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Hemolysis during Pediatric Extracorporeal Membrane Oxygenation: Associations with Circuitry, Complications, and Mortality

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Abstract

Objective: To describe factors associated with hemolysis during pediatric extracorporeal membrane oxygenation (ECMO), and the relationships between hemolysis, complications and mortality.

Design: Secondary analysis of data collected prospectively by the Collaborative Pediatric Critical Care Research Network (CPCCRN) between December 2012 and September 2014.

Setting: Three CPCCRN-affiliated hospitals.

Patients: Age <19 years and treated with ECMO.

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Interventions: None.

Measurements and Main Results: Hemolysis was defined based on peak plasma free hemoglobin levels during ECMO and categorized as none (<0.001 g/L), mild (0.001 to <0.5 g/L), moderate (0.5 to <1.0 g/L) or severe (≥ 1.0 g/L). Of 216 patients, 4 (1.9%) had no hemolysis, 67 (31.0%) had mild, 51 (23.6%) had moderate, and 94 (43.5%) had severe. On multivariable analysis, variables independently associated with higher daily plasma free hemoglobin concentration included the use of in-line hemofiltration or other continuous renal replacement therapy (CRRT), higher hemoglobin concentration, higher total bilirubin concentration, lower mean heparin infusion dose, lower body weight, and lower platelet count. Using multivariable Cox modeling, daily plasma free hemoglobin was independently associated with development of renal failure during ECMO (defined as creatinine >2 mg/dL (>176.8 $\mu\text{mol/L}$) or use of in-line hemofiltration or CRRT) (hazard ratio 1.04, 95% CI 1.02, 1.06, $p<0.001$), but not mortality (hazard ratio 1.01, 95% CI 0.99, 1.04, $p=0.389$).

Conclusions: Hemolysis is common during pediatric ECMO. Hemolysis may contribute to the development of renal failure, and therapies used to manage renal failure such as in-line hemofiltration and other forms of CRRT may contribute to hemolysis. Hemolysis was not associated with mortality after controlling for other factors. Monitoring for hemolysis should be a routine part of ECMO practice, and efforts to reduce hemolysis may improve patient care.

Keywords

Extracorporeal Membrane Oxygenation; hemolysis; plasma free hemoglobin; child; infant; neonate

INTRODUCTION

Extracorporeal membrane oxygenation (ECMO) is an invasive treatment modality used for patients with respiratory and cardiac failure refractory to maximal medical therapy. Despite technologic advances, hemostatic complications remain common during pediatric ECMO (1). Hemolysis, the lysis of red blood cells and subsequent release of hemoglobin into the plasma, remains a major problem (1–5). Hemolysis is measured by elevated plasma free hemoglobin (PFH) concentration. Hemolysis occurs due to mechanical trauma and complement activation during ECMO (6–9). The frequency of hemolysis is likely under-recognized and underreported because PFH is not routinely measured at many centers (10). In the Collaborative Pediatric Critical Care Research Network (CPCCRN) Bleeding and Thrombosis during ECMO (BATE) study, 32.9% of the entire cohort had hemolysis and at sites where PFH was routinely measured, 57.5% had hemolysis (1). Free hemoglobin in the plasma is cytotoxic, causes endothelial dysfunction, and consumes nitric oxide leading to vasoconstriction (9, 11). Hemolysis during ECMO has been associated with renal injury, need for renal replacement therapy, thrombotic events, need for circuit component change, and mortality (1, 5, 8, 9).

Risk factors for hemolysis are likely multifactorial but reports delineating these factors have been inconsistent. Concerns have been raised about pump-related factors (oxygenator type, venous inlet pressure, pump speed, cavitation, priming solution) (5, 8–9, 11–14) and patient-

related factors (high hemoglobin) (15, 16). Most reports of hemolysis during pediatric ECMO are in vitro laboratory simulations, retrospective single-center audits, or based on Extracorporeal Life Support Organization (ELSO) registry data. Prospective, multicenter data are needed to gain more accurate and generalizable knowledge. The objectives of this study were to describe factors associated with hemolysis during pediatric ECMO, and the relationships between hemolysis, complications, and mortality.

METHODS

Design and Setting

The study was a secondary analysis of data from the BATE study (1) which described the incidence of bleeding and thrombosis in neonatal and pediatric ECMO patients. The BATE study collected prospective observational data at eight CPCCRN-affiliated hospitals between December 2012 and September 2014. Of these eight sites, only three routinely measured PFH during ECMO on at least 80% of study days; therefore, only these three sites were included in this analysis. The Institutional Review Boards for each hospital and the Data Coordinating Center at the University of Utah approved the study with waiver of parental permission.

Study Subjects

All patients <19 years old treated with ECMO in a neonatal, pediatric or cardiac intensive care unit (ICU) were included in the BATE study (n=514) (1). Only the initial ECMO course was included for patients who required multiple courses of ECMO. The three sites contributing data to this analysis recruited 218 patients. Two of these patients had no PFH measurements and were excluded, leaving 216 patients.

Data Collection

Research coordinators collected data daily via direct observation, discussion with bedside clinicians, and review of medical records. Pre-ECMO data included demographics; body weight; history of prematurity; acute and chronic diagnoses; occurrence of an operative procedure or cardiopulmonary bypass (CPB) in the 24 hours prior to ECMO initiation; primary indication for ECMO; placement on ECMO directly from CPB or via an ex utero intrapartum treatment (EXIT) procedure; and clinical site. Demographics included age at ECMO initiation, sex, race and ethnicity. Age was categorized as neonate 30 days, infant >30 days to 12 months, child >1 year to 12 years, and adolescent >12 years to <19 years. Prematurity was <37 weeks gestational age at birth and collected for neonates only. Primary indication for ECMO was categorized as respiratory, cardiac or extracorporeal cardiopulmonary resuscitation (ECPR).

ECMO set-up and management data included mode of ECMO; type of pump; use of a venous reservoir; circuit or oxygenator biocompatibility coating; circuit priming method; heparin bolus dose for cannulation; total daily heparin dose; ECMO flow rates; use of therapeutic hypothermia; transfusion volumes; use of plasmapheresis; use of in-line hemofiltration or other continuous renal replacement therapy (CRRT); and location of ECMO within the hospital. Mode of ECMO was categorized as venoarterial (VA) or

venovenous (VV). VV ECMO that was converted to VA was categorized as VA ECMO. Type of pump was categorized as roller head or centrifugal. Circuit priming method was categorized as blood or non-blood (clear) prime. ECMO flow rate and core body temperature were collected daily at 7 AM. Because the use of therapeutic hypothermia was not recorded in the BATE study, therapeutic hypothermia was defined as a core body temperature $\geq 34^{\circ}\text{C}$ for two consecutive days during the first three days of ECMO (17). Transfusion volumes included daily volumes of red blood cells, platelets and fresh frozen plasma administered. Location of ECMO was neonatal, pediatric or cardiac ICU.

Laboratory data included arterial blood gases, complete blood count, blood urea nitrogen (BUN), creatinine, total bilirubin, lactate, prothrombin time (PT), partial thromboplastin time (PTT), international normalization ratio (INR), fibrinogen, activating clotting time (ACT), antithrombin III (ATIII), anti-factor Xa, and PFH. Baseline laboratory values were obtained closest and prior to ECMO initiation; daily laboratory values were obtained closest to 7 AM on each ECMO day. Hemolysis was defined based on peak PFH levels and categorized as none (<0.001 g/L), mild (0.001 to <0.5 g/L), moderate (0.5 to <1.0 g/L) or severe (≥ 1.0 g/L).

Outcomes included complications during ECMO; duration of ECMO, ICU and hospital stay; and in-hospital mortality. Complications included bleeding events, thrombotic events, neurologic events, hepatic dysfunction, renal failure, and new infection. Bleeding events were defined as blood loss requiring a transfusion or intracranial hemorrhage. Thrombotic events included intracranial infarction, limb ischemia, pulmonary embolus, intracardiac thrombus, aorto-pulmonary shunt thrombus, other sites of thrombosis, and circuit thrombosis requiring replacement of a circuit component. Neurologic events included seizures, intracranial hemorrhage or infarction, and brain death. Hepatic dysfunction was defined as an INR >2 . Renal failure was defined as a creatinine >2 mg/dL (>176.8 $\mu\text{mol/L}$) or use of in-line hemofiltration or other form of CRRT. New infection was defined as a new culture- or polymerase chain reaction-proven infection diagnosed after ECMO initiation.

Statistical Analysis

Demographics, pre-ECMO status, ECMO system setup and management, and outcome variables were summarized by peak level of hemolysis (Tables 1, 2, 3, and 5, respectively). Counts and percentages are reported for categorical variables while the median and interquartile range are reported for continuous variables. Percentages are based on row totals. P-values for the associations of variables with peak hemolysis level were based on statistical tests that take advantage of the ordered nature of the peak hemolysis categories. The Cochran-Armitage trend test was used for binary variables, the Kruskal-Wallis test for nominal variables with more than two levels, and the Jonckheere-Terpstra test for continuous variables.

Multivariable models for daily PFH, renal failure, and mortality on ECMO are presented in Tables 4, 6, and 7 respectively. A multivariable model was developed for each of these three outcomes independently. Multivariable model selection was done in two steps. First, univariable models were created for each candidate predictor. Variables were considered potential predictors if they were associated with the outcome being modeled in univariable

analysis ($p < 0.10$) and available for at least 90% of the study days (Supplemental Digital Content 1, 2, and 3). Second, the final model for each outcome was selected using bi-directional stepwise selection on the potential predictors with a significance criterion of $p < 0.05$ to enter and stay in the final model. PFH was forced into the final multivariable models of renal failure and mortality on ECMO, but no variables were forced into the final multivariable model of daily PFH.

Daily PFH was modeled with linear regression using the identity link function, Gaussian errors, and robust error estimates (Table 4). In order to account for the temporality of predictor variables with PFH, PFH was considered as a daily outcome and was modeled based on patient factors and status on that day. For example, we demonstrated that PFH was 16.3 mg/dL higher on days when hemofiltration or CRRT was used. An autoregressive covariance structure of order 1 was specified to account for correlation between PFH on different study days from the same subject. In particular, this accounts for a higher correlation between PFH measurements on study days that are temporally close together but relatively lower correlation between PFH measurements on study days that are far apart. Mortality and renal failure modeling also incorporated the temporality of predictor variables by using time-varying covariates in the Cox models. This allows the models to account for mortality and renal failure hazards that change from day to day corresponding with changes in the predictor variables (Table 6 and 7). Daily data collection was discontinued after decannulation, which leads to censoring in the model of mortality. In particular, deaths occurring after the calendar day of decannulation are not considered by this model. All reported p -values were based on two-sided alternatives and considered statistically significant if less than 0.05. Analyses were performed using SAS 9.4 (SAS Institute; Cary, NC).

RESULTS

Of 216 patients, 4 (1.9%) had no hemolysis, 67 (31.0%) had mild, 51 (23.6%) had moderate, and 94 (43.5%) had severe hemolysis during ECMO. Neonatal age group, Asian race, and lower body weight were associated with increased peak level of hemolysis (Table 1). Congenital diaphragmatic hernia and persistent pulmonary hypertension of the newborn were also associated with increased peak level of hemolysis (Table 2). Regarding ECMO system set-up, use of a roller head pump and blood circuit prime were associated with increased peak level of hemolysis (Table 3). Regarding ECMO management, use of in-line hemofiltration or other form of CRRT, higher mean daily ECMO flow rate, higher mean daily heparin dose, and higher mean daily red blood cell, platelet and plasma transfusion volumes were associated with increased peak level of hemolysis (Table 3).

Univariable associations with daily PFH during ECMO are shown in Supplemental Digital Content 1. On multivariable analysis, variables independently associated with higher daily PFH (Table 4) included the use of in-line hemofiltration or CRRT, higher hemoglobin, higher total bilirubin, lower heparin infusion dose, lower body weight, lower platelet count, presence of an acute neurologic condition, and absence of acute non-septic shock, chronic immune dysfunction and chronic neurologic conditions.

Complications and outcomes by peak level of hemolysis are shown in Table 5. Bleeding events, thrombotic events, neurologic events, hepatic dysfunction, and renal failure were associated with increased peak level of hemolysis. Longer duration of ECMO and in-hospital mortality were also associated with increased peak level of hemolysis.

Univariable Cox models of renal failure are shown in Supplemental Digital Content 2. On multivariable Cox analysis, variables independently associated with renal failure during ECMO included higher PFH, higher hemoglobin, higher lactate, and location of ECMO in a pediatric ICU (Table 6). Univariable Cox models of mortality are shown in Supplemental Digital Content 3. Variables independently associated with mortality included ECPR as the indication for ECMO, higher red blood cell transfusion volume, lower heparin infusion dose and lower pH (Table 7). Mortality was not associated with PFH on multivariable analysis.

DISCUSSION

Nearly all pediatric patients in our ECMO cohort had some degree of hemolysis, and 67.1% had moderate to severe hemolysis (PFH ≥ 0.5 g/L). Using the ELSO registry, O'Brien et al reported hemolysis (PFH >0.5 g/L) occurring in 10.6% of pediatric ECMO runs between 2010 and 2015 (18). In a single center retrospective review, Lou et al reported hemolysis (< 0.5 g/L) in 19.3% of pediatric ECMO patients between 2005 and 2011 (5). Differences in study design, and the frequency and consistency of obtaining PFH measurements likely account for the differences in rates observed. Patient-related factors, ECMO set-up and management factors, and various laboratory values were found to be associated with the severity of hemolysis during ECMO in our study. Hemolysis was also found to be associated with several complications during ECMO including renal failure. Whether hemolysis results in renal failure or occurs as a consequence of treatment modalities such as CRRT could not be determined. Hemolysis was not associated with mortality after controlling for other factors.

Among patient-related factors, lower body weight was independently associated with higher daily PFH in our study. This finding on multivariable analysis is consistent with the neonatal and infant age groups being associated with higher daily PFH on univariable analyses. Lower body weight is likely associated with increased fetal red blood cells which show a greater susceptibility to mechanical stress than adult red blood cells (19). The mechanism for greater hemolysis with lower body weight may also be related to more shear stress with flows through smaller caliber cannulas and blood vessels (19–21). Other patient-related factors such as various acute and chronic diagnoses were also found to be independently associated with daily PFH levels. Establishing reasons for these associations is outside the scope of this report; some may represent spurious findings.

Aspects of ECMO set-up and management were associated with PFH levels. Lower heparin infusion dose adjusted for body weight (units/kg/min) was independently associated with higher daily PFH. Higher heparin infusion doses could reflect a more aggressive management style with some centers titrating heparin dose to higher levels to achieve better anticoagulation, or to be consistent with laboratory monitoring algorithms. Another possibility is that the non-anticoagulant effects of heparin including anti-inflammatory

properties, inhibition of reactive oxygen species generation, tissue protection and repair properties, and cardiovascular protective effects also decrease hemolysis (22, 23). Use of in-line hemofiltration or other forms of CRRT was independently associated with higher daily PFH levels. These therapies may contribute to hemolysis by diverting venous flow away from the ECMO circuit thereby contributing to negative inlet pressure (5), providing additional areas of turbulent flow at connector sites, or increasing red cell destruction by mechanical stresses within the CRRT system. CRRT itself is associated with hemolysis (24). Unlike previous reports we did not find a clear association between hemolysis and use of centrifugal or roller head pumps (18, 25); however, roller head pumps were used in only 15 (6.9%) patients. Of 201 patients with centrifugal pumps, over 60% had moderate to severe hemolysis based on peak PFH levels. Using the ELSO registry, O'Brien et al found more hemolysis with centrifugal than roller pumps although hemolysis was reported less frequently in the ELSO registry overall (18). Other potential factors for differences include the type of centrifugal pump used (older versions were known to be associated with hemolysis), and the fact that many patients during the time period in the ELSO report received centrifugal support following cardiac arrest or cardiac surgery. Such factors may also influence hemolysis regardless of pump type.

Higher hemoglobin concentration during ECMO was independently associated with higher daily PFH consistent with a recent single center report (16). Higher hemoglobin levels increase blood viscosity (26–28) which may result in more red cell damage as blood traverses the ECMO pump head and oxygenator. In adults, increased hemoglobin and blood viscosity have been associated with cardiovascular and cerebrovascular ischemic events (26–28). Although hemoglobin level was an independent predictor of PFH in our study, the volume of red blood cells transfused was not an independent predictor. Hemoglobin level and red cell transfusion volume are clinically interrelated; however, our findings suggest that hemoglobin level is the stronger predictor of hemolysis. On the other hand, the volume of red blood cells transfused and not daily PFH level was an independent predictor of mortality. Red cell transfusion likely contributes to mortality by mechanisms other than or in addition to hemolysis such as transfusion-related immune dysfunction or lung injury (29–31). The optimal hemoglobin level for pediatric ECMO patients is unclear but additional studies focused on transfusion thresholds may improve rates of hemolysis and mortality.

Higher bilirubin concentration and lower platelet count were independently associated with higher daily PFH levels. Hyperbilirubinemia is a known complication of ECMO and hemolysis contributes to an increase in bilirubin production (32–34). At high levels, bilirubin can induce apoptosis, inflammation and oxidative stress which can lead to thrombocytopenia (32, 35). In addition, hemoglobin-mediated nitric oxide scavenging and reduced plasma nitric oxide can cause thrombocytopenia (11, 36). Therefore, whereas higher hemoglobin may predispose to hemolysis, higher bilirubin level and lower platelet count likely occur as a result of hemolysis.

Our findings suggest that hemolysis is associated with complications during ECMO including renal failure. PFH in sufficient amounts can be damaging to the kidney and other organs because of its bioreactivity and pro-oxidant effects (37). Similar to our findings, others have shown that PFH predicts acute renal failure during VA ECMO (36, 38).

Hemolysis during combined ECMO and CRRT has been shown to be increased compared to ECMO alone (39). These reciprocal relationships suggest that use of in-line hemofiltration or CRRT may contribute to worsening renal failure by promoting hemolysis, although the extent is difficult to determine. Our findings also suggest associations between hemolysis and bleeding and thrombotic events, other organ failures, and duration of ECMO. Thrombosis may also be a cause of elevated PFH complicating our understanding of the relationship between hemolysis and thrombotic events.

Hemolysis was not an independent predictor of mortality in our multivariable Cox model; this is in contrast to other research suggesting an association between hemolysis and mortality (5).

PFH was not routinely monitored across all eight CPCCRN-affiliated centers. Consistent monitoring and further inspection of site-specific factors such as circuit set-up, laboratory testing or anticoagulation algorithms may identify best practices that can be prospectively evaluated. The ability to refine ECMO practices to reduce hemolysis and associated morbidities would benefit the field.

Strengths of this study include the multicenter design and daily prospective collection of data. Limitations include recording the PFH levels and other daily data (i.e., laboratory studies, body temperature, and ECMO flow rate) that were obtained closest to 7 AM rather than all values, and the lack of a standardized protocol for the timing and frequency of PFH levels. While our definition of renal failure was a creatinine level of >2 mg/dL (>176.8 $\mu\text{mol/L}$), it also included the use of in-line hemofiltration, which some practitioners employ to manage fluid status even in the absence of renal failure. This factor is another potential limitation. Missing data for some variables prevented their inclusion in multivariable models. Although many variables were evaluated, potential unmeasured confounders exist. Importantly, this is an observational study and the associations observed do not infer causation.

CONCLUSIONS

Our findings suggest that nearly all pediatric patients undergoing ECMO have some degree of hemolysis. Hemolysis may contribute to the development of renal failure, and therapies used to manage renal failure such as in-line hemofiltration and CRRT may contribute to hemolysis. Hemolysis was also associated with other morbidities. Our findings suggest that monitoring for hemolysis should be a routine component of ECMO practice, and efforts to reduce hemolysis may improve patient care.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1.

Demographic by Peak Level of Hemolysis

Variable ^a	Peak Hemolysis				P-value
	None (N = 4)	Mild (N = 67)	Moderate (N = 51)	Severe (N = 94)	
Age					<.001 ^b
Pre-Term Neonate	0 (0.0%)	8 (27.6%)	3 (10.3%)	18 (62.1%)	
Full-Term Neonate	0 (0.0%)	14 (17.3%)	25 (30.9%)	42 (51.9%)	
Infant	1 (1.8%)	19 (33.3%)	14 (24.6%)	23 (40.4%)	
Child	3 (10.3%)	14 (48.3%)	6 (20.7%)	6 (20.7%)	
Adolescent	0 (0.0%)	12 (60.0%)	3 (15.0%)	5 (25.0%)	
Male	3 (2.4%)	36 (28.8%)	26 (20.8%)	60 (48.0%)	0.284 ^c
Race					0.007 ^d
Asian	0 (0.0%)	0 (0.0%)	3 (23.1%)	10 (76.9%)	
Black or African American	2 (4.5%)	18 (40.9%)	11 (25.0%)	13 (29.5%)	
White	2 (1.9%)	32 (30.5%)	21 (20.0%)	50 (47.6%)	
Unknown or Not Reported	0 (0.0%)	17 (31.5%)	16 (29.6%)	21 (38.9%)	
Ethnicity					0.388 ^d
Hispanic or Latino	1 (2.6%)	13 (33.3%)	11 (28.2%)	14 (35.9%)	
Not Hispanic or Latino	3 (2.4%)	38 (30.9%)	30 (24.4%)	52 (42.3%)	
Unknown or Not Reported	0 (0.0%)	16 (29.6%)	10 (18.5%)	28 (51.9%)	
Weight (kg)	13.2 [8.1, 17.7]	6.7 [3.1, 26.0]	3.7 [2.8, 6.3]	3.2 [3.0, 4.3]	<.001 ^b

^aVariables reported had no missing values.^bJonckheere-Terpstra test.^cCochran-Armitage trend test.^dKruskal-Wallis test.

Table 2.

Pre-ECMO Status by Peak Level of Hemolysis

Variable ^{a,b}	Peak Hemolysis				P-value
	None (N = 4)	Mild (N = 67)	Moderate (N = 51)	Severe (N = 94)	
Primary ECMO indication					0.851 ^f
Respiratory	1 (1.0%)	34 (34.3%)	16 (16.2%)	48 (48.5%)	
Cardiac	2 (2.2%)	24 (25.8%)	33 (35.5%)	34 (36.6%)	
ECPR ^c	1 (4.2%)	9 (37.5%)	2 (8.3%)	12 (50.0%)	
Meconium aspiration syndrome	0 (0.0%)	3 (17.6%)	4 (23.5%)	10 (58.8%)	0.123 ^g
Congenital diaphragmatic hernia	0 (0.0%)	2 (9.1%)	1 (4.5%)	19 (86.4%)	<.001 ^g
Persistent pulmonary hypertension of the newborn	0 (0.0%)	3 (10.0%)	10 (33.3%)	17 (56.7%)	0.013 ^g
Operative procedure in the 24 hours prior to ECMO initiation	1 (1.2%)	30 (34.9%)	27 (31.4%)	28 (32.6%)	0.074 ^g
CPB ^d in the 24 hours prior to ECMO	1 (1.4%)	21 (30.4%)	24 (34.8%)	23 (33.3%)	0.326 ^g
Placed on ECMO directly from CPB ^d	0 (0.0%)	6 (17.6%)	12 (35.3%)	16 (47.1%)	0.146 ^g
Placed on ECMO via EXIT ^e procedure	0 (0.0%)	1 (100.0%)	0 (0.0%)	0 (0.0%)	0.226 ^g
Baseline pH in arterial blood	7.3 [7.2, 7.6]	7.3 [7.1, 7.3]	7.3 [7.1, 7.4]	7.3 [7.1, 7.4]	0.967 ^h
Baseline lactate (mmol/L)	3.6 [1.8, 7.9]	4.0 [1.6, 8.0]	3.0 [1.9, 8.0]	5.4 [1.8, 8.2]	0.328 ^h
Baseline creatinine (mg/dL) (μmol/L)	0.6 [0.3, 0.7] 53 [26, 62]	0.6 [0.5, 1.0] 53 [44, 88]	0.7 [0.5, 0.9] 62 [44, 79]	0.5 [0.4, 0.8] 44 [35, 71]	0.188 ^h
Baseline blood urea nitrogen (mg/dL) (mmol/L)	28 [7, 30] 10.0 [2.5, 10.7]	15 [10, 24] 5.3 [3.5, 8.5]	18 [13, 23] 6.4 [4.6, 8.2]	17 [9, 26.] 6.0 [3.2, 9.2]	0.579 ^h

^aPercentages reported are based on row totals.

^bpH, blood urea nitrogen, creatinine, and lactate had missingness rates of 13%, 19%, 19%, and 17% respectively; other variables had no missing values.

^cECPR is extracorporeal cardiopulmonary resuscitation.

^dCPB is cardiopulmonary bypass.

^eEXIT is ex utero intrapartum treatment.

^fKruskal-Wallis test.

^gCochran-Armitage trend test.

^hJonckheere-Terpstra test.

Table 3.**ECMO System Setup and Management by Peak Level of Hemolysis**

Variable ^a	Peak Hemolysis				P-value
	None (N = 4)	Mild (N = 67)	Moderate (N = 51)	Severe (N = 94)	
ECMO system Setup					
Mode of ECMO					0.921 ^b
Venoarterial	3 (1.6%)	56 (29.9%)	50 (26.7%)	78 (41.7%)	
Venovenous	1 (3.4%)	11 (37.9%)	1 (3.4%)	16 (55.2%)	
Type of Pump					0.047 ^b
Roller Head	0 (0.0%)	0 (0.0%)	7 (46.7%)	8 (53.3%)	
Centrifugal	4 (2.0%)	67 (33.3%)	44 (21.9%)	86 (42.8%)	
Setup includes bladder / venous reservoir	4 (2.9%)	39 (27.9%)	31 (22.1%)	66 (47.1%)	0.290 ^b
Oxygenator biocompatibility coating	4 (1.9%)	64 (30.8%)	49 (23.6%)	91 (43.8%)	0.778 ^b
Circuit tubing biocompatibility coating	4 (3.2%)	33 (26.8%)	28 (22.8%)	58 (47.1%)	0.346 ^b
Method for priming the circuit					0.009 ^b
Non-blood (clear)	1 (5.6%)	10 (55.6%)	3 (16.7%)	4 (22.2%)	
Blood	3 (1.5%)	57 (28.8%)	48 (24.2%)	90 (45.5%)	
Heparin bolus for cannulation	4 (2.2%)	61 (33.3%)	39 (21.3%)	79 (43.2%)	0.201 ^b
Heparin bolus dose (IU/kg) ^c	50.0 [50.0, 75.0]	100.0 [50.0, 100.0]	75.0 [1.0, 100.0]	89.0 [50.0, 100.0]	0.420 ^d
ECMO Management ^e					
Plasmapheresis	0 (0.0%)	4 (21.1%)	5 (26.3%)	10 (52.6%)	0.248 ^b
In-line hemofiltration	1 (1.5%)	7 (10.8%)	17 (26.2%)	40 (61.5%)	<.001 ^b
Continuous renal replacement therapy	0 (0.0%)	10 (15.9%)	16 (25.4%)	37 (58.7%)	<.001 ^b
Therapeutic hypothermia	0 (0.0%)	3 (30.0%)	2 (20.0%)	5 (50.0%)	0.687 ^b
Mean daily ECMO flow rate (mL/kg/min)	62.3 [56.4, 88.0]	89.2 [71.6, 103.9]	93.3 [79.3, 112.5]	102.8 [89.1, 120.5]	<.001 ^d
Mean daily RBC transfusion (mL/kg)	12.9 [10.8, 15.2]	27.3 [12.6, 58.9]	35.5 [23.4, 57.3]	35.8 [26.8, 58.7]	0.007 ^d
Mean daily heparin dose (units/kg/min)	0.4 [0.3, 0.5]	0.3 [0.2, 0.4]	0.3 [0.2, 0.5]	0.4 [0.3, 0.6]	<.001 ^d
Mean daily platelet transfusion (mL/kg)	3.1 [2.2, 3.9]	8.7 [4.6, 14.6]	16.2 [11.7, 23.8]	19.8 [12.6, 28.3]	<.001 ^d
Mean daily plasma transfusion (mL/kg)	6.4 [2.7, 9.0]	5.3 [1.3, 14.7]	8.7 [3.0, 17.9]	8.8 [5.0, 16.4]	0.010 ^d

^aVariables reported had no missing values.^bCochran-Armitage trend test.^cHeparin bolus dose (IU/kg) is summarized only for those who receive heparin.^dJonckheere-Terpstra test.^eA limitation is that the timing of intervention in relation to hemolysis is not considered.

Table 4.**Multivariable Model for Plasma Free Hemoglobin**

Variable ^a	Plasma Free Hemoglobin (0.01 g/L)	
	Effect (95% CI)	P-value
Chronic neurologic condition	−11.84 (−21.88, −1.81)	0.021
Chronic immune dysfunction	−30.35 (−47.42, −13.29)	<.001
Acute non-septic shock	−33.79 (−51.87, −15.72)	<.001
Acute neurologic condition	52.38 (9.05, 95.71)	0.018
Hemofiltration or CRRT ^b	16.30 (3.23, 29.37)	0.014
Total bilirubin (mg/dL) (17.10 μmol/L)	4.15 (3.19, 5.12)	<.001
Hemoglobin (g/dL) (10 g/L)	6.01 (3.51, 8.50)	<.001
Weight (kg)	−0.34 (−0.51, −0.17)	<.001
Heparin (units/kg/min)	−35.64 (−58.10, −13.18)	0.002
Platelets (10 ³ /μL)	−0.15 (−0.27, −0.03)	0.016

^a Rates of missingness for all predictors considered for modeling are included in Supplemental Digital Content 1. The outcome, plasma free hemoglobin, was missing on 8% of study days.

^b CRRT is continuous renal replacement therapy

Table 5.

Complications and Outcomes by Peak Level of Hemolysis

Variable ^{a,b}	Peak Hemolysis				P-value
	None (N = 4)	Mild (N = 67)	Moderate (N = 51)	Severe (N = 94)	
Thrombocytopenia	0 (0.0%)	21 (25.6%)	21 (25.6%)	40 (48.8%)	0.067 ^c
New documented infection	1 (1.7%)	17 (29.3%)	14 (24.1%)	26 (44.8%)	0.746 ^c
Bleeding event	3 (1.7%)	47 (26.9%)	42 (24.0%)	83 (47.4%)	0.005 ^c
Thrombotic event	0 (0.0%)	19 (17.9%)	28 (26.4%)	59 (55.7%)	<.001 ^c
Neurologic organ failure	1 (1.4%)	15 (20.5%)	17 (23.3%)	40 (54.8%)	0.008 ^c
Renal organ failure	1 (1.1%)	13 (14.0%)	22 (23.7%)	57 (61.3%)	<.001 ^c
Hepatic organ failure	1 (1.2%)	20 (23.3%)	21 (24.4%)	44 (51.2%)	0.026 ^c
Duration of ECMO (days)	4.3 [2.8, 5.8]	3.7 [2.5, 6.2]	5.4 [2.9, 9.0]	8.6 [4.3, 12.3]	<.001 ^d
Length of ICU stay (days)	33.3 [16.3, 107.0]	29.6 [14.1, 48.2]	29.9 [17.1, 48.9]	29.4 [15.7, 50.8]	0.692 ^d
Length of hospital stay (days)	40.7 [16.3, 127.8]	46.1 [18.7, 83.4]	41.6 [19.9, 77.9]	36.4 [16.1, 61.1]	0.210 ^d
In-hospital mortality	1 (1.0%)	24 (23.3%)	26 (25.2%)	52 (50.5%)	0.010 ^c

^aVariables reported had no missing values.

^bA limitation in this table is that the timing of some outcomes relative to hemolysis is not considered.

^cCochran-Armitage trend test.

^dJonckheere-Terpstra test.

Table 6.**Multivariable Cox Model of Renal Failure**

Variable^a	Renal Failure	
	Hazard ratio (95% CI)	P-value
Plasma free hemoglobin (for each 0.1 g/L increase)	1.04 (1.02, 1.06)	<.001
Location of ECMO care		0.014
PICU	1.28 (0.60, 2.75)	
NICU	0.36 (0.17, 0.76)	
CICU	Reference	
Hemoglobin (g/dL) (10 g/L)	1.20 (1.03, 1.40)	0.019
Lactate (mmol/L)	1.06 (1.00, 1.11)	0.035

This analysis includes only the 184 subjects without renal failure at baseline; of those, 59 developed renal failure. In addition to the variables used in the model for plasma free hemoglobin, this also uses daily bleeding and thrombosis as potential predictors and forces plasma free hemoglobin in as a predictor. Importantly, need for in-line hemofiltration and continuous renal replacement therapy is not considered as a potential predictor.

^aRates of missingness for all predictors considered for modeling are included in Supplemental Digital Content 2. The outcome, renal failure, was never missing.

Table 7.**Multivariable Cox Model of Morality**

Variable^a	Mortality	
	Hazard ratio (95% CI)	P-value
Plasma free hemoglobin (for each 0.1 g/L increase)	1.01 (0.99, 1.04)	0.389
Primary ECMO indication		0.001
Respiratory	Reference	
Cardiac	1.56 (0.70, 3.44)	
ECPR ^b	5.35 (2.15, 13.33)	
Heparin (0.01 units/kg/min)	0.94 (0.92, 0.96)	<.001
Red blood cells transfused (10 mL/kg)	1.02 (1.01, 1.04)	0.009
pH in arterial blood (0.05 increase)	0.72 (0.58, 0.90)	0.003

In addition to the variables used in the model for plasma free hemoglobin, this also uses daily bleeding and thrombosis as potential predictors and forces plasma free hemoglobin in as a predictor. The population at risk consists of subjects while on ECMO and thus does not capture death occurring after the last day of ECMO.

^aRates of missingness for all predictors considered for modeling are included in Supplemental Digital Content 3. The outcome, mortality, was never missing.

^bECPR is extracorporeal cardiopulmonary resuscitation