lung injury biomarkers	Primary process evaluated	Supporting Evidence
Interleukin-6	Inflammation	44-46
Interleukin-8	Inflammation	44-48
Plasma activator inhibitor-1	Dysregulated coagulation	46,47,49-51
von Willebrand Factor	Endothelial injury	52-57
sICAM-1	Endothelial injury	44,47,48,58-60

Surfactant protein D	Epithelial injury	44,48,61,62
Receptor of advanced glycation end products	Epithelial injury	48,63,64
Exploratory pathogenic BRMs	**Primary process evaluated**	**Supporting Evidence**
Neutral lipids	Lung inflammation	10,65
sCD40L	Lung inflammation	12
CCL5/RANTES	Lung inflammation	15,66,67
RBC-derived microparticles	NO scavenging	21,23,68
Cell-free hemoglobin	NO scavenging	18,22,68
N-terminal brain natriuretic peptide	Ventricular stretch/volume-overload	69-71

ICAM-1, intercellular adhesion molecule-1; BRMs, biologic response modifiers; sCD40L, soluble CD40 ligand; CCL5/RANTES, chemokine ligand 5/regulated on activation, normal T-cell expressed and secreted; NO, nitric oxide.

As study participants are expected to receive variable numbers of RBC transfusions at inconsistent times, four discrete time points have been chosen for assessment of these lung injury biomarkers. The first and second samples will be obtained from the recipient prior to transfusion and within 30 minutes following the first intervention or control RBC unit administered. For the third and fourth assessments, samples will be obtained 6 hours (± 30 minutes) and 18 hours (± 30 minutes) from the end of the first study RBC transfusion.

Secondary analyses will include detailed assessment of cardiopulmonary responses to RBC transfusions, with specific variables to be assessed shown in Table 3.

Table 3. Physiologic assessments during the study intervention period.

Respiratory Variables	**Hemodynamic Variables**
Arterial partial pressure of oxygen	Mean arterial pressure
Arterial oxygen saturation	Heart rate

Fraction of inspired oxygen	Cardiac output
Tidal volume	Right atrial pressure
Peak airway pressure	Pulmonary artery wedge pressure
Plateau airway pressure	Systemic vascular resistance*
Positive end-expiratory pressure	

*Systemic vascular resistance (SVR) will be calculated using the following equation: SVR (dyns/cm^5) = [(Mean arterial pressure – right arterial pressure)/cardiac output] x 80.

Each of these physiologic variables will be assessed and recorded immediately prior to the study RBC transfusion and again immediately after the transfusion (within 30 minutes). Standard operating procedures for these cardiopulmonary assessments will be defined prior to study onset according to previously established recommendations.[72] These secondary outcomes will allow a more detailed assessment of the cardiopulmonary response to RBC transfusion and will be expected to provide important insight into the pathophysiology of TACO. Of note, pulmonary artery catheter placement is standard of care for this patient population at both enrolling institutions.

In an attempt to evaluate specific potential mechanistic pathways for TRALI and TACO, exploratory putative BRMs have also been selected (Study aim 2; Table 2). In the recipient, samples for the putative BRM assessments will be obtained at the same time points outlined above for the lung injury biomarker samples. To better elucidate the relationship between the dose of these soluble BRMs in the RBC components, their subsequent concentration in the recipient, and their ultimate relationship to the recipient's cardiopulmonary response to transfusion, levels of these potential putative agents will be determined in the RBC components prior to transfusion in both the washed and standard issue cohorts as well as in the transfusion

recipient. As enrolled patients are expected to receive 4 or more units of allogeneic RBCs, samples will be obtained from the RBC component for all RBC units administered up to and including the fourth unit for each study participant. For those in the intervention arm, this will be a post-wash sample. Of note, there exists the potential for incomplete capture of relevant information in those who receive larger volumes of RBC transfusion. However, a four-unit cutoff represents a compromise between study feasibility and scientific validity.

Clinical Outcomes (Study aim 3): To facilitate the design and conduct of future clinical trials, we will also pursue a number of exploratory clinical outcomes, with study coordinators collecting data daily until hospital discharge or death. The primary clinical outcome will be the duration of postoperative mechanical ventilation for each study participant, determined by subtracting the time of ICU admission from the time of endotracheal extubation. If the study participant is extubated prior to ICU admission, the duration of mechanical ventilation will be assigned as 0 hours. Recognizing the potential for early death (intraoperative or early postoperative) biasing the primary clinical outcome, the number of ventilator-free days (VFD) at postoperative day 28 will also be determined, with those who die prior to day 28 being assigned zero VFD. Participants discharged from the hospital alive prior to day 28 will be assumed to have had no additional days of mechanical ventilation following hospital discharge. Additional secondary clinical outcomes will include evaluations of hypoxemia including oxygen saturation measured by pulse oximetry (SpO_2) and the ratio of partial pressure of oxygen (PaO_2) to fraction of inspired oxygen (FiO_2), duration of oxygen supplementation, clinical diagnoses of TRALI, possible TRALI, and/or TACO, sequential organ failure assessment (SOFA) scores, and durations of ICU and hospital stay.

Sample size estimation

The sample size for this clinical trial is based on the aforementioned mechanistic outcomes (study aim 2), with estimates of the range of effect sizes for biomarkers considered in this study derived from a previous investigation.[48] Using an approximation of the standard deviation (SD) derived from the interquartile range (IQR; i.e. SD ≈ IQR/1.35), the median effect size was found to be 0.4, a magnitude of change that is considered relevant and appropriate to power this study. With equal allocation between groups, the sample size is estimated to be 78 participants per group. This assumes a type 1 error rate (alpha) = 0.10 (two-sided) and a power of 80%. Actual power is expected to be higher due to the repeated measures design. To allow for drop out and non-feasible cases, 170 total participants will be randomized with approximately 85 per treatment arm.

Randomization and blinding

Randomization in a 1:1 fashion will occur following the acquisition of signed informed consent. Randomization to the RBC washing or control group will be conducted by the study's electronic data management system's Balance (Medidata) algorithm. This software uses dynamic minimization stratified by clinical center. The software will return a confirmation of the randomization indicating the study participant's treatment allocation status. A note will be placed into the electronic health record (EHR) identifying the patient as a study participant.

In light of the time-sensitive, point-of-care nature of the intervention, the patient, clinical team, and study team will not be blinded to the patient's treatment allocation status. Additionally, the transfusion medicine service will have unblinded, electronic access to the treatment assignment. Blinding, however, will be ensured for the physicians and laboratory

personnel involved in biomarker analyses.

Statistical methods

Aim 1 of the protocol is centered on the feasibility, safety, and efficacy of POC allogeneic RBC washing. Feasibility is defined as the administration of protocol RBCs instead of off-protocol standard-issue RBCs. At the patient level, a washed arm patient is considered feasible if at least 50% of administered RBCs are washed per-protocol. Simon's optimal two-stage design will be used to determine if the protocol needs to be modified to prepare RBCs prior to the surgical procedure. The null hypothesis feasibility rate, p_0=0.75, will be tested against a one-sided alternative that feasibility is higher. In the first stage, 16 patients will be accrued in the RBC washing arm with at least one RBC unit transfused on the operative day. If 12 or fewer patients were deemed feasible, the protocol will be modified to pre-wash RBCs (see limitations section of discussion for more details). If 13 or more are feasible, 32 additional patients in the RBC washing arm will be evaluated. The null hypothesis will be rejected if 40 or more of the 48 studied patients are considered feasible and the study will continue as originally planned. Else if 39 or fewer patients are deemed feasible, the protocol will be modified to pre-wash. This design yields a type I error rate of 0.10 and at least 90% power when the true patient feasibility rate is 0.90 or higher. The change in hemoglobin after the first transfused unit will be used as the primary safety measure. Additional safety endpoints including CFH and haptoglobin levels will be collected and analyzed at multiple time points as described previously. Wilcoxon rank sum tests will be used to compare changes in these continuous outcome measures between randomized groups. These analyses will be conducted utilizing 'as-treated' principles. Specifically, a participant who has received one or more units of CATS washed allogeneic RBC

transfusion(s) on the day of surgery will be assigned and analyzed as a member of the washed cohort. Those who received allogeneic RBC transfusions on the day of surgery, none of which were washed, will be assigned to the standard-issue cohort. Categorical variables (e.g., development of acute kidney injury) will be assessed with chi-square tests. The change in concentrations of soluble BRMs pre- to post-wash (washed arm, washed RBC units only) will be assessed using paired t-tests or Wilcoxon signed-rank tests. To balance assay costs while ensuring scientific success, pre- and post-wash samples will be obtained and analyzed for the first 75 washed RBC units. If efficacy is not clearly established, pre- and post-wash samples will be obtained for an additional 75 washed units. As leukocyte reduction methods may vary between the two clinical sites depending on the use of whole blood-derived or apheresis RBCs, sensitivity analyses will be performed stratified by clinical site.

Aim 2 examines the changes in RBC recipient's intermediate markers of respiratory injury or dysfunction (biomarkers) over four time points relative to the patient's first transfusion as described above. Mixed models will be fit to model the linear trajectory of these biomarkers. A model with a random slope and intercept will be considered initially, and the primary parameter of interest will be the treatment group by time interaction. For these analyses, an as-treated principle will be considered. Patients who receive at least one unit of washed cells will be included in the RBC washing group. Those not receiving RBC washed cells will be in the control group, as this aim is focused on mechanistic action and demonstrating biologic plausibility prior to formal evaluation of clinical outcomes, which would be analyzed using traditional intention to treat (ITT) considerations. Since the functional form of the changes in biomarkers over time is not known, a discrete (3 D.F. test) representation of time will also be used to gauge the linearity assumption, as well as provide a sensitivity analysis to the primary

regression model. Standard mixed modeling practices will be utilized (e.g., assessment of residuals, verification of variance components, nested modeling to simply variance components and covariance patterns). This modeling scenario will be conducted for each biomarker of interest. Since prior research has noted that these outcomes are clustered, the previously described methodology by Shi et al. will be used to adjust for multiple comparisons.[73] We will also compute O'Brien's nonparametric global test statistic to provide an overall measure of treatment effect between the two treatment groups.

Due to our desire to evaluate the impact of RBC washing in a pragmatic and clinically relevant setting, effect modification by RBC storage duration will be assessed. For each patient, separately mean and maximum RBC storage duration (among transfused RBC units) will be considered as effect modifiers using interaction terms in the above models. Similarly, total number of transfused RBC units will be considered as a potential effect modifier.

Cardiopulmonary response values are measured pre- and post-transfusion for each transfused unit. Linear regression with generalized estimating equations (GEE) will assess the relationship between randomized group and change in cardiopulmonary response, accounting for the correlation of observations within individuals receiving multiple transfusions.

As a final component of aim 2, we will test the hypothesis that lower levels of putative BRMs (neutral lipids, sCD40L, CCL5, RBC-MPs, CFH) in transfused RBC components (and in the RBC recipient) will be associated reduced levels of lung injury biomarkers and an attenuated cardiopulmonary response to RBC transfusion. We will specifically quantify the relationships of the putative BRMs as measured in the post-wash bag or unwashed bag (as well as in the recipient) with measures of lung injury and cardiopulmonary response. Multiple linear regression models will test for the joint effect of randomized group and the randomized group by

BRM interaction term in order to determine if the relationships of BRMs with markers of lung injury and cardiopulmonary response are co-incident (similar relationship) between study groups. Validated lung-injury associated biomarkers levels are measured at 4 time points relative to a patient's first RBC transfusion [pre-transfusion (but after the decision to transfuse is made), within 30 minutes post-transfusion, 6 hours post, and 18-hours post (all relative to first transfusion)]. Mixed models will be fit to model the linear trajectory of these biomarkers. Cardiopulmonary response is measured before and immediately after each RBC Unit transfused; linear regression using GEE will assess this relationship.

Aim 3 will utilize standard analytical measures for comparing randomized treatment groups under the ITT paradigm. Continuous outcomes will be analyzed using t-tests, or, for skewed data such as duration of mechanical ventilation, Wilcoxon rank sum tests will be used to compare groups. Binary outcomes will be analyzed using Pearson chi-square or exact tests. Serial measurements (e.g. arterial oxygen saturation) will be analyzed using longitudinal summary statistics. Of note, this study is not powered for these intermediate clinical outcomes. Estimates of precision with confidence intervals along with the range of responses will be used to guide subsequent trial designs, including a larger phase II/III trial with clinical outcomes as the primary outcome of interest.

Consistent with early phase clinical trials, a higher level of significance than 0.05 is selected and we consider p-values less than 0.10 to be significant. This will facilitate advancement of the technique should it prove feasible with potential efficacy. Multiple testing may also increase the overall family wise error rate, so further research, particularly with clinical events, may be needed to quantify clinical efficacy of the approach. Missing data is expected to be minimal given the close surveillance provided in the surgical and ICU environments.

However, missing specimens may occur in the event of patient discharge, death or administrative issues. Initial analyses will be conducted with the assumption of missing completely at random. Sensitivity analyses using multiple imputation and pattern mixture models will be used to assess the robustness of the model assumptions.

Data quality and management

Data quality and safety will be monitored by each site's principal investigator (PI). In addition, strategies to achieve a high level of protocol adherence will include: (1) refresher education sessions for study coordinators, (2) weekly checks of protocol compliance by the Mayo Clinic research coordinators, and (3) computerized identification of protocol violations in the database. Mayo Clinic has implemented an enterprise-wide Clinical Trials Management System (CTMS). CTMS is a data management infrastructure that operates in compliance with 21 Code of Federal Regulations (CFR) Part 11 to support multicenter clinical trials and participant registries. The core of the CTMS project is the Medidata RAVE product, which will serve as the electronic data capture and randomization system for the study. The system has comprehensive audit trails, user authentication, security and disaster plans, and standardized training for users. The system provides real-time data integrity checks, maximizing data integrity while lessening the need for on-site source document verification. Protocol amendments will be fully vetted by the site's principal investigators (Mayo Clinic: Daryl J. Kor, MD; Duke University Medical Center: Ian Welsby, MBBS) prior to submission for approval by each site's IRB. The investigation's final trial dataset will be available to both sites principal investigators (Mayo Clinic: Daryl J. Kor, MD; Duke University Medical Center: Ian Welsby, MBBS). Study data will be available for secondary use by contacting the Mayo Clinic principal investigator, Dr. Daryl J. Kor, MD.

Access to study data will be made available only for the subset of trial participants who have consented to the use of their study data for this purpose.

ETHICS AND DISSEMINATION

Adverse outcomes

Safety data including adverse events such as the development of TRALI, TACO, organ failure (including acute kidney injury), prolonged hospitalization, ICU admission, and mortality will be recorded. Other adverse events will be monitored by the site PI and research specialist in real time from the start of randomization to hospital discharge or death. Adverse events will be defined as "unexpected," "expected" and "serious." As our patient population is by definition "critically ill" due to their high-risk surgical procedure, it is expected that they will have a number of unrelated adverse health events during the course of their hospital stay. Therefore, we will limit the scope of our adverse event monitoring and recording to the following:

1. Serious adverse events (SAEs) will be defined as:
    - Death, believed to be related to the study procedures or a death that is unexpected considering the acuity of a patient.
    - A life-threatening experience believed to be related to the study procedures.
    - Persistent or significant disability or incapacity that is of greater frequency or severity than what would be normally expected in the perioperative course.
    - An event that jeopardizes the human subject and may require medical or surgical treatment to prevent one of the preceding outcomes and is not expected in the perioperative course.
2. Adverse events possibly related to the study procedures will be defined as:
    - Profound anemia (hemoglobin < 7 g/dL).
    - Renal failure requiring renal replacement therapy.
    - Myocardial infarction.

- Non-hemorrhagic stroke.
- Mesenteric ischemia requiring laparotomy (ischemic events secondary to anemia).
- Bloodstream infections.

Role of the data safety and monitoring board

All serious adverse events will be reported to the site institutional review board (IRB) within 24 hours of discovery followed by a more detailed written report to the IRB. The following information about adverse events will be collected: (1) the onset and resolution of the event, (2) an assessment of the severity or intensity of the event, (3) an assessment of the relationship of the event to the intervention, and (4) any action taken because of event. Reporting of SAEs to the respective IRBs will be conducted by the PI at each site. All potentially related SAEs will be reported to the data safety monitoring board (DSMB) and to NHLBI within 7 days of discovery. Additionally, a summary report will be provided to the DSMB prior to each DSMB meeting, at least every 6 months. Safety oversight will be performed by a DSMB, whose members will be independent from the study investigators. Safety endpoints consisting of expected clinical events, including death, will be assessed for all participants who are enrolled in the study on an intent-to-treat basis. Safety endpoints, as well as all serious and unexpected adverse events, will be summarized by treatment group. Trial conduct will be audited by the DSMB at least every 6 months.

Ethics approval

Prior to enrollment of the first study participant, protocol approval was obtained from the DSMB, each participating institutional IRB, and the NHLBI. Compliance of informed consent

forms with NHLBI requirements and the CFRs Title 21 Part 50 Section 50.25 was ensured. Documentation of all IRB approvals, including all finalized consent forms, have been collected and stored by the study team.

Considerations for continuation to a phase II/III clinical trial

This phase I/II clinical trial is not powered to detect subtle differences in clinical outcomes, which would be more adequately addressed in a much larger phase II/III clinical trial. Nonetheless, the clinical evaluations outlined in this protocol will provide essential preliminary data that can inform the merit and feasibility of a future phase II/III clinical trial. Moreover, if POC RBC-washing is determined not to be feasible, safe, or efficacious (aim 1), then this would provide evidence against pursuit of a larger clinical trial. Additionally, if no substantial impact is seen in the intermediate markers of respiratory injury/dysfunction (aim 2), there would be limited benefit in pursuing a larger trial.

Protocol amendments

Protocol amendments will be fully vetted by the site's principal investigators (Mayo Clinic: Daryl J. Kor, MD; Duke University Medical Center: Ian Welsby, MBBS) prior to submission for approval by each site's IRB.

Access to Data

The investigation's final trial dataset will be available to both sites principal investigators (Mayo Clinic: Daryl J. Kor, MD; Duke University Medical Center: Ian Welsby, MBBS). Study data will be available for secondary use by contacting the Mayo Clinic principal investigator, Dr.

Daryl J. Kor, MD. Access to study data will be made available only for the subset of trial participants who have consented to the use of their study data for this purpose.

Dissemination Policy

Study findings, including those of associated ancillary studies, will be disseminated to the scientific community in abstract and oral presentation formats at major national and international medical specialty meetings. All published manuscripts will be submitted to Pub Med Central in accordance with the National Institute of Health Public Access Policy.

Ancillary studies

Ancillary study proposals that complement or advance the specific proposals of this study protocol will be encouraged. Proposals will be reviewed by the Co-PIs of this protocol (Drs. Daryl Kor and Ian Welsby), both to ensure scientific merit and validity as well as ensuring consistency with the goals and conduct of the main study. Such ancillary studies may utilize data and/or samples accrued during the clinical trial or, when feasible, additional data may be collected. All statistical plans will be reviewed a priori and approved before data analysis is initiated. All presentations and manuscripts will require explicit review and approval by this investigation's Co-PIs.

Protocol funding

This study is supported by the NIH-NHLBI (Grant Number: R01 HL121232), the Mayo Clinic Critical Care and Anesthesiology and Perioperative Medicine Research Committees, as well as the Duke Clinical Anesthesia Research Endeavors (CARE). Funding and time allotment has

been provided by each of these entities to support study personnel, protocol development and data management (Medidata Rave), sample acquisition, transfusion procedures, sample and data processing and storage, and statistical support. There is no influence exerted by funding sources on the scientific conduct of the study protocol including data collection, analyses, or interpretation. Additionally, funding sources will play no role in the preparation of study results for presentation or publication.

DISCUSSION

Strategies that may decrease the incidence of RBC-associated pulmonary complications, particularly those that can be rapidly disseminated to clinical practice, remain undefined. We have presented the study protocol and data analysis plans for a phase I/II, multicenter, randomized clinical trial that seeks to test the feasibility, safety, and efficacy of POC washing of allogeneic RBCs in cardiac surgery with the goal of attenuating transfusion-related pulmonary complications. Specifically, we hypothesize that POC washing of allogeneic RBCs in cardiac surgery patients will be feasible, safe, and efficacious for the removal of soluble BRMs. Additionally, we hope to gain important mechanistic information regarding the relationship between these potentially pathogenic BRMs and intermediate markers of both TRALI (lung injury biomarkers) and TACO (cardiopulmonary physiologic indices) in transfused patients undergoing cardiovascular surgery. Finally, important clinical outcomes will also be assessed in order to provide essential information in determining the value and feasibility of a larger phase II/III clinical trial of RBC-washing for the reduction of transfusion-related pulmonary complications.

Limitations

Despite notable strengths of this study protocol including a large and accessible at-risk population, an established clinical trial infrastructure, and multidisciplinary experience and expertise in translational, patient-centered transfusion research, there are also limitations. The first relates to the feasibility of point-of-care RBC washing in a time-sensitive environment such as cardiac surgery. Though experience regarding the feasibility of washing allogeneic RBC units in this patient environment is limited, both centers have substantial experience with the

successful implementation of autotransfusion practices. This will be invaluable in ensuring the feasibility of the study protocol. In the unlikely circumstance that feasibility becomes a barrier to progress, we will perform pre-washing of two units of allogeneic RBCs at the time of OR entry for those adjudicated to the washing arm of the trial. These pre-washed RBC units would be stored in appropriate blood coolers until the time of RBC need is determined by the clinical team. Of note, washed RBCs can be stored in coolers for up to 18 hours, as the coolers have been validated to maintain a temperature range between 1 and 6 degrees Centigrade for this length of time. All subsequent units could then be washed as described above. Of note, storage of allogeneic RBCs in blood coolers in the OR for the duration of the surgical procedure is standard of care at the two participating institutions for patients who are predicted at high-risk for RBC transfusion. It should be noted the proposed design to evaluate feasibility of real-time washing preserves the blood product supply and minimizes waste in the event RBCs are not required by the patient.

As a second limitation, our candidate biomarkers may not represent or capture true causal pathways. If promising alternative biomarkers and mechanistic pathways are identified, our stored blood samples from this investigation will be available for future analyses for all study participants providing consent for the use of their specimens for this purpose. Additionally, while we are measuring the concentrations of relevant BRMs from both the RBC unit and the transfusion recipient, we are not measuring the hematocrit of the RBC unit, which may result in incomplete characterization of the total dose of transfused BRMs. Importantly, it is also possible that the putative agents are the RBCs themselves rather than contaminants of the RBC supernatant. Indeed, if washed RBC transfusions show no impact on recipient responses, this may in fact support a key role for the RBC itself rather than BRMs in the RBC storage

supernatant. Although an unexpected finding, this would provide essential insight guiding future research on mitigating RBC-associated TRALI and TACO.

A third concern is that the inflammatory response seen in cardiac surgery may mask between-group differences in our analyses. Previous evaluations of patients undergoing cardiac surgery have identified a significant increase in IL-6 concentrations in those who receive RBC transfusion versus those who do not.[74] However, IL-6 concentrations following cardiac surgery have been shown to remain under 200 pg/ml.[75,76] This concentration falls well below levels typically encountered with lung injury, which are frequently greater than 500 pg/mL.[46,48] Additionally, we expect an even greater separation of biomarkers evaluating specific lung-injury pathways rather than markers of general inflammation.

Another area of concern relates to RBC storage duration. Although equipoise remains, clinical data suggests the potential importance of RBC storage duration on patient-important outcomes.[77-80] Previous work has also shown clear temporal changes in the biochemical profile of stored RBC supernatant.[19,21,22,65,81,82] Recent evidence suggests that RBC storage age beyond 6 weeks results in increased extravascular hemolysis but storage age of 5 weeks or less does not.[83] Therefore, it is possible that variability in RBC storage duration may impact our results. However, we have outlined a statistical plan to address this potential concern (see statistical considerations). In addition, we hypothesize that the washing protocol will attenuate the effects of storage duration. It should also be mentioned that washed RBC units outdate after 24 hours. Hence, any washed RBCs not transfused within 24 hours will be discarded and the incidence of this occurrence recorded. However, as washed RBCs will only be administered on the day of surgery, this should not impact study results.

Finally, the study protocol is not adequately powered to fully evaluate clinical outcomes (Aim 3). Such hypotheses would more adequately be addressed in a larger phase II/III clinical trial. Nonetheless, we believe the clinical evaluations outlined in this protocol will provide essential preliminary data that can inform the merit and feasibility of a future more definitive phase II/III clinical trial.

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Contributorship Statement.

Matthew A. Warner, MD. Contributed to the study design and conduct, and writing of the manuscript.

Ian J. Welsby, MBBS. Contributed to the conception of the work, study design and conduct, acquisition and interpretation of data, and writing of the manuscript.

Philip J. Norris, MD. Contributed to the study design and conduct, acquisition and interpretation of data, and writing of the manuscript.

Christopher C. Silliman, MD, PhD. Contributed to the study design and conduct, and writing of the manuscript.

Sarah Armour, MD. Contributed to the study design and conduct, and writing of the manuscript.

Erica D. Wittwer, MD, PhD. Contributed to the study design and conduct, and writing of the manuscript.

Paula J. Santrach, MD. Contributed to the study design and conduct, and writing of the manuscript.

Laurie A. Meade, RN. Contributed to the study design and conduct, acquisition and interpretation of data, and writing of the manuscript.

Lavonne M. Liedl, RRT. Contributed to the study design and conduct, acquisition and interpretation of data, and writing of the manuscript.

Chelsea M. Nieuwenkamp, MLS(ASCP). Contributed to the study design and conduct, acquisition and interpretation of data, and writing of the manuscript.

Brian Douthit, RN. Contributed to the study design and conduct, acquisition and interpretation of data, and writing of the manuscript.

Camille M. van Buskirk, MD. Contributed to the study design and conduct, and writing of the manuscript.

Phillip J. Schulte, PhD. Contributed to the study design and conduct, acquisition and interpretation of data, and writing of the manuscript.

Rickey E. Carter, PhD. Contributed to the conception of the work, study design and conduct, acquisition and interpretation of data, and writing of the manuscript.

Daryl J. Kor, MD, MSc. Contributed to the conception of the work, study design and conduct, acquisition and interpretation of data, and writing of the manuscript.

All co-authors have provided final approval of the current manuscript version and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Competing Interests. The authors declare that they have no conflicts of interest.

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Data Sharing Statement. Study data will be available for secondary use by contacting the Mayo Clinic principal investigator, Dr. Daryl J. Kor, MD. Access to study data will be made available only for the subset of trial participants who have consented to the use of their study data for this purpose.

References

1. Vlaar AP, Binnekade JM, Prins D, et al. Risk factors and outcome of transfusion-related acute lung injury in the critically ill: a nested case-control study. Crit Care Med 2010;38:771-8.

2. Gajic O, Rana R, Winters JL, et al. Transfusion-related acute lung injury in the critically ill: prospective nested case-control study. Am J Respir Crit Care Med 2007;176:886-91.

3. Silliman CC, Fung YL, Ball JB, Khan SY. Transfusion-related acute lung injury (TRALI): current concepts and misconceptions. Blood Rev 2009;23:245-55.

4. Popovsky MA. The Emily Cooley Lecture 2009 To breathe or not to breathe-that is the question. Transfusion 2010.

5. Popovsky MA. Transfusion and the lung: circulatory overload and acute lung injury. Vox Sang 2004;87 Suppl 2:62-5.

6. Li G, Kojicic M, Reriani MK, et al. Long-term survival and quality of life after transfusion-associated pulmonary edema in critically ill medical patients. Chest 2010;137:783-9.

7. Bux J, Sachs UJ. The pathogenesis of transfusion-related acute lung injury (TRALI). Br J Haematol 2007;136:788-99.

8. Rana R, Fernandez-Perez ER, Khan SA, et al. Transfusion-related acute lung injury and pulmonary edema in critically ill patients: a retrospective study. Transfusion 2006;46:1478-83.

9. Kopko PM, Popovsky MA. Pulmonary injury from transfusion-related acute lung injury. Clin Chest Med 2004;25:105-11.

10. Silliman CC, Moore EE, Kelher MR, Khan SY, Gellar L, Elzi DJ. Identification of lipids that accumulate during the routine storage of prestorage leukoreduced red blood cells and cause acute lung injury. Transfusion 2011;51:2549-54.

11. Silliman CC. The two-event model of transfusion-related acute lung injury. Crit Care Med 2006;34:S124-31.

12. Khan SY, Kelher MR, Heal JM, et al. Soluble CD40 ligand accumulates in stored blood components, primes neutrophils through CD40, and is a potential cofactor in the development of transfusion-related acute lung injury. Blood 2006;108:2455-62.

13. Bierbaum BE, Callaghan JJ, Galante JO, Rubash HE, Tooms RE, Welch RB. An analysis of blood management in patients having a total hip or knee arthroplasty. J Bone Joint Surg Am 1999;81:2-10.

14. Popovsky MA, Audet AM, Andrzejewski C, Jr. Transfusion-associated circulatory overload in orthopedic surgery patients: a multi-institutional study. Immunohematology 1996;12:87-9.

15. Bless NM, Huber-Lang M, Guo RF, et al. Role of CC chemokines (macrophage inflammatory protein-1 beta, monocyte chemoattractant protein-1, RANTES) in acute lung injury in rats. J Immunol 2000;164:2650-9.

16. Andrzejewski C, Jr., Popovsky MA, Stec TC, et al. Hemotherapy bedside biovigilance involving vital sign values and characteristics of patients with suspected transfusion reactions associated with fluid challenges: can some cases of transfusion-associated circulatory overload have proinflammatory aspects? Transfusion 2012;52:2310-20.

17. Klein HG, Anstee DJ, eds. Mollison's Blood Transfusion in Clinical Medicine. 11 ed. Malden, MA: Oxford: Blackwell Publishing; 2006.

18. Vermeulen Windsant IC, de Wit NC, Sertorio JT, et al. Blood transfusions increase circulating plasma free hemoglobin levels and plasma nitric oxide consumption: a prospective observational pilot study. Crit Care 2012;16:R95.

19. Stapley R, Owusu BY, Brandon A, et al. Erythrocyte storage increases rates of NO and nitrite scavenging: implications for transfusion-related toxicity. Biochem J 2012;446:499-508.

20. Singel DJ, Stamler JS. Chemical physiology of blood flow regulation by red blood cells: the role of nitric oxide and S-nitrosohemoglobin. Annu Rev Physiol 2005;67:99-145.

21. Jy W, Ricci M, Shariatmadar S, Gomez-Marin O, Horstman LH, Ahn YS. Microparticles in stored red blood cells as potential mediators of transfusion complications. Transfusion 2011;51:886-93.

22. Gladwin MT, Kim-Shapiro DB. Storage lesion in banked blood due to hemolysis-dependent disruption of nitric oxide homeostasis. Curr Opin Hematol 2009;16:515-23.

23. Burnier L, Fontana P, Kwak BR, Angelillo-Scherrer A. Cell-derived microparticles in haemostasis and vascular medicine. Thromb Haemost 2009;101:439-51.

24. Westphal-Varghese B, Erren M, Westphal M, et al. Processing of stored packed red blood cells using autotransfusion devices decreases potassium and microaggregates: a prospective, randomized, single-blinded in vitro study. Transfus Med 2007;17:89-95.

25. Rosolski T, Matthey T, Frick U, Hachenberg T. Blood separation with two different autotransfusion devices: effects on blood cell quality and coagulation variables. Int J Artif Organs 1998;21:820-4.

26. Gruber M, Breu A, Frauendorf M, Seyfried T, Hansen E. Washing of banked blood by three different blood salvage devices. Transfusion 2013;53:1001-9.

27. Booke M, Fobker M, Fingerhut D, Storm M, Mortlemans Y, Van Aken H. Fat elimination during intraoperative autotransfusion: an in vitro investigation. Anesth Analg 1997;85:959-62.

28. Blumberg N, Heal JM, Gettings KF, et al. An association between decreased cardiopulmonary complications (transfusion-related acute lung injury and transfusion-associated circulatory overload) and implementation of universal leukoreduction of blood transfusions. Transfusion 2010;50:2738-44.

29. Blumberg N, Heal JM, Rowe JM. A randomized trial of washed red blood cell and platelet transfusions in adult acute leukemia [ISRCTN76536440]. BMC Blood Disord 2004;4:6.

30. Biffl WL, Moore EE, Offner PJ, Ciesla DJ, Gonzalez RJ, Silliman CC. Plasma from aged stored red blood cells delays neutrophil apoptosis and primes for cytotoxicity: abrogation by poststorage washing but not prestorage leukoreduction. J Trauma 2001;50:426-31; discussion 32.

31. Silliman CC, Moore EE, Johnson JL, Gonzalez RJ, Biffl WL. Transfusion of the injured patient: proceed with caution. Shock 2004;21:291-9.

32. The 2011 National Blood Collection and Utilization Survey. Washington, DC: Department of Health and Human Services, 2011.

33. Reeves BC, Murphy GJ. Increased mortality, morbidity, and cost associated with red blood cell transfusion after cardiac surgery. Curr Opin Cardiol 2008;23:607-12.

34. Koch CG, Li L, Sessler DI, et al. Duration of red-cell storage and complications after cardiac surgery. N Engl J Med 2008;358:1229-39.

35. Koch CG, Li L, Duncan AI, et al. Morbidity and mortality risk associated with red blood cell and blood-component transfusion in isolated coronary artery bypass grafting. Crit Care Med 2006;34:1608-16.

36. Koch C, Li L, Figueroa P, Mihaljevic T, Svensson L, Blackstone EH. Transfusion and pulmonary morbidity after cardiac surgery. Ann Thorac Surg 2009;88:1410-8.

37. Goudie R, Sterne JA, Verheyden V, Bhabra M, Ranucci M, Murphy GJ. Risk scores to facilitate preoperative prediction of transfusion and large volume blood transfusion associated with adult cardiac surgery. Br J Anaesth 2015;114:757-66.

38. Murphy GJ, Reeves BC, Rogers CA, Rizvi SI, Culliford L, Angelini GD. Increased mortality, postoperative morbidity, and cost after red blood cell transfusion in patients having cardiac surgery. Circulation 2007;116:2544-52.

39. Li G, Rachmale S, Kojicic M, et al. Incidence and transfusion risk factors for transfusion-associated circulatory overload among medical intensive care unit patients. Transfusion 2011;51:338-43.

40. O'Leary MF, Szklarski P, Klein TM, Young PP. Hemolysis of red blood cells after cell washing with different automated technologies: clinical implications in a neonatal cardiac surgery population. Transfusion 2011;51:955-60.

41. Mehta RL, Kellum JA, Shah SV, et al. Acute Kidney Injury Network: report of an initiative to improve outcomes in acute kidney injury. Critical care 2007;11:R31.

42. Bakkour S, Acker JP, Chafets DM, et al. Manufacturing method affects mitochondrial DNA release and extracellular vesicle composition in stored red blood cells. Vox Sang 2016;111:22-32.

43. Danesh A, Inglis HC, Jackman RP, et al. Exosomes from red blood cell units bind to monocytes and induce proinflammatory cytokines, boosting T-cell responses in vitro. Blood 2014;123:687-96.

44. Ware LB, Koyama T, Billheimer DD, et al. Prognostic and pathogenetic value of combining clinical and biochemical indices in patients with acute lung injury. Chest 2010;137:288-96.

45. Parsons PE, Eisner MD, Thompson BT, et al. Lower tidal volume ventilation and plasma cytokine markers of inflammation in patients with acute lung injury. Crit Care Med 2005;33:1-6; discussion 230-2.

46. Meduri GU, Headley S, Kohler G, et al. Persistent elevation of inflammatory cytokines predicts a poor outcome in ARDS. Plasma IL-1 beta and IL-6 levels are consistent and efficient predictors of outcome over time. Chest 1995;107:1062-73.

47. McClintock D, Zhuo H, Wickersham N, Matthay MA, Ware LB. Biomarkers of inflammation, coagulation and fibrinolysis predict mortality in acute lung injury. Crit Care 2008;12:R41.

48. Fremont RD, Koyama T, Calfee CS, et al. Acute lung injury in patients with traumatic injuries: utility of a panel of biomarkers for diagnosis and pathogenesis. J Trauma 2010;68:1121-7.

49. Ware LB, Matthay MA, Parsons PE, et al. Pathogenetic and prognostic significance of altered coagulation and fibrinolysis in acute lung injury/acute respiratory distress syndrome. Crit Care Med 2007;35:1821-8.

50. Prabhakaran P, Ware LB, White KE, Cross MT, Matthay MA, Olman MA. Elevated levels of plasminogen activator inhibitor-1 in pulmonary edema fluid are associated with mortality in acute lung injury. Am J Physiol Lung Cell Mol Physiol 2003;285:L20-8.

51. Christie JD, Robinson N, Ware LB, et al. Association of protein C and type 1 plasminogen activator inhibitor with primary graft dysfunction. Am J Respir Crit Care Med 2007;175:69-74.

52. Ware LB, Koyama T, Billheimer DD, et al. Prognostic and pathogenetic value of combining clinical and biochemical indices in patients with acute lung injury. Chest 2010;137:288-96.

53. Fremont RD, Koyama T, Calfee CS, et al. Acute lung injury in patients with traumatic injuries: utility of a panel of biomarkers for diagnosis and pathogenesis. J Trauma 2010;68:1121-7.

54. Ware LB, Eisner MD, Thompson BT, Parsons PE, Matthay MA. Significance of von Willebrand factor in septic and nonseptic patients with acute lung injury. American journal of respiratory and critical care medicine 2004;170:766-72.

55. Ware LB, Conner ER, Matthay MA. von Willebrand factor antigen is an independent marker of poor outcome in patients with early acute lung injury. Crit Care Med 2001;29:2325-31.

56. Rubin DB, Wiener-Kronish JP, Murray JF, et al. Elevated von Willebrand factor antigen is an early plasma predictor of acute lung injury in nonpulmonary sepsis syndrome. J Clin Invest 1990;86:474-80.

57. Moss M, Ackerson L, Gillespie MK, Moore FA, Moore EE, Parsons PE. von Willebrand factor antigen levels are not predictive for the adult respiratory distress syndrome. American journal of respiratory and critical care medicine 1995;151:15-20.

58. Calfee CS, Eisner MD, Parsons PE, et al. Soluble intercellular adhesion molecule-1 and clinical outcomes in patients with acute lung injury. Intensive Care Med 2009;35:248-57.

59. Covarrubias M, Ware LB, Kawut SM, et al. Plasma intercellular adhesion molecule-1 and von Willebrand factor in primary graft dysfunction after lung transplantation. Am J Transplant 2007;7:2573-8.

60. Agouridakis P, Kyriakou D, Alexandrakis MG, et al. The predictive role of serum and bronchoalveolar lavage cytokines and adhesion molecules for acute respiratory distress syndrome development and outcome. Respir Res 2002;3:25.

61. Eisner MD, Parsons P, Matthay MA, Ware L, Greene K. Plasma surfactant protein levels and clinical outcomes in patients with acute lung injury. Thorax 2003;58:983-8.

62. Greene KE, Wright JR, Steinberg KP, et al. Serial changes in surfactant-associated proteins in lung and serum before and after onset of ARDS. American journal of respiratory and critical care medicine 1999;160:1843-50.

63. Christie JD, Shah CV, Kawut SM, et al. Plasma levels of receptor for advanced glycation end products, blood transfusion, and risk of primary graft dysfunction. Am J Respir Crit Care Med 2009;180:1010-5.

64. Calfee CS, Ware LB, Eisner MD, et al. Plasma receptor for advanced glycation end products and clinical outcomes in acute lung injury. Thorax 2008;63:1083-9.

65. Silliman CC, Clay KL, Thurman GW, Johnson CA, Ambruso DR. Partial characterization of lipids that develop during the routine storage of blood and prime the neutrophil NADPH oxidase. J Lab Clin Med 1994;124:684-94.

66. Grommes J, Alard JE, Drechsler M, et al. Disruption of platelet-derived chemokine heteromers prevents neutrophil extravasation in acute lung injury. Am J Respir Crit Care Med 2012;185:628-36.

67. Conti P, DiGioacchino M. MCP-1 and RANTES are mediators of acute and chronic inflammation. Allergy Asthma Proc 2001;22:133-7.

68. Donadee C, Raat NJ, Kanias T, et al. Nitric oxide scavenging by red blood cell microparticles and cell-free hemoglobin as a mechanism for the red cell storage lesion. Circulation 2011;124:465-76.

69. Li G, Daniels CE, Kojicic M, et al. The accuracy of natriuretic peptides (brain natriuretic peptide and N-terminal pro-brain natriuretic) in the differentiation between transfusion-related acute lung injury and transfusion-related circulatory overload in the critically ill. Transfusion 2009;49:13-20.

70. Tobian AA, Sokoll LJ, Tisch DJ, Ness PM, Shan H. N-terminal pro-brain natriuretic peptide is a useful diagnostic marker for transfusion-associated circulatory overload. Transfusion 2008;48:1143-50.

71. Zhou L, Giacherio D, Cooling L, Davenport RD. Use of B-natriuretic peptide as a diagnostic marker in the differential diagnosis of transfusion-associated circulatory overload. Transfusion 2005;45:1056-63.

72. Bridges EJ, Woods SL. Pulmonary artery pressure measurement: state of the art. Heart Lung 1993;22:99-111.

73. Shi Q, Pavey ES, Carter RE. Bonferroni-based correction factor for multiple, correlated endpoints. Pharmaceutical statistics;11:300-9.

74. Fransen E, Maessen J, Dentener M, Senden N, Buurman W. Impact of blood transfusions on inflammatory mediator release in patients undergoing cardiac surgery. Chest 1999;116:1233-9.

75. Hirai S. Systemic inflammatory response syndrome after cardiac surgery under cardiopulmonary bypass. Ann Thorac Cardiovasc Surg 2003;9:365-70.

76. Allan CK, Newburger JW, McGrath E, et al. The relationship between inflammatory activation and clinical outcome after infant cardiopulmonary bypass. Anesth Analg 2010;111:1244-51.

77. Koch CG, Li L, Sessler DI, et al. Duration of red-cell storage and complications after cardiac surgery. The New England journal of medicine 2008;358:1229-39.

78. Eikelboom JW, Cook RJ, Liu Y, Heddle NM. Duration of red cell storage before transfusion and in-hospital mortality. American heart journal 2010;159:737-43.e1.

79. Manlhiot C, McCrindle BW, Menjak IB, et al. Longer blood storage is associated with suboptimal outcomes in high-risk pediatric cardiac surgery. The Annals of thoracic surgery 2012;93:1563-9.

80. Basran S, Frumento RJ, Cohen A, et al. The association between duration of storage of transfused red blood cells and morbidity and mortality after reoperative cardiac surgery. Anesthesia and analgesia 2006;103:15-20, table of contents.

81. Kristiansson M, Soop M, Saraste L, Sundqvist KG. Cytokines in stored red blood cell concentrates: promoters of systemic inflammation and simulators of acute transfusion reactions? Acta Anaesthesiol Scand 1996;40:496-501.

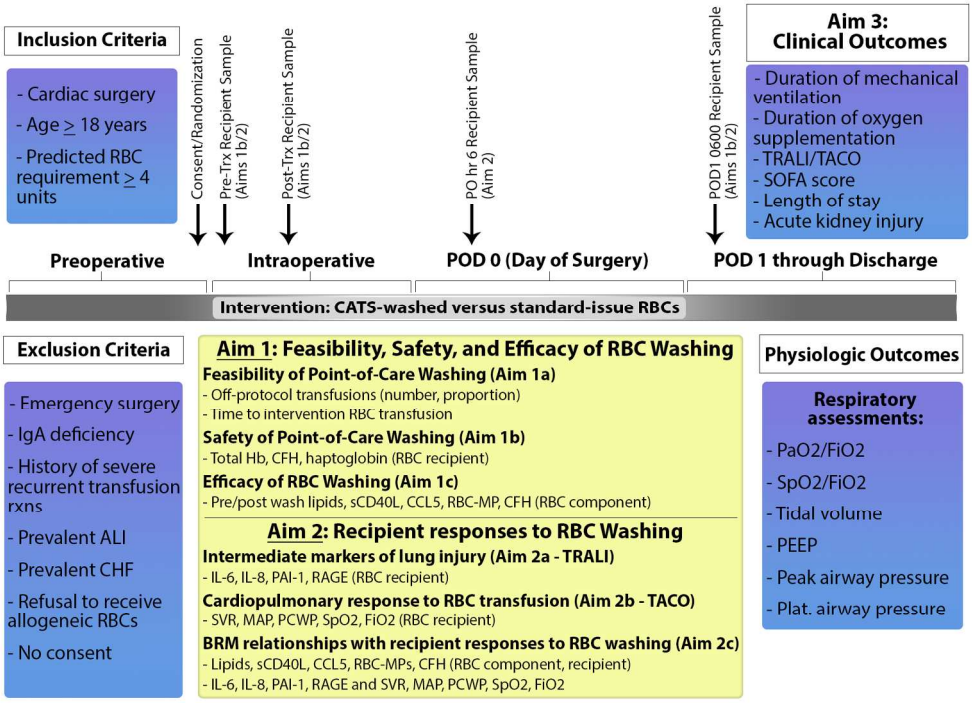
82. Nielsen HJ, Reimert CM, Pedersen AN, et al. Time-dependent, spontaneous release of white cell- and platelet-derived bioactive substances from stored human blood. Transfusion 1996;36:960-5.

83. Rapido F, Brittenham GM, Bandyopadhyay S, et al. Prolonged red cell storage before transfusion increases extravascular hemolysis. J Clin Invest 2017;127:375-82.

Figure Legends

Figure 1. Schematic of the planned study procedures.

ALI – acute lung injury; CATS - Continuous Autotransfusion System; CCL5, chemokine ligand 5; CFH – cell free hemoglobin; CHF – congestive heart failure; FiO2 – fraction of inspired oxygen; Hb – hemoglobin; MAP – mean arterial pressure; PAI-1 – plasminogen activator inhibitor 1; PaO2 – arterial partial pressure of oxygen; PCWP – pulmonary capillary wedge pressure; PEEP – positive end expiratory pressure; PO – postoperative; POD – postoperative day; RAGE – receptor of advanced glycation end-products; RBC – red blood cell; RBC-MP – red blood cell microparticle; Rxs – reactions; sCD40L – soluble CD40 ligand; SOFA – sequential organ failure assessment; SpO2 – oxygen saturation by pulse oximetry; SVR – systemic vascular resistance; TACO – transfusion-associated circulatory overload; TRALI – transfusion-related acute lung injury; Trx – transfusion.



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177x127mm (300 x 300 DPI)

Supplemental materials:

RBC washing protocol with the CATS device.

Responsible	Step	Action
OPERATING ROOM/PATIENT ROOM SET-UP		
Autotransfusion Personnel	1.	Enter OR or patient room with CATS (designated for study) and perform patient ID per Patient Identification.
	2.	Press the I (power on) key until the screen turns on.
	3.	Press the Select Program key to choose the desired wash program. a. Use the ↓ key to find the High Quality Wash program. b. Once the High Quality Wash program is selected, press the Enter key.
	4.	Continue with disposable set-up per CATS.
	5.	Press the Prime key. NOTE: The CATS device recognizes High Quality Wash as an adult prime and will prime with approximately 283 mL of saline.
	6.	Record patient/surgery information on yellow AT worksheet.
	7.	Record lot numbers of disposables on reverse side of yellow AT worksheet.
	8.	Record the AT tech pager number that is responsible for case on RBC Study Sheet for In-Room Provider (see attachment)
PRE – SAMPLE COLLECTION		
	9.	Anesthesia will hand over one unit of RBCs to Autotransfusion personnel.
	10.	Record time of RBC unit request on RBC Study Sheet for In-Room Provider
	11.	Place one unit number label on RBC Study Sheet for In-room Provider and one unit number label on RBC BLOOD BAG PLASMA COLLECTION FORM
	12.	Spike one port of the RBC unit with a sterile plasma transfer set device.
	13.	Attach a sterile 10 mL syringe to plasma transfer set device.
	14.	Draw 6mL of RBCs into syringe.

	15.	Dispense contents of syringe into 10 mL EDTA tube provided by research coordinator.
	16.	Place one patient ID label on EDTA tube and place in biohazard bag.
	17.	Fill out required sections of RBC BLOOD BAG PLASMA COLLECTION FORM: a. Site ID = 001 b. Subject ID = 4 digit # located on In-Room provider sheet c. Check the box next to Plasma #1 Pre-wash d. Date/time of sample collection
	18.	Fill out pink study card with the following information: a. Place one patient ID label in upper left corner b. Record Subject ID c. Record Date/time sample was collected
	19.	Place the RBC BLOOD BAG PLASMA COLLECTION FORM and pink study card in biohazard bag with EDTA sample.
	20.	Hang RBC unit on CATS pole.
PRE-DILUTION OF RBCS BEFORE WASHING		
	21.	Open one 1000 mL bag of saline and hang on CATS pole
	22.	Open Y-type Blood Set with Pump and close both roller clamps.
	23.	Attach female end of Y-type blood set to the male port on the side of the blood collection reservoir.
	24.	Spike 1000 mL bag of saline and open roller clamp.
	25.	Drain entire volume of saline bag into blood collection reservoir.
	26.	Disconnect empty bag and attach another 1000 mL bag of saline.
	27.	Drain 200 mL of saline into blood collection reservoir and close clamp.
	28.	Spike one unit of RBCs with remaining spike.
	29.	Drain contents of RBCs bag into blood collection reservoir. NOTE: The pre-dilution of the RBCs/saline mixture is 4:1.
	30.	Swirl the contents of the reservoir to ensure proper mixing.
	31.	Track the total amount of saline bags used on Comments section of AT worksheet.

WASHING (PROCESSING)		
	32.	Press the start key to begin processing.
	33.	Record the following information on the yellow AT worksheet: a. Unit number (take label from original bag) b. Processed by c. Processing time (Time processing began until processing finished. E.g. 0915-0930)
	34.	Once blood reservoir is empty, press Save Final PRC key.
	35.	Press Save Final PRC key on next screen.
	36.	Record the remaining information in the processing section of the yellow AT worksheet: a. RBC volume recovered b. Transfer pack volume c. Comments section: Track number of saline bags used.
POST - SAMPLE COLLECTION		
	37.	Attach a sterile plasma transfer set device to one port on the reinfusion bag and close clamp.
	38.	Attach a sterile 10 mL syringe to plasma transfer set device and open clamp.
	39.	Draw 6mL of RBCs into syringe.
	40.	Dispense contents of syringe into 10 mL EDTA tube provided by research coordinator
	41.	Place one patient ID label on EDTA tube and place in biohazard bag.
	42.	Fill out required sections of RBC BLOOD BAG PLASMA COLLECTION FORM: e. Site ID = 001 f. Subject ID = 4 digit # located on In-Room provider sheet g. Check the box next to Plasma #2 Post-wash h. Date/time of sample collection
	43.	Fill out pink study card with the following information: d. Place one patient ID label in upper left corner e. Record Subject ID f. Record Date/time sample was collected
	44.	Place the RBC BLOOD BAG PLASMA COLLECTION FORM and pink study card in biohazard bag with EDTA sample.

	45.	Open clamp and drain contents of reinfusion bag into transfer pack.
	46.	Expel air from transfer pack.
	47.	Seal transfer pack using the hand sealer and two hand sealer clips. Cut between the clips.
LABELING		
	48.	Complete Research Only - Washed Allogenic Blood label in the following manner a. Retrieve unit number label from original RBC unit and place in the upper left corner of blood label. b. Place a patient ID label on the lower left corner. c. Record the volume of the washed unit. d. Record the time (hh:mm) that the CATS began washing the RBC unit. e. Record the expiration date/time (4 hours from beginning of wash).
	49.	Affix label to transfer pack.
ADMINISTRATION		
	50.	Perform visual inspection of unit and release unit to Anesthesia/nursing/Perfusion personnel. a. Record initials in the “Inspected and Release by” box on the AT worksheet. b. Record Time transfused/volume transfused.
SENDING SAMPLES		
	51.	OPERATING ROOM: a. Place samples in OR window for lab personnel. b. Press the LAB button the communication panel. PATIENT ROOM: a. Using the nearest small tube station, send all collected samples to 4th tower.
ADDITIONAL WASHED RBCS DURING STUDY TIME PERIOD		
	52.	If additional units are requested to be washed in the current OR or patient room, repeat the following sections in this procedure: a. Pre-sample collection b. Pre-dilution of RBCs before washing c. Processing d. Post-Sample Collection e. Labeling

		f. Administration
	53.	After surgery is complete, perform tear-down/cleaning of CATS device per procedure.
	54.	Transport CATS device and yellow AT worksheet with patient information to patient room.
	55.	Once an order for the first RBC unit is received, go to patient room and perform steps in OPERATING ROOM/PATIENT ROOM SET-UP section.
	56.	Complete all steps in the following sections in this procedure for all subsequent RBC orders: a. Pre-sample collection b. Pre-dilution of RBCs before washing c. Processing d. Post-Sample Collection e. Labeling f. Administration
	57.	After the last order for RBCs and the washing process has been completed, perform tear-down/cleaning of CATS device per procedure.
	58.	Transport CATS device to Autotransfusion office.
	59.	Place yellow AT worksheet on Quality Specialist desk.

Supplemental Materials:

Laboratory sample and biomarker handling.

Aim 1b: 8.5 ml of blood will be drawn from each study participant prior to transfusion, within 30 minutes following the first RBC transfusion, as well as 6 hours (± 30 minutes) and 18 hours (±30 minutes) after the end of the first RBC transfusion for all study participants. If the patient remains in the hospital, safety labs will also be drawn on study day number 5. These safety laboratory assessments (total hemoglobin, CFH, haptoglobin) will be analyzed locally at the enrolling sites using standard clinical assays.

Aim 1c: A 6 ml sample will be taken pre- and post-wash from the already anticoagulated intervention RBC units. An additional aliquot of the RBC unit will be sealed in a capillary tube, centrifuged at 2000 g, and expressed as a decimal fraction using a micro-hematocrit reader. A single 6 ml sample will also be drawn from the standard-issue RBC units prior to administration.

Aim 2: 10 ml of blood will be drawn from each study participant at baseline prior to transfusion, within 30 minutes following the first RBC transfusion, as well as 6 hours (± 30 minutes) and 18 hours (±30 minutes) after the end of the first RBC transfusion. At each time point, blood will be placed in a 10-ml EDTA tube. All samples will be centrifuged at 2500 g for 20 minutes at 20°C within 4 hours of blood draw. The platelet-poor plasma will then be stored in 1.8 ml cryotubes at -80°C. Samples will be batch shipped and analyzed in at Blood Systems Research Institute (San Francisco, CA, USA).

Biomarkers: 240 µl of thawed plasma will be diluted with assay buffers and measured on the Milliplex multi- and singleplex assay platforms (a**ims 1c/2c**: sCD40L, CCL5/RANTES; **aims 2a/2c**: IL-6, IL-8, PAI-1; Millipore, Billerica, MA). Washed, incubated, and labeled samples will be acquired on a Labscan 200 analyzer (Luminex, Austin, TX) and analyzed using Bio-Plex

manager 6.1 software (Bio-Rad). A further 150 µl of plasma will used to perform ELISA-based measurements of RAGE (a**im 2a/2c**; R&D Systems, Minneapolis, MN). NT-proBNP (a**im 2c**) will be measured using a clinical diagnostic system (Novus Biologicals, Littleton, CO). RBC-derived microparticles **(aims 1c/2c):** Thawed platelet-poor plasma will be spun at 13,000 g for 10 minutes at 20°C, then labeled in preparation for flow cytometric measurement (BD LSR II flow cytometer, San Jose, CA). Vesicles will be lysed with NP-40 detergent and samples re-run to confirm results and allow setting of gates. Free hemoglobin **(aims 1c/2c):** The Human Hemoglobin ELISA Kit will be utilized for the detection of free hemoglobin in plasma (Bethyl Laboratories, Inc., Montgomery, TX). Neutral lipids **(aims 1c/2c):** Following the addition of ice-cold methanol, proteins will be precipitated, and non-polar lipids will be extracted/analyzed using high-pressure liquid chromatography (LC) interfaced into the electrospray source of a triple quadrupole mass spectrometer (MS) (liquid chromatography coupled to electrospray ionization mass spectrometry [LC/MS/MS]). Lipid concentrations will be estimated using ratios to an internal standard ($^{2}H^{8}$-5-HETE), as previously described.[1-3]

References:

1. Gijón MA, Zarini S, Murphy RC. Biosynthesis of eicosanoids and transcellular metabolism of leukotrienes in murine bone marrow cells. Journal of lipid research 2007;48:716-25.
2. Jordan JR, Moore EE, Sarin EL, et al. Arachidonic acid in postshock mesenteric lymph induces pulmonary synthesis of leukotriene B4. Journal of applied physiology (Bethesda, Md : 1985) 2008;104:1161-6.
3. Zarini S, Gijón MA, Ransome AE, Murphy RC, Sala A. Transcellular biosynthesis of cysteinyl leukotrienes in vivo during mouse peritoneal inflammation. Proceedings of the National Academy of Sciences of the United States of America 2009;106:8296-301.

Supplemental material:

Standard-issue allogeneic RBC characteristics.

- Mayo Clinic:
  - RBC type: 91% whole blood-derived, 9% apheresis.
  - Additive: 13% AS-1, 87% AS-3, <1% other (e.g., CPD, CPD-1)
  - Leukoreduction:
    - Apheresis: The Fenwal ALYX Component Collection System (Fenwal™) is used to collect double red blood cell product on eligible donors. All RBC units are leukocyte reduced during collection/processing via in-line filters.
    - Whole blood-derived: Whole blood is leukocyte reduced prior to further processing into RBCs, plasma, and cryoprecipitate utilizing the Pall filter, which is part of the Pall whole blood collection bag set with residual leukocyte content < 5×10^6.
- Duke:
  - RBC type: 80% whole blood-derived, 20% apheresis.
  - Additive: 66% AS-1, 5% AS-3, and 29% other (e.g. CPDA).
  - Leukoreduction:
    - Apheresis: Apheresis units are leukoreduced intrinsically by the Trima Accel® (Teruma BCT, Inc.) apheresis system.
    - Whole blood-derived: Whole blood-derived RBCs are passed through a leukocyte reduction filter (Sepacell Flex-Excel for AS-1 and CPDA units; Haemonetics BPF4 for AS-3) after separation of whole blood into its components and after combination with the additive solution. They must have a residual leukocyte content < 5×10^6.

MAYO CLINIC

Name and Clinic Number

Approval Date: **January 27, 2017**
Not to be used after: **September 15, 2017**

RESEARCH PARTICIPANT CONSENT AND PRIVACY AUTHORIZATION FORM

Study Title: Point-of-Care RBC Washing to Prevent Transfusion-Related Pulmonary Complications

IRB#: 13-005965

Principal Investigator: Dr. D. Kor and Colleagues

Please read this information carefully. It tells you important things about this research study. A member of our research team will talk to you about taking part in this research study. If you have questions at any time, please ask us.

Take your time to decide. Feel free to discuss the study with your family, friends, and healthcare provider before you make your decision.

To help you decide if you want to take part in this study, you should know:

- Taking part in this study is completely voluntary.
- You can choose not to participate.
- You are free to change your mind at any time if you choose to participate.
- Your decision won't cause any penalties or loss of benefits to which you're otherwise entitled.
- Your decision won't change the access to medical care you get at Mayo Clinic now or in the future if you choose not to participate or discontinue your participation.

For purposes of this form, Mayo Clinic refers to Mayo Clinic in Arizona, Florida and Rochester, Minnesota; Mayo Clinic Health System; and all owned and affiliated clinics, hospitals, and entities.

If you decide to take part in this research study, you will sign this consent form to show that you want to take part. We will give you a copy of this form to keep. A copy of this form will be put in your medical record.

MAYO CLINIC

Name and Clinic Number

Approval Date: **January 27, 2017**
Not to be used after: **September 15, 2017**

CONTACT INFORMATION

You can contact …	At …	If you have questions about …
Principal Investigator: Dr. Daryl Kor **Study Team Contact:** Laurie Meade, RN	**Phone:** (507) 255-6051 **Phone:** (507) 255-1829 **Address:** 200 First Street SW Rochester, MN 55905	▪ Study tests and procedures ▪ Research-related injuries or emergencies ▪ Any research-related concerns or complaints ▪ Withdrawing from the research study ▪ Materials you receive ▪ Research-related appointments
Mayo Clinic Institutional Review Board (IRB)	**Phone:** (507) 266-4000 **Toll-Free:** (866) 273-4681	▪ Rights of a research participant
Research Subject Advocate **(The RSA is independent of the Study Team)**	**Phone:** (507) 266-9372 **Toll-Free:** (866) 273-4681 **E-mail:** researchsubjectadvocate@mayo.edu	▪ Rights of a research participant ▪ Any research-related concerns or complaints ▪ Use of your Protected Health Information ▪ Stopping your authorization to use your Protected Health Information
Research Billing	**Rochester, MN:** (507) 266-5670	▪ Billing or insurance related to this research study

MAYO CLINIC

Name and Clinic Number

Approval Date: **January 27, 2017**
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A description of this clinical trial will be available on http://www.ClinicalTrials.gov, as required by U.S. Law. This Website will not include information that can identify you. At most, the Website will include a summary of the results. You can search this Website at any time.

1. Why are you being asked to take part in this research study?

You are being asked to take part in this research study because you are having cardiovascular surgery at Mayo Clinic. About 170 people will take part in this research study. The plan is to have about 85 people take part in this study at Mayo Clinic.

2. Why is this research study being done?

The purpose of this study is to determine if washing red blood cells just before blood transfusion prevents pulmonary complications in patients undergoing cardiovascular surgery.

3. Information you should know

Who is Funding the Study?

The National Heart, Lung, and Blood Institute is funding the study. National Heart, Lung, and Blood Institute will pay the Principal Investigator or the institution to cover costs related to running the study.

4. How long will you be in this research study?

You will be in the study until you are discharged from the hospital, or day 28, whichever comes first.

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5. What will happen to you while you are in this research study?

If you agree to be in the study, you will be asked to participate in the following: If you are eligible for the study, we will assign you by chance (like a coin toss) to the *standard red blood cell (RBC)* group or a *washed RBC* group. You and the Principal Investigator can't choose your study group. You will have *an equal chance* of being assigned to the *washed RBC* group. *The decision to transfuse with red blood cells will be left up to your surgical team.* A total of about 4 tablespoons of blood will be drawn from you for the study. Blood will be drawn at four different time points: during your surgery, six hours after your first blood transfusion, 18 hours after your first blood transfusion, and on study day 5, if you are still in the hospital. This blood will be used to look for markers in blood that are associated with lung injury. Your care team will check twice daily to ensure that you are receiving an appropriate level of oxygen supplementation up to day 28 or hospital discharge, whichever comes first.

6. What are the possible risks or discomforts from being in this research study?

The risks of drawing blood include pain, bruising, lightheadedness, and/or fainting, or rarely, infection at the site of the needle stick.

Your doctor will discuss the risks of blood transfusions with you as these procedures are part of your standard clinical care. The purpose of this study is to determine if washed red blood cells are safer than unwashed red blood cells. Although unlikely, it is possible that the washing procedures in this study could damage the red blood cells that are planned to be transfused. If this were to happen, it may make the transfusion less effective.

Many side effects that occur with red blood cell transfusions go away shortly after a transfusion is stopped. However, in some cases side effects can be serious, long lasting, or may never go away. Some side effects may not be known. Side effects may range from mild to life-threatening. Other drugs may be given to make side effects less serious and less uncomfortable. Talk to the researcher and/or your healthcare provider about side effects and ask any other questions.

MAYO CLINIC

Name and Clinic Number

Approval Date: **January 27, 2017**
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7. Are there reasons you might leave this research study early?

You may decide to stop at any time. You should tell the Principal Investigator if you decide to stop and you will be advised whether any additional tests may need to be done for your safety.

In addition, the Principal Investigator, the NIH or Mayo Clinic may stop you from taking part in this study at any time:

- if it is in your best interest,
- if the study is stopped.

If you leave this research study early, or are withdrawn from the study, no more information about you will be collected; however, information already collected about you in the study may continue to be used.

We will tell you about any new information that may affect your willingness to stay in the study.

8. What if you are injured from your participation in this research study?

Where to get help:

If you think you have suffered a research-related injury, you should promptly notify the Principal Investigator listed in the Contact Information at the beginning of this form. Mayo Clinic will offer care for research-related injuries, including first aid, emergency treatment and follow-up care as needed.

Who will pay for the treatment of research related injuries:

Care for such research-related injuries will be billed in the ordinary manner, to you or your insurance. You will be responsible for all treatment costs not covered by your insurance, including deductibles, co-payments and coinsurance.

MAYO CLINIC

Name and Clinic Number

Approval Date: **January 27, 2017**
Not to be used after: **September 15, 2017**

9. What are the possible benefits from being in this research study?

This study may or may not make your health better. However, it may provide important information on how to best manage blood transfusions of patients undergoing cardiovascular surgery in the future.

10. What alternative do you have if you choose not to participate in this research study?

You don't have to be in this study to receive treatment for your condition. Your other choices may include receiving the standard blood transfusion. Talk to the Principal Investigator or your doctor if you have any questions about any of these treatments or procedures.

11. What tests or procedures will you need to pay for if you take part in this research study?

You won't need to pay for tests and procedures which are done just for this research study. These tests and procedures are:

- Washing of the RBCs
- Study labs and processing

However, you and/or your insurance will need to pay for all other tests and procedures that you would have as part of your clinical care, including co-payments and deductibles.

If you have billing or insurance questions call Research Billing at the telephone number provided in the Contact Information section of this form.

MAYO CLINIC

Name and Clinic Number

Approval Date: **January 27, 2017**
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12. Will you be paid for taking part in this research study?

You won't be paid for taking part in this study.

13. What will happen to your samples?

For this study, your de-identified blood samples will be sent to two different external laboratories to look for markers that may indicate pulmonary complications.

We would like to keep your sample for future research. You can still take part in this current study even if you don't want your sample used for future research. If you agree to give your sample, it will be the property of Mayo Clinic.

Other researchers at Mayo Clinic who aren't involved with this study may ask to use your sample for future research. Researchers at other institutions may also ask for a part of your sample for future studies. Your sample will be sent to researchers in a coded format, which protects your identity.

Please read the following statements and mark your choices:

1. I permit my sample to be stored and used in future research of critical illness and lung injury at Mayo Clinic:

☐ Yes ☐ No Please initial here: _____Date: _____

2. I permit my sample to be stored and used in future research at Mayo Clinic to learn about, prevent, or treat any other health problems:

☐ Yes ☐ No Please initial here: _____Date: _____

3. I permit Mayo Clinic to give my sample to researchers at other institutions:

☐ Yes ☐ No Please initial here: _____Date: _____

There is a very small chance that some commercial value may result from the use of your donated sample. If that happens, you won't be offered a share in any profits.

MAYO CLINIC

Name and Clinic Number

Approval Date: **January 27, 2017**
Not to be used after: **September 15, 2017**

You may request to have your sample destroyed by writing to the Principal Investigator. The address is found in the "Contact Information" section of this consent form.

Because we cannot predict how your sample will be used in the future, we cannot promise that samples can be retrieved and destroyed.

14. How will your privacy and the confidentiality of your records be protected?

Mayo Clinic is committed to protecting the confidentiality of information obtained about you in connection with this research study. We will not publish personal identifying information and we use a code to help protect your identity.

During this research, information about your health will be collected. Under Federal law called the Privacy Rule, health information is private. However, there are exceptions to this rule, and you should know who may be able to see, use and share your health information for research and why they may need to do so. Information about you and your health cannot be used in this research study without your written permission. If you sign this form, it will provide that permission.

Health information may be collected about you from:

- Past, present and future medical records.
- Research procedures, including research office visits, tests, interviews and questionnaires.

Why will this information be used and/or given to others?

- To do the research.
- To report the results.
- To see if the research was done correctly.

If the results of this study are made public, information that identifies you will not be used.

Who may use or share your health information?

- Mayo Clinic research staff involved in this study.
- National Institutes of Health (NIH).

MAYO CLINIC

Name and Clinic Number

Approval Date: **January 27, 2017**
Not to be used after: **September 15, 2017**

With whom may your health information be shared?

- The Mayo Clinic Institutional Review Board that oversees the research.
- Researchers involved in this study at other institutions.
- Federal and State agencies (such as the Food and Drug Administration, the Department of Health and Human Services, the National Institutes of Health and other United States agencies) or government agencies in other countries that oversee or review research.
- The sponsor(s) of this study and the people or groups it hires to help perform this research.
- A group that oversees the data (study information) and safety of this research.

Is your health information protected after it has been shared with others?

Mayo Clinic asks anyone who receives your health information from us to protect your privacy; however, once your information is shared outside Mayo Clinic, we cannot promise that it will remain private and it may no longer be protected by the Privacy Rule.

Your Privacy Rights

You do not have to sign this form, but if you do not, you cannot take part in this research study.

If you cancel your permission to use or share your health information, your participation in this study will end and no more information about you will be collected; however, information already collected about you in the study may continue to be used.

If you choose not to take part or if you withdraw from this study, it will not harm your relationship with your own doctors or with Mayo Clinic.

You can cancel your permission to use or share your health information at any time by sending a letter to the address below:

Mayo Clinic
Office for Human Research Protection
ATTN: Notice of Revocation of Authorization
200 1st Street SW
Rochester, MN 55905

Alternatively, you may cancel your permission by emailing the Mayo Clinic Research Subject Advocate at: researchsubjectadvocate@mayo.edu

MAYO CLINIC

Name and Clinic Number

Approval Date: **January 27, 2017**
Not to be used after: **September 15, 2017**

Please be sure to include in your letter or email:

- The name of the Principal Investigator,
- The study IRB number and /or study name, and
- Your contact information.

Your permission lasts forever, unless you cancel it.

ENROLLMENT AND PERMISSION SIGNATURES:

Your signature documents your permission to take part in this research.

	/ /	: AM/PM
Printed Name	Date	Time

Signature

Person Obtaining Consent

- I have explained the research study to the participant.
- I have answered all questions about this research study to the best of my ability.

	/ /	: AM/PM
Printed Name	Date	Time

Signature

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description
Administrative information		
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym (Page 1)
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry (Page 2)
	2b	All items from the World Health Organization Trial Registration Data Set (Complete, throughout manuscript)
XProtocol version	3	Date and version identifier (Page 2)
XFunding	4	Sources and types of financial, material, and other support (Page 2, 8)
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors (Page 2, 35)
	5b	Name and contact information for the trial sponsor (Page 2)
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities (Page 36)
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee) (Page 10, 23, 24, 26)
Introduction		
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention (Page 6-8)
	6b	Explanation for choice of comparators (Page 8, 11)
Objectives	7	Specific objectives or hypotheses (Page 8, 14-18)

Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory) (Page 9, 19)
Methods: Participants, interventions, and outcomes		
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained (Page 10)
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists) (Page 9-10)
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered (Page 10-13)
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease) (Page 12)
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests) (Page 14, 19-20)
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial (Page 12-13)
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended (Page 14-18)
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure) (Figure 1, Page 10-12)
Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations (Page 18)
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size (Page 9-10)
Methods: Assignment of interventions (for controlled trials)		
Allocation:		

Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions (Page 19)
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned (Page 19)
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions (Page 10)
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how (Page 9, 19)
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial (N/A)
Methods: Data collection, management, and analysis		
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol (Page 14-18, 23-24; Supplemental materials)
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols (Page 14-18, 23-24; Supplemental materials)
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol (Page 14-18, 23-24; Supplemental materials)
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol (Page 19-23)
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses) (Page 19-23)

	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation) (Page 19-24)
Methods: Monitoring		
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed (Page 26)
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial (Page 26)
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct (Page 25-26)
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor (Page 26)
Ethics and dissemination		
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval (Page 26-27)
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators) (Page 24, 27)
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32) (Page 10, 26, supplemental consent form)
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable (Page 28)
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial (Page 23-24)
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site (Page 2, 36)
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators (Page 27-28, 36)

Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation (N/A)
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions (Page 28)
	31b	Authorship eligibility guidelines and any intended use of professional writers (N/A)
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code (Page 27-28)
Appendices		
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates (Supplemental materials)
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable (Supplemental materials)

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.