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Plasma Free Hemoglobin Generation Using the EOS PMP™ Oxygenator and the CentriMag® Blood Pump

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Abstract: Hemolysis is a known consequence of extracorporeal membrane oxygenation (ECMO) resulting from shear force within the different components of the extracorporeal circuit. The primary aim of this study was to evaluate the EOS PMP™ oxygenator for generation of plasma free hemoglobin (PfHg) over 24 hours at nominal operating range flow rates. The EOS ECMO™ (LivaNova, Inc.; formerly Sorin, Arvada, CO) is equipped with a plasma tight polymethylpentene (PMP) hollow fiber oxygenator. We hypothesized that PfHg generation would be elevated in circuits with higher flow rates, because of the significant pressure drop across the oxygenator according to manufacturer provided flow charts. Generated PfHg concentrations were compared with PfHg concentrations from blood not exposed to an ECMO circuit. The secondary aim was to evaluate circuit flow-rate-induced changes in platelet count and platelet function over 24 hours. Circuits contained a CentriMag® (St. Jude Medical, St. Paul, MN) blood pump and an EOS ECMO PMP™ oxygenator. Circuits in triplicate were run continuously for 24 hours at three flow rates [1, 3, and 5 liters per minute {LPM}]. PfHg was analyzed at baseline, 6, 12, 18, and 24 hours. Platelet count and function were measured at baseline and 24 hours. Concentrations of PfHg at baseline for circuits operating at 1, 3, and 5 LPM were 24.4 ± 4.0, 38.4 ± 28.6, and 26.7 ± 6.9 mg/dL, respectively. PfHg concentrations after 24 hours were statistically compared for the three flow rates using analysis of variance; PfHg concentrations at 1 LPM (181.4 ± 29.1 mg/dL), 3 LPM (145.9 ± 8.7 mg/dL), and 5 LPM (100.1 ± 111.3 mg/dL) circuits. The *F*-test was not statistically significant (p = .632), indicating that PfHg generation at 24 hours was similar among the three flow rates. Excessive hemolysis using PfHg levels in the EOS PMP™ membrane oxygenator was not observed.
Keywords: plasma free hemoglobin, hemolysis, CentriMag®, EOS PMP™, pressure drop. *J Extra Corpor Technol. 2018;50:94–8*

As the demand for extracorporeal membrane oxygenation (ECMO) support increases across the country, the medical device industry is challenged to produce new products that are approved for long-term support. Additional challenges have recently arisen with regard to availability of disposable ECMO components because of increased patient volumes and product supply shortages. Newer ECMO product designs have transitioned to preassembled circuits to allow the clinician immediate use. A shortage of these products makes it more challenging for centers to find replacement circuits, forcing clinicians to seek additional modalities of support that are both efficacious and safe for patient care.

The newly released EOS PMP™ (LivaNova, Inc.) polymethylpentene (PMP) hollow fiber oxygenator that provides up to 5 liters per minute (LPM) of support and is approved by

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the Food and Drug Administration (FDA) for 6 hours of support. Unlike other newer commercially available devices that are designed with lower shear stress and pressure drops, the EOS PMP™ oxygenator has a large pressure drop. The possibility of increased red blood cell fragmentation due to the large pressure drop in the EOS PMP™ could result in a greater generation of plasma free hemoglobin (PfHb), which elevates the risk of end organ injury, perturbation of vascular nitric oxide (NO) processes, and morbidity (1,2).

Previous studies have been inconclusive regarding the relative contribution of oxygenator components to PfHg generation. In a study by Williams et al. (3), the larger pressure gradients associated with smaller oxygenator dimensions led to increased PfHg generation; likely due to hemolysis from the increased shear forces through these oxygenators. Another study with membrane oxygenators suggested that the surface area, prime volume, and blood path length, rather than the pressure drop, were the major contributors to hemolysis (4).

An additional potential source of PfHg generation in the ECMO circuit is the pump. The debate between centrifugal vs. roller head pumps has been going on for decades (5–7). Some ECMO centers have chosen to use a centrifugal pump head because of hemolysis. The CentriMag® (St. Jude Medical) blood pump has been shown to produce only minimal amounts of hemolysis which is why the pump was chosen for this study (8). In addition, pump flow time did not impact the generation of PfHg (4,9). The CentriMag®, designed with large gaps between the impeller and the pump housing, minimizes shear forces and allows high blood flow rates with minimal hemolysis while maintaining a low prime volume (31 mL). These newer generation centrifugal pumps use magnetic levitation to suspend and spin the impeller, which eliminates bearings and further reduces frictional heat generation. The maximum revolutions per minute (RPM) is 5,500 with 10 L/min maximum flow. Because of these features the CentriMag® pump was chosen for our study (4,10).

Traditionally the only PMP oxygenators used across the United States are the Quadrox D and Quadrox iD, which includes the integrated CardioHelp system (Maquet Gentige Group, Rastatt, Germany). FDA recently approved the LivaNova EOS PMP™ oxygenator, which introduces a different design than the Quadrox, with a longer blood path and a larger pressure drop. At the tested flow rates the pressure drop is 55 and 200 mmHg at flow rates of 1 and 3 LPM, and 5 LPM is off the chart. For comparison, the Quadrox D pressure drop is approximately 12, 25, and 45 mmHg at flow rates of 1, 3, and 5 LPM, respectively. We hypothesized that PfHg generation through the EOS PMP™ oxygenator with the CentriMag® pump would increase in circuits in direct correlation with flow rates.

The primary aim of this study, therefore, was to evaluate the EOS ECMO™ equipped with a plasma tight PMP hollow fiber oxygenator for the generation of PfHg over 24 hours at three different flow rates. Concentrations of generated PfHg were compared with PfHg concentrations from blood not exposed to an ECMO circuit. The secondary aim of the study was to correlate circuit flow rate to changes in platelet count and platelet function from baseline to 24 hours.

MATERIALS AND METHODS

This study was deemed exempt by the local Institutional Review Board. Human volunteers donated fresh whole blood, which was collected into transfusion bags containing the anticoagulant citrate dextrose-A (Fenwal, Inc., Lake Zurich, IL) (8 mL per 52 mL of blood). Blood was mixed well during collection to ensure proper anticoagulation and used within 1 hour of collection. Circuits were primed with Normosol-R® (Hospira, Inc., Lake Forest, IL) to a final prime volume of 350 mL. Whole blood was then introduced into the ECMO circuits displacing all crystalloid with whole blood. Sodium bicarbonate (Hospira, Inc.) was added up to a concentration of 22–26 mEq/L, and heparin (Sagent Pharmaceuticals, Schamburg, IL) was added to achieve an anti-factor Xa of 0.5 IU/mL of blood. This heparin concentration, which reflects the goal of our clinical practice, was maintained for the study duration.

In each of three separate experiments, three circuits were assembled, consisting of a hard shell Capiox® Advance Reservoir (Terumo Cardiovascular, Ann Arbor, MI), 3/8 inch Carmeda® coated tubing (Medtronic, Minneapolis, MN) to the inlet and outlet of the CentriMag® blood pump, which was then connected with 3/8 inch tubing into the LivaNova EOS PMP™ oxygenator. Luer lock connectors, placed at the inlet and outlet of the oxygenator and at the inlet of the centrifugal pump, allowed continuous pre- and post-pressure monitoring of the circuit. Circuits were evaluated in three test groups (1, 3, and 5 LPM) over 24 hours at a constant 37°C. The three flow rates represented the low, mid, and maximal flow rates of the device. Blood was tested for anti-factor Xa at baseline and 18 hours; heparin was administered to maintain an anti-factor Xa of .3–.8 IU/mL. Blood gases were sampled from each circuit at baseline, 6, 12, 18, and 24 hours and measured with an i-Stat® system (Abbott Laboratories, Abbott Park, IL). A gas mixture of 5% CO_2 and 21% O_2 (Praxair, Inc., Danbury, CT) was used to normalize blood pCO_2 and pO_2 values and bicarbonate was administered to maintain physiologic blood gas parameters. For platelet count and function tests, blood was collected from each circuit in ethylenediaminetetraacetic acid (EDTA) blood collection tube or citrate vacutainer tubes, respectively (Becton, Dickinson and Co., Franklin Lakes, NJ) at baseline and 24 hours. For PfHg assays, blood (1 mL) was collected from each circuit in EDTA vacutainer tubes at baseline (0), 6, 12, 18, and 24 hours. Control blood,

not exposed to a circuit, was maintained in the transfusion bag at ambient temperature for the duration of the study and sampled at the same intervals as the test circuits. Temperature, pre-oxygenator pressure, post-oxygenator pressure, delta pressure, flow, RPM, and inlet pressure were monitored hourly for each circuit to assure no deviation from study protocol.

Collected blood samples for PfHg analysis were immediately centrifuged at 1,500 × *g*, at 4°C for 10 minutes. Plasma (350 μL) was removed, being careful to avoid cellular layers, and stored at −80°C. Plasma was analyzed for free hemoglobin content using a Human Hemoglobin enzyme linked immunosorbent assay (ELISA) Quantitation Set and protocol (Cat. No. E80-134; Bethyl Laboratories, Inc., Montgomery, TX). Before assay, plasma was thawed on ice and diluted both 20,000-fold and 80,000-fold in MSD Blocker Solution A (Meso Scale Discovery, Inc., Rockville, MD) containing .05% Tween® 20. Both diluted plasma sample sets were analyzed in duplicate. Only those plasma samples with A_{450} values within the linear range of the standard curve (.35–1.70 AU) were used for analysis. PfHg levels were extrapolated from the standard curve by four-parameter logistic analysis, and averaged per interval in the study using GraphPad Prism 6.07 software (La Jolla, CA).

Whole blood platelet counts were determined using the XN-2000™ Hematology System (Sysmex America, Inc., Lincolnshire, IL) by means of electronic resistance detection. Briefly, whole blood is passed through a detection aperture in the presence of an electric current, and the differences in magnitude of the electrical resistance allows for distinction of blood cell types.

Platelet function was determined using the platelet function analysis-100® System (Siemens Healthineers USA, Malvern, PA). Briefly, whole blood is aspirated at high shear rates through a test cartridge containing an aperture within a membrane coated with either collagen and epinephrine (Col/EPI) or collagen and ADP (Col/ADP). These agonists induce platelet adhesion, activation, and aggregation leading to rapid occlusion of the aperture and cessation of blood flow, measured as closure time (CT). CT are reported in seconds with a reference range.

Data are summarized as means with SE. PfHg at 24 hours, compared across circuit flow rates, was analyzed using analysis of variance (ANOVA). Average PfHg at 24 hours from each set of circuits was compared with reference PfHg at 24 hours from the control blood using two-sample *t* tests. Change in platelet function from baseline to 24 hours was evaluated using repeated-measures ANOVA with an interaction between time point and flow rate, with the *F*-test on the interaction term determining whether this change differed according to circuit flow rate. Data were analyzed using Stata/IC 13.1 (StataCorp, LP, College Station, TX), and $p < .05$ was considered statistically significant.

RESULTS

The measured operating characteristics of each circuit, summarized in Table 1, were held in close tolerance throughout the 24-hour test period. Circuit hematocrit levels (25 ± 3%) and the anti-factor Xa (.34–.74 IU/mL) were also maintained throughout the test. PfHg concentrations (mg/dL) at baseline (0), 6, 12, 18, and 24 hours for each flow rate (n = 3 circuits per flow rate tested) are summarized in Table 2. At baseline, average PfHg concentrations at 1, 3, and 5 LPM flow rates were 24 ± 2, 38 ± 17, and 27 ± 4 mg/dL, respectively (Table 2). Although the individual circuits for each flow rate generated variable PfHg concentrations per interval (Figure 1), the ANOVA *F*-test on PfHg concentrations at 24 hours was not statistically significant between flow rates ($p = .632$), indicating no significant differences in generated PfHg. The PfHg generated from baseline to 24 hours at each flow rate ranged from 3.8 to 7.5 fold increase from baseline (Figure 1 and Table 2).

The PfHg concentrations at 24 hours were compared between each group of circuits and control blood (left at ambient temperature for 24 hours, but not run through an ECMO circuit). PfHg generated in the control blood after 24 hours was 101.3 mg/dL. Two-sample *t* tests found a statistically significant increase in PfHg generation at 24 hours

Table 1. Hourly operating characteristics (means and SE) in each ECMO circuit over 24 hours.

Circuit	Flow (LPM)	Temperature (°C)	Pre-Oxygenator Pressure (mmHg)	Post-Oxygenator Pressure (mmHg)	Delta Pressure (mmHg)	RPM	Inlet Pressure (mmHg)
1	1.0 ± .03	37 ± .0	17 ± .5	4 ± .6	13 ± .9	1,600 ± 0	−37 ± .7
2	1.0 ± .02	37 ± .0	19 ± .3	8 ± 2.5	11 ± 2.5	1,600 ± 0	−39 ± 8.5
3	1.0 ± .03	37 ± .0	19 ± .7	6 ± 3.1	14 ± 3.5	1,600 ± 0	−42 ± 7.0
4	3.0 ± .10	37 ± .1	54 ± 3.0	11 ± 2.4	43 ± 2.3	2,336 ± 31	−35 ± 3.0
5	3.1 ± .04	37 ± .1	42 ± 4.1	7 ± .0	35 ± 4.1	2,200 ± 0	−35 ± 6.8
6	3.0 ± .10	37 ± .1	59 ± 12.6	11 ± 2.0	49 ± 10.8	2,380 ± 92	−48 ± 4.1
7	5.0 ± .03	37 ± .0	114 ± 2.7	2 ± 2.9	113 ± 5.1	2,886 ± 23	−49 ± 4.0
8	5.0 ± .03	37 ± .0	119 ± 2.4	5 ± 2.3	114 ± 4.3	2,986 ± 23	−50 ± 3.9
9	5.0 ± .03	37 ± .0	175 ± 5.5	3 ± .7	173 ± 4.9	3,370 ± 46	−58 ± 2.4

Numbers reported as Mean ± SD. LPM, liters per minute; RPM, revolutions per minute.

Table 2. PfHg concentrations per interval in ECMO circuits at each of three flow rates.

Time	1 LPM Circuits (n = 3)	3 LPM Circuits (n = 3)	5 LPM Circuits (n = 3)
PfHg at baseline	24 ± 2	38 ± 17	27 ± 4
PfHg at 6 hours	92 ± 4	54 ± 4	102 ± 23
PfHg at 12 hours	127 ± 16	74 ± 20	123 ± 14
PfHg at 18 hours	172 ± 15	112 ± 18	138 ± 39
PfHg at 24 hours	181 ± 17	146 ± 5	199 ± 64

Numbers reported in mg/dL; Mean ± SE. PfHg, plasma free hemoglobin; LPM, liters per minute.

relative to the control blood for the 1 LPM ($p = .041$) and 3 LPM ($p = .013$) circuits, but not for the 5 LPM ($p = .268$) circuits (Figure 1).

To address the secondary aim of the study, platelet count and function with either Col/EPI or Col/ADP were measured at baseline and at 24 hours for each group of circuits (Table 3). Among all circuits, platelet count decreased by 39 ± 29 thousand/mm^3. Over the 24-hour test period, platelet function by Col/EPI increased by 26 ± 18% and platelet function by Col/ADP decreased by 2 ± 36%. Repeated-measures ANOVA found no statistically significant variability among circuit flow rates in each of these changes over time (F-test $p = .469$, $p = .110$, and $p = .316$, for 1, 3, and 5 LPM, respectively). Therefore, changes in platelet count and platelet function over 24 hours were similar at each circuit flow rate.

DISCUSSION

Although improvements in extracorporeal device design have been vast, the extracorporeal community of clinicians still strives to minimize PfHg generation because of circuit design. The clinical sequelae of hemolysis and more specifically PfHg generation are significant and continue to plague the ECMO patient population (11–13). Hemolysis may be generated from multiple components in the ECMO circuit. One application where hemolysis may be generated is at the inlet to the blood pump. Red cells exposed to excessive negative pressure are subject to hemolysis (14). However, the primary culprit identified by the exact process or component in the circuit responsible for hemolysis is still unknown.

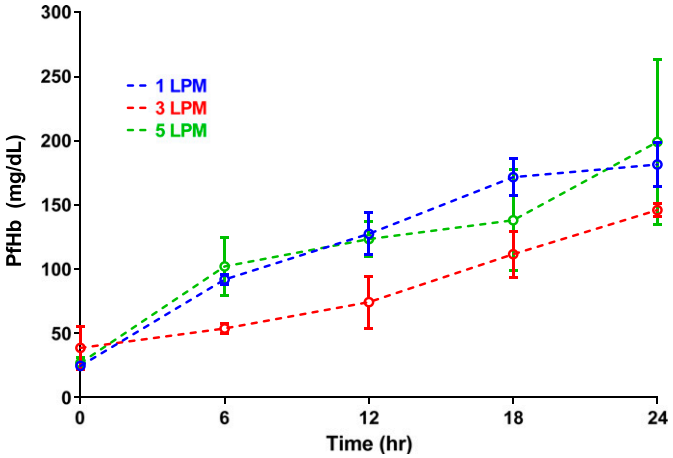

Figure 1. PfHg concentrations per interval in ECMO circuits testing three flow rates over 24 hours.

Neonatal respiratory support comprises 37% of all international ECMO support runs (15). Some of these respiratory ECMO runs involve neonates having persistent pulmonary hypertension (PPHN), a severe pulmonary disorder and life threatening condition (15). PfHg generation becomes especially important in this patient population because of subsequent alterations in NO levels (16). When hemoglobin-scavenging mechanisms are saturated, levels of cell-free hemoglobin increase in the circulating plasma. PfHg depletes endothelial NO because of its high affinity (10^7 M^{-1} s^{-1}) and irreversible binding (17). The rapid binding of NO to free hemoglobin depletes NO at a rate approximately 1,000-fold greater than hemoglobin sequestered in erythrocytes (16). In addition, hemolysis also releases erythrocyte arginase, which degrades arginine, the sole substrate for NO synthesis, thus causing a further reduction of endothelial NO (17). Another important factor for ECMO patients is that NO also hinders coagulation. NO chemically modifies and inhibits Factor XIII, (fibrin stabilizing factor) retarding clot formation (17). Elevated levels of PfHg and the infusion of cross-linked hemoglobin drastically reduce NO levels resulting in increased platelet adhesion and aggregation on prothrombotic surfaces such as the extracorporeal circuit (17). Thus, elevated levels of hemolysis and PfHg contribute to morbidity and mortality while on extracorporeal support, specifically in the neonatal PPHN population.

Previous studies examining PfHg generation during ECMO, such as Williams et al. (3), were limited to shorter durations. Our study with 24-hour continuous flow at three different rates was designed to better mimic clinical ECMO situations and allow us to measure cumulative PfHg generation (17). The effect of increased pressure drops for extended periods on erythrocyte stability was also important to evaluate. With the recent rapid advancements in oxygenator development, available designs vary greatly in an effort to balance performance, efficiency and ease of use.

This study was limited to 24 hours and a longer time interval would be helpful in further demonstrating the long-term clinical impact of a higher pressure drop oxygenator. The study design included three circuits in each flow rate group; however, the SD in the 3 LPM control and the 5 LPM at 24 hours were large and may have led to the discrepancy

Table 3. Platelet count and platelet function in ECMO circuits at baseline and 24 hours at each of three flow rates.

Time	1 LPM Circuits (n = 3)	3 LPM Circuits (n = 3)	5 LPM Circuits (n = 3)
Platelet count			
At baseline	141 ± 19	215 ± 0	152 ± 0
At 24 hours	115 ± 25	149 ± 27	120 ± 0
Platelet function (Col/EPI)			
At baseline	250 ± 35	223 ± 0	187 ± 0
At 24 hours	267 ± 34	237 ± 21	244 ± 0
Platelet function (Col/ADP)			
At baseline	254 ± 19	231 ± 0	227 ± 0
At 24 hours	231 ± 29	230 ± 21	270 ± 0

Numbers reported in thousands/mm^3, Mean ± SE. LPM, liters per minute; Col/EPI, collagen and epinephrine; Col/ADP, collagen and ADP.

of PfHg generation of the 3 LPM group. Additional studies are needed with additional oxygenators to compare PfHb generation.

Despite reducing available choices, design differences remain. Perceptions about these differences are not always accurate and should be tested. As we have demonstrated in this study using the LivaNova EOS PMP™ oxygenator, an oxygenator with a higher pressure drop does not always increase shear stresses or hemolysis. Users should consider internal design elements (e.g., port configuration, manifolds that increase and decrease flow, blood path length, oxygenator, and heat exchanger blood path) when selecting a device for a particular application. Indeed, the longer blood flow path design in the EOS PMP™ may mitigate the effect of higher pressure drops with regard to PfHg generation, even at elevated flow rates.

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