Heparin concentration in cell salvage during heparinization: a pilot study

Takahiro Tamura1,2, Jonathan H Waters2, and Kimitosi Nishiwaki1

1Department of Anesthesiology, Nagoya University Graduate School of Medicine, Nagoya, Japan
2Department of Anesthesiology and Perioperative Medicine, University of Pittsburgh Medical Center, PA, USA

ABSTRACT

Cell salvage is frequently used to avoid unnecessary allogeneic blood transfusions, which results in a reduction in blood transfusion volume and cost. The aspirated blood is washed with normal saline and centrifuged to recover only blood cells, salvaged blood is then made. In cardiovascular surgery, heparin is used to maintain activated clotting time over 400 seconds. Some practitioners believe that heparin remains in the salvaged blood. Therefore, we hypothesized that salvaged blood during cardiovascular surgery includes heparin. A pilot study was conducted to evaluate our hypothesis using three different salvage systems. This study was a prospective, observational, pilot study, with patients aged 20–85 years old who were scheduled for cardiovascular surgery from May 2018 to October 2018. The intent of this study was to evaluate whether salvaged blood with three different devices includes large enough quantities of heparin to influence activated clotting time in cardiovascular surgery. Between May and October 2018, 12 samples during heparinization were collected, and 12 samples of salvaged blood from 3 devices were collected after administering protamine. The heparin concentration of the 24 samples was measured. All heparin concentrations in salvage blood sample from two devices was below the limit of measurement (0.10 IU/mL). Slightly measurable heparin was detected in salvaged blood sample from one device (mean 0.15 IU/mL). Salvaged blood during cardiovascular surgery intervention does not contain enough heparin to influence activated clotting time.

Keywords: cell salvage, heparin, heparinization, salvaged blood, concentration

Abbreviations:
ACT: activated clotting time
XTRA: XTRA®, manufactured by Liva Nova Japan K.K., Tokyo, Japan
ELITE: Cell Saver ELITE®, manufactured by HAEMONETICS Japan GK, Tokyo, Japan
AUTOLOG: AUTOLOG®, manufactured by Medtronic Japan Co. Ltd, Japan

This is an Open Access article distributed under the Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License. To view the details of this license, please visit (http://creativecommons.org/licenses/by-nc-nd/4.0/).

INTRODUCTION

Cardiovascular surgery is associated with high rates of transfusion. There are multiple factors,
such as hypothermia, acidosis, heparinization and induction of the inflammatory cascade, that are responsible for hemostatic disorders after bypass.\textsuperscript{1-3} Cell salvage is frequently used to avoid unnecessary allogeneic blood transfusions. While allogeneic transfusion has minimal complications, it leads to an increased cost.\textsuperscript{4-6} Cell salvage removes most of the unwanted components, such as free hemoglobin, and provides concentrated and washed red blood cell with a 50–60% hematocrit level. It is well known that the recovered blood contains almost no coagulation factors. However, because of using heparin to maintain activated clotting time (ACT) during bypass, some practitioners believe that heparin remains in the salvaged blood, and that heparin in salvaged blood causes bleeding. Therefore, we hypothesized that salvaged blood during cardiovascular surgery with heparinization includes heparin in large enough quantities to extend the ACT. Here, a pilot study was conducted to evaluate our hypothesis using three different salvage systems.

METHODS

This prospective study was approved by the Institutional Review Board of the Nagoya University Hospital (IRB # 2018–0084). Written informed consent was obtained from all subjects participating in the trial. The trial was registered prior to patient enrollment at the University Hospital Medical Information Network (UMIN000031125). Twelve patients aged 20–85 years who were scheduled for cardiovascular surgery with heparinization from May 2018 to October 2018 were enrolled. The twelve patients were randomly allocated by computer-generated randomization to one of 3 machines so that each machine was tested with 4 patients. The cell salvage machines used were as follows: XTRA\textsuperscript{®}, manufactured by Liva Nova Japan K.K., Tokyo, Japan; Cell Saver ELITE\textsuperscript{®}, manufactured by HAEMONETICS Japan G.K., Tokyo, Japan; AUTOLOG\textsuperscript{®} manufactured by Medtronic Japan Co. Ltd., Japan. The AUTOLOG was employed only for aortic surgery in accordance with our facility usage agreement. The automatic washing mode for each device was used for washing. Each machines processing settings are shown in Table 1. The primary study endpoint was the heparin concentration in salvaged blood.

Non-invasive arterial blood pressure measurements, electrocardiography, pulse oximetry, bispectral index monitoring, and radial artery cannulation for blood pressure monitoring and sampling were performed in all patients. Fentanyl and midazolam were administered intravenously to induce general anesthesia. Remifentanil and rocuronium were additionally used to facilitate tracheal intubation. General anesthesia maintenance was performed using air, oxygen, remifentanil, and volatile anesthetics. In cardiac surgery (for XTRA, ELITE), porcine heparin (300 IU/kg) was administered before starting cannulation for cardiopulmonary bypass (CPB), and additional heparin boluses (50 IU/kg) were administered to maintain an ACT of at least 400 seconds. Ventilation of both lungs was initiated before separating from CPB. Protamine (3 mg/kg) was administered to antagonize the heparin effect. Red blood cell concentrates were transfused to maintain a hemoglobin level over 8 mg/dl during CPB. CPB was terminated with inotropic drug support, and the patient was intubated and admitted to the ICU. In aortic surgery (AUTOLOG), porcine heparin (300 IU/kg) was administered before aortic clamping, and additional heparin boluses (50 IU/kg) were administered to maintain an ACT of at least 400 seconds. Protamine (3 mg/kg) was administered to antagonize the heparin effect. Packed red blood cells were transfused to maintain a hemoglobin level over 8 mg/dl. In each patient, both the blood collected via radial artery catheter at the same time as measuring the first ACT and the completed cell salvage blood were collected and submitted for heparin concentration measurement and blood count. After blood collection, they were quickly centrifuged at 2500 rpm for 15 minutes, the serum was collected, and was stored at –80°C until heparin measurement.
Table 1 Summary of blood count and each device setting

| Blood count   | XTRA (n=4) | ELITE (n=4) | AUTOLOG (n=4) |
|---------------|------------|-------------|---------------|
|               | Heparinization | Salvage | Heparinization | Salvage | Heparinization | Salvage |
| Hb (g/dl)     | 9.3 ± 0.6   | 18.2 ± 1.2  | 9.1 ± 0.7     | 19.8 ± 2.1 | 9.0 ± 0.7     | 20.0 ± 0.8 |
| Ht (%)        | 29.0 ± 0.8  | 55.7 ± 1.2  | 29.2 ± 1.2    | 56.1 ± 1.7 | 28.7 ± 0.8    | 58.3 ± 1.7 |
| Platelet (×10⁴/µl) | 8.5 ± 1.6   | 1.5 ± 5.1   | 8.6 ± 1.2     | 3.4 ± 6.2  | 7.2 ± 1.1     | 0.9 ± 3.2  |
| Potassium (mmol/l) | 4.2 ± 0.3   | 0.9 ± 0.2   | 4.2 ± 0.2     | 1.0 ± 0.2  | 4.1 ± 0.2     | 1.3 ± 0.3  |

| Settings of Cell salvage devices | XTRA | ELITE | AUTOLOG |
|----------------------------------|------|-------|--------|
| Bowl size (ml)                   | 125  | 125   | 135    |
| Washing quantity (ml)            | 500  | 500   | 250    |
| Washing flow rate (ml/min)       | 300  | 300   |        |
| Return flow rate (ml/min)        | 300  | 100   | Not disclosed by the manufacturer* |
| Concentration flow rate (ml/min) | 300  | 250   |        |
| Heparin in normal saline for aspiration of blood (IU/ml) | 30  |       |        |
| Dropping rate in normal saline for aspiration of blood (ml/h) | 90  |       |        |

Data are expressed as means ± standard deviation (SD), number of volume (ml), international unit (IU/ml), or flow rate (ml/min) and dropping rate (ml/h) (n = 12). Hb, hemoglobin; Ht, hematocrit; ml, milliliter; min, minutes; h, hours; Salvage, the blood collected in holding bag of salvaged blood. XTRA, XTRA®, manufactured by Liva Nova Japan K.K., Tokyo, Japan; ELITE, Cell Saver ELITE®, manufactured by HAEMONETICS Japan GK, Tokyo, Japan; AUTOLOG, AUTOLOG® manufactured by Medtronic Japan Co. Ltd., Tokyo, Japan. *We used the standard settings of this device.
Based on a measurement error less than 10% in quantitative measurement of heparin concentration, and minimum detection limit of 0.10 IU/mL, a pilot study was planned in which over three samples were measured and the power was compared. Parametric tests were used for statistical analysis because the Levene test showed that variances were homogeneous. Each group was compared using the one-way analysis of variance. A p-value of <0.05 indicated statistical significance and all p-values were two-tailed. All data were analyzed using SPSS software (version 26, IBM Japan Ltd., Tokyo, Japan).

RESULTS

Between May and October 2018, 12 patients were randomly allocated to each group, with 4 patients in XTRA, 4 in ELITE, and 4 in AUTOLOG groups, respectively. Demographic information for these patients is shown in Table 2. Data from 4 patients per group were included in the final analysis. Heparin concentrations of the 24 samples were measured.

All heparin concentrations in the salvaged blood from two devices (XTRA and ELITE) were below the lower limit of measurement (0.10 IU/mL). Slightly measurable heparin was detected in blood from one device (AUTOLOG, mean 0.15 IU/mL), F(2, 9) = 4.3, p = 0.11). Mean heparin concentrations of blood during heparinization were 3.78 IU/mL, 3.64 IU/mL, and 3.70 IU/mL, respectively, F(2, 9) = 4.3, p = 0.90). (Figure 1)

Table 1 shows the summary of blood counts in the salvaged blood and the blood collected via radial artery catheter. Potassium was almost completely removed. Interestingly, some platelets were also present in the washed salvaged blood.

| Table 2 | Patients demographic and surgical characteristics |
|---------|--------------------------------------------------|
| Total n=12 | XTRA (n=4) | ELITE (n=4) | AUTOLOG (n=4) |
| Demographic information | mean ± SD | mean ± SD | mean ± SD |
| Age (years) | 62.1 ± 9.1 | 68.5 ± 3.9 | 63.5 ± 13.5 |
| Height (cm) | 165.2 ± 8.6 | 164.1 ± 5.3 | 167.4 ± 3.3 |
| Body weight (kg) | 66.1 ± 7.4 | 58.9 ± 5.5 | 64.1 ± 17.1 |
| Body mass index (kg/m²) | 24.2 ± 1.3 | 21.1 ± 2.3 | 22.9 ± 6.3 |
| Male:female | 3:1 | 3:1 | 3:1 |
| Surgical information | | | |
| On-pump CAGB | 1 | 0 | 0 |
| Single valve | 1 | 3 | 0 |
| Aorta | 2 | 1 | 4 |

Data are expressed as means ± standard deviation (SD) or number of patients. On-pump CAGB, coronary artery bypass graft with cardio-pulmonary bypass. XTRA, XTRA®, manufactured by Liva Nova Japan K.K., Tokyo, Japan; ELITE, Cell Saver ELITE®, manufactured by HAEMONETICS Japan GK, Tokyo, Japan; AUTOLOG, AUTOLOG® manufactured by Medtronic Japan Co. Ltd., Tokyo, Japan.
DISCUSSION

Some practitioners believe that heparin remains in salvaged blood collected during CPB, and believe that it is one of the causes for massive transfusion and bleeding. This is sometimes controversial in clinical situation. This current study was a pilot study to analyze heparin concentrations in cell salvaged blood. The results of this study did not support our hypothesis which was that significant, residual heparin would be present in a salvaged blood product. Our results suggest that almost all of the heparin salvage blood is below the limit of measurement (0.10 IU/mL). Even if heparin is detected, it is only an average of 0.15 IU/mL which is well below that which would alter coagulation function. In comparison, mean heparin concentrations of blood during heparinization were 3.78 IU/mL, 3.64 IU/mL, and 3.70 IU/mL, respectively. In each cell salvage device, blood collected from the patient are washed in heparin-free normal saline, and are centrifuged. Cell salvage removes most of the unwanted components, such as free hemoglobin, and provides concentrated and washed red blood cells with a 50–60% hematocrit. It is safe to assume that there is almost no heparin contamination when blood is processed with standard cell salvage equipment and standard operating procedures. When 500 ml of salvaged blood is administered, only 0–100 units of heparin could be given which would be below the detection limit. It is well known that heparin less than 100 units is not involved in APTT prolongation.7

When red blood cell transfusion, crystalloid, and/or colloid are given to bleeding patients, it is likely causing a dilutional coagulopathy. In addition, hemorrhagic shock and associated hypothermia and acidosis exacerbate consumptive and dilutional coagulopathy, resulting in a vicious circle that impairs patient prognosis.8 Therefore, at the time of cardiovascular surgery,
there are several factors that facilitate bleeding other than just surgical bleeding.\textsuperscript{1–3} Previous reports indicated that salvaged blood collected from the surgical field during CPB increased postoperative bleeding and increased blood transfusion volume.\textsuperscript{9} The main reasons that were proposed was that the salvaged blood prolonged activated partial thromboplastin time, prothrombin time and international normalized ratio, and decreased fibrinogen levels. It is highly unlikely that coagulation factors or fibrinogen were adequately replaced in this report. In addition, multiple factors such as hemorrhagic shock, associated hypothermia and dilutional coagulopathy could be related. Therefore, it was considered that the administration of salvaged blood was not the sole cause. Recently, the optimal administration of blood products according to the situation is recommended such as using TEG6s.\textsuperscript{10–12} If larger amounts of salvaged blood without coagulation factors are transfused, it may result in a dilutional coagulopathy. In addition, the production of large amounts of salvaged blood suggests that bleeding is proportionately high. In order to replenish this, a large dose of salvaged blood, crystalloid or colloid also can cause dilutional coagulopathy. In order to solve this dilutional effect, it is absolutely recommended that appropriate amounts of plasma and platelets should be administered according to the results from point of care devices such as thromboelastography (TEG6s).\textsuperscript{13–17} Thus, various factors are involved in bleeding, however, it can be concluded from this study that one of the causes is not heparin from salvaged blood.

This study has two major limitations that should be addressed. First, this study was a pilot study, and the number of cases was small. All of the heparin concentrations in the salvaged blood samples were too low to measure. As result, the power is sufficient to reject the hypothesis that cell salvage blood contains heparin. Thus, a larger study to demonstrate that heparin in salvaged blood is the cause of bleeding is unnecessary. Second, the results of this study may vary depending on the processing settings. Therefore, the processing settings for each facility should be adjusted and confirmed.

In conclusion, almost all of the heparin in salvaged blood as evaluated by quantitative measurements was below the lower limit of measurement (0.10 IU/mL). Salvaged blood during cardiovascular surgery intervention does not contain enough heparin to influence ACT.

ACKNOWLEDGEMENT

We thank all anesthesiology medical members, and all extra-corporeal technology members at Nagoya University Hospital. The authors also thank Christine Burr (Scientific Writer, Department of Anesthesiology and Perioperative Medicine/Department of Surgery, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA) for her editorial assistance with the manuscript.

DISCLOSURE STATEMENT

None of the authors has any conflicts of interest to declare in relation to this work.

REFERENCES

1. Westaby S. Anti-fibrinolytic therapy in thoracic aortic surgery. Ann Thorac Surg. 1999;67(6):1983–1985, discussion 1997–1998. doi: 10.1016/s0003-4975(99)00354-9.
2. Nomura F, Tamura K, Yoshitatsu M, Katayama A, Katayama K, Ihara K. Changes in coagulation condition, cytokine, adhesion molecule after repair of type A aortic dissection. Eur J Cardiothorac Surg. 2004;26(2):348–350. doi: 10.1016/j.ejcts.2004.05.001.
3. DeAndrade D, Waters JH, Triulzi DJ, et al. Very low rate of patient-related adverse events associated with
the use of intraoperative cell salvage. *Transfusion*. 2016;56(11):2768–2772. doi: 10.1111/trf.13791.

4. Colson PH, Gaudard P, Fellahi JL, et al. Active bleeding after cardiac surgery: a prospective observational multicenter study. *PLoS One*. 2016;11(9):e0162396. doi: 10.1371/journal.pone.0162396.

5. Carless PA, Henry DA, Moxey AJ, O’Connell D, Brown T, Fergusson DA. Cell salvage for minimising perioperative allogeneic blood transfusion. *Cochrane Database Syst Rev*. 2010;(4):CD001888. doi: 10.1002/14651858.CD001888.pub4.

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. White RH1, Zhou H, Woo L, Mungall D. Effect of weight, sex, age, clinical diagnosis, and thromboplastin reagent on steady-state intravenous heparin requirements. *Arch Intern Med*. 1997;157(21):2468–2472.

8. Mazzefi MA, Chriss E, Davis K, et al. Optimal plasma transfusion in patients undergoing cardiac operations with massive transfusion. *Ann Thorac Surg*. 2017;104(1):153–160. doi: 0.1016/j.athoracsur.2016.09.071.

9. Rubens FD, Boodhwani M, Mesana T, et al. The cardiotomy trial: a randomized, double-blind study to assess the effect of processing of shed blood during cardiopulmonary bypass on transfusion and neurocognitive function. *Circulation*. 2007;116(11)(Suppl):I89–97. doi: 10.1161/CIRCULATIONAHA.106.678987.

10. Holcomb JB, Tilley BC, Baraniuk S, et al. Transfusion of plasma, platelets, and red blood cells in a 1:1:1 vs a 1:1:2 ratio and mortality in patients with severe trauma: the PROPR randomized clinical trial. *JAMA*. 2015;313(5):471–482. doi: 10.1001/jama.2015.10.

11. Karkouti K, Callum J, Wijeysundera DN, et al. Point-of-care hemostatic testing in cardiac surgery: a stepped-wedge clustered randomized controlled trial. *Circulation*. 2016;134(16):1152–1162. doi: 10.1161/CIRCULATIONAHA.116.023956.

12. Fominskiy E, Nepomniashchikh VA, Lomivorotov VV, et al. Efficacy and safety of fibrinogen concentrate in surgical patients: a meta-analysis of randomized controlled trials. *J Cardiothorac Vasc Anesth*. 2016;30(5):1196–1204. doi: 10.1053/j.jvca.2016.04.015.

13. Westbrook AJ, Olsen J, Bailey M, Bates J, Scully M, Salamonsen RF. Protocol based on thromboelastograph (TEG) out-performs physician preference using laboratory coagulation tests to guide blood replacement during and after cardiac surgery: a pilot study. *Heart Lung Circ*. 2009;18(4):277–288. doi: 10.1016/j.hlc.2008.08.016.

14. Girdauskas E, Kempfert J, Kuntze T, et al. Thromboelastometrically guided transfusion protocol during aortic surgery with circulatory arrest: a prospective, randomized trial. *J Thorac Cardiovasc Surg*. 2010;140(5):1117–1124.e2. doi: 10.1016/j.jtcvs.2010.04.043.

15. Kozek-Langenecker SA, Afshari A, Albaladejo P, et al. Management of severe perioperative bleeding: guidelines from the European Society of Anaesthesiology. *Eur J Anaesthesiol*. 2013;30(6):270–382. doi: 10.1097/EJA.0b013e32835f4d5b.

16. American Society of Anesthesiologists Task Force on Perioperative Blood Management. Practice guidelines for perioperative blood management: an updated report by the American Society of Anesthesiologists Task Force on Perioperative Blood Management. *Anesthesiology*. 2015;122(2):241–275. doi: 10.1097/ALN.0000000000000463.

17. Tamura T. Predicting results of fibrinogen and platelet levels by TEG6s during cardiopulmonary bypass: a pilot study. *J Clin Anesth*. 2019;58:59–60. doi: 10.1016/j.jclinane.2019.05.012.