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Evaluation of coagulation factors and platelet function from an off-line modified ultrafiltration technique for post-cardiopulmonary bypass circuit blood recovery

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Abstract

Modified ultrafiltration (MUF) is a technique that hemoconcentrates residual CPB circuit blood and the patient at the same time. Hemoconcentration and MUF are Class 1-A recommendations in the anesthesia and surgical blood conservation guidelines. This study evaluated the off-line MUF process of the Hemobag (HB, Global Blood Resources, Somers, CT, USA) to quantitate coagulation factor levels, platelet (PLT) count and function in one facility and cellular growth factor concentrations of the final product that were transfused to the patient in another facility

In two cardiac surgery facilities, after decannulation, the extracorporeal circuit (ECC) blood from 22 patients undergoing cardiac surgery was processed with the HB device. In eleven patients from the first facility by the study design, blood samples for coagulation factor levels and PLT aggregation were drawn from the reservoir of the MUF device pre- and post-processing. The samples (n = 11) were sent to a reference laboratory where testing for prothrombin time (PT), international normalized ratio (INR), activated partial thromboplastin time (aPTT), reptilase time, fibrinogen, clotting factors II,V,VII,VIII, IX, X,ADAMTS-13, protein C, protein S, antithrombin III, von Willebrand Factor (vWF), and platelet (PLT) aggregation were performed. A portion of the final concentrated HB blood samples (n = 5-10) from the second facility by design were evaluated for transforming and platelet-derived cellular growth factor concentrations.

On average, approximately 800 – 2000 mls of whole blood were removed from the ECC post-CPB for processing in the HB device. After processing, there was, on the average, approximately 300 – 950 mls of concentrated whole blood salvaged for reinfusion. The PT and INR were significantly lower in the post-processing product compared to the pre-processing samples while the aPTT times were not significantly different. All coagulation factors and natural anticoagulants were significantly increased in the final product.The PLT number, although increased by 24%, was not statistically significant. While PLT function assays showed a statistically significant decrease in the levels post-processing, there was substantial platelet function in the MUF product. Overall, the decrease in function was in the range of 10% to 15%. Final product PDGF-αβ and TGF-β1 averaged 11,048 and 2,040 pg/ml, respectively.

In these two case series, (ECC) circuit blood concentrated using the HB device showed coagulation studies with significantly lower PT and INR and significantly increased levels of all clotting factors. The findings are similar to trends reported in other studies utilizing conventional MUF and the HB. Functioning platelets remain in the final product, with growth factor concentrations similar to some methods employed to create platelet concentrates to enhance coagulation. Based on the ability of the HB off-line MUF procedure to concentrate circuit blood, the clinical utility of the HB device to decrease allogeneic blood product exposure should be evaluated in a prospective randomized clinical trial.

Keywords

modified ultrafiltration; Hemobag; cell salvage; cardiac surgery; cardiopulmonary bypass; blood management; blood conservation

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Introduction

As the risks of adverse events from allogeneic transfusion become better elucidated and the problem of blood product shortages seems to grow more acute, the techniques of perioperative blood management are becoming more imperative. Cardiac surgery patients are at a high risk for the transfusion of blood products perioperatively.[1] Blood management can begin prior to a surgical procedure by addressing and treating preoperative anemia as well as discontinuing medications that may interfere with coagulation. During the surgical procedure and use of cardiopulmonary bypass (CPB), meticulous minimally invasive surgical techniques, acute normovolemic hemodilution, tolerance of anemia, pharmacotherapy, judicious fluid administration, hemofiltration and cell washing procedures can all be employed to avoid allogeneic donor exposure.[2]

Up to two liters of whole dilute blood can, potentially, remain in the CPB circuit post-protamine sulfate infusion. In many instances, the residual blood in the CPB circuit is transferred to the reservoir of a cell processing device and then the washed red blood cells (RBC) are transfused back to the patient. The washing step removes potential contaminants, but also viable platelets, plasma proteins and clotting factors, and returns only the RBCs.[3-5] Another technique is transfusion of the circuit blood to the patient without washing by direct infusion; this technique would preserve PLTs, plasma and RBCs; however, it does not change coagulation nor remove the excess water or inflammatory mediators and relies on the kidneys to restore fluid homeostasis over an extended period of time.[4,6]

Modified ultrafiltration (MUF) is a technique to remove excess plasma water and inflammatory mediators while concentrating the blood in the patient and the CPB circuit, simultaneously, at the conclusion of surgery. MUF was recently recommended as a Class 1-A guideline by the STS/SCA for blood conservation in cardiac surgery.[7] MUF, which is performed while the patient is still connected to the CPB circuit, has been shown to decrease transfusion requirements and is effective in removing mediators while retaining coagulation factors and platelets.[8,9] The levels of some clotting factors and other plasma proteins have been shown to be higher post-ultrafiltration with the MUF technique compared to that in pre-ultrafiltration samples or when measured directly in the blood from the extracorporeal circuit.[10,11] The technique of MUF has never become a mainstream practice because it is cumbersome and time-consuming, which increases the amount of time that the patient is connected to the CPB circuitry and remains heparinized.[12]

To overcome some of the drawbacks of conventional MUF, a novel device has been developed for recovering and processing residual blood in the extracorporeal circuit after the patient has been disconnected from the CPB machine. The off-line MUF device, called the Hemobag® (HB, Global Blood Resources LLC, Somers, CT), has US Food and Drug Administration clearance, CE Marking ("Conformité Européene") and Canadian Registration. The HB collects the blood in the CPB circuit post-CPB and removal of cannulas from the patient.[10,11] This is accomplished by purging the circuit with crystalloid fluid and capturing the blood in the HB. The whole blood in the circuit with the HB is then recirculated through the HB at a rate of 400-500 ml/min through the device, its tubing set and through any commercially available microporous hollow-fiber hemoconcentrator. The concentrator fibers have pores ranging in size from 15,000-55,000 Daltons.[13] Non-cellular water and small blood-borne substances, including inflammatory mediators and platelet inhibitors, exit the fibers through the pores and enter an effluent line and are discarded, while the larger cellular substances that remained in the fibers, such as RBCs, PLTs, clotting factors and plasma proteins, are recirculated back to the HB reservoir. The cycle of filtration is repeated until the pressure proximal to the hemoconcentrator reaches approximately 400-450 mmHg, at which point the filtration lines are returned to the reservoir, thereby, terminating the procedure. The hemoconcentrated whole blood is then available for reinfusion.

The purpose of this observational study was to comprehensively evaluate the changes in the levels of clotting factors and PLT quantity and function in the post-CPB circuit blood. Samples collected before and after processing with the novel HB off-line MUF device were drawn to determine if viable clotting factors and PLTs are available to be returned to the patient. The blood samples were collected from two groups of patients from two cardiac surgical centers. The samples were analyzed by three accredited laboratories. From one facility, platelet-derived and transforming growth factor concentrations were measured in the MUF final product to compare to other methods to create platelet or whole blood concentrates.

Materials and Methods

Two groups of eleven patients from two separate institutions who were scheduled to undergo cardiac surgery requiring a CPB circuit were prospectively identified between January and April 2009, with the same surgeon at each institution performing the eleven procedures. At one facility, baseline PT, INR, aPTT and PLT counts were obtained on all patients as part of routine testing. Patients

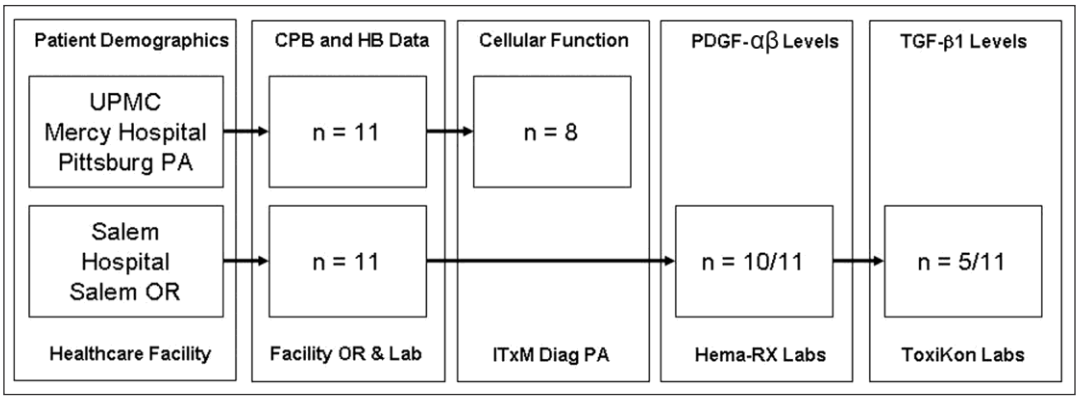

Figure 1. Clinical sites and patient data.
n: number of patients or samples; HB: is the Hemobag; Cellular function and growth factor data are from the HB final MUF product.

with INR ≤1.3, normal aPTT, and normal PLT counts were selected for participation in this study. All peri-operative surgical and transfusion decisions, including the use of the off-line MUF device for salvaged post-CPB blood, were made by the anesthesiologist and surgeon, according to their routine practice. This study protocol was approved by the Quality Improvement Review Committee of the University of Pittsburgh Medical Center (UPMC) and the Internal Review Board at the Salem Hospital (SHO) in Oregon as a minimally invasive quality improvement study, thus, obtaining patient informed consent for participation was not necessary. Figure 1 maps the facility and patient blood sampling collection and analysis design.

After the completion of CPB and decannulation, a pre-measured volume of the residual CPB circuit blood was displaced with crystalloid solution into the HB off-line MUF device. At the UPMC, a sample was drawn for the pre-processing coagulation factor levels and PLT function tests, using sterile technique, through a port on the device designed for this purpose. Some samples were prepared for transport to an outside lab for further analysis. After processing was completed in the HB off-line MUF device, another sample was drawn for post-processing tests. Typically, the HB technique required about 5-10 minutes to complete its ultrafiltration and hemoconcentration processing cycle. The pre- and post-processing samples were immediately shipped to one of three coagulation reference laboratories: (ITxM Diagnostics, Pittsburgh, PA, USA; Hema-RX Labs, Colorado Springs CO, USA and ToxiKon Labs, Bedford, MA, USA). PLT function testing on 8 of the 11 UPMC samples occurred within 4 hours of sample collection. For other clotting factor tests at UPMC, the plasma was separated and stored at -70°C until coagulation factor testing was performed.

Some or all of the following parameters were measured on the pre- and post-processing samples: PT/INR, aPTT, reptilase time, fibrinogen level, factors II, V, VII, VIII, IX, X levels, protein C (PC), protein S (PS), antithrombin (AT), von Willebrand factor (vWF), ADAMTS-13 (a disintegrin and metalloproteinase with thrombospondin type 1 motif), and hematocrit (Hct). The pre- and post-processing samples underwent Hepzyme (Dade Behring, Deerfield, IL, USA) treatment as necessary to remove the heparin added to the CPB circuit. The quantity of Hepzyme added to each sample has been validated to neutralize 4 IU/ml of heparin.

Prothrombin time/extrinsic pathway coagulation factor activity testing

Thromboplastin (Thromborel S; Siemens Healthcare Diagnostics Inc., New Castle, DE, USA) and calcium ions were combined with test plasma at 37°C; the PT was the time in seconds required for a fibrin clot to form. The INR was calculated using ISI=1.09. The activity of the extrinsic factors (II, V, VII, and X) were determined from a standard curve by the addition of the appropriate factor-deficient plasma (HRF, Inc., Raleigh, NC, USA).

Activated partial thromboplastin time (aPTT)/ intrinsic pathway coagulation factor activity testing

The aPTT was performed by re-calcifying the test plasma in the presence of a standardized amount of platelet-like phosphatides (Pathromptin SL, Siemens Healthcare Diagnostics Inc. New Castle, DE, USA) and an activator of the contact factors of the intrinsic coagulation pathway. The aPTT was the time in seconds required for a fibrin clot to form. The activity of the intrinsic factors (VIII and IX) was determined from a standard curve by the addition of the appropriate factor-deficient plasma (HRF Inc.).

Fibrinogen

The fibrinogen determination was based on a modification of the Clauss Fibrinogen method (Multifibrin U Kit, Siemens Healthcare Diagnostics Inc.). Excess thrombin was added to citrated plasma and the time required for clot formation was recorded.

Protein C (PC) and Protein S (PS) activity

Both of these tests were performed using commercially available kits (Protein C Reagent, Coagulometric Assay Kit; Protein S Activity Kit, Siemens Healthcare Diagnostics Inc.), according to the manufacturer's instructions.

Antithrombin (AT) activity

The AT chromogenic assay (IL Hemosil Antithrombin, Instrumentation Laboratories, Lexington, MA, USA) is specific for factor Xa activity and was carried out in two steps, according to the manufacturer's instructions, on an ACL10,000 instrument (Beckman Coulter Inc., Miami, FL, USA). The AT level was expressed as a percentage of normal human plasma calculated from a stored calibration curve.

The PT, aPTT, individual coagulation factor activity levels, fibrinogen, PC, and PS levels were all performed on a BCS instrument (Siemens Healthcare Diagnostics Inc.).

ADAMTS-13 activity assay

ADAMTS-13 activity was assessed using a manual test based on fluorescence resonance energy transfer (FRET) technology. A synthetic fragment (FRETS-VWF73) of the vWF protein was used as the substrate. Cleavage of the peptide released a fluorescent signal, which was quantified against standards to determine protease activity (GTI Inc., Milwaukee, WI, USA).

Reptilase time

This assay measured the speed of polymerization of fibrinogen to fibrin, utilizing a snake venom enzyme (Batroxobin; Siemens Healthcare Diagnostics, Inc.) which cleaved fibrinopeptide A from fibrinogen. Reptilase time is not sensitive to the presence of heparin and is used in lieu of thrombin time.

vWF antigen

Quantitation of vWF antigen was performed using a commercially available kit (Liatest vWF kit; Diagnostica Stago, Parsippany, NJ, USA).

Platelet aggregations

Platelet studies utilized a quantitative method that measured changes in light transmission of a sample of platelet-rich plasma (PRP). A known quantity of aggregating agent or agonist is added to citrated PRP in a cuvette in a light-recording instrument (Bio-Data Platelet Aggregation Profiler PAP-4, Bio-Data, Horsham, PA, USA) under conditions of constant temperature with constant stirring. The final concentration and agonists used included: 20 µM, 10 µM and 5 µM ADP (Bio-Pool, West Chester, PA, USA); 2 µg/ml collagen (Bio-Data) and 5 µg/ml arachidonic acid (Bio-Data).

Platelet-derived and transforming growth factors

The human platelet-derived growth factor (PDGF-αβ) and transforming growth factor (TGF-β1) analysis kits were enzyme-linked immunosorbent assays (ELISA). Test samples and standards of known PDGF and TGF concentrations were added to the ELISA wells and allowed to bind with PDGF and TGF antibodies for analysis.

Statistical analysis of continuous variables was performed with a 2-tailed Wilcoxon matched pairs test (Graphpad Software or JMP 8.0). A p-value <0.05 was considered statistically significant. Descriptive statistics were employed for demographic data.

Results

The demographics of the two groups of 11 patients from each facility in this study are presented in Tables 1A and 1B. At UPMC Mercy Hospital, six patients underwent coronary artery bypass graft procedures, three had coronary artery bypass grafting and valve replacement, while two underwent valve replacements. Two patients required allogeneic transfusion during their surgery prior to returning blood from the HB MUF device. One patient required a single unit of RBCs while another required two units of RBCs and two doses of platelets. Some patients had been receiving low-dose aspirin therapy until the night before their surgery. No patient received clopidogrel within one week of the study. At Salem Hospital, the 11 patients underwent coronary artery bypass graft procedures, one of these being a re-operation. Two patients required allogeneic transfusion during cardiopulmonary bypass.

Table 2 demonstrates the pre- and post-ultrafiltration coagulation factor levels for the UPMC blood samples. The reduction in the PT/INR between the two sample collections was significant while there was no significant difference in the aPTT times between samples. The levels of all measured clotting factors and natural anticoagulants, ATIII, ADAMTS-13 and vWF, increased significantly after

Table 1A. Demographics and surgical parameters of the UPMC patients.

Statistic	Age (year)	Wt (kg)	PT (s)	INR	aPTT (s)	Hb (g/dl)	Hct (%)	PLT	Time on CPB circuit (min)	Surgical blood loss (ml)	Post-process volume in MUF device (ml)
Mean	67	100	12.9	1.12	29.6	12.7	38.9	201	124	668	398
SD	9	17	1.62	0.09	3.6	1.9	5.9	48	43	356	88.6
Median	67	102	13.2	1.1	29	13.5	41.2	192	117	600	400

Patient pre-cardiopulmonary bypass values from the UPMC (University of Pittsburgh Mercy Hospital). SD: standard deviation; PT: prothrombin time; INR: international normalized ratio; aPTT: partial thromboplastin time; Hb: hemoglobin; Hct: hematocrit; PLT: platelets; CPB: cardiopulmonary bypass; MUF: modified ultrafiltration process.

Table 1B. Demographics and surgical parameters of the SHO patients.

Statistic	Age (year)	Wt (kg)	Baseline ACT (s)	Time on CPB circuit (min)	Pre-process volume in MUF device (ml)	Post-process volume in MUF device (ml)
Mean	72	84	114	95	1990	922
SD	7	19	12	24	373	263
Median	69	76	112	100	2100	1000

Patient values from the Salem Hospital in Oregon (SHO). SD: standard deviation; ACT: activated clotting time; CPB: cardiopulmonary bypass; MUF: modified ultrafiltration process.

Table 2. Coagulation factor values pre- and post-processing with the MUF device.

Coagulation parameter	n	Pre-processing mean	SD	Post-processing mean	SD	Change	p	FFP Study(21)	FP24 Study(22)
PT	10	23.32	6.63	15.02	2.04	–8.30	<0.01		12.3 (0.29)
INR	10	2.03	0.63	1.28	0.18	–0.75	<0.01		0.99 (0.03)
aPTT	10	63.58	24.96	57.67	7.16	–5.91	0.36		36.3 (2.12)
Reptilase Time	11	18.25	1.87	19.20	1.85	0.95	0.02		
Fibrinogen	11	216.64	110.08	366.00	157.26	132.70	0.01	290 (255–308)	313 (42.1)
F II	11	0.49	0.17	1.30	0.46	0.80	<0.01	0.83 (0.77–0.86)	1.20 (0.11)
F V	11	0.43	0.27	1.15	0.71	0.72	<0.01	1.16 (1.00–1.26)	1.31 (0.08)
F VII	11	0.54	0.20	1.31	0.52	0.78	<0.01	1.15 (0.97–1.31)	1.17 (0.06)
F X	11	0.45	0.14	1.11	0.36	0.65	<0.01	0.93 (0.87–1.01)	
F VIII	11	1.03	0.46	2.71	1.17	1.69	<0.01	1.25 (1.03–1.34)	0.72 (0.14)
F IX	11	0.79	0.30	2.00	0.79	1.21	<0.01	1.14 (1.05–1.22)	1.14 (0.11)
ADAMTS-13	9	48.00	20.45	75.67	30.36	27.67	<0.01		84 (10)
vWF	11	1.53	0.68	2.86	0.27	1.32	<0.01	1.32 (0.99–1.55)	
PC	11	57.64	25.39	155.27	51.37	97.64	<0.01	1.00 (0.85–1.10)	1.04 (0.06)
PS	11	52.55	26.13	178.45	73.58	125.91	<0.01	0.95 (0.88–1.03)	0.74 (0.05)
ATIII	11	41.64	13.93	117.64	33.94	76.00	<0.01	1.01 (0.98–1.10)	0.88 (0.05)
Hct %	9	20.93	9.07	49.97	7.80	29.03	<0.01		

All data from UPMC. For comparison, reported coagulation factor levels immediately after the thawing of FFP and FP24 are also presented. In the FFP Study[21] (von Heymann C, Keller MK, Spies C et al. Transfusion 2009;49:913-205), data are presented as median (range) while, in the FP24 Study[22] (Yazer MH, Cortese-Hassett A, Triulzi DJ. Transfusion 2008;48:2525-2530), data are presented as mean (SD). Blanks spaces indicate testing not performed. The ADAMTS-13 level in the FP24 study was measured on day 2 post-thaw. The p-value compares the differences in the coagulation parameters between the pre- and post-processing samples.
MUF: modified ultrafiltration process; FFP: fresh frozen plasma; FP: frozen plasma; PT: prothrombin time; INR: international normalized ratio; aPTT: activated partial thromboplastin time; F: factor; vWF: von Willebrand factor; PC: protein C; PS: protein S; AT: antithrombin.

processing with the MUF device. The post-processing Hct increased more than two-fold over the pre-processing level. For comparison, in the same table, the reported levels of some clotting factors in both FFP (plasma frozen within 8 hours of collection) and FP24 (plasma frozen within 24 hours of collection), measured immediately after thawing, are also provided.

Table 3 demonstrates the results of PLT aggregation studies (n=8) performed on pre- and post-ultrafiltration samples at UPMC. Despite a higher mean PLT count

Table 3. Platelet concentration and percentage aggregation values pre- and post-processing with the MUF device.

PLT agonist	Reference range	n	Pre-processing mean	SD	Post-processing mean	SD	Change	p
PLT count (x10³/µl)	150–370	9	157	56.0	181	96.7	24.2	0.43
20 µM ADP	60–100%	8	71	29.2	60	25.6	–11.5	0.04
10 µM ADP	60–100%	8	67	26.8	52	22.9	–14.8	0.03
5 µM ADP	50–100%	8	64	28.2	51	26.2	–12.9	0.02
Collagen	60–100%	8	73	27.2	60	26.5	–13.9	0.02
Arachadonic Acid	60–100%	8	29	20.0	20	14.7	–9.9	0.25

Percentage platelet aggregation data were collected from eight University of Pittsburgh Medical Center patients. SD is one standard deviation. MUF: modified ultrafiltration process; PLT: platelets; SD: standard deviation; ADP: adenosine diphosphate;

Table 4. Cellular and platelet growth factor levels in post off-line MUF process product.

Patient	Final Vol	WBC	NEUT	Hb	Hct	PLT	FIB	PDGF-αβ	TGF-β1
1	1200	30.5	21.6	20.0	59.8	292	679	12579.2	2985.7
2	1250	18.2	10.7	14.5	42.1	197	460	5307.7	*
3	700	19.9	16.0	16.7	48.0	185	704	3306.6	1440.0
4	1250	13.0	8.0	12.5	35.8	181	594	31882.1	*
5	1000	18.5	15.1	15.2	44.1	236	376	5983.0	1891.6
6	600	18.8	17.1	14.0	41.2	250	734	7391.2	*
7	1000	10.7	8.0	14.8	41.9	252	268	5999.5	1793.1
8	950	30.8	20.1	16.0	47.1	191	485	6584.2	*
9	500	21.5	16.8	14.3	40.2	260	460	4970.1	2090.1
10	700	18.9	12.8	14.4	41.5	369	420	26479.9	*
11	1000	15.8	10.7	17.2	50.3	182	637	*	*
Mean	923	19.7	14.3	15.4	44.7	236	529	11048.3	2040.1
SD	263	6.2	4.6	2.0	6.4	58	150	9937.6	578.8
Median	1000	18.8	15.1	14.8	42.1	236	485	6291.8	1891.6

Data from 11 Salem Hospital in Oregon (SHO) patients. Levels measured in Hemobag final off-line MUF product. MUF: modified ultrafiltration process; WBC is total white blood cell count x10⁹/l, NEUT is neutrophil count x10⁹/l, Hb and Hct are hemoglobin and hematocrit. PLT is platelet count x10³/µl. FIB is fibrinogen level in mg/dl. Platelet-derived growth factor αβ (PDGF) is pg/ml and transforming growth factor β1 (TGF) is pg/ml. * samples were not evaluated. SD is one standard deviation.

post-processing, it was not statistically significant. There was a decrease in post-ultrafiltration PLT aggregation levels. However, the PLT function levels post-processing were either at or slightly below the lower normal range (adenosine diphosphate (ADP) and collagen tests) or only slightly decreased compared to the pre-processing level (arachadonic acid).

Table 4 lists the results of the post-HB MUF final product platelet-derived and transforming growth factor levels in the off-line MUF process final product. Platelet-derived and transforming growth factor concentrations were present in the HB MUF final product at levels comparable to normal blood.

Discussion

This study showed that the technique of off-line MUF post-CPB after arterial and venous decannulation, using the HB MUF device, significantly improved the coagulation parameters (PT and INR), prevented platelets from discard and concentrated all functional coagulation factors. The finding of elevated functional platelet-derived growth factor concentrations in the HB MUF product presents a unique insight into the off-line MUF technique. In addition, hemoconcentration was effectively achieved as the Hct increased by more than two-fold. The ultimate goal is to apply the HB technique as part of a multi-modality approach to blood conservation, coagulation timing and healing to ultimately help patients avoid allogeneic blood product transfusion.[14]

The residual whole blood from the CPB circuit at the end of the operation can be recovered by direct infusion, classic MUF, MUF using the HB or by cell washer.[15] Direct infusion requires significant skill as, potentially, there can be a significant amount of dilute whole blood transfused back to the patient over a short period of time and reliance on the kidneys to return to homeostasis

over an extended period of time. Patients may experience transfusion-associated circulatory overload or renal compromise with the infusion of residual circuit blood, which is predominately hemodiluted plasma.[16] If a cell washer device is used, the blood is washed and most of the contaminants and inflammatory mediators are removed, but so will any residual functioning clotting factors, plasma proteins and PLTs; only the RBCs are retained.[4] Classic MUF requires the patient to remain cannulated for an additional amount of time and is cumbersome as the patient must remain heparinized and attached to the CPB circuit, restricting the advancement of surgery. The HB MUF is a modification of traditional MUF in that the procedure is performed after CPB is discontinued and the patient is decannulated from the CPB circuit. The total time for processing using the HB in these observations was 5-10 minutes.

This study revealed that, similar to classic MUF, there was a significant decrease in PT and INR, along with a significant increase in all the levels of functional clotting factors.[14,15] The PLT number, although increased by 24%, was not statistically significant. Additionally, PLT function assays showed a statistically significant decrease in the levels post-processing. Overall, though, the total decrease in functions was in the range of 10% to 15% and these absolute levels were either at or slightly below the lower normal range (ADP and collagen tests) or only slightly decreased compared to the pre-processing level. The PLT function levels were on the low side of normal prior to processing for the ADP and collagen studies and were considerably abnormal for arachadonic acid to begin with. This effect on the arachadonic acid assay was most likely the result of the aspirin that all of the patients had been taking until the night before their surgery. The clinical relevance needs to be elucidated.[17]

In this analysis, the volume of blood returned to the patient after processing with the MUF device went from 800-2000 mL of 21% Hct blood to approximately 400-1000 mL with an Hct of approximately 50%. Since the red cells would be returned to the patient regardless of which post-CPB blood recovery technique was employed, salvaging the coagulation factors and platelets using the HB MUF device is equivalent to approximately 200-400 mL of autologous plasma added to the patient's red cells in this study. While some factors, like fibrinogen, factor VIII and Antithrombin 3 (ATIII), were particularly concentrated after the ultrafiltration procedure, most were equivalent to or slightly higher than the levels seen in both FFP and frozen plasma 24 hours (FP24) immediately after thawing (Table 3). Thus, it can be concluded from this study that the processed whole blood volume unit-equivalent of plasma recovered by the MUF device was equivalent to a full unit of concentrated plasma. Recovering larger quantities of post-CPB blood from the extracorporeal circuit would improve the plasma yield and have a potentially greater reduction in donor exposures in cardiac surgery.

Table 5 compares the Hemobag final product cellular and growth factor concentrations to recent reports for growth factor levels achieved by several methods to create platelet concentrates and gels.[18,19] There is a high variability in growth factor and cellular concentrations between current methods to create PRP. It is useful to compare the final MUF product to PRP in the hopes that, in future studies, the concentrated residual pump blood may be employed to form a coagulation gel similar to concentrated PRP. The Hemobag (HB) method yields higher or similar final product concentrations of hemoglobin, fibrinogen, WBCs and TGF-β1 compared to centrifuged fresh PRP, normal whole blood or hemodiluted CPB ECC blood. Many PRP methodologies yield higher concentrations of platelets, WBCs and PDGF-βα compared to the HB, which yields higher or similar values compared to normal whole blood or ECC blood.

Table 5. Hemobag final product cellular and growth factor concentrations compared to published platelet-rich plasma values.

Parameter	Normal Blood	CPB Residual Blood	Hemobag Product	Platelet-rich Plasma
Hb g/dl	13.5–17.0	6.5–11.5	15.4 ± 2.0	N/A
Hct %	36–54	18–35	44.7 ± 6.4	N/A
WBCs x10⁹/l	4.2–11.5	1.5–7.5	19.7 ± 1.9	1.1–34.4
Platelets x10³/mm³	150–450	60–190	236 ± 18	443–780
Fibrinogen mg/dl	150–270	75–225	529 ± 45	254 ± 31
PDGF-βα ng/ml	10–29	N/A	11.0 ± 3.0	33–133
TGF-β1 pg/ml	334–2382	N/A	2040 ± 175*	755 ± 370

All data from SHO (Salem Hospital in Oregon) and reference publications. PRP is platelet-rich plasma. CPB is cardiopulmonary bypass circuit residual blood; N/A is not applicable or available; Values reported are for serum and are the mean ± one standard error of the mean; PRP data is from concentrate volumes of about 7-10 ml; Hyphenated numbers are high and low limits of observations from multiple published methods;
* n = 5 samples.
Hb: hemoglobin; Hct: hematocrit; WBC: white blood cell; PDGF: platelet-derived growth factor; TGF: transforming growth factor.

Perhaps the TGF levels are high due to the ability of the HB to concentrate WBCs which are a source for TGF. PDGFs are lower in the HB product because post-CPB platelets have been exposed to dilution, the hemoconcentrator and the extracorporeal circuit. PRP created with fresh whole citrated blood should have greater PLT counts and higher growth factor concentrations. The growth factor levels measured in this study should encourage the possible evaluation of concentrated residual circuit blood as a hemostatic gel.

This study has several limitations. First, only a limited portion of the post-CPB circuit volume was processed at one of the facilities. On average, CPB circuits have a volume of approximately 1200 to 1800 mL of whole blood volume that may be processed. In this study, the different average volumes that were processed were different between facilities due to institutional preferences. Recovering larger quantities of post-CPB blood from the extracorporeal circuit should improve the amount of plasma, RBC and PLTs recovered. This could, potentially, lead to a greater reduction in donor exposures to allogeneic blood and blood products in cardiac surgery. The number of patients in this study was small and was only powered to evaluate coagulation factor levels. Post-processing platelet function decreased. However, only 8 of the 11 PLT function tests were available for analysis. The total decrease in function was only in the range of 10% to 15% while the platelet count, although not statistically significant, increased 24%.

This data is an observation of the patient's diluted whole blood concentrated within the HB MUF device. With no data collected on chest tube drainage and post-operative allogeneic blood product exposure, further studies are required to analyze the clinical significance of these findings. Thus, no conclusions about the effect of MUF on post-operative hemostasis, donor exposures, or other clinical outcomes can be drawn from this data. Furthermore, this was not a randomized study with a matched group where blood was processed by other techniques to compare the effectiveness of the HB MUF technique on preserving platelets and coagulation factors. However, a meta-analysis of ultrafiltration and MUF in adult cardiac surgical patients yielded positive outcomes of less post-operative bleeding and fewer transfusions versus the few drawbacks of the cost of the equipment, the lack of blinded researchers and the need for more reporting of long-term outcome measures in future studies.[20] The positive effects were greater in studies analyzing MUF versus simple use of UF during cardiopulmonary bypass.

Conclusion

In this observational study, the HB off-line MUF device was able to quickly concentrate whole blood post-CPB with the patient decannulated and protamine administered while keeping the CPB circuit safely primed. There was a significant increase in concentration of all clotting factors post-processing. The PT and INR statistically improved while the aPTT, although improved, was statistically unchanged. Overall, the PLT number improved; however, it was not statistically significant and the platelet function was slightly decreased yet near normal limits for several activators. Platelet-derived and transforming growth factors were concentrated and present in the final MUF product. In summary, not only were RBCs salvaged using the HB off-line MUF device, but also the patient's autologous platelets and plasma constituents. The amount of autologous clotting factors recovered by the HB MUF device, concentrating about 800-1000 mL of residual circuit diluted whole blood, was equivalent to one full unit or more of concentrated autologous plasma for reinfusion after cardiopulmonary bypass.

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Conflict of Interest Statement

The authors declare that there are no conflicts of interest.

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