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Original Articles

Effect of Partial-Filling Autotransfusion Bowls on the Quality of Reinfused Product

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Abstract: Intraoperative autotransfusion is used in a variety of surgical procedures with the quantity of blood loss dependent upon numerous factors. These procedures may or may not produce a full autotransfusion bowl. The inadequate removal of contaminants has been correlated to the incomplete filling of bowls, resulting in a condition called "Salvaged Blood Syndrome." The purpose of this study was to assess the quality of aspirated whole blood after processing with an autotransfusion system using various fill volumes and two wash volumes. An in vitro circuit was designed to mimic the mechanical effects of extracorporeal flow on blood. Twenty-four Baylor-style bowls were filled at 400 mL min^{-1} and washed at 300 mL min^{-1}. Two wash volumes, 1000 and 2000 mL, and three bowl volumes; low, mid, and full, were used in this study. The bowl volumes were determined by using red cell quantities of 60, 100, and 135 mL for the low-fill, mid-fill, and full bowls, respectively. Samples were drawn pre-autotransfusion and post-autotransfusion and analyzed for plasma-free hemoglobin, IL-8, white blood cell count, platelet count, albumin, and total protein. All data were analyzed using one-way analysis of variance (ANOVA) with significance accepted at $p \geq .05$. Plasma-free hemoglobin levels and hematocrit were concentrated significantly ($p < .05$) as bowl volume increased. A significant difference in IL-8 levels was found in the wash volumes in the low-fill bowls ($p < .02$). Platelet count was significantly decreased between the full bowl with 1000 mL wash and the full bowl with 2000 mL wash ($p < .0004$). Total protein reduction was significantly less in the low-fill bowl with 1000 mL wash as compared to the other bowl treatments ($p < .05$). In conclusion, the quality of the washed product did not vary significantly between fill or wash volumes, with the exception of the low-fill bowl with 1000 mL wash. **Keywords:** partial-filled bowls, platelets, white blood cells, IL-8. *JECT. 2001;33:80–85*

Despite advances in blood bank technology in screening for transmittable diseases, the general public remains hesitant to accept homologous sources of blood. One of the alternatives, autotransfusion, has continued to maintain a strong foothold in surgical centers worldwide. The benefits of washing salvaged blood with a centrifuge have been proved in various surgical procedures where significant blood loss is expected (1). These procedures include cardiac, vascular, and orthopedic surgery, as well as certain trauma situations. However, additional surgical patients may benefit from the utilization of intraoperative autotransfusion.

The quality, and ultimate benefit, of infusing blood products is dependent upon a number of factors. These include the health of the donor from which the blood was taken, the collection, processing, and storage of the product, and the infusion technique to the patient. During autotransfusion, the quality of the reinfused product is directly related to mechanical forces, such as air-to-blood interface (2), suction and blood-to-foreign surface contact (3) involved in the collection and processing sequence. As autotransfusion is increasingly utilized in such surgeries as orthopedic and neurosurgery, a concern with autotransfusion is the effect of partially filled bowls on the quality of

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the end-product. When autotransfusion bowls are partially filled, inadequate washing of the red blood cells occurs (4–6). This may reduce the quality of the product and limit the over-all benefits of autotransfusion. The purpose of this study was to assess the quality of aspirated whole blood after processing with an automated cell-washing autotransfusion system at various fill conditions and two wash volumes.

METHODS

Experimental Circuit

An in vitro circuit was prepared to mimic the mechanical effects of extracorporeal blood salvage. A previously reported model (2) was modified to incorporate a Baylor-style bowl (COBE BRAT 2® Autologous Transfusion System, COBE Laboratories, Arvada, CO). This cell activation model was composed of recirculation, aspiration, and autotransfusion circuits (Figure 1). The extracorporeal circuit was constructed using a 20-L reservoir, ¼-inch polyvinyl chloride (PVC) tubing, and two positive displacement twin roller pumps.

Recirculation Circuit

The recirculation portion of the circuit was composed of a 20-L reservoir connected to the blood source twin roller pump (RP #1) (Sorin Stockert, Sorin Biomedical, Irvine, CA). The outlet of RP #1 was wyed to the recirculation line, which was connected to the top of the 20-L reservoir. This circuit ran continuously throughout the study.

Aspiration Circuit

The aspiration portion of the circuit was composed of a second twin roller pump (RP #2) with dual shims, ¼-inch PVC tubing, two cardiotomies (COBE Laboratories, Arvada, CO), and rigid intracardiac suckers with fluted tips. RP #2 was used to pump blood from the recirculation circuit, at the wye distal to RP #1, into one of the cardiotomies. Within the cardiotomy, the intracardiac sucker was positioned so that only 1 cm of the tip was submerged in the blood. RP #2 was used to aspirate blood from the cardiotomy through the intracardiac sucker and then to transfer it back into a filtered port on the cardiotomy reservior.

Autotransfusion Circuit

The autotransfusion system was connected to a wye connector at the outlet of the cardiotomy. Two sample ports were inserted: pre-autotransfusion (sample port A) and post-autotransfusion (sample port B). To keep the desired levels consistent, the tally screen was utilized when filling the bowls.

The entire circuit was primed with buffered physiologic saline solution. To the prime, 5000 units of bovine lung heparin were added. Sixteen units of less than 3-day-old CPDA-1 whole human blood were obtained from Interstate Blood Bank, Inc., Memphis, TN. To simulate clinical conditions, the blood source starting hematocrit was 23 ± 2%. Acid-base balance was maintained with a pH of 7.40 ± 0.05, and base excess at 0 ± 5 mEq. Blood temperature was maintained at 22 ± 2°C.

Experimental Protocol

The blood source pump (RP #1) was set at 1 L min^{-1}, and the second roller pump (RP #2) was held constant at

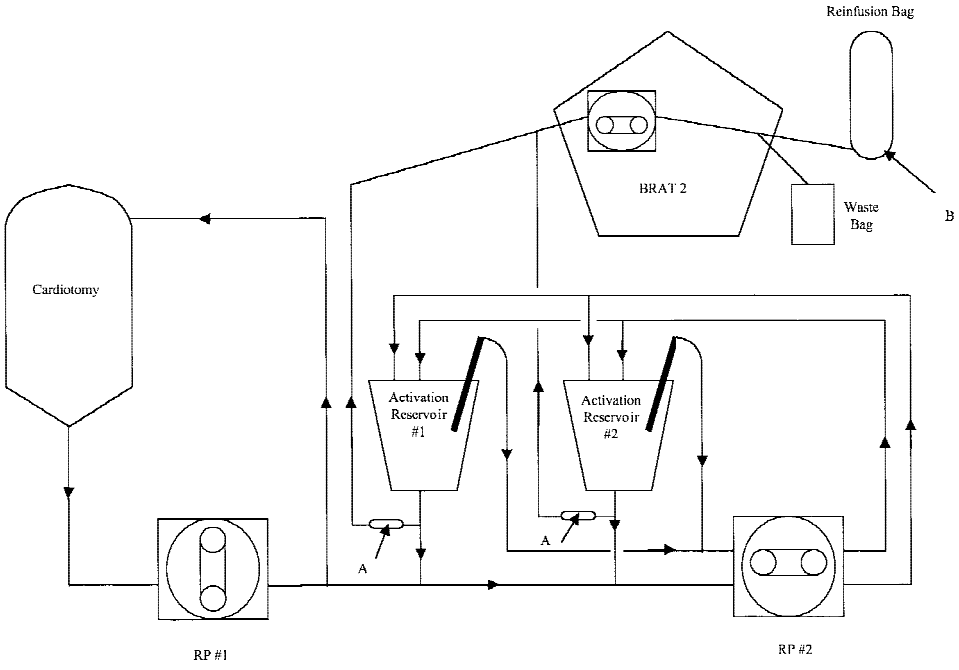

Figure 1. Experimental circuit. A: Sample port pre-autotransfusion; B: Sample port post-autotransfusion.

500 mL min^{-1}. The autotransfusion line was clamped off until sampling began. The blood was run through one of the two aspiration circuits for 1 h to activate the blood. After this time, that aspiration circuit was clamped out while the autotransfusion line was unclamped to allow sampling and to fill the Baylor bowl. To simulate clinical conditions, the fill rate of the autotransfusion roller pump was maintained at 400 mL min^{-1}, and the wash rate was 300 mL min^{-1} throughout the entire study.

A total of six experiments, using 24 (250 mL) bowl sets, were performed in the manual program mode, with the centrifuge running at 4400 rpm. Three bowl volumes and two wash volumes, 1000 mL and 2000 mL, were used. To determine the fill volume for the low-fill bowl, the bowl was filled until a visual spillover of plasma was noted. The mid-fill bowl volume was determined mathematically to be the mean difference between the low-fill and full bowl volumes. The order in which the fill volumes were processed was randomized.

LABORATORY ANALYSIS

Samples were drawn at two points. The first sample was drawn at the outlet of the cardiotomy reservoir before the autotransfusion device (sample port A) to establish baseline values. The second sample was drawn from the washed product (sample port B) in the re-infusion bag. The samples were assayed for plasma-free hemoglobin, IL-8, white blood cells, platelet count, albumin, total protein, and hematocrit. Laboratory analyses were performed by NHS Clinical Laboratory. For the IL-8 ELISA (enzyme-linked immunoassay) (Cytoscreen, Biosource International, Camarillo, CA) test, blood samples were drawn into laboratory red top tubes and stored on ice. The blood samples were centrifuged at 4000 rpm for 15 min at 4°C. The plasma was stored for up to 60 days at –20°C in 2-mL aliquots until the ELISAs could be completed.

Statistical Analysis

All data were loaded onto a personal computer in a spreadsheet format. The data were then analyzed using a one-way ANOVA. Significant differences ($p \leq .05$) were further evaluated with the use of a multiple comparison test (Fisher's least-significant difference). Statistical significance was accepted at $p \leq .05$ level. All data were expressed as mean ± SD of the mean.

RESULTS

The adequacy of the removal of the measured variables from the washed red blood cells was determined by evaluating the percentage change between baseline and washed product raw data (Table 1). The percentage changes of plasma free hemoglobin, hematocrit, IL-8, white blood cell count, platelet count, albumin, and total protein were compared between experiments. Figure 2 shows the percentage change in plasma-free hemoglobin and hematocrit. Plasma-free hemoglobin levels significantly increased as the fill volume increased. No significance was found between wash volumes at the same fill volume. The percentage change in hematocrit also significantly increased as the bowl volume increased.

Figure 3 shows the percentage change in IL-8 and white blood cell count. There was no significance found in IL-8 levels between fill volumes. However, significance was found in IL-8 levels between 1000 mL wash and 2000 mL wash. No significance was found in the white blood cell count between wash volumes or between fill volumes. However, it was trending toward a concentrating effect as bowl volume increased.

Table 1. Baseline and washed product raw data.

	PFHb (mg/dL)	IL-8 (pcg/mL)	WBC (10^3/mcL)	Platelet Count (10^3/mcL)	Albumin (g/dL)	Total Protein (g/dL)	HCT (%)
Low-fill 1000 mL							
Baseline	19.20 ± 2.07	106.95 ± 21.25	0.83 ± 0.23	37.00 ± 18.17	1.45 ± 0.06	2.30 ± 0.92	21.38 ± 0.25
Washed product	30.3 ± 9.89	116.50 ± 77.83	0.70 ± 0.17	33.50 ± 17.25	0.00 ± 0.00	0.99 ± 0.13	24.67 ± 2.25
Low-fill 2000 mL							
Baseline	24.90 ± 2.20	216.98 ± 113.22	0.65 ± 0.33	72.00 ± 11.34	1.55 ± 0.06	2.97 ± 0.09	21.37 ± 0.48
Washed product	27.90 ± 3.96	78.42 ± 29.39	0.35 ± 0.25	59.50 ± 18.84	0.00 ± 0.00	0.53 ± 0.37	27.13 ± 1.18
Mid-fill 1000 mL							
Baseline	24.00 ± 2.90	247.98 ± 194.89	0.70 ± 0.08	58.00 ± 21.40	1.53 ± 0.10	2.94 ± 0.14	21.63 ± 0.63
Washed product	43.10 ± 11.30	163.00 ± 102.98	0.85 ± 0.30	74.00 ± 27.98	0.00 ± 0.00	0.73 ± 0.23	38.00 ± 8.40
Mid-fill 2000 mL							
Baseline	21.90 ± 1.83	164.27 ± 63.77	0.70 ± 0.12	61.00 ± 23.66	1.55 ± 0.06	3.10 ± 0.08	22.38 ± 1.44
Washed product	55.80 ± 29.48	101.64 ± 28.61	0.85 ± 0.57	65.75 ± 23.17	0.00 ± 0.00	0.29 ± 0.58	40.33 ± 3.70
Full Bowl 1000 mL							
Baseline	21.60 ± 3.62	179.99 ± 11.65	0.60 ± 0.22	52.00 ± 25.61	1.48 ± 0.10	2.89 ± 0.20	21.25 ± 0.29
Washed product	83.80 ± 16.72	121.92 ± 67.22	1.98 ± 0.21	55.25 ± 20.42	0.00 ± 0.00	0.03 ± 0.05	68.04 ± 12.33
Full Bowl 2000 mL							
Baseline	26.40 ± 3.50	321.88 ± 182.45	0.70 ± 0.35	77.25 ± 11.44	1.58 ± 0.05	2.99 ± 0.03	21.50 ± 0.71
Washed product	82.60 ± 19.01	191.93 ± 77.11	2.00 ± 0.55	53.00 ± 4.76	0.00 ± 0.00	0.00 ± 0.00	77.88 ± 10.31

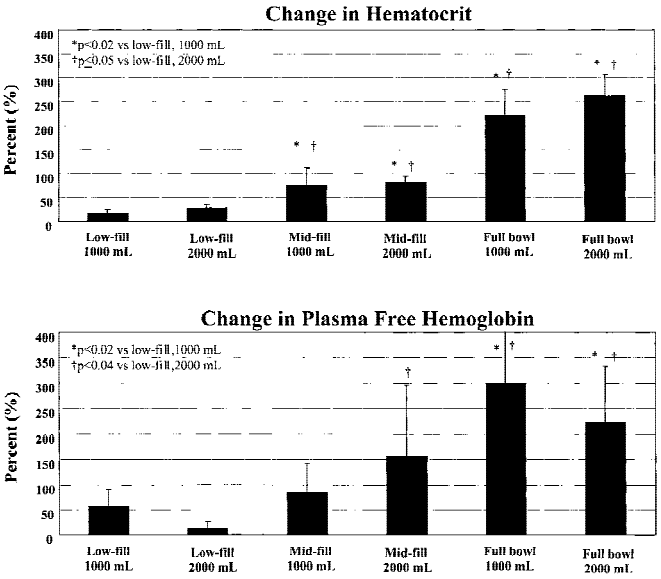

Figure 2. Data expressed as percentage change from baseline.

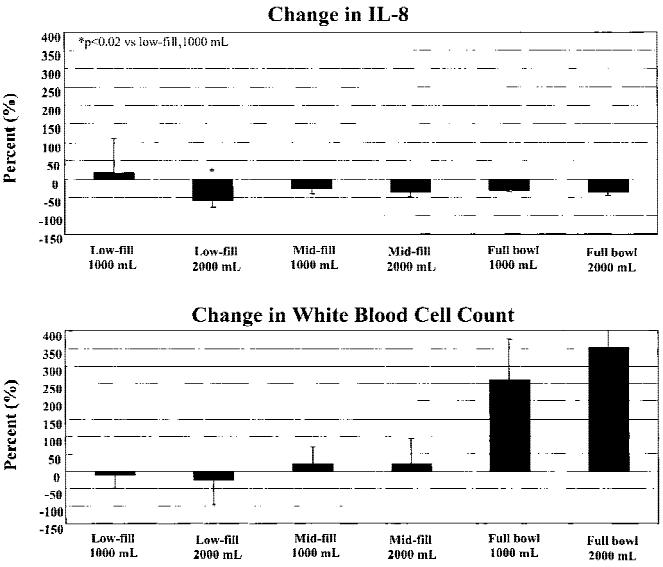

Figure 3. Data expressed as percentage change from baseline.

No significance was found in the percentage change of platelet count between the wash volumes in the low-fill and mid-fill bowls. However, it was found that the full bowl with 1000 mL wash significantly removed fewer platelets (12.26 ± 16.73%) than with 2000 mL wash (–30.33 ± 11.77%, $p < .0004$). It was also found that the mid-fill bowls did not reduce platelets as well as low-fill bowls with 1000 mL wash ($p < .05$) or full bowls with 2000 mL wash ($p < .0001$).

Figure 4 shows the percentage change in albumin and total protein. Albumin was completely removed in all fill volumes and wash volumes. Total protein was reduced significantly less in the low-fill bowl with 1000 mL wash than the low-fill bowl with 2000 mL wash. In the remaining bowl treatments, no difference was observed in total protein removal.

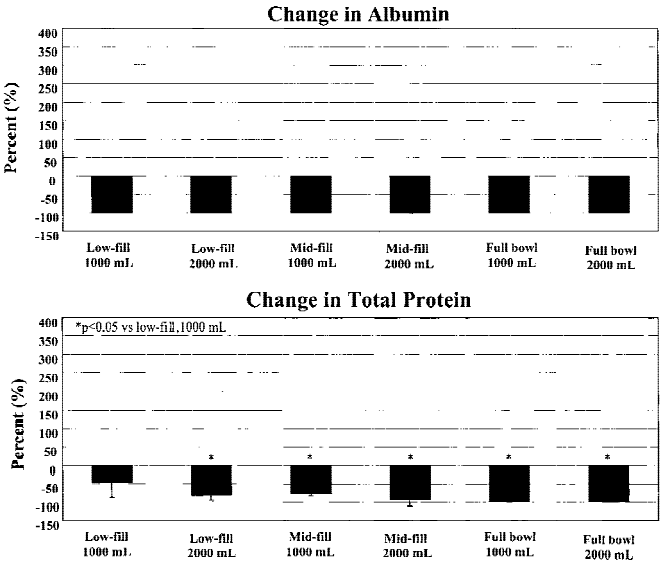

Figure 4. Data expressed as percentage change from baseline.

DISCUSSION

Although autotransfusion has reduced patient dependency on autogeneic transfusions, the costs associated with this technology have spurred a re-evaluation of its utilization. Today, a unit of packed red blood cells in the United States costs around $113 to administer; however, this does not take into consideration the hidden costs of transfusions, which include screening, handling costs, the risk of infectious disease transmission, and inflammation from tissue activation (7).

Autotransfusion, therefore, has become an attractive alternative. The collection of shed blood with direct reinfusion, using a gross transfusion filter, is termed whole blood autotransfusion. This process returns all components of aspirated blood along with activated coagulation factors, solutions, medications, debris, and activated white blood cells and platelets. The activation of these factors occurs when they interact with subendothelial surfaces, foreign synthetic material, and the blood-to-air interfaces (3) of extracorporeal collection. When reinfused, these substances can induce an inflammatory response, affecting the pulmonary system, kidney and splanchnic circulation, cerebral circulation, and cardiac function (8). Activation of platelets results in their adherence to surfaces and aggregation. As a result, the number of platelets decrease, which may result in increased postoperative bleeding or in severe cases, disseminated intravascular coagulation (4). When activated, white blood cells can behave in three ways. First, the activated cells become adherent to the vascular endothelium, which may result in organ dysfunc-

tion (9). Second, white blood cells can be stimulated to secrete such chemotoxins as oxygen-free radicals and proteolytic enzymes (10,11). Last, the clumped white blood cells can break apart, which may cause reperfusion injury (8). The effects on the body to these three mechanisms involve all the microvasculature and organ beds. For these reasons, further processing of the collected blood becomes necessary.

An adjunctive technique for whole blood autotransfusion is the use of automated systems that incorporate centrifugation and cell washing. This process removes plasma proteins, platelets, clotting factors, as well as such toxic products as plasma-free hemoglobin and activators of white and red blood cells (12). However, several studies by Bull and associates have shown that platelets and leukocytes are concentrated and form a deposit on the inner surface of the bowl (4–6). These findings have resulted in a closer scrutiny of the quality of washed blood products.

Autologous cell washing is used in several operative procedures where the surgical procedure and technique influence the quantity of blood loss. Therefore, there may or may not be enough blood loss to produce a full autotransfusion bowl. The incomplete filling of autotransfusion bowls has been correlated to the inadequate removal of contaminants, resulting in a condition called "Salvaged Blood Syndrome." This condition occurs with the reinfusion of red cell suspensions contaminated with activated leukocytes and platelets (6).

The results of this study showed that the amount of white blood cells in the washed product trended upward as the bowl volumes increased. We hypothesize that white blood cell removal was impaired, because, when activated, they become adherent and form clumps, increasing their density to levels similar to red blood cells. Instead of rising to the top of the bowl, where they can be removed, the clumps of white blood cells are concentrated at the bottom. This study also found that platelets were present in the washed product, but they had been significantly reduced. The platelets remaining in the washed red blood cells may also be attributed to density. New platelets are larger in size than older platelets; thus, they are not as easily removed. In addition, younger platelets are more readily activated than older platelets.

The mechanical forces involved in the collecting and processing sequence directly affect the quality of the reinfused product. Suction creates a large air-to-blood interface, which then causes red cell activation, hemolysis (13), and the liberation of plasma-free hemoglobin. Plasma-free hemoglobin is removed through the kidneys; therefore, high levels of plasma-free hemoglobin may cause renal dysfunction in older patients and those with renal insufficiency. There is some disagreement on whether plasma-free hemoglobin is removed through cell washing. A study by Walpoth et al. found that plasma-free hemoglobin was removed when the Fresenius continuous autologous transfusion system (CATS) was utilized (14). However, several other studies have found that washing of salvaged blood using a Latham bowl does not remove plasma-free hemoglobin (15,16). This study found that plasma-free hemoglobin was not removed in any of the bowl treatments. A possible reason for the lack of plasma free hemoglobin removal may also be contributed to similarities in density between red cells and plasma free hemoglobin.

Interleukin-8 (IL-8) is a cytokine, which is a small protein produced and released by monocytes, endothelial cells, macrophages, and fibroblasts. It is released because of the inflammatory response and causes upregulation of neutrophil adhesion molecules, stimulates neutrophil degranulation, and generates oxygen radicals (17). One study found that IL-8 had the lowest elimination rate in the washed cells as compared to the other blood parameters measured (14). Tilg and associates attributed the low elimination rate to IL-8 binding to red blood cells (18). Our results support the theory of IL-8 binding to red blood cells. IL-8 is a protein that weighs 8–10 kD (19), and our results showed significant removal of albumin and total protein, but only a slight reduction in IL-8 levels. Therefore, the remaining IL-8 measured could be attributed to binding to red blood cells.

Future studies should include increasing the sample size, which could allow significance to be reached easier; whereas, in this study, there was only a trend toward significance. Another interesting study improvement would be to compare the removal of the blood parameters between the different bowl configurations at various fill volumes to determine whether processing type influences removal. Also, recreating this study in a clinical setting would be beneficial.

The manufacturers of centrifugal cell washing systems recommend only full bowls should be reinfused into the patient. However, the results of this study found that low-filled bowls significantly reduced the endpoints measured, especially when washed with 2000 mL. In addition, the mid-filled bowls at both wash volumes reduced the parameters measured comparably to full bowls except in platelet removal, where the mid-filled bowls did not decrease the platelet count. There was also no significance found between the two wash volumes in the mid-filled bowls. There was a significant difference in the percentage removal of platelets between wash volumes in the full bowls. Therefore, this study found that the low-fill bowl with 2000 mL wash and both the mid-filled bowls adequately removed most of the endpoints measured in this study.

In conclusion, the quality of the washed product did not vary significantly between fill or wash volumes, with the exception of the low-filled bowl with 1000 mL wash; therefore, the results of the study showed that the reinfusion of

partially filled autotransfusion bowls can be recommended.

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