

In Vitro and In Vivo Comparison of Hemoglobin and Electrolytes Following the Collection of Cell Saver Blood Washed with Either Normal Saline or Plasma-Lyte A

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Abstract: Cell saver blood is typically washed with normal saline (NS); however, recent studies have reported decreased red blood cell hemolysis and increased platelet function when a more physiologic washing solution, such as Plasma-Lyte A (PL-A) is used. We evaluated the in vitro and in vivo effects of NS compared to PL-A as washing solutions for cell saver blood in pediatric cardiac surgery. Cell saver blood was re-infused for up to 24 hours post-collection. Laboratory and clinical data were collected from infants receiving cell saver washed with either NS ($n = 20$) or PL-A ($n = 21$). Compositions of the cell saver blood

were compared between groups at 5 in vitro time points and in vivo patient blood at 24 hours post-bypass. Although there were differences in in vitro laboratory values between groups; 24 hours post-bypass, in vivo results were similar. Our data supports 24-hour reinfusion of cell saver washed with either NS versus PL-A in pediatric cardiac surgery patients, and provides data on the differences in cell saver composition to guide future studies. **Keywords:** hemolysis, cell saver, cardiopulmonary bypass, hemoglobin, renal function. *J Extra Corpor Technol.* 2021;53:214–9

Cell saver blood reinfusion has been shown to reduce allogenic red blood cell (RBC) transfusions and hospital-acquired infections (1) but is typically reserved for the first 4–6 hours after processing. Allogeneic RBC and coagulation product transfusions can also be reduced with cell saver blood reinfusion in pediatric cardiac surgery (2). Although historically normal saline (NS) is used as the washing solution for autotransfusion/cell saver products, more physiologic solutions may prevent electrolyte disturbances (3). Plasma-Lyte A (PL-A) (Baxter Healthcare Corporation, Deerfield, IL), is a buffered isotonic crystalloid, approved by the U.S. Food and Drug

Administration (FDA) for injection as a source of fluid and electrolytes replacement, to use as alkalinizing agent, or with blood or blood components. Unlike NS, PL-A contains lower concentrations of sodium and chloride and at ambient temperature has a physiologic pH (4).

Fewer acid–base and electrolyte derangements and decreased RBC fragility have been found in cell saver blood washed with PL-A as compared to NS (5). When comparing autotransfusion product washing/processing with .9% NaCl, Normosol-R™ (Abbott Labs, Abbott Park, IL), another low chloride solution, PL-A produces the most physiologic solution with the highest hemoglobin and lowest chloride and sodium levels (3). Refaai et al. washed RBC aliquots with either NS or PL-A. They measured free hemoglobin and heme levels in each aliquot at 24, 48, and 72 hours after washing. They found significantly greater RBC hemolysis in the RBC aliquots washed with NS at each time point, as compared to the RBC aliquots washed with PL-A. They found decreased platelet function at each time point in the platelet aliquots washed with NS as compared to PL-A (6).

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Our aim was to understand the impact of NS versus PL-A washing solutions on infants undergoing pediatric cardiac surgery receiving cell saver blood. We examined both the in vitro product from the cell saver, and the in vivo effects following cell saver transfusion at 24 hours for measurable differences in hemoglobin, electrolyte, acid base composition, and hemolysis.

DESCRIPTION

Our medical center Research Subject Review Board approved this quality assurance retrospective study. Data were collected from 41 children weighing ≤ 8 kg who had cell saver collected during cardiac surgery with cardiopulmonary bypass (CPB) between September 2018 and January 2020. To limit confounders we chose < 8 kg to ensure uniform CPB circuit and procedures, and to limit case heterogeneity and variation in blood product utilization and post-operative (post-op) management. We collected data from the first sequential 20 cases who had blood processed with the cell saver washed with NS, and the subsequent 21 sequential cases who had blood processed with the cell saver washed with PL-A.

All cases used the Livanova S5 Heart Lung Machine (LivaNova PLC, London, United Kingdom) with an Fx05 oxygenator (with Terumo X-coating) with hard shell reservoir and integrated arterial line filter (Terumo Corporation, Tokyo, Japan). The bypass circuit consisted of 3/16" arterial and 1/4" venous tubing (Medtronic Inc., Minneapolis, MN), and a roller pump with 1/4" raceway in the arterial position. Conventional ultrafiltration (CUF) was used on all cases. The bypass prime consists of 200 mg/kg mannitol (Hospira, Inc., Lake Forest, IL), 1,000 units heparin (2,000 u/2 mL) (Fresenius Kabi, Lake Zurich, IL), 25 mg/kg cefazolin (West Ward Pharmaceutical Corp, Eatontown, NJ), methylprednisolone (Pharmacia & UpJohn Co., New York, NY) 30 mg/kg (for patients < 6 months or if antegrade cerebral perfusion was used), 20 mg tranexamic acid (Apotex Corp., Weston, FL), 6–10 mEq 8.4% sodium bicarbonate (IMS Ltd., So. El Monte,

CA), and 20 mL of 25% albumin (Baxter US Inc., Lexington, MA). All patients received washed RBC in their prime (volumes ranging from 50 to 60 mL). Following termination of CPB and sequestration of the volume in the venous line, the residual pump volume was hemoconcentrated (Hemocor HPH™ 400 hemoconcentrator, Minntech Inc., Minneapolis, MN) to the lowest possible level (pump salvage) and transferred into syringes for possible re-infusion until aortic cannula removal.

Remaining pump salvage was infused into the Fresenius Continuous AutoTransfusion System® (CATS) (Terumo Corporation, Tokyo, Japan), with the resulting processed and washed volume collected. The cell saver blood was maintained up to 24 hours after its collection in a designated temperature controlled (1–6°C) and monitored cooler (Igloo Ice cube™ 12 quart cooler, Igloo Products Corp., Katy, TX) in keeping with New York State blood bank regulation 58–2.25 (7). Aliquots for transfusion were drawn off aseptically using a needleless adapter used for RBC transfusion if the patient's Hb was < 13 g/dL to maintain hemodynamic stability, improve oxygenation and/or treat anemia. Transfusion of RBC, platelets, fresh frozen plasma (FFP), and cryoprecipitate was per pediatric cardiac intensive care unit (PCICU) transfusion guideline.

Data from arterial blood gas analysis and panel were collected from samples obtained at five time points in the operating room (OR) and post-op in the PCICU (Table 1). Clinical data were collected from the patient's medical record including blood product transfusions, hemoglobin levels, serum creatinine, cardiac lesion, surgical procedure, acute kidney injury, renal replacement therapy, mortality, and hospital length of stay.

STATISTICAL ANALYSIS

Data is presented as mean $\pm$ standard deviation, median with interquartile range, or frequency with percentage. Categorical variables were examined using a Fisher's exact test. Continuous variables were evaluated using a Wilks–Shapiro test and either two tailed Students

Table 1. Sample collection details.

Sample Draw Location	Sample Name	Sample Collection Details
Operating room (intra-operatively)	First CPB	Sample drawn from CPB circuit within 5 minutes of initiation of CPB.
	CPB salvage	Sample drawn from the CPB "salvage" blood (blood collected from the CPB circuit after patient separation that was then ultrafiltered.
	Cell saver	Sample drawn from cell saver blood once processing and hemoconcentration completed.
Pediatric cardiac ICU (post-operatively)	24-hour cell saver	Sample drawn from the cell saver blood 24 hours after its collection.
	24-hour patient	Arterial blood gas and panel from patient 24 hours after coming off CPB and receiving cell saver blood throughout that time period.

CPB, cardiopulmonary bypass; ICU, intensive care unit.

t test or Mann–Whitney test performed when appropriate. An analysis of variance (ANOVA) or Kruskal–Wallis test were used to compare laboratory data over multiple time points between groups. All statistics were performed using Graphpad Prism™ (GraphPad software, San Diego, CA) 8.1 where a *p*-value of < .05 was considered statistically significant.

RESULTS

Patient characteristics are presented in Table 2; there was no significant difference between the two groups regarding age, sex, weight, and surgical procedures. One patient in the NS group, following palliation for complete atrioventricular septal defect (AVSD) and Tetralogy of Fallot, suffered a cardiac arrest his first post-op night, required extracorporeal membrane oxygenation but survived to discharge. Two patients in the PL-A group died

before discharge. One death occurred post-operative day 1 following modified Norwood after sudden cardiac arrest. The second death occurred on post-op day 26 after successful arch reconstruction and PA banding when the diagnosis of congenital pulmonary lymphangiectasis was made and the family decided to re-direct care. No patients of either cohorts required re-operation or re-exploration for bleeding.

Laboratory data are presented in Figure 1. Plasma-free hemoglobin was significantly lower in the PL-A group following CPB ($p < .0001$), but was similar at all other time points, and remained low following cell saver processing and at 24 hours after collection. Hemoglobin values were similar at all time points between groups and increased in the hemoconcentrated washed cell saver blood and remained elevated at 24 hours.

In vitro sodium levels were significantly higher in cell saver blood washed with NS at processing ($p < .0001$) and at 24 hours ($p < .0001$) but were similar between

Table 2. Patient characteristics.

	NS Group (N = 20)	PL-A Group (N = 21)	<i>p</i> -Value
Age, days (median range)	35.5 (7–195)	42 (6–185)	.801
Sex, male (%)	14 (70)	11 (52)	.341
Weight, kg (mean $\pm$ SD)	4.51 $\pm$ 1.29	4.18 $\pm$ 1.16	.392
Single-ventricle physiology (%)	4 (20)	4 (19)	>.999
Cardiac lesion (%)			
Tetralogy of Fallot	5 (25)	6 (29)	>.999
Tetralogy of Fallot + complete atrioventricular septal defect	1 (5)	0 (0)	.488
Double outlet right ventricle	1 (5)	0 (0)	.488
Hypoplastic left heart syndrome	2 (10)	2 (10)	>.999
Atrioventricular septal defect	1 (5)	3 (14.2)	.606
Atrioventricular septal defect + aortic coarctation	2 (10)	1 (4.7)	.606
Ventricular septal defect	1 (5)	1 (4.7)	>.999
Transposition of the great arteries	2 (10)	3 (14.2)	>.999
Double outlet right ventricle + transposition of the great arteries	0 (0)	1 (4.7)	>.999
Cor triatrium sinister	1 (5)	0 (0)	.488
Hypoplastic aortic arch/coarctation	2 (10)	0 (0)	.232
Aortic coarctation + ventricular septal defect	1 (5)	1 (4.7)	>.999
Total anomalous pulmonary venous return	1 (5)	1 (5)	>.999
Aorto-pulmonary window	0 (0)	1 (5)	>.999
Truncus arteriosus	0 (0)	1 (5)	>.999
Cardiac Surgical Procedure (%)			
Arch reconstruction + ventricular septal defect repair	4 (20)	1 (5)	.184
Tetralogy of Fallot repair	5 (25)	5 (24)	>.999
Atrial septal defect and/or ventricular septal defect repair	2 (10)	2 (10)	>.999
Modified Norwood	2 (10)	3 (14)	>.999
Arch/coarctation repair + pulmonary artery band	1 (5)	1 (5)	>.999
Atrioventricular septal defect repair	2 (10)	2 (10)	>.999
Arterial switch operation	2 (10)	4 (19)	.663
Total anomalous venous return repair	1 (5)	1 (5)	>.999
Truncus arteriosus repair	0 (0)	1 (5)	>.999
Aortopulmonary window repair	0 (0)	1 (5)	>.999
Cor triatrium repair	1 (5)	0 (0)	>.999
Post-operative morbidity and mortality (%)			
ECMO	1 (5)	0 (0)	>.999
Deceased	0 (0)	2 (10)	>.999
Renal replacement therapy	0 (0)	0 (0)	>.999

ECMO, extra corporeal membrane oxygenation; NS, normal saline; PL-A, – Plasma-Lyte-A; SD, standard deviation.

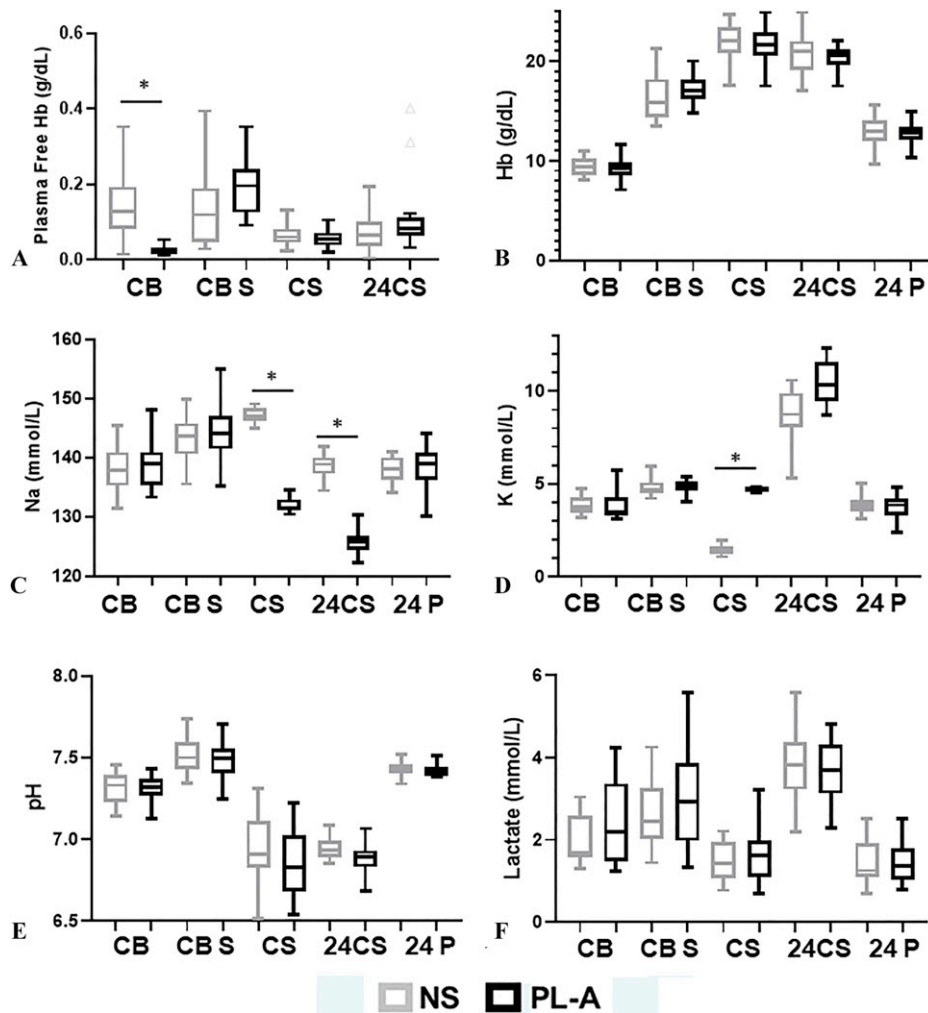


Figure 1. Box and whisker plots representing median and 95% confidence intervals for laboratory values of in vitro and in vivo measurements following the washing of cell saver blood with either normal saline (NS)-gray and plasma-Lyte (PL-A)-black. (1A) Plasma-free hemoglobin (HB). (1B) Hemoglobin. (1C) Na (Sodium). (1D) K (Potassium). (1E) pH. (1F) Arterial lactate. CB, values were obtained after 5 minutes of cardiopulmonary bypass; CB S, cardiopulmonary bypass salvage; CS, cell saver; 24 CS, in vitro cell saver blood at 24 hours; 24 P, in vivo patient values at 24 hours. *Denotes statistical significance.

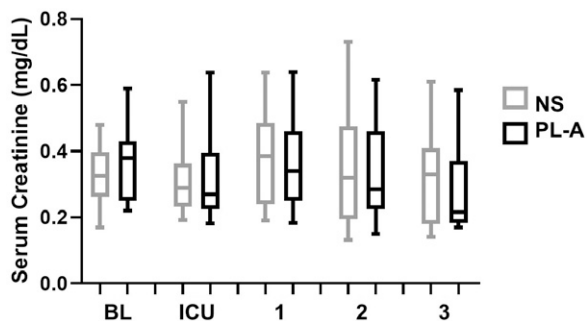


Figure 2. Box and whisker plots representing median and 95% confidence intervals for serum creatinine values at baseline, ICU admission, and post-operative day 1–3 from patients transfused with cell saver blood washed with either normal saline—gray or plasma-Lyte—black. BL, baseline; ICU, intensive care unit; NS, normal saline; PL-A, Plasma-Lyte A.

groups in patient samples. In vitro potassium levels were significantly higher in cell saver blood immediately washed with PL-A ($p < .0001$) and were increased in both groups at 24 hours. However, in vivo potassium levels from 24-hour patient samples were similar, and remained within normal range. The pH was decreased in cell saver blood compared to pump salvage and remained lower 24 hours after collection/processing. However, patient levels at 24 hours were normal (7.4 range) and were similar regardless of the washing solution. Lactate levels were also elevated in both groups of in vitro 24-hour cell saver blood but did not appear to significantly impact in vivo patient samples at 24 hours. Serum creatinine levels were similar throughout the immediate post-op period between the groups ($p = .28$) (Figure 2).

Table 3. Cell saver and transfusion data from first cardiopulmonary bypass through 24 hours post-op.

	NS Group	PL-A Group	<i>p</i> -Value
CPB time (min)	114.45 ± 32.99	145.71 ± 52.25	.028
Clamp time (min)	66.05 ± 29.60	86.24 ± 40.78	.079
Intraoperative red blood cells transfused (mL)	135.75 ± 32.29	148.00 ± 29.49	.212
Intraoperative platelets transfused (mL)	.00 ± .00	.00 ± .00	>.999
24-hour cell saver total volume (non-OR) (mL)	64.35 ± 44.59	61.76 ± 38.19	.843
24-hour cell saver volume (mL/kg)	14.80 ± 11.21	14.45 ± 8.01	.861
24-hour mediastinal tube total volume (mL)	112.15 ± 97.41	120.24 ± 45.98	.089
24-hour mediastinal tube volume (mL/kg)	28.38 ± 32.24	29.99 ± 12.42	.071

NS, normal saline; PL-A, Plasma-Lyte A; OR, operating room; CPB, cardiopulmonary bypass. Data shown in mean ± SD.

There was a significant difference between groups regarding CPB time, with significantly longer duration in the PL-A group ($p = .028$). Each group had one patient transfused with platelets within 24 hours post-op ($p > .999$). One patient in the NS group received FFP within 24 hours of surgery ($p > .999$), while two patients in the PL-A group received RBC transfusions within 48 hours ($p = .487$). Of the 41 infants, blood product utilization and mediastinal tube drainage were similar between groups (Table 3).

DISCUSSION

Electrolyte and acid–base derangements are common in critically ill neonates and infants following cardiac surgery requiring CPB (8). The composition of resuscitative fluid and blood product transfusions in response to bleeding, altered hemostasis and/or hemodilution can impact acid–base balance, electrolyte levels, renal function, and mortality (9). Therefore, it is critical to understand whether a more physiologic washing solution for cell saver processing/suspension results in a more physiologic blood product, as well as the resultant effect on patient laboratory values. Huber et al. performed an *in vitro* study comparing acid–base balance and RBC stability in cell saver blood washed with NS versus a bicarbonate buffered hemofiltration solution. Cell saver blood washed with the bicarbonate buffered solution not only was associated with improved electrolyte and acid–base balance, but also decreased RBC lysis, as evidenced by decreased plasma-free hemoglobin, lactate dehydrogenase (LDH), and higher concentrations of erythrocyte adenosine triphosphate (ATP; marker of RBC stability). These authors concluded that clinical studies are necessary to verify the influence of the washing solution *in vivo* (10).

In this study, expected significant differences in sodium and potassium levels were found between groups in cell saver blood at its collection (higher sodium levels in NS, and higher potassium levels in PL-A). The difference in sodium remained significant at 24 hours; but though elevated, the higher potassium levels in cell saver blood

washed with PL-A was not significant at 24 hours. Despite the higher potassium levels in cell saver blood at 24 hours in both groups, normal potassium levels were maintained *in vivo* at 24 hours. Plasma-free hemoglobin was not consistently decreased or elevated across the stages of processing in the OR (first CPB, CPB salvage, and initial cell saver) in either group. The initial cell saver blood had lower plasma-free hemoglobin in the PL-A group but did not reach significance, and was not maintained at 24 hours, indicating that potential benefit of the washing solution on hemolysis was not sustained at 24 hours.

Our study shows the significance of plasma-free hemoglobin as a quality marker of hemolysis in washed RBCs. Exploring a better “novel” washing solution is substantial. Nevertheless, the on board possible ongoing hemolysis is more crucial to be investigated. Our work did not fully investigate all markers of hemolysis (such as LDH, haptoglobin, erythrocyte ATP, or plasma-free hemoglobin levels) in the patients, as this is not collected as part of our current standard of care. Additional exploration of hemolysis in both cell saver blood and the subjects, would have provided more information regarding the impact of washing solution on RBC lysis over time and is an important limitation of this work. Future investigations aimed at identifying and reducing hemolysis are critical in determination of the optimal washing solution for these vulnerable patients.

Impact of hemolysis as a potential contributor to post-op acute renal injury led us to examine creatinine levels in our subjects; however, these values were similar between groups during the initial post-op period. Additional data collection regarding known contributors to acute renal injury (hypotension, nephrotoxic medications, etc.) would be required to further explore for a between-group difference in those receiving cell saver washed with NS versus PL-A. Furthermore, creatinine is known to be a poor (insensitive and late) measure of impaired renal function (11). Although serum creatinine elevation is strongly associated with acute renal injury, additional urinary biomarkers of renal injury (neutrophil gelatinase-associated lipocalin [N-GAL]) and insulin-like growth factor binding protein 7 (IGFBP7), and serum cystatin-C would likely be more sensitive indicator of

renal injury and more reflective of the impact of RBC hemolysis (12,13).

We are not aware of studies comparing cell saver washed with NS versus PL-A after its initial collection. This is the first work to present laboratory values from cell saver maintained for 24 hours following its collection. More importantly, we are not aware of any human studies that examine the impact of washing solution on acid–base or electrolyte composition in the transfusion recipient. Furthermore, this work is first to explore for possible impact of the washing solution on RBC hemolysis and renal function, particularly relevant at our institution where cell saver blood is reinfused up to 24 hours after processing.

This pilot study provides evidence of potential benefit from washing cell saver blood with PL-A as compared to NS, though larger subject numbers are needed to support this. Importantly, it provides reassuring data that reinfusion of cell saver blood for up to 24 hours after its processing when maintained with tight temperature regulation does not adversely impact the acid–base or electrolyte composition of the recipient. Larger controlled trials for volume of cell saver blood received would be required to confirm our findings. Data regarding measures of RBC stability and hemolysis would be needed to discern any clinical benefit from PL-A washing in regards to acute kidney injury and clinical outcomes.

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