

Hemolysis-Associated Nitric Oxide Dysregulation during Extracorporeal Membrane Oxygenation

Jason P. Sulkowski, MD;* Jennifer N. Cooper, PhD;* Erik G. Pearson, MD;†
James T. Connelly;† Natalie Rintoul, MD;† Todd J. Kilbaugh, MD;‡
Katherine J. Deans, MD, MHSc;* Peter C. Minneci, MD, MHSc*

*Center for Surgical Outcomes Research, The Research Institute at Nationwide Children's Hospital, Columbus, Ohio; and the Departments of †Neonatology and ‡Critical Care Medicine, Children's Hospital of Philadelphia, Philadelphia, Pennsylvania

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Abstract: Acute intravascular hemolysis during extracorporeal membrane oxygenation (ECMO) leads to increased levels of cell-free hemoglobin (Fhb). Our aim was to investigate whether Fhb levels are associated with nitric oxide (NO) consumption and clinical outcomes. A prospective observational study was performed involving pediatric patients on ECMO. Blood samples were collected before, during, and after the ECMO run, and plasma was evaluated for Fhb, oxyhemoglobin, and NO consumption. Clinical data were collected including baseline patient characteristics, indications for ECMO, circuit changes, and mortality. Correlations between laboratory measures and associations between laboratory measures and clinical observations were evaluated. Twenty-three patients (11 male, 17 neonates) were enrolled with a median weight of 3.1 kg (interquartile range, 2.8–14.0 kg) and median ECMO run of 12 days (interquartile range, 5–19 day). There was a significant increase in Fhb over time on ECMO ($p = .007$), and significant correlations were present between NO consumption and both Fhb ($r = .41, p = .01$) and oxyhemoglobin

levels ($r = .98, p < .0001$). Patients on ECMO for sepsis ($n = 6$) had lower average levels of oxyhemoglobin (mean [standard deviation {SD}] 14.5 [4.4] versus 19.0 [5.0] μM , $p = .07$) and NO consumption (mean [SD] 15.8 [4.1] versus 19.8 [3.7] μM , $p = .04$) during ECMO than patients with other indications. In the 3 days leading up to a circuit change, there were increases in mean total cell-free hemoglobin levels (24%/day, $p = .08$), oxyhemoglobin (37%/day, $p = .005$), and NO consumption (40%/day, $p = .006$) ($n = 5$). There were no significant associations identified between peak or average plasma measures of hemolysis and type of ECMO (venovenous versus venoarterial) or mortality. For children on ECMO, we observed a strong correlation between increased levels of plasma Fhb and elevations in oxyhemoglobin and NO consumption; however, these changes were not associated with increased mortality. Increased hemolysis before circuit changes may be both a marker and a contributor to circuit failure. **Keywords:** extracorporeal membrane oxygenation, ECMO, nitric oxide, consumption, hemolysis. *JECT. 2014;46:217–223*

Intravascular hemolysis is thought to play a role in a number of different disease processes as a result of the release of free hemoglobin (Fhb) from erythrocytes into the plasma (1). In particular, Fhb interacts rapidly and

irreversibly with nitric oxide (NO), attenuating the effects of NO, which include vasodilation, inhibition of platelet aggregation, and inactivation of superoxide molecules (2–5). In a large animal model, a mechanistic link has been demonstrated between NO consumption during intravascular hemolysis and pathophysiologic changes including vasoconstriction with hypertension and cardiac and renal dysfunction (6).

Hemolysis resulting from a number of different etiologies has been shown to be associated with adverse outcomes (7–11). In particular, hemolysis during extracorporeal membrane oxygenation (ECMO) is a result of interactions between the red blood cells and priming solutions along with the mechanical and accelerative forces associated with the circuit pumps (12). For pediatric patients on ECMO, hemolysis-related sequelae include prolonged ECMO

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Address correspondence to: Peter C. Minneci, MD, MHSc, Co-Director, Center for Surgical Outcomes Research, Assistant Professor, Department of Surgery, Center for Innovation in Pediatric Practice, The Research Institute at Nationwide Children's Hospital, 700 Children's Drive, JW4914, Columbus, OH 43205. E-mail: peter.minneci@nationwidechildrens.org
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courses, longer hospital stays, renal failure, and increased mortality (10,11). The objectives of this observational study were: 1) to explore the mechanism of hemolysis-associated NO dysregulation in children treated with ECMO by determining the correlations between plasma Fhb and NO consumption in children on ECMO; and 2) to identify any associations between these factors and clinical parameters and observations.

METHODS

Study Design

A prospective observational study was performed that included patients who were placed on ECMO at a free-standing academic children's hospital between July 2008 and July 2010. Once the decision to initiate ECMO was made, collection of blood samples was initiated with samples collected before, during, and after the ECMO run. Informed consent was obtained from the guardians of all patients in a delayed fashion given the severity of each patient's clinical status, but always within 24 hours of ECMO cannulation. Blood sample collection was initiated pre-emptively for all patients placed on ECMO and continued for those whose guardians agreed to participate. Samples were discarded for those who declined to participate. The Institutional Review Board of the Children's Hospital of Philadelphia approved this protocol.

Extracorporeal Membrane Oxygenation Circuit

While the study was being performed, the ECMO circuit at our institution consisted of a Sorin S5 roller pump (Sorin S.p.A., Milan, Italy), a Quadrox iD oxygenator (Quadrox USA, Henderson, NV), Medtronic tubing packs (Medtronic, Minneapolis, MN) with a BetterBladder compliance chamber (Circulatory Technology Inc., Oyster Bay, NY), and Edwards Lifesciences cannulae for venoarterial (VA) ECMO (Edwards Lifesciences Corp., Irvine, CA) and Avalon Bicaval cannulae for venovenous (VV) ECMO (Avalon Laboratories LLC, Rancho Dominguez, CA). "Walks" were performed every 7–10 days when ¼-inch circuits were used and every 4–6 days when 3/8-inch circuits were used. The "walks" refer to a procedure that entails moving or "walking" the tubing worn down by the roller head's compression to an area of prepump tubing that has been under lower pressure; this places a fresh segment of tubing in contact with the roller heads in an effort to minimize spallation or cracking of the tubing segments by the roller head. Quarter-inch circuits were used for patients less than 10 kg and 3/8-inch circuits for patients >10 kg. The typical circuit configuration would place all infusions postpump and preoxygenator with the exception of a single postoxygenerator port for platelet infusions.

Sample and Data Collection

The schedule of blood sample collection during the ECMO run was as follows: every 2 hours for the first 6 hours followed by daily collections for the next 7 days and weekly thereafter. In addition, samples were taken before and after any circuit changes. Plasma was isolated from each sample and evaluated for the following markers of hemolysis: total plasma Fhb, oxyhemoglobin (oxy-Hb), met-hemoglobin (met-Hb), and NO consumption. Total plasma Fhb was determined by visible absorbance spectrophotometry and oxy-Hb and met-Hb fractions were determined by deconvoluting the spectrum into components, as has been previously described (13). NO consumption was determined using a NO chemiluminescence analyzer (6).

Demographic and clinical data, including age, gender, previous medical history, and indication for ECMO, were collected and serial objective measurements and observations including vital signs, laboratory values, and medications or blood products administered were collected concurrently with blood samples. In particular, renal function was assessed by monitoring daily urine output (volume per unit weight per hour) and serum creatinine levels. Acute renal insufficiency was defined as an increase in creatinine by 1.5 mg/dL or more above normal levels (or by 25% above baseline when pre-ECMO levels were already elevated) or the need for renal replacement therapy during the ECMO course. Also, important events were documented such as circuit component changes, addition or replacement of ECMO cannulae, and other major clinical events.

Statistical Analysis

Baseline demographic and clinical characteristics of study patients as well as summary measures of plasma markers of hemolysis during ECMO were reported as medians and 25th and 75th percentiles for continuous data and frequencies and percentages for categorical data. Because the plasma markers of hemolysis (total plasma Fhb, oxy-Hb, met-Hb, and NO consumption) all had positively skewed distributions, these were log-transformed to achieve normal distributions. Total plasma Fhb, oxy-Hb, and NO consumption were also analyzed as the following values during the entire ECMO course: peak, time-weighted average, and whether the patient was a high hemolyzer (defined as greater than the 75th percentile of the peak value of that plasma marker across all patients). The time-weighted average value of the plasma marker over a patient's ECMO course was determined by first calculating the area under the curve (AUC) of each patient's measurements. The AUC was determined using linear mixed models (growth curves) for the serial measurements during ECMO. These models included fixed and random intercepts and linear slopes and quadratic and cubic time effects were kept when

significant at $p < .10$. The time-weighted average was then calculated by integrating each patient's estimated growth curve over his or her total time on ECMO (the AUC) and finally dividing this value by the patient's time on ECMO (14). Correlations between the plasma markers of hemolysis were evaluated using bivariate linear mixed models for the two plasma markers under consideration with random intercepts and a first-order autoregressive residual error covariance structure (15). Probability values for the correlation coefficients came from likelihood ratio tests. Bias-corrected bootstrap 95% confidence intervals were calculated for the correlation coefficients from 5000 bootstrap samples of the original data set (16). Because the between-patient and within-patient correlation coefficients were not significantly different in any of these bivariate models, we report only the overall correlation coefficient, which represents weighted averages of the between-patient and within-patient correlation coefficients. Associations between in-hospital mortality and markers of hemolysis were evaluated using a Wilcoxon rank sum test for nonnormally distributed continuous variables, a t test for normally distributed continuous data, or the Fisher's exact test for categorical data. The primary associations of interest were the relationships between markers of hemolysis and mortality. The associations between markers of hemolysis and other clinical characteristics and outcomes, including indication for ECMO, circuit changes, and ECMO type (VV versus VA), were also explored. To analyze the trends in hemolysis factors before circuit changes, linear mixed models for the hemolysis factor measurements in the 3 days before the first circuit change were fit, including only patients with a circuit change. In the analyses involving ECMO type, for patients who switched from VV to VA or VA to VV, only measurements before the switch were used. All analyses were performed using SAS Version 9.3 (SAS Institute, Cary, NC). No adjustments were made for multiple testing. Probability values $< .05$ were considered statistically significant. All hemoglobin measurements are expressed in terms of heme groups.

RESULTS

Patient Characteristics

Twenty-three patients (11 males, 47.8%) were enrolled during the study period. Table 1 lists baseline characteristics of the study patients. Of note, 17 (73.9%) were neonates and the median weight at the time of ECMO was 3.1 kg (interquartile range [IQR], 2.8–14.0). Most patients were placed on ECMO at least in part because of respiratory distress (65.2%), and six (26.1%) had sepsis as their indication. One patient had a transient serum creatinine

Table 1. Baseline characteristics of study patients and summary of plasma markers of hemolysis.

Variable	No. (%) or Median (interquartile range)
Neonate (≤ 30 days)	17 (73.9)
Male	11 (47.8)
Race	
White	13 (56.5)
Black	6 (26.1)
Asian	2 (8.7)
Unknown	2 (8.7)
Hispanic	
Yes	1 (4.4)
No	20 (87.0)
Unknown	2 (8.7)
Weight (kg)	3.1 (2.8–14.0)
ICU LOS (days)	48 (25–83)
ECMO indication	
Respiratory distress	15 (65.2)
PPHN	4 (17.4)
CDH	4 (17.4)
Meconium aspiration	4 (17.4)
Sepsis-related cause	6 (26.1)
Cardiac failure	2 (8.7)
Days on ECMO	12 (5–19)
ECMO circuit type	
VA	11 (47.8)
VV	9 (39.1)
VV converted to VA	2 (8.7)
VA converted to VV	1 (4.4)
At least one circuit change during ECMO course	8 (34.8)
Laboratory measures	
Cell-free hemoglobin (mg/dL)*	540.1 (388.7–712.5)
Oxyhemoglobin (μM)*	17.5 (13.7–23.7)
Oxyhemoglobin (%)*	23.4 (15.6–30.1)
Met-hemoglobin (%)*	74.4 (66.1–78.5)
NO consumption (μM)*	19.2 (15.1–24.0)
Peak cell-free hemoglobin (mg/dL)†	1091.9 (841.8–1454.5)
Peak oxyhemoglobin (μM)†	55.0 (43.5–91.7)
Peak NO consumption (μM)†	57.0 (45.8–80.0)
Average cell-free hemoglobin (mg/dL)‡	613.0 (449.2–805.6)
Average oxyhemoglobin (μM)‡	18.7 (13.9–21.3)
Average NO consumption (μM)‡	19.4 (15.0–22.8)
Died§	6 (26.1)

*Median of patient-specific medians.

†Median of patient-specific maxima.

‡Time-weighted averages were calculated as the area under the patient's growth curve divided by the patient's time on ECMO.

§During same ECMO hospitalization.

ICU, intensive care unit; LOS, length of stay; ECMO, extracorporeal membrane oxygenation; PPHN, persistent pulmonary hypertension of the newborn; CDH, congenital diaphragmatic hernia; VV, venovenous; VA, venoarterial; NO, nitric oxide.

elevation of 1.5 mg/dL and another required renal replacement therapy; both of these patients ultimately died.

Cell-free Hemoglobin Is Correlated with Nitric Oxide Consumption during Extracorporeal Membrane Oxygenation

A summary of plasma markers of hemolysis during ECMO is shown in Table 1. Overall, total Fhb increased significantly by 6% per day (95% confidence interval [CI], 3–10, $p = .0003$) over time during the course of a patient's

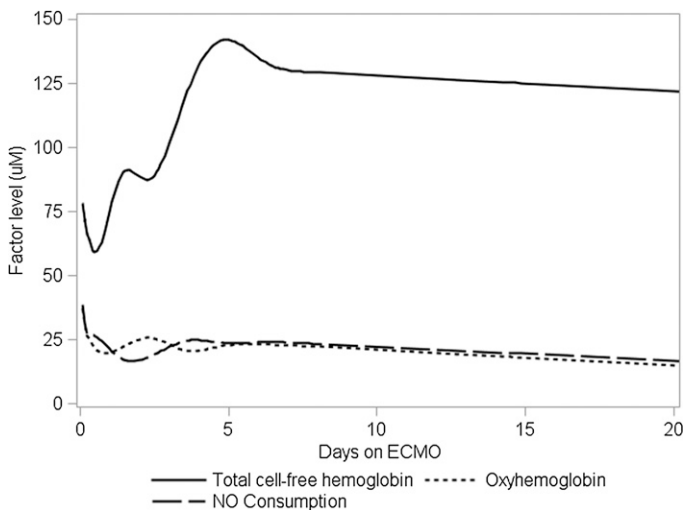


Figure 1. Average plasma levels of free hemoglobin, oxyhemoglobin, and nitric oxide (NO) consumption in pediatric patients on extracorporeal membrane oxygenation (ECMO) over time. These curves were estimated by averaging all individual patients' growth curves, which were estimated using linear mixed effects models with continuous time fit using restricted cubic splines with a knot at each decile of the time distribution. Over the entire ECMO run for all patients, the average total cell-free hemoglobin concentration (solid line) increased significantly by 6% per day (95% confidence interval [CI], 3–10, $p = .0003$), oxyhemoglobin (dotted line) decreased by an average of 5% per day (95% CI, 1–9), and NO consumption (dashed line) decreased by an average of 2% per day (95% CI, .3–4). However, during the first 5 days on ECMO, total cell-free hemoglobin increased significantly by 16% per day (95% CI, 10–23, $p < .0001$), whereas oxyhemoglobin and NO consumption did not demonstrate significant changes.

ECMO run (Figure 1). Oxy-Hb decreased by an average of 5% per day (95% CI, 1–9), and NO consumption decreased by an average of 2% per day (95% CI, .3–4) during ECMO. However, as Figure 1 shows, there were particular periods of time during which each of three factors changed more rapidly. A significant overall correlation with NO consumption was identified for both total FHb ($r = .41$, $p < .0001$) and oxy-Hb ($r = .98$, $p < .0001$) (Figure 2). These correlations with NO consumption remained significant after log transformation of all variables, which reduced the influence of outliers and improved the normality of the distributions (log total FHb: $r = .45$, $p < .0001$ and log oxy-Hb: $r = .63$, $p < .0001$). Furthermore, over time the composition of hemoglobin species found in the plasma changed, with the percentage of FHb that was oxy-Hb decreasing by approximately 2.2% per day (95% CI, 1.4–3.0, $p < .0001$) and met-Hb increasing on average by 1.4% per day (95% CI, .8–2.0, $p = .0002$).

No Significant Associations Detected between Markers of Hemolysis and Mortality

Six patients (26.1%) died during the ECMO run or shortly after decannulation. There was no difference between those who survived and those who died in the peak value of any of the three plasma markers of hemoly-

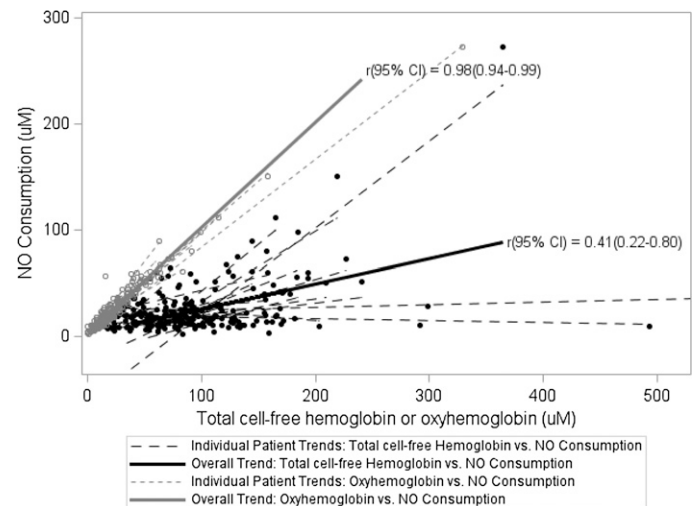


Figure 2. Correlations among cell-free hemoglobin, oxyhemoglobin, and nitric oxide (NO) consumption in pediatric patients on extracorporeal membrane oxygenation (ECMO). Individual patient trends and overall trend lines for NO consumption with correlation coefficients derived from bivariate linear mixed models for the two plasma markers under consideration using a random intercept and a first-order autoregressive residual error covariance structure. Confidence intervals are bias-corrected bootstrap 95% confidence intervals based on 5000 bootstrap samples of the original data set. A significant overall correlation with NO consumption was identified for both total cell-free hemoglobin (FHb) (solid black line; $r = .41$, $p < .0001$) and oxyhemoglobin (oxy-Hb) (solid gray line; $r = .98$, $p < .0001$).

sis (median [IQR] FHb: 1053 mg/dL [926–1180] versus 1173 mg/dL [842–1455], $p = .60$; oxy-Hb: 56.0 μM [50.0–62.8] versus 55.0 μM [40.3–91.7], $p = .81$; NO consumption: 57.2 μM [51.8–88.7] versus 57.0 μM [40.4–66.7], $p = .70$). Also, there was not a significant difference between patients who did and did not die in the hospital in the proportion of high hemolyzers (FHb: 16.7% versus 29.4%, $p = 1.0$; oxy-Hb: 16.7% versus 29.4%, $p = 1.0$; NO consumption: 33.3% versus 23.5%, $p = .63$) or in the time-weighted average measures of hemolysis (mean \pm standard deviation [SD] FHb: 643 \pm 217 versus 601 \pm 166 mg/dL, $p = .67$; oxy-Hb: 18.6 \pm 5.1 versus 15.6 \pm 5.1 μM , $p = .22$; NO consumption: 19.3 \pm 3.5 versus 17.2 \pm 5.5, $p = .28$).

Patients on Extracorporeal Membrane Oxygenation for Sepsis Had Lower Average Levels of Oxyhemoglobin and Nitric Oxide Consumption

Those patients who were placed on ECMO for sepsis ($n = 6$) had similar peak levels of all three measures as those whose indication was not sepsis related (median [IQR] FHb: 1136 mg/dL [926–1539] versus 1056 mg/dL [842–1405], $p = .81$; oxy-Hb: 50.7 μM [46.0–62.8] versus 60.5 μM [43.5–91.7], $p = .70$; NO consumption: 53.1 μM [47.9–88.7] versus 59.9 μM [45.8–66.7], $p = .86$). There was also no difference in the proportion of high hemolyzers with a sepsis-related indication (FHb: 33.3% versus 23.5%, $p = .63$; oxy-Hb: 16.7% versus 29.4%, $p = 1.0$; NO

consumption: 33.3% versus 23.5%, $p = .63$). On the other hand, aside from total Fhb, which was similar between groups (692 ± 263 versus 611 ± 181 mg/dL, $p = .41$), patients with a sepsis-related indication had lower average levels of oxy-Hb (14.5 ± 4.4 versus 19 ± 5.0 μ M, $p = .07$) and NO consumption (15.8 ± 4.1 versus 19.8 ± 3.7 μ M, $p = .04$) over their ECMO course than those on ECMO for nonsepsis indications.

Markers of Hemolysis Increased before Circuit Changes

Five patients had two or more samples collected in the 72 hours before their first circuit change. According to the linear mixed models used to identify trends in the plasma markers of hemolysis during this period, total Fhb increased by an average of 24% per day ($p = .08$), oxy-Hb increased by 37% per day ($p = .005$), and NO consumption increased by 40% per day ($p = .006$) in the 72 hours before a circuit change.

No Significant Association Was Identified between Hemolysis and Extracorporeal Membrane Oxygenation Type

Twelve patients (52.2%) were initially placed on VA ECMO and 11 (47.8%) on VV ECMO. There were no significant differences between VA and VV ECMO for peak markers of hemolysis (median [IQR] Fhb: 1005 mg/dL [626–1354] versus 1056 mg/dL [842–1791], $p = .64$; oxy-Hb: 57.7 μ M [41.9–73.7] versus 50.0 μ M [35.0–115.4], $p = .83$; NO consumption: 60.4 μ M [50.2–65.1] versus 50.1 μ M [35.1–97.2], $p = .78$); the proportion of high hemolyzers in each group (Fhb: 25.0% versus 27.3%, $p = 1.00$; oxy-Hb: 16.7% versus 36.4%, $p = .37$; NO consumption: 16.7% versus 36.4%, $p = .37$); and average levels of markers of hemolysis (Fhb: 457 ± 190 versus 532 ± 176 mg/dL, $p = .34$; oxy-Hb: 17.6 ± 3.8 versus 17.7 ± 6.3 μ M, $p = .97$; NO consumption: 20.0 ± 4.2 versus 18.1 ± 4.1 μ M, $p = .29$).

DISCUSSION

In this study, measures of plasma Fhb and oxy-Hb in patients on ECMO were directly related to NO consumption, but there was no significant correlation between elevated markers of hemolysis and mortality. However, several other associations were found, namely that Fhb, oxy-Hb, and NO consumption tended to increase before circuit changes, and average levels of oxy-Hb and NO consumption were higher in patients who had nonsepsis-related indications for ECMO.

Plasma Fhb resulting from hemolysis is thought to play a role in the mechanisms behind a number of different disease processes as a result of the direct toxicity of Fhb and the downstream effects of its role as a scavenger of NO (1). The iatrogenic hemolysis that results from placement on mechanical cardiopulmonary support, including

ECMO, has been implicated in a number of the deleterious effects associated with these interventions (10,11). In a review of children younger than 3 years old who were placed on ECMO after cardiac surgery, elevations in Fhb were associated with renal dysfunction and death (11). Another study that stratified patients on ECMO according to the amount of hemolysis demonstrated an association between death and severe hemolysis, which was defined as Fhb levels >100.0 mg/dL (10). Even young children placed transiently on cardiopulmonary bypass were shown to have increased Fhb and decreased haptoglobin, which was associated with a fivefold increase in acute kidney injury after controlling for on-pump time (11). An analysis of data from the Extracorporeal Life Support Organization database showed use of centrifugal pumps was associated with an increased risk of hemolysis and acute renal failure; however, no direct association between hemolysis and outcomes was examined (17). Many other studies have examined the relationship between different types of pumps or circuit components and the magnitude of associated hemolysis; however, there is little other research demonstrating any association between negative outcomes and ECMO-related intravascular hemolysis. Furthermore, these prior studies of children on ECMO did not examine the mechanism for the association between hemolysis and poor outcomes.

One potential mechanism for the dysfunction associated with high Fhb during ECMO is hemolysis-associated NO dysregulation resulting from rapid NO consumption by Fhb. In a large animal model of intravascular hemolysis, rapid NO consumption by Fhb led to hemodynamic and renal dysfunction, which were attenuated by the administration of NO donor agents (6). This study suggests that hemolysis-associated NO dysregulation may in part explain the pathophysiologic changes that occur during intravascular hemolysis. In addition, several human studies have shown a link between elevated NO consumption and hemodynamic disruptions further supporting the hypothesis that hemolysis-associated NO dysregulation may be mechanistically associated with pathophysiologic derangements and potentially affect outcomes (6–8). Taken together, these studies support the hypothesis that there is a link between NO consumption and physiologic derangements; however, they were either carried out in a highly controlled experimental environment or are just descriptive reports of one specific disease state. On the other hand, the results of this study, which involved a heterogeneous group of highly complex patients, did not support this hypothesis. Although we identified strong positive correlations between NO consumption and both Fhb and oxy-Hb, which supports the occurrence of hemolysis-associated NO dysregulation, no associations were found between peak or average levels of any of these three measures during ECMO and mortality or renal dysfunction. This may in part be explained by the

findings that although total Fhb increased throughout the ECMO run, the proportion that was oxy-Hb (the form of Fhb responsible for NO consumption) rose initially and then slowly decreased. Unlike the animal studies in which there were linear increases in both Fhb and oxy-Hb over hours, in patients on ECMO in which intravascular hemolysis occurs over days, total Fhb continued to increase, but oxy-Hb did not. This suggests that during ECMO, oxy-Hb is rapidly converted to other inactive forms of hemoglobin, which do not consume NO. Taken together, these results suggest that the mechanism of NO scavenging by Fhb does not play a significant role in development of poor outcomes from hemolysis during ECMO.

We also observed that patients with a sepsis-related indication had similar overall levels of hemolysis but had lower levels of oxy-Hb and NO consumption. A possible explanation for this finding may be that the inflammatory response led to elevated levels of haptoglobin, an acute phase reactant (18). In addition, we demonstrated that levels of Fhb and the other markers of hemolysis increased in the 3 days before circuit changes. With the available data, there is no way to determine causality in this finding as to whether elevations in hemolysis led to circuit failure or vice versa; however, routine monitoring of Fhb might serve as a marker to predict the impending need for a circuit change. Allowing clinicians to anticipate impending circuit failure may improve care by assisting with decisions to either push for a trial off of ECMO or to perform a circuit change before reaching complete circuit failure with disseminated intravascular coagulation (19).

The results of this study should be considered in the context of its limitations. First, as a single-center study with only 23 patients, this study had limited power to find significant relationships of interest, and multivariable modeling of outcomes was not possible. In addition, the results may not be generalizable to all neonatal and pediatric patients on ECMO. Also, the clinical outcomes that we were able to evaluate were limited in scope. Children placed on ECMO are severely ill and extremely complex, in contrast with the highly controlled environments of the animal studies that have been done to explore this disease mechanism. There are innumerable confounders to consider in any attempt to understand a single component of their physiology. To date, there have been no randomized studies in humans that have focused on a single disease state and attempted to identify mechanisms of hemolysis or methods to attenuate it. Furthermore, in clinical care, interventions are being made on a minute-by-minute basis to maintain the patient's physiology, which further complicates the interpretation of clinical data. In this study, large amounts of clinical data were collected for each patient; however, we noticed very small fluctuations in many clinical parameters such as blood pressure, which we attributed in large part to the interventions made by

the care providers. In fact, because the care of many critical patients such as those on ECMO is based on following protocols with targets for various parameters, it is of little surprise that there was minimal variability observed. On the other hand, the multiple laboratory measures included to evaluate hemolysis and the potential role of plasma NO consumption in poor outcomes associated with hemolysis is a strength of this study.

In conclusion, this study demonstrated a strong correlation between both plasma Fhb and oxy-Hb with NO consumption, which had been hypothesized to play a role in the development of hemolysis-associated complications. However, although we did notice a trend toward lower NO consumption in patients with sepsis and an increase in hemolysis in the 3 days before a circuit change, we did not demonstrate an association between any clinical outcomes, including mortality, with higher levels of hemolysis and NO consumption. In these extremely complicated patients, it is likely that the influence of hemolysis-associated NO dysregulation is overwhelmed by numerous other pathophysiologic processes associated with the patient's underlying critical illness or treatment with ECMO.

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