ANESTHESIOLOGY

A Pilot Trial of Platelets Stored Cold *versus* at Room Temperature for Complex Cardiothoracic Surgery

Geir Strandenes, M.D., Joar Sivertsen, B.Sc., Christopher K. Bjerkvig, M.D., Theodor K. Fosse, M.D., Andrew P. Cap, M.D., Ph.D., Deborah J. del Junco, Ph.D., Einar Klæboe Kristoffersen, M.D., Ph.D., Rune Haaverstad, M.D., Ph.D., Venny Kvalheim, M.D., Ph.D., Hanne Braathen, B.Sc., Turid Helen Felli Lunde, M.Sc., Tor Hervig, M.D., Ph.D., Karl Ove Hufthammer, Ph.D., Philip C. Spinella, M.D., Torunn Oveland Apelseth, M.D., Ph.D.

ANESTHESIOLOGY 2020; 133:1173–83

ABSTRACT

Background: This pilot trial focused on feasibility and safety to provide preliminary data to evaluate the hemostatic potential of cold-stored platelets (2° to 6°C) compared with standard room temperature–stored platelets (20° to 24°C) in adult patients undergoing complex cardiothoracic surgery. This study aimed to assess feasibility and to provide information for future pivotal trials.

Methods: A single center two-stage exploratory pilot study was performed on adult patients undergoing elective or semiurgent complex cardiothoracic surgery. In stage I, a two-armed randomized trial, platelets stored up to 7 days in the cold were compared with those stored at room temperature. In the subsequent single-arm stage II, cold storage time was extended to 8 to 14 days. The primary outcome was clinical effect measured by chest drain output. Secondary outcomes were platelet function measured by multiple electrode impedance aggregometry, total blood usage, immediate and long-term (28 days) adverse events, length of stay in intensive care, and mortality.

Results: In stage I, the median chest drain output was 720 ml (quartiles 485 to 1,170, n = 25) in patients transfused with room temperature–stored platelets and 645 ml (quartiles 460 to 800, n = 25) in patients transfused with cold-stored platelets. No significant difference was observed. The difference in medians between the room temperature– and cold-stored up to 7 days arm was 75 ml (95% CI, –220, 425). In stage II, the median chest drain output was 690 ml (500 to 1,880, n = 15). The difference in medians between the room temperature arm and the nonconcurrent cold-stored 8 to 14 days arm was 30 ml (95% CI, –1,040, 355). Platelet aggregation *in vitro* increased after transfusion in both the room temperature– and cold-stored platelet study arms. Total blood usage, number of adverse events, length of stay in intensive care, and mortality were comparable among patients receiving cold-stored and room temperature–stored platelets.

Conclusions: This pilot trial supports the feasibility of platelets stored cold for up to 14 days and provides critical guidance for future pivotal trials in high-risk cardiothoracic bleeding patients.

(ANESTHESIOLOGY 2020; 133:1173–83)

EDITOR'S PERSPECTIVE

What We Already Know about This Topic

- Platelets are stored at room temperature (20° to 24°C) with constant agitation for 5 to 7 days to maximize recovery and survival *in vivo* after transfusion
- Cold storage of platelets at 2° to 6°C would provide extended shelf life, and laboratory investigations indicate improved hemostatic function, which may be beneficial for treatment of bleeding

What This Article Tells Us That Is New

- No significant difference was observed between cold-stored and room temperature–stored platelets for hemostatic function assessed by chest drainage, total blood usage, platelet function, and clinical outcomes
- This pilot trial supports the potential feasibility of cold-stored platelets for clinical use and provides potential guidance for future pivotal trials

Platelets for transfusion are stored at room temperature (20° to 24°C) with gentle agitation for 4 to 7 days depending on national regulations. The resulting functional storage lesion and risk of bacterial growth limit shelf life.[1] Short shelf life causes outdates, and increased storage time is warranted.[1,2] Room temperature storage was implemented as routine for prophylactic transfusion in hypoproliferative thrombocytopenia because recovery and survival studies demonstrated longer circulation time of room temperature–stored platelets compared with refrigerated or cold-stored (2° to 6°C) platelets.[3] For platelet transfusion in actively bleeding patients, multiple lines of evidence provide the biologic rationale to compare cold- *versus* room temperature–stored platelets. *In vitro* studies demonstrate better preserved platelet aggregation and mitochondrial function in cold-stored *versus* room temperature–stored platelets and hemostatic function in cold-stored platelets for up to 22 days.[4–8] Clinical studies show decreased bleeding time and reduced bleeding in patients who received cold-stored platelets or refrigerated whole blood.[9–11] These findings suggest that cold-stored platelets may be more hemostatically active in bleeding patients after transfusion.

Patients undergoing prolonged cardiopulmonary bypass (CPB) for complex cardiothoracic surgery develop thrombocytopathy and thrombocytopenia requiring platelet transfusion. Evaluations of platelet products have focused on this population because postoperative bleeding measured by chest

Copyright © 2020, the American Society of Anesthesiologists, Inc. All Rights Reserved. Anesthesiology 2020; 133:1173–83. DOI: 10.1097/ALN.0000000000003550

Copyright © 2020, the American Society of Anesthesiologists, Inc. Unauthorized reproduction of this article is prohibited.

<zdoi;. DOI: 10.1097/ALN.0000000000003550>

Downloaded from http://asa2.silverchair.com/anesthesiology/article-pdf/133/6/1173/513308/20201200.0-00011.pdf by guest on 24 October 2021

tube output and blood usage provide measures of hemostatic clinical effect.[12] This pilot trial focused on feasibility and safety to provide preliminary data to evaluate hemostatic potential of cold-stored platelets (2° to 6°C) compared with standard room temperature–stored platelets (20° to 24°C) in adult patients undergoing complex cardiothoracic surgery.

Materials and Methods

Study Design

The Norwegian Regional Ethics Committee North (Tromsø, Norway) approved the study (identifier 2014/692). The trial was registered on ClinicalTrials.gov on July 8, 2015, with identifier NCT02495506. This two-stage pilot trial was designed to inform future pivotal trials by providing a preliminary test of this hypothesis: Relative to transfusion with room temperature–stored platelets, cold-stored platelets reduce postoperative bleeding with sufficient platelet aggregation in patients who develop indications for platelet transfusion during complex cardiothoracic surgery. Stage I was a two-arm randomized trial that compared transfusion with cold-stored and room temperature–stored platelet concentrates stored for up to 7 days. An external safety monitor was appointed, and interim analysis on clinical effect and safety was performed during the study. Based on acceptable safety and potential for clinical effect after inclusion of 25 patients in the cold-stored arm, an exploratory, single-arm prospective observational study, stage II, extended the duration of platelet cold storage to 8 to 14 days. Stage I was conducted from March 2015 to October 2017, and the observational stage II extension was conducted from October 2017 to August 2018.

Study Participants

Adult patients hospitalized at Haukeland University Hospital, Bergen, Norway, for elective and semiurgent (within 24 h from hospital admission) cardiothoracic surgery were identified and screened for eligibility by their surgeons. The inclusion screening criteria were scheduled elective or semiurgent cardiothoracic surgery with either expected CPB time over 120 min or use of dual platelet inhibition drugs less than 48 h before surgery. Patients with congenital coagulopathies or hemostatic disorders were ineligible. Informed consent was obtained from patients who fulfilled the screening criteria; however, only the subset of consented patients who developed indications for platelet transfusion and had orders for study platelets received by the blood bank were enrolled into the study.

Indications for platelet, erythrocyte, and plasma transfusion, as well as hemostatic adjuncts, was decided according to standard practice at our institution in collaboration between the surgeon and anesthesiologist. The decision to transfuse platelets was based on prolonged CPB time, excessive bleeding needing a balanced transfusion approach, and/or problems achieving surgical hemostasis. Excessive bleeding is present when the patient is hemodynamically unstable because of blood loss and requiring ongoing transfusion of blood products to maintain mean arterial blood pressure above 60 mmHg and end tidal carbon dioxide above 3.5 kPa combined with too much bleeding to close the chest. After chest closure, chest tube output and hemodynamic parameters as described above were used to guide transfusion needs in the postsurgical phase. A transfusion episode was defined as ended if no additional platelet transfusions were given within 30 min after completion of the previous platelet transfusion. As an additional safety precaution, because the clinical performance of platelets stored in the cold beyond 7 days was unknown, a maximum of two units of study platelets per patient was enforced in stage II. If prolonged bleeding with additional need for platelet transfusion occurred in stage II, additional nonstudy platelets were transfused. A bolus of tranexamic acid (10 mg/kg IV) was given to patients during induction of anesthesia before surgery and followed by continuous infusion ($10\,mg \cdot kg^{-1} \cdot h^{-1}$) until the end of surgery.

Study Intervention

For stage I, patients in the cold-stored arm received leukocyte-depleted apheresis platelets in platelet additive solution stored at 2° to 6°C under constant agitation for up to 7 days. Patients in the room temperature–stored arm received leukocyte-depleted apheresis platelets in platelet additive solution stored under constant agitation for up to 7 days in room temperature (20° to 24°C). For stage II, patients received leukocyte-depleted apheresis platelets

This article is featured in "This Month in Anesthesiology," page 1A. This article is accompanied by an editorial on p. 1161. This article has a related Infographic on p. 17A. Supplemental Digital Content is available for this article. Direct URL citations appear in the printed text and are available in both the HTML and PDF versions of this article. Links to the digital files are provided in the HTML text of this article on the Journal's Web site (www.anesthesiology.org). This article has an audio podcast. This article has a visual abstract available in the online version. Preliminary data were presented at the American Association of Blood Banks annual meetings October 22 to 25, 2016, in Orlando, Florida, and October 7 to 10, 2017, in San Diego, California. In relation to these conferences, an abstract with preliminary results was published. Further, preliminary results were presented without published abstracts at the American Association of Blood Banks–Trauma, Hemostasis, Oxygenation Resuscitation Network preconference for the American Association of Blood Banks annual meeting October 12, 2018, in Boston, Massachusetts, and at the Trauma, Hemostasis, Oxygenation Resuscitation Network annual symposium June 17 to 20, 2018 in Bergen, Norway.

Submitted for publication November 22, 2019. Accepted for publication August 11, 2020. Published online first on September 9, 2020. From the Departments of Immunology and Transfusion Medicine (G.S., J.S., E.K.K., H.B., T.H.F.L., T.H., T.O.A.), Anesthesia and Intensive Care (C.K.B., T.K.F.), Heart Disease (R.H., V.K.), and Clinical Biochemistry and Pharmacology (T.O.A.) and the Centre for Clinical Research (K.O.H.), Haukeland University Hospital, Bergen, Norway; the Department of War Surgery and Emergency Medicine, Norwegian Armed Forces Medical Services, Oslo, Norway (G.S.); the Institute of Clinical Science, University of Bergen, Bergen, Norway (C.K.B., T.K.F., E.K.K., R.H., V.K., T.H.); the Norwegian Naval Special Operations Command, Bergen, Norway (C.K.B., T.K.F.); the U.S. Army Institute of Surgical Research (A.P.C.) and the Joint Trauma System, Department of Defense Center of Excellence for Trauma (D.J.d.J.), Joint Base San Antonio–Fort Sam Houston, San Antonio, Texas; and the Division of Pediatric Critical Care, Department of Pediatrics, Washington University in. St Louis, St. Louis, Missouri (P.C.S.).

Downloaded from http://asa2.silverchair.com/anesthesiology/article-pdf/133/6/1173/513308/20201200.0-00011.pdf by guest on 24 October 2021

Copyright © 2020, the American Society of Anesthesiologists, Inc. Unauthorized reproduction of this article is prohibited.

in platelet additive solution stored at 2° to 6°C for 8 to 14 days without agitation. The platelets were suspended in 63% PAS III (InterSol, Fresenius Kabi, Germany) up to March 11, 2016, and thereafter PAS IIIM (T-PAS+, Terumo BCT, USA) and 37% donor plasma according to standard procedures in our institution. Bacterial testing of the platelet concentrates was performed on day 1 according to procedures in our department and the Norwegian requirements. All study platelet concentrates showed negative results.

Study Outcomes

The trial was registered on ClinicalTrials.org with an original plan to use evidence of altered platelet function confirmed by point-of-care measurements as the primary outcome. However, during data collection, we replaced aggregometry with chest drain output as the primary outcome to better address the pilot study's hypothesis based on a reduction of clinically significant bleeding. We evaluated aggregometry as a secondary outcome.

Chest drain output was measured from chest closure until 8:00 AM the next morning as the best estimate of cumulative postoperative blood loss. The secondary outcomes of platelet function were measured as changes in multiple electrode impedance aggregometry (Multiplate, Roche Diagnostics, Germany) after platelet transfusion, blood cell counts, conventional coagulation tests, blood usage, and hemostatic viscoelastic assays up until 8:00 AM the next morning. Adverse events (venous or arterial thromboembolism, transfusion reactions), length of stay in intensive care, and mortality were measured up to 28 days.

Post Hoc Analysis

Study patients who developed indications for platelet transfusion during complex cardiothoracic surgery were expected to be heterogeneous in terms of their baseline risks of surgical outcomes and complications. In a small pilot study, between-group variations in underlying disease severity or other potential confounders (*e.g.*, intraoperative administration of anticoagulants, other medications, or transfusion with nonassigned platelets) could produce biased treatment effect estimates and misinterpretations, especially for a single primary outcome measured by postoperative chest tube drainage. In addition to the standard intention-to-treat analysis, we conducted a *post hoc* analysis restricted to the more homogeneous subset of study patients who received the correctly randomized treatment and had surgical bleeding more amenable to treatment with platelet transfusion. Patients were excluded from the *post hoc* analysis if they (1) were reoperated within 24 h because of excessive bleeding uncontrolled by the cardiothoracic surgical procedures, (2) were anticoagulated with heparin because of the need for venoarterial extracorporeal membrane oxygenation, (3) had iatrogenic spleen bleeding, or 4) received nonassigned platelets (fig. 1). Chest drain output of more than 200 ml/h for more than 3 to 4 h or 500 ml/h for 2 h were the indications for reopening the chest. All patients that were removed from the *post hoc* analysis because excessive bleeding and reoperation had an identified surgically correctable bleeding.

Randomization and Blinding

For stage I, the study weeks were randomized to maintain an inventory for the study without excessive waste. A randomization list for study weeks was generated by use of electronic software. The randomization list was only available to study coworkers at the Department of Immunology and Transfusion Medicine. Surgeons screening the patients for eligibility were blinded to the randomization, as were the clinical personnel responsible for the treatment and follow up of the patients. Clinicians were blinded to patient randomization when deciding whether to transfuse and when ordering platelets. Enrolled stage I study patients were randomized to either the cold-stored or room temperature–stored arms based on the week of their procedure. In the room temperature arm, there were 19 weeks with one patient/week and 3 weeks with two patients/week. In the cold-stored arm, there were 17 weeks with one patient/week and 4 weeks with two patients/week. For stage II, all enrolled patients were assigned to receive platelets stored cold for 8 to 14 days.

The personnel handling platelet concentrates during transfusion could observe both temperature and labeling of the product. Clinicians reported the effect of transfusion and potential adverse events after procedure in the electronic medical records. Study coworkers from the Department of Immunology and Transfusion Medicine who obtained written informed consent, collected data from electronic medical records, and registered information in the study database were not blinded.

Data Collection

Clinical data were extracted by study personnel from electronic medical records. All transfusions were registered with start time and location (operating theater or intensive care unit), end time and location, and component details. Only transfused blood components were reported. Blood samples for laboratory analyses were collected from a radial artery catheter at baseline day of surgery, immediately after weaning CPB, 1 h after arrival at the intensive care unit, immediately before and 20 min after platelet transfusion episodes, and the following morning.

Statistical Analysis

The trial was initially registered on ClinicalTrials.gov with a plan to use noninferiority testing of between-arm differences in platelet function. Because of the lack of evidence needed to set acceptable, nonarbitrary tolerance margins for

Downloaded from http://asa2.silverchair.com/anesthesiology/article-pdf/133/6/1173/513308/20201200.0-00011.pdf by guest on 24 October 2021

Copyright © 2020, the American Society of Anesthesiologists, Inc. Unauthorized reproduction of this article is prohibited.

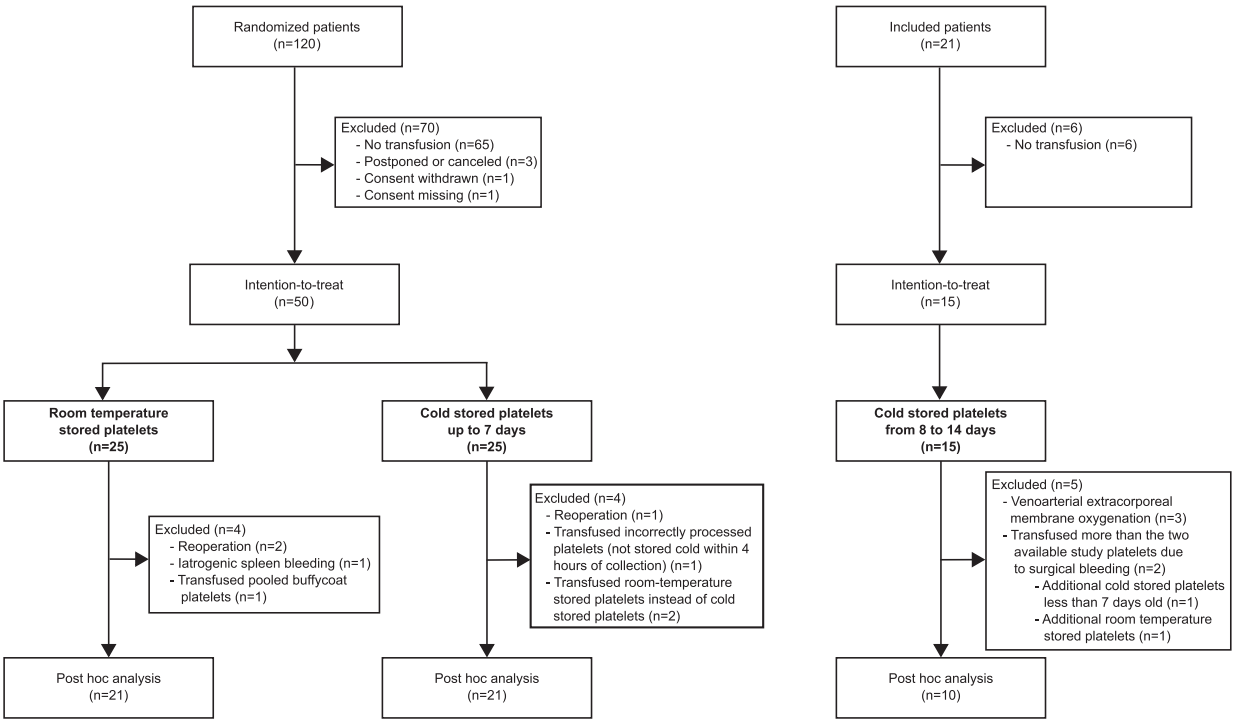

Fig. 1. Flow diagram showing inclusion, exclusion, and randomization of patients.

the noninferiority testing, we conducted standard tests of superiority, commensurate with the early phase of the trial.

A formal sample size calculation was not performed for this pilot study. Instead, the sample size was based on previous publications investigating the clinical effect of cold-stored compared to room temperature–stored platelets (n = 7 to 14,[9] n = 8,[3] and n = 12[13]). A previous clinical study in complex cardiothoracic surgery patients investigating post-CPB platelet dysfunction and chest tube output in normovolemic ultrafiltration included 25 patients.[14] We concluded that 20 patients or more per arm in stage I would provide sufficient statistical precision for the preliminary estimation of effect size of our primary outcome to inform future pivotal trials while minimizing risks in a pilot trial. For stage II, a sample size of 10 was chosen.

Statistical analyses were performed using IBM SPSS Statistics for Windows, version 24.0 (IBM, USA). For the primary outcome, means with 95% CI, standard deviations with one-sided 95% upper confidence limits, medians, lower and upper quartiles, and 95% CI for the difference in medians are presented. As recommended for pilot studies,[15] the CI for the SD requires only an upper bound not a lower bound. We also report differences in median chest drain output with 95% CI of the difference using the room temperature arm as the comparison. The CIs for differences in medians were calculated using the percentile bootstrap with 99,999 replications, using *R*, version 4.0.0 (R Core Team 2020, https://www.r-project.org/, accessed June 19, 2020).[16]

For the secondary outcomes, the results are given as medians (quartiles, minimum, maximum) or count (percentage). Because the study period was limited to 28 days, the length of stay for patients remaining in intensive care at this point was defined as 28 days in the statistical analysis.

In the randomized stage I, primary and secondary outcomes were compared using two-tailed Mann–Whitney U tests. Pre- and posttransfusion measurements were compared using the Wilcoxon signed-rank test. Linear regression analysis of chest drain output with the predictors study arm and time from finished surgery until next morning at 8:00 AM was performed to determine the association of time with bleeding volume. It has been shown that multiple electrode impedance aggregometry response depends on platelet count.[17–19] For this reason, we performed *post hoc* adjustment for platelet count by dividing aggregometry response by platelet count. A *P* value less than 0.05 was considered significant.

Results

Study Participants

From March 2015 through August 2018, 141 patients were provisionally assigned to one of the three treatment arms according to the study's randomization protocol at the start of surgery. The exclusions and inclusions are described in detail

Downloaded from http://asa2.silverchair.com/anesthesiology/article-pdf/133/6/1173/513308/20201200.0-00011.pdf by guest on 24 October 2021

Copyright © 2020, the American Society of Anesthesiologists, Inc. Unauthorized reproduction of this article is prohibited.

in figure 1. Only the randomized candidates who developed indications for platelet transfusion and survived long enough for the surgical team to initiate transfusion of the assigned study product were enrolled as study patients in this pilot trial. Enrollment ceased when the target sample size was obtained. 65 consented patients in stage I and 6 consented patients in stage II were ineligible for the study because they did not receive platelet transfusion. The intention-to-treat population included 65 patients receiving platelet transfusion: 25 received room temperature–stored, 25 received cold-stored for up to 7 days, and 15 received cold-stored for 8 to 14 days. Patient characteristics are presented in table 1. The *post hoc* analysis included 21 patients who received room temperature–stored platelets, 22 patients who received platelets stored cold for up to 7 days and 10 patients who received platelets stored cold for 8 to 14 days (fig. 1).

Platelet Transfusion Episodes

Most patients received their first platelet transfusion in the operating room during surgical hemostasis, before chest closure (22 of 25 patients in the room temperature arm, 23 of 25 patients in cold-stored up to 7 days arm, and 15 of 15 patients in the cold-stored for 8 to 14 days arm). No platelet transfusions were given during CPB. Six patients in the room temperature arm, two patients in the cold-stored up to 7 days arm, and four patients in the cold-stored for 8 to 14 days arm received more than one transfusion episode.

Blood Loss

As shown in figure 2, postoperative blood loss measured as median chest drain output was 720 ml (485 to 1,170, 180 to 2,340) for room temperature, 645 ml (460 to 800, 90 to 1,785) for cold-stored up to 7 days, and 690 ml (500 to 1,880, 100 to 9,210) for 8 to 14 days. In the *post hoc* analysis, the median chest drain output was 720 ml (490 to 1,020, 180 to 2,340), 590 ml (450 to 720, 90 to 960), and 595 ml (460 to 800, 100 to 1,550) for room temperature, cold storage for up to 7 days and cold storage for 8 to 14 days, respectively. Relative to the room temperature arm, the median chest drain output in the cold-stored up to 7 days arm was not statistically significant different ($P_{\text{intention-to-treat}} = 0.735$, $P_{\text{post hoc analysis}} = 0.154$).

The difference in medians between the room temperature– and cold-stored up to 7 days arm was 75 ml (95% CI, −220, 425). The difference in medians between the room temperature arm and the nonconcurrent cold-stored 8 to 14 days arm was 30 ml (95% CI, −1,040, 355). Means with standard deviations and 95% upper confidence limits are shown in table 2.

The median time from chest closure until postoperative chest drain output registration next morning was 16 (15 to 17, 12 to 19), 16 (16 to 18, 10 to 19), and 16 (13.5 to 17, 11 to 17) hours in the room temperature, cold-stored up to 7 days, and cold-stored for 8 to 14 days arms, respectively. No significant association between the length of this time period and chest drain output was observed ($P = 0.458$).

Table 1. Patient Characteristics

	Room Temperature–stored up to 7 Days (n = 25)	Cold-stored up to 7 Days (n = 25)	Cold-stored for 8 to 14 Days (n = 15)
Age, yr	65 (57–71, 38–79)	61 (46–67, 27–82)	67 (56–73, 44–78)
Sex (male)	17 (68%)	16 (64%)	13 (87%)
Height, cm	179 (175–185, 163–192)	176 (170–182, 153–192)	178 (173–187, 161–200)
Weight, kg	82 (72–95, 51–108)	78 (69–91, 51–120)	84 (72–102, 63–118)
Body mass index	26,3 (24–27,2, 16,7–31,7)	26,6 (21,8–31,6, 17,9–34,1)	27,4 (23,8–31,1, 21,8–32,1)
Logistic EuroSCORE, %	13 (5–23, 2,44–82,81)	18 (13,01–40,34, 5,96–93,1)	28,6 (19,4–44,31, 3,5–82,02)
Ejection fraction	60 (55–60, 30–65)	60 (50–60, 29–65)	50 (40–56, 25–60)
Cardiopulmonary bypass, min	220 (155–270, 76–418)	203 (143–249, 80–304)	210 (166–297, 78–428)
≥ 34°C	11 (44%)	11 (44%)	5 (33%)
< 34°C	14 (56%)	14 (56%)	10 (67%)
Aorta clamp time, min	131 (87–163, 51–227)	124 (90–168, 47–240)	143 (121–175, 62–228)
Platelet inhibitors			
Single	13 (52%)	11 (44%)	4 (29%)
Dual	0 (0%)	0 (0%)	0 (0%)
Redo surgery*	9 (36%)	18 (72%)	10 (67%)
Surgical procedure			
Aneurismectomy	1 (4%)	0 (0%)	0 (0%)
Aorta	16 (64%)	15 (63%)	9 (64%)
Coronary artery bypass grafting	4 (16%)	4 (17%)	1 (7%)
Pericardectomy	0 (0%)	0 (0%)	1 (7%)
Valve	15 (60%)	17 (71%)	11 (79%)

The results are given as median (interquartile range, minimum, maximum) or count (percentage).
*Redo surgery is defined as previous sternotomy.

Downloaded from http://asa2.silverchair.com/anesthesiology/article-pdf/133/6/1173/513308/20201200.0-00011.pdf by guest on 24 October 2021

Copyright © 2020, the American Society of Anesthesiologists, Inc. Unauthorized reproduction of this article is prohibited.

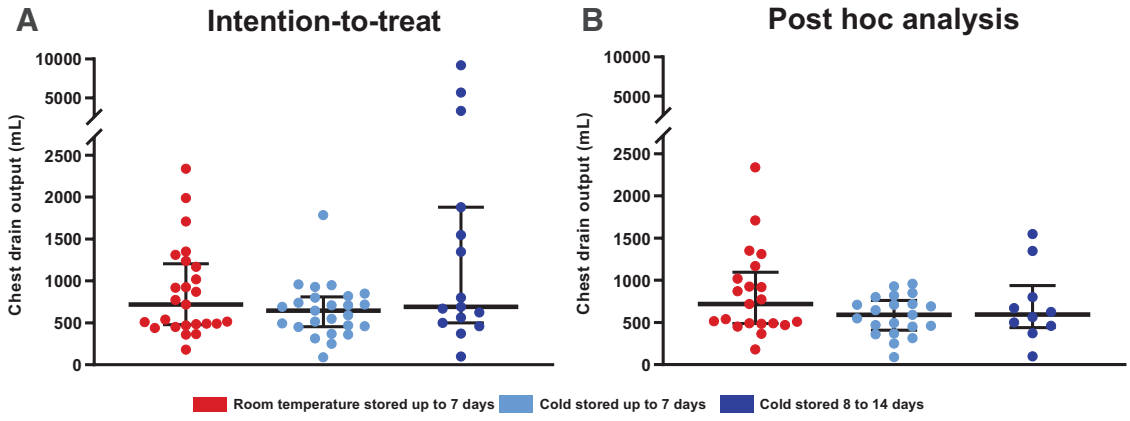

Fig. 2. Postoperative blood loss as measured by chest drain output for intention-to-treat patients (*A*) and *post hoc* analysis patients receiving room temperature–stored platelets, 7 days cold-stored platelets, and 8 to 14 days cold-stored platelets (*B*). The *dots* represent the individual study patients. The *lines* show median with interquartile range. The difference between the room temperature– and cold-stored for 7 days arms was not statistically significant ($P_{\text{intention-to-treat}} = 0.265$, $P_{\text{post hoc}} = 0.115$, two-tailed Mann–Whitney U test; SPSS Statistics for Windows, version 24.0, IBM, USA). The nonconcurrent stage II cold-stored for 8 to 14 days arm was not compared with the two stage I arms.

Table 2. Chest Drain Output

	N	Mean (95% CI)	SD (95% CL)	25%	Median	75%
Intention-to-treat						
Room temperature–stored up to 7 days	25	865 (640–1,091)	546 (719)	485	720	1,170
Cold–stored up to 7 days	25	649 (514–784)	328 (432)	460	645	800
Cold–stored 8–14 days	15	1,851 (467–3,234)	2,498 (3,646)	500	690	1,880
Post hoc analysis						
Room temperature–stored up to 7 days	21	838 (604–1,073)	514 (698)	490	720	1,020
Cold–stored up to 7 days	21	583 (477–688)	232 (315)	450	590	720
Cold–stored 8–14 days	10	699 (384–1,014)	440 (724)	460	595	800

The values are given as the number of patients (N), mean with 95% CI, SD with (one-sided) 95% upper confidence limit (CL), 25% percentile, median, 75% percentile. Chest drain output is measured in ml.

Blood Product Usage and Hemostatic Adjunct Treatments

Blood usage is reported in figure 3. There were no statistically significant differences in total units of transfused platelets ($P = 0.124$), plasma ($P = 0.079$), or red cells ($P = 0.543$) in the room temperature– and cold-stored up to 7 days arms. In total, 51% of room temperature–stored units transfused were 5 to 7 days old, *versus* 67% of cold-stored units, whereas 83% of the cold-stored for 8 to 14 days units were transfused beyond storage day 10.

Fibrinogen concentrate (RiaSTAP, CSL Behring, USA) and prothrombin complex concentrate (Octaplex, Octapharma, Switzerland) were administered after CPB. Overall, 35 patients received fibrinogen: 15 (60%) patients in the room temperature arm, 11 (44%) patients in the cold-stored up to 7 days arm, and 9 (60%) patients in the cold-stored for 8 to 14 days arm. In total, 10 patients received prothrombin complex concentrate, 2 (8%) patients in the room temperature arm, 4 (16%) patients in the cold-stored up to 7 days arm, and 4 (26%) patients in the cold-stored for 8 to 14 days arm (Supplemental Digital Content, http://links.lww.com/ALN/C476). There was no statistically significant difference in chest drain output between patients receiving either fibrinogen concentrates or prothrombin complex concentrate compared with patients who did not receive either of these hemostatic adjuncts within each study group ($P_{\text{room temperature}} = 0.540$, $P_{\text{cold-stored 7 days}} = 0.340$, $P_{\text{cold-stored for 8 to 14 days}} = 0.272$).

Platelet Function and Coagulation

Platelet counts and function are presented in figure 4 and in the Supplemental Digital Content (http://links.lww.com/ALN/C476). Because of the low number of patients with more than one platelet transfusion episode, the results of platelet count and function are presented for the first

Downloaded from http://asa2.silverchair.com/anesthesiology/article-pdf/133/6/1173/513308/20201200.0-00011.pdf by guest on 24 October 2021

Copyright © 2020, the American Society of Anesthesiologists, Inc. Unauthorized reproduction of this article is prohibited.

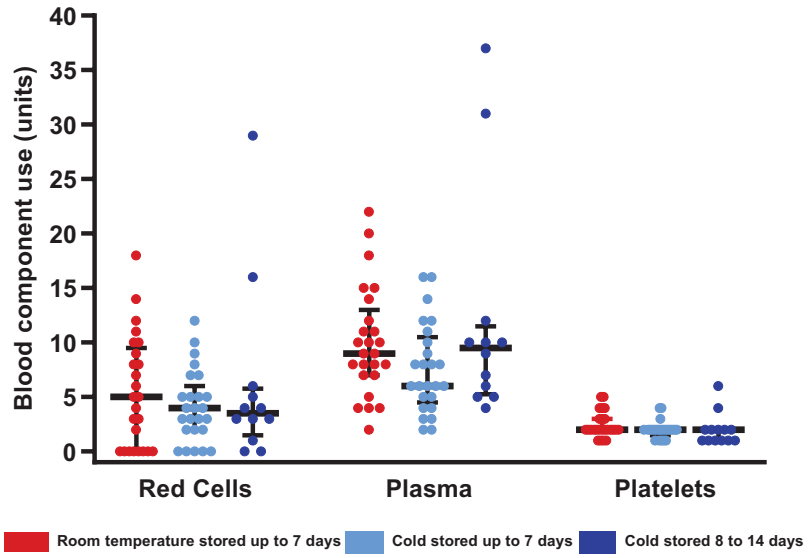

Fig. 3. Overall blood component use in patients receiving room temperature–stored platelets, 7 days cold-stored platelets, and 8 to 14 days cold-stored platelets. The *dots* represent the individual study patients. The *lines* show median with interquartile range. The difference between the room temperature– and cold-stored for 7 days arms was not statistically significant for number of transfused red cells (P = 0.543), plasma units (P = 0.079), or platelets (P = 0.124; two-tailed Mann–Whitney U test; SPSS Statistics for Windows). The nonconcurrent stage II arm was not compared with the two stage I arms.

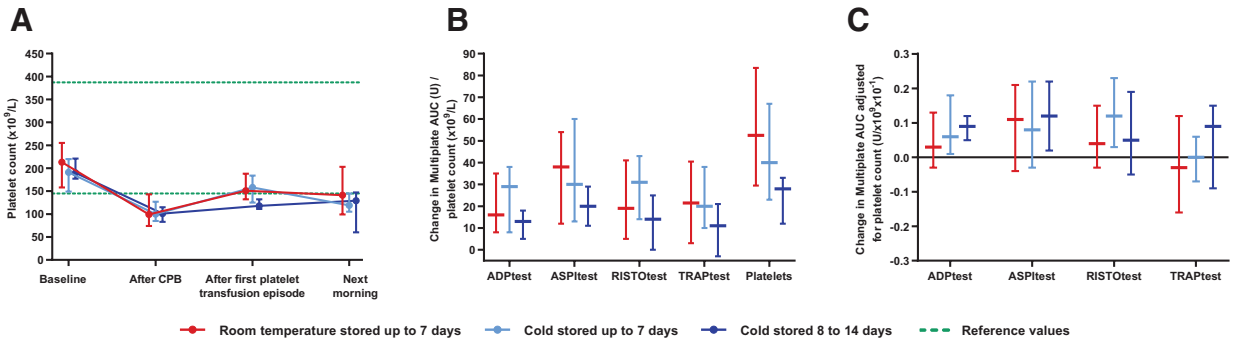

Fig. 4. (*A*) Platelet count at baseline before the start of surgery, after cardiopulmonary bypass (CPB), after the first platelet transfusion episode, and at 8:00 AM the following morning for patients receiving room temperature–stored platelets, 7 days cold-stored platelets, and 8 to 14 days cold-stored platelets. Points show median with interquartile range. *Green dotted lines* indicate reference values. There was no statistically significant difference between the two stage I arms at any sample point (one-tailed Mann–Whitney U test; SPSS Statistics for Windows, IBM). (*B*) Change in multiple electrode aggregation response and platelet count after the first platelet transfusion episode. Platelet count and multiple electrode aggregation response to all agonists increased after platelet transfusion for all study arms (P < 0.05), with the exception of RISTOtest (ristocetin) in the stage II arm (P = 0.137; two-tailed Wilcoxon signed-rank test). The level of change was not statistically significant different between the stage I arms for any agonist (two-tailed Mann–Whitney U test). (*C*) Change in multiple electrode aggregation response adjusted for platelet count. The nonconcurrent stage II arm was not compared to the two stage I arms. ADPtest, adenosine diphosphate; ASPItest, arachidonic acid; AUC, area under the curve; TRAPtest, thrombin receptor-activating peptide 6.

transfusion episode only. Platelet count increased after platelet transfusion for all study arms (P < 0.05). Multiple electrode aggregation response to all agonists increased after platelet transfusion for all study arms (P < 0.05), with the exception of RISTOtest (ristocetin) in the cold-stored for 8 to 14 days arm (P = 0.137l fig. 4).

Median thromboelastography maximum amplitude remained within normal ranges in all study arms. No

Downloaded from http://asa2.silverchair.com/anesthesiology/article-pdf/133/6/1173/513308/20201200.0-00011.pdf by guest on 24 October 2021

Copyright © 2020, the American Society of Anesthesiologists, Inc. Unauthorized reproduction of this article is prohibited.

significant difference were observed between the study arms in stage I. Similar, no differences were observed between the two randomized study arms for rotational thromboelastometry in-tem and ex-tem maximum clot firmness at any time point (Supplemental Digital Content, http://links.lww.com/ALN/C476). Information on coagulation and hemoglobin concentration is presented in the Supplementary Digital Content (http://links.lww.com/ALN/C476).

Clinical Safety

No transfusion reactions related to platelet transfusions were observed. The complexity of the surgical interventions performed in these high-risk patients resulted in an overall 28-day hospital mortality of three deaths (12%) in the room temperature group, two (8%) in the cold-stored 7 days arm and two (13%) in the cold-stored 8 to 14 days arm. Information on thromboembolic events and other safety parameters are shown in table 3. For the evaluation of length of stay in intensive care, one patient in the room temperature arm, two patients in the cold-stored up to 7 days arm, and two patients in the cold-stored for 8 to 14 days arm remained in intensive care at the conclusion of the 28-day study period.

Discussion

In complex cardiothoracic surgery with prolonged CPB circulation time, thrombocytopenia and platelet dysfunction cause excessive perioperative bleeding and increased utilization of blood products.[20,21] Achieving postoperative hemostasis often requires platelet transfusion.[22] Optimization of blood components for bleeding patients is therefore needed.[7]

This pilot study examined the safety and feasibility of cold-stored platelets stored for up to 14 days in patients undergoing complex cardiothoracic surgery. The preliminary tests of the hypothesis showing that cold-stored platelets are sufficiently functional to participate in hemostasis suggest a feasible alternative product for treatment of postoperative bleeding and provide critical information to proceed with pivotal trials. No statistically significant difference in chest drain output was observed between room temperature–stored platelets and cold-stored platelets stored for up to 7 days. We have reported the effect size of our primary outcome measure chest drain output to be used for sample size calculation in future studies (table 2). We recommend that sample size calculations for future studies should be based on what the investigators regard as a clinically significant blood loss, along with the reported upper confidence limits for the standard deviations.

Impedance aggregometry is an established functional analysis for detecting platelet dysfunction that can affect hemostasis. This includes dysfunction caused by platelet disorders, antiplatelet therapy, bleeding, transfusion, and medical interventions. In our study we quantified the dysfunction caused by CPB and surgery by first performing a preoperative test to establish baseline measurement for the patient and then retesting just before platelet transfusion. To investigate to what degree the dysfunction could be reversed through transfusion of cold-stored compared with room temperature–stored platelets, we performed another test after the platelets were transfused. Our findings of significant increase in aggregation response suggest that platelets that have been stored cold for up to 14 days remain functionally active. These results are in correspondence with previous published *in vitro* results.[5,23]

Postoperative thromboembolic event rates were similar between cold-stored and room temperature–stored platelets. Postoperative complications in complex cardiothoracic surgery are often unsuspected and multifactorial; evaluating thromboembolic events and their relationship to platelet transfusion is difficult.[24] The patient population in this study had high logistic euroSCORE, indicating high risk of mortality. Even though we observed no difference in complications, the number of patients in the study is too low to definitively conclude similarity in risk profile between cold-stored and room temperature–stored platelets.

Table 3. Safety Endpoints

	Room Temperature–stored up to 7 Days (n = 25)	Cold-stored up to 7 Days (n = 25)	Cold-stored for 8 to 14 Days (n = 15)
Arterial thromboembolism*	6 (24%)	6 (24%)	2 (13%)
Venous thromboembolism†	2 (8%)	0 (0%)	2 (13%)
Transfusion reaction caused by platelet transfusion	0 (0%)	0 (0%)	0 (0%)
Time to extubation, h	1.6 (5.08–21.37, 3.37–212.72)	6.6 (5.12–19.45, 3.67–349.02)	19.3 (4.28–27.27, 3.28–135.58)
Length of stay in intensive care unit, days‡	2 (1–5, 1–28+)	2 (1–6, 1–28+)	4 (2–14, 1–28+)
28-Day mortality	3 (12%)	2 (8%)	2 (13%)

The results are given as median (interquartile range, minimum, maximum) or count (percentage).

* Days from surgery: room temperature–stored up to 7 days: 3, 9, 10, 13, 16, and 22. Cold-stored up to 7 days: 2, 4, 4, 7, 8, and 14. Cold-stored from 8 to 14 days: 2 and 6,

†Days from surgery: room temperature–stored up to 7 days: 1 and 6; cold-stored for 8 to 14 days: 1 and 9.

‡One patient in the group with room temperature–stored up to 7 days, one patient in the cold-stored up to 7 days group, and two patients in the cold-stored for 8 to 14 days remained in intensive care at the conclusion of the 28-day study period. These were defined as 28 days for statistical analysis.

Downloaded from http://asa2.silverchair.com/anesthesiology/article-pdf/133/6/1173/513308/20201200.0-00011.pdf by guest on 24 October 2021

Copyright © 2020, the American Society of Anesthesiologists, Inc. Unauthorized reproduction of this article is prohibited.

Limitations

When the study was designed, only room temperature–stored platelet concentrates were available for routine use in our hospital. Cold-stored platelets had to be specially prepared for the study. To ensure the ready supply of cold-stored platelets for the study, we had to randomize the availability of either cold-stored or room temperature–stored platelets per week. The randomization schedule enabled us to collect the appropriate type of apheresis platelets in the week(s) before study patients were enrolled. The purpose of randomization is to maximize the chances for balance in the baseline covariates (potentially important confounders) between groups at the time of intervention. If unconcealed, randomization before the decision to transfuse (enrollment) could increase susceptibility to selection bias. To minimize the risk of between-group dissimilarity in the indications for platelet transfusion, all clinical personnel treating our study patients (surgeons, anesthesiologists, nursing staff) were blinded to the randomization both when deciding to transfuse and when ordering platelets. Once study platelets were ordered, no enrolled patients were withdrawn from the study, and no randomized treatment was withheld after either the order or delivery of study product. We believe our randomization scheme and concealment strategies achieved an appropriate balance between scientific rigor and practical logistics.

Most patients received the first transfusion episode during surgical hemostasis when multiple blood components and hemostatic products were transfused simultaneously. This might influence results. The number of patients having previous sternotomies was higher in the cold-stored up to 7 days arm. History of previous sternotomies is associated with increased bleeding and might influence the results; especially chest drain output and blood product use.

The stage II cold-stored arm was different from the stage I cold-stored arm in that the number of platelet units was restricted to two because of safety concerns for their extended storage time. The stage II and stage I inclusion criteria did not differ; however, sometime between stage I and stage II, the surgeons identifying patients for screening and informed consent improved in their selection of the surgical candidates at higher risk for platelet transfusion.

Although the causes of postoperative bleeding measured by chest tube output are multifactorial and difficult to disentangle, we chose to report chest tube output and total blood products transfused as sensitive, if not necessarily specific measures of the contribution of platelet transfusion to postsurgical hemostasis. Postsurgical hemostasis depends on the combination of patients' intrinsic hemostatic capabilities, preceding and interim medical treatments including transfusion, and the degree of surgical hemostasis. In our study, the majority of blood products was transfused during the surgical hemostatic procedures before chest closure, and study platelet transfusions were initiated before the col lection of postoperative chest tube output. We acknowledge that other measures of hemostasis may be more accurate than chest tube drainage, *e.g.*, hemostasis score,[25] and should be considered in future studies.

Conclusions

This pilot trial supports the feasibility of platelets stored cold for up to 14 days and provides critical guidance for future pivotal trials in high-risk cardiothoracic bleeding patients.

Acknowledgments

The study was performed by the Blood Far Forward research group, a collaboration between military and civilian healthcare services, including the Norwegian Armed Forces Medical Services, Sessvollmoen, Norway, and the Departments of Immunology and Transfusion Medicine, Anesthesia and Intensive Care, and Heart Disease at Haukeland University Hospital, Bergen, Norway. Collaborators at the U.S. Army Institute of Surgical Research contributed to study design, data analysis, data interpretation, and article preparation. The Blood Far Forward Research Group thanks all personnel at the Departments of Immunology and Transfusion Medicine, Anesthesia and Surgical Services, and Heart Disease of Haukeland University Hospital. This study has been our collaborative effort. Finally, the authors thank the members of the Trauma, Hemostasis, and Oxygenation Research Network for inspiring discussions and encouragement.

Research Support

Supported by the Norwegian Armed Forces Medical Services, Sessvollmoen, Norway, and the Department of Immunology and Transfusion Medicine at Haukeland University Hospital, Bergen, Norway.

Competing Interests

Dr. Spinella reports ongoing financial relationships with Secure Transfusion Services (South San Francisco, California), Cerus (Concord, California), Entegrion (Durham, North Carolina), KaloCyte (Baltimore, Maryland), and Haima (Cleveland, Ohio), and a past financial relationship with Hemanext (Lexington, Massachusetts). The opinion or assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the U.S. Department of the Army, the U.S. Department of Defense or the Norwegian Armed Forces Medical Services. The remaining authors declare no competing interests.

Reproducible Science

Full protocol available at: geir@docfish.no. Raw data available at: geir@docfish.no.

Correspondence

Address correspondence to Dr. Strandenes: Norwegian Armed Forces Medical Services, Forsvarsveien 75, N-2085

Downloaded from http://asa2.silverchair.com/anesthesiology/article-pdf/133/6/1173/513308/20201200.0-00011.pdf by guest on 24 October 2021

Copyright © 2020, the American Society of Anesthesiologists, Inc. Unauthorized reproduction of this article is prohibited.

Sessvollmoen, Norway. geir@docfish.no. This article may be accessed for personal use at no charge through the Journal Web site, www.anesthesiology.org.

References

1. Ng MSY, Tung JP, Fraser JF: Platelet storage lesions: What more do we know now? Transfus Med Rev 2018; 46:1111–13
2. Ellingson KD, Sapiano MRP, Haass KA, Savinkina AA, Baker ML, Chung KW, Henry RA, Berger JJ, Kuehnert MJ, Basavaraju SV: Continued decline in blood collection and transfusion in the United States 2015. Transfusion 2017; 57:1588–98
3. Murphy S, Gardner FH: Effect of storage temperature on maintenance of platelet viability: Deleterious effect of refrigerated storage. N Engl J Med 1969; 280:1094–8
4. Bynum JA, Meledeo MA, Getz TM, Rodriguez AC, Aden JK, Cap AP, Pidcoke HF: Bioenergetic profiling of platelet mitochondria during storage: 4°C storage extends platelet mitochondrial function and viability. Transfusion 2016; 56:S76–84
5. Getz TM, Montgomery RK, Bynum JA, Aden JK, Pidcoke HF, Cap AP: Storage of platelets at 4°C in platelet additive solutions prevents aggregate formation and preserves platelet functional responses. Transfusion 2016; 56:1320–8
6. Pidcoke HF, McFaul SJ, Ramasubramanian AK, Parida BK, Mora AG, Fedyk CG, Valdez-Delgado KK, Montgomery RK, Reddoch KM, Rodriguez AC, Aden JK, Jones JA, Bryant RS, Scherer MR, Reddy HL, Goodrich RP, Cap AP: Primary hemostatic capacity of whole blood: A comprehensive analysis of pathogen reduction and refrigeration effects over time. Transfusion 2013; 53:137–49S
7. Pidcoke HF, Spinella PC, Ramasubramanian AK, Strandenes G, Hervig T, Ness PM, Cap AP: Refrigerated platelets for the treatment of acute bleeding: A review of the literature and reexamination of current standards. Shock 2014; 41:51–3
8. Wood B, Padula MP, Marks DC, Johnson L: Refrigerated storage of platelets initiates changes in platelet surface marker expression and localization of intracellular proteins. Transfusion 2016; 56:2548–59
9. Becker GA, Tuccelli M, Kunicki T, Chalos MK, Aster RH: Studies of platelet concentrates stored at 22 °C and 4 °C. Transfusion 1973; 13:61–8
10. Manno CS, Hedberg KW, Kim HC, Bunin GR, Nicolson S, Jobes D, Schwartz E, Norwood WI: Comparison of the hemostatic effects of fresh whole blood, stored whole blood, and components after open heart surgery in children. Blood 1991; 77:930–6
11. Valeri CR: Blood components in the treatment of acute blood loss: Use of freeze-preserved red cells, platelets, and plasma proteins. Anesth Analg 1975; 54:1–14
12. Khuri SF, Healey N, MacGregor H, Barnard MR, Szymanski IO, Birjiniuk V, Michelson AD, Gagnon DR, Valeri CR: Comparison of the effects of transfusions of cryopreserved and liquid-preserved platelets on hemostasis and blood loss after cardiopulmonary bypass. J Thorac Cardiovasc Surg 1999; 117:172–84
13. Filip DJ, Aster RH: Relative hemostatic effectiveness of human platelets stored at 4 degrees and 22 degrees C. J Lab Clin Med 1978; 91:618–24
14. Papadopoulos N, Bakhtiary F, Grün V, Weber CF, Strasser C, Moritz A: The effect of normovolemic modified ultrafiltration on inflammatory mediators, endotoxins, terminal complement complexes and clinical outcome in high-risk cardiac surgery patients. Perfusion 2013; 28:306–14
15. Moore CG, Carter RE, Nietert PJ, Stewart PW: Recommendations for planning pilot studies in clinical and translational research. Clin Transl Sci 2011; 4:332–7
16. R Core Team: R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria, 2020
17. Ranucci M, Baryshnikova E, Soro G, Ballotta A, De Benedetti D, Conti D; Surgical and Clinical Outcome Research (SCORE) Group: Multiple electrode whole-blood aggregometry and bleeding in cardiac surgery patients receiving thienopyridines. Ann Thorac Surg 2011; 91:123–9
18. Hanke AA, Roberg K, Monaca E, Sellmann T, Weber CF, Rahe-Meyer N, Görlinger K: Impact of platelet count on results obtained from multiple electrode platelet aggregometry (Multiplate). Eur J Med Res 2010; 15:214–9
19. Henriksen HH, Grand AG, Viggers S, Baer LA, Solbeck S, Cotton BA, Matijevic N, Ostrowski SR, Stensballe J, Fox EE, Chen TA, Holcomb JB, Johansson PI, Cardenas JC, Wade CE: Impact of blood products on platelet function in patients with traumatic injuries: A translational study. J Surg Res 2017; 214:154–61
20. Hartmann M, Sucker C, Boehm O, Koch A, Loer S, Zacharowski K: Effects of cardiac surgery on hemostasis. Transfus Med Rev 2006; 20:230–41
21. Velik-Salchner C, Maier S, Innerhofer P, Kolbitsch C, Streif W, Mittermayr M, Praxmarer M, Fries D: An assessment of cardiopulmonary bypass-induced changes in platelet function using whole blood and classical light transmission aggregometry: The results of a pilot study. Anesth Analg 2009; 108:1747–54

Downloaded from http://asa2.silverchair.com/anesthesiology/article-pdf/133/6/1173/513308/20201200.0-00011.pdf by guest on 24 October 2021

Copyright © 2020, the American Society of Anesthesiologists, Inc. Unauthorized reproduction of this article is prohibited.

22. Ranucci M, Baryshnikova E, Pistuddi V, Menicanti L, Frigiola A; Surgical and Clinical Outcome REsearch (SCORE) Group: The effectiveness of 10 years of interventions to control postoperative bleeding in adult cardiac surgery. Interact Cardiovasc Thorac Surg 2017; 24:196–202
23. Braathen H, Sivertsen J, Lunde THF, Kristoffersen EK, Assmus J, Hervig TA, Strandenes G, Apelseth TO: *In vitro* quality and platelet function of cold and delayed cold storage of apheresis platelet concentrates in platelet additive solution for 21 days. Transfusion 2019; 59:2652–61
24. Mueller J, Jeudy J, Poston R, White CS: Cardiac CT angiography after coronary bypass surgery: Prevalence of incidental findings. AJR Am J Roentgenol 2007;189:414–9
25. Greilich PE, Edson E, Rutland L, Jessen ME, Key NS, Levy JH, Faraday N, Steiner ME: Protocol adherence when managing massive bleeding following complex cardiac surgery: A study design pilot. J Cardiothorac Vasc Anesth 2015; 29:303–10

ANESTHESIOLOGY REFLECTIONS FROM THE WOOD LIBRARY-MUSEUM

Laughing Gas Kings of Penguin Guano…or Is It GuaN_2O?

Lying roughly equidistant from the Antarctic peninsula and the tip of South America, South Georgia Island shelters a huge colony of King Penguins. These massive birds are celebrated on 43-pence stamps issued by South Georgia and the South Sandwich Islands (*above*). To pack on blubber, King Penguins ingest krill and fish that have dined on nitrogen-rich phytoplankton. Then the penguins excrete kingly amounts of guano. For South Georgia, global warming has increased both glacial retreat and penguin activity. Consequently, there is more open land from which soil microbes can release even greater amounts of nitrous oxide from deposited guano. With the "intense" levels of laughing gas, Danish scientist and permafrost expert Bo Eberling has observed that, "after nosing about in guano for several hours, one goes completely cuckoo." (Copyright © the American Society of Anesthesiologists' Wood Library-Museum of Anesthesiology.)

Melissa L. Coleman, M.D., Penn State College of Medicine, Hershey, Pennsylvania, and George S. Bause, M.D., M.P.H., Case Western Reserve University, Cleveland, Ohio.

Downloaded from http://asa2.silverchair.com/anesthesiology/article-pdf/133/6/1173/513308/20201200.0-00011.pdf by guest on 24 October 2021

Copyright © 2020, the American Society of Anesthesiologists, Inc. Unauthorized reproduction of this article is prohibited.