Hemolysis During Pediatric Extracorporeal Membrane Oxygenation: Associations With Circuitry, Complications, and Mortality

Heidi J. Dalton, MD[1]; Katherine Cashen, DO[2]; Ron W. Reeder, PhD[3]; Robert A. Berg, MD[4]; Thomas P. Shanley, MD[5]; Christopher J. L. Newth, MD[6]; Murray M. Pollack, MD[7]; David Wessel, MD[7]; Joseph Carcillo, MD[8]; Rick Harrison, MD[9]; J. Michael Dean, MD[2]; Kathleen L. Meert, MD[2]; for the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development Collaborative Pediatric Critical Care Research Network (CPCCRN)

[1]Department of Pediatrics, Inova Fairfax Hospital, Falls Church, VA.

[2]Division of Critical Care, Department of Pediatrics, Children's Hospital of Michigan/Wayne State University, Detroit, MI.

[3]Department of Pediatrics, University of Utah, Salt Lake City, UT.

[4]Department of Anesthesiology and Critical Care, Children's Hospital of Philadelphia, Philadelphia, PA.

[5]Department of Pediatrics, Ann & Robert H. Lurie Children's Hospital of Chicago/Northwestern University Feinberg School of Medicine, Chicago, IL.

[6]Department of Anesthesiology and Critical Care Medicine, Children's Hospital Los Angeles, Los Angeles, CA.

[7]Department of Pediatrics, Children's National Medical Center, Washington, DC.

[8]Department of Critical Care Medicine, Children's Hospital of Pittsburgh, Pittsburgh, PA.

[9]Department of Pediatrics, Mattel Children's Hospital UCLA, Los Angeles, CA.

Supplemental digital content is available for this article. Direct URL citations appear in the printed text and are provided in the HTML and PDF versions of this article on the journal's website (http://journals.lww.com/pccmjournal).

Supported, in part, by the following cooperative agreements from the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development, National Institutes of Health, Department of Health and Human Services: U10HD050096, U10HD049981, U10HD049983, U10HD050012, U10HD063108, U10HD063106, U10HD063114, and U01HD049934. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Drs. Dalton's, Reeder's, Berg's, Shanley's, Wessel's, Harrison's, Dean's, and Meert's institutions received funding from the National Institutes of Health (NIH). Drs. Dalton, Reeder, Berg, Shanley, Newth, Pollack, Wessel, Carcillo, Harrison, Dean, and Meert received support for article research from the NIH. Dr. Dalton received funding from Innovative Extracorporeal membrane oxygenation (ECMO) Concepts and Maquet speakers bureau, and she disclosed off-label product use of ECMO equipment. Dr. Shanley received funding from Springer Publishing, Pediatric Academic Societies Operating and Executive Committees, and International Pediatric Research Fund. Dr. Newth received funding from Philips Research North American (consulting services for data analysis). Dr. Carcillo's institution received funding from the National Institute of Child Health and Human Development Collaborative Pediatric Critical Care Research Network U10. Dr. Cashen has disclosed that she does not have any potential conflicts of interest.

For information regarding this article, E-mail: kmeert@med.wayne.edu

DOI: 10.1097/PCC.0000000000001709

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

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

Setting: Three Collaborative Pediatric Critical Care Research Network-affiliated hospitals.

Patients: Age less than 19 years and treated with extracorporeal membrane oxygenation.

Interventions: None.

Measurements and Main Results: Hemolysis was defined based on peak plasma free hemoglobin levels during extracorporeal membrane oxygenation 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, four (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, 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 extracorporeal membrane oxygenation (defined as creatinine > 2 mg/dL [> 176.8 μmol/L] or use of in-line hemofiltration or continuous renal replacement therapy) (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 extracorporeal membrane oxygenation. 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 continuous renal replacement therapy may contribute to hemolysis. Hemolysis was not associated with mortality after controlling for

other factors. Monitoring for hemolysis should be a routine part of extracorporeal membrane oxygenation practice, and efforts to reduce hemolysis may improve patient care. (*Pediatr Crit Care Med* 2018; 19:1067–1076)

Key Words: child; extracorporeal membrane oxygenation; hemolysis; neonate; plasma free hemoglobin

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 RBCs 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 underrecognized 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 frequency 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 less than 19 years old treated with ECMO in a neonatal, pediatric, or cardiac 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 procedure; and clinical site. Demographics included age at ECMO initiation, sex, race, and ethnicity. Age was categorized as neonate less than or equal to 30 days, infant greater than 30 days to less than or equal to 12 months, child greater than 1 year to less than or equal to 12 years, and adolescent greater than 12 years to less than 19 years. Prematurity was less than 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 setup 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 or venovenous. Venovenous ECMO that was converted to venoarterial was categorized as venoarterial ECMO. Type of pump was categorized as roller head or centrifugal. Circuit priming method was categorized as blood or nonblood (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 less than or equal to 34°C for two consecutive days during the first 3 days of ECMO (17). Transfusion volumes included daily volumes of RBCs, 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, creatinine, total bilirubin, lactate, prothrombin time, partial thromboplastin time, international

normalization ratio (INR), fibrinogen, activating clotting time, antithrombin III, antifactor 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 inhospital 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, aortopulmonary 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 greater than 2. Renal failure was defined as a creatinine greater than 2 mg/dL (> 176.8 µmol/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 1–4**). Counts and percentages are reported for categorical variables whereas 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 5–7**. 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

TABLE 1. Demographics by Peak Level of Hemolysis

Variables[a]	Peak Hemolysis None (*n* = 4)	Mild (*n* = 67)	Moderate (*n* = 51)	Severe (*n* = 94)	*p*
Age, *n* (%)					< 0.001[b]
Preterm 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, *n* (%)	3 (2.4)	36 (28.8)	26 (20.8)	60 (48.0)	0.284[c]
Race, *n* (%)					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, *n* (%)	1 (2.6)	13 (33.3)	11 (28.2)	14 (35.9)	
Not Hispanic or Latino, *n* (%)	3 (2.4)	38 (30.9)	30 (24.4)	52 (42.3)	
Unknown or not reported, *n* (%)	0 (0.0)	16 (29.6)	10 (18.5)	28 (51.9)	
Weight (kg), median (IQR)	13.2 (8.1–17.7)	6.7 (3.1–26.0)	3.7 (2.8–6.3)	3.2 (3.0–4.3)	< 0.001[b]

IQR = interquartile range.
[a]Variables reported had no missing values.
[b]Jonckheere-Terpstra test.
[c]Cochran-Armitage trend test.
[d]Kruskal-Wallis test.

TABLE 2. Preextracorporeal Membrane Oxygenation Status by Peak Level of Hemolysis

Variables[a, b]	Peak Hemolysis				p
	None (*n* = 4)	Mild (*n* = 67)	Moderate (*n* = 51)	Severe (*n* = 94)	
Primary ECMO indication, *n* (%)					0.851[c]
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)	
Extracorporeal cardiopulmonary resuscitation	1 (4.2)	9 (37.5)	2 (8.3)	12 (50.0)	
Meconium aspiration syndrome, *n* (%)	0 (0.0)	3 (17.6)	4 (23.5)	10 (58.8)	0.123[d]
Congenital diaphragmatic hernia, *n* (%)	0 (0.0)	2 (9.1)	1 (4.5)	19 (86.4)	< 0.001[d]
Persistent pulmonary hypertension of the newborn, *n* (%)	0 (0.0)	3 (10.0)	10 (33.3)	17 (56.7)	0.013[d]
Operative procedure in the 24 hr prior to ECMO initiation, *n* (%)	1 (1.2)	30 (34.9)	27 (31.4)	28 (32.6)	0.074[d]
CPB in the 24 hr prior to ECMO, *n* (%)	1 (1.4)	21 (30.4)	24 (34.8)	23 (33.3)	0.326[d]
Placed on ECMO directly from CPB, *n* (%)	0 (0.0)	6 (17.6)	12 (35.3)	16 (47.1)	0.146[d]
Placed on ECMO via ex utero intrapartum treatment procedure, *n* (%)	0 (0.0)	1 (100.0)	0 (0.0)	0 (0.0)	0.226[d]
Baseline pH in arterial blood, median (IQR)	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[e]
Baseline lactate (mmol/L), median (IQR)	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[e]
Baseline creatinine (mg/dL), median (IQR)	0.6 (0.3–0.7)	0.6 (0.5–1.0)	0.7 (0.5–0.9)	0.5 (0.4–0.8)	0.188[e]
Baseline creatinine, (μmol/L), median (IQR)	53 (26–62)	53 (44–88)	62 (44–79)	44 (35–71)	
Baseline blood urea nitrogen (mg/dL), median (IQR)	28 (7–30)	15 (10–24)	18 (13–23)	17 (9–26)	0.579[e]
Baseline blood urea nitrogen (mmol/L), median (IQR)	10.0 (2.5–10.7)	5.3 (3.5–8.5)	6.4 (4.6–8.2)	6.0 (3.2–9.2)	

CPB = cardiopulmonary bypass, ECMO = extracorporeal membrane oxygenation, IQR = interquartile range.

[a]Percentages reported are based on row totals.

[b]pH, blood urea nitrogen, creatinine, and lactate had missingness rates of 13%, 19%, 19%, and 17%, respectively; other variables had no missing values.

[c]Kruskal-Wallis test.

[d]Cochran-Armitage trend test.

[e]Jonckheere-Terpstra test.

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 Table 1**, Supplemental Digital Content 1, http://links.lww.com/PCC/A742; **Supplemental Table 2**, Supplemental Digital Content 2, http://links.lww.com/PCC/A743; and **Supplemental Table 3**, Supplemental Digital Content 3, http://links.lww.com/PCC/A744). Second, the final model for each outcome was selected using bidirectional stepwise selection on the potential predictors with a significance criterion of *p* value of less than 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 5). 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 (Tables 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).

TABLE 3. Extracorporeal Membrane Oxygenation System Setup and Management by Peak Level of Hemolysis

Variables[a]	Peak Hemolysis				p
	None (*n* = 4)	Mild (*n* = 67)	Moderate (*n* = 51)	Severe (*n* = 94)	
ECMO system setup					
Mode of ECMO, *n* (%)					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, *n* (%)					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, *n* (%)	4 (2.9)	39 (27.9)	31 (22.1)	66 (47.1)	0.290[b]
Oxygenator biocompatibility coating, *n* (%)	4 (1.9)	64 (30.8)	49 (23.6)	91 (43.8)	0.778[b]
Circuit tubing biocompatibility coating, *n* (%)	4 (3.2)	33 (26.8)	28 (22.8)	58 (47.1)	0.346[b]
Method for priming the circuit					0.009[b]
Nonblood (clear), *n* (%)	1 (5.6)	10 (55.6)	3 (16.7)	4 (22.2)	
Blood, *n* (%)	3 (1.5)	57 (28.8)	48 (24.2)	90 (45.5)	
Heparin bolus for cannulation, *n* (%)	4 (2.2)	61 (33.3)	39 (21.3)	79 (43.2)	0.201[b]
Heparin bolus dose (IU/kg)[c], median (IQR)	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, *n* (%)	0 (0.0)	4 (21.1)	5 (26.3)	10 (52.6)	0.248[b]
In-line hemofiltration, *n* (%)	1 (1.5)	7 (10.8)	17 (26.2)	40 (61.5)	< 0.001[b]
Continuous renal replacement therapy, *n* (%)	0 (0.0)	10 (15.9)	16 (25.4)	37 (58.7)	< 0.001[b]
Therapeutic hypothermia, *n* (%)	0 (0.0)	3 (30.0)	2 (20.0)	5 (50.0)	0.687[b]
Mean daily ECMO flow rate (mL/kg/min), median (IQR)	62.3 (56.4–88.0)	89.2 (71.6–103.9)	93.3 (79.3–112.5)	102.8 (89.1–120.5)	< 0.001[d]
Mean daily RBC transfusion (mL/kg), median (IQR)	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 (U/kg/min), median (IQR)	0.4 (0.3–0.5)	0.3 (0.2–0.4)	0.3 (0.2–0.5)	0.4 (0.3–0.6)	< 0.001[d]
Mean daily platelet transfusion (mL/kg), median (IQR)	3.1 (2.2–3.9)	8.7 (4.6–14.6)	16.2 (11.7–23.8)	19.8 (12.6–28.3)	< 0.001[d]
Mean daily plasma transfusion (mL/kg), median (IQR)	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]

ECMO = extracorporeal membrane oxygenation, IQR = interquartile range.
[a]Variables reported had no missing values.
[b]Cochran-Armitage trend test.
[c]Heparin bolus dose (IU/kg) is summarized only for those who receive heparin.
[d]Jonckheere-Terpstra test.
[e]A limitation is that the timing of intervention in relation to hemolysis is not considered.

RESULTS

Of 216 patients, four (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).

TABLE 4. Complications and Outcomes by Peak Level of Hemolysis

Variables[a, b]	Peak Hemolysis				p
	None (*n* = 4)	Mild (*n* = 67)	Moderate (*n* = 51)	Severe (*n* = 94)	
Thrombocytopenia, *n* (%)	0 (0.0)	21 (25.6)	21 (25.6)	40 (48.8)	0.067[c]
New documented infection, *n* (%)	1 (1.7)	17 (29.3)	14 (24.1)	26 (44.8)	0.746[c]
Bleeding event, *n* (%)	3 (1.7)	47 (26.9)	42 (24.0)	83 (47.4)	0.005[c]
Thrombotic event, *n* (%)	0 (0.0)	19 (17.9)	28 (26.4)	59 (55.7)	< 0.001[c]
Neurologic organ failure, *n* (%)	1 (1.4)	15 (20.5)	17 (23.3)	40 (54.8)	0.008[c]
Renal organ failure, *n* (%)	1 (1.1)	13 (14.0)	22 (23.7)	57 (61.3)	< 0.001[c]
Hepatic organ failure, *n* (%)	1 (1.2)	20 (23.3)	21 (24.4)	44 (51.2)	0.026[c]
Duration of extracorporeal membrane oxygenation (d), median (IQR)	4.3 (2.8–5.8)	3.7 (2.5–6.2)	5.4 (2.9–9.0)	8.6 (4.3–12.3)	< 0.001[d]
Length of ICU stay (d), median (IQR)	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 (d), median (IQR)	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]
Inhospital mortality, *n* (%)	1 (1.0)	24 (23.3)	26 (25.2)	52 (50.5)	0.010[c]

IQR = interquartile range.

[a]Variables reported had no missing values.

[b]A limitation in this table is that the timing of some outcomes relative to hemolysis is not considered.

[c]Cochran-Armitage trend test.

[d]Jonckheere-Terpstra test.

Regarding ECMO system setup, use of a roller head pump and blood circuit prime was 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 RBC, platelet, and plasma transfusion volumes were associated with increased peak level of hemolysis (Table 3).

Complications and outcomes by peak level of hemolysis are shown in Table 4. Bleeding events, thrombotic events, neurologic events, hepatic dysfunction, and renal failure were associated with increased peak level of hemolysis. Longer duration of ECMO and inhospital mortality were also associated with increased peak level of hemolysis.

Univariable associations with daily PFH during ECMO are shown in Supplemental Table 1 (Supplemental Digital Content 1, http://links.lww.com/PCC/A742). On multivariable analysis, variables independently associated with higher daily PFH (Table 5) 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 nonseptic shock, chronic immune dysfunction, and chronic neurologic conditions.

Univariable Cox models of renal failure are shown in Supplemental Table 2 (Supplemental Digital Content 2, http://links.lww.com/PCC/A743). 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 PICU (Table 6). Univariable Cox models of mortality are shown in Supplemental Table 3 (Supplemental Digital Content 3, http://links.lww.com/PCC/A744). Variables independently associated with mortality included ECPR as the indication for ECMO, higher RBC 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 (18) reported hemolysis (PFH > 0.5 g/L) occurring in 10.6% of pediatric ECMO runs between 2010 and 2015. In a single-center retrospective review, Lou et al (5) reported hemolysis (≥ 0.5 g/L) in 19.3% of pediatric ECMO patients between 2005 and 2011. 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 setup 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.

TABLE 5. Multivariable Cox Model of Renal Failure

Variables[a]	Renal Failure Hazard Ratio (95% CI)	*p*
Plasma free hemoglobin (for each 0.1 g/L increase)	1.04 (1.02–1.06)	< 0.001
Location of extracorporeal membrane oxygenation care		0.014
PICU	1.28 (0.60–2.75)	
Neonatal ICU	0.36 (0.17–0.76)	
Cardiac ICU	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

[a]Rates of missingness for all predictors considered for modeling are included in Supplemental Table 2 (Supplemental Digital Content 2, http://links.lww.com/PCC/A743). The outcome, renal failure, was never missing.

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.

TABLE 6. Multivariable Cox Model of Mortality

Variables[a]	Mortality Hazard Ratio (95% CI)	*p*
Plasma free hemoglobin (for each 0.1 g/L increase)	1.01 (0.99–1.04)	0.389
Primary extracorporeal membrane oxygenation indication		0.001
Respiratory	Reference	
Cardiac	1.56 (0.70–3.44)	
Extracorporeal cardiopulmonary resuscitation	5.35 (2.15–13.33)	
Heparin (0.01 U/kg/min)	0.94 (0.92–0.96)	< 0.001
RBCs 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

[a]Rates of missingness for all predictors considered for modeling are included in Supplemental Table 3 (Supplemental Digital Content 3, http://links.lww.com/PCC/A744). The outcome, mortality, was never missing.

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 extracorporeal membrane oxygenation (ECMO) and thus does not capture death occurring after the last day of ECMO.

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 RBCs which show a greater susceptibility to mechanical stress than adult RBCs (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 setup and management were associated with PFH levels. Lower heparin infusion dose adjusted for body weight (U/kg/min) was independently associated with higher daily PFH. Higher heparin infusion doses could reflect a more

TABLE 7. Multivariable Model for Plasma Free Hemoglobin

	Plasma Free Hemoglobin (0.01 g/L)	
Variables[a]	Effect (95% CI)	*p*
Chronic neurologic condition	−11.84 (−21.88 to −1.81)	0.021
Chronic immune dysfunction	−30.35 (−47.42 to −13.29)	< 0.001
Acute nonseptic shock	−33.79 (−51.87 to −15.72)	< 0.001
Acute neurologic condition	52.38 (9.05–95.71)	0.018
Hemofiltration or continuous renal replacement therapy	16.30 (3.23–29.37)	0.014
Total bilirubin (mg/dL) (17.10 μmol/L)	4.15 (3.19–5.12)	< 0.001
Hemoglobin (g/dL) (10 g/L)	6.01 (3.51–8.50)	< 0.001
Weight (kg)	−0.34 (−0.51 to −0.17)	< 0.001
Heparin (U/kg/min)	−35.64 (−58.10 to −13.18)	0.002
Platelets (10^3/μL)	−0.15 (−0.27 to −0.03)	0.016

[a]Rates of missingness for all predictors considered for modeling are included in Supplemental Table 1 (Supplemental Digital Content 1, http://links.lww.com/PCC/A742). The outcome, plasma free hemoglobin, was missing on 8% of study days.

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 nonanticoagulant 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 patients (6.9%). 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 (18) found more hemolysis with centrifugal than roller pumps although hemolysis was reported less frequently in the ELSO registry overall. 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 RBCs 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 RBCs 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 concertation 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 prooxidant effects (37). Similar to our findings, others have shown that PFH predicts acute renal failure during venoarterial ECMO (36, 38). Hemolysis during combined ECMO and CRRT has been shown to be increased compared with ECMO alone (10). 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 setup, 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. Although our definition of renal failure was a creatinine level of greater than 2 mg/dL (> 176.8 μmol/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.

ACKNOWLEDGMENTS

We acknowledge the contributions of the following research coordinators and data coordinating center staff: Stephanie Bisping, BSN, RN, CCRP, Alecia Peterson, BS, and Jeri Burr, MS, RN-BC, CCRC, from University of Utah, Salt Lake City, UT; Mary Ann DiLiberto, BS, RN, CCRC and Carol Ann Twelves, BS, RN, from The Children's Hospital of Philadelphia, Philadelphia, PA; Jean Reardon, MA, BSN, RN and Elyse Tomanio, BSN, RN, from Children's National Medical Center, Washington, DC; Aimee Labell, MS, RN, from Phoenix Children's Hospital, Phoenix, AZ; Margaret Villa, RN and Jeni Kwok, JD, from Children's Hospital Los Angeles, Los Angeles, CA; Mary Ann Nyc, BS, from UCLA Mattel Children's Hospital, Los Angeles, CA; Ann Pawluszka, BSN, RN and Melanie Lulic, BS, from Children's Hospital of Michigan, Detroit, MI; Monica S. Weber, RN, BSN, CCRP and Lauren Conlin, BSN, RN, CCRP, from University of Michigan, Ann Arbor, MI; Alan C. Abraham, BA, CCRC, from University of Pittsburgh Medical Center, Pittsburgh, PA. We also acknowledge the contributions of Robert Tamburro, MD, and Tammara Jenkins, MSN, RN, from the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development Collaborative Pediatric Critical Care Research Network.

REFERENCES

1. Dalton HJ, Reeder R, Garcia-Filion P, et al; *Eunice Kennedy Shriver* National Institute of Child Health and Human Development Collaborative Pediatric Critical Care Research Network: Factors associated with bleeding and thrombosis in children receiving extracorporeal membrane oxygenation. *Am J Respir Crit Care Med* 2017; 196:762–771
2. Annich GM, Meinhardt JP, Mowery KA, et al: Reduced platelet activation and thrombosis in extracorporeal circuits coated with nitric oxide release polymers. *Crit Care Med* 2000; 28:915–920
3. Thiara AP, Hoel TN, Kristiansen F, et al: Evaluation of oxygenators and centrifugal pumps for long-term pediatric extracorporeal membrane oxygenation. *Perfusion* 2007; 22:323–326
4. Talor J, Yee S, Rider A, et al: Comparison of perfusion quality in hollow-fiber membrane oxygenators for neonatal extracorporeal life support. *Artif Organs* 2010; 34:E110–E116
5. Lou S, MacLaren G, Best D, et al: Hemolysis in pediatric patients receiving centrifugal-pump extracorporeal membrane oxygenation: Prevalence, risk factors, and outcomes. *Crit Care Med* 2014; 42:1213–1220
6. Annich GM: Extracorporeal life support: The precarious balance of hemostasis. *J Thromb Haemost* 2015; 13(Suppl 1):S336–S342
7. Gorbet MB, Sefton MV: Biomaterial-associated thrombosis: Roles of coagulation factors, complement, platelets and leukocytes. *Biomaterials* 2004; 25:5681–5703
8. Steinhorn RH, Isham-Schopf B, Smith C, et al: Hemolysis during long-term extracorporeal membrane oxygenation. *J Pediatr* 1989; 115:625–630
9. Gbadegesin R, Zhao S, Charpie J, et al: Significance of hemolysis on extracorporeal life support after cardiac surgery in children. *Pediatr Nephrol* 2009; 24:589–595
10. Betrus C, Remenapp R, Charpie J, et al: Enhanced hemolysis in pediatric patients requiring extracorporeal membrane oxygenation and continuous renal replacement therapy. *Ann Thorac Cardiovasc Surg* 2007; 13:378–383
11. Rother RP, Bell L, Hillmen P, et al: The clinical sequelae of intravascular hemolysis and extracellular plasma hemoglobin: A novel mechanism of human disease. *JAMA* 2005; 293:1653–1662
12. Barrett CS, Jaggers JJ, Cook EF, et al: Outcomes of neonates undergoing extracorporeal membrane oxygenation support using centrifugal versus roller blood pumps. *Ann Thorac Surg* 2012; 94:1635–1641
13. Palanzo DA, El-Banayosy A, Stephenson E, et al: Comparison of hemolysis between CentriMag and RotaFlow rotary blood pumps during extracorporeal membrane oxygenation. *Artif Organs* 2013; 37:E162–E166
14. Lehle K, Philipp A, Zeman F, et al: Technical-induced hemolysis in patients with respiratory failure supported with veno-venous ECMO - prevalence and risk factors. *PLoS One* 2015; 10:e0143527
15. Sulkowski JP, Cooper JN, Pearson EG, et al: Hemolysis-associated nitric oxide dysregulation during extracorporeal membrane oxygenation. *J Extra Corpor Technol* 2014; 46:217–223
16. Jenks CL, Zia A, Venkataraman R, et al: High hemoglobin is an independent risk factor for the development of hemolysis during pediatric extracorporeal life support. *J Intensive Care Med* 2017 Jan 1: 885066617708992.. [Epub ahead of print]
17. Cashen K, Reeder RW, Shanti C, et al: Is therapeutic hypothermia during neonatal extracorporeal membrane oxygenation associated with intracranial hemorrhage? *Perfusion* 2018; 33:354–362
18. O'Brien C, Monteagudo J, Schad C, et al: Centrifugal pumps and hemolysis in pediatric extracorporeal membrane oxygenation (ECMO) patients: An analysis of Extracorporeal Life Support Organization (ELSO) registry data. *J Pediatr Surg* 2017; 52:975–978
19. Vercaemst L: Hemolysis in cardiac surgery patients undergoing cardiopulmonary bypass: A review in search of a treatment algorithm. *J Extra Corpor Technol* 2008; 40:257–267
20. Hessel EA: Cardiopulmonary bypass circuitry and cannulation techniques. *In*: Cardiopulmonary Bypass Principles and Practice. Gravlee GP, Davis RF, Utley JR (Eds). Baltimore, MD, Williams & Wilkins, 1993, pp 55–92
21. Kameneva MV, Burgreen GW, Kono K, et al: Effects of turbulent stresses upon mechanical hemolysis: Experimental and computational analysis. *ASAIO J* 2004; 50:418–423
22. Wildhagen KC, García de Frutos P, Reutelingsperger CP, et al: Nonanticoagulant heparin prevents histone-mediated cytotoxicity in vitro and improves survival in sepsis. *Blood* 2014; 123:1098–1101
23. Cassinelli G, Naggi A: Old and new applications of non-anticoagulant heparin. *Int J Cardiol* 2016; 212(Suppl 1):S14–S21
24. Finkel KW, Podoll AS: Complications of continuous renal replacement therapy. *Semin Dial* 2009; 22:155–159
25. Horton AM, Butt W: Pump-induced haemolysis: Is the constrained vortex pump better or worse than the roller pump? *Perfusion*1992; 7:103–108
26. Salazar Vázquez BY, Martini J, Chávez Negrete A, et al: Cardiovascular benefits in moderate increases of blood and plasma viscosity surpass those associated with lowering viscosity: Experimental and clinical evidence. *Clin Hemorheol Microcirc* 2010; 44:75–85
27. Lowe GD, Lee AJ, Rumley A, et al: Blood viscosity and risk of cardiovascular events: The Edinburgh Artery Study. *Br J Haematol* 1997; 96:168–173
28. Bonithon-Kopp C, Levenson J, Scarabin PY, et al: Longitudinal associations between plasma viscosity and cardiovascular risk factors in a middle-aged French population. *Atherosclerosis* 1993; 104:173–182
29. Muszynski JA, Reeder RW, Hall MW, et al: RBC transfusion practice in pediatric extracorporeal membrane oxygenation support. *Crit Care Med* 2018; 46:e552–e559
30. Muszynski JA, Spinella PC, Cholette JM, et al; Pediatric Critical Care Blood Research Network (Blood Net): Transfusion-related immunomodulation: Review of the literature and implications for pediatric critical illness. *Transfusion* 2017; 57:195–206
31. Kleiber N, Lefebvre É, Gauvin F, et al: Respiratory dysfunction associated with RBC transfusion in critically ill children: A prospective cohort study. *Pediatr Crit Care Med* 2015; 16:325–334
32. Walsh-Sukys MC, Cornell DJ, Stork EK: The natural history of direct hyperbilirubinemia associated with extracorporeal membrane oxygenation. *Am J Dis Child* 1992; 146:1176–1180

33. Maisels MJ, McDonagh AF: Phototherapy for neonatal jaundice. *N Engl J Med* 2008; 358:920–928
34. Abbasi S, Stewart DL, Radmacher P, et al: Natural course of cholestasis in neonates on extracorporeal membrane oxygenation (ECMO): 10-year experience at a single institution. *ASAIO J* 2008; 54:436–438
35. Somanathapura K, Kumar N, Thushara RM, et al: Unconjugated bilirubin exerts proApoptotic effect on platelets via p38-MAPK activation. *Sci Rep* 2015; 5:15045
36. Lyu L, Long C, Hei F, et al: Plasma free hemoglobin is a predictor of acute renal failure during adult venous-arterial extracorporeal membrane oxygenation support. *J Cardiothorac Vasc Anesth* 2016; 30:891–895
37. Tracz MJ, Alam J, Nath KA: Physiology and pathophysiology of heme: Implications for kidney disease. *J Am Soc Nephrol* 2007; 18:414–420
38. Lv L, Long C, Liu J, et al: Predictors of acute renal failure during extracorporeal membrane oxygenation in pediatric patients after cardiac surgery. *Artif Organs* 2016; 40:E79–E83