ANESTHESIOLOGY

Combined Platelet and Erythrocyte Salvage: Evaluation of a New Filtration-based Autotransfusion Device

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EDITOR'S PERSPECTIVE

What We Already Know about This Topic

- Autotransfusion is frequently used intraoperatively for patient blood management, with devices selectively able to salvage and wash red blood cells but not platelets.

What This Article Tells Us That Is New

- A novel filtration-based autotransfusion device salvaged both red blood cells and platelets, without significantly impacting cell integrity and function, with the recovery of 88.1% and 36.8%, respectively. The filtration and washing prevented reinfusion of high concentrations of heparin and did not activate leukocytes.

ABSTRACT

Background: The SAME device (i-SEP, France) is an innovative filtration-based autotransfusion device able to salvage and wash both red blood cells and platelets. This study evaluated the device performances using human whole blood with the hypothesis that the device will be able to salvage platelets while achieving a erythrocyte yield of 80% and removal ratios of 90% for heparin and 80% for major plasma proteins without inducing signification activation of salvaged cells.

Methods: Thirty healthy human whole blood units (median volume, 478 ml) were diluted, heparinized, and processed by the device in two consecutive treatment cycles. Samples from the collection reservoir and the concentrated blood were analyzed. Complete blood count was performed to measure blood cell recovery rates. Flow cytometry evaluated the activation state and function of platelets and leukocytes. Heparin and plasma proteins were measured to assess washing performance.

Results: The global erythrocyte yield was 88.1% (84.1 to 91.1%; median [25th to 75th]) with posttreatment hematocrits of 48.9% (44.8 to 51.4%) and 51.4% (48.4 to 53.2%) for the first and second cycles, respectively. Ektacytometry did not show evidence of erythrocyte alteration. Platelet recovery was 36.8% (26.3 to 43.4%), with posttreatment counts of 88 × 10^9/l (73 to 101 × 10^9/l) and 115 × 10^9/l (95 to 135 × 10^9/l) for the first and second cycles, respectively. Recovered platelets showed a low basal P-selectin expression at 10.8% (8.1 to 15.2%) and a strong response to thrombin-activating peptide. Leukocyte yield was 93.0% (90.1 to 95.7%) with no activation or cell death. Global removal ratios were 98.3% (97.8 to 98.9%), 98.2% (96.9 to 98.8%), and 88.3% (86.6 to 90.7%) for heparin, albumin, and fibrinogen, respectively. The processing times were 4.4 min (4.2 to 4.6 min) and 4.4 min (4.2 to 4.7 min) for the first and second cycles, respectively.

Conclusions: This study demonstrated the performance of the SAME device. Platelets and red blood cells were salvaged without significant impact on cell integrity and function. In the meantime, leukocytes were not activated, and the washing quality of the device prevented reinfusion of high concentrations of heparin and plasma proteins.

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Autotransfusion or cell salvage devices allow processing of blood shed from the surgical field and transfusion of red blood cells back to the patient.[1,2] They play an important role in patient blood management and are recommended by international guidelines.[1,3] Cell salvage has proven a reduction in the need for perioperative allogeneic blood transfusion in high hemorrhagic risk surgery, such as cardiac, orthopedic, gynecologic, and abdominal surgery, and might

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also improve clinical postoperative outcomes in cardiac surgery, including postoperative infections.[1,2,4–6] Finally, the cost-effectiveness of cell salvage has been demonstrated.[5,6] In addition to erythrocyte salvage, current devices can also effectively wash erythrocytes to remove cell breakdown products and activated plasma proteins, to reduce the risk of induced coagulopathy and inflammation.[2,6,7]

However, centrifugation-based autotransfusion devices can only salvage red blood cells, whereas blood platelets are removed during the process.[6] Hence, it has been shown that large amounts of intraoperative cell salvage blood transfusion can be associated with thrombocytopenia and increased use of allogeneic platelet transfusion.[8] Further, thrombocytopenia and platelet function disorders are known conditions associated with perioperative bleeding.[9–11] Although platelet transfusion is commonly used to treat thrombocytopenia-induced bleeding, it is also associated with an increase in postoperative complications including infections and increased length of stay.[3,12–14]

The SAME device (Smart Autotransfusion for Me; i-SEP, France) was designed as an innovative filtration-based autotransfusion device able to salvage and wash both red blood cells and platelets. This new autotransfusion system integrates a hollow fiber filtration technology, comparable to the filters used for plasmapheresis or for ultrafiltration during cardiopulmonary bypass. Using a combination of washing and filtration of salvage blood, the device allows the concentration of red blood cells and platelets within the concentrated blood product, as well as the removal of heparin, free hemoglobin, coagulation factors, and inflammatory mediators such as complement proteins.

We evaluated the i-SEP new autotransfusion device using human whole blood. The objectives of the study were to determine the performance of the device in terms of platelets and erythrocyte yield and function recovery, removal of heparin and of major plasma proteins, and potential impact on blood cell activation. We hypothesized that the device will be able to salvage platelets while achieving a erythrocyte minimal yield of 80% and a minimal removal ratio of 90% for heparin and 80% for major plasma proteins, without significant blood cell activation, with a fast treatment time of less than 5 min.

Materials and Methods

Autotransfusion Device

The SAME device is a medical device consisting of reusable equipment and disposable consumables (Supplemental Digital Content 1, http://links.lww.com/ALN/C621). The device innovative technology and process are described in the following patents: PCT/FR2018/053500 published as WO 2019/129973 on July 4, 2019 (corresponding to U.S. application no. 16/958,473); PCT/FR2018/053501 published as WO 2019/129974 on July 4, 2019 (corresponding to U.S. application no. 16/958,458); and PCT/FR2020/051115 published as WO 2020/260836 on December 30, 2020. Consumables include a dual-lumen suction line (allowing both collection and anticoagulation of shed blood), a blood collection reservoir (including a 40-μm filter), and a treatment set. The treatment set includes tubing, a polyethersulfone hollow fiber cartridge that separates the blood cells from the plasma, a compliant blood treatment bag that ensures the blood washing, a waste bag that receives the plasma and contaminants, and a reinfusion bag that stores the filtered, washed, and concentrated blood cells (Supplemental Digital Content 2, http://links.lww.com/ALN/C622). The reusable equipment is an electromedical medical device composed by several systems required for blood circulation and continuous measurements, including a continuous in-line hematocrit monitor. The i-SEP device is associated with a specific software to drive the different steps of the device installation and blood treatment.

Blood Processing by the i-SEP Device

During clinical use, the first stage of cell salvage with the i-SEP device is the collection of shed blood from the surgical field by the dual-lumen suction line, allowing the anticoagulation of shed blood by a heparinized saline drip. The shed blood is collected in the collection reservoir in which it undergoes a first filtration by the included 40-μm filter, allowing the removal of bone debris and microaggregates before blood treatment by the device. In the current experimental study, the suction line is used without heparinized saline drip as the whole blood is already heparinized (see below, under "Blood Preparation").

Then the treatment set is filled with anticoagulated salvaged blood transferred from the blood collection reservoir when a sufficient volume is collected. During the treatment phase, the blood is processed by the i-SEP device, with simultaneous filtration and washing (Supplemental Digital Content 2, http://links.lww.com/ALN/C622). The volume of the treatment set (300 to 1,000 ml thanks to the compliant treatment bag) limits the amount of collected blood that can be processed in one time, hence defining a treatment cycle. Thus, for research purpose, the volume of a cycle can be programed in the software between 300 and 1,000 ml.

Several simultaneous steps then constitute the innovative i-SEP process: wash solution (normal saline) is pumped into the treatment set; diluted salvaged blood circulates within the treatment set between the treatment bag and the polyethersulfone hollow fiber to allow microfiltration to occur; and fluid is continuously discarded from the treatment circuit into the waste bag through the effluent line. Once the continuously monitored hematocrit reaches the prespecified target, the device automatically transfers the processed blood from the treatment set into the reinfusion bag.

Blood Preparation

Thirty whole human blood units were obtained from the French Blood Bank Institute (Etablissement Français du Sang, Rennes, France, convention and ethical approval

reference No. 79/2019-2022) after obtaining donor written informed consent. Whole blood was collected in citrate–phosphate–dextrose anticoagulant and stored less than 24 h at room temperature before processing. Whole blood unit volume was 478 ml (461 to 511 ml) with a hematocrit of 38.6% (36.2 to 39.9%). Blood preparation is described in figure 1. Blood units were diluted in normal saline (0.9% NaCl; Macopharma, France) up to 1,200 ml (including 200 ml of collection reservoir priming) to obtain clinically relevant hematocrits of 14.4% (13.1 to 15.3%), corresponding to initial hematocrits measured in blood collection reservoir during cardiac and orthopedic surgeries (ranging between 10 and 20%) while preserving between-subject heterogeneity.[7,15–17] A high concentration of unfractionated heparin (Choay heparin; Sanofi-Aventis, France) was added to whole blood before dilution in saline (fig. 1); the final concentration of heparin in the collection reservoir was 12 IU/ml, to evaluate heparin washout in worst-case clinical conditions.

Experimental Procedure for *In Vitro* Study

All experiments were conducted in the Department of Hematology of the University Hospital of Rennes (France). After collection reservoir priming (200 ml of 0.9% NaCl; Macopharma), the blood was collected into the blood collection reservoir under controlled depression level using the suction line, with a vacuum level of −250 mbar (fig. 1). Then the experimental procedure consisted of two consecutive treatment cycles using the i-SEP device standard program. The choice of a two-treatment cycle procedure allows the evaluation of the impact of two consecutive cycles on the same filtering membrane and surface pacification. The first cycle was programed to treat 700 ml, and the second cycle treated 500 ml (total volume, 1,200 ml), using 600 ml of washing volume (0.9% NaCl; Macopharma) for each cycle. This allowed evaluation of the impact of blood volume to washing volume ratio on cell yield and washing performance. A different reinfusion bag was used for each cycle to facilitate the posttreatment sampling. The processing time of each cycle was recorded to be used as a device performance endpoint.

Blood Sample Collection during Processing

Four sample series were realized for each blood unit: (1) blood in the reservoir after collection and before first cycle (first cycle pretreatment), (2) blood cell concentrate in the reinfusion bag at the end of first cycle (first cycle posttreatment), (3) blood in the reservoir before second cycle (second cycle pretreatment), and (4) blood cell concentrate in the reinfusion bag at the end of second cycle (second cycle posttreatment).

The sampling procedure was identical for all tests. Blood was gently homogenized in both the collection reservoir and the transfusion bag before taking samples to ensure homogeneous sampling. Pretreatment samples were taken from the line between the collection reservoir and the treatment set during the transfer of diluted blood into the treatment set, for each cycle. Tubes were cautiously filled in up to the volume indicator. When a centrifugation was needed, the tubes were handled a maximum of 1 h after their sampling. The samples were distinctly stored at room temperature, 4°C, or −80°C or sent, when necessary, at room temperature, in ice, or in dry ice following recommendations.

Laboratory Analyses

Laboratory analyses were performed on all blood units for each sample series (30 blood units, 60 cycles), except for ektacytometry (9 blood units, 18 cycles) and CD64 cytometric analysis (14 blood units, 28 cycles). Complete blood count (including erythrocyte count, leukocyte count, platelet count, hematocrit, and total hemoglobin level) was performed on an EDTA tube using UniCel DxH 800 (Beckman Coulter, USA). Albumin, lactate deshydrogenase, and immunoglobulins were measured in a lithium heparin tube using a Cobas 8000 modular analyzer (Roche Diagnostics, Switzerland). Free hemoglobin was assessed using spectrophotometry (SAFAS, Monaco). Two citrate (109 mM) tubes were taken for each cycle and centrifuged at 2,000*g* to obtain plasma. All plasma samples were frozen (–80°C) before performing the following assays: unfractionated heparin anti-Xa activity using STA-Liquid-anti-Xa (Stago, France), factors II, V, and X coagulant activity using factors II-, V- and X-deficient plasma (Stago) after 1/10 dilution and Neoplastin CI+ (Stago), fibrinogen von Clauss activity (Stago) on a STA R Max coagulometer (Stago), and complement factor 3 assays (Siemens BN nephelometer). Anti-Xa activity was measured in samples diluted to 1:20 or 1:2 in normal pool plasma to allow measurement of high heparin concentration. Blood cell yield was calculated using the following formula: cell yield = [(posttreatment blood volume × posttreatment cell concentration)/(pretreatment blood volume × pretreatment cell concentration)] × 100. Hemolysis was calculated as follows: (100 − hematocrit) × free hemoglobin/total hemoglobin. Removal ratios of major blood proteins were measured as follows: (initial quantity of protein − final quantity of protein)/initial quantity of protein.

Ektacytometry

Osmotic gradient ektacytometry allows measurement of red cell deformability in response to alterations in medium osmolality.[18] It is a useful technique for the diagnosis of inherited red cell membrane disorders and characterization of blood storage lesions.[18–21] We used a LORCA ektacytometer (Centre Hospitalier Universitaire Bicêtre, Le Kremlin Bicêtre, France) to assess the impact of the i-SEP device process on red cell membrane integrity. Samples were run at 37°C. Three parameters were used: osmolality

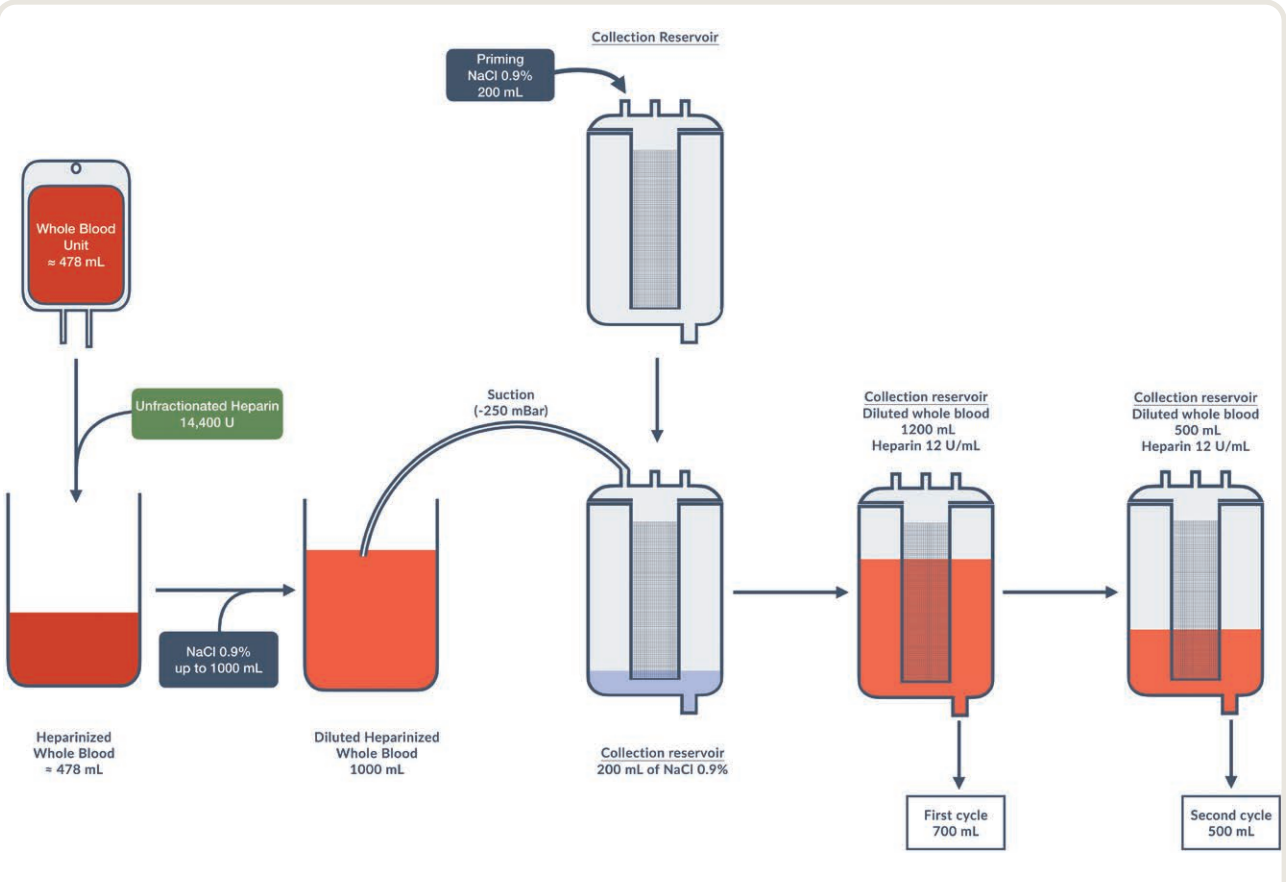

Fig. 1. Blood preparation and experimental procedure for *in vitro* study. Whole human blood units were obtained from the French Blood Bank Institute (Rennes, France) after obtaining donor written informed consent. Whole blood unit volume was 478 ml (461 to 511 ml). Citrate–phosphate–dextrose anticoagulated blood units were diluted in normal saline (0.9% NaCl; Macopharma, France) up to 1,200 ml (including 200 ml of collection reservoir priming). Unfractionated heparin (Choay heparin; Sanofi-Aventis, France) at high concentration was added to whole blood before dilution in saline. The final heparin concentration in the collection reservoir was 12 IU/ml. After collection reservoir priming (200 ml of 0.9% NaCl; Macopharma), the blood was collected into the blood collection reservoir under controlled depression level using the suction line, with a vacuum level of −250 mbar. Then the experimental procedure consisted of two consecutive treatment cycles using the i-SEP device. The first cycle was programed to treat 700 ml and the second cycle treated 500 ml (total volume, 1,200 ml).

corresponding to minimum elongation index, which reflects osmotic fragility; maximum elongation index, which assesses membrane flexibility; and osmololality corresponding to half-maximal elongation in the hypertonic arm of the osmotic gradient, which gives information on intracellular viscosity of the red cell.

Flow Cytometric Evaluation of Platelet Activation

Platelet activation was evaluated by measuring surface expression of three main physiologic platelet glycoproteins: P-selectin, GPIb, and GPIIb. Unlike P-selectin, GPIb and GPIIb are constitutively expressed at the surface of quiescent platelets. Upon activation, surface expression of P-selectin and GPIIb is increased, whereas GPIb surface exposure is reduced. Quantitation of GPIb, GPIIb, and P-selectin on platelet surface was measured using flow cytometry (DxFLEX; Beckman Coulter) and the PLT GP/receptors kit from Stago.[22,23] GPIb and GPIIb expression was reported as the number of receptors per platelet. P-selectin expression was reported as the percentage of P-selectin–positive platelets. Glycoprotein expression at rest, in pretreated and posttreated blood, was used to measure platelet activation as a potential side effect induced by the i-SEP device. Then stimulation by thrombin receptor PAR1-activating peptide 6 (TRAP6) was used as a surrogate to determine whether posttreatment platelets can be fully activated after being recovered by the i-SEP device.

Flow Cytometric Analysis of Leukocyte Viability and Activation State

Leukocyte subset viability and activation states were measured using flow cytometry (DxFLEX; Beckman Coulter). DuraClone IM phenotyping panel (Beckman Coulter) was used to identify leukocyte subpopulations in whole blood

samples. Leukocyte viability was evaluated using 7-amino-actinomycin D antibodies.[24] Alive leukocytes were defined as CD45+/7-amino-actinomycin D and leukocyte viability was reported as a percentage of alive white blood cells among CD45-positive cells. Neutrophils and monocytes were respectively defined as high side scatter size and CD16+/CD14− and CD16−/CD14+ cells. An increase in CD64 surface expression was used as a marker of neutrophil and monocyte activation and was reported as mean fluorescence intensity.[25] The T-cell activation state was evaluated using HLA-DR antibodies.[26] Activated CD4-positive and CD8-positive T cells were defined as CD4+/HLA-DR+ or CD8+/HLA-DR+ double-positive cells and expressed as percentages of HLA-DR–positive cells, respectively, among CD4- or CD8-positive cells, respectively. The data were analyzed with Kaluza software (Beckman Coulter).

Definition of Endpoints

The main goal of this study was to evaluate performance of blood cell salvage by the i-SEP device. Given the lack of consensual guidelines on preclinical evaluation of autotransfusion systems and the low level of evidence in the current literature, we determined prespecified performance criteria based on *in vitro* and clinical assessment of commercially available devices and international guidelines on evaluation of blood products.[27,28] Hence, the erythrocyte minimal recovery rate and hematocrit were respectively set at 80 and 40%, corresponding to 1 SD less than the mean values published for centrifugation-based devices. Washout quality was defined as a minimal removal ratio of 90 and 80%, respectively, for heparin and major plasma proteins. Maximal hemolysis was set at 0.8% in accordance with European guidelines on packed red cell evaluation.[27] Being a major innovative characteristic of the device, no minimal platelet yield criteria was defined. The activation state and function of platelets and leukocytes were considered as exploratory secondary endpoints. The protocol and the choice of endpoints were approved by the French National Agency for Medicines and Health Products Safety (Agence Nationale de Sécurité du Médicament et des Produits de Santé, Saint-Denis, France).

Statistics

Sample size selection was based on the French National Agency for Medicines and Health Products Safety guidelines for therapeutic blood product quality evaluation and was set at 30 replicates (60 cycles).[28] Therefore, no *a priori* statistical power calculation was conducted. *Post hoc* statistical analyses were conducted using Prism 8 (GraphPad Software, USA). The data were tested for normality using the D'Agostino and Pearson normality test. All measured parameters did not show normal distribution. Kruskal–Wallis and Friedman's test with *post hoc* Dunn's correction test were used for multiple comparisons between treatment phases, cycles, and TRAP6 stimulation in the flow cytometric analysis of platelet glycoproteins. The Mann–Whitney test was used for single comparisons between: (1) the first and second cycles for erythrocyte yield, platelet yield, and leukocyte yield and (2) pre- and posttreated blood for erythrocyte lysis markers, ektacytometric parameters, leukocytes viability, and leukocytes activation, with independent analysis of the first and second cycles. All tests used two-tailed hypothesis. Statistical significance was achieved for $P < 0.05$. Statistical analyses used limit-of-quantification values as substitute for values inferior to the limit of quantification. The data are presented as medians with interquartile ranges. The differences between two conditions are reported as actual differences between medians with Hodges–Lehman computed 95% CI. Outliers were not excluded from the analyses. There were no missing data.

Results

Erythrocyte Yield and Impact on Cell Integrity

Whole blood processing using the i-SEP autotransfusion device produced a high recovery rate of red blood cells with a global yield of 88.1% (84.1 to 91.1%). The second cycle steadily achieved significantly higher RBC yield compared to the first cycle (16.3% [12.3 to 18.9%]; $P < 0.001$; fig. 2). The erythrocyte counts in the pretreated blood were 1.5 × 10^{12}/l (1.3 to 1.6) for the first cycle and 1.4 × 10^{12}/l (1.3 to 1.6) for the second cycle. Erythrocyte counts in the posttreated blood were 4.8 × 10^{12}/l (4.4 to 5.1 × 10^{12}/l) for the first cycle and 5.1 × 10^{12}/l (4.8 to 5.3 × 10^{12}/l) for the second cycle. The final posttreatment concentrate volume was 186 ml (152 to 217 ml) for the first cycle and 105 ml (87 to 112 ml) for the second cycle. Likewise, posttreatment hematocrit was consistently above 40%, with 48.9% (44.8 to 51.4%) for the first cycle and 51.4% (48.4 to 53.2%) for the second cycle (fig. 3). Assessment of erythrocyte lysis markers demonstrated a significant increase in global LDH (107 UI/ml [98 to 124 UI/ml]; $P < 0.001$), free hemoglobin (54 mg/dl [46 to 56 mg/dl]; $P < 0.001$), and hemolysis (0.12% [0.11 to 0.14%]; $P < 0.001$) in the posttreated compared to the pretreated blood (table 1).

Ektacytometric analysis of red blood cells demonstrated that blood processing through the i-SEP device did not induce alterations in erythrocyte integrity and deformability, neither between pre- and posttreated blood nor between the first and second cycles for the three parameters analyzed (table 1). Hence, no statistical differences were measured in global osmolality at minimum elongation index (−5 mOsm/kg [−9 to 1 mOsm/kg]; $P = 0.089$), maximum elongation index (0.00 [−0.01 to 0.01]; $P = 0.879$) and osmolality at half-maximal elongation in the hypertonic arm (−1 mOsm/kg [−17 to 13 mOsm/kg]; $P = 0.683$) in the posttreated compared to the pretreated blood (table 1).

Platelet Recovery and Platelet Function Analysis

The device achieved a global platelet recovery rate of 36.8% (26.3 to 43.4%) with significantly higher platelet yield during

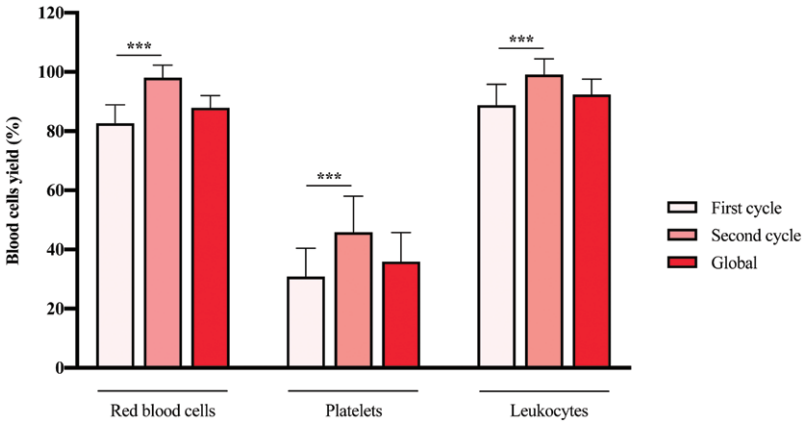

Fig. 2. Blood cell yield, including red blood cells, platelets, and white blood cells, calculated for the first cycle (n = 30) and the second cycle (n = 30). The global yield includes all the results (cycles 1 and 2; n = 30): it represents the global yield of all red blood cells obtained in the concentrated blood compared to all red blood cells from diluted blood. ***$P < 0.001$.

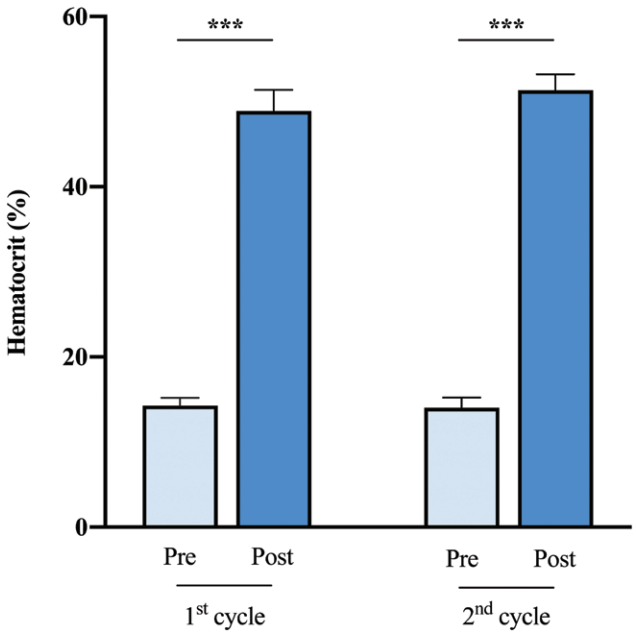

Fig. 3. Hematocrit measurement between pretreatment (Pre) and posttreatment (Post; concentrated) blood for each cycle (n = 30 for cycle 1 and n = 30 for cycle 2). ***$P < 0.001$.

the second cycle compared to the first cycle (14.3% [9.3 to 21.0%]; $P < 0.001$; fig. 2). Platelet counts in the pretreated blood were 74 × 10^9/l (59 to 89 × 10^9/l) and 72 × 10^9/l (60 to 82 × 10^9/l) for the first and second cycles, respectively. Platelet counts in the posttreated blood were 88 × 10^9/l (73 to 101 × 10^9/l) and 115 × 10^9/l (95 to 135 × 10^9/l) for the first and second cycles, respectively. The global number of salvaged platelets was 28.7 × 10^9 platelets (24.2 to 35.4 × 10^9).

Flow cytometric analysis of glycoproteins revealed a limited platelet activation induced by the device, as demonstrated by a significant increase in the percentage of P-selectin–positive platelets in posttreated compared to pretreated blood for both cycles. The percentages of P-selectin–positive platelets were 2.3% (1.5 to 3.4%) and 10.8% (7.2 to 12.6%; $P < 0.001$; fig. 4A) during the first cycle in the pretreated and posttreated blood, respectively, and 3.5% (2.5 to 4.2%) and 10.8% (8.1 to

Table 1. Impact of Blood Processing on Erythrocyte Integrity

	First Cycle		Second Cycle		Global	
	Pretreatment	Posttreatment	Pretreatment	Posttreatment	Pretreatment	Posttreatment
Hemolysis, %	0.03 (0.02 to 0.05)	0.18 (0.14 to 0.21)*	0.04 (0.02 to 0.05)	0.15 (0.13 to 0.19)*	0.03 (0.02 to 0.05)	0.16 (0.15 to 0.21)*
Hemoglobin, g/dl	4.8 (4.4 to 5.2)	15.9 (14.7 to 16.5)*	4.7 (4.3 to 5.1)	16.5 (16.0 to 17.0)*	4.8 (4.4 to 5.4)	16.2 (15.6 to 16.7)*
Free hemoglobin, mg/dl	2 (1 to 3)	58 (47 to 65)*	2 (1 to 3)	53 (44 to 65)*	2 (1 to 3)	56 (44 to 64)*
Lactate dehydrogenase, U/l	43 (37 to 57)	145 (123 to 163)*	45 (37 to 50)	169 (150 to 206)*	44 (39 to 58)	151 (140 to 182)*
Ektacytometry						
Osmolality at minimal elongation index, mOsm/kg	154 (151 to 159)	151 (150 to 159)	156 (131 to 158)	148 (147 to 155)	155 (153 to 159)	150 (148 to 155)
Maximal elongation index	0.61 (0.61 to 0.62)	0.62 (0.61 to 0.62)	0.61(0.61 to 0.62)	0.62 (0.61 to 0.62)	0.61(0.61 to 0.62)	0.62 (0.61 to 0.62)
Osmolality at half-maximal elongation in the hypertonic arm, mOsm/kg	464 (449 to 478)	461 (449 to 474)	465 (450 to 477)	461 (445 to 472)	463 (449 to 477)	462 (447 to 473)

The data are expressed as medians and interquartile ranges 25 to 75%. N = 30, except for ektacytometric parameters, for which N = 9. The global results were obtained by pooling the observations of the two cycles for each repetition.

*$P < 0.001$ *versus* pretreatment.

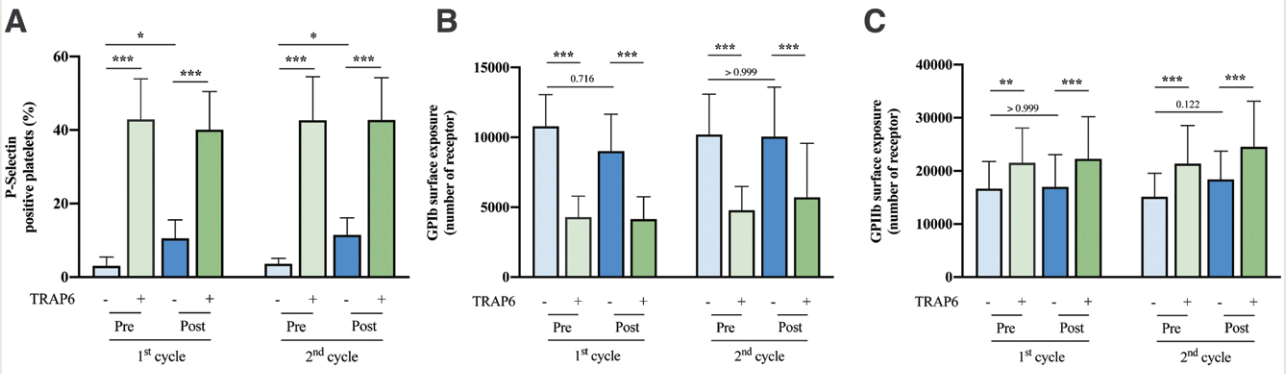

Fig. 4. Effect of i-SEP device processing on P-selectin, GPIb, and GPIIb platelet expression and evaluation of platelet activation potential by thrombin receptor pathway stimulation using thrombin receptor-activating peptide 6 (TRAP6). All flow cytometry experiments were performed on DxFLEX (Beckman Coulter, USA), using the PLT Gp/receptors kit from Stago (Biocytex, France). (A) P-selectin expression reported as the percentage of P-selectin–positive platelets. P-selectin expression was measured between pretreatment (Pre) and posttreatment (Post; concentrated) blood for each cycle (n = 30 for cycle 1 and n = 30 for cycle 2). *$P < 0.05$; ***$P < 0.001$, measurements were performed before (–) or after TRAP6-induced platelet activation (+). (B) GPIb expression reported as the number of receptors per platelet. GPIb expression was measured between pretreatment and posttreatment (concentrated) blood for each cycle (n = 30 for cycle 1 and n = 30 for cycle 2). ***$P < 0.001$. +, platelet stimulation by TRAP6; –, absence of platelet stimulation by TRAP6. (C) GPIIb expression reported as number of receptors per platelet. GPIIb expression was measured between pretreatment and posttreatment (concentrated) blood for each cycle (n = 30 for cycle 1 and n = 30 for cycle 2). **$P < 0.01$; ***$P < 0.001$. +, platelet stimulation by TRAP6; –, absence of platelet stimulation by TRAP6. GpIb and GpIIb expressions were performed before (–) or after TRAP6-induced platelet activation (+).

15.2%; $P < 0.001$; fig. 4A) during the second cycle in the pretreated and posttreated blood, respectively. However, no significant change between pretreated and posttreated blood was found during both cycles regarding GPIb surface expression (fig. 4B) and GPIIb surface expression (fig. 4C), in line with a limited platelet activation.

Compared to unstimulated posttreated blood, TRAP6 stimulation of posttreated blood induced a significant change in platelet glycoprotein surface expression, with increased percentage of P-selectin–positive platelets, decreased GPIb surface expression, and increased GPIIb surface expression. These results clearly demonstrate that recovered platelets retain a high potential of activation (fig. 4, A, B, and C). The percentage of P-selectin–positive platelets was 10.8% (7.2 to 12.6%) and 43.0% (32.6 to 46.7%) during the first cycle in the unstimulated and TRAP6-stimulated posttreated

blood ($P < 0.001$; fig. 4A), respectively, and 10.8% (8.1 to 15.2%) and 47.6% (38.2 to 50.5%) during the second cycle in the unstimulated and TRAP6-stimulated posttreated blood ($P < 0.001$; fig. 4A), respectively. GPIb surface expression was 8,632 (7,611 to 10,849) and 3,809 (3,043 to 5,337) during the first cycle in the unstimulated and TRAP6-stimulated posttreated blood ($P < 0.001$; fig. 4B), respectively, and 9,956 (7,996 to 13,100) and 5,189 (4,067 to 6,152) during the second cycle in the unstimulated and TRAP6-stimulated posttreated blood ($P < 0.001$; fig. 4B), respectively. GPIIb surface expression was 16,297 (13,547 to 20,273) and 23,616 (17,790 to 27,429) during the first cycle in the unstimulated and TRAP6-stimulated posttreated blood ($P < 0.001$; fig. 4C), respectively, and 18,863 (15,047 to 21,424) and 24,892 (19,421 to 30,579) during the second cycle in the unstimulated and TRAP6-stimulated posttreated blood ($P < 0.001$; fig. 4C), respectively.

Leukocyte Yield and Activation State

The device produced a global leukocyte recovery rate of 93.0% (90.1 to 95.7%) with significantly higher leukocyte yield during the second cycle compared to the first cycle (12.4% [7.0 to 13.6%]; $P < 0.001$; fig. 2). Leukocyte counts in the pretreated blood were 2.3×10^9/l (1.9 to 2.7×10^9/l) and 2.3×10^9/l (1.9 to 2.6×10^9/l) for the first and second cycles, respectively. Leukocyte counts in the posttreated blood were 8.3×10^9/l (6.6 to 9.4×10^9/l) and 8.2×10^9/l (6.5 to 9.3×10^9/l) for the first and second cycles, respectively.

Leukocyte viability in pretreated blood was 97.6% (97.0 to 98.5%) for the first cycle and 97.7% (97.3 to 98.5%) for the second cycle. Regarding basal activation of leukocytes in the pretreated blood, respectively, for the first and second cycles: the percentages of HLA-DR positive/CD4-positive cells were 4.4% (3.1 to 6.2%) and 4.4% (3.2 to 6.0%); the percentages of HLA-DR positive/CD8-positive cells were 13.2% (5.5 to 16.3%) and 12.7% (5.2 to 17.5%); CD64 surface expression levels on neutrophils were 1,420 (1,257 to 1,600) and 1,463 (1,297 to 1,721); and CD64 surface expression levels on monocytes were 11,122 (8,900 to 13,157) and 12,219 (9,408 to 14,536).

Blood processing through the device was not associated with leukocyte cell death, as demonstrated by flow cytometric measurement of leukocyte viability in posttreated compared to pretreated blood (0.5% [−0.1 to 1.0%], $P = 0.096$ for the first cycle; 0.4% [−0.1 to 0.6%], $P = 0.281$ for the second cycle). Likewise, cell recovery was not associated with significant leukocyte activation, either regarding CD4-positive cells (−0.1% [−1.2 to 0.7%], $P = 0.535$ for the first cycle; 0.0% [−1.2 to 0.7%], $P = 0.620$ for the second cycle) or CD8-positive cells (−3.2% [−4.6 to 2.2%]; $P = 0.443$ for the first cycle; −2.0% [−4.6 to 1.8%], $P = 0.406$ for the second cycle). Last, blood treatment did not induce any significant increase in CD64 surface expression in posttreated compared to pretreated blood for neutrophils (8 [−119 to 203], $P = 0.701$ for the first cycle; −30 [−238 to 166], $P = 0.635$ for the second cycle) or monocytes (1,905 [−441 to 3,522], $P = 0.125$ for the first cycle; 794 [−1,443 to 3,829], $P = 0.427$ for the second cycle).

Washout Quality

The i-SEP device exhibited a high heparin washing capacity, demonstrated by a global heparin removal ratio of 98.3% (97.8 to 98.9%), despite very high median heparin concentration in the pretreated blood of 11.7 U/ml (11.0 to 13.3 U/ml) for the first cycle and 12.2 U/ml (11.2 to 12.8 U/ml) for the second cycle (table 2). Still, the second cycle achieved a better removal of heparin with a final median concentration of 0.2 U/ml (0.1 to 0.4 U/ml) in the treated-blood and a removal ratio of 99.7% (99.6 to 99.9%), compared to the first cycle with a final median concentration of 1.8 U/ml (1.4 to 2.17 U/ml) and a removal ratio of 97.8% (96.8 to 98.5%).

Likewise, a high washing quality of major plasma proteins was obtained, including albumin, immunoglobulins G, complement factor 3, fibrinogen, and coagulation factors II and VII, demonstrated by global removal ratios > 88% as reported in table 2.

Processing Time

The i-SEP device achieved regular and short median processing times of 4.4 min (4.2 to 4.6 min) for the first cycle and 4.4 min (4.2 to 4.7 min) for the second cycle.

Discussion

This study demonstrates the ability of a filtration-based autotransfusion device to recover and wash both red blood cells and platelets from diluted whole human blood with a fast processing time of less than 5 min. Recovery rates and final hematocrit demonstrated a high quality of erythrocyte salvage by the i-SEP device, comparable to commercially available centrifugation-based devices.[7,16,29–31] Because high suction forces greater than −200 mbar during cell salvage are associated with erythrocyte hemolysis, current centrifugation-based devices use standard vacuum levels of approximately −150 mmHg (−200 mbar). However, those levels can be increased up to −300 mmHg (−400 mbar), in manual mode. Although the i-SEP device is intended to be clinically used with standard vacuum levels of −150 mbar, we decided to increase it to −250 mbar to measure hemolysis in a worst-case clinical scenario. Hemolysis was limited and remained far below acceptable levels for packed red blood cells, according to European and U.S. guidelines, of 0.8 and 1%, respectively.[27,32] Measured hemolysis (0.16%) was comparable to fresh packed red blood cells (less than 7 days of storage).[32] Unlike blood storage, the i-SEP device had no impact on erythrocyte deformability and membrane integrity, as demonstrated by ektacytometry and is therefore comparable to current centrifugation-based devices.[19,33,34]

Table 2. Removal Ratios for Heparin and Major Plasma Proteins

	First Cycle			Second Cycle			Global
	Pretreatment	Posttreatment	Removal Ratio	Pretreatment	Posttreatment	Removal Ratio	Removal Ratio
Heparin, U/ml	11.7 (11.0 to 13.3)	1.8 (1.4 to 2.17)*	97.8 (96.8 to 98.5)	12.2 (11.2 to 12.8)	0.2 (< 0.1 to 0.4)*	99.7 (99.6 to 99.9)	98.3 (97.8 to 98.9)
Albumin, g/l	9.0 (7.9 to 10)	1.5 (0.7 to 1.9)*	97.9 (96.6 to 98.8)	9.3 (8.0 to 10.7)	0.7 (0.2 to 1.1)*	98.8 (97.9 to 99.7)	98.2 (96.9 to 98.8)
Immunoglobulin G, g/l	2.2 (1.8 to 2.9)	0.4 (0.3 to 0.5)*	97.6 (96.7 to 98.7)	2.2 (1.9 to 2.7)	0.1 (0.1 to 0.3)*	99.2 (98.2 to 99.4)	98.1 (97.0 to 98.8)
Complement component 3, g/l	0.25 (0.19 to 0.29)	All values < 0.18*	> 88.4 (86.2 to 90.6)	0.23 (0.19 to 0.29)	All values < 0.18*	> 87.3 (85.4 to 89.6)	> 87.6 (87.0 to 89.4)
Fibrinogen, g/l	0.60 (< 0.4 to 0.69)	All values < 0.4*	> 88.7 (87.3 to 91.2)	0.59 (< 0.4 to 0.64)	All values < 0.4*	> 88.5 (84.8 to 90.7)	> 88.3 (86.6 to 90.7)
Factor II, U/ml	19.5 (16.0 to 24.0)	All values < 10*	> 92.7 (90.9 to 93.4)	20.0 (17.0 to 23.3)	All values < 10*	> 92.3 (91.4 to 93.1)	> 92.4 (91.6 to 93.0)
Factor VII, U/ml	26.5 (23.0 to 30.3)	All values < 10*	> 94.3 (92.7 to 95.7)	27.0 (22.8 to 32.5)	All values < 10*	> 94.4 (92.7 to 95.6)	> 94.3 (92.5 to 95.3)

Blood parameters are expressed as medians and interquartile ranges 25 to 75%. Removal ratios (%) are expressed as medians and interquartile ranges 25 to 75%. Statistical analyses use limit-of-quantification values as substitute for values inferior to the limit of quantification (N = 30).

*$P < 0.001$ *versus* pretreatment.

Platelet recovery is a major innovative feature of the i-SEP autotransfusion device. Overall, the processing of a median 478 ml of whole blood by the device allowed the salvage of 28.7×10^9 platelets, which exceeds minimum platelet content requirement for 1 unit equivalent of platelet concentrate (single-donor whole blood-derived platelet or one sixth of single donor apheresis platelet concentrate). Additionally, recovered platelet function was not altered by the device, as demonstrated by limited platelet activation and strong response to thrombin pathway stimulation. Still, the number of P-selectin positive platelets in the treated blood remained greatly inferior to that of blood bank platelet concentrates, including 1-day storage concentrates (5 to 25% of P-selectin–positive platelets).[35,36] It should be noted that mean platelet GpIb and GpIIb surface expression was inferior to reference values in adult.[37] Blood processing by the i-Sep device cannot be accounted for with this phenomenon, because it was already observed in the pretreated blood. It could rather be explained by platelet activation and glycoprotein shedding during the initial steps of the study, including blood storage, blood dilution with normal saline, and blood suction–induced shear stress, as previously described.[38–40] During blood processing by the i-SEP device, the loss of platelets is probably multifactorial and might involve platelet activation induced by inflammation or shear stress, mechanical destruction during suction and processing, and platelet adhesion to tubing and filtering membranes. We can hypothesize that some mechanisms may be saturable, thanks to tubing and membrane pacification, and will allow for improvement of platelet yield in future device developments. Although centrifugation-based devices theoretically remove platelets from salvaged blood, studies demonstrated that small amounts of platelets remained in the treated blood; however, these platelets were not evaluated in terms of function and activation state.[15] Compared to these centrifugation-based devices, the i-SEP device demonstrated a 6- to 7-fold higher platelet yield. Overall, these results allow further clinical evaluation of the potential benefits of platelet recovery, because we can now hypothesize (1) that the device might decrease perioperative bleeding in the setting of nonmassive surgical bleeding for which platelet transfusion is unlikely and (2) that the processing of undiluted shed blood by the i-SEP device might be sufficient to decrease or overcome the need for platelet transfusion in the setting of massive surgical bleeding.

Although centrifugation-based devices theoretically remove leukocytes from salvaged blood, several studies demonstrated that a significant amount of white blood cells remained in the treated blood, with recovery rates between 27 and 81%.[15,16,29,41] This represents a major concern because leukocyte damage and activation can occur during centrifugation and washing and might induce a systemic inflammatory response.[41–43] We therefore evaluated the impact of i-SEP processing on leukocytes and demonstrated that filtration-based cell salvage and washing did not induce significant leukocyte cell death or activation.

Compared to the first cycle, the second cycle of treatment was steadily associated with a significantly higher erythrocyte, platelet, and leukocyte yield, with second cycle recovery rates sometimes above 100%. This phenomenon can be entirely explained by the fact that a substantial amount of treated blood from first cycle is staying in the i-SEP device circuit and is only released at the end of the second cycle.

Regarding washing quality, the i-SEP device achieved high removal ratios of heparin and major plasma proteins (including albumin, immunoglobulins, complement, and coagulation factors), in the same manner as current centrifugation-based devices.[7,15,16,44,45] This considerably reduces the risk of induced coagulopathy and inflammation. The second cycle steadily exhibited better washing quality as compared to the first cycle, essentially as a result of a smaller treated blood volume (700 ml for the first cycle and 500 ml for the second cycle) for the same processing time and washing volume.

Despite a high heparin removal ratio (98.3%), a substantial heparin concentration remained in the treated blood after the first cycle, with a heparin level greater than 0.5 U/ml.

This phenomenon is explained by the choice of the addition of a high final unfractionated heparin concentration of 12 U/ml, considered to be a worst-case operative condition, whereas studies of centrifugation-based devices commonly used lower heparin concentrations of 5 U/ml.[7,15,16] Indeed, during cardiopulmonary bypass in patients, the heparin concentration in circulating blood frequently exceeds 5 U/ml.[46,47] Also, accidental overheparinization of salvaged blood can occur if the heparinized saline drip is unintentionally increased in the dual-lumen suction tip. In these settings, heparin removal by cell savers might be insufficient to prevent significant heparin reinfusion by commercially available devices in the clinical operative setting, despite high heparin removal ratios.[45] The recently developed cell salvage system HemoSep (Brightwake, United Kingdom) greatly differs from i-SEP device by producing blood cell filtration, without washing, using long processing time (more than 15 min), and was therefore not included in this discussion.[48,49]

A few points have to be considered to evaluate the clinical relevance of our results. First, this study was conducted using diluted whole human blood units, and the results might therefore not be generalized to the clinical setting. Hence, the study was not designed to evaluate clinical efficacy and safety of the device. Multiple factors might indeed interfere with the filtration process during perioperative use, including preexistent coagulopathy, drug-induced platelet dysfunction, cardiopulmonary bypass, suction-induced hemolysis, or systemic inflammatory response. Second, given the nature of posttreated blood cell concentrate, composed only of blood cells and traces of proteins suspended in normal saline, functional analysis of platelets using aggregometry, although considered as the reference test, was impossible. We therefore used thrombin receptor stimulation in combination with flow cytometric analysis as a surrogate for the platelet function test.

This study reports the performance evaluation of a filtration-based autotransfusion device, able to simultaneously recover and wash human platelets and red blood cells. It also provides a detailed cytometric analysis of salvaged platelet and leukocyte viability and activation state. With a fast processing time of less than 5 min, the device was able to recover 88% of red blood cells with minimal hemolysis and without inducing alteration in membrane integrity and deformability. The device achieved 37% of platelet recovery with minimal platelet activation while maintaining platelet ability to be activated by thrombin receptor–activating peptide. The washing process allowed high heparin and plasma protein removal ratios. Together, these results demonstrate the *in vitro* performance of i-SEP new autotransfusion technology. Future trials will be necessary to assess the clinical efficacy and safety of the device.

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Competing Interests

Dr. Decouture is currently employed as project manager by i-SEP (Nantes, France). Dr. Skreko is currently employed as research and development engineer by i-SEP. Dr. Ouattara received expertise fees from i-SEP. Dr. Bachelot-Loza received expertise fees from i-SEP. Dr. Gaussem received expertise fees from Aspen France (Rueil Malmaison, France) and i-SEP. The other authors declare no competing interests.

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References

1. Klein AA, Bailey CR, Charlton AJ, Evans E, Guckian-Fisher M, McCrossan R, Nimmo AF, Payne S, Shreeve K, Smith J, Torella F: Association of Anaesthetists guidelines: Cell salvage for peri-operative blood conservation 2018. Anaesthesia 2018; 73:1141–50
2. Frank SM, Sikorski RA, Konig G, Tsilimigras DI, Hartmann J, Popovsky MA, Pawlik TM, Waters JH: Clinical utility of autologous salvaged blood: A review. J Gastrointest Surg 2020; 24:464–72
3. Boer C, Meesters MI, Milojevic M, Benedetto U, Bolliger D, Heymann C von, Jeppsson A, Koster A, Osnabrugge RL, Ranucci M, Ravn HB, Vonk ABA, Wahba A, Pagano D: 2017 EACTS/EACTA Guidelines on patient blood management for adult cardiac surgery. J Cardiothorac Vasc Anesth 2018; 32:88–120
4. Ashworth A, Klein AA: Cell salvage as part of a blood conservation strategy in anaesthesia. Br J Anaesth 2010; 105:401–16
5. Meybohm P, Choorapoikayil S, Wessels A, Herrmann E, Zacharowski K, Spahn DR: Washed cell salvage in surgical patients: A review and meta-analysis of prospective randomized trials under PRISMA. Medicine (Baltimore) 2016; 95:e4490

6. Sikorski RA, Rizkalla NA, Yang WW, Frank SM: Autologous blood salvage in the era of patient blood management. Vox Sang 2017; 112:499–510
7. Seyfried TF, Gruber M, Streithoff F, Mandle RJ, Pawlik MT, Busse H, Hansen E: The impact of bowl size, program setup, and blood hematocrit on the performance of a discontinuous autotransfusion system. Transfusion 2017; 57:589–98
8. Al-Riyami AZ, Al-Khabori M, Baskaran B, Siddiqi M, Al-Sabti H: Intra-operative cell salvage in cardiac surgery may increase platelet transfusion requirements: A cohort study. Vox Sang 2015; 109:280–6
9. Holm M, Biancari F, Khodabandeh S, Gherli R, Airaksinen J, Mariscalco G, Gatti G, Reichart D, Onorati F, De Feo M, Santarpino G, Rubino AS, Maselli D, Santini F, Nicolini F, Zanobini M, Kinnunen EM, Ruggieri VG, Perrotti A, Rosato S, Dalén M: Bleeding in patients treated with ticagrelor or clopidogrel before coronary artery bypass grafting. Ann Thorac Surg 2019; 107:1690–8
10. Hansson EC, Jidéus L, Åberg B, Bjursten H, Dreifaldt M, Holmgren A, Ivert T, Nozohoor S, Barbu M, Svedjeholm R, Jeppsson A: Coronary artery bypass grafting-related bleeding complications in patients treated with ticagrelor or clopidogrel: A nationwide study. Eur Heart J 2016; 37:189–97
11. Nammas W, Dalén M, Rosato S, Gherli R, Reichart D, Gatti G, Onorati F, Faggian G, De Feo M, Bancone C, Chocron S, Khodabandeh S, Santarpino G, Rubino AS, Maselli D, Nardella S, Salsano A, Gherli T, Nicolini F, Zanobini M, Saccocci M, Bounader K, D'Errigo P, Kiviniemi T, Kinnunen EM, Perrotti A, Airaksinen J, Mariscalco G, Ruggieri VG, Biancari F: Impact of preoperative thrombocytopenia on the outcome after coronary artery bypass grafting. Platelets 2019; 30:480–6
12. Raphael J, Mazer CD, Subramani S, Schroeder A, Abdalla M, Ferreira R, Roman PE, Patel N, Welsby I, Greilich PE, Harvey R, Ranucci M, Heller LB, Boer C, Wilkey A, Hill SE, Nuttall GA, Palvadi RR, Patel PA, Wilkey B, Gaitan B, Hill SS, Kwak J, Klick J, Bollen BA, Shore-Lesserson L, Abernathy J, Schwann N, Lau WT: Society of Cardiovascular Anesthesiologists clinical practice improvement advisory for management of perioperative bleeding and hemostasis in cardiac surgery patients. Anesth Analg 2019; 129:1209–21
13. Hout FMA van, Hogervorst EK, Rosseel PMJ, Bom JG van der, Bentala M, Dorp ELA van, Geloven N van, Brand A, Meer NJM van der, Watering LMG van de: Does a platelet transfusion independently affect bleeding and adverse outcomes in cardiac surgery? Anesthesiology 2017; 126:441–9
14. Ming Y, Liu J, Zhang F, Chen C, Zhou L, Du L, Yan M: Transfusion of red blood cells, fresh frozen plasma, or platelets is associated with mortality and infection after cardiac surgery in a dose-dependent manner. Anesth Analg 2020; 130:488–97
15. Lindau S, Kohlhaas M, Nosch M, Choorapoikayil S, Zacharowski K, Meybohm P: Cell salvage using the continuous autotransfusion device CATSmart: An observational bicenter technical evaluation. BMC Anesthesiol 2018; 18:189
16. Overdevest EP, Lanen PW, Feron JC, van Hees JW, Tan ME: Clinical evaluation of the Sorin Xtra® autotransfusion system. Perfusion 2012; 27:278–83
17. Vieira SD, da Cunha Vieira Perini F, de Sousa LCB, Buffolo E, Chaccur P, Arrais M, Jatene FB: Autologous blood salvage in cardiac surgery: Clinical evaluation, efficacy and levels of residual heparin. Hematol Transfus Cell Ther 2021; 43:1–8
18. Llaudet-Planas E, Vives-Corrons JL, Rizzuto V, Gómez-Ramírez P, Sevilla Navarro J, Coll Sibina MT, García-Bernal M, Ruiz Llobet A, Badell I, Velasco-Puyó P, Dapena JL, Mañú-Pereira MM: Osmotic gradient ektacytometry: A valuable screening test for hereditary spherocytosis and other red blood cell membrane disorders. Int J Lab Hematol 2018; 40:94–102
19. Salaria ON, Barodka VM, Hogue CW, Berkowitz DE, Ness PM, Wasey JO, Frank SM: Impaired red blood cell deformability after transfusion of stored allogeneic blood but not autologous salvaged blood in cardiac surgery patients. Anesth Analg 2014; 118:1179–87
20. Parrow NL, Violet P-C, Tu H, Nichols J, Pittman CA, Fitzhugh C, Fleming RE, Mohandas N, Tisdale JF, Levine M: Measuring deformability and red cell heterogeneity in blood by ektacytometry. J Vis Exp 2018; 131:56910
21. Xu Z, Zheng Y, Wang X, Shehata N, Wang C, Sun Y: Stiffness increase of red blood cells during storage. Microsystems Nanoeng 2018; 4:17103
22. Goodall AH, Appleby J: Flow-cytometric analysis of platelet-membrane glycoprotein expression and platelet activation, Platelets and Megakaryocytes, Volume 1. Functional Assays. Edited by Gibbins JM, Mahaut-Smith MP. Clifton, New Jersey, Humana Press, 2004, pp 225–53
23. Pasalic L, Pennings GJ, Connor D, Campbell H, Kritharides L, Chen VM: Flow cytometry protocols for assessment of platelet function in whole blood. Methods Mol Biol 2017; 1646:369–89
24. Shenkin M, Babu R, Maiese R: Accurate assessment of cell count and viability with a flow cytometer. Cytometry B Clin Cytom 2007; 72:427–32
25. Sack U: CD64 expression by neutrophil granulocytes. Cytometry B Clin Cytom 2017; 92:189–91
26. Caruso A, Licenziati S, Corulli M, Canaris AD, De Francesco MA, Fiorentini S, Peroni L, Fallacara F, Dima F, Balsari A, Turano A: Flow cytometric analysis of activation markers on stimulated T cells and their correlation with cell proliferation. Cytometry 1997; 27:71–6

27. European Directorate for the Quality of Medicines and HealthCare of the Council of Europe (EDQM): Guide to the Preparation, Use and Quality Assurance of Blood Components, 20th edition. Strasbourg, France, Council of Europe Publishing, 2020
28. Agence Nationale de Sécurité du Médicament et des produits de santé: Avis aux demandeurs. Evaluation des produits sanguins labiles, version 1.1. 2012
29. Alberts M, Groom RC, Walczak R Jr, Kramer R, Karpiel A, Dieter J, Sheth L, Greene NH, Jooste EH: *In vitro* evaluation of the Fresenius Kabi CATSmart autotransfusion system. J Extra Corpor Technol 2017; 49:107–11
30. Naumenko KS, Kim SF, Cherkanova MS, Naumenko SE: The Haemonetics® Cell Saver 5 washing properties: Effect of different washing pump and centrifuge speeds. Interact Cardiovasc Thorac Surg 2008; 7:759–63
31. Wang X, Ji B, Zhang Y, Zhu X, Liu J, Long C, Zheng Z: Comparison of the effects of three cell saver devices on erythrocyte function during cardiopulmonary bypass procedure–a pilot study. Artif Organs 2012; 36:931–5
32. Makroo RN, Raina V, Bhatia A, Gupta R, Majid A, Thakur UK, Rosamma NL: Evaluation of the red cell hemolysis in packed red cells during processing and storage. Asian J Transfus Sci 2011; 5:15–7
33. Frank SM, Abazyan B, Ono M, Hogue CW, Cohen DB, Berkowitz DE, Ness PM, Barodka VM: Decreased erythrocyte deformability after transfusion and the effects of erythrocyte storage duration. Anesth Analg 2013; 116:975–81
34. Gu YJ, Vermeijden WJ, de Vries AJ, Hagenaars JA, 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; 86:1570–5
35. Bontekoe IJ, van der Meer PF, Verhoeven AJ, de Korte D: Platelet storage properties are associated with donor age: *In vitro* quality of platelets from young donors and older donors with and without type 2 diabetes. Vox Sang 2019; 114:129–36
36. Sperling S, Vinholt PJ, Sprogøe U, Yazer MH, Frederiksen H, Nielsen C: The effects of storage on platelet function in different blood products. Hematology 2019; 24:89–96
37. Hézard N, Potron G, Schlegel N, Amory C, Leroux B, Nguyen P: Unexpected persistence of platelet hyporeactivity beyond the neonatal period: A flow cytometric study in neonates, infants and older children. Thromb Haemost 2003; 90:116–23
38. Chen Z, Mondal NK, Ding J, Koenig SC, Slaughter MS, Griffith BP, Wu ZJ: Activation and shedding of platelet glycoprotein IIb/IIIa under non-physiological shear stress. Mol Cell Biochem 2015; 409:93–101
39. Hosseini E, Mohtashami M, Ghasemzadeh M: Down-regulation of platelet adhesion receptors is a controlling mechanism of thrombosis, while also affecting post-transfusion efficacy of stored platelets. Thrombosis J 2019; 17:20
40. Bender M, Stegner D, Nieswandt B: Model systems for platelet receptor shedding. Platelets 2017; 28:325–32
41. Cross MH: Cell salvage and leucodepletion. Perfusion 2001; 16:61–6
42. Connall TP, Zhang J, Vaziri ND, Kaupke CJ, Wilson SE: Leukocyte CD11b and CD18 expression are increased in blood salvaged for autotransfusion. Am Surg 1994; 60:797–800
43. Kirkpatrick UJ, Adams RA, Lardi A, McCollum CN: Rheological properties and function of blood cells in stored bank blood and salvaged blood. Br J Haematol 1998; 101:364–8
44. Rougé P, Fourquet D, Depoix-Joseph JP, Nguyen F, Barthélémy R: Heparin removal in three intraoperative blood savers in cardiac surgery. Appl Cardiopulm Pathophysiol 1993; 5:5–8
45. Buys WF, Buys M, Levin AI: Reinfusate heparin concentrations produced by two autotransfusion systems. J Cardiothorac Vasc Anesth 2017; 31:90–8
46. Lennon MJ, Thackray NM, Gibbs NM: Anti-factor Xa monitoring of anticoagulation during cardiopulmonary bypass in a patient with antiphospholipid syndrome. Anaesth Intensive Care 2003; 31:95–8
47. Hellstern P, Bach J, Simon M, Saggau W: Heparin monitoring during cardiopulmonary bypass surgery using the one-step point-of-care whole blood anti-factor-Xa clotting assay heptest-POC-Hi. J Extra Corpor Technol 2007; 39:81–6
48. Gunaydin S, Gourlay T: Novel ultrafiltration technique for blood conservation in cardiac operations. Ann Thorac Surg 2013; 95:2148–51
49. Hogan M, Needham A, Ortmann E, Bottrill F, Collier TJ, Besser MW, Klein AA: Haemoconcentration of residual cardiopulmonary bypass blood using Hemosep®: A randomised controlled trial. Anaesthesia 2015; 70:563–70