Published in final edited form as:

Pediatr Crit Care Med. 2015 March ; 16(3): 227–235. doi:10.1097/PCC.0000000000000320.

Longer red blood cell storage duration is associated with increased post-operative infections in pediatric cardiac surgery

Jill M. Cholette, MD¹, Anthony P. Pietropaoli, MD², Kelly F. Henrichs, MT(ASCP)³, George M. Alfieri, MD⁴, Karen S. Powers, MD¹, Richard Phipps, PhD⁵, Sherry L. Spinelli, PhD³, Michael Swartz, PhD⁴, Francisco Gensini, MD⁴, L. Eugene Daugherty, MD¹, Emily Nazarian, MD¹, Jeffrey S. Rubenstein, MD, MBA¹, Dawn Sweeney, MD⁵, Michael Eaton, MD⁵, and Neil Blumberg, MD³

¹Department of Pediatrics, University of Rochester, Rochester New York

²Department of Medicine, University of Rochester, Rochester New York

³Department of Pathology and Laboratory Medicine, University of Rochester, Rochester New York

⁴Department of Congenital Cardiac Surgery, University of Rochester, Rochester New York

⁵Department of Environmental Medicine, ⁵Anesthesiology, University of Rochester, Rochester New York

Abstract

Background—Infants and children undergoing open heart surgery routinely require multiple red blood cell (RBC) transfusions. Children receiving greater numbers of RBC transfusions have increased post-operative complications and mortality. Longer RBC storage age is also associated with increased morbidity and mortality in critically ill children. Whether the association of increased transfusions and worse outcomes can be ameliorated by use of fresh RBCs in pediatric cardiac surgery for congenital heart disease is unknown.

Methods—128 consecutively transfused children undergoing repair or palliation of congenital heart disease with cardiopulmonary bypass who were participating in a randomized trial of washed v. standard RBC transfusions were evaluated for an association of RBC storage age and clinical outcomes. To avoid confounding with dose of transfusions and timing of infection versus timing of transfusion, a subgroup analysis of patients only transfused 1–2 units on the day of surgery was performed.

Results—Mortality was low (4.9%) with no association between RBC storage duration and survival. The post-operative infection rate was significantly higher in children receiving the oldest

Corresponding author and address for reprints: Jill M. Cholette MD, Associate Professor of Pediatrics, University of Rochester Medical Center, Box 667, 601 Elmwood Avenue, Rochester, NY 14642, (p) 585-275-0189; (f) 585-275-0707, Jill.Cholette@urmc.rochester.edu.

Reprints will not be ordered.

Conflict of interest statement: Dr. Blumberg has served as a consultant to, and research grant recipient, from manufacturers of leukoreduction filters (Pall Biomedical, Fenwal) and cell washing devices (Caridian-now Terumo BCT) (Haemonetics). Caridian provided a small proportion of the cell washing sets for patients in the washed arm of the study. No other author has any financial or personal relationship with other people or organizations that could inappropriately influence his/her work.

blood (25–38 days) compared to those receiving the freshest RBCs (7–15 days); (34% v. 7%; $p = 0.004$). Subgroup analysis of subjects receiving only 1–2 RBC transfusions on the day of surgery ($n=74$) also demonstrates a greater incidence of infections in subjects receiving the oldest RBC units [0 of 33 (0%) with 7–15 day storage, 1 of 21 (5%) with 16–24 day storage and 4 of 20 (20%) with 25–38 day storage; ($p=0.01$)]. In multivariate analysis, RBC storage age and corticosteroid administration were the only predictors of post-operative infection. Washing the oldest RBCs (>27 days) was associated with a higher infection rate and increased morbidity compared with unwashed RBCs.

Discussion—Longer RBC storage duration was associated with increased postoperative nosocomial infections. This association may be secondary in part, to the large doses of stored RBCs transfused, from single donor units. Washing the oldest RBCs was associated with increased morbidity, possibly from increased destruction of older, more fragile erythrocytes incurred by washing procedures. Additional studies examining the effect of RBC storage age on post-operative infection rate in pediatric cardiac surgery are warranted.

Keywords

congenital heart disease; cardiac surgery; cardiopulmonary bypass; transfusion; nosocomial infection; red cell storage

INTRODUCTION

Blood transfusions alter humoral and cellular immunity in the recipient, particularly in the setting of cardiac surgery with cardiopulmonary bypass (CPB) (1–6); a procedure which in children, routinely requires red blood cell (RBC) transfusion (7,8). Children receiving greater numbers of RBC transfusions have increased post-operative complications and mortality (9–14). Longer RBC storage age is also associated with increased morbidity and mortality in critically ill children (15–17). Whether the association of increased transfusions and worse outcomes can be ameliorated by use of fresh RBCs is unknown. One of the best characterized signals of blood transfusion-associated immunomodulation is the dose dependent increased risk of post-operative infections and multi-organ failure seen in patients undergoing cardiac surgery, which is partially mitigated by leukoreduction of transfused RBC (18–21).

It is unknown whether transfusion-associated immunomodulation is mediated by the allogeneic, storage altered RBC themselves, the supernatant, or both (22,23). Washed RBC transfusions have been associated with reduced cardiopulmonary complications in aggregate hospital data (24), and reduced inflammation and improved survival in adults with acute leukemia (25). More recently, the RBC storage age has emerged as another factor that may be responsible for post-transfusion morbidity and mortality (26,27). All studies to date are retrospective analyses of databases including very heterogeneous patients treated by multiple physicians. These studies are confounded in most cases by additional transfusions of platelets and plasma which may have independent and differing effects on host immunity, hemostasis and thrombosis (28).

Existing retrospective, observational studies of the role of RBC storage duration in pediatric cardiac surgery outcomes found evidence for increased organ failure and mortality (29–31). Prospective studies examining the association between RBC storage duration and clinical outcomes that are not confounded by concurrent transfusion of other blood products are lacking. We recently completed a randomized clinical trial of washed v. standard RBC transfusions in pediatric cardiac surgery that presents a unique opportunity to assess the relationship between storage age and clinical outcomes (5). Novel advantages of this dataset include prospective, blinded data collection from consecutively enrolled subjects in a controlled clinical trial, exclusive transfusion of RBC (no other blood product transfusions) in >95% of patients, and receipt of RBCs from two donors in the majority of patients. Although each subject received multiple aliquots from each RBC unit (providing few donor exposures), the small size of these infants (mostly neonates) provided a very high ml/kg dose of transfused RBC. Thus, the “oldest” unit received by a patient was often the only unit they received, providing a unique opportunity to focus on the relationship between storage duration and clinical outcomes.

We analyzed our cohort of 128 consecutively transfused pediatric patients to determine whether the storage duration of the RBC transfusion was associated with increased mortality or major morbidity (post-operative infection, thrombosis, duration of mechanical ventilatory or inotropic/vasopressor support). We also determined whether post-storage saline washing of RBC prior to transfusion influenced these associations.

MATERIALS AND METHODS

Subjects

This cohort of transfused children up to 18 years presenting to the University of Rochester Medical Center (URMC) for cardiac surgical repair/palliation with CPB has been previously described (5). The protocol was approved by the URMC Research Subjects Review board and registered with ClinicalTrials.gov (NCT00693498). Subjects were enrolled at their pre-anesthesia visit, with properly witnessed and documented informed consent. Once enrolled, subjects were block-randomized according to age and the presence of cyanosis to the unwashed or washed transfusion strategy maintained throughout their hospital admission.

Transfusions and Surgical Techniques

All blood products were pre-storage leukoreduced, irradiated and ABO identical, without restrictions on storage age. Peri-operative management including anesthesia, CPB technique, surgical repair and postoperative management was performed per our current standard of care. The cardiac surgeons (GMA, FG) were blinded to study assignment to prevent any potential bias on the surgical or CPB management. Obvious differences in packaging and labeling of washed blood products prevented blinding of the perfusionists and anesthesiologists. The Terumo RX05 oxygenator (Terumo Cardiovascular Systems, Ann Arbor, MI) was used for subjects weighing 10kg, and the Terumo RX15 oxygenator for subjects >10kg. RBCs were used in CPB prime for infants weighing <10kg. Fresh frozen plasma (FFP), platelets and/or cryoprecipitate were never included in CPB prime, nor given routinely in the operating room. All patients were managed intra-operatively on CPB with

unfractionated heparin adjusted according to activated clotting times and reversed with protamine. All patients received epsilon aminocaproic acid. Conventional ultrafiltration was employed for all study subjects. All subjects undergoing deep hypothermic circulatory arrest and children six months of age received intravenous corticosteroids on CPB (solumedrol 0.5 mg/kg). Vasoactive medications were utilized to maintain hemodynamics during transition off CPB at the discretion of the cardiac surgical team.

PCICU Management

All patients remained intubated and sedated for transfer to the pediatric cardiac intensive care unit (PCICU). Comprehensive clinical and transfusion details were collected. Subjects were weaned from mechanical ventilation as their cardiopulmonary status allowed. Crystalloid and/or 5% albumin were infused in 10–20mL/kg boluses for clinical findings of hypovolemia and poor cardiac output as needed to maintain hemodynamic stability. Vasoactive medications were adjusted to maintain hemodynamics and end-organ perfusion.

Transfusion of RBC, platelet and hemostatic products were based upon standard URM C PCICU practice that takes into account: 1) the type of cardiac defect and repair; 2) the overall hemodynamic stability of the patient; and 3) the presence/absence of active bleeding/MT output, in addition to the hemoglobin, platelet or coagulation profile. RBC are transfused in 10 mL/kg volumes at a rate at the discretion of the prescribing physician. Subjects received anticoagulation (i.e., aspirin, enoxaparin, warfarin) as appropriate for the type of cardiac defect and surgical palliation/repair.

Blood Bank Blood Component Therapy Procedures

All blood products were prestorage leukoreduced with a PALL BPF High Efficiency Leukocyte removal filter that removes leukocyte, platelets (from the RBC units) and micro-aggregates from the RBC or platelet units. All platelets and RBC were irradiated using a CIS-US IBL 437 Blood Irradiator delivering 2500 centigray. The “age” (number of stored days) of RBC and platelet products prepared for each group was recorded. All blood products were ABO identical.

Washing procedures

A COBE (Gambro/Caridian/Terumo, Lakewood, CO) 2991 Blood Cell Processor separated and removed supernatant plasma proteins, preservative agents and microaggregates from the RBC or platelet unit by centrifugation using a previously published method (32). After separation and concentration of the RBCs, the supernatant was expressed into a waste bag and two additional washing cycles were repeated by adding 0.9% saline solution.

Clinical Outcome Measures

Subjects were monitored daily for clinical complications including requirement of mechanical ventilation or inotropic support, re-operation, hemodynamically significant bleeding or arrhythmias, seizures, sepsis (33), venous thrombosis (34) and/or nosocomial infection as defined below adapted from the CDC surveillance definitions for specific types of infections (35). All information was based upon clinical observations in the medical record.

Nosocomial pneumonia required chest radiograph demonstrating new or progressive infiltrate or consolidation, and at least two of the following: fever $>38^{\circ}\text{C}$, leukocytosis ($\text{WBC} > 20\text{K}$), decline in oxygenation, new onset of purulent sputum or change in sputum character, isolation of an etiologic agent from a tracheal aspirate or isolation of virus or detection of viral antigen in respiratory secretions, or organism cultured from blood, and institution of antimicrobial therapy.

Mediastinitis was diagnosed if the patient has a fever ($>38^{\circ}\text{C}$) and at least one of the following: sternum instability, purulent discharge from the mediastinal area, organisms cultured from blood or mediastinum, spontaneous dehiscence or surgical opening by surgeon for localized signs and symptoms of sternal pain, tenderness, fluctuance and/or erythema, and institution of antimicrobial therapy.

Urinary tract infection was defined by fever $>38^{\circ}\text{C}$ in a patient with an existing or history of an indwelling urinary catheter and either a positive urine culture or urinalysis with leukocyte esterase or nitrites or pyuria or micro-organisms on gram stain and institution of antimicrobial therapy. Bacteremia was defined when a recognized pathogen was identified in one or more cultures and the organism is not related to an infection at another site, in the setting of fever $>38^{\circ}\text{C}$ and institution of antimicrobial therapy. Necrotizing enterocolitis is diagnosed if the infant has at least two of the following signs or symptoms with no other recognized cause: abdominal distention, persistent vomiting, microscopic or gross blood in stools and at least one radiographic abnormality (pneumoperitoneum, pneumatosis intestinalis, unchanging “rigid” loops of small bowel), and institution of antimicrobial therapy. Suspicion of infection without confirmation and treatment was considered “no infection.”

Statistical Analysis

All subjects were randomized to their transfusion group pre-operatively to allow for preparation of the RBC units for the operating room blood cooler prior to surgery. Student's t-test or the Mann-Whitney test was used for continuous outcome data, as appropriate. The chi-square or Fisher's exact test were used for categorical data, as appropriate. Multivariate logistic regression was used to determine whether comorbid conditions, surgical variables, or other transfusion variables confounded the association between transfused RBC age and clinical outcomes. We did not adjust for multiple comparisons as all secondary analyses are considered exploratory in nature. JMP and Statview (SAS Institute, Cary, NC) were used for all statistical analyses. A p value < 0.05 was considered statistically significant.

When it became apparent that the metric of oldest red cell transfused was most strongly associated with clinical outcomes, rather than median or mean age of red cells received, we proceeded to perform an analysis of a subset of the majority ($n=74$ of 128) of patients who received only 1–2 transfusions containing an oldest red cell only on the day of surgery. This restriction minimizes confounding due to transfusion dose (the more difficult the surgery/sicker the patient, the more red cells given, and the more likely an older red cell might be given by chance) and timing (a patient with post-operative adverse event is more likely to receive, after the event, a subsequent transfusion, which may by chance, and irrelevant, be an older red cell)

RESULTS

Subjects

Characteristics of the patients in this study have been previously published (5). In brief, median age was 7 months (range: 2 days to 17 years) and median weight was 6.75 kg (2.2–106.8kg). Thirty-six (23%) were neonates (age <31 days), 39 (24.1%) had single ventricle physiology, 78 (48%) had cyanotic cardiac lesions, and 31 (19%) had diagnosed chromosomal abnormalities or syndromes.

Blood transfusions

Of 162 subjects that completed study procedures, 34 (21%) did not receive any blood products, 17 in each group. Patients received a median of about 3 RBC transfusions, with the majority (58%) receiving 2. Fewer than 5% of subjects received platelets or plasma transfusions.

Mortality

There were eight (4.9%) deaths overall (2 in the washed group, 6 in the unwashed group), with a higher mortality rate in the unwashed (7.4%) than the washed arm (2.5%), however this difference was not statistically significant ($p=0.15$). There was no relationship between duration of RBC storage (oldest, mean, median) and mortality (data not shown).

Infection

Initial analysis showed no relationship between average or median duration of RBC storage and rate of post-operative infection. However, when postoperative infection versus RBC storage age was analyzed by tertiles or quartiles, there was a progressive, proportionally increase in infection rate with longer storage duration. The post-operative infection rate was significantly higher in children receiving the oldest blood (third tertile: 25–38 days) compared to those receiving the freshest RBCs (first tertile: 7–15 days); (34% v. 7%; $p = 0.004$) (Figure 1a). Similar results were found employing quartiles (data not shown), with infection rates progressively increasing from 3% (first quartile: oldest unit 7–14 days in storage) to 34% (fourth quartile: oldest unit 28–38 days in storage) ($p = 0.004$). The types of infection are presented in Table 1. All 26 infections are included in Figure 1a, and the five infections in patients receiving no more than 2 transfusions, only on the day of surgery in our subset analysis in Figure 1b.

Some of the oldest stored RBC were given to patients receiving larger transfusion volumes later in their post-operative course. We chose to analyze as a subset only an oldest RBC unit that was given on the day of surgery to patients receiving a maximum of 1–2 RBC units to minimize confounding from transfusions given after the onset of infection, and from transfusion dose (i.e., the more RBC transfused the more likely an older RBC unit would be transfused).

Post-operative infections occurred in none of 33 subjects whose oldest RBC unit was stored 7–15 days. Infections occurred in 1 of 21 (5%) receiving a RBC of 16–24 days storage, and in 4 of 20 (20%) that received RBC stored 25–38 days ($p=0.01$) (Figure 1b).

Demographic, surgical, and clinical characteristics of this 74 patient subgroup analysis are presented in Table 2. Patients receiving "younger" RBC were younger, smaller, received fewer ml/kg of red cells, and were disproportionately neonates with cyanotic or single ventricle lesions, primarily characteristics that increase the likelihood of infection and morbidity. However, this group had fewer infections and overall less morbidity suggesting a possible protective benefit from the shorter storage duration. In multivariate analysis, maximal storage duration of transfused RBC and corticosteroid administration (more prevalent in the shorter storage group) were the only independent variables predicting post-operative infection after adjustments including age, weight, gender, duration of surgery, duration of cardiopulmonary bypass, ml/kg transfused, type of surgery and other potentially relevant clinical variables (Table 2).

Clinical outcomes for each red cell storage age group in the subset analysis are displayed in Table 3. Despite having more significant risk factors for infection (e.g., central venous catheter days, corticosteroid administration, longer hospital stay), the children receiving a fresher RBC transfusion had significantly fewer infections and less antibiotic administration. When patients receiving corticosteroids were excluded from the analysis, the association between oldest red cell transfused and infection remained significant (data not shown). Likewise, when factors measuring comorbidity/acuity of illness (e.g. RACHS-1 score) and complexity of surgery (e.g., duration of surgery; dose of red cells) were included in multivariate models along with oldest red cell transfused, they were not significant predictors of clinical outcomes, including infection. When neonatal patients, such as those undergoing arterial switch procedures, stage 1 palliation and truncus repairs, were excluded from the analysis, the only significant predictor of infection remained oldest red cell transfused. When these analyses were repeated on the larger, more highly confounded entire patient population, the independent prognostic association of oldest red cell transfused and clinical outcomes was replicated (data not shown).

Impact of Washed Transfusions on Post-operative Infection and Morbidity

When the entire transfused population was evaluated, and patients transfused with RBC that were <15 days storage were analyzed those receiving unwashed blood, experienced statistically significantly longer intubation and inotropic support than patients receiving washed blood of similar age (<15 days maximal storage). In contrast, when patients whose oldest transfusion was stored >28 days were analyzed, those receiving washed blood had more post-operative infections, longer duration of mechanical ventilation and inotrope support compared with patients in the unwashed group (Table 4).

DISCUSSION

Children undergoing palliation or repair of congenital heart defects with CPB routinely require large numbers and volume of RBC transfusions (7–14). Multiple studies have found an association between larger numbers of RBC transfusions and worse clinical outcomes in a wide variety of settings (21,36–38). Whether the storage age of the RBC units transfused is responsible, at least in part, on this association has not been definitively determined. Furthermore, whether transfusion of "fresh" (typically <15 days storage) RBC can

ameliorate the effect of the larger number of transfusions has yet to be studied. The current study would suggest that children undergoing open heart surgery are exposed to progressively increasing risk of post-operative infection as the storage age of the RBC units they receive increases. Remarkably, we found an order of magnitude increase in the association between RBC storage duration and post-operative infection when "fresh" RBC in the first quartile of storage duration were transfused (< 14 days) as compared to older blood in the fourth quartile (> 28 days storage). Associations of this magnitude are rarely due solely to confounding.

We hypothesize that these findings are in part due to a causal relationship between RBC storage duration and post-operative infection in this specific setting. However cautious interpretation is warranted due to the observational design of this study. In addition, all our red cells were irradiated prior to storage, which likely leads to additional damage, and may limit the generalizability of our results in clinical settings where irradiation is not in use. Some of our patients received a single dose of corticosteroids peri-surgically, and while this did not explain our findings, could be a relevant co-factor in the high infection rate seen in some patients. Previous epidemiologic studies are heavily confounded by transfusion timing and dose, but on balance suggest a possible role for storage duration of RBC contributing to nosocomial infections and mortality in critically ill patients. Animal models are also persuasive that prolonged duration of storage, and a large dose of prolonged storage RBC may be deleterious (39). A number of randomized clinical trials are under way to attempt to clarify this issue.

In our patients, washing of RBC appeared to potentially minimize morbidity if storage duration was < 15 days) and exacerbated morbidity when storage age was >15 days (Figures 2 and 3). If the supernatant of the older RBC unit were primarily responsible for the adverse clinical impact of the transfusion, saline washing (and thus removal of the supernatant) of the oldest RBCs would presumably mitigate this effect. Our results suggest that washing of older RBC might not be helpful, but instead might increase the risk for adverse clinical outcomes. If true, this would suggest that the deleterious impact of the RBC transfusion is due to changes in the erythrocyte itself. As storage age increases, so does the fragility of the RBC, with decrease in RBC deformity and making the red blood cell itself vulnerable to the process of washing (40, 41).

This is consonant with the hemolysis-iron hypothesis suggesting that excess free iron mediates the increased risk of nosocomial infection after allogeneic transfusion (42,43). However, these post-hoc unplanned analyses should be considered hypothesis-generating, leading to further investigation. Our findings contrast with a recently reported dog model in which washing exacerbated mortality for shorter storage RBC transfusions and mitigated it for longer storage RBC transfusions (39). This may reflect the difference in the setting (bacterial pneumonia in a dog model, versus cardiac surgery in small children) or in the properties of dog and human red cells subjected to saline washing. Previous work suggests that washed transfusions can improve cure rates in adult leukemia, regardless of red cell storage duration (25). Further study of plasma depletion by washing in critically ill patients and animal model systems are warranted to determine whether this strategy ameliorates the toxic effects of stored red cell transfusions and improves clinical outcomes.

The strengths of this study relate in large part to a patient population that is uniquely suited to analysis of the relationship between RBC transfusion and postoperative complications. Pediatric cardiac surgery patients are particularly vulnerable to post-operative morbidity because of the extensive and technically difficult surgery, and the intensity of required post-operative treatment. Moreover, nearly all require RBC transfusion at predictable and similar time points in their perioperative course (i.e. prime on CPB, in OR, on first post-operative day). Importantly, the RBC (in mL) per kilogram dose for these small patients is remarkably large, and comes in many cases from a single RBC unit. This provides an opportunity to observe the effect of transfusion of older RBC at high dose, and minimizes any confounding effects that might stem from exposure to allogeneic transfusion from multiple donors (usually the case in adults receiving large volume transfusions). Likewise, almost none of the patients received confounding infusions of platelets or plasma, which may independently predispose to increased morbidity or mortality. An additional strength of the current study is that our patient sample was recruited prospectively and consecutively as part of a controlled clinical trial.

Our patient population may also be considered a limitation, since these small subjects received massive weight-based irradiated RBC transfusion dosages far exceeding those received by the vast majority of transfused adult or pediatric patients, thus constraining the generalizability of these findings. Our study is also limited by its observational design that ultimately can only prove associations, not causality.

CONCLUSION

In summary, children undergoing open heart surgery experience a progressively rising risk of post-operative infection as the storage duration of the RBC transfusions they receive increases. The risks associated with older RBC are exacerbated, not mitigated, by pre-transfusion saline washing, which may provide a clue to which component of the RBC unit has greatest impact on the recipient. Clinical trials that test the benefits of shorter RBC storage duration are underway and warranted. Additional research is required to determine whether alternative pre-transfusion washing strategies are beneficial.

Acknowledgments

Financial support: This study was funded in part by a Strong Children's Research Development award from the URM-Department of Pediatrics (JMC), and from the National Institute of Environmental Health Sciences/National Institute of Health (ES01247) and the National Heart Lung and Blood Institute/National Institute of Health (HL100051, HL095467) (RP and NB). These funding sources did not have any role in the study design, collection, analysis, and/or in the decision to submit the manuscript for publication. There are no relationships with industry.

We wish to thank the children and families who participated in this study. We also thank Regina Cable, PNP, for her assistance with consent procedures. We thank the pediatric cardiac operating room staff and perfusionists for their assistance with study procedures. We thank the PCICU attendings, fellows and staff for their assistance with laboratory data collection.

References

1. Wan S, LeClerc JL, Vincent JL. Inflammatory response to cardiopulmonary bypass: Mechanisms involved and possible therapeutic strategies. *Chest*. 1997; 112:676–92. [PubMed: 9315800]

2. Roth-Isigkeit A, Hasselbach L, Ocklitz E, et al. Inter-individual differences in cytokine release in patients undergoing cardiac surgery with cardiopulmonary bypass. *Clin Exp Immunol*. 2001; 125:80–88. [PubMed: 11472429]
3. Amand T, Pincemail J, Blaffart F, et al. Levels of inflammatory markers in the blood processed by autotransfusion devices during cardiac surgery associated with cardiopulmonary bypass circuit. *Perfusion*. 2002; 17:117–23. [PubMed: 11958302]
4. Levy JH, Tanaka KA. Inflammatory response to cardiopulmonary bypass. *Ann Thorac Surg*. 2003; 75:S715–20. [PubMed: 12607717]
5. Cholette JM, Henrichs KF, Alfieri GM, et al. Washing red blood cells and platelets transfused in cardiac surgery reduces postoperative inflammation and number of transfusions: Results of a prospective, randomized, controlled clinical trial. *Pediatric Crit Care Med*. 2012; 13(3):290–9.
6. Prieto MA, Guash S, Mendez JC, et al. Does use of cell saver decrease the inflammatory response in cardiac surgery? *Asian Cardiovascular & Thoracic Annals*. 2012; 21(1):37–42. [PubMed: 23430418]
7. Willems A, Harrington K, Lacroix J, et al. TRIPICU investigators; Canadian Critical Care Trials Group; Pediatric Acute Lung Injury and Sepsis Investigators (PALISI) Network: Comparison of two red cell transfusion strategies after pediatric cardiac surgery: a subgroup analysis. *Crit Care Med*. 2010; 38:649–56. [PubMed: 19789443]
8. Whitney G, Daves S, Hughes A, et al. Implementation of a transfusion algorithm to reduce blood product utilization in pediatric cardiac surgery. *Pediatric Anesthesia*. 2013; 23(7):639–46. [PubMed: 23506389]
9. Iyengar A, Scipione CN, Sheth P, et al. Association of complications with blood transfusions in pediatric cardiac surgery patients. *Ann Thorac Surg*. 2013; 96(3):910–6. [PubMed: 23866807]
10. Redlin M, Kukucka M, Boettcher W, et al. Blood transfusion determines postoperative morbidity in pediatric cardiac surgery applying a comprehensive blood-sparing approach. *J Thorac Cardiovasc Surg*. 2013; 146(3):537–42. [PubMed: 23228399]
11. Kipps AK, Wypij D, Thiagarajan RR, et al. Blood transfusion is associated with prolonged duration of mechanical ventilation in infants undergoing reparative cardiac surgery. *Pediatric Crit Care Med*. 2011; 12:52–56.
12. Szekely A, Sapi E, Kiraly L, et al. Intraoperative and postoperative risk factors for prolonged mechanical ventilation after pediatric cardiac surgery. *Pediatric Anesthesia*. 2006; 16:1166–75. [PubMed: 17040306]
13. Szekely A, Cserep Z, Sapi E, et al. Risks and predictors of blood transfusion in pediatric patients undergoing open heart operations. *Ann Thorac Surg*. 2009; 87:187–97. [PubMed: 19101294]
14. Salvin JW, Scheurer MA, Lauseen PC, et al. Blood transfusion after pediatric cardiac surgery is associated with prolonged hospital stay. *Ann Thorac Surg*. 2011; 91:204–10. [PubMed: 21172513]
15. Gauvin F, Spinella PC, Lacroix J, et al. Association between length of storage of transfused red blood cells and multiple organ dysfunction syndrome in pediatric intensive care patients. *Transfusion*. 2010; 50(9):1902–13. [PubMed: 20456697]
16. Karam O, Tucci M, Bateman ST, et al. Association between length of storage of red blood cell units and outcome of critically ill children: a prospective observational study. *Crit Care*. 2010; 14(2):R57. [PubMed: 20377853]
17. Baltasavias I, Faraoni D, Willems A, et al. Blood storage duration and morbidity and mortality in children undergoing cardiac surgery: A retrospective analysis. *Eur J Anaesthesiol*. 2014; 31(6):310–6. [PubMed: 24492183]
18. Bilgin YM, van de Watering LM, Eijssman L, et al. A Double-blind, randomized controlled trial on the effect of leukocyte-depleted erythrocyte transfusions in cardiac valve surgery. *Circulation*. 2004; 109(22):2755–60. [PubMed: 15148271]
19. Lannan KL, Sahler J, Spinelli SL, et al. Transfusion immunomodulation--the case for leukoreduced and (perhaps) washed transfusions. *Blood Cells Mol Dis*. 2013; 50(1):61–8. [PubMed: 22981700]
20. Murphy GJ, Angelini GD. Indications for blood transfusion in cardiac surgery. *Ann Thorac Surg*. 2006; 82:2323–34. [PubMed: 17126171]
21. Hajjar LA, Vincent JL, Galas FR, et al. Transfusion requirements after cardiac surgery: The TRACS randomized controlled trial. *JAMA*. 2010; 304(14):1559–67. [PubMed: 20940381]

22. Blumberg N. Deleterious clinical effects of transfusion immunomodulation: proven beyond a reasonable doubt. *Transfusion*. 2005; 45:33S–39S. [PubMed: 16086785]
23. Vamvakas EC, Blajchman MA. Deleterious clinical effects of transfusion-associated immunomodulation: fact or fiction? *Blood*. 2001; 97:1180–1195. [PubMed: 11222359]
24. 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. [PubMed: 20561296]
25. Blumberg N, Heal JM, Rowe JM. A randomized trial of washed red blood cell and platelet transfusions in adult acute leukemia. *BMC Blood Dis*. 2004;4–6.
26. Weinberg JA, MacLennan PA, Vandromme-Cusick MJ, et al. The deleterious effect of red blood cell storage on microvascular response to transfusion. *The Journal of Trauma and Acute Care Surgery*. 2013; 75(5):807–12. [PubMed: 24158198]
27. Robinson SD, Janssen C, Fretz EB, et al. Red blood cell storage duration and mortality in patients undergoing percutaneous coronary interventions. *Am Heart J*. 2010; 159(5):876–81. [PubMed: 20435199]
28. Refaai MA, Blumberg N. Transfusion immunomodulation from a clinical perspective: an update. *Expert Rev Hematol*. 2013; 6(6):653–63. [PubMed: 24168641]
29. Manlhiot C, McCrindle BW, Menjak IB, et al. Longer blood storage is associated with suboptimal outcomes in high-risk pediatric cardiac surgery. *Ann Thorac Surg*. 2012; 93(5):1563–9. [PubMed: 22137242]
30. Ranucci M, Carlucci C, Isgrò G, et al. Duration of red blood cell storage and outcomes in pediatric cardiac surgery: an association found for pump prime blood. *Crit Care*. 2009; 13(6):R207. [PubMed: 20025760]
31. Redlin M, Habazettl H, Schoenfeld H, et al. Red blood cell storage duration is associated with various clinical outcomes in pediatric cardiac surgery. *Transfus Med Hemother*. 2014; 41(2):146–51. [PubMed: 24847191]
32. Kalmin ND, Brown DJ. Platelet washing with a blood cell processor. *Transfusion*. 1982; 22:125–7. [PubMed: 7071915]
33. Proulx F, Fayon M, Farrell CA, et al. Epidemiology of sepsis and multiple organ dysfunction in children. *Chest*. 1996; 109:1033–37. [PubMed: 8635327]
34. Monagle P, Chan A, Massicotte P, et al. Antithrombotic therapy in children. The Seventh ACCP Conference on Antithrombotic and Thrombolytic Therapy. *Chest*. 2004; 126:645S–687S. [PubMed: 15383489]
35. CDC surveillance definitions for specific types of infections. *Jan.2014* 17-1 to 17–63.
36. Wang D, Sun J, Solomon SB, et al. Transfusion of older stored blood and risk of death: a meta-analysis. *Transfusion*. 2012; 52(6):1184–95. [PubMed: 22188419]
37. Walsh TS, Boyd JA, Watson D, et al. Restrictive versus liberal transfusion strategies for older mechanically ventilated critically ill patients: A randomized pilot trial. *Crit Care Med*. 2013; 41:2354–63. [PubMed: 23939351]
38. Villanueva C, Colomo A, Bosch A, et al. Transfusion Strategies for Acute Upper Gastrointestinal Bleeding. *NEJM*. 2013; 368(1):11–21. [PubMed: 23281973]
39. Solomon SB, Wang D, Sun J, et al. Mortality increases after massive exchange transfusion with older stored blood in canines with experimental pneumonia. *Blood*. 2013; 121(9):1663–72. [PubMed: 23255558]
40. Harm SK, Raval JS, Cramer J, et al. Haemolysis and sublethal injury of RBCs after routine blood bank manipulations. *Transfus Med*. 2012; 22(3):181–5. [PubMed: 22188550]
41. Kim-Shapiro DB, Lee J, Gladwin MT. Storage lesion: Role of red blood cell breakdown. *Transfusion*. 2011; 51(4):844–51. [PubMed: 21496045]
42. Hod EA, Brittenham GM, Billote GB, et al. Transfusion of human volunteers with older, stored red blood cells produces extravascular hemolysis and circulating non-transferrin-bound iron. *Blood*. 2011; 118(25):6675–82. [PubMed: 22021369]
43. Ozment CP, Turi JL. Iron overload following red blood cell transfusion and its impact on disease severity. *Biochim Biophys Acta*. 2009; 1790(7):694–701. [PubMed: 18992790]

Figure 1.

Figure 1a and 1b. The proportion of patients, with post-operative infection according to red blood cell storage age of the oldest unit received. The population was divided into tertiles, determined by the the oldest unit received (Figure 1a). Similar results were found employing quartiles (data not shown), with infection rates progressively increasing from 3% (oldest unit 7–14 days in storage) to 33% (oldest unit 28–38 days in storage). Figures 1a includes all transfused patients in the original study (n=128). Figure 1b is restricted to patients receiving only 1–2 transfusions which were given only on the day of surgery (n=74).

Table 1

Infections according to red blood cell storage age.

Oldest RBC (Days)	POD Oldest RBC	Total # RBC	Subject Age (mo.)	Surgical Procedure	Steroids Given	Type of Infection	POD of +Culture	Survival
11	5	5	6	ToF repair/PA unifocal.	Yes	MRSA tracheitis/PNA	4	Yes
15	0	3	0.3	Arch recon. +VSD	Yes	E Coli UTI	5	Yes
15	8	6	8	RV to PA Conduit	Yes	S pneumo PNA	7	Yes
18	6	3	6	AVSD repair	Yes	Staph/enterobacter PNA	3	Yes
19	6	5	0.56	Norwood (Sano)	No	S aureus mediastinitis	19	Yes
19	10	13	0.2	Norwood (Sano)	Yes	pseudomonas UTI	29	No
20*	0	1	7	BDG and TVR	Yes	Group A strep bacteremia	11	Yes
21	1	41	24	TVR in BDG	No	pseudomonas PNA	5	No
21	3	4	0.16	Norwood (Sano)	No	enterobacter UTI	9	Yes
22	10	19	0.43	Norwood (Sano)	No	E. Coli peritonitis/NEC	0	Yes
23	0	3	5	AVSD repair	Yes	S aureus/moraxella PNA	3	Yes
23	11	3	9	BDG	No	Enterovirus pneumonia	4	Yes
25	0	3	2	ToF repair	No	S aureus PNA/bacteremia	9	Yes
26	7	6	0.2	Arch recon. + ASD	No	EColi PNA and bacteremia	7	Yes
27	0	3	5	AVSD repair	No	hemophilus bacteremia	5	Yes
27*	0	2	21	Bental + Arch repair	No	H influenza PNA	4	Yes
28	0	6	0.23	Arterial Switch	No	Group b strep bacteremia	15	Yes
28	16	9	2	ToF repair	Yes	Pseudomonas/enterobacter PNA	5	Yes
30	60	15	0.33	Arterial Switch + VSD	Yes	MRSA bacteremia/mediastinitis	3	Yes
33*	0	2	6	VSD repair	Yes	Hemophilus + Ecoli PNA	4	Yes
33	0	3	24	ToF repair	Yes	Moraxella + H influenza PNA	2	Yes
34*	0	1	48	Sub-Aortic membrane	No	Influenza A PNA	1	Yes
34*	0	1	17	VSD repair	Yes	UTI	-	Yes
35	0	5	12	ToF repair	Yes	MRSA mediastinitis	6	Yes
37	0	8	3	Central shunt	Yes	S pneumo PNA	8	Yes
38	42	14	0.23	Arterial Switch + VSD	Yes	S aureus empyema/bacteremia	4	Yes

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

ASD = Atrial septal defect; AVSD = atrioventricular septal defect; BDG = Bidirectional Glenn; MRSA = methicillin resistant staph aureus; NEC = necrotizing enterocolitis; PA = Pulmonary Artery; PNA = pneumonia; POD = post operative day; RBC = red blood cell; RV = right ventricle; ToF = Tetralogy of Fallot; TVR = tricuspid valve replacement; UTI = urinary tract infection; VSD = Ventricular septal defect.

* Patients included in the 74 patient subset analysis who received no more than 2 transfusions, with the oldest transfusion given on the day of surgery (POD = 0).

Table 2

Subject characteristics and intra-operative results by oldest red cell transfused; (1–2 transfusions maximum; oldest red cell on day of surgery).

	Red Blood Cell Age (Days)			p value
	7–15 (n=33)	16–24 (n=21)	25–38 (n=20)	
Subject Age (mo.)	16 (5.8–26)	18 (4.8–31)	21 (11–31)	0.01
RBC Transfusions	1.3 (1.2–1.5)	1.1 (0.9–1.3)	1.4 (1.3–1.7)	0.06
RBC Dose (mL/kg)	83 (42–124)	102 (77–125)	101 (74–125)	0.02
Age of oldest RBC	12 (11–13)	20 (19–21)	29 (28–31)	<0.001
Male sex	20 (61%)	9 (43%)	15 (79%)	0.06
Weight (kg)	8.3 (5.3–11.3)	9.6 (5.8–13.4)	10.2 (6.2–14.2)	0.02
Neonate (< 30 days)	8 (24%)	0	0	0.001
Genetic syndrome	7 (21%)	5 (24%)	5 (26%)	0.91
Cyanotic	18 (55%)	7 (33%)	4 (21%)	0.04
Single Ventricle	4 (12%)	2 (10%)	1 (5%)	0.70
RACHS-1 score	2.8 (2.5–3.1)	2.5 (2.1–2.9)	2.4 (2.1–2.7)	0.56
Procedure				0.11
Stage 1 palliation	2 (6%)	0	0	
Arterial switch	4 (12%)	0	0	
Truncus + IAA repair	1(3%)	0	0	
BDG or Fontan	1 (3%)	2 (10%)	1 (5%)	
Tetralogy of Fallot	13 (39%)	5 (24%)	3 (16%)	
RV to PA conduit	2 (6%)	5 (24%)	2 (11%)	
Aortic root or Ross	0	1 (5%)	1 (5%)	
ASD, VSD or AVSD	7 (21%)	7 (33%)	10 (53%)	
Arch ± ASD/VSD	1 (3%)	1 (5%)	0	
Other*	2 (6%)	0	2 (11%)	
OR duration (min)	216 (189–243)	201 (173–230)	210 (175–245)	
CPB duration (min)	112 (94–130)	95 (73–118)	96 (71–120)	0.84
Aortic cross clamp	26 (79%)	17 (81%)	16 (84%)	0.40
Aortic cross clamp (min)	78 (60–95)	62 (39–84)	65 (42–88)	0.89
DHCA	4 (12%)	0 (0%)	1 (5%)	0.35
DHCA duration (min)	28 (21–34)	0	14	0.12
RBC in prime	27 (82%)	15 (71%)	14 (74%)	0.16
CPB prime vol. (mL)	217 (162–273)	281 (211–352)	201 (123–280)	0.63
Hemoconc. vol. (mL)	599 (442–757)	682 (480–885)	794 (587–1,002)	0.49

Values expressed as: number (%) or mean ± 95% confidence interval. p values were derived from Chi-square and the likelihood ratio for proportions and the Kruskal-Wallis test for continuous data.

* Other surgeries include: subaortic membrane resection, pulmonary artery unifocalization, coronary re-implantation and repair of partial or total anomalous pulmonary venous return.

CPB = cardiopulmonary bypass; RACHS = Risk adjusted congenital heart surgery; IAA = interrupted aortic arch; BDG = bidirectional Glenn; PA = pulmonary artery; ASD = atrial septal defect; VSD = ventricular septal defect; AVSD = atrioventricular septal defect; DHCA = deep hypothermic circulatory arrest; RV = right ventricle; RBC = red blood cell; OR = operating room.

Table 3

Clinical outcomes in subjects receiving less than or equal to 2 total red blood cell transfusions.

	Oldest Red Blood Cell Storage Age (days)			p value
	7–15 (n=33)	16–24 (n=21)	25–38 (n=20)	
Platelet transfusions	0	0	0	-
FFP transfusions	0	0	0	-
Cryo. transfusions	0	0	0	-
Mechanical ventilation (hrs)	39 (8–55)	24 (3.4–44)	48 (28–70)	0.43
Inotropes/pressors (hrs)	49 (33–64)	39 (19–59)	55 (34–76)	0.88
CVC (days)	3.6 (3.0–4.1)	2.8 (2.1–3.6)	3.1 (2.4–3.9)	0.11
Mediastinal tube (days)	6.7 (5.8–7.6)	5.8 (4.6–6.9)	6.7 (5.5–8.0)	0.44
Antibiotics	2 (6%)	1 (5%)	5 (26%)	0.044
Infection	0	1 (5%)	4 (21%)	0.01
Steroids	10 (30%)	8 (40%)	5 (26%)	0.63
Thrombosis	0	0	0	-
Arrhythmias	8 (24%)	2 (9.5%)	5 (26%)	0.29
PICU Admission lactate	2.3 (2.0–2.6)	1.7 (1.3–2.1)	2.1 (1.7–2.6)	0.07
Peak lactate	3.1 (2.6–3.5)	2.4 (1.9–3.0)	2.7 (2.1–3.3)	0.41
Crystalloid/Albumin (cc)	76 (0–152)	116 (20–212)	178 (77–279)	0.66
PICU complication	3 (9%)	1 (5%)	4 (21%)	0.25
PICU LOS (days)	3.9 (3.2–4.5)	3.6 (2.7–4.4)	4.4 (3.5–5.3)	0.92
Hospital LOS (days)	8.5 (7.3–9.6)	6.2 (4.7–7.6)	7.8 (6.3–9.3)	0.11
ECMO	0	0	0	-
Death	0	0	0	-

Values expressed as: number (%) or mean $\pm$ 95% confidence interval. p values were derived from Chi-square and the likelihood ratio for proportions and the Kruskal-Wallis non-parametric test for continuous data,

CVC = central venous catheter; ECMO = extra-corporeal membrane oxygenation; FFP = fresh frozen plasma; ICU = intensive care unit; LOS = length of stay

Table 4

Effect of washing according to red blood cell storage age.

Red cells	15 days	Unwashed (n = 22)	Washed (n = 22)	p-value
Transfusion dose		520 cc/kg	995 cc/kg	0.026
IL-6 at 6 hours		180 ± 25	92 ± 25	0.006
Mechanical ventilation (hrs)		104 ± 21	41 ± 21	0.008
Inotropic/Pressors (hrs)		94 ± 17	58 ± 17	0.028
Thrombosis		9%	4.5%	0.5
Infection		14%	0%	0.23

Red cells	28 days	Unwashed (n = 17)	Washed (n = 14)	p-value
Transfusion dose		1180 cc/kg	660 cc/kg	0.31
IL-6 at 6 hours		200 ± 64	230 ± 71	0.92
Mechanical ventilation (hrs)		128 ± 58	239 ± 66	0.03
Inotropic/Pressors (hrs)		83 ± 37	234 ± 43	<0.01
Thrombosis		18%	29%	0.89
Infection		18%	57%	0.05