Changes in Mechanical Fragility and Free Hemoglobin Levels after Processing Salvaged Cardiopulmonary Bypass Circuit Blood with a Modified Ultrafiltration Device

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Abstract: Modified ultrafiltration (MUF) is available for the salvage of post-cardiopulmonary bypass circuit blood. This study evaluated the extent of hemolysis, the mechanical fragility index (MFI), and the amount of plasma free hemoglobin (PFHb) created after processing with the MUF device. Several RBC parameters were measured on pre- and post-MUF device processed samples of blood from 12 patients undergoing cardiac surgery. The MFI and total amount of PFHb did not change significantly between the pre- and post-processing samples: MFI, pre: .19 ± .06 versus post: .19 ± .06, p = .76; total amount of PFHb, pre: .24 ± .21 g versus post: .20 ± .12 g, p = .42. There was significantly more hemolysis in the post-processing samples compared with the pre-processing samples, .33 ± .24% versus .96 ± .48%, respectively, p < .001. Although percent hemolysis was increased following processing with the MUF device, the total amount of PFHb and RBC sublethal injury were not increased. The clinical significance of these findings needs to be determined. Keywords: modified ultrafiltration, cardiac surgery, hemolysis, cell salvage, red blood cell.

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Allogeneic transfusion is a lifesaving procedure in many medical and surgical situations. However, the transfusion of blood products is not without potential adverse events, some of the products can be subject to shortages, and there are financial implications each time a transfusion is ordered. Thus, techniques that either avoid blood utilization or minimize the use of allogeneic products should be investigated.

Cardiopulmonary bypass (CPB) is an integral part of cardiac surgery, and these patients frequently receive allogeneic transfusions. At the conclusion of bypass the perfusion circuit can contain a residual blood volume of up to 2000 mL and there are several options for returning it to the recipient once the circuit has been discontinued. Commonly, the residual blood is transferred to the cell washer for processing and return of the red blood cells (RBCs). Washing the residual blood removes most of the plasma and a portion of the contaminants from the product. It is also possible to return the post-bypass salvaged blood to the patient after filtering but without additional processing: this technique would preserve all of the plasma and platelets that were in the circuit but would not remove cytokines or other plasma borne contaminants. This technique would also return significant quantities of crystalloid with a low hematocrit.

Another technique for processing the post-CPB blood before it is returned to the recipient is modified ultrafiltration (MUF). A U.S. Food and Drug Administration-approved and CE marked device, the Hemobag (MUF device; Global Blood Resources, LLC, Somers, CT), is available for this purpose. The MUF device uses a hemocentrator to remove extracellular water thereby concentrating the blood in the CPB circuit. The clinical use of the MUF device during cardiac surgery has been described previously (1–3). While the device is concentrating the blood, it repetitively passes the RBCs through the hemocentrator/ultrafilter under pressure over a period of several minutes, which could potentially lead to both lethal (hemolysis) and sublethal injury to the RBCs.

Our laboratory uses the mechanical fragility (MF) test to evaluate the extent of sublethal injury that has occurred to RBCs. In this test, RBCs are exposed to shear stress.
Some of the cells that were intact before the application of the shear stress will lyse during the experiment presumably because they had accumulated more injury than the cells that did not lyse, hence the term sublethal injury. The output of the MF test, the mechanical fragility index (MFI), is thus an overall measure of the extent of sublethal injury sustained by a population of RBCs. Higher MFI values reflect greater RBC susceptibility to lysis under shear stress conditions, which likely indicates that greater degrees of sublethal injury had been inflicted on the RBCs. The MF test has been used to demonstrate that RBCs tend to accumulate sublethal injury during the 42 days of routine blood bank storage, that is, the MFI of the stored RBCs increases over the storage period (4–6). The increasing MFI values during storage correlated well with an in vivo study that demonstrated significantly higher recovery of 5-day-old (mean storage length) RBCs compared with 30-day-old (mean storage length) RBCs 24-hours post-transfusion (7).

The MF test has also been used to evaluate the extent of damage inflicted on the RBCs after suctioning from surgical fields (6,8). The purpose of this study was to evaluate the extent of both lethal and sublethal injury on the RBCs, and to determine how much plasma free hemoglobin (PFHb) was returned to the recipient after CPB salvaged blood was processed with the MUF device.

MATERIALS AND METHODS

After the protocol was approved by the University of Pittsburgh Medical Center’s Quality Improvement Review Committee, 12 patients who met the following criteria were entered into the study: undergoing on-pump cardiac surgery, not transfused with allogeneic blood products before discontinuation of the CPB circuit, and scheduled to receive a reinfusion of residual CPB blood after processing with the MUF device. In this study, each patient’s RBCs served as their own control as the blood samples that were taken both before and after processing with the MUF device were compared with each other.

At the time the CPB circuit was disconnected, the residual circuit blood was drained into the MUF device’s reservoir. To determine the volume of blood, the reservoir was weighed. Using aseptic technique, a 20 mL aliquot of blood was removed from the reservoir before the processing commenced (pre-processing sample). The MUF device then began processing the blood according to the hospital’s standard operating procedure. Briefly, the recovered post-CPB whole blood flowed at a rate of 400–500 mL/min through a hemoconcentrator/ultrafilter (Sorin DHF0.6, Arvada, CO) composed of microporous hollow fibers. These fibers had pores ranging in size from 15,000–55,000 Da. Non-cellular water and small blood borne substances that exited the fibers through the pores entered an effluent line and were discarded, while the larger cellular substances that remained in the fibers recirculated back to the reservoir. From four patients a 20 mL sample of the effluent line was collected for PFHb testing. At this hospital, the MUF device is processed with a target of doubling the starting hematocrit (HCT). To this end the pre-processing volume of the MUF device and its hematocrit were measured. The volume of the RBCs is calculated using the following formula:

\[
\text{RBC volume} = \frac{\text{Pre-processing MUF device volume}}{\text{pre-processing HCT}}
\]

As the desired end point of processing is a doubling of the pre-processing RBC volume, the amount of ultrafiltrate to be removed is calculated as follows:

\[
\text{Ultrafiltrate to be removed} = \frac{\text{pre-processing MUF device volume}}{2} - \text{pre-processing RBC volume}
\]

The MUF device then processed the blood until the calculated amount of ultrafiltrate had been removed. The hemoconcentrated whole blood was then available for transfusion. At this time the reservoir was weighed again and another 20 mL specimen was drawn using aseptic technique (post-processing sample) before the contents were returned to the patient. The samples were then immediately subjected to the MF test, as described below.

Mechanical Fragility Test

After thorough mixing, both the pre- and post-processing samples were adjusted to a standard hematocrit of 20% using Dulbecco’s phosphate buffered saline (Lonza BioWhittaker DPBS w/ Calcium and Magnesium, Fisher Scientific, Pittsburgh, PA). As the MFI and percent hemolysis are proportionate to the hematocrit, the MF test on both the pre- and post-processing samples were performed at a hematocrit of 20% to accommodate the relatively dilute nature of the pre-processing samples. After dilution the hemoglobin (Hb) concentration of each aliquot was measured (HemoCue, Inc., Lake Forest, CA).

Following dilution and Hb measurement, 3 mL from each of the samples were placed into five test tubes (7 mL serum glass vacutainers, Fisher Scientific, Pittsburgh, PA) to assess the free hemoglobin concentration and mechanical fragility. Three of these tubes contained five 3.2 mm stainless steel ball bearings (BBs, BNMX-2, Type 316 balls, Small Parts, Inc., Miami Lakes, FL). These three tubes were subsequently rocked for 1 hour on a rocker platform (Type M79700 Platform Vari-Mix rocker, Barnstead Thermolyne Corp., Dubuque, IA) at 18 cycles per minute and a rocking angle of ±17° from horizontal. The remaining two tubes were not rocked and did not contain BBs; they were used as controls to determine the baseline level of PFHb.
After 1 hour, all test tubes were centrifuged at 2750 \( \times \) g for 15 minutes at room temperature. The supernatants were then transferred to 1.5 mL microcentrifuge tubes and re-centrifuged to ensure the complete sedimentation of intact RBCs or their membrane fragments at 20,800 \( \times \) g for 20 minutes at room temperature. The supernatants were then transferred into 1.5 mL spectrophotometer cuvettes (1.5-mL semimicro UV methacrylate cuvette, Fisher Scientific, Pittsburgh, PA), and the PFHb concentration was measured by light absorbance at 540 nm (Spectronic Genesys 5 spectrophotometer, Spectronic Instruments, Inc., Columbus, OH).

### MFI Calculation

The mechanical fragility index was calculated on both the pre- and post-processing samples using the formula:

\[
MFI = \frac{(PFH_{\text{brocked}} - PFH_{\text{control}})}{(Hb_{\text{ aliquot}} - PFH_{\text{control}})} \times 100,
\]

where PFH_{\text{brocked}} is the mean plasma free hemoglobin concentration in the supernatants of the rocked specimens, PFH_{\text{control}} is the mean plasma free hemoglobin concentration in the supernatants of the control (unrocked) samples, and Hb_{\text{ aliquot}} is the mean hemoglobin concentration of the RBC aliquots at a hematocrit of 20%.

Percent hemolysis was calculated as follows:

\[
\left(100 - \frac{\text{Hematocrit}}{\text{Plasma Volume in MUF device's reservoir}}\right) \times \left(\text{Plasma Volume in MUF device's reservoir} \times \text{Hct} \times 0.2\right)
\]

As PFHb is only located in the plasma component, the total amount of PFHb in each MUF device’s reservoir was calculated as follows:

\[
\text{PFHb}_{\text{control}} \times \left(\text{plasma volume in MUF device’s reservoir}\right)
\]

The total amount of hemoglobin to be returned to the patient after processing with the MUF device was calculated as follows:

\[
\left(\text{Hb}\right)_{\text{of post-processing sample}} \times \left(\text{RBC volume in MUF device’s reservoir}\right)
\]

### Statistical Analysis

Descriptive statistics were used for continuous variables (GraphPad Prism software). The D’Agostino and Pearson omnibus normality test was used to determine if the distribution of values for each parameter was normal. The significance of the differences between the pre- and post-processing variables was assessed with the Wilcoxon-matched-pairs signed rank test or a 2-tailed paired t test as appropriate. Results are presented as mean ± standard deviation.

### RESULTS

The average age of the 12 patients in this study was 66 ± 15 years, and 8/12 (67%) were male. There were six coronary artery bypass graft patients, five valve replacement patients, and one ascending aorta repair patient. The average length of time on bypass was 114.0 ± 25.5 minutes, and the average MUF device processing time was 5.73 ± 1.01 minutes. The pre- and post-processing characteristics of the MUF device’s contents are shown in Table 1. Due to a malfunction of the scale on one occasion, the exact reservoir weights were not available for one patient; the average reservoir weights of the remaining 11 patients were used for the calculations in this patient.

Table 2 demonstrates the RBC parameters evaluated in this study. The mean post-processing MFI and total amount of PFHb were not significantly different compared with their corresponding pre-processing values. There was considerable variability in the total amount of PFHb, especially in the pre-processing samples, which was caused by variations in patient’s volume status and by the amount of blood that remained in the reservoir when the circuit was discontinued. The mean percent hemolysis was significantly higher in the post-processing specimens. That the concentration of PFHb was higher in the post-processing MUF reservoir compared with the pre-processing reservoir was expected given that the MUF device concentrates

<table>
<thead>
<tr>
<th>Pre-processing</th>
<th>Post-processing</th>
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<tbody>
<tr>
<td>Hct (%)</td>
<td>21.27 ± 3.90</td>
</tr>
<tr>
<td>Hb (g/dL)</td>
<td>7.34 ± 1.26</td>
</tr>
<tr>
<td>Volume of MUF device reservoir (mL)</td>
<td>1027.17 ± 296.26</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Pre-processing</th>
<th>Post-processing</th>
</tr>
</thead>
<tbody>
<tr>
<td>MFI</td>
<td>19 ± 6</td>
</tr>
<tr>
<td>Percent hemolysis</td>
<td>33 ± 24</td>
</tr>
<tr>
<td>Total amount of PFHb (g)</td>
<td>24 ± 21</td>
</tr>
<tr>
<td>PFHb concentration (mg/dL)</td>
<td>28.31 ± 19.96</td>
</tr>
<tr>
<td>Total amount of Hb returned (g)</td>
<td>NC</td>
</tr>
<tr>
<td>Total amount of PFHb post-processing</td>
<td>.0060</td>
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NC, not calculated.
the blood during processing. The average total quantity of PFHb in the effluent fluid was 9.59 ± 8.04 mg in the four samples tested.

**DISCUSSION**

In this study we demonstrated that although the MUF device repetitively passes the post-CPB RBCs through a hemoconcentrator/ultrafilter during processing, and despite increases in the percent hemolysis observed in the post-processing samples, the total PFHb load to the recipient after reinfusion of the contents of the MUF device was not increased over the pre-processing level. This was likely explained by the removal of some of the PFHb that was generated during the MUF device’s processing through the ultrafilter. Excess PFHb can be problematic for a recipient as it rapidly scavenges nitric oxide (NO), which is an important local regulator of blood pressure (9), and has other systemic functions (10). In addition to its role as a NO scavenger, PFHb has also been demonstrated to cause platelet activation in models of sickle cell disease and pulmonary arterial hypertension (11,12). The amount of PFHb that is returned to the recipient with the MUF device appears to be less than that returned by two devices used to salvage shed blood after orthopedic surgery. In a recent study, which compared a device that washed and concentrated recovered shed blood following total knee arthroplasty (Ortho-PAT, Haemonetics, Braintree, MA) to a device that simply filtered the shed blood (Suretrans, Davol, Inc., Warwick, RI) before it was returned to the patient, the total amounts of PFHb that were reinfused to the patient were higher at .51 ± .12 g and .55 ± .35 g, respectively (13), compared with the mean amount of PFHb that was returned after processing with the MUF device in the current study. In fact, the average total amount of PFHb returned to the recipient with the MUF device in this study, .20 ± .12 g, is nearly equivalent to the average amount of PFHb in a 39-day-old allogeneic RBC unit stored in the commonly used AS-5 solution (9). Furthermore, the ratio of PFHb returned/total Hb returned for the MUF device, .0060 ± .0031, was approximately equal to that of the filtered device used after orthopedic surgery although it was more than double that of the washed device. Although one of the markers for acceptable wash quality in the AABB (formerly known as the American Association for Blood Banks) standards for perioperative autologous blood collection is a PFHb concentration of <100 mg/dL (14), the clinical significance of reinfusing even this small quantity of PFHb after processing with the MUF device is unknown and should be considered in future studies of this device. A recent study demonstrated that a peak post-reperfusion PFHb level of approximately .02 g/dL in patients who underwent on-pump aortic aneurysm repair was prognostic for acute kidney injury following the surgery (15). Furthermore, the average concentration of PFHb in the post-processing samples of the MUF device was 87.9 mg/dL, which is in the range of post-wash PFHb levels reported over a 7-year period using two different intraoperative cell salvage devices which washed the RBCs before they were returned to the recipients (16).

That the MFI did not appear to be increased in the post-processing samples should not necessarily be interpreted to mean that the MUF device does not inflict injury on the RBCs. In light of the increased percent hemolysis in the post-processing samples, it is likely that the MUF device destroyed the cells that had sustained the most damage during the surgery, when passing through the CPB circuit, or in the hemoconcentrator/ultrafilter itself. Thus the cells that remained intact were likely those that had sustained the least amount of injury before the MF test was performed. A similar phenomenon was observed when RBCs in reconstituted whole blood were suctioned from a simulated surgical field using different suction devices – the MFI of the suctioned RBCs was actually lower (likely indicating less sublethal injury had been accumulated) than that of the unsuctioned RBCs leading the investigators to presume that the cells that did not lyse during the suctioning were those with the least amount of injury in the first place and thus they were the most resistant to lysis during the experiment (8). In this light, a direct comparison between the MFI of the RBCs post-MUF device processing and that with the two orthopedic shed blood recovery systems is hampered by the fact that MFI values and percent hemolysis before washing or filtering with the latter two devices were not presented in the report (13). Thus it cannot be determined if these two post-operative blood salvage devices had simply lysed the most fragile RBCs leaving behind a relatively healthy population of RBCs or whether the post-processing MFI reflects increased sublethal injury on the remaining intact RBCs. Furthermore, in the post-orthopedic blood salvage study the MFI was calculated at a Hb concentration of 10 g/dL (Hct 31%), whereas in the current study the MFI was calculated at Hct = 20% due to the dilute nature of the pre-processing samples (Table 1). Thus, assuming that hemolysis and MFI are proportionate to the sample’s Hct, the MFI of the RBCs in this study cannot be directly compared with that from the post-orthopedic study. Additionally, as plasma proteins confer protection on the RBCs from shear stress, it is not surprising that the mean MFI of the RBCs that were washed after salvage was higher than when they were processed with the MUF device, regardless of the starting Hct (17).

It is also difficult to compare the findings in this study to those that had been previously published using the MUF device because in the two studies where similar parameters were measured, only animal blood was processed (18,19). The source of the blood notwithstanding, in a
study where citrated bovine blood was evaluated, the post-processing “hemolysis index” was reported to be either “slightly” or “moderately” elevated although exact quantities or concentrations of PFHb were not reported (19). In another study of four Yorkshire pigs, the reported post-processing concentration of PFHb was 49.5 ± 42.5 mg/dL (18), while in a study of three human subjects the post-processing concentration of PFHb was 49.5 ± 42.5 mg/dL (1). In our human study, which featured 12 recipients, the average post-processing PFHb concentration was 87.94 ± 43.78 mg/dL, which, like the percent hemolysis value, was also quite variable between our human patients. The etiology of this variability is unknown but could relate to inter-personal differences in RBC shear stress tolerance, which would be manifested in different percent hemolysis values and, by extension, PFHb concentrations. As alluded to above, the maximum or safe level of reinfused PFHb has not been determined.

Previous methods of performing MUF have been reported to take between 13–20 minutes to concentrate the residual volume by 50% (20). Others have reported processing with the MUF device for approximately 10–13 minutes (1,18). In our hands it took the MUF device 5.73 ± 1.01 minutes to achieve similar outcomes. During the immediate post-bypass period, the reduction in processing time can quickly provide much needed blood components for improved hemodynamic stability.

In this study we demonstrated that the processing of CPB salvaged blood with the MUF device increases the extent of hemolysis. However, the amount of PFHb returned to the recipient and the extent of sublethal RBC injury were not greater than what was present in the blood before it was processed. The cost effectiveness and the potential clinical benefits for the recipients of using the MUF device instead of a cell washer for the salvage of post-CPB blood remain to be determined.

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REFERENCES