Original article

Optimal 6-hour window for salvaged mediastinal blood retransfusion after cardiovascular surgery: an *in vitro* quality and safety analysis

Jiachen Shan[1], Jie Gao[1], Yuye Chen[1], Hongwen Ji[1,2]

[1]*Department of Anesthesiology, Fuwai Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China;*
[2]*Department of Anesthesiology, Chinese Academy of Medical Sciences Fuwai Hospital Shenzhen Hospital, Guangdong, China*

Background - Postoperative hemorrhage is a significant complication of cardiovascular surgery. Although autologous cell salvage is safe and effective for preserving patients' blood, its use in the postoperative context remains underexplored. We, therefore, evaluated the quality and safety of mediastinal blood collected for different periods at room temperature after cardiac surgery.

Materials and methods - In this preclinical, in vitro investigation, 60 patients who lost ≥500 mL mediastinal blood within 6 hours after cardiovascular surgery were randomized into three groups (6H, 8H, 12H) according to the time the shed mediastinal blood was processed. The quality of the salvaged blood was evaluated through measurements of hematocrit, pH, electrolyte and lactate levels, 2,3-diphosphoglycerate (2,3-DPG) and adenosine triphosphate (ATP) content, and red blood cell (RBC) deformability and morphology. The safety evaluations included hemolysis, contamination, and levels of inflammatory cytokines.

Results - With regard to quality, the median [IQR] values for the 6H group were hematocrit 56.3 [46.5-59.0] %, pH 7.34 [7.31-7.40]), potassium 1.10 [0.91-1.25] mmol/L, lactate 0.45 [0.20-0.90] mmol/L, 2,3-DPG 12.00 [9.24-13.32] µmol/gHb), ATP 5.59 [5.27-5.93] µmol/gHb and deformability 0.88 [0.77-0.93]. The values were similar across the three groups, except for pH, which decreased over collection period. RBC morphology transitioned from biconcave in the 6H and 8H groups to spiny forms in the 12H group. Hemolysis and inflammatory cytokine levels were low in all groups. Only the 6H group achieved total microbial sterility, with 0% bacterial contamination, compared to 10% (No.=2) in the 8H group and 5% (No.=1) in the 12H group.

Discussion - Our study establishes a 6-hour window as the optimal period for ensuring microbial sterility and preserving the quality and safety of salvaged mediastinal blood postoperatively. Although blood quality remained stable for up to 12 hours, the heightened risk of contamination beyond 6 hours necessitates rigorous microbiological monitoring.

Keywords: *cardiovascular surgery, patient blood management, shed mediastinal blood, postoperative salvaged blood, autologous cell salvage.*

Arrived: 6 April 2025
Revision accepted: 18 June 2025
Correspondence: Hongwen Ji
e-mail: 13701229553@163.com

INTRODUCTION

Postoperative bleeding is one of the most frequent complications following cardiovascular surgery, with nearly 50% of patients undergoing different degrees of bleeding and transfusion after surgery[1-3]. Simultaneously, the rates of allogeneic blood utilization are higher for cardiovascular surgery than for other surgical categories, accounting for 10 to 15% of national blood use in the United States and the United Kingdom[3-5]. Autologous cell salvage, as part of a strategy of Patient Blood Management (PBM), has been extensively demonstrated to be safe and effective, reducing exposure to allogeneic blood by 40%[6], with particularly robust evidence in cardiovascular surgery[7,8]. Building on this, cell salvage of shed mediastinal blood may provide a feasible solution for postoperative bleeding and minimizing allogeneic blood transfusions following cardiovascular surgery.

The direct reinfusion of shed mediastinal blood from postoperative chest tube drainage has been extensively studied in the past[9-12]. Nevertheless, several medical organizations, notably the Society of Thoracic Surgeons (STS) and the Society of Cardiovascular Anesthesiologists (SCA), categorize this practice as a class III intervention, citing the potential increased risk of complications, such as fat embolism, acute kidney injury, coagulopathy, and wound infection[13,14]. Current blood salvage technologies, with their filtration and washing steps, may reduce the incidence of these complications by improving blood purity. However, the application of cell salvage for postoperative shed mediastinal blood remains underexplored, and existing clinical guidelines lack specific recommendations for its use in this context[13]. The aim of this study was to enhance the current body of knowledge by assessing the quality and safety of salvaged mediastinal blood and exploring the safe collection period at room temperature after surgery.

To explore the comprehensive properties of salvaged mediastinal blood after cardiovascular surgery, we conducted a preclinical in *vitro* study to: (i) evaluate the quality and safety of salvaged blood in the postoperative period; and (ii) explore the viable period of collection of shed mediastinal blood at room temperature.

MATERIALS AND METHODS

This preclinical *in vitro* investigation, conducted at Fuwai Hospital, Chinese Academy of Medical Sciences, assessed the quality and safety of salvaged mediastinal blood from patients following cardiovascular surgery. The study was conducted in accordance with the principles of the Declaration of Helsinki, following approval from the institutional ethics committee and registration of the trial (Ethics number: 2022-1805, clinicaltrials.gov registration: ChiCTR2200065958). Written informed consent was obtained prospectively from all participants enrolled in the study.

Study population

The participant enrollment process is illustrated in **Figure 1**. Adult patients scheduled for cardiovascular surgery between June 1 and September 30, 2024, were initially screened. Eligibility required meeting all baseline

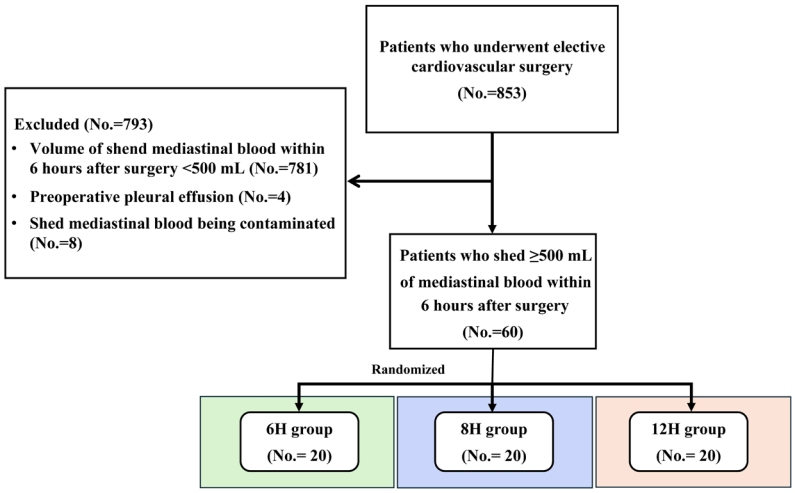

Figure 1 - Flow chart of enrollment and grouping

inclusion criteria: (i) age 18-70 years; (ii) undergoing coronary artery bypass grafting, heart valve, aortic, or combined surgery. From this cohort, patients who shed more than 500 mL of mediastinal blood within 6 hours after surgery underwent further evaluation for final enrollment.

The exclusion criteria comprised: (i) clinically diagnosed preoperative pleural effusion; (ii) sepsis, bacteriemia, or hematological disease during the perioperative period; (iii) contaminated shed mediastinal blood samples.

Operative techniques

All patients received general anesthesia with a combination of intravenous opioids, inhaled agents, and neuromuscular blockade, along with intravenous antibiotics administered according to a standardized hospital protocol. After closing the sternum, three 28Fr soft sump drains were placed as usual: one each in the pericardial cavity, mediastinum, and pleural space. The pericardial and mediastinal drains were joined with a Y-connector to a sterile negative pressure drainage bottle, pre-filled with 20 mL of heparin- saline solution (12,500 IU of heparin in 20 mL of 0.9% NaCl) to prevent clotting and collect blood after surgery.

Study design

This *in vitro* study assessed the quality and safety of salvaged mediastinal blood over extended postoperative collection intervals ranging from 6 to 12 hours. Sixty patients who underwent elective cardiovascular surgery and shed ≥500 mL via their mediastinal drains within the first 6 hours after surgery were randomly assigned, using opaque envelopes, to one of three processing timepoints: 6 hours, 8 hours, and 12 hours, thereby forming three groups, the 6H, 8H and 12H groups (**Figure 1**). Blood samples were subjected to standardized washed cell salvage protocols at their respective processing time points to systematically evaluate the impact of prolonged storage at room temperature on blood quality and safety. If a patient required urgent reoperation due to excessive postoperative bleeding (>150 mL/h), the drainage bottle was disconnected from the patient prior to the reoperation. It was then aseptically stored at ambient Intensive Care Unit temperature until cell salvage processing at the assigned group timepoint (6, 8, or 12 hours postoperatively).

The shed blood was transferred to the same cell saver (Sorin Xtra, Munchen, Germany) for processing. The system used a 225 mL collection bowl and default settings (Popt program, FSF Inc., Boston, MS, USA). Initially, the collection reservoir was primed with normal saline at twice the blood volume to wet the walls and dilute the blood. Blood was then manually transferred from the drainage bottle into the reservoir under sterile conditions, using negative pressure suction at 100 mmHg to prevent hemolysis. To address the potential presence of inflammatory cytokines, 5,000 mL of normal saline were used to wash the shed mediastinal blood[15]. Following the default program, with a prime rate of 400/250 mL/min, wash rate of 500 mL/min, and empty rate of 400 mL/min, the salvaged blood was collected in a reinfusion bag for subsequent laboratory analysis. Details of the laboratory analyses and specific methods for each indicator can be found in *Online Supplementary Content*.

Outcomes

The outcomes were categorized into two primary domains: blood quality and safety. Blood quality was evaluated through hematologic parameters, including hematocrit and hemoglobin (Hb); metabolic profiles, such as pH, electrolyte levels and lactate levels; and red blood cell (RBC) function, which encompassed the levels of 2,3-diphosphoglycerate (2,3-DPG) and adenosine triphosphate (ATP), hemorheology (aggregation, rigidity and deformability index) and morphology. Safety was assessed by measuring free hemoglobin (fHb) levels, performing microbiological cultures, and assaying pro- and anti-inflammatory cytokine levels.

Sample size and statistical analysis

Due to the exploratory nature of this study and lack of prior data, a sample size of 60 was chosen, with 20 participants per group[16]. Therefore, no *a priori* statistical power calculation was performed. *Post hoc* statistical analyses were carried out. The Shapiro-Wilk test showed non-normal data distribution, so results are presented as medians with interquartile ranges (IQR), and categorical variables as numbers and percentages. The Kruskal-Wallis test with Dunn's correction was used for comparing the three groups. All tests were two-tailed, with p values <0.05 indicating statistical significance. Analyses were conducted using R (version 4.3.2 [R Foundation, Wien, Austria]) and Prism 10 (GraphPad Software, Boston, MA, USA).

Table I - *Perioperative characteristics of patients in the 6H, 8H, and 12H groups*

Characteristics	6H (No.=20)	8H (No.=20)	12H (No.=20)	p value
Female sex, No. (%)	4 (20)	3 (15)	1 (5)	0.36
Age, years, median [IQR]	61 [54-66]	61[55-64]	65 [55-68]	0.43
Height, cm, median [IQR]	170 [162-174]	170 [166-175]	170 [166-174]	0.76
BMI, kg/m^2, median [IQR]	24.55 [22.86-27.22]	24.46 [22.86-26.34]	25.31 [24.43-26.14]	0.75
Coexisting illness, N (%)				
Hypertension	8 (40)	14 (70)	12 (60)	0.15
Diabetes mellitus	3 (15)	9 (45)	7 (35)	0.12
Dyslipidemia	10 (50)	9 (45)	12 (60)	0.63
Atrial fibrillation	4 (20)	2 (10)	4 (20)	0.62
Chronic kidney disease	1 (5)	0 (0)	2 (10)	0.35
History of stroke	1 (5)	2 (10)	1 (5)	0.77
Active smoking	8 (40)	8 (40)	10 (50)	0.80
Laboratory values before surgery, median [IQR]				
WBC count, 10^9/L	5.80 [5.32-6.89]	6.26 [5.47-6.84]	5.67 [5.21-6.93]	0.64
RBC count, 10^{12}/L	4.62 [4.24-5.07]	4.74 [4.36-4.96]	4.70 [4.43-4.90]	0.97
Hemoglobin, g/dL	14.1 [12.9-15.2]	13.9 [13.2-15.3]	13.5 [13.0-15.1]	0.93
Hematocrit, %	42.1 [39.5-46.3]	43.5 [40.3-44.9]	42.0 [39.3-45.9]	0.96
Platelet count, 10^9/L	184 [148-233]	203 [156-228]	183 [169-208]	0.93
NYHA class, N (%)				0.18
I	0 (0.0)	0 (0.0)	1 (5.3)	
II	15 (75.0)	17 (85.0)	10 (52.6)	
III	5 (25.0)	3 (15.0)	8 (42.1)	
Surgery type, N (%)				0.48
Only CABG	9 (45)	14 (70)	12 (60)	
On-pump	2 (10)	7 (35)	9 (45)	
Off-pump	7 (35)	7 (35)	3 (15)	
Only valve	5 (25)	3 (15)	2 (10)	
Only aorta	1 (5)	1 (5)	1 (5)	
Combined	5 (25)	2 (10)	5 (25)	
Surgery procedure, median [IQR]				
Procedure duration, min	242.50 [216.00-292.50]	219.00 [203.00-236.25]	248.50 [212.25-291.75]	0.08
Cardiopulmonary bypass duration, min	168 [117-184]	96 [81-130]	135 [105-175]	0.08
Aortic cross-clamping duration, min	89 [80-140]	82 [59-101]	111 [71-121]	0.31
Lowest temp, °C	32.0 [31.8-32.0]	32.0 [32.0-32.8]	32.0 [32.0-32.8]	0.06
Perioperative outcomes				
RBC transfusion, N (%)	1 (5)	1 (5)	1 (5)	>.99
Reoperation, N (%)	6 (30)	3 (15)	1 (5)	0.10
ICU stay, hours, median [IQR]	42.3 [23.9-58.0]	39.3 [26.5-62.0]	40.3 [16.5-75.8]	0.93
Ventilation time, hours, median [IQR]	11.0 [9.0-13.2]	9.0 [7.0-11.0]	7.0 [6.0-10.5]	0.06
Hospital stays, days, median [IQR]	12 [9-13]	11 [9-13]	13 [10-17]	0.40
Mortality in 30 days, N (%)	0 (0)	0 (0)	0 (0)	

IQR: interquartile range; BMI: body mass index; WBC: white blood cell; RBC: red blood cell; NYHA: New York Heart Association; CABG: coronary artery bypass graft; ICU: Intensive Care Unit.

RESULTS

Sixty patients scheduled for elective cardiovascular surgery from June 1 to September 30, 2024 were enrolled. From these patients, a total of 60 shed mediastinal blood samples (one per patient) were collected and allocated to three processing timepoints –6, 8 and 12 hours– resulting in 20 samples per group (**Figure 1**).

Baseline characteristics

Table I provides a summary of the patients' baseline characteristics, laboratory test results, and surgical attributes across the three salvaged blood groups. Statistical analysis revealed no significant differences among the three groups.

Quality of the salvaged blood

The quality parameters of samples from the three groups are presented in **Table II**. The salvaged blood of the 6H group had a median [IQR] hematocrit of 56.3 [46.5-59.0]% and Hb of 19.4 [16.0-19.7] g/dL with no significant differences (p=0.96 and p=0.93) observed among groups. However, white blood cell counts exhibited significant differences (p=0.013), showing elevated levels in the 8H (9.20 [7.51-10.90]×10^9/L) and 12H (9.28 [8.00-12.14]×10^9/L) groups compared with the baseline 6H group (5.34 [3.95-10.03]×10^9/L) (p=0.026, *Z*=2.626 and p=0.027, *Z*=2.616). Metabolic profiles demonstrated a gradual decline in pH, with statistically significant decreases observed in the 8H group (p=0.005, *Z*=3.151) and 12H group (p<0.001, *Z*=4.129), although all values remained within the range of physiological compensation. Electrolyte and glucose homeostasis was preserved across all groups.

Parameters associated with 2,3-DPG and ATP, exhibited no statistically significant differences across the groups (p=0.95 and p=0.77, respectively). Similarly, hemorheological parameters, including the aggregation index (p=0.91), rigidity index (p=0.43), and elongation

Table II - *The quality parameters in the 6H, 8H, and 12H groups*

Parameters	6H (No.=20)	8H (No.=20)	12H (No.=20)	p value
Hematologic performance				
RBC count, 10^{12}/L	6.57 [5.08-6.67]	6.30 [5.48-6.66]	6.04 [5.07-6.67]	0.80
Hemoglobin, g/dL	19.4 [16.0-19.7.]	18.6 [17.1-19.9]	18.5 [16.0-20.4]	0.93
Hematocrit, %	56.3 [46.5-59.0]	55.8 [48.9-59.3]	56.6 [44.9-61.1]	0.96
WBC count, 10^9/L	5.34 [3.95-10.03]	9.20 [7.51-10.90]	9.28 [8.00-12.14]	0.013
Metabolic profiles				
pH	7.34 [7.31-7.40]	7.27 [7.24-7.34]	7.26 [7.24-7.30]	<0.001
PCO_2, mmHg	5.80 [5.57-6.62]	5.90 [5.68-6.50]	6.30 [5.57-7.55]	0.40
PO_2, mmHg	224.1 [219.4-228.8]	224.8 [221.4-230.1]	226.1 [213.5-238.7]	0.81
SO2, %	91.8 [89.2-99.8]	92.5 [89.9-99.7]	99.6 [89.5-99.8]	0.56
Na^+, mmol/L	146.8 [143.8-152.7]	147.3 [145.7-153.9]	146.3 [145.8-153.8]	0.76
K^+, mmol/L	1.10 [0.91-1.25]	1.11 [0.84-1.25]	0.98 [0.87-1.22]	0.82
Ca^{2+}, mmol/L	0.20 [0.16-0.21]	0.18 [0.16-0.22]	0.20 [0.16-0.24]	0.65
Glucose, mmol/L	0.88 [0.84-0.98]	0.88 [0.82-0.93]	0.95 [0.82-1.11]	0.37
Lactate, mmol/L	0.45 [0.20-0.90]	0.65 [0.40-1.20]	1.30 [0.45-2.62]	0.12
2,3-DPG, µmol/gHb	12.00 [9.24-13.32]	11.94 [7.42-14.80]	12.10 [7.52-15.65]	0.95
ATP, µmol/gHb	5.59 [5.27-5.93]	5.29 [5.15-5.69]	5.65 [5.22-6.13]	0.77
Hemorheology				
Aggregation index	5.25 [4.92-5.56]	5.03 [4.77-5.50]	5.10 [4.78-5.53]	0.91
Rigidity index	5.11 [3.39-5.63]	5.21 [4.18-6.40]	5.26 [3.77-6.35]	0.43
Deformability index	0.88 [0.77-0.93]	0.80 [0.70-0.88]	0.76 [0.70-0.87]	0.11

Values are median [interquartile range]. RBC: red blood cell; WBC: white blood cell; 2,3-DPG, 2,3- diphosphoglycerate; ATP, adenosine triphosphate.

index (p=0.11), remained consistent across the groups. As depicted in **Figure 2**, RBC in the 6H and 8H groups largely retained their characteristic biconcave-discoid morphology, with minimal morphological changes observed. In contrast, RBC in the 12H group predominantly exhibited a marked increase in spiny forms.

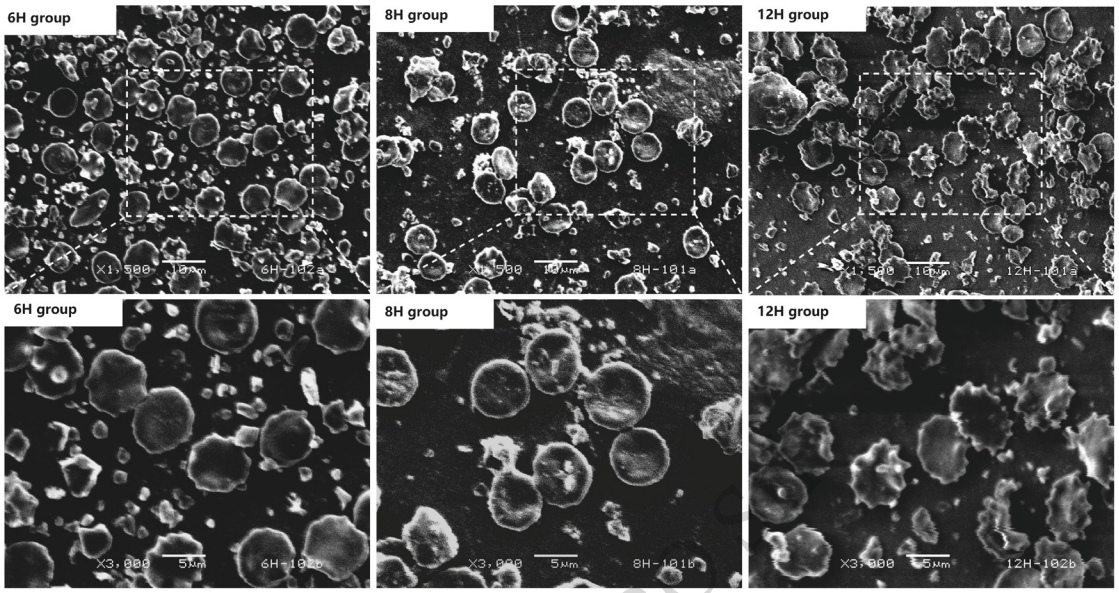

Figure 2 - Red blood cell morphology in the 6H, 8H, and 12H groups at different magnifications in scanning electron microscope images
The first row (1,500×, scale bar = 10 μm) provides an overview of the structural features, while the second row (3,000×, scale bar = 5 μm) highlights finer surface detail.

Table III - *The safety parameters in the 6H, 8H, and 12H groups*

Parameters	6H (No.=20)	8H (No.=20)	12H (No.=20)	p value
Hemolysis				
fHb, g/L	0.55 [0.30-0.80]	0.50 [0.40-0.92]	0.50 [0.30-0.77]	0.76
Hemolysis rate, %	0.15 [0.09-0.18]	0.16 [0.11-0.22]	0.14 [0.09-0.21]	0.74
Inflammatory cytokines, pg/mL				
IL-1β	3.90 [1.93-16.29]	7.13 [4.03-12.89]	8.05 [4.08-20.26]	0.19
IL-2	1.77 [1.25-3.07]	2.39 [1.69-3.23]	2.36 [1.98-2.81]	0.26
IL-4	1.61 [0.75-3.25]	2.15 [0.86-2.71]	2.33 [2.01-2.67]	0.44
IL-5	1.88 [1.15-2.87]	1.98 [1.48-2.73]	2.5 [1.63-2.84]	0.44
IL-6	14.23 [11.76-21.28]	20.45 [11.62-44.01]	17.06 [12.11-25.87]	0.28
IL-10	3.75 [3.14-4.79]	3.86 [3.28-4.55]	4.90 [4.09-5.96]	0.009
IL-12p70	2.57 [1.56-3.57]	3.43 [2.71-3.85]	3.04 [2.27-3.69]	0.16
IL-17	2.07 [0.99-3.15]	2.43 [1.58-3.26]	2.35 [1.57-2.88]	0.90
TNF-α	2.31 [1.70-4.11]	3.77 [2.40-5.29]	3.73 [2.67-4.04]	0.17
IFN-a	3.41 [2.46-4.50]	4.08 [3.22-6.09]	4.66 [3.79-5.71]	0.11
IFN-γ	1.27 [0.93-2.31]	1.77 [1.31-2.26]	1.62 [0.95-2.32]	0.53

Values are median [interquartile range]. fHb: free hemoglobin; IL: interleukin; TNF: tumor necrosis factor; IFN: interferon.

Safety of salvaged blood

Table III presents the results of the safety assessments, including hemolysis, microbiological culture, and inflammatory cytokines, across the three groups. The hemolysis parameters, including the hemolysis index and fHb concentrations, were not significantly different between the groups (p=0.74 and p=0.76, respectively). Regarding microbiological cultures, 5% (3/60) of the salvaged blood samples yielded positive cultures, with no contamination in the 6H group, two cases (10%) in the 8H group (*Staphylococcus epidermidis*, *Corynebacterium afermentans*), and one case (5%) in the 12H group (*Staphylococcus epidermidis*). Analysis of inflammatory cytokines revealed that interleukin (IL)-10 was the only analyte with a statistically significant increase in the 12H group compared to both the 6H (p=0.05, *Z*=2.395) and 8H (p=0.012, *Z*=2.875) groups. Importantly, despite these variations, all IL-10 concentrations remained within physiological reference ranges (<5 pg/mL), indicating that the changes were not clinically significant. The other inflammatory cytokines showed no statistically significant differences among the three groups.

DISCUSSION

This study was a preliminary assessment of the quality and safety of salvaged mediastinal blood collected 6 to 12 hours after cardiac surgery. The findings indicated that shed mediastinal blood, after being stored at room temperature within this timeframe and subsequently undergoing a cell salvage process, preserved its quality and RBC functions. Devices for prompt processing are already available and have been proven to be safe, also in cardiac surgery within a 6-hour timeframe[17]. While all salvaged blood maintained acceptable levels of fHb and inflammatory factors, only the 6H group achieved complete microbial sterility. The findings offer insights to inform clinical decision-making regarding the duration of collection of shed blood following surgery, a critical concern due to the prolonged durations frequently observed in real-world surgical settings.

While existing research has focused on intraoperative salvage, our findings underscore the untapped potential of postoperative cell salvage in the advancement of PBM. By exploring the quality and safety of postoperative salvaged blood, this study seeks to provide preliminary evidence for extending PBM protocols into the postoperative phase. Moreover, these insights have the potential to optimize transfusion practices associated with a high risk of postoperative bleeding and to enhance preparedness for patients with rare blood phenotypes.

The quality of salvaged blood is a primary consideration in postoperative retransfusion. In this study, salvaged blood samples exhibited promising hematocrits (45 to 60%), closely resembling those achieved with intraoperative cell salvage[18]. Moreover, samples from all groups maintained near normal pH, as well as lower potassium, glucose, and lactate levels. The cell salvage washing process has been shown to reduce lactate, glucose, and potassium concentrations[19], and these parameters exhibited consistent levels in this study.

We also assessed RBC function through hemorheological parameters, which are essential determinants of effective oxygen delivery to tissues and organs[20,21]. Previous research indicated that RBC deformability and structural integrity remain stable for up to 6 hours at room temperature and longer at 4°C, with no significant decline of deformability even after 5 weeks of storage under blood bank conditions[21-23]. Consistently, our findings indicated that the aggregation and deformability of RBC in shed mediastinal blood stored at room temperature for 6 to 12 hours did not alter, and were comparable to those in previous studies on intraoperative cell salvage blood[24-26]. Additionally, in this study, the values of the RBC deformability index across the three groups were consistent with established measurements from blood in the systemic circulation reported in previous human studies (0.79±0.04)[25]. This may suggest that the salvaged RBC maintained intrinsic deformability comparable to that of systemic circulating RBC throughout the 6- to 12-hour postoperative collection period.

The levels of 2,3-DPG and ATP remained stable in the salvaged blood across all three groups. As a critical biomarker of RBC functionality, 2,3-DPG is a significant determinant of hemoglobin-oxygen affinity, thereby influencing the oxygen delivery capacity of RBC, while ATP serves as an essential energy source for the overall functioning of RBC. Under normal systemic circulation, baseline levels are maintained at 12-15 µmol/g Hb for 2,3-DPG and 4±1 µmol/g Hb for ATP[27]. However, during prolonged storage of up to 4 weeks, these levels decline to 1-2 µmol/g Hb for 2,3-DPG and 3 µmol/g Hb for ATP[27-30].

Our findings suggest that the collection of shed blood at room temperature for 6 to 12 hours aftervsurgery did not lead to a significant depletion of either 2,3-DPG or ATP within RBC.

Regarding morphology, the biconcave shape of RBC is essential for the cells' physiological function, as it optimizes gas exchange, preserves membrane flexibility, and enhances microvascular perfusion[31]. Our findings indicate that during the collection of shed blood, RBC underwent a morphological transformation from discocytes to echinocytes. Previous studies have demonstrated that changes in RBC morphology do not significantly affect deformability, as echinocytes can gradually regain their biconcave shape when placed in a systemic environment[20,32]. This may explain why RBC deformability did not show significant differences in our study, despite the notable morphological differences. Therefore, our findings suggest that salvaged mediastinal blood collected at room temperature for 6 to 12 hours does not significantly impair RBC deformability, thereby supporting the adequate microcirculatory function and oxygen delivery of the cells once reinfused.

The safety of salvaged blood is assessed by evaluating the hemolysis rate, microbiological contamination, and inflammatory cytokine levels. It has been demonstrated that the washing process in cell salvage effectively removes plasma chemical and biological contaminants, such as activated complement proteins, fHb, and inflammatory cytokines, thereby supporting our findings[33,34]. In our study, samples from all groups exhibited low hemolysis rates that comply with international blood product quality standards mandating that the hemolysis rate of the blood product is less than 0.8%[35]. Furthermore, the salvaged blood demonstrated low concentrations of pro- and anti-inflammatory cytokines. Surgical trauma induces the localized release of pro-inflammatory cytokines and previous studies reported IL-6 levels in shed blood as high as 1,300 pg/mL[10]. Encouragingly, it has been demonstrated that the washing process effectively removes inflammatory cytokines, with varying removal rates based on initial levels[15]. Our findings contribute to the limited evidence on inflammatory cytokine concentrations in blood salvaged postoperatively.

Importantly, only the 6H salvaged blood was free of contamination, whereas one or two cases of contamination were found in other groups. Previous randomized controlled trials have demonstrated that the reinfusion of shed blood within 4 to 24 hours following cardiac surgery does not increase postoperative complication rates, particularly with respect to infections[11,36,37]. Concerns have, however, been raised regarding intraoperative cell salvage. A recent study in cardiac surgery reported positive bacterial cultures in 49% of intraoperatively salvaged blood samples, with *Staphylococcus epidermidis* identified as the predominant pathogen[38]. Furthermore, research across various surgical disciplines has documented variable contamination rates in intraoperatively salvaged blood[39-41]. Nonetheless, there remains a lack of consensus on whether such contamination is associated with an increased incidence of postoperative infections in patients[41], a phenomenon that may be influenced by the perioperative administration of prophylactic antibiotics. A significant challenge is to identify the source of bacterial contamination in intraoperatively salvaged blood. Potential sources, including intraoperative airborne exposure, skin flora, preexisting subclinical infections, or the salvage procedures themselves, are complex and difficult to differentiate. In this *in vitro* study, we performed manual aseptic transfer of shed blood to the cell saver. While strict protocols were followed, this manual step cannot exclude the potential introduction of contaminants during handling. Observed bacterial growth in the 8H and 12H groups may therefore reflect either intrinsic properties of shed blood stored for a prolonged period or extrinsic factors related to transfer. To eliminate this transfer-associated risk in future clinical applications, we recommend a technical modification: using the cell saver reservoir itself as the postoperative drainage bottle. This closed-system approach would allow shed blood to drain directly into the salvage device reservoir, bypassing manual transfer steps and significantly reducing opportunities for contamination. Comprehensive cohort studies and prospective clinical trials remain necessary to systematically explore the incidence and etiology of bacterial contamination in postoperative salvaged blood. In the absence of definitive evidence, maintaining a 6-hour collection window as the optimal timeframe for microbial sterility and blood quality remains a prudent clinical practice.

Blood Transfus 2026; 24(2):129-138. doi: 10.2450/BloodTransfus.1073

Notably, our findings supporting this 6-hour window align well with existing clinical practices in orthopedic and cardiac surgery[42,43]. Indeed, devices such as the OrthoPAT (Haemonetics, Boston, MA, USA)[44], XTRA (Sorin)[45], and CATS (Fresenius, Bad Homburg, Germany)[36] have already been successfully used in this timeframe, underscoring the broader clinical feasibility of postoperative processing and retransfusion of shed blood.

Limitations

Our study has several limitations. Firstly, given that this is an observational study with a relatively small sample size, our findings should be interpreted with caution. Although each group comprised 20 participants, aligning with sample sizes in prior exploratory studies, larger-scale investigations are necessary to validate the results. Secondly, as this was an *in vitro* study, the findings may not entirely capture the complexities inherent in clinical practice. A forthcoming study is intended to conduct a comprehensive evaluation of the safety, efficacy, and cost-effectiveness of postoperative salvaged mediastinal blood for clinical practice, with the goal of providing more robust evidence for its use in postoperative autotransfusion.

CONCLUSIONS

Our study establishes a 6-hour collection window as the optimal period for ensuring microbial sterility and preserving the quality and safety of salvaged mediastinal blood. Although the blood maintains stable quality for up to 12 hours, the heightened risk of contamination beyond the 6-hour window necessitates rigorous microbiological monitoring.

Acknowledgements

The Authors thank the Departments of Anesthesiology and Intensive Care Unit in Fuwai Hospital for their invaluable support in facilitating this study.

Funding

This work was supported by grants from the Chinese Academy of Medical Sciences Innovation Fund for Medical Sciences (CIFMS :2021-I2M-1-60).

Authorship contribution

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

The Authors declare no conflicts of interest.

REFERENCES

1. Dyke C, Aronson S, Dietrich W, Hofmann A, Karkouti K, Levi M, et al. Universal definition of perioperative bleeding in adult cardiac surgery. J Thorac Cardiovasc Surg. 2014; 147(5): 1458-1463.e1. doi: 10.1016/j.jtcvs.2013.10.070.
2. Pereira KMFSM, De Assis CS, Cintra HNWL, Ferretti-Rebustini REL, Püschel VAA, Santana-Santos E, et al. Factors associated with the increased bleeding in the postoperative period of cardiac surgery: a cohort study. J Clinical Nurs. 2019; 28(5-6): 850-861. doi: 10.1111/jocn.14670.
3. Whitlock R, Crowther MA, Ng HJ. Bleeding in cardiac surgery: its prevention and treatment– an evidence-based review. Crit Care Clin. 2005; 21(3): 589-610. doi: 10.1016/j.ccc.2005.04.003.
4. Ohmes LB, Di Franco A, Guy TS, Lau C, Munjal M, Debois W, et al. Incidence, risk factors, and prognostic impact of re-exploration for bleeding after cardiac surgery: a retrospective cohort study. Int J Surg. 2017; 48: 166-173. doi: 10.1016/j.ijsu.2017.10.073.
5. Mauney C, Etchill E, Rea A, Edwin Fonner C, Whitman G, Salenger R. What drives variability in postoperative cardiac surgery transfusion rates. J Thorac Cardiovasc Surg. 2025; 169(2) :667-674.e1. doi: 10.1016/j.jtcvs.2024.02.004.
6. Wang G, Bainbridge D, Martin J, Cheng D. The efficacy of an intraoperative cell saver during cardiac surgery: a meta-analysis of randomized trials. Anesth Analg. 2009; 109(2): 320-330. doi: 10.1213/ane.0b013e3181aa084c.
7. Sadana D, Pratzer A, Scher LJ, Saag HS, Adler N, Volpicelli FM, et al. Promoting high-value practice by reducing unnecessary transfusions with a patient blood management program. JAMA Intern Med. 2018; 178(1): 116. doi: 10.1001/jamainternmed.2017.6369.
8. Shander A, Hardy JF, Ozawa S, Farmer SL, Hofmann A, Frank SM, et al. A global definition of patient blood management. Anesth Analg. 2022; 135(3): 476-488. doi: 10.1213/ANE.0000000000005873.
9. Tripkovic B, Bukovic D, Sakic K, Sakic S, Bukovic N, Radakovic B. Quality of the blood sampled from surgical drainage after total hip arthroplasty. Coll Antropol 2008; 32(1): 153-160. PMID: 18494201.
10. Sebastián C, Romero R, Olalla E, Ferrer C, García-Vallejo JJ, Muñoz M. Postoperative blood salvage and reinfusion in spinal surgery: blood quality, effectiveness and impact on patient blood parameters. Eur Spine J. 2000; 9(6): 458-465. doi: 10.1007/s005860000167.
11. Cholette JM, Powers KS, Alfieris GM, Angona R, Henrichs KF, Masel D, et al. Transfusion of cell saver salvaged blood in neonates and infants undergoing open heart surgery significantly reduces RBC and coagulant product transfusions and donor exposures: results of a prospective, randomized, clinical trial*. Pediatr Crit Care Med. 2013; 14(2): 137-147. doi: 10.1097/PCC.0b013e31826e741c.
12. Schaff HV, Hauer JM, Bell WR, Gardner TJ, Donahoo JS, Gott VL, et al. Autotransfusion of shed mediastinal blood after cardiac surgery: a prospective study. J Thorac Cardiovasc Surg. 1978; 75(4): 632-641. PMID: 642556.
13. Martin J, Robitaille D, Perrault LP, Pellerin M, Pagé P, Searle N, et al. Reinfusion of mediastinal blood after heart surgery. J Thorac Cardiovasc Surg. 2000;120(3):499-504. doi: 10.1067/mtc.2000.108691.
14. Schmidbauer SL, Seyfried TF. Cell salvage at the ICU. JCM. 2022; 11(13): 3848. doi: 10.3390/jcm11133848.
15. Amand T, Pincemail J, Blaffart F, Larbuisson R, Limet R, Defraigne JO. Levels of inflammatory markers in the blood processed by autotransfusion devices during cardiac surgery associated with cardiopulmonary bypass circuit. Perfusion. 2002; 17(2): 117-123. doi: 10.1191/0267659102pf544oa.
16. Eldridge SM, Chan CL, Campbell MJ, Bond CM, Hopewell S, Thabane L, et al. CONSORT 2010 statement: extension to randomised pilot and feasibility trials. BMJ. 2016; 355: i5239. doi: 10.1136/bmj.i5239.
17. Weltert L, Nardella S, Rondinelli MB, Pierelli L, De Paulis R. Reduction of allogeneic red blood cell usage during cardiac surgery by an integrated intra- and postoperative blood salvage strategy: results of a randomized comparison. Transfusion (Paris). 2013; 53(4): 790-797. doi: 10.1111/j.1537-2995.2012.03836.x.

18. Overdevest E, Lanen P, Feron J, Van Hees J, Tan M. Clinical evaluation of the Sorin Xtra(R) autotransfusion system. Perfusion. 2012; 27(4): 278-283. doi: 10.1177/0267659112442222.
19. Liu J, Ji B, Feng Z, Zhao J, Li C, Li B, et al. The effect of preprocessing stored red blood cells on neonates undergoing corrective cardiac surgery. ASAIO J. 2007; 53(6): 680-683. doi: 10.1097/MAT.0b013e31815a5edb.
20. Barshtein G, Pajic-Lijakovic I, Gural A. Deformability of stored red blood cells. Front Physiol. 2021; 12: 722896. doi: 10.3389/fphys.2021.722896.
21. Uyuklu M, Cengiz M, Ulker P, Hever T, Tripette J, Connes P, et al. Effects of storage duration and temperature of human blood on red cell deformability and aggregation. Clin Hemorheol Microcirc. 2009; 41(4): 269-278. doi: 10.3233/CH-2009-1178.
22. Cluitmans JCA, Chokkalingam V, Janssen AM, Brock R, Huck WTS. Alterations in red blood cell deformability during storage: a microfluidic approach. Biomed Res Int. 2014; 2014: 764268. doi: 10.1155/2014/764268.
23. Zheng Y, Chen J, Cui T, Shehata N, Wang C, Sun Y. Characterization of red blood cell deformability change during blood storage. Lab Chip. 2014; 14(3): 577-583. doi: 10.1039/c3lc51151k.
24. Liu X, Fan R, Lu Y, Kuang L, Yuan Q, Chen Y, et al. Influence of autologous blood transfusion in liver transplantation in patients with hepatitis B on the function and hemorheology of red blood cells. Exp Ther Med. 2017; 14(2): 1205-1211. doi: 10.3892/etm.2017.4587.
25. Ichikawa J, Koshino I, Arashiki N, Nakamura F, Komori M. Storage-related changes in autologous whole blood and irradiated allogeneic red blood cells and their ex vivo effects on deformability, indices, and density of circulating erythrocytes in patients undergoing cardiac surgery with cardiopulmonary bypass. J Cardiothorac Vasc Anesth. 2022; 36(3): 855-861. doi: 10.1053/j.jvca.2021.06.016.
26. Yalcin O, Ortiz D, Tsai AG, Johnson PC, Cabrales P. Microhemodynamic aberrations created by transfusion of stored blood. Transfusion (Paris) 2014; 54(4): 1015-1027. doi: 10.1111/trf.12361.
27. Hess JR. Measures of stored red blood cell quality. Vox Sang. 2014; 107(1): 1-9. doi: 10.1111/vox.12130.
28. Vonk AB, Muntajit W, Bhagirath P, van Barneveld LJ, Romijn JW, de Vroege R, et al. Residual blood processing by centrifugation, cell salvage or ultrafiltration in cardiac surgery: effects on clinical hemostatic and ex-vivo rheological parameters. Blood Coagul Fibrinolysis. 2012; 23(7): 622-628. doi: 10.1097/MBC.0b013e328356d2cc.
29. Gu YJ, Vermeijden WJ, De Vries AJ, Hagenaars JAM, Graaff R, Van Oeveren W. Influence of mechanical cell salvage on red blood cell aggregation, deformability, and 2,3-diphosphoglycerate in patients undergoing cardiac surgery with cardiopulmonary bypass. Ann Thorac Surg. 2008; 86(5): 1570-1575. doi: 10.1016/j.athoracsur.2008.07.052.
30. Scott AV, Nagababu E, Johnson DJ, Kebaish KM, Lipsitz JA, Dwyer IM, et al. 2,3- diphosphoglycerate concentrations in autologous salvaged versus stored red blood cells and in surgical patients after transfusion. Anesth Analg. 2016; 122(3): 616-623. doi: 10.1213/ANE.0000000000001071.
31. Uzoigwe C. The human erythrocyte has developed the biconcave disc shape to optimise the flow properties of the blood in the large vessels. Med Hypotheses. 2006; 67(5): 1159-1163. doi: 10.1016/j.mehy.2004.11.047.
32. Lu M, Shevkoplyas SS. Dynamics of shape recovery by stored red blood cells during washing at the single cell level. Transfusion (Paris). 2020; 60(10): 2370-2378. doi: 10.1111/trf.15979.
33. Klodell CT, Richardson JD, Bergamini TM, Spain DA. Does cell-saver blood administration and free hemoglobin load cause renal dysfunction? Am Surg. 2001; 67(1): 44-47. PMID: 11206896.
34. Spain DA, Miller FB, Bergamini TM, Montgomery RC, Richardson JD. Quality assessment of intraoperative blood salvage and autotransfusion. Am Surg. 1997; 63(12): 1059-1063; discussion 1063- 1064. PMID: 9393253.
35. Participation E. The blood safety and quality regulations 2005. Avalilable at: https://www.legislation.gov.uk/uksi/2005/50. Accessed on 9/12/2024.
36. Dalrymple-Hay MJR, Pack L, Deakin CD, Shephard S, Ohri SK, Haw MP, et al. Autotransfusion of washed shed mediastinal fluid decreases the requirement for autologous blood following cardiac surgery: a prospective randomized trial. Eur J Cardiothorac Surg. 1999; 15(6): 830-834. doi: 10.1016/s1010-7940(99)00112-8.
37. Murphy GJ, Allen SM, Unsworth-White J, Lewis CT, Dalrymple-Hay MJR. Safety and efficacy of perioperative cell salvage and autotransfusion after coronary artery bypass grafting: a randomized trial. Ann Thorac Surg. 2004; 77(5): 1553-1559. doi: 10.1016/j.athoracsur.2003.10.045.
38. Zhou Y, Chen T, Yang C, Liu J, Yang X, Zhang B, et al. Risk factors associated with positive bacterial culture in salvaged red blood cells during cardiac surgery and postoperative infection incidence: A prospective cohort study. Front Med. 2023; 10: 1099351. doi: 10.3389/fmed.2023.1099351.
39. Geldof WC, Brom AG. Infections through blood from heart-lung machine. Thorax. 1972; 27(4): 395-397. doi: 10.1136/thx.27.4.395.
40. Bland LA, Villarino ME, Arduino MJ, McAllister SK, Gordon SM, Uyeda CT, et al. Bacteriologic and endotoxin analysis of salvaged blood used in autologous transfusions during cardiac operations. J Thorac Cardiovasc Surg. 1992; 103(3): 582-588. doi: 10.1016/S0022- 5223(19)35002-0.
41. Schwieger IM, Gallagher CJ, Finlayson DC, Daly WL, Maher KL. Incidence of Cell-Saver contamination during cardiopulmonary bypass. Ann Thorac Surg. 1989; 48(1): 51-53. doi: 10.1016/0003-4975(89)90175-6.
42. Weltert L, Nardella S, Rondinelli MB, Pierelli L, De Paulis R. Reduction of allogeneic red blood cell usage during cardiac surgery by an integrated intra- and postoperative blood salvage strategy: results of a randomized comparison. Transfusion (Paris). 2013; 53(4): 790-797. doi: 10.1111/j.1537-2995.2012.03836.x.
43. Xie H, Pan JK, Hong KH, Guo D, Fang J, Yang WY, et al. Postoperative autotransfusion drain after total hip arthroplasty: a meta-analysis of randomized controlled trials. Sci Rep. 2016;6(1):27461. doi: 10.1038/srep27461.
44. Nemani VM, Kim HJ, Mina CA, Sheha ED, Ross T, Boachie-Adjei O. Postoperative blood salvage and autotransfusion for adult spinal deformity: a randomized controlled trial. Spine. 2020; 45(18): 1247-1252. doi: 10.1097/BRS.0000000000003176.
45. Hao X, Chen Y, Wang L, Jia M, Lu Y. Sodium citrate effectively used in shed mediastinal blood autotransfusion after cardiac surgery. Perfusion. 2024; 39(6): 1106-1112. doi: 10.1177/02676591231171271.