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Bedside allogeneic red blood cell washing with a cell saver to remove cytokines, chemokines and cell-derived microvesicles, a clinical feasibility study.

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Abstract

Background—Removal of cytokines, chemokines and microvesicles from the supernatant of allogeneic red blood cells may help mitigate adverse transfusion reactions. Blood bank based washing procedures present logistical difficulties; therefore, we tested the hypothesis that on-demand, bedside washing of allogeneic red cell units is capable of removing soluble factors and is feasible in a clinical setting.

Methods—There were in vitro and prospective, observation cohort components to this a priori planned sub-study evaluating bedside allogeneic red cell washing, with a cell saver, during cardiac surgery. Laboratory data were collected from the first 75 washed units given to a subset of patients nested in the intervention arm of a parent clinical trial. Paired pre- and post-wash samples from the blood unit bags were centrifuged, supernatant aspirated and frozen at −70°C then batch tested for cell-derived microvesicles, soluble CD40 ligand, chemokine ligand 5 and neutral lipids (all previously associated with transfusion reactions) and cell free hemoglobin (possibly increased by washing). From the entire cohort randomized to the intervention arm of the trial, bedside washing was defined as feasible if at least 75% of prescribed units were washed per protocol.

Results—Paired data were available for 74 units. Washing reduced soluble CD40 ligand (from 143[1–338]ng/ml to zero), chemokine ligand 5 (from 1314[715–2551] to 305[179–488]ng/ml),

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and microvesicle numbers (from 6.90[4.10–20.0] to 0.83[0.33–2.80] x10^6), while cell free hemoglobin concentration increased from 72.6 [53.6–171.6]mg/dl to 210.5[126.6–479.6]mg/dl ($p<0.0001$ for each). There was no effect on neutral lipids.

Bedside washing being determined as feasible for 80 of 81 patients (99%) patients; overall, 293 of 314 (93%) of units were washed per protocol.

Conclusions—Bedside RBC washing was clinically feasible and greatly reduced concentrations of soluble factors thought to be associated with transfusion-related adverse reactions, increasing concentrations of cell free hemoglobin while maintaining acceptable (<0.8%) hemolysis.

Summary statement

We demonstrated the feasibility and capability of bedside washing to remove cytokines, chemokines and microvesicles from red blood cell supernatant prior to transfusion during cardiac surgery.

Keywords

Thoracic Surgery; Transfusion-Related Acute Lung Injury; Transfusion-Associated Circulatory Overload; Transfusion-Associated Dyspnea; Erythrocyte Transfusion

Introduction

Until recently, transfusion-related acute lung injury (TRALI) was the leading cause of transfusion-related death. Fortunately, recent mitigation strategies have substantially reduced the frequency of this life-threatening transfusion complication.[1] However, their impact is primarily limited to the high plasma volume products (plasma and platelets). In contrast, little has been done to address the rate of red cell-mediated adverse transfusion reactions. While the donor antibody-recipient antigen mechanism is classically ascribed as the mechanism underlying transfusion-related acute lung injury resulting from the transfusion of high plasma volume products[2], an alternate mechanism has been proposed after red cell units[3]. Here, it has been postulated that infused chemokines, cytokines or microvesicles (plasma membrane derived vesicular structures 0.1 to 1.0 micrometers in size) in the infused donor red cell unit supernatant (collectively referred to as soluble biological response modifiers), especially soluble CD40 ligand, may activate the recipient's immune system resulting in adverse transfusion reactions[4], including allergic or febrile, non-hemolytic reactions.

With transfusion-related acute lung injury mitigation efforts, transfusion-associated circulatory overload (TACO) has now surpassed the former as the leading cause of transfusion-related death in many countries.[5] The only mitigation strategies suggested has been, diuretic therapy[6]. Accumulating evidence suggests a potential role for soluble mediators in the red cell supernatant in this and other serious transfusion complications,[7,8] which may be particularly relevant in recipients with a significant risk at baseline for volume overload or congestive heart failure, such as in those with cardiac or renal disease. Importantly, soluble mediators are present in the supernatant of red cell products, which may directly cause pro-inflammatory changes in the recipients' innate immune system.

The persistence of red cell-associated reactions presents a significant unmet challenge in transfusion medicine. If the etiological factors underlying red cell-mediated respiratory complications reside within the donor unit supernatant, cell washing prior to transfusion may negate the potential pathophysiologic effects of mediators in the supernatant. Specifically, provision of over 28,000 red cell units washed in the blood bank was associated with a zero incidence of adverse pulmonary reactions[9], albeit in a retrospective analysis with associated limitations, such as under-reporting of adverse outcomes. Unfortunately, the logistical challenges of providing washed red cell units are particularly relevant in the dynamic and time sensitive cardiothoracic surgery environment, while the risk for transfusion-related respiratory complications are likely the greatest.[10] To address these knowledge gaps and feasibility concerns, we hypothesize that on-demand, bedside red cell washing is clinically feasible and effectively removes cytokines, chemokines and red cell derived microparticles from the supernatant of red cell units.

Methods

Study design:

This was a planned sub-study of the parent clinical trial, completed prior to the end of subject enrollment, that addressed the specific question of the feasibility and effectiveness of bedside red cell washing in removing potentially injurious soluble biologic response modifiers from allogeneic red cell units. This is distinct from the comparison of patient level biomarkers and recipient clinical outcomes, powering the parent clinical trial, that we plan to report as a separate manuscript once the data and laboratory analyses are complete.

There were in vitro and prospective, observation cohort components. Firstly, laboratory data were collected from the first 75 washed units given to a sub-set of patients in the intervention arm of a clinical trial evaluating bedside RBC washing, with a cell saver, during cardiac surgery (NCT02094118).[11] Paired pre- and post-wash samples from these blood unit bags were centrifuged, supernatant aspirated and frozen at −70°C, then subsequently batch tested for cell-derived microvesicles, soluble CD40 ligand, chemokine ligand 5 or “Regulated on Activation, Normal T Expressed and Secreted” (often referred to as CCL5/RANTES) and neutral lipids (previously associated with transfusion reactions) and cell free hemoglobin (possibly increased by washing). Secondly, from the entire cohort randomized to the washing arm of the trial, bedside washing was defined as feasible if at least 75% of prescribed units were able to be washed per protocol. IRB approval for this study protocol was obtained from both participating institutions prior to enrolling the first study subject in the parent trial.

Study population:

All adult patients scheduled for cardiac surgery via sternotomy at one of two tertiary referral, academic heart centers (Duke University Medical Center, Durham, NC or Mayo Clinic, Rochester, MN) between May 2015 and June 2019 were screened for study eligibility. Those projected to require 4 or more units of allogeneic RBCs, based on a published scoring system (supplemented by the opinion of experienced cardiac anesthesiologists – IW, WM), were further assessed for trial eligibility.[11] Those meeting

inclusion criteria and lacking all exclusions were approached for consent to participate. Exclusion criteria were emergency surgery, history of severe recurrent transfusion reaction, refusal to receive allogeneic red cells or provide informed consent, prevalent acute lung injury or congestive heart failure prior to randomization, expected hospital stay < 48 hours, not anticipated to survive > 48 hours or a re-operation in a patient previously enrolled in this trial. Following written informed consent, patients were randomized to either the intervention arm or to the control arm for the entire day of surgery. Our laboratory and feasibility data all came from the intervention arm. A Data Safety Monitoring Board met every 6 months to determine the suitability of continuing the study for reasons of safety or futility.

Study intervention:

For transfusions deemed necessary by the clinical team, units were handed to the study team from the 4 red cell units typically provided in a cooler, under ice, for all cardiac cases. These were washed on-demand using a continuous automated transfusion system (CATS, Fresenius Kabi USA LLC, Lake Zurich, IL) with the FDA-approved 0.9% saline wash solution, following a 4:1 dilution with 0.9% saline in the cell-saver reservoir, as previously described.[12] Pre-dilution was included to reduce the hematocrit of packed RBCs to better approximate that of the salvaged blood mixed with heparin anticoagulant that cell-savers were designed to process. This automated washing procedure has been shown to more efficiently remove contaminants from the supernatant.[12,13] This cell-saver system was chosen as the g-force applied to the cells is less for this apheresis belt system (~800G) than for the Latham bowl design favored by most cell-saver devices (~2000 G),[14] and has been reported to induce less hemolysis when compared to a standard blood bank based cell washing device (Cobe 2991, Terumo BCT, Lakewood, CO).[15] Staff trained to use this cell-saver included research personnel, anesthesia technicians and perfusionists (Duke), and transfusion medicine personnel with specific expertise in cellular washing techniques (Mayo).

Laboratory testing:

From the group randomized to receive allogeneic units washed with the cell-saver, 6 ml samples were taken from each unit of blood before and after the washing procedures. Samples were acquired from the first 75 washed units (no more than 4 units from the same individual were included in this aim for the logistics of sample processing), as defined a priori in the study protocol.[11] These paired samples contained red cells and supernatant (plasma, additive solution and anticoagulant pre-wash, and mostly saline post-wash). Samples were stored on ice for less than 2 hours and centrifuged at 2000*g* in refrigerated centrifuges with supernatant removed for a second 2000*g* centrifugation prior to dividing into aliquots for frozen storage (−70°C). Subsequent batch analysis tested the removal of the following biological response modifiers: chemokine ligand 5, a T cell derived chemokine that recruits leucocytes (other than neutrophils) to sites of inflammation; soluble CD40 ligand, a platelet and T cell derived protein that has pro-aggregatory effects on platelets;[16] the neutrophil priming activity of neutral lipids; and cell-derived microparticles present in the unit. The mediators have been shown to accumulate in stored blood products and have previously been associated with lung injury.[10,17–22]

Assays performed included Luminex Assays, Minneapolis and flow cytometry (Professor Philip Norris, Vitalant Research Institute, San Francisco, CA). Flow cytometry characterized the number and cellular origin of microparticles in pre and post-wash samples, with the following pairing: CD62p (P-selectin), activated endothelial cell or platelet derived; CD 235a (Glycophorin A), red cell derived; CD108 (Semaphorin-7A), activated lymphocytes or red cells; CD41a (Glycoprotein IIb), megakaryocyte/platelet derived. Due to the potential for inducing hemolysis with manipulation of the units,[23] cell free hemoglobin was also measured in pre and post wash samples by ELISA (Bethyl Laboratories, Inc., Montgomery, TX). Specific lipid priming assays were performed as previously described (Professor Christopher Silliman, University of Denver, Denver, CO).[24,25] All measurements were performed in triplicate.

Due to concerns about excess sample volume being taken from the 75 patient units, additional data required to calculate % hemolysis (pre- and post-wash hematocrits, product and supernatant volumes and measures of the total amount of cell free hemoglobin present in the supernatants) were only obtained in a post-hoc QC study using 5 test units that had been stored for 18 days. For % hemolysis calculations, cell free hemoglobin was measured by a point of care analyzer (Hemocue Plasma/Low Hb system, HemoCue America, Brea, CA) after immediate, point of care processing to account for the potential for the storage on ice and the processing necessary for microparticle separation inducing hemolysis in the sample from the bags (especially compounding the stress of blood washing). The ratio of the amounts of cell free hemoglobin to total unit hemoglobin (in milligram) was used to derive % hemolysis.

Feasibility of providing washed units:

Provision was made for the clinical teams to administer “off-protocol”, standard issue units should the bedside washing process be expected to or be incurring an unacceptable delay based on the clinical situation. Washing RBC units according to protocol determined feasibility; if an individual patient received 75% of prescribed units on protocol then bedside washing for that patient was considered feasible.

Statistical analysis:

A power and sample size calculation for the overall WAR-PRC trial was reported previously, [11] which was based on biomarker outcomes in patients randomized to control/intervention groups. A power calculation was not performed for this specific aim *a priori*, but a sample size of 75 washed bags with pre- and post-wash samples was thought to be feasible and within budget, based on the clearance of mediators in preliminary data. A total 75 bags with pre- and post-wash paired samples provides 90% power to detect an effect size (location shift divided by the standard deviation of the paired differences) of 0.39 using a Wilcoxon signed-rank test with two-sided alpha level 0.05.

For the primary pre-specified analysis, pre- and post-wash bag samples were compared for each of the primary outcomes using a paired Wilcoxon signed-rank test. Change in HCT was analyzed similarly. In secondary, *post-hoc* analyses, we assess the association between red cell storage additive solutions and outcomes. We calculated estimates that reflect the

estimated multiplicative increase in geometric mean of each mediator, microvesicle sub-type and total count, as associated with a one day increase in storage duration. We also assessed whether there was a non-linear relationship between storage duration and outcomes. Pre-wash outcomes are compared across additive solutions using Kruskal-Wallis tests. Post-wash outcomes are analyzed using linear regression, adjusted for pre-wash values. Log transformations were applied to satisfy assumptions for normality of residuals and results transformed to report the association between additive solutions and multiplicative change in the geometric mean outcome. The association between storage age and outcomes was assessed similarly.

Applying Bonferroni correction to our 9 total a *priori* outcomes analyzed would specify $p<0.0055$ for significance. Post-hoc analyses are identified as such. Statistical test were two-tailed and data were analyzed in using R software (version 3.6.1)

Results

Of 314 red cell transfusions prescribed by the clinical team on the operative day among 81 patients in the washed arm, we were able to complete the bedside washing procedures per protocol on 293 units (93%). Feasibility was defined at the patient level, with patients considered feasible if at least 75% of prescribed units were washed per protocol; all transfused patients were evaluated for the feasibility of providing units washed at the bedside with a delay acceptable to the clinical team. By this definition, of 81 patients receiving a transfusion on the operative day, 80 (99%) were considered feasible. A total of 21 non-washed units were provided off-protocol to 11 patients randomized to the washing arm of the trial. The time from decision to transfuse to start of transfusion (median (25^{th} – 75^{th}) percentiles) for all units transfused on the day of surgery was 5 (3–12) minutes for the standard and 31 (23–50) minutes for the washed arms. This suggests the washing process approximately incurs a 25 minute delay in transfusion initiation, which was acceptable to the clinical team for 99% of patients.

Complete data from paired pre- and post-wash samples, with sufficient volume to test all proposed biological response modifiers and cell free hemoglobin, were available from 74 of the 75 units tested (39 from the Duke site and 35 from Mayo). The distribution of storage ages of transfused units (standard blood bank issue) are illustrated in Figure 1 with a median storage duration (median (25^{th} – 75^{th}) percentile) of 15.5 (12–23) days (max 41 days); the majority of units (65%) had been stored for 20 days or less. While storage age duration was unrelated to any biomarker or cell free hemoglobin concentrations, there were additive solution dependent differences. Most units (n=41, 55%) were stored in additive solution-3 with the remainder in the mannitol containing additive solution-1 (n=24, 32%) or additive solution-5 (n=8, 11%); there was one citrate/phosphate/dextrose/adenine-1 unit (not analyzed). All units from the Mayo Clinic were stored in additive solution-3. After adjusting for pre-wash cell free hemoglobin, there was still evidence of post-wash differences by additive solution group (overall p=0.021). Specifically, in *post hoc* pairwise comparisons, there is evidence that for post-wash, additive solution-3 was associated with 53% higher cell free Hb compared to additive solution-1 units (estimated ratio [95%CI] 1.53 [1.06, 2.22], p=0.027) and 91% higher concentrations compared to additive solution-5 units (1.91 [1.11,

3.28], p=0.022). There were also significantly more platelet derived microvesicles post-wash in the additive solution-3 units (11.19 [2.8–44.67]; p=0.001 and 2.08 [1.34–3.25]; p=0.002 respectively) and overall more microparticles in the additive solution-3 units (1.66 [1.03–2.69]; p=0.043) compared to additive solution-1 units.

Combining the data from both sites, we identified a wide range of concentrations of soluble mediators in the individual units prior to washing varying over 1000-fold for chemokine ligand 5 over 10,000-fold for soluble CD40 ligand and almost 100-fold for total microvesicle counts between units. These data are detailed in Table 1. As shown in Figures 2 and 3, there were significant reductions in the amount of each marker after washing with a clearance of 93, 76, 86% for soluble CD40 ligand, chemokine ligand 5 and total microvesicles respectively (significant p-value after Bonferroni correction < 0.0055). Only 1% of the median counts of microvesicles were identified by a cell specific marker, but microvesicles from all cell lines were significantly reduced (p<0.004 for all), as shown in Table 1. In terms of neutral lipids, there was no difference in the neutrophil priming ability of the red cell supernatants before or after washing (p=0.694).

From a safety standpoint, there was an overall increase in median cfHb concentration in the supernatant from ~70mg/dl to ~210mg/dl as measured by ELISA after two 20 minute, 2000g centrifugations. In a post-hoc study of purchased additive solution-3 units (n=20), we measured a median (IQR) increase in Hct from 62 (58–64) to 66 (60–70)% (p=0.004) after the washing procedure, and an expected decrease in product volume from 330ml to 262ml (n=5 test units, not compared); after a 800g centrifugation to separate cells and supernatant, the average dose of cell free hemoglobin from the supernatant of these 5 test units was less post wash than pre wash (85mg versus 148mg), mostly due to hemoconcentration. The % hemolysis of these 5 test units ranged from 0.1 to 0.3% pre-wash to 0.1 to 0.2% post-wash, all less than the acceptable upper limit of 0.8%. The % hemolysis was not calculated for all 75 washed patient units, as hematocrit and volume pre and post wash were not able to be measured with the time pressure of the clinical workflow.

Discussion

The reported data support the feasibility of bedside washing of allogeneic RBC units in the setting of elective major cardiac surgery both in the operating room and in the early ICU course, with nearly all of the units assigned to the washed group being washed. Additionally, bedside washing of pre-diluted, allogeneic RBC units was able to eliminate the vast majority of cytokines, chemokines and microvesicles. This study suggests bedside washing with this cell-saver is feasible, safe, and greatly reduces the concentrations of potentially injurious factors in the red cell supernatant.

Transfusion-related hyperkalemic cardiac arrest has previously been described in adult and pediatric patients[26] and bedside washing has successfully reduced hyperkalemia in small case series[27] (useful especially in pediatric cases, recipients with renal failure or massive transfusions). Washing cellular blood products in pediatric cardiac surgery has also been shown to reduce lactate concentrations[28] and pro-inflammatory markers[29] in transfusion recipients. The removal of cytokines, chemokines and microvesicles that have previously

been associated with transfusion-associated respiratory complications supports the hypothesis that bedside red cell washing may provide a means for abrogating the effect of injurious mediators in the supernatant, especially in at risk patients. In a study of adverse reactions to platelet transfusions[4] (the product most commonly associated with reactions), Cognasse et al identified that most patients with an adverse reaction had received a higher concentration of soluble CD40 ligand and the "pathogenic threshold" they calculated was 6.4 ng/ml. In contrast, the concentrations in our units before washing (albeit using a different assay platform) were higher, with median [IQR] 143[1–338] ng/ml pre-wash and 0[0–0] ng/ml post-wash. Based on this, we believe our washing procedure is capable of removing enough of this cytokine to possibly be able to reduce transfusion reactions with the potential to improve clinical outcomes in a larger, suitably powered study. By effectively removing the vast majority of protein,[14,30] bedside washing offers the possibility of also reducing antibody-mediated transfusion-related acute lung injury. We did not specifically test for immunoglobulins, though, so removal of these antibodies remains speculative.

The elimination of neutral lipids was ineffective with our protocol, which was surprising, as this has previously been demonstrated, albeit with a blood bank washing protocol and using blood stored for 42 days.[8] Whether residual lipids remained after washing the stored units or were released as a result of hemolysis induced by washing is unknown. Regardless, the remaining lipids were capable of neutrophil priming and, therefore, theoretically capable of inducing lung injury or activating endothelium and predisposing to later injury.[10]

As has been previously reported,[31] red cell washing incurred an 3-fold increase in cell free hemoglobin concentration in the reduced volume supernatant, although, when calculated, %hemolysis remained within acceptable range (<0.8%).[33] Our findings regarding additive solutions were unexpected, although the mannitol in additive solution-1 may promote membrane stability[32] and, ideally, reduce hemolysis. They could also reflect variations in red cell processing techniques or inter-donor variability associated with the blood centers that favor additive solution-3. As mentioned, the extent of hemolysis, in the small sub-set we tested, was well below the acceptable level of 0.8%.[33] Similar findings were reported in the REDWASH study in that cell free hemoglobin in the supernatant of washed units was higher than controls, but there was no increase detected after transfusion in vivo, likely due to the avid scavenging of free hemoglobin by haptoglobin in the plasma.[34] There are also some potentially important differences between the REDWASH study and our trial: we prediluted red cell units containing additive solutions-1, 3 and 5 with saline, whereas they used undiluted units with a different additive solution. We also used a more rigorous washing setting on the same cell-saver device. The physiological relevance of the balance between increased cell free hemoglobin and removal of nitric oxide scavenging microvesicles[35] will await future comparisons of the clinical outcomes between the washed versus standard cohorts in the parent study, although there is no reason to expect this to differ from REDWASH. Similarly, lung injury biomarkers were not included in this manuscript as measuring the recipients of these 75 units only from the intervention arm of the parent trial were not powered or designed to detect any difference. The results of the clinical biomarkers and outcomes associated with the parent clinical trial have not yet been analyzed or published.

Regarding the microvesicle evaluations, their characterization revealed an identifiable cell lineage in only ~1% of detected signals, consistent with prior publications from our group and others.[36,37] Whether they lost the antigens we targeted during storage, the level of antigen per microvesicle was not high enough to detect by flow cytometry, or whether we should have used a broader panel to identify relevant antigens is not clear. Alternatively, it is possible that the majority of microvesicles detected represent cell debris or vesicles derived from internal multivesicular bodies rather than vesicles that bear cell surface markers as has been shown previously.[38] Of note, whether the physiological effect of cell debris versus specific cell-derived encapsulated vesicles is equivalent or not, is unknown. However, total microvesicle content derived from red cell units has been shown to support prothrombinase activity and thrombin generation,[39,40] so all may be biologically relevant[41]. Regardless, there were substantial and significant decreases in microvesicle counts for those with identifiable cell lineage as well as for those without.

Our study has important limitations. First, our exclusive use of an apheresis belt system on a high quality wash program setting precludes generalization of our study findings to other cell washing devices. Regarding the more common Latham bowl design, certain models have been used successfully to wash red cell units,[42] while others appear to induce excessive hemolysis.[43] Secondly, we were limited to the FDA approved saline washing solution, whereas the balanced solution Plasmalyte-A (Baxter, Deerfield, IL), appears to be associated with less hemolysis than saline, which has an un-physiologically high chloride level of 154 millimoles per liter and an acidic pH of 5.5.[44,45] Future bedside washing protocols to be evaluated include simplifying the workflow by dilution with 1000 (~1:3) rather than the current 1200ml (1:4), using other cell-saver devices, optimizing red cell by diluting and washing with Plasmalyte A or other balanced, neutral solutions or even reversing aspects of the red cell storage lesion by replenishing 2,3 diphoshoglycerate or removing toxic phthalates.[46] While our median post wash cell free hemoglobin value of 210 mg/dl is similar to a mean post-wash value of 212 mg/dl previously reported using a spectrophotometric technique,[43] the extreme values seen with our ELISA platform that required serial dilutions for most of our samples, present the possibility of error. We only systematically calculated % hemolysis in a small sub-set of washed units, but this would be an essential component of a future quality control process and using spectrophotometric techniques such as that used to measure hemoglobin in our QC sub-set, may offer a simpler solution. Our reported median value of 210mg/dl would result in ~3–5mg/dl increase in cell free hemoglobin (normal value <~15mg/dl), assuming a 5000ml circulating volume and Hct 0.25, but only if the natural hemoglobin scavenger, haptoglobin, were absent. While an increase in cell-free hemoglobin above the normal limit is not expected, confirmation of the safety of this bedside washing procedure will require sampling recipients' plasma.

A third limitation relates to the external validity of our feasibility findings. The two enrolling institutions leveraged research personnel, anesthesia technicians, perfusionists, and transfusion medicine specialists to oversee the bedside washing procedures for the purposes of the study. However, study procedures can be simplified and plausibly performed by existing personnel in the operating room. Allogeneic units can be washed in the cell-saver being routinely used and the only additional workflow would involve gravity draining the saline and red cell unit into the reservoir of the cell-saver using a standard "Y-spike" set.

Further processing is automated and the staff perfusionist (assisted by anesthesia personnel during bypass) could realistically perform this task. This scenario is limited to the OR environment and our data are limited to the CATS, as we did not test other Latham bowl cell-saver designs. Extending the process into the ICU would be more complex and likely to require additional staffing with variable, local, economic implications.

Finally, we did not correlate the present study results with clinical outcomes, as this study was not designed or powered to do so. To be clear, this report is limited to the assessment of our ability to wash allogeneic red blood cell units in the setting of elective cardiac surgery and to quantify the removal of soluble mediators from the allogeneic red blood cell units. It is an a-priori planned sub-study of a parent clinical trial that has completed enrollment, but is pending recipient outcome assessments, data analysis and publication. While this is an important first step in understanding the potential role of bedside allogeneic RBC washing, it is insufficient evidence to support bedside washing for the prevention of transfusion-related respiratory complications. The primary aims of the parent trial, from which the present work arises, will address this question.[11]

In summary, this study demonstrates that bedside washing of allogeneic red cells with a familiar cell-saver technology is feasible and effective in removing soluble mediators immediately prior to transfusion. In acute care environments such as the operative setting, where autologous cell salvage is frequently employed, this technology may offer a practical approach to removing mediators associated with adverse transfusion reactions.

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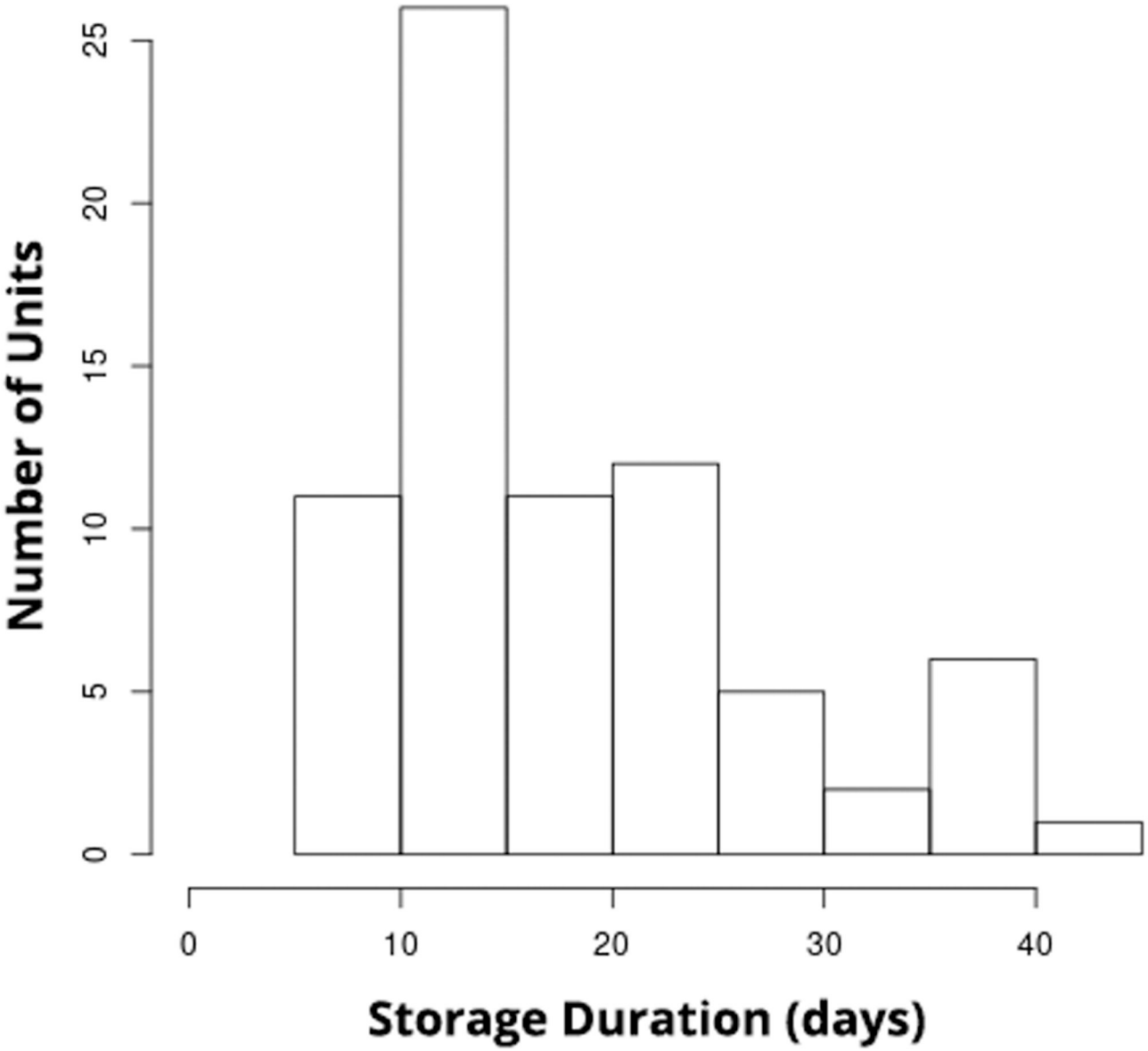
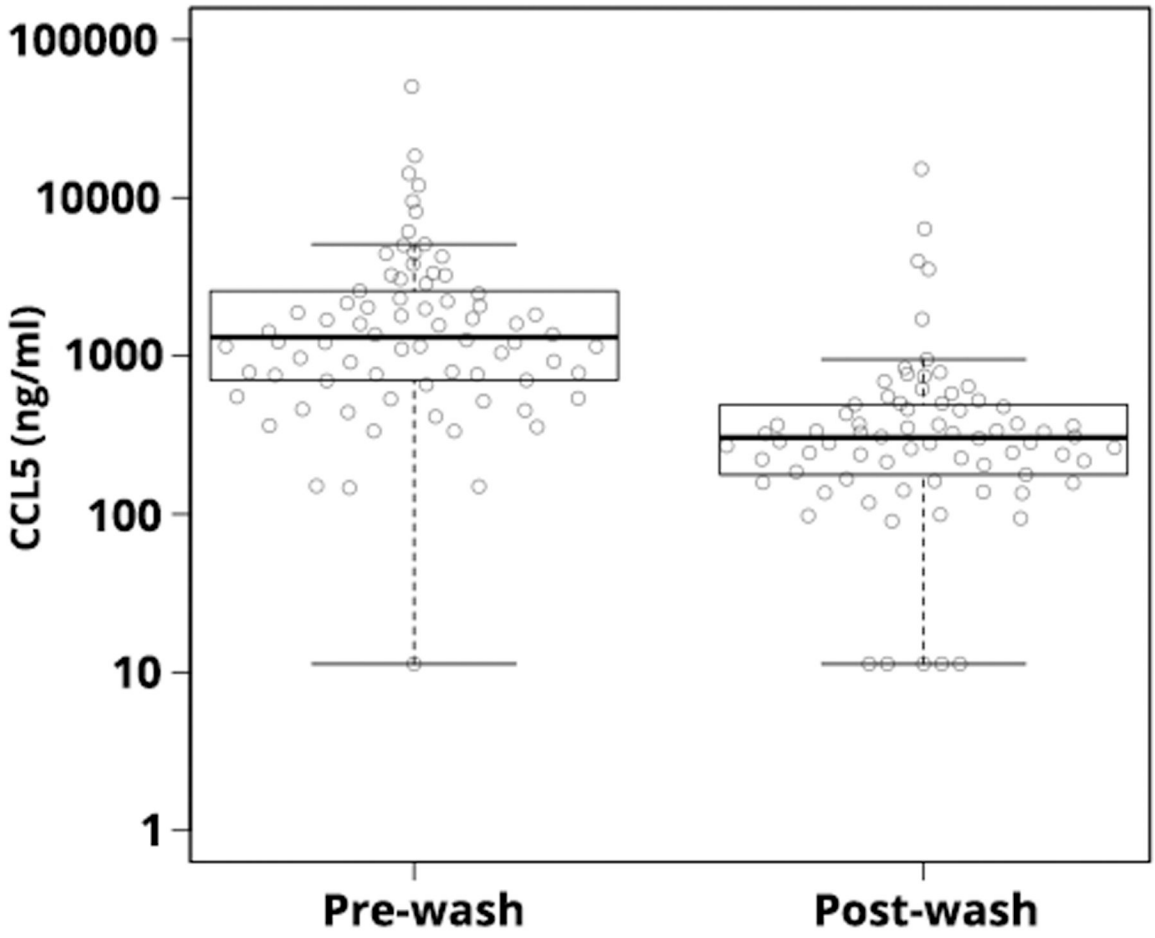


Figure 1.
Histogram of RBC storage duration with storage age ranges on the x-axis and number of units in those categories on the y-axis.



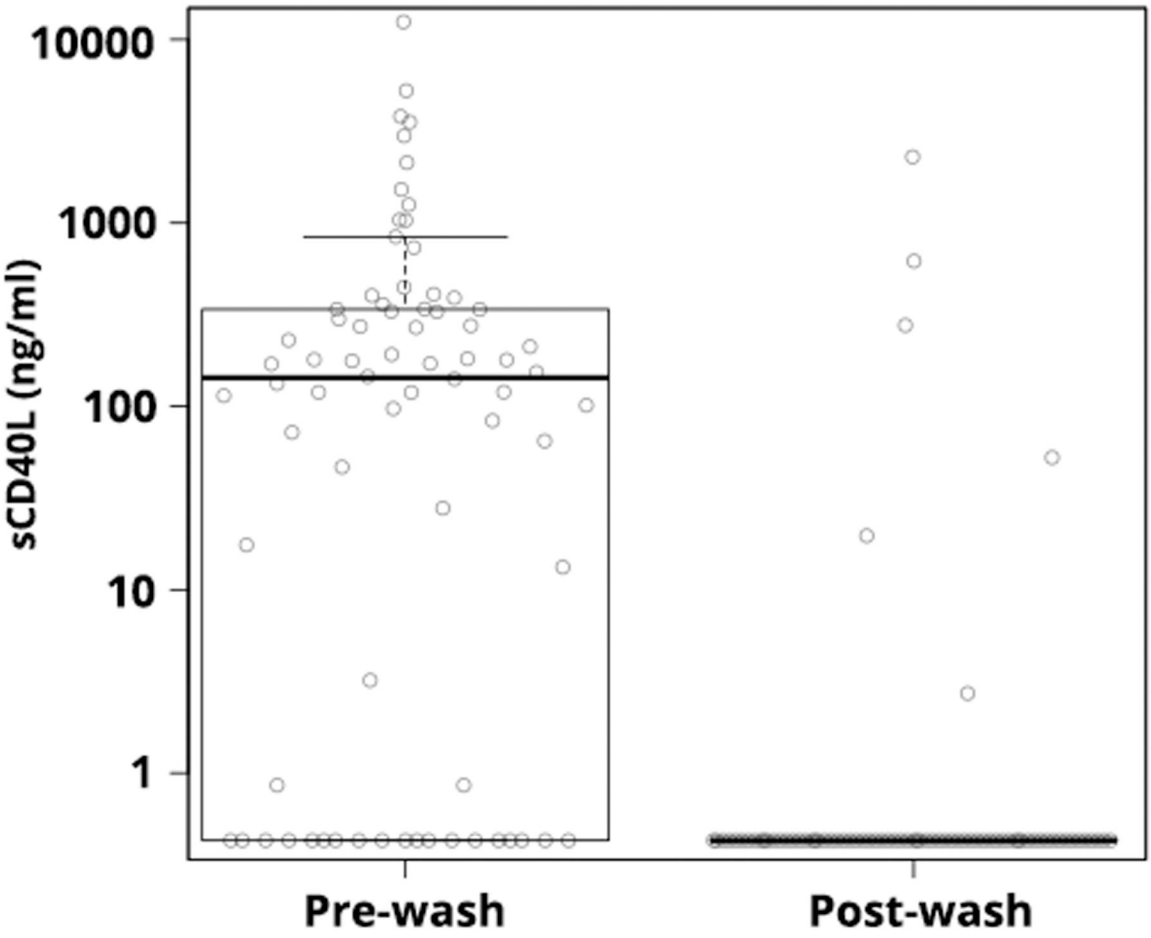


Figure 2.

Concentrations of CCL5 (2A) and sCD40L (2B) in blood units pre- and post-wash. The axis reflects a logarithmic scale. Box and whiskers represent median (IQR) and 10^{th}-90^{th} percentiles in ng/ml.

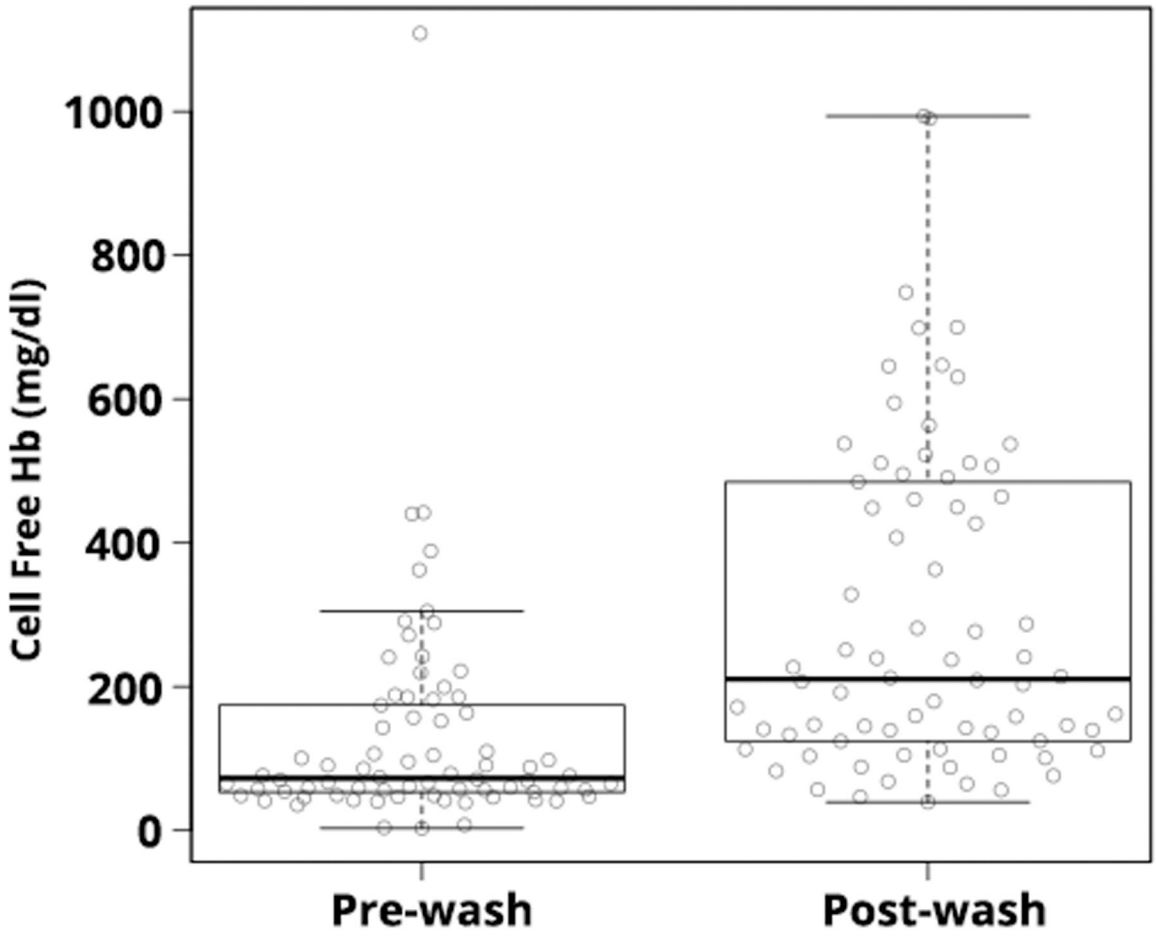
Abbreviations:

CCL5 – chemokine ligand 5/RANTES, a leucocyte derived pro-inflammatory chemokine.

sCD40L – soluble CD40 ligand, a leucocyte derived platelet pro-aggregatory protein.

IQR – Interquartile range

ng/ml – nanograms per milliliter



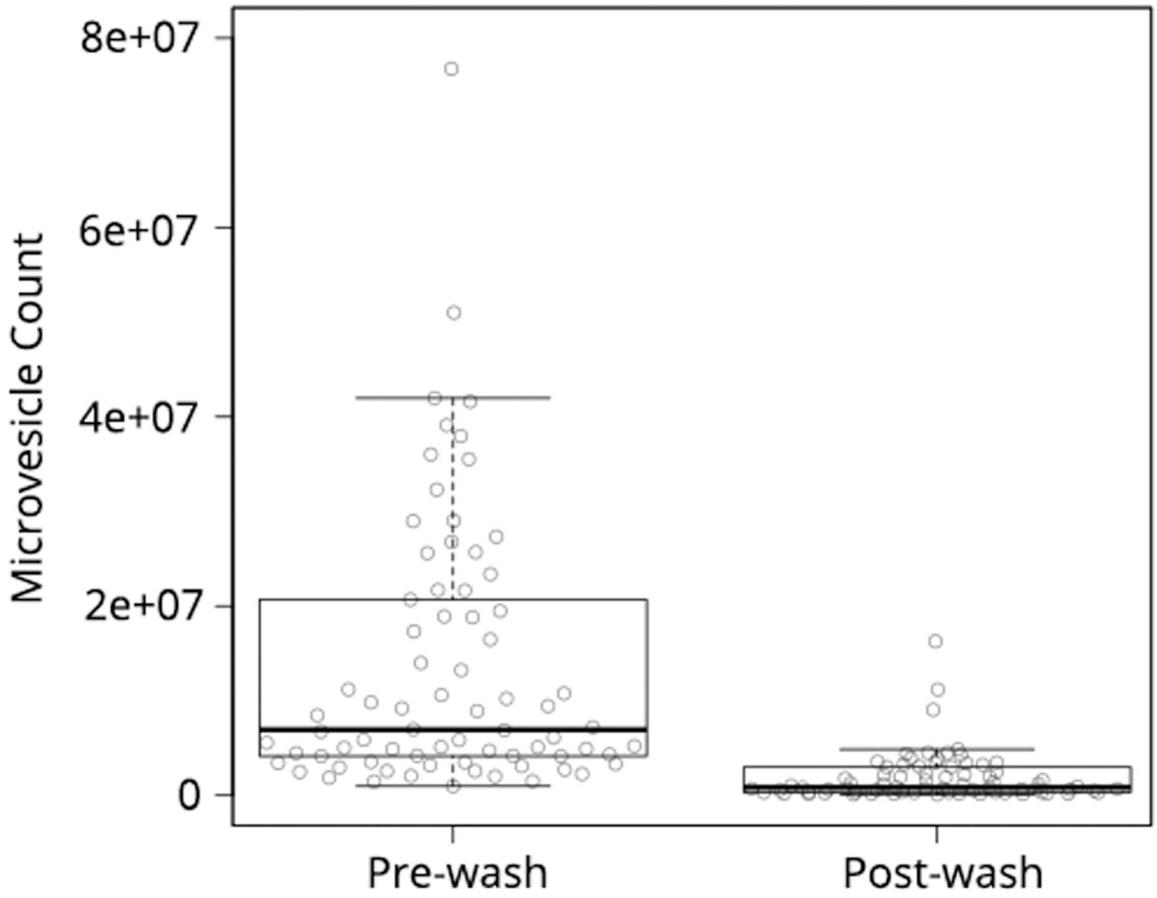


Figure 3.

Total microvesicle count (3A) and cell free Hb concentrations (3B) in mg/dl from blood units pre- and post-wash. The axis reflects a linear scale.

Abbreviations:

Cell free Hb – cell free hemoglobin concentration.

MV – Total microvesicle count.

IQR – Interquartile range

ng/ml – milligrams per deciliter

Table 1

Microvesicle counts and details of the microvesicle cellular origin from the 74 paired red cell units pre- and post-washing in a cell-saver; most are of indeterminate cellular origin. After Bonferroni correction a significant p-value is < 0.0055.

	Pre wash Median (IQR)	Post wash Median (IQR)	p-value
Total count per microliter	6,900,000 (4,100,000–20,000,000)	830,000 (330,000–2,800,000)	p<0.0001
Cell markers			
CD62P	99 (0–350)	20 (0–56)	p<0.0001
CD235a	13,000 (5,400–32,000)	4,600 (2,400–15,000)	p=0.004
CD108	730 (300–1,800)	130 (43–310)	p<0.0001
CD41a	33,000 (21,000–48,000)	6,900 (3,200–17,000)	p<0.0001

Abbreviations:

IQR – Interquartile range

MV - microvesicle

CD62p marker - P-selectin, activated endothelial cell or platelet derived MV

CD235a marker - Glycophorin A, erythrocyte derived MV

CD108 marker - Semaphorin-7A, activated lymphocytes or erythrocyte derived MV

CD41a marker - Glycoprotein IIb, megakaryocyte/platelet derived MV