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An In-Vitro Comparison between Hemobag® and Non-Hemobag® Ultrafiltration Methods of Salvaging Circuit Blood Following Cardiopulmonary Bypass

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Abstract: Ultrafiltration of the residual cardiopulmonary bypass circuit blood has become one of the most advantageous procedures to maximize autologous whole blood recovery and coagulation management in cardiovascular surgery. In this in-vitro study, the Hemobag® technique (HB) was compared to the most common non-Hemobag® method (NHB) of hemoconcentrating residual circuit blood. The residual bovine blood from 10 identical extracorporeal circuits was processed by the recirculating HB technique or by a venous reservoir NHB concentration method. Blood component concentrations and hemolysis levels were measured before and after processing. The HB method yielded significantly higher hemoglobin, hematocrit, fibrinogen, albumin, and total protein levels in the final product. There was no significant difference in final product platelet and white blood cell counts, or hemolysis index. HB processing times were substantially shorter at all residual circuit volumes tested. The HB technique resulted in significantly less wasted red blood cells at the end of processing. The recirculating HB method to process residual extracorporeal circuit blood is consistent and superior to the most common single pass concentrating method. **Keywords:** extracorporeal circuit, Hemobag®, hemoconcentration, ultrafiltration, coagulation factors, autologous blood conservation, cardiac surgery.
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Perfusionists are faced with optimizing whole blood management within the surgical environment of invasive surgery. Autologous blood conservation and optimal fluid and coagulation management compliment safe surgical procedures in cardiac surgery. Ultrafiltration helps to attenuate fluid overload, electrolyte imbalances, and exposure to foreign surfaces (1,2). Ultrafiltration is used to manage blood volume, hemoglobin, protein, platelet, and certain electrolyte concentrations.

Ultrafiltration during cardiopulmonary bypass (CPB) and post-CPB has evolved into techniques such as zero-balance ultrafiltration, modified ultrafiltration, and now the Hemobag® (Global Blood Resources LLC, Somers, CT) technique that facilitates post bypass ultrafiltration for whole blood salvaging and conservation. Each ultrafiltration strategy utilizes a different technique but has similar goals to remove unnecessary plasma water, to avoid discarding plasma protein and coagulation factors, and to minimize patient exposure to allogeneic blood products.

The Hemobag® or HB provides each patient with a concentrated autologous whole blood product which will enhance post-operative hemostasis, vascular blood volume, and homeostasis in a rapid, safe, and reproducible manner. Our study was designed to compare the modern Hemobag® technology and method and its technique, to the traditional technique utilizing post-cardiopulmonary bypass ultrafiltration on an existing "in-use" perfusion circuit. The technique to hemoconcentrate post-bypass residual circuit blood was probably first described in 1983 (3).

This research focuses on the end points of time to blood product completion, bovine blood component lab analysis, and completeness of circuit blood recovery. The null hypothesis was that between the two extracorporeal circuit (ECC) residual blood processing methods there is no difference in final blood product cell and protein concentrations, no difference in the time to process to the final product, and no difference in the wasted residual blood cells in the two CPB circuit blood concentrating methods.

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METHOD

The researchers constructed 10 ECCs for five separate trials using two identical circuits and reservoir blood volumes for each trial. For each trial the researchers used new circuits and hemoconcentrators. No devices were reused. The bypass circuits were composed of Terumo Capiox SX18 open venous reservoir and Sarns Delphin centrifugal pump (Terumo Cardiovascular Systems Corp., Ann Arbor, MI), Gish tubing pack with arterial filter (Gish Biomedical, Rancho Santa Margarita, CA), and Fresenius hemoconcentrator (HF5000, Fresenius Hemocare, Boston, MA). Less than 1-day-old citrated bovine blood was maintained at a temperature of 37°C for each trial. Figure 1 outlines the in-vitro study design.

Non-Hemobag® Method

The non-Hemobag® (NHB) method used the venous reservoir as an NHB alternative. A hemoconcentrator was wyed distal to the arterial filter in a recirculation line back to the venous reservoir. The circuit was primed with one 1000 mL of .9% NaCl and circulated at 4 L/min. Citrated bovine whole blood was added to achieve a starting hemoglobin concentration of about 7.2 g/dL. Hemoglobin tests were repeatedly performed with the HemoCue® analyzer (Hb 201+, HemoCue, Inc., Lake Forest, CA, www.hemocue.com). Circulating volume was controlled to produce five specific venous reservoir test blood levels of 550 mL, 450 mL, 350 mL, 250 mL, and 150 mL. Each trial volume was circulated at 4 L/min at 37°C for 5 minutes with a hemoglobin concentration of about 7.2 g/dL to start. Hematology and chemistry labs were drawn for laboratory analysis and an additional hemoglobin measurement was performed using the HemoCue to confirm the starting hemoglobin. After the samples were taken the circulating flow was terminated. A timer was initiated once the venous line was drained to the venous reservoir. A clamp was placed on the arterial line and the recirculation line to the hemoconcentrator was opened to flow at 500 mL/min. No suction was applied to the hemoconcentrator.

Each circuit was concentrated to a venous reservoir level of approximately 25 mL and then the distal end of the hemoconcentrator was connected to a labeled blood transfer bag. NaCl .9% was used to chase the concentrated blood through the circuit at 50 mL intervals until all of the concentrated blood was evacuated to the blood transfer bag. The timer was then stopped. On the blood transfer bag final product, hematology and chemistry samples as well as plasma free hemoglobin concentrations were drawn for laboratory analysis and hemoglobin analysis was performed on the blood transfer bag. Volumes were measured and the residual contents of the CPB circuit were drained into a basin and sampled for total hemoglobin. Pathology assigned plasma free hemoglobin levels to a nominal value of 0 = none, 1 = slightly elevated, 2 = moderately elevated, 3 = elevated, or 4 = severely elevated.

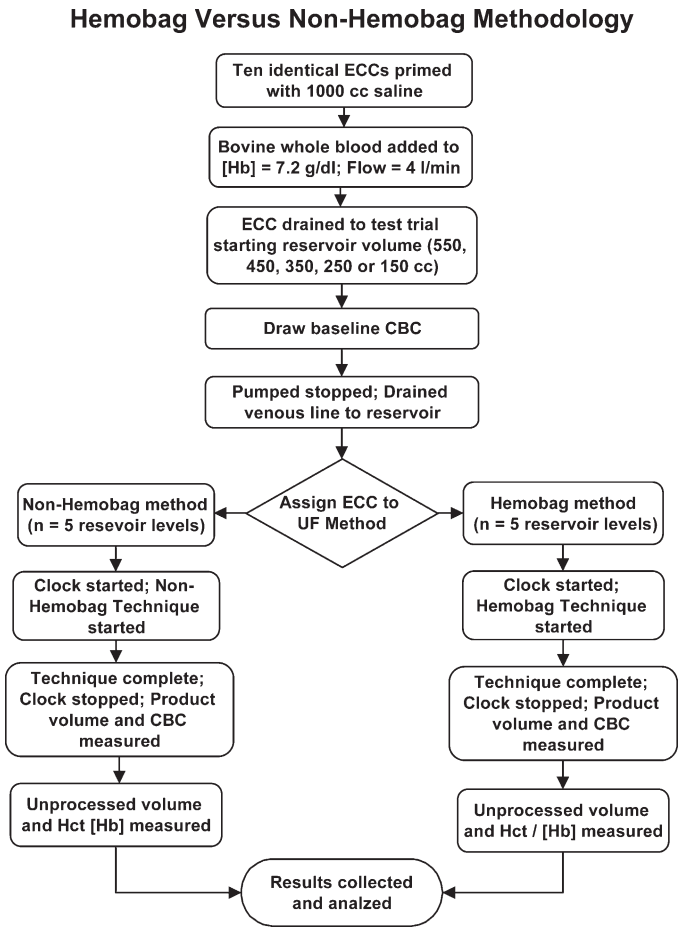

Figure 1. The design of the experiment. CBC, complete blood count; ECC, extra-corporeal circuit; [Hb], hemoglobin concentration g/dL; Hct, percent hematocrit. The unprocessed volume is the wasted volume after the technique is completed.

Hemobag® Method

The HB method used the Hemobag® and TS3 Tubing Set® (Global Blood Resources, Somers, CT, www.mybloodfirst.com). The circuit was primed with one liter of .9% NaCl and circulated at 4 L/min. Citrated bovine whole blood was added to achieve an initial hemoglobin concentration of about 7.2 g/dL in an identical manner to the NHB method. Hemoglobin tests were repeatedly performed with the HemoCue Hb 201+ to confirm identical starting blood values. Five test ECCs were constructed with the same reservoir levels as used in the NHB trials. Each trial volume was circulated at 4 L/min at 37°C for 5 minutes with a hemoglobin concentration of about 7.2 g/dL to start. Baseline hematology and chemistry labs were drawn for laboratory analysis, and after the samples were taken the circulating flow was terminated. A timer was initiated once the venous line was drained to the venous reservoir and the arterial line was connected to the Hemobag®. The entire contents of the circuit were pumped forward into the Hemobag® using a .9% NaCl chase. A 1/4 inch inside diameter (ID) tubing

roller pump was initiated to flow at 500 mL/min to concentrate the contents of the Hemobag®. No suction was applied to the hemoconcentrator. At a pre-hemoconcentrator line pressure of 450 mmHg the Hemobag® technique was stopped. The TS3 Tubing Set® circuit was chased through back to the Hemobag® and the pump and timer were then stopped. On the Hemobag®, hematology and chemistry samples and plasma free hemoglobin concentrations were then drawn again for laboratory analysis and hemoglobin values were performed. Volumes were measured and the residual contents of the CPB circuit were drained into a basin and sampled for total hemoglobin and plasma free hemoglobin concentrations. Pathology assigned plasma free hemoglobin levels to the same nominal value listed above.

Statistical Analysis

Normally distributed parametric data were compared using two-way (concentration method and sample type) analysis of variance. If the data were not normally distributed, the Wilcoxon Rank Sum test was performed. Nonparametric data was compared using Chi-Square analysis or simple descriptive statistics. Multiple linear regression was used to describe the association between specific parameters. Statistical software JMP® 7.0 (SAS Institute Inc., Cary, NC, www.jmp.com) was used to analyze the data. Statistical significance was set at a p-value less than .05.

RESULTS

Table 1 presents the results of hematologic measurements from the circuit blood and the final concentrated product. Hematocrit and protein concentrations were significantly higher in the Hemobag® process final product. White blood cell and platelet counts were not significantly different in the final products. Figure 2 illustrates the magnitude and experimental interaction of protein and cellular concentration changes between the two concentrating methods. The platelet count started significantly higher (interaction was not statistically significant) in the non-Hemobag technique test circuits. The average percent increase of 36% in the non-Hemobag method was lower than the average platelet count increase of 55% measured in the Hemobag process circuits.

Figure 2 illustrates the higher Hemobag final product hemoglobin concentration and the significantly lower circuit waste solution hemoglobin concentration with the Hemobag. Table 2 lists the hemolysis levels reported in the blood samples. The change in observed hemolysis between the two methods resulted in no significant difference in the distribution of hemolysis levels between the two methods in the final product ($\chi^2 = 4.133$, df = 2, $p = .127$).

Figure 3 depicts the concentrating method processing times at five different test circuit residual blood levels. The more volume in the test circuit, the longer it will take to process for both methods. The coefficients of association (r2) for the polynomial fit between processing time and reservoir level were statistically significant for both methods and are listed in Table 3. Multiple linear regression revealed that the Hemobag method had significantly ($p = .046$) shorter processing times than the non-Hemobag technique at the same starting reservoir level (see Figure 4).

Table 1. Blood parameters from circuit residual blood and final concentrated product by non-Hemobag and Hemobag methodology.

		Non-Hemobag®		Hemobag®		Method
Parameter	ECC/Product	Mean	SD	Mean	SD	p-value
Hematocrit %	Circuit ($n = 5$)	22.8	.5	22.4	.2	
	Product ($n = 5$)	33.6	5.2	50.7	4.2	$p = .005$
	Sample p	$p < .001$; INT < .0001				
Platelets Count K/mm^3	Circuit ($n = 5$)	152	24	123	12	
	Product ($n = 5$)	206	35	191	54	NS
	Sample p	$p = .0003$; INT = NS				
Total Protein gm/dL	Circuit ($n = 5$)	2.88	.08	2.90	0	
	Product ($n = 5$)	5.16	1.32	9.38	1.08	$p < .0001$
	Sample p	$p < .0001$; INT < .0001				
Fibrinogen mg/dL	Circuit ($n = 5$)	180	45	160	55	
	Product ($n = 5$)	260	89	400	71	$p = .0346$
	Sample p	$p < .0001$; INT = .0067				
Albumin gm/dL	Circuit ($n = 5$)	1.5	0	1.5	0	
	Product ($n = 5$)	2.7	.7	4.8	.4	$p = .004$
	Sample p	$p < .001$; INT < .0001				
White blood cells K/μL	Circuit ($n = 5$)	2.9	.6	2.8	.3	
	Product ($n = 5$)	4.6	1.6	6.2	.8	NS
	Sample p	$p < .0001$; INT = .0380				

General linear model p-values are for main effects between residual blood treatment method and between circuit and final concentrated product sample. INT, statistical interaction; NS, not significant.

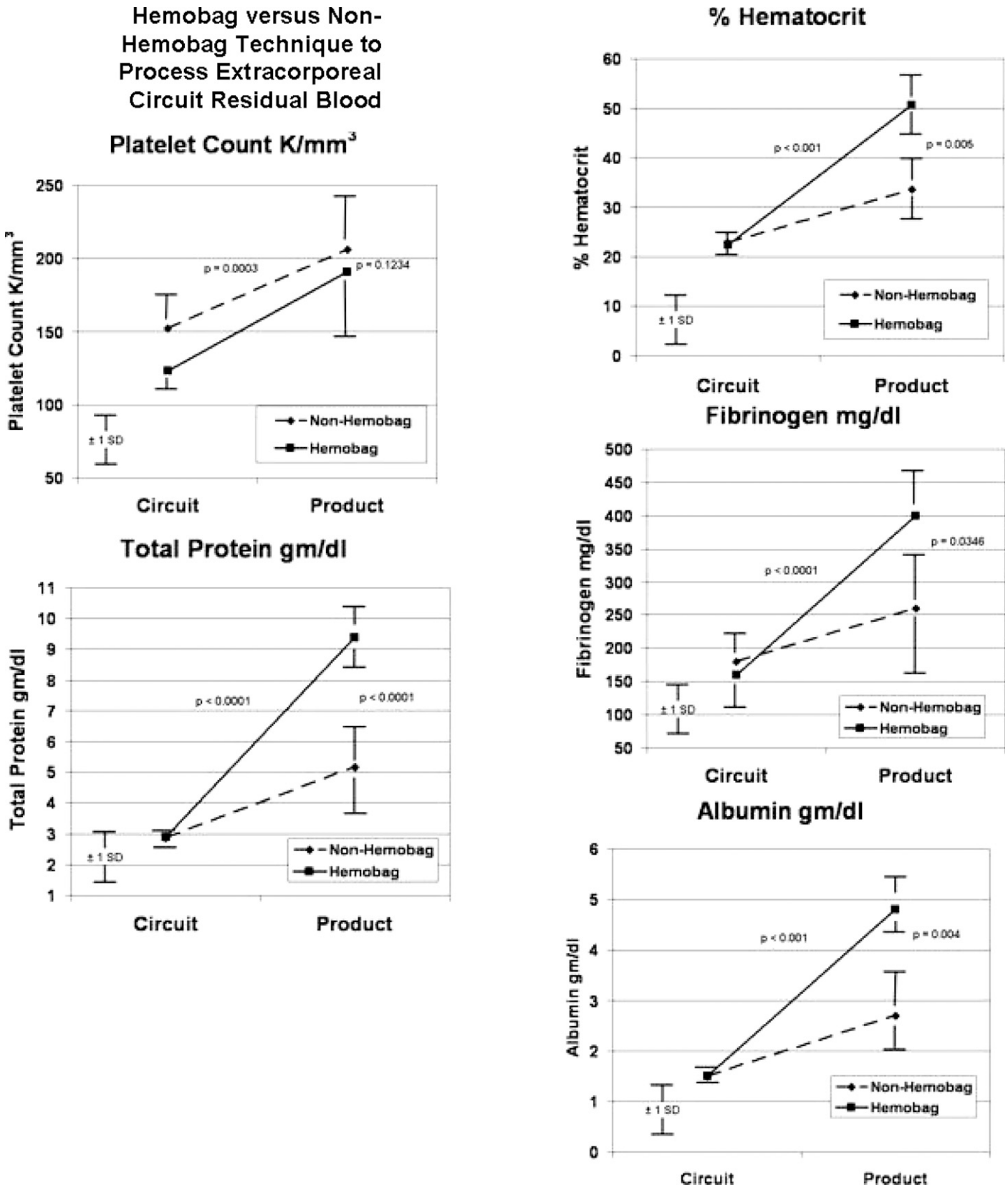

Figure 2. See Table 1 for standard deviations. *P*-values are for the main effects between treatment method and between circuit and concentrated product.

Table 2. Contingency table for hemolysis index levels in final concentrated product.

Treatment	Time	Hemolysis Index Counts N	1	2	3	4
Non-Hemobag	Baseline	0	3	2	0	0
	Product	0	2	1	0	2
Hemobag	Baseline	3	1	1	0	0
	Product	0	1	4	0	0

Five observations are used for each circuit concentration method. Nominal hemolysis levels are 0 = none, 1 = slightly elevated, 2 = moderately elevated, 3 = elevated, or 4 = severely elevated. Analyzed by Chi Square, the distribution of hemolysis indices is not significantly different between methods.

Table 3. Timing parameters comparing two methods to concentrate residual ECC blood volume.

Treatment	Process Second M	SD	REG Sec/ML m	r^2	p
Non-Hemobag	473	186	1.154	.945	< .001
Hemobag	407	146	.901	.945	< .001
p-value	NS (.5537)		REG p = .046		

Process second is the time to process the residual ECC volume. REG sec/mL is the regression of process time in seconds versus the starting reservoir volume (mL). REG p-value is for the treatment effect in multiple linear regression. M, mean; SD, standard deviation.

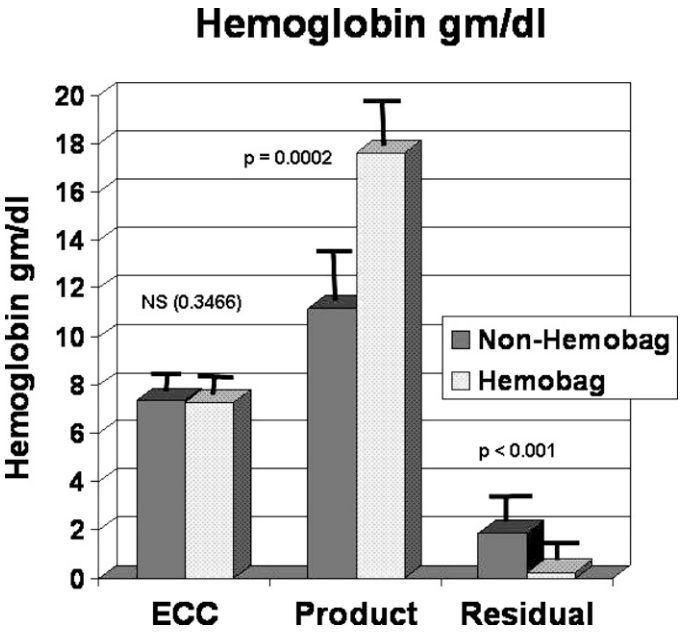

Figure 3. Bars represent one standard deviation. Hemoglobin values during processing changed significantly between processing steps. Hemobag was significantly higher in the concentrated product and lower in the ECC waste blood after processing.

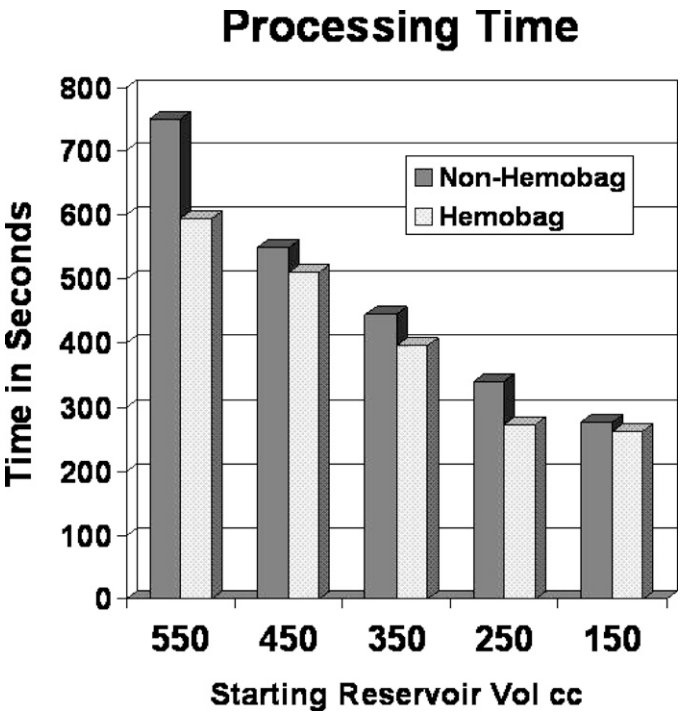

Figure 4. The concentrating time observations significantly fit a polynomial model. The method effect is significant at p = .046 with the Hemobag yielding shorter processing times at all reservoir volumes.

The time to completion of blood product for administration was statistically faster for the Hemobag technique.

DISCUSSION

The Hemobag® method employs a collapsible reservoir that contains all of the residual ECC blood that can be concentrated quickly to a target volume and hemoglobin concentration. Circuit recirculation methods are limited by the ECC fixed volume and the constraint to retrieve the entire concentrated product. Because of these differences, the HB method results in higher cell concentrations, more rapid processing, and less lost final product.

The role of the perfusionist to optimize blood and fluid management during cardiac surgery is more relevant than ever (1). Research over the past 10 years has demonstrated a direct and significant association in overall negative outcomes in cardiac surgical patients with exposure to allogeneic blood products (4,5). One often overlooked method to help reduce allogeneic blood use and to conserve and preserve autologous plasma proteins, coagulation factors, platelets, white cells, and red cells is to concentrate the residual extracorporeal circuit blood immediately after CPB (1,6,7). Perceived drawbacks to the concentration method are the time it takes to process the residual blood, and the belief that the final concentrated product is inconsistent and may do more harm than good. However,

these perceptions have not been substantiated in clinical studies (8). The Hemobag® technique is a coagulation and fluid homeostasis tool that enhances outcomes in a safe, simple, and reproducible manner (6).

Anticoagulation strategies and CPB management can also negatively impact patient hemostasis which can further be confounded by autologous cell salvage (9). Nutritional challenges related to blood volume, total protein, serum albumin, osmolarity, and colloidal oncotic pressure are fast becoming the new frontier of enhanced CPB and patient management (10). Appreciating the physiologic role of whole blood from the macro to micro circulatory domain along with lymphatics is critical to end-point perfusion of oxygen delivery to cells and effective homeostasis. The reproducibility of the HB end-product revealed consistent improvement in whole blood markers for coagulation and concentrated whole blood components such as platelets, fibrinogen, serum albumin, and total protein.

Colloid osmotic pressure impacts physiologic vascular and extravascular volume balance intraoperatively and post operatively (11). The concentrated residual extracorporeal circuit blood is a rich source of colloidal proteins. In our study, the physiologic fluid balance parameters that contribute to maintenance of intravascular blood volume were superior in the Hemobag® group versus the modified CPB circuit techniques. The concentrating method results show substantial elevated platelet counts and reasonable free hemoglobin levels. Autologous cell salvage and the washing away of residual circuit blood plasma proteins and platelets can negatively impact patient hemostasis (9). Autologous cell washing, though successful at preserving red blood cell mass and reducing plasma heparin levels, wastes the plasma component of whole blood contributing to reduced serum albumin, total protein, colloid oncotic pressure, platelets, and coagulation factors (12).

Surgical teams need to develop a strategic balance between vascular tone and volume administration (13,14). Upon arrival to the operating room, anesthesiologists must remain diligent in optimizing volume administration. Perfusionists need to develop the techniques and judgment to contribute to team whole blood management and vascular volume during CPB and in the early post bypass period. The results of our study demonstrated that time to completion of blood product for administration was statistically faster for the Hemobag® technique.

The results of our study demonstrate that the Hemobag® method preserves sterile technique and yields higher hemoglobin, clotting factor, and plasma protein concentrations, with less remaining blood cell waste, and in a shorter period of time than alternative circuit concentrating methods. The Hemobag® is an effective tool and is the preferred modality to assist in the management of each patient's circulating whole blood remaining in the extracorporeal circuit.

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