

Beyond the operating room: preservation of blood quality and erythrocyte function in postoperative salvaged mediastinal blood

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Background - Postoperative hemorrhage is a major complication in cardiovascular surgery, and whether to salvage and reinfuse postoperative shed blood remains controversial. The quality and erythrocyte function of this blood have not been well defined, making clinical decision-making challenging. This study compared postoperative autologous cell-salvaged blood (Postop-ACS) with intraoperative salvaged blood (ICS) and stored packed red blood cells (PRBC).

Materials and methods - This *in vitro* observational study analyzed 60 blood samples (20 per group): Postop-ACS, ICS, and mid-storage (stored for 15-20 days) PRBC. The salvaged samples were processed using a cell saver. Assessed parameters included hemoglobin, hematocrit, pH, potassium, glucose, lactate, hemolysis rate, erythrocyte deformability, morphology, 2,3-diphosphoglycerate (2,3-DPG) and ATP levels.

Results - All groups met international hemolysis standards (<0.8%). Regarding blood quality, the median [interquartile range] Postop-ACS hemoglobin (19.4 [16.0-19.7] g/dL) and hematocrit (56.3 [46.5-59.0]%) levels were comparable to those of PRBC (18 [17-19] days), with near physiological pH (7.34 [7.31-7.40]); potassium (1.10 [0.91-1.25] mmol/L), glucose (0.88 [0.84-0.98] mmol/L), and lactate (0.45 [0.20-0.90] mmol/L) levels were significantly lower than those of mid-storage PRBC (all $p < 0.001$) and similar to ICS (all $p > 0.05$). Regarding erythrocyte function, Postop-ACS had significantly higher 2,3-DPG (12.00 [9.24-13.32] $\mu\text{mol/gHb}$), ATP (5.59 [5.27-5.93] $\mu\text{mol/gHb}$), and deformability (0.88 [0.77-0.93]) than PRBC (all $p < 0.05$), but comparable to those of ICS (all $p > 0.05$). Erythrocyte morphology remained biconcave, discoid in all groups.

Discussion - Postop-ACS showed comparable quality and more physiological metabolic and erythrocyte functional profiles relative to mid-storage PRBC, with results similar to ICS. These findings support postoperative autologous cell salvage as a physiologically favorable strategy to enhance patient blood management in cardiovascular surgery.

Keywords: Patient Blood Management, postoperative cell salvage, shed mediastinal blood, intraoperative cell salvage, packed red blood cell.

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INTRODUCTION

Postoperative bleeding remains a substantial challenge in cardiovascular surgery, with approximately 50% of patients requiring varying degrees of hemostatic intervention



or transfusion¹⁻³. Moreover, cardiovascular procedures consume 10% to 15% of national blood supplies in both the USA and the United Kingdom, which is more than any other surgical category³⁻⁵. Intraoperative cell salvage (ICS), a component of Patient Blood Management (PBM), reduces the need for allogeneic blood transfusions by as much as 40%, with strong evidence supporting its efficacy and safety in cardiovascular surgery⁶⁻⁸. Building on this, salvaging shed mediastinal blood postoperatively may provide a feasible strategy to address bleeding and reduce allogeneic transfusions requirements in cardiovascular surgery.

The reinfusion of unwashed shed mediastinal blood has been investigated in recent decades but has been associated with risks such as thromboembolism, coagulopathy, and renal injury⁹⁻¹³. Consequently, several medical organizations have classified this practice as a Class III intervention, indicating its potential risks¹⁴. In contrast, modern washing cell salvage techniques can effectively remove activated platelets, free hemoglobin, and metabolic byproducts while preserving blood quality and erythrocyte function¹⁵⁻¹⁷. Paradoxically, these technological advances have not been widely applied in the postoperative setting, particularly in hemorrhage-prone cardiovascular surgical procedures, in which the use of cell salvage for shed blood remains limited². Whether postoperative autologous cell salvage (Postop-ACS) yields comparable quality and erythrocyte function to ICS or allogeneic packed red blood cells (PRBC) remains unclear. This is especially pertinent given that postoperative shed blood is collected at room temperature for extended periods before processing, raising concerns about its impact on red blood cell viability and function, factors which are critical for oxygen delivery and tissue perfusion in the vulnerable postoperative period. This is particularly relevant for patients undergoing cardiac surgery, who are prone to hemodynamic instability and low cardiac output syndrome. For example, erythrocyte deformability, a marker of red cell flexibility, is essential for navigating the microcirculation and ensuring adequate oxygen delivery. Likewise, intracellular 2,3-diphosphoglycerate (2,3-DPG) modulates hemoglobin's oxygen affinity and promotes oxygen release to peripheral tissues, while adenosine triphosphate (ATP) provides the energy needed to maintain red cell structural integrity and

their biconcave discoid shape. This shape, far from being a mere morphological feature, is vital for optimal gas exchange and microcirculatory flow in postoperative cardiovascular patients.

Building on these considerations, in this study we undertook a comprehensive assessment of postoperative salvaged blood by comparing it with intraoperative salvaged blood and PRBC across two principal dimensions: (i) blood quality, evaluated by hematologic performance, hemolysis, and metabolic profiles; and (ii) erythrocyte function, evaluated by intracellular 2,3-DPG and ATP levels, hemorheology properties, and cellular morphology.

MATERIALS AND METHODS

This study was conducted at the Chinese Academy of Medical Sciences Fuwai Hospital and in accordance with the ethical principles outlined in the Declaration of Helsinki. Approval for the study was granted by the Ethics Committee of Fuwai Hospital (Ethics number: 2022-1805, clinicaltrials.gov registration ChiCTR2200065958), and written informed consent was obtained from all participants.

Study design

This *in vitro* observational study assessed blood quality and erythrocyte function in postoperative autologous cell-salvaged blood (Postop-ACS group), with comparative analyses conducted against intraoperatively salvaged blood (ICS group) and stored allogeneic packed red blood cells (PRBC group). Twenty samples were collected for each group, based on a predefined sample size, with all collections completed between June 1 and December 31, 2024. Each sample in the Postop-ACS and ICS groups was obtained from a distinct patient undergoing cardiovascular procedures, with no repeated sampling or overlap. Samples in the PRBC group were obtained from anonymous blood bank donors. As this was a preclinical *in vitro* investigation focused exclusively on laboratory evaluation; the postoperative salvaged blood was not re-infused into any patients.

In the Postop-ACS group, postoperative shed blood was collected using a standard chest tube drainage system in accordance with routine clinical practice. After sternal closure, three 28 Fr soft sump drains were placed: one in the pericardial cavity, one in the mediastinum, and one in the pleural space. The pericardial and mediastinal

drains were connected via a Y-connector to a 1,000 mL sterile negative-pressure drainage bottle, which was prefilled with 20 mL of heparinized saline (12,500 IU of heparin in 20 mL of 0.9% NaCl) to prevent clotting. At the end of the 6-hour postoperative period, the shed blood was aseptically transferred to a Sorin Xtra cell salvage system (München, Germany) for processing. The 6-hour collection window was adopted based on our previously published study, which established this time frame as optimal for preserving microbial sterility, blood quality, and erythrocyte function in postoperative salvaged mediastinal blood¹⁸.

In the ICS group, after the administration of protamine, shed blood was collected and processed intraoperatively using a Sorin Xtra cell salvage system.

Both Postop-ACS and ICS samples were processed using the same standardized washing protocol with a 225 mL wash set and predefined parameters: prime rate 400/250 mL/min, wash rate 500 mL/min, and empty rate 400 mL/min. Blood from the ICS group was processed using approximately 1,000 mL of normal saline per cycle, consistent with routine intraoperative clinical practice. In contrast, 5,000 mL of normal saline per cycle was used for the Postop-ACS group to more effectively remove inflammatory mediators and accumulated metabolic byproducts, based on the washing protocol established in our previous study¹⁸.

For the PRBC group, 10 mL aliquots were aseptically drawn from the standard PRBC units obtained from the blood bank.

For laboratory analyses, 10 mL samples were taken from the salvaged blood reinfusion bags (Postop-ACS and ICS groups) or directly from the PRBC aliquots. All samples were analyzed immediately after collection.

Blood source

Both the Postop-ACS and ICS groups consisted of patients aged 18-70 years who were undergoing cardiovascular procedures, including coronary artery bypass grafting, heart valve surgery, aortic surgery, or combined surgeries. Individuals with preoperative pleural effusion, perioperative sepsis/bacteremia, hematologic disorders, or contaminated shed blood samples were excluded. The Postop-ACS group specifically required the collection of ≥ 500 mL of postoperative mediastinal blood within the first 6 hours, which was processed using an autologous

cell saver system. In contrast, the ICS group included patients from routine clinical practice who met the shared inclusion and exclusion criteria.

The PRBC group consisted of banked packed red blood cells preserved in mannitol-adenine-phosphate (MAP) additive solution, stored under standardized conditions for 15-20 days without irradiation.

Endpoints

The evaluation of Postop-ACS blood by comparing it with ICS blood and PRBC focused on two primary domains: (i) blood quality, assessed by hematologic performance, hemolysis rates, and metabolic profiles, including blood gas parameters, electrolyte, glucose and lactate concentrations; and (ii) erythrocyte function, assessed through the levels of 2,3-DPG and ATP, hemorheology (rigidity and deformability index), and morphology.

Laboratory analyses

Routine hematological parameters, including hemoglobin (Hb), hematocrit (Hct), mean corpuscular volume (MCV) and white blood cell (WBC) count, were measured using an automated hematology analyzer (XN2000, Sysmex, Kobe, Japan). Blood pH, potassium levels (K⁺), lactate, and glucose levels were assessed with a blood gas analyzer (ABL300, Radiometer, Copenhagen, Denmark).

Free hemoglobin (fHb) concentration was determined by a colorimetric method (HemoCue Plasma/Low Hb System, Ängelholm, Sweden) following sample centrifugation at $1,020 \times g$ for 5 minutes. The hemolysis rate was calculated using the formula: $(100 - \text{Hct}) \times \text{fHb} / \text{total Hb}$.

Red blood cell rigidity and deformability were evaluated at room temperature using an automated hemorheology analyzer (SA-6600, Succeder, Beijing, China) employing a cone-plate rotational viscometry system. For each sample, whole blood viscosity was measured at a shear rate of 200 s^{-1} . The rigidity index was calculated as $(\eta_{\text{whole blood}} - \eta_{\text{plasma}}) / (\eta_{\text{plasma}} \times \text{Hct})$, and the deformability index as $(1 - \eta_r^{-0.4}) / \text{Hct}$, where $\eta_r = \eta_{\text{whole blood}} / \eta_{\text{plasma}}$.

Erythrocyte morphology was examined by scanning electron microscopy (JSM-5510, JEOL, Tokyo, Japan) after standard fixation, dehydration, and metal coating.

The intracellular levels of 2,3-DPG and ATP were measured by hydrophilic interaction liquid chromatography (HILIC) coupled with high-resolution mass spectrometry (Q Exactive HF, Thermo Fisher, Bremen, Germany). Red blood cell metabolites were extracted with

methanol-acetonitrile-water (4:4:2, v/v/v) by overnight protein precipitation, followed by centrifugation, evaporation, and reconstitution. Chromatographic separation was performed on a BEH Amide HILIC column (50 × 1 mm, 1.7 µm; Waters, Milford, MA, USA) at 0.3 mL/min with gradient elution (mobile phase A: 95% water/5% acetonitrile/5 mM ammonium formate; B: 95% acetonitrile/5% water/5 mM ammonium formate). Detection was performed on a Q Exactive HF mass spectrometer in negative ESI mode (spray voltage: 3 kV; capillary temperature: 350°C) using targeted MS/MS (t-MS²; resolution: 15,000; stepped collision energies: 20, 40, 60). Quantification was based on precursor-to-product ion transitions (2,3-DPG: m/z 264.95 → 166.97; ATP: m/z 506.99 → 79.02) with external calibration using commercially available reference standards (Sigma-Aldrich, St. Louis, MO, USA) and quality control samples.

Sample size and statistical analysis

Due to the exploratory nature of this study and lack of prior data, a sample size of 20 per group was chosen. The normality of the data was assessed using the Shapiro-Wilk test. Normally distributed continuous variables are presented as mean ± standard deviation (SD), and non-normally distributed variables as median [interquartile range, IQR]. Categorical variables are summarized as counts and percentages. For group comparisons, analysis of variance (ANOVA) or Kruskal-Wallis tests were applied based on data distribution. Post-hoc analyses were selected accordingly: Tukey's test following ANOVA for normally distributed variables with equal variances, and Dunn's test following Kruskal-Wallis for non-normally distributed variables. Since the primary objective was to compare Postop-ACS with ICS and with PRBC, pairwise comparisons were limited to these two comparisons; no statistical testing was conducted between ICS and PRBC. All tests were two-tailed with significance at $p < 0.05$, using R (version 4.3.2) and GraphPad Prism (Boston, MA, USA) 10 for the analyses.

RESULTS

Table I provides a summary of the patients' baseline characteristics, laboratory test results, and surgical attributes across the Postop-ACS and ICS groups. Statistical analysis revealed no significant differences between the two groups.

Table I - Perioperative characteristics of patients in the groups whose postoperative autologous cell salvaged blood or intraoperative salvaged blood was studied

Characteristics	Postop-ACS No.=20	ICS No.=20	P
Female sex, No. (%)	4 (20)	5 (25)	0.72
Age, years	61 [54-66]	55 [52-66]	0.60
BMI, kg/m ²	24.6 [22.9-27.2]	24.2 [23.0-24.9]	0.50
Coexisting illness, No. (%)			
Hypertension	8 (40)	11 (55)	0.53
Diabetes mellitus	3 (15)	2 (10)	0.77
Dyslipidemia	10 (50)	14 (70)	0.33
Atrial fibrillation	4 (20)	1 (5)	0.34
Chronic kidney disease	1 (5)	0 (0)	0.36
History of stroke	1 (5)	0 (0)	0.60
Active smoking	8 (40)	5 (25)	0.50
Laboratory values before surgery			
WBC count, 10 ⁹ /L	5.8 [5.3-6.9]	6.6 [5.4-7.3]	0.47
Hemoglobin, g/dL	14.1 [12.9-15.2]	13.7 [12.8-14.4]	0.18
Platelet count, 10 ⁹ /L	184 [148-233]	189 [179-221]	0.56
NYHA class, No. (%)			0.12
I	0 (0)	3 (15)	
II	15 (75)	10 (50)	
III	5 (25)	7 (35)	
IV	0 (0)	0 (0)	
Surgery type, No. (%)			0.14
Only CABG	9 (45)	15 (75)	
Only valve/aorta	6 (30)	2 (10)	
Combined	5 (25)	3 (15)	
Perioperative outcomes			
Procedure duration, min	243 [216-293]	223 [210-245]	0.19
RBC transfusion, No. (%)	1 (5)	1 (5)	0.88
ICU stay, hours	42 [24-58]	36 [29-45]	0.85
Ventilation time, hours	11.0 [9.0-13.2]	9.5 [7.0-11.2]	0.13
Hospital stay, days	12 [9-13]	12 [11-16]	0.19
Mortality in 30 days, No. (%)	0 (0)	0 (0)	/

Data are presented as median [interquartile range], or number (percentage). Postop-ACS: postoperative autologous cell-salvaged blood; ICS: intraoperative salvaged blood; BMI: body mass index; WBC: white blood cell; NYHA class: New York Heart Association class; CABG: coronary artery bypass grafting; RBC: red blood cell; ICU: intensive care unit.

Hematologic performance

The median storage duration of PRBC units was 18 [17-19] days (range: 15-20 days). **Table II** presents the hematologic parameters across the three groups. Compared to the ICS group, the Postop-ACS group had significantly elevated

Table II - The quality of the postoperative autologous cell-salvaged blood, the intraoperative salvaged blood, and the packed red blood cells

Parameters	Postop-ACS No.=20	ICS No.=20	PRBC No.=20	pa	p1	p2
Hemoglobin, g/dL	19.4 [16.0-19.7]	12.2 [10.6-15.2]	18.1 [17.5-18.5]	<0.001	<0.001	>0.99
Hematocrit, %	56.3 [46.5-59.0]	37.6 [30.3-46.8]	57.2 [55.6-59.5]	<0.001	<0.001	>0.99
MCV, fL	88.7 [86.5-90.8]	88.8 [86.7-90.0]	88.4 [87.8-88.9]	0.81	-	-
WBC, 10 ⁹ /L	5.34 [3.95-10.03]	3.08 [1.49-4.66]	3.01 [2.20-4.52]	<0.001	0.001	0.003
Hemolysis						
fHb, g/L	0.55 [0.30-0.80]	0.45 [0.31-0.70]	0.40 [0.30-0.70]	0.84	-	-
hemolysis rate (%)	0.15 [0.09-0.18]	0.23 [0.14-0.30]	0.16 [0.09-0.19]	<0.001	0.03	0.41
Physical and chemical properties						
pH	7.34 [7.31-7.40]	7.42 [7.33-7.46]	6.51 [6.41-6.60]	<0.001	0.16	<0.001
K ⁺ , mmol/L	1.10 [0.91-1.25]	1.03 [0.73-1.56]	21.13 [18.13-27.37]	<0.001	>0.99	<0.001
Glu, mmol/L	0.88 [0.84-0.98]	0.97 [0.71-1.12]	20.86 [19.08-23.00]	<0.001	>0.99	<0.001
Lac, mmol/L	0.45 [0.20-0.90]	0.50 [0.13-0.84]	16.15 [14.45-18.65]	<0.001	>0.99	<0.001

Data are presented as median [interquartile range]. Postop-ACS: postoperative autologous cell-salvaged blood; ICS: intraoperative salvaged blood; PRBC: packed red blood cells; MCV: mean corpuscular volume; WBC: white blood cells; fHb: free hemoglobin; K⁺: potassium; Glu: glucose; Lac: lactic acid. pa: comparison of differences among the three groups; p1: comparison of differences between the Postop-ACS group and ICS group; p2: comparison of differences between the Postop-ACS group and PRBC group.

Hb ($p_1 < 0.001$) and Hct ($p_1 < 0.001$). There were no significant differences in Hb and Hct (both $p_2 > 0.99$) in comparisons between Postop-ACS and PRBC group and no significant difference in MCV between Postop-ACS and either ICS or PRBC ($p_a > 0.05$).

The Postop-ACS group demonstrated significantly elevated WBC counts compared to both the ICS and PRBC groups (5.34 [3.95-10.03] vs 3.08 [1.49-4.66] and 3.01 [2.20-4.52] $\times 10^9/L$, respectively; $p_1 = 0.001$, $p_2 = 0.003$).

Hemolysis

The Postop-ACS group showed no significant differences in fHb levels compared to both the ICS and PRBC groups ($p_a > 0.05$ for all). The ICS group exhibited a significantly higher hemolysis rate (0.23% [0.14-0.30]) than the Postop-ACS group (0.15% [0.09-0.18], $p_1 = 0.03$), while the Postop-ACS and PRBC groups had comparable rates of hemolysis (0.15% [0.09-0.18] vs 0.16% [0.09-0.19], $p_2 = 0.41$) (Table II).

Metabolic profiles

The Postop-ACS group maintained physiological pH levels of 7.34 [7.31-7.40], distinct from the severe acidosis in mid-storage PRBC (6.51 [6.41-6.60], $p_2 < 0.001$). In the Postop-ACS group, the concentrations of potassium (1.10 [0.91-1.25] mmol/L), glucose (0.88 [0.84-0.98] mmol/L), and lactate (0.45 [0.20-0.90] mmol/L) were significantly

lower compared to those in the PRBC group ($p_2 < 0.001$). In contrast, the Postop-ACS group and the ICS group showed no differences in these parameters ($p_1 > 0.05$ for all) (Table II).

Hemorheology

As shown in Figure 1, the Postop-ACS group exhibited a rigidity index of 5.11 [3.39-5.63] and a deformability index of 0.88 [0.77-0.93], which were comparable to those observed in the ICS group, with values of 4.33 [3.42-6.59] and 0.90 [0.73-1.06], respectively ($p_1 > 0.05$ for all parameters). Notably, the Postop-ACS group demonstrated higher deformability relative to the PRBC group (0.88 [0.77-0.93] vs 0.68 [0.51-0.81], $p_2 = 0.003$), while the rigidity index remained comparable (5.11 [3.39-5.63] vs 5.71 [4.34-8.82], $p_2 > 0.05$).

2,3-diphosphoglycerate and ATP

As shown in Figure 1, the Postop-ACS group maintained similar levels of 2,3-DPG (12.00 [9.24-13.32] $\mu\text{mol/gHb}$) and ATP (5.59 [5.27-5.93] $\mu\text{mol/gHb}$) compared to the ICS group (2,3-DPG: 13.90 [9.57-17.25] $\mu\text{mol/gHb}$, $p_1 > 0.99$; ATP: 5.21 [4.34-5.87] $\mu\text{mol/gHb}$, $p_1 > 0.05$). However, the Postop-ACS group demonstrated markedly higher 2,3-DPG ($p_2 < 0.001$) and ATP ($p_2 < 0.001$) levels than the PRBC group, which had 2,3-DPG levels of 2.43 [1.55-3.21] $\mu\text{mol/gHb}$ and ATP levels of 4.24 [3.21-4.92] $\mu\text{mol/gHb}$.

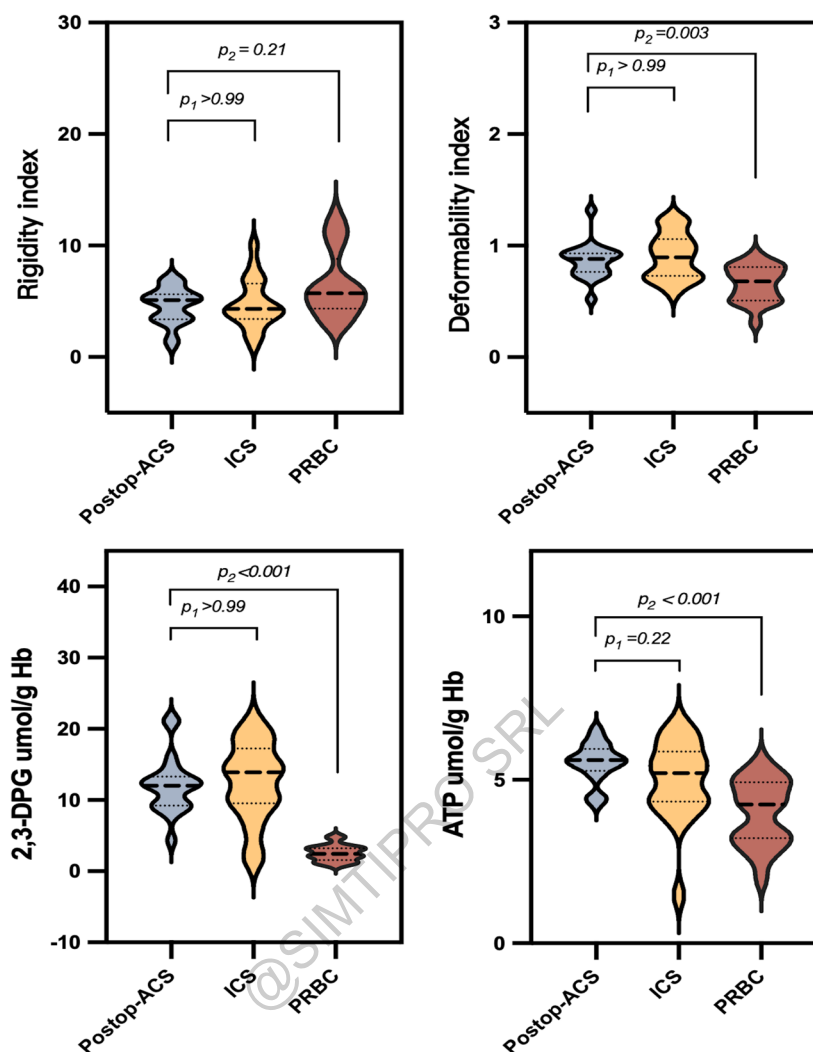


Figure 1 - The hemorheological parameters and levels of 2,3-diphosphoglycerate and adenosine triphosphate in the postoperative autologous cell-salvaged blood, the intraoperative salvaged blood, and the packed red blood cells

Postop-ACS: postoperative autologous cell-salvaged blood; ICS: intraoperative salvaged blood; PRBC: packed red blood cells; 2,3-DPG: 2,3-diphosphoglycerate; ATP: adenosine triphosphate. p₁: comparison of differences between the Postop-ACS group and ICS group; p₂: comparison of differences between the Postop-ACS group and PRBC group.

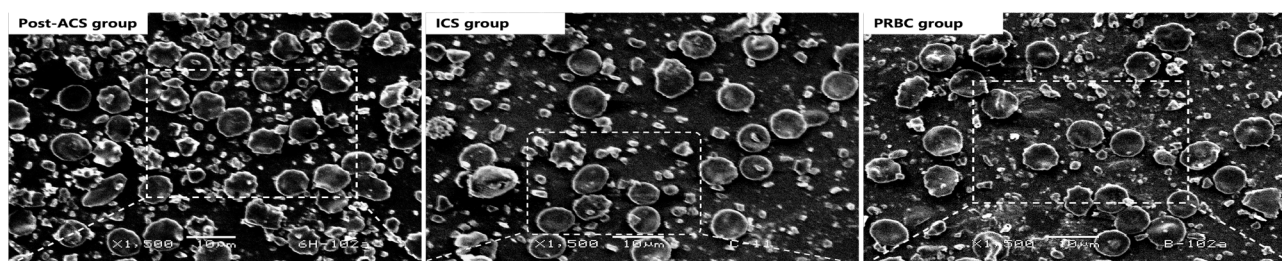


Figure 2 - Scanning electron microscopy images of red blood cell morphology in the postoperative autologous cell-salvaged blood, the intraoperative salvaged blood, and the packed red blood cells

Original magnification, ×1,500; scale bar = 10 μm. Postop-ACS: postoperative autologous cell-salvaged blood; ICS: intraoperative salvaged blood; PRBC: packed red blood cells.

Morphology

As illustrated in **Figure 2**, erythrocytes within the three groups largely preserved their characteristic biconcave-disk morphology, displaying minimal morphological alterations.

DISCUSSION

This study provides the first comprehensive comparison of Postop-ACS blood against ICS blood and PRBC, focusing on blood quality and erythrocyte function. Our findings demonstrate that Postop-ACS blood exhibits comparable quality and function to ICS blood while exhibiting more physiological metabolic and erythrocyte characteristics compared to mid-storage PRBC.

Prior research has mainly emphasized the importance of intraoperative blood salvage, but has often overlooked the potential of postoperative blood salvage, especially in cardiovascular surgery in which the risk of postoperative bleeding is high. Postoperative blood salvage aligns with PBM strategies, which advocate for the use of autologous blood and the reduction of allogeneic transfusions.

A major concern regarding postoperative autologous cell salvage is the potential for bacterial contamination during the prolonged collection of mediastinal shed blood outside the operating room. In our previous preclinical investigation, we systematically evaluated this issue using standardized aerobic and anaerobic bacterial cultures. Under a protocol incorporating sterile collection, a restricted 6-hour collection window, and extensive washing with the cell salvage system, all processed samples remained microbiologically sterile. Moreover, that study demonstrated preservation of blood quality and erythrocyte function within the same collection window, thereby establishing the methodological basis for the present investigation¹⁸ and the rationale for selecting the 6-hour collection window in the present study and supporting further investigation of postoperative autologous cell salvage in cardiovascular surgery.

Beyond microbiological assessment of the collected blood itself, the clinical safety of postoperative autologous reinfusion has also been evaluated in previous studies. Previous clinical studies showed that reinfusion of postoperative shed blood collected within predefined postoperative periods after cardiac surgery was not associated with an increased incidence of infectious

complications^{12,19,20}. Similar findings have been made in other surgical settings, including orthopedic procedures, in which postoperative autologous reinfusion has been performed without evidence of increased infection risk under standardized clinical protocols^{11,10,21}. However, most available evidence originates from non-cardiac surgery populations or earlier clinical protocols, and contemporary cardiovascular surgery-specific evidence remains limited. Therefore, prospective studies evaluating contamination control strategies and infection-related outcomes are still required before widespread adoption.

In this study, the Postop-ACS group exhibited favorable Hct levels, closely approximating those of the PRBC group. Notably, in clinical practice, the performance of ICS may occasionally be limited by insufficient shed blood volume, preventing completion of a full washing cycle and leading to lower-than-expected Hct levels. The Postop-ACS group specifically enrolled patients with ≥ 500 mL of postoperative mediastinal drainage within the first 6 hours, a volume threshold that has been recommended for justifying postoperative autotransfusion¹⁸. This volume typically allows at least one complete washing cycle, consistently yielding a higher Hct in the salvaged product and offering a practical means to support Hb recovery in patients with significant postoperative bleeding.

All groups demonstrated hemolysis rates below the internationally accepted safety threshold of 0.8%, thereby meeting international standards for blood product safety²². Hemolysis rates above this threshold are known to indicate excessive red blood cell destruction, which can increase the risk of transfusion-related complications such as hyperkalemia, acute kidney injury, and disseminated intravascular coagulation. The hemolysis rate of the ICS group is consistent with the values of $0.51\% \pm 0.27\%$ reported by Li *et al*¹⁶. In contrast, the Postop-ACS group showed an even lower hemolysis rate of 0.15% [0.09–0.18], which was likely attributable to the use of 5,000 mL of saline during the washing process²³. This enhanced washing protocol was intended to more effectively eliminate inflammatory mediators, such as fHb and other soluble components commonly present in postoperative shed blood, along with accumulated metabolic by products^{24,25}. Similar washing processes have been employed prior to transfusing allogeneic red blood cells in pediatric patients, aiming to reduce cytokines and fHb concentrations, thereby

mitigating the risk of transfusion-related complications. It should be noted that the differential wash volume between the Postop-ACS group (5,000 mL) and the ICS group (1,000 mL) may represent a potential confounder when comparing metabolic and hemolysis parameters between the two groups.

The Postop-ACS group exhibited a more favorable metabolic profile than PRBC, including near-physiologic pH and substantially reduced concentrations of potassium, glucose, and lactate. While these characteristics are hypothesized to mitigate transfusion-related risks such as hyperkalemia, acidosis, and hyperglycemia –common concerns associated with PRBC transfusion– this study did not directly evaluate clinical outcomes related to these biochemical differences²⁶. Therefore, further dedicated studies are needed to determine whether these improvements in metabolic profile translate into measurable clinical benefits.

The elevated WBC count in the Postop-ACS group most likely reflects two concurrent mechanisms: local recruitment of activated leukocytes to the surgical wound surface²⁷ and the inherent concentration effect of centrifugation-based cell salvage systems, which have limited capacity to remove leukocytes during processing²⁸. These factors together explain the significantly higher WBC counts in Postop-ACS blood compared to both ICS blood and PRBC. The clinical significance of this elevation should be interpreted within the framework of transfusion immunology. Transfusion-related immunomodulation –encompassing febrile non-hemolytic transfusion reactions, alloimmunization, and transfusion-related acute lung injury– is predominantly associated with allogeneic transfusion, being driven by non-self antigens and bioactive substances that accumulate during donor blood storage²⁹. Because the patient's own blood in Postop-ACS, the mechanisms underlying transfusion-related immunomodulation do not apply, and the enhanced washing protocol further removes cytokines, chemokines, and cell-derived microvesicles, providing additional protection against inflammatory transfusion responses³⁰. Notably, despite being significantly higher than in the ICS and PRBC groups, WBC counts in the Postop-ACS group remained within the normal physiological range, even after centrifugation-induced concentration. This

increase likely reflects concentration during processing rather than an increase in total leukocyte exposure. Nevertheless, these leukocytes are activated in nature, and their potential to contribute to local or systemic inflammatory responses should not be dismissed, particularly in patients with preoperative inflammation or infection. In such higher-risk populations, the selective integration of leukoreduction filters into the Postop-ACS circuit may offer additional safety benefits, as such filters have been shown to reduce leukocyte concentrations and proinflammatory mediators in autologous salvaged blood and to improve pulmonary ventilation, gas exchange, and inflammatory and oxidative stress outcomes in surgical patients^{31–33}. While routine leukoreduction may not be necessary for all patients, it represents a practical, evidence-based option that can be applied selectively according to individual clinical risk profiles.

In addition, we assessed erythrocyte function through measurements of 2,3-DPG and ATP levels, along with hemorheological and morphological characteristics, all of which are critical determinants of effective oxygen delivery to tissues and organs^{34,35}. The levels of 2,3-DPG and ATP in the Postop-ACS group suggest more favorable oxygen-carrying capacity and metabolic energy compared to those in the PRBC group. Specifically, 2,3-DPG facilitates oxygen release by reducing hemoglobin's affinity for oxygen, while ATP supports membrane integrity and flexibility³⁶. In fresh blood, these markers are typically maintained at 12–15 $\mu\text{mol/gHb}$ for 2,3-DPG and 3–5 $\mu\text{mol/gHb}$ for ATP. However, prolonged storage –particularly after 4 weeks– leads to a significant depletion of 2,3-DPG to 1–2 $\mu\text{mol/gHb}$, while ATP levels decrease to approximately 3 $\mu\text{mol/gHb}$ ^{15,17,37,38}. These reference values are consistent with our findings, with Postop-ACS and ICS blood maintaining physiologic levels of both markers, while PRBC exhibited marked reductions, likely due to cumulative storage lesions caused by oxidative stress and refrigeration^{16,38}. The less favorable erythrocyte functional parameters observed in the mid-storage PRBC group are consistent with the well-characterized red blood cell storage lesion, a constellation of progressive biochemical and structural changes that accumulate during hypothermic *ex vivo* storage. As comprehensively reviewed by Yoshida *et al.*, the root causes of the storage lesion can be classified into two categories: metabolic

impairments arising from the isolation of red blood cells in acidic additive solutions, and oxidative damage resulting from prolonged exposure to oxygen during refrigerated storage³⁹. The acidic pH of additive solutions rapidly depletes 2,3-DPG, while ATP is gradually consumed as energy-dependent membrane maintenance mechanisms progressively fail. Concurrently, lactic acid accumulates and further acidifies the storage medium, potassium leaks out of red blood cells down its concentration gradient, and glucose is consumed at supraphysiological rates, all of which were reflected in our PRBC measurements. These metabolic impairments, compounded by oxidative cross-linking of cytoskeletal proteins and membrane lipid peroxidation, reduce erythrocyte deformability and may promote a morphological transition from biconcave discocytes toward echinocytes and spherocytes. Importantly, the storage lesion accumulates non-linearly, with an exponential increase in oxidative damage after approximately 2 weeks of storage in standard additive solutions. The PRBC units in our study, stored for 15-20 days in MAP additive solution, fall within this critical window, likely accounting for the marked reductions observed in 2,3-DPG, ATP, and deformability. In contrast, the preserved erythrocyte functional parameters in both the Postop-ACS and ICS groups reflect the absence of hypothermic storage-induced lesions, as these autologous products were processed and would be reinfused within hours of collection.

Regarding hemorheology, the rigidity index reflects the stiffness of the erythrocyte membrane, which can affect microcirculatory flow and efficiency of oxygen delivery. Conversely, the deformability index measures the ability of red blood cells to adapt their shape to pass through narrow capillaries, a critical feature for ensuring effective tissue perfusion and gas exchange. In this study, the rigidity index and deformability index did not differ significantly between Postop-ACS and ICS blood. This suggests that processing within 6 hours of collection preserves erythrocyte structural integrity, an observation consistent with prior studies indicating that erythrocyte deformability and membrane stability are generally maintained for up to 6 hours at room temperature^{12,19,20}. Compared to PRBC, the Postop-ACS group exhibited statistically significant yet clinically modest differences in deformability. Importantly, this variation did not translate

into morphological deterioration. All three groups preserved the typical biconcave discocyte morphology, a critical feature for maintaining gas exchange and optimal microvascular flow. Previous studies have shown that erythrocytes stored under standard blood bank conditions (4°C for up to 5 weeks) retain their structural morphology, which is consistent with our observations in the PRBC group^{35,40}. Notably, hemorheological parameters—especially erythrocyte deformability—have been proposed as crucial markers for erythrocyte integrity and post-transfusion viability³⁴. In this context, the preserved deformability, morphology, and near-physiologic 2,3-DPG and ATP levels observed in the Postop-ACS group suggest that autologous salvaged blood maintains functional erythrocyte integrity, supporting its potential clinical utility.

Limitations

This pilot study has several limitations. First, the study was limited by its single-center design and small sample size, which may restrict the generalizability of the findings. Second, erythrocyte morphology was assessed qualitatively from representative scanning electron microscopy images; quantitative morphological distribution analysis was not performed, and future studies should incorporate systematic morphological scoring for a more objective evaluation. Third, the PRBC comparator was restricted to a mid-storage age of 15-20 days, and findings may differ with fresher units or those stored for a longer time; moreover, donor-specific characteristics and processing strategies are known to substantially influence red blood cell quality and post-transfusion outcomes, and these sources of variability were not controlled for in the present study. Finally, as an *in vitro* investigation, this study did not directly assess clinical outcomes such as postoperative complications, transfusion reactions, or patients' survival; whether the observed differences in blood quality and erythrocyte function translate into measurable clinical benefits remains to be determined in prospective studies. To validate and extend these findings, additional preclinical investigations are warranted to further characterize microbiological safety, contamination-control strategies, inflammatory responses, and protocol reproducibility. Upon completion of these studies, a multicenter randomized controlled trial will be conducted to evaluate

the clinical safety, efficacy, and cost-effectiveness of postoperative autologous cell salvage.

CONCLUSIONS

Postop ACS blood demonstrated comparable quality and erythrocyte function to ICS blood, with more physiologic metabolic and functional parameters relative to mid-storage PRBC. These findings provide preliminary evidence supporting postoperative autologous cell salvage as a physiologically favorable transfusion strategy, with the potential to enhance PBM and reduce allogeneic transfusion requirements in cardiovascular surgery.

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Authors' contributions

HWJ designed and planned the study. JCS and YYC conducted the study. GJ analyzed data. JCS wrote the paper.

The Authors declare no conflicts of interest.

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