

# Correlation among Hemolysis Biomarkers in Pediatric Patients Undergoing Extracorporeal Membrane Oxygenation

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**Abstract:** Hemolysis is a common complication associated with mortality on extracorporeal membrane oxygenation (ECMO). Plasma-free hemoglobin (PFH) is the most commonly used biomarker reported for hemolysis on ECMO. This test is not readily available at all institutions, and other more readily available tests may indicate hemolysis nearly as well or as well as PFH. The purpose of this study was to study the correlation of other biomarkers of hemolysis to PFH on ECMO. All patients younger than 21 years placed on ECMO in a quaternary children's hospital between January 2013 and December 2016 were included in the study; biomarkers (urine hemoglobin [U-Hb], PFH, lactate dehydrogenase [LDH], aspartate aminotransferase [AST], gross hemolysis, and red cell distribution width (RDW)) were collected from the medical record. Descriptive statistics and repeated bivariate analyses were determined using SPSS 22.0. The median age on day 0 of ECMO was 29 days (.08 years) (IQR: 2; 319 days

(.005; .875 years)). The median weight was 3.9 kg (IQR: 2.8; 8.6), and the median total duration of the ECMO run was 10.48 days (IQR: 4.25; 14), with 82% of all the patients being on venoarterial ECMO. There was no correlation between hematuria on urinalysis and the level of PFH ( $p = .338$ ). There was a statistically significant positive correlation between PFH and the following respective biomarkers: gross hemolysis on the routine chemistry studies ( $p < .01$ ,  $Rho = .439$ ), AST ( $p < .01$ ,  $Rho = .439$ ), RDW ( $p < .01$ ,  $Rho = .190$ ), LDH ( $p < .01$ ,  $Rho = .584$ ), and AST (when associated elevated alanine transaminase (ALT) levels were censored) ( $p < .01$ ,  $Rho = .552$ ). U-Hb correlated poorly with PFH. The serum biomarkers AST (in the absence of ALT elevation) and LDH can be useful surrogates for PFH to quantify hemolysis on ECMO in pediatric patients. **Keywords:** extracorporeal membrane oxygenation, hemolysis, plasma free hemoglobin, biomarker. *J Extra Corpor Technol. 2021;53:125–9*

Mechanical circulatory support (MCS) is a life-saving therapy used to bridge patients to recovery from illness or, in some cases, transplantation. However, MCS is not without risk of significant complications that contribute to both morbidity and mortality. Despite the benefits of MCS in bridging patients to recovery or transplant, this patient population is exposed to significant morbidity including MCS-related complications such as pump thrombosis, hemolysis, bleeding, loss of sites of arteries and veins used

for access, coagulopathy, and stroke (1–11). These morbidities are in turn strongly related to mortality on MCS (15,8-10). Hematologic biomarkers of hemolysis have been shown to better predict pump thrombosis than echocardiographic findings or actual pump parameters (11,12). Biomarkers selected for screening vary based on center preference, but often include plasma-free hemoglobin (PFH), lactate dehydrogenase (LDH), aspartate transaminase (AST), reticulocyte count, and bilirubin. Hematologic biomarkers of hemolysis have been shown to better predict pump thrombosis than echocardiographic findings or actual pump parameters, but there are multiple confounders that may limit direct causal relationship with hemolysis (11,12). Hemolysis screening is not well standardized and is currently based on measurement of plasma biomarkers. Even when screening, pathologic levels of

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**Table 1.** Demographic features of patients placed on ECMO (n = 117).

Variable	Value
Age on ECMO day zero, years	2.18 (.005, .875)
Female gender, n (%)	55 (47)
Nonwhite race, n (%)	85 (72)
Body mass index, m <sup>2</sup>	14.95 (11.87, 16.40)
Total duration of ECMO run, days	10.48 (4.25, 14.00)
Venoarterial ECMO, n (%)	97 (82)

Data presented as median (interquartile range).

hemolysis are not well defined with thresholds of 50 mg/dL and 100 mg/dL previously applied in studies and databases. In practice, PFH levels combined with end organ function are taken into consideration. Multiple studies have evaluated, separately, the ability of those biomarkers to detect hemolysis, but a comparative study among those biomarkers is lacking; therefore, the studies are not necessarily comparable (1,3,8,12-15). To address this gap in the literature, we retrospectively collected hemolysis biomarkers in patients on extracorporeal membrane oxygenation (ECMO).

## MATERIALS AND METHODS

### Study Population and Data Source

With the IRB approval, we included all pediatric patients (defined as patients < 21 years of age at the time of ECMO cannulation) at a quaternary children's hospital who required ECMO support between January 2013 and December 2016 and who had concurrent measurements of PFH, LDH, AST, general blood chemistries, hemograms, and urinary hemoglobin (U-Hb). Concurrent measurements were defined as blood samples collected within four hours of each other consisting of the following laboratory tests: AST, PFH, LDH, hemograms, and general blood chemistries as well as a urine sample collected for U-Hb analysis within 24 hours of the collection of blood samples. Patients were identified through query of the electronic medical record. The final analysis included 1,069 measurements of PFH, 967 measurements of AST, 116 measurements of LDH, 945 measurements of red cell width

distribution (RDW), and 60 measurements of U-Hb. Aspartate aminotransferase (AST) and LDH were analyzed using uncentrifuged blood samples using the AST assay and the LDH assay, respectively, on a Siemens Atellica analyzer. Gross hemolysis was determined optically on the Siemens Atellica analyzer as well in a semiquantitative manner (none, slight, moderate, and gross). PFH was analyzed using a heparinized plasma (lithium only) sample that was separated from cells as soon as possible (or within 2 hours of collection) and measured by spectrophotometry, using a Hemocue plasma/low hemoglobin system<sup>®</sup> (Hemocue, Lake Forest, CA). For the U-Hb analysis, a fresh urine sample of 4 mL (collected within 2 hours of the analysis completion) was analyzed using the iChem VELOCITY which is an in vitro urine chemistry analyzer that measures the chemical constituents of the urine to perform a complete routine urinalysis profile.

A positive U-Hb was defined as any level of hemoglobin detected in the urine. When positive, U-Hb was stratified in five categories based on the amount of urinary red blood cells per high power field. These categories were as follows: negative, trace (1+), small (2+), moderate (3+), and large (4+). PFH measurements were categorized by increments of 50 (0–50 = 1, 51–100 = 2, etc.) The presence of hemolysis was stratified based on the amount of hemolysis described: negative (0), mild (1), moderate (2), and gross (3).

### Statistical Analysis

The data were collected and collated, and all analyses were conducted in SPSS 24.0, IBM Corp. (Armonk, NY). Because of the semiquantitative or ordinal nature of the variables and the non-normal distribution of the PFH assay, Spearman's correlation coefficient was used to conduct repeated bivariate analyses. Correlation was considered significant at the .01 level (two-tailed). Correlation among PFH and hemolysis, PFH and AST, PFH, U-Hb, and LDH were performed separately.

### Patient Characteristics

Data abstracted from electronic medical record included patient demographics as well as results of measurements of PFH, LDH, AST, and urinary hemoglobin measured

**Table 2.** Summary of bivariate analyses of correlation among hematuria on urinalysis\*, hemolysis on chemistry†, AST‡, LDH‡, and RDW§ with PFH.

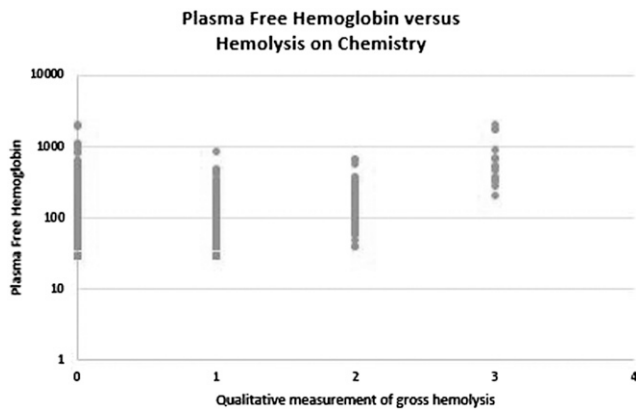
PFH Ordinal	Urine Blood*	Hemolysis Scalar†	AST‡	LDH‡	RDW§
Spearman's rho coefficient	.126	.439*	.487*	.584*	.190
Sig (2-tailed)	.338	.000	.000	.000	.000
n = 1,109	60	1,069	967	116	945

\*Scalar variable of red blood cells per high power field: negative (0), trace (1+), small (2+), moderate (3+), and large (4+).

†0–50 mg/dL = 1, 51–100 mg/dL = 2, 101–150 mg/dL = 3, 151–200 mg/dL = 4, 201–250 mg/dL = 5, 251–300 mg/dL.

‡Units per liter.

§Red cell distribution width.



**Figure 1.** Association between categories of PFH measurements and categories of gross hemolysis on chemistry panel.

within 24 hours of one another when available daily during the total duration of the ECMO. Patient age was defined as the age at the time of ECMO cannulation. Age, body mass index, hemograms, and duration of ECMO were analyzed as continuous variables. U-Hb, degree of hemolysis, and schistocyte presence were analyzed as ordinal variables. PFH and AST were analyzed separately as both ordinal variables and continuous variables.

## RESULTS

### Study Cohort

A total of 117 patients met our inclusion criteria. The cohort was predominately male (53%) and of a nonwhite race background (72%; Table 1). The median age on day zero of ECMO was 29 days (.08 years) (IQR 2 days; 319 days (.005 years; .875 years)). The median weight was 3.9 kg (2.8; 8.6). The median total duration of the ECMO run was 10.48 days (4.25; 14), with 82% of all the patients being on venoarterial ECMO (Table 1). PFH was measured

1,069 times with 967 concurrent measurements of AST, 116 concurrent measurements of LDH, 945 concurrent measurements of red cell width distribution (RDW), and 60 concurrent measurements of U-Hb.

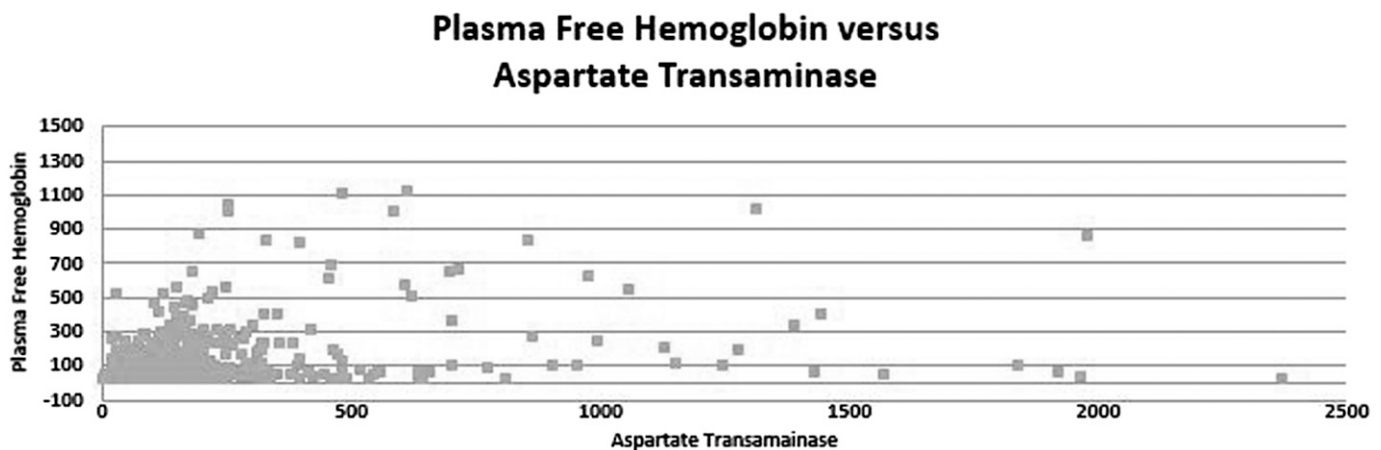
### Correlation between PFH and the Different Hemolysis Biomarkers

Table 2 summarizes PFH measurements when analyzed in comparison to the different hemolysis biomarkers. There was no correlation between hematuria on urinalysis and the level of PFH ( $p = .338$ ; Table 2). There was a correlation among PFH and gross hemolysis on the routine chemistry studies ( $Rho = .439$ ,  $p < .001$ ; Figure 1), AST ( $Rho = .487$ ,  $p < .001$ ; Figure 2), and LDH ( $Rho = .584$ ,  $p < .001$ ; Figure 3). Although weak, there was a correlation between PFH and RDW ( $Rho = .190$ ,  $p < .001$ ). When AST values that had concurrent pathologic elevation of alanine transaminase were censored, the association strengthened ( $Rho = .552$ ,  $p < .01$ ).

## DISCUSSION

Hemolysis is a common complication in pediatric patients who are placed on ECMO, which is frequently associated with more serious complications such as pump thrombosis, renal failure, coagulopathy, and multi-organ dysfunction, all of which are associated with significant morbidity and mortality (4–11). Despite widespread use of ECMO in national and international intensive care units, hemolysis screening continues to be nonuniform, heterogeneous, and dependent on each institution's internal guidelines and available laboratory tests.

This study demonstrated that the use of routine, widely available biomarkers of hemolysis (gross hemolysis, AST, LDH, and RDW) correlated with PFH levels, which indicates that these biomarkers may be suitable alternatives



**Figure 2.** Association between categories of PFH measurements and aspartate aminotransferase measurements.

### Plasma Free Hemoglobin versus Lactate Dehydrogenase

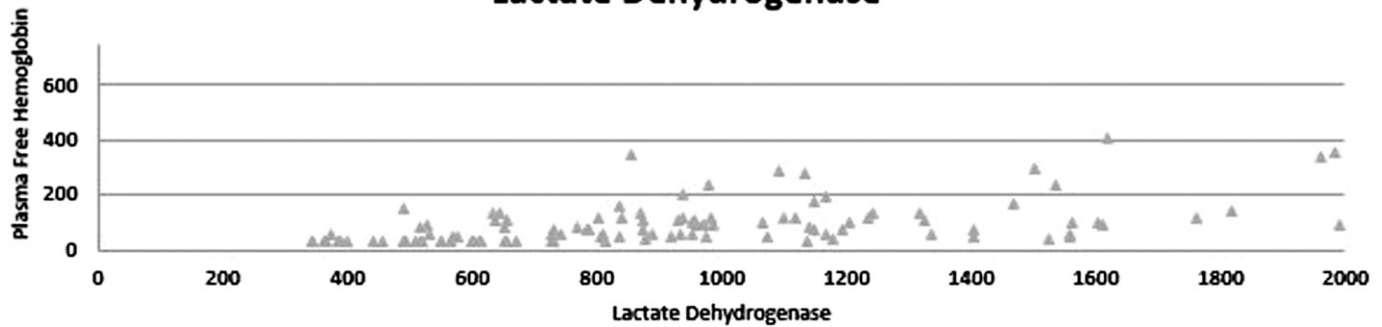


Figure 3. Association between categories of PFH measurements and lactate dehydrogenase measurements.

to PFH levels for the detection of hemolysis in pediatric patients requiring MCS. This information is clinically important because not all centers conducting ECMO have access to PFH measurements. In addition, PFH measurements are exquisitely sensitive to the method of sample acquisition and delays of analysis, which both could falsely elevate the PFH. This emphasizes the utility of having corroborating biomarkers (16). In addition, these biomarkers are not only readily available at most hospitals, but the results should be rapidly available after collection. Early detection of hemolysis may enable physicians to eliminate the source of hemolysis, such as pump thrombosis, before the development of organ dysfunction that results from the oxidative and microangiopathic changes that occur with hemolysis. The weak correlation between PFH and RDW suggests that RDW, which is part of a standard complete blood count, may also provide some benefit in determining the presence of hemolysis in this patient population.

Last, basing the diagnosis of hemolysis solely on the PFH results is not optimal, given that the diagnosis of significant hemolysis and pump thrombosis is one of utmost urgency, and even at large, tertiary care centers, PFH levels do not result quickly. Therefore, even at centers where PFH levels are available, the utilization of other biomarkers to correlate to the previously used “gold-standard” of PFH can potentially decrease time to life-saving alterations in care.

On another level but of the same importance, correlation between U-Hb (on the urinalysis) and PFH was analyzed, but no correlation was denoted in this study. The absence of correlation could have been due to the small number of U-Hb measurements obtained during the period of our study (60 U-Hb measurements vs. 1069 PFH measurements). In the absence of an adequate number of measurements, a correlation is difficult to be observed because of lack of power. This is even more important as U-Hb is a commonly available, noninvasive test that, if a correlation had been shown, could be used as a surrogate for other laboratory blood values in an effort to minimize phlebotomy

in patients on ECMO. One explanation in addition to our lack of samples is that hemolysis, if severe, can rapidly lead to kidney injury and anuria before screening laboratories detect hemolysis, particularly in individuals without a Foley catheter where it would be difficult to discern a change in the color of the urine.

This study has several limitations related to the retrospective nature of its study design. Misclassification is a potential inherent bias to retrospective studies and could have happened during the data collection or gathering. The retrospective nature of the study also affected the data that we were available to collect, as not all the alternate hemolysis biomarkers were collected daily, which led to a decrease in the pool of the data available and might have affected the presence or absence of correlation and the strength or weakness of a correlation. Also, the measurements the U-Hb were collected within 24 hours of the rest of the hemolysis biomarkers (the U-Hb collection and analysis were not always concomitant to the laboratory draws), which increases the error margin and decreases the chance that actual correlations can be demonstrated.

PFH should remain the gold standard for the diagnosis of hemolysis for patients on MCS as the association of mortality with hemolysis in the ECMO population has been demonstrated repeatedly (13). However, the results of this study suggest that the use of widely available biomarkers can aid physicians in early recognition of hemolysis in pediatric patients on ECMO, especially when PFH is not readily available. Further prospective study with rigid sampling will be needed to understand the correlation of serum hemolysis biomarkers with U-Hb.

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