Vitamin D deficiency is associated with an oxidized plasma cysteine redox potential in critically ill children

Jessica Alvarez, Emory University  
Jocelyn Grunwell, Emory University  
Scott E. Gillespie, Emory University  
Vin Tangpricha, Emory University  
Kiran Hebbar, Emory University

Journal Title: Journal of Steroid Biochemistry and Molecular Biology  
Volume: Volume 175  
Publisher: Elsevier: 12 months | 2018-01-01, Pages 164-169  
Type of Work: Article | Post-print: After Peer Review  
Publisher DOI: 10.1016/j.jsbmb.2016.09.013  
Permanent URL: https://pid.emory.edu/ark:/25593/tmssm

Final published version: http://dx.doi.org/10.1016/j.jsbmb.2016.09.013

Copyright information:  
© 2016 Elsevier Ltd  
Accessed November 19, 2019 4:56 AM EST
Vitamin D Deficiency is Associated with an Oxidized Plasma Cysteine Redox Potential in Critically Ill Children

Jessica A. Alvarez, PhD, RD1,2, Jocelyn R. Grunwell, MD, PhD3,4, Scott E. Gillespie, MS2,5, Vin Tangpricha, MD, PhD1,2,6, and Kiran B. Hebbar, MD, FCCM2,3,4

1Emory University School of Medicine, Department of Medicine, Division of Endocrinology, Metabolism & Lipids, Atlanta, GA
2Emory + Children’s Pediatric Research Center, Atlanta, GA
3Emory University School of Medicine, Department of Pediatrics, Division of Pediatric Critical Care Medicine, Atlanta, GA
4Children’s Healthcare of Atlanta at Egleston, Atlanta, GA
5Emory University School of Medicine, Department of Pediatrics, Division of Infectious Disease; Emory + Children’s Pediatric Research Center, Atlanta, GA
6Atlanta Veterans Affairs Medical Center, Section of Endocrinology, Atlanta, GA

Abstract

Critically ill populations incur high levels of oxidative stress and commonly present with vitamin D deficiency. This study aimed to investigate the relationship between vitamin D status and plasma markers of glutathione (GSH) and cysteine (Cys) redox and immunity in critically ill children. This was a cross-sectional study of n = 50 PICU patients. Subjects were categorized according to their plasma 25-hydroxyvitamin D [25(OH)D] concentrations: (<20, 20–30, and ≥30 ng/dL). Plasma GSH, glutathione disulfide (GSSG), Cys, and cystine (CySS) were measured with high-performance liquid chromatography, and their associated redox potentials determined (E_hGSSG and E_hCySS, respectively). Plasma LL-37, an indicator of innate immune function, was assayed with ELISA. Data were analyzed using general linear regression before and after adjustment for age, sex, and race. Results showed that E_hCySS was more reduced in subjects with plasma 25(OH)D concentrations ≥30 ng/mL compared to those with 25(OH)D concentrations <20 ng/mL (P=0.009). Plasma GSH, GSSG, and total GSH decreased with increasing 25(OH)D category (P=0.06, 0.03, and 0.01, respectively), and plasma glutamine levels were lowest in subjects with

Corresponding Author: Jessica A. Alvarez, 101 Woodruff Cr NE, WMRB 1330, Atlanta, GA 30329, jessica.alvarez@emory.edu, Phone: (404) 727-1549, Fax: (404) 727-1390.

Publisher’s Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Conflict of Interest
The authors report no conflict of interest.

Authorship: Author contributions were as follows: conception and design of the study (JAA, KBH, VT); generation, collection assembly, analysis and/or interpretation of data (JAA, KBH, JRG, SEG); drafting or revision of manuscript (JAA, KBH, VT, JRG, SEG); and approval of final version of the manuscript (JAA, KBH, VT, JRG, SEG).
plasma 25(OH)D concentrations ≥30 ng/mL (P=0.004). Plasma LL-37 concentrations did not significantly differ by vitamin D status (P=0.08). In conclusion, vitamin D sufficiency was associated with more reduced plasma $\text{E}_2\text{CySS}$, indicative of lower oxidative stress in critically ill children. Plasma GSH, GSSG, and glutamine, however, were lower in the vitamin D sufficient group. The role of vitamin D in maintaining redox status during pediatric critical illness requires further study.

**Keywords**

calcidiol; oxidative stress; cathelicidin; LL-37; critical care; pediatrics

1. **Introduction**

The widespread prevalence of low circulating concentrations of 25-hydroxyvitamin D (25(OH)D), an indicator of vitamin D status, is well-documented in both pediatric and adult critically-ill populations. Less is known about the implications of concurrent vitamin D deficiency and critical illness. The ubiquitous presence of vitamin D receptors and vitamin D activating enzyme, $\alpha$-hydroxylase, throughout tissues and cells, as well as several studies reporting an association between vitamin D status and clinical outcomes, provide support for a role in the pathophysiology of critical illness. To date, however, there are no published studies evaluating the effects of vitamin D repletion in critically-ill children, and limited available data in adults are suggestive that vitamin D may have benefit, but are not conclusive.

The systemic response to critical illness involves complex shifts in circulating markers of oxidative stress, antioxidant status and function, inflammation, immunity, and substrate metabolism. Research in critical illness has focused on the role of vitamin D on immunity and inflammation. Indeed, experimental studies show that vitamin D in its active form, calcitriol, downregulates pro-inflammatory cytokines and upregulates expression of the antimicrobial peptide LL-37 of the cathelicidin gene. In studies of critically ill adults with sepsis, circulating plasma LL-37 concentrations were shown to positively correlate with plasma 25(OH)D concentrations, and supplementation with vitamin D increased both plasma LL-37 and select proinflammatory cytokines. The relationship between vitamin D status and inflammation and immunity in critically ill children is not clear. An understanding of the role of vitamin D across the spectrum of the physiological changes that occur during critical illness is needed to determine optimal recommendations for vitamin D supplementation.

Oxidative stress has been implicated in the progression of organ dysfunction and failure in critical illness. In addition to measurement of byproducts of oxidative damage or specific antioxidants, oxidative stress can be assessed in terms of reduction-oxidation (redox) balance. Glutathione (GSH) and cysteine (Cys) and their respective disulfides [glutathione disulfide (GSSG) and cystine (CySS)] represent major intracellular and extracellular redox systems, respectively. Plasma measures of these low molecular weight thiol redox systems were recently shown to differ in critically ill children compared to...
In a separate study, plasma glutamine concentrations, a precursor of GSH, were depleted in pediatric intensive care unit (PICU) patients. *In vitro*, calcitriol directly upregulates key enzymes involved in GSH redox homeostasis, glutathione cysteine ligase and glutathione reductase. Furthermore, in an ambulatory adult population, vitamin D status was significantly associated with biomarkers of plasma GSH/GSSG and Cys/CySS redox. Whether vitamin D plays a role in maintaining redox balance in critical illness is not known.

In this study, we aimed to investigate the relationship between vitamin D status and biomarkers of key players in the stress response to critical illness, specifically redox and immunity, in children admitted to a pediatric intensive care unit (PICU). We hypothesized that patients with adequate vitamin D status would have more negative (i.e., less oxidized) plasma redox potentials for the GSH/GSSG and Cys/CySS systems and higher plasma LL-37 compared to vitamin D insufficient patients.

### 2. Materials and Methods

#### 2.1 Subjects

This was a cross-sectional study of n = 50 PICU patients presenting to Children’s Healthcare of Atlanta at Egleston between January 2010 and April 2012. Patients ages 0–18 years and weighing ≥6 kg were eligible for study inclusion. Patients were excluded if they had the following: chronic renal disease, gastrointestinal malabsorption conditions, post-operative state following an elective surgery, trauma as related to possible abuse, or weight < 6kg. Blood was drawn within 24 hours of admission to PICU. Disease severity was assessed using the Pediatric Risk of Mortality III (PRISM III) score and the Pediatric Logistic Organ Dysfunction (PELOD) score. The study was approved by both the Emory University and Children’s Healthcare of Atlanta Institutional Review Boards, and informed consent was obtained from patients’ guardians prior to any study procedures. In this sub-set of a larger cohort study ([clinicaltrials.gov](https://clinicaltrials.gov) identifier NCT01052207), we only included PICU subjects with available plasma redox and 25(OH)D data.

#### 2.2 Measurement of Redox States, Plasma Glutamine, and Plasma LL-37

Quantitation of GSH, GSSG, Cys, and CySS was performed with high-performance liquid chromatography (HPLC) with fluorescence detection. Details for sample collection and processing and subsequent thiol/disulfide measurement and determination of redox states have been previously described. In brief, whole blood was collected and transferred to microcentrifuge tubes with a preservative solution containing iodoacetic acid to minimize autooxidation. Samples were centrifuged and the supernatant mixed with a solution containing perchloric acid, boric acid, and γ-L-glutamyl-L-glutamate as an internal standard and frozen at −80°C until ready for analysis. Thawed samples were treated with a dansyl chloride solution for derivatization prior to HPLC analysis. Quantification of analytes occurred by integration relative to the internal standard. Total Cys was calculated as [Cys] + 2[CySS], and total GSH as [GSH] + 2[GSSG]. The ratios of the reduced and oxidized aminothiols (Cys:CySS and GSH:GSSG) were determined. The redox potentials, $E_h^{GSSG}$ and $E_h^{CySS}$ were calculated using the Nernst equation with $E_o$ values of −264 mV and −250 mV.
mV, respectively, at a pH of 7.4\textsuperscript{23}. Higher (less negative) redox potentials, expressed in mV, are indicative greater levels of oxidative stress. For measurement of plasma glutamine, EDTA plasma (100 ul) was first treated with glutaminase to convert the glutamine to ammonia and then treated with glutamate dehydrogenase, α-ketoglutanate and NADH to generate L-glutamate and NAD. The remaining NADPH concentration is inversely proportional to the glutamine concentration as determined on an Analox GM7 MicroStat analyzer (London, UK). Plasma LL-37 concentrations were determined with ELISA (Hycult Biotech, Uden, Netherlands).

2.3 Determination of Vitamin D Status

Blood was collected in EDTA vacutainer tubes, separated, and stored at –80°C until ready for analysis. Plasma 25-hydroxyvitamin D [25(OH)D] was measured using a chemiluminescent automated technique (iSYS analyzer, Immunodiagnostic Systems, Inc., Fountain Hills, AZ) in a laboratory certified by the Vitamin D External Quality Assessment Scheme (DEQAS). The laboratory inter- and intra-assay CVs for measurement of 25(OH)D are 10.1–13.0% and 1.8–4.0%, respectively. As vitamin D adequacy levels have not been specifically defined for PICU patients and cut-off values for adequate vitamin D status have been debated to be ≥20 ng/mL or ≥30 ng/mL\textsuperscript{24, 25}, study participants were categorized based on plasma 25(OH)D concentrations <20 ng/mL, 20–30 ng/mL, and ≥30 ng/mL\textsuperscript{2, 3}. Classification of plasma 25(OH)D levels using <12 ng/mL as an indicator of severe vitamin D deficiency was also explored.

2.4 Statistical Analyses

Data analyses were performed using SAS 9.3 (Cary, NC), and statistical significance was evaluated at the 0.05 level. Demographics and clinical summaries were calculated using counts and frequencies, medians and ranges, or means and standard deviations where appropriate. Plasma biomarkers were assessed by vitamin D category (< 20, 20 – 30, and ≥30 ng/dL) using general linear regression. Residual errors for each modeled biomarker outcome were gauged for normality via histograms, boxplots, and quantile-quantile probability plots. With the exception of the redox potentials, the circulating biomarkers were not normally distributed, and were thus transformed using the natural log. Analyses were conducted both unadjusted and adjusted for patient age, gender and race. Back-transformed least-squares means and 95% confidence intervals were reported. When overall significant differences were found, pairwise comparisons were considered using the Tukey-Kramer method.

3. Results

Demographic and clinical characteristics are presented in Table 1. The mean age was approximately 12 years, and over half of the patients were male. The majority of the cohort was African American. Over 60% of subjects required mechanical ventilation. The median plasma 25(OH)D was in the deficient range (< 20 ng/mL); 58% of subjects presented to the PICU with plasma 25(OH)D < 20 ng/mL, and 74% of patients had plasma 25(OH)D < 30 ng/mL. There were no statistically significant differences in PRISM III or PELOD scores between the vitamin D status groups (\(P = 0.50\) and 0.44, respectively). Use of advanced
technologies (extracorporeal membrane oxygenation, continuous venovenous hemodialysis, and plasma exchange) also did not significantly differ between the vitamin D status groups ($P = 0.33, 0.23$, and $0.54$, respectively).

### 3.1 Relationship between vitamin D status and plasma aminothiol redox

Differences in plasma GSH/GSSH and Cys/CySS redox data and glutamine based on plasma $25(OH)D$ concentrations are shown in Table 2. Plasma Cys, CySS, and total Cys did not significantly differ by vitamin D status ($P > 0.05$ for all). The ratio Cys:CySS was higher and $E_h$CySS was significantly lower (more reduced) in subjects with plasma $25(OH)D$ concentrations $\geq 30$ ng/mL compared to those with $25(OH)D$ concentrations $<20$ ng/mL. As shown in Fig. 1A, the group difference in $E_h$CySS remained statistically significant after adjustment for age, sex, and race. Plasma GSH, GSSG, and total GSH decreased with increasing $25(OH)D$ category (Table 2; $P = 0.06, 0.03,$ and $0.01$, respectively); differences remained or were more pronounced after adjustment for age, sex, and race (Fig. 1B–D). Plasma glutamine levels were lowest in subjects with plasma $25(OH)D$ concentrations $\geq 30$ ng/mL before and after adjustment for covariates (Table 2, Fig. 1E, $P = 0.004$ for both). Results were similar if a lower threshold for severe vitamin D deficiency ($25(OH)D < 12$ ng/mL) was compared to milder vitamin D deficiency ($25(OH)D 12–30$ ng/mL, Supplemental Table 1).

### 3.2 Relationship between vitamin D status and plasma LL-37

Group differences in plasma LL-37 based on vitamin D status were not statistically significant (Table 2, $P = 0.08$), although plasma LL-37 was quantitatively higher in subjects with plasma $25(OH)D \geq 30$ ng/mL. Findings were similar after adjustment for age, sex, and race (Fig. 1F), or if different thresholds for vitamin D status were compared ($25(OH)D < 12$ ng/mL vs $12–30$ ng/mL vs $30$ ng/mL, Supplemental Table 1).

### 4. Discussion

Vitamin D has been previously suggested to play a role in regulation of oxidative stress, inflammation, and the immune response. The purpose of this study was to examine the associations between vitamin D status and plasma markers of redox and immunity in PICU patients. Our data showed variable relationships between vitamin status and redox biomarkers, depending on whether Cys/CySS or GSH/GSSG was assessed.

Given their abundance and central roles in redox signaling pathways, quantification of the major plasma intracellular (Cys/CySS) and extracellular (GSH/GSSG) thiol pools and their respective redox potentials ($E_h$CySS and $E_h$GSSG) can be useful clinical indicators of systemic oxidative stress$^{16, 23}$. In this cohort of critically-ill children and compared to healthy controls, Grunwell et al.$^{17}$ reported a more oxidized plasma $E_h$GSSG, higher plasma GSSG, and lower plasma Cys and CySS, with no difference in plasma $E_h$CySS. These data suggested a role of the plasma GSH redox system in maintaining the extracellular redox balance of the Cys thiol system in critically-ill children. In our study, low plasma $25(OH)D$ concentrations, which have been previously reported in this cohort$^2$ and other pediatric ICU cohorts$^1, 3$, was associated with more oxidized plasma $E_h$CySS, thus suggesting a role for...
vitamin D in control of oxidative stress in pediatric critical illness. Furthermore, subjects with higher 25(OH)D levels presented with lower plasma GSH, GSSG, total GSH and glutamine concentrations. Although mechanisms remain to be elucidated, it is possible that vitamin D amplifies the plasma GSH-mediated effects on extracellular Cys redox during critical illness. Lower GSH and glutamine concentrations in vitamin D sufficient patients may reflect enhanced plasma substrate utilization for the maintenance of the plasma Cys redox potential during critical illness.

This is the first study, to our knowledge, to investigate a relationship between vitamin D status and oxidative stress in a critically-ill cohort. The associations differ from those of a previous study of generally healthy, ambulatory adults in which we described an independent, inverse relationship of plasma 25(OH)D with plasma E_ShGSSG (but not E_0CySS) and a positive relationship with GSH. Clinical trials in chronic diseases, such as diabetes, have shown vitamin D to increase circulating GSH concentrations. In vitro and animal studies show vitamin D, as calcitriol (1,25(OH)_2D) or vitamin D_3, to alter the expression or action of redox regulating proteins, such as glutathione peroxidase, glutamate cysteine ligase, glutathione reductase, and thioredoxin binding protein-2 (also known as vitamin D_3 upregulating protein 1), although results have not been consistent. For example, George et al. reported an increase in hepatic glutathione peroxidase gene expression in streptozotocin-induced diabetic rats. In contrast, Bhat et al. reported a decrease in the activity of GSH-dependent enzymes glutathione peroxidase and glutathione reductase in rat muscle following vitamin D repletion. The influence of disease state (e.g., acute, chronic, healthy) and tissue system (plasma, muscle, liver) on the relationship between vitamin D status and the GSH/GSSG and Cys/CySS redox pairs will require additional investigation to establish whether vitamin D may be a useful adjunctive therapy to reduce oxidative stress in pediatric critical illness.

In this cohort, subjects with plasma 25(OH)D concentrations ≥30 ng/mL had the highest plasma LL-37 concentrations, although the relationship was not statistically significant. Our small sample size likely limited the power to produce a statistically significant result; nevertheless, the biologic plausibility for a role of vitamin D on this marker of innate immunity is strong. LL-37 is an anti-microbial peptide of the human cathelicidin gene, hCAP18, which contains a vitamin D response element on its promoter region, and calcitriol directly upregulates the expression of cathelicidin. Although we do not have plasma calcitriol available in our subjects for comparison, it is possible that plasma LL-37 is more influenced by plasma calcitriol. On the other hand, in vitro studies suggest upregulation of cathelicidin only occurs in the setting of adequate serum 25(OH)D concentrations. In critically ill adults with sepsis, higher plasma 25(OH)D concentrations correlated with higher plasma LL-37. Furthermore, in a recent randomized, placebo-controlled trial in adult ICU patients with sepsis, high-dose supplementation with cholecalciferol (400,000 IU) resulted in a 30% increase in plasma LL-37 concentrations. Randomized, controlled trials in critically ill children are needed to verify an effect of vitamin D on plasma LL-37. Importantly, whether an increase in plasma LL-37 will translate to improved clinical outcomes in critical illness, in either adults or children, will need to be determined.
There is on-going debate regarding the specific circulating 25(OH)D concentrations that define an “adequate” or “deficient” vitamin D status\(^\text{24, 25}\). Optimal concentrations for PICU populations are not known. Our data show complex relationships between vitamin D status and the major constituents of intra- and extracellular aminothiol redox and immunity. Additional studies are clearly required to describe the clinical and metabolic effects of vitamin D supplementation in pediatric critical illness in order to determine the optimal level of supplementation needed. It is also possible that repletion of vitamin D following the initiation of critical illness may not prove to be efficacious, rather adequate stores of vitamin D may be necessary prior to the onset of critical illness to reap the benefits of reduced oxidative stress and increased levels of LL-37.

A major strength of this study was the novel investigation of the major intracellular and extracellular redox systems in association with vitamin D status during critical illness in children. However, given the limitations in sample size, this can only be considered a pilot study. As this is a sub-study based off of a previous study\(^\text{2, 17}\), sample size calculations were not conducted specifically to assess the relationship between vitamin D status and plasma redox. We were limited in power to adjust for numerous covariates, such as severity or duration of illness, underlying diagnosis, diagnosis of an acute event vs. exacerbation of chronic disease, or medications. It is possible that the heterogeneity of the cohort in terms of diagnosis and severity further, as well as medication use, influenced statistical power and the ability to make clinical inferences. As this was a cross-sectional study, one cannot infer cause-effect relationships, although the data are hypothesis-generating. We do not have available measurements of plasma calcitriol or free or bioavailable 25(OH)D. The predictive value of bioavailable 25(OH)D may be greater than total circulating 25(OH)D\(^\text{12}\), particularly in critically ill patients, in whom the major 25(OH)D carriers (vitamin binding protein and albumin) are acute phase reactants\(^\text{31}\). Finally, we do not have measurements of vitamin D, redox, or nutrition status prior to reporting to the ICU, or measurements of other plasma oxidants and antioxidants, for comparison.

### 4.1 Conclusions

In critically ill children, vitamin D sufficiency was associated with less oxidized plasma $\text{E}_\text{h}$\(_\text{CySS}\), the redox state of the primary extracellular redox couple (Cys and CySS). In contrast, vitamin D sufficiency was associated with lower plasma GSH, GSSG, and glutamine, suggesting complex relationships between vitamin D and aminothiol redox during critical illness in children. Limitations of this pilot study elicit a need for additional detailed investigation to verify a specific role of vitamin D in aminothiol redox balance. Clinical trials of vitamin D supplementation will be required to elucidate the various ways vitamin D may be impacting the stress response to critical illness.

### Supplementary Material

Refer to Web version on PubMed Central for supplementary material.
Acknowledgments

This study was supported by the Emory+Children’s Statistical Core and the Emory+Children’s Biomarkers Core. Funding was provided by the Children’s Healthcare of Atlanta Friends (grant number 38234), the National Institutes of Health (UL1 TR000454 and K01 DK102851). Funding sources had no involvement in the study design; the collection, analysis or interpretation of the data; the writing of the report; or the decision to submit the article for publication.

Abbreviations

25(OH)D  25-hydroxyvitamin D
CySS    cystine
Cys     cysteine
EhCySS  redox potential for the Cys/CySS couple
EhGSSG  redox potential for the GSH/GSSG couple
GSH     glutathione
GSSG    glutathione disulfide
HPLC    high-performance liquid chromatography
PICU    pediatric intensive care unit
PELOD   Pediatric Logistic Organ Dysfunction
PRISM III Pediatric Risk of Mortality III

References


HIGHLIGHTS

• We examined the link between vitamin D, oxidative stress, and immunity in the PICU.

• Vitamin D deficiency was associated with a more oxidized plasma cysteine redox.

• Plasma glutathione was higher in vitamin D deficient subjects.

• The role of vitamin D in regulating plasma redox in the PICU needs further study.
Fig. 1.
Plasma redox and LL-37 concentrations by vitamin D status, after adjustment for age, sex, and race. Data are shown as back-transformed, model-based mean estimates and 95% CI (redox potential data were not transformed). Abbreviations: 25(OH)D, 25-hydroxyvitamin D; Cys, cysteine; CySS, cystine; $E_h$, redox potential; GSH, glutathione; GSSG, glutathione disulfide. Total Cys was calculated as $[\text{Cys}] + 2[\text{CySS}]$, and total GSH as $[\text{GSH}] + 2[\text{GSSG}]$. Bold values indicate statistical significance; values not connected by same superscript are significantly different. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$
<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Mean ± SD or n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>11.8 ± 5.2</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>46.8 ± 25.2</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>28 (56%)</td>
</tr>
<tr>
<td>Female</td>
<td>22 (44%)</td>
</tr>
<tr>
<td>Hispanic</td>
<td>2 (4%)</td>
</tr>
<tr>
<td>Race</td>
<td></td>
</tr>
<tr>
<td>African American</td>
<td>34 (68%)</td>
</tr>
<tr>
<td>Caucasian</td>
<td>11 (22%)</td>
</tr>
<tr>
<td>Other</td>
<td>5 (10%)</td>
</tr>
<tr>
<td>Primary Diagnosis</td>
<td></td>
</tr>
<tr>
<td>Shock</td>
<td>24 (48%)</td>
</tr>
<tr>
<td>Sepsis</td>
<td>22 (44%)</td>
</tr>
<tr>
<td>Septic Shock</td>
<td>18 (36%)</td>
</tr>
<tr>
<td>Asthma</td>
<td>11 (22%)</td>
</tr>
<tr>
<td>Other</td>
<td>32 (64%)</td>
</tr>
<tr>
<td>PRISM III Score</td>
<td>11.2 ± 5.8</td>
</tr>
<tr>
<td>PELOD Score</td>
<td>16.9 ± 14.2</td>
</tr>
<tr>
<td>Hospital length of stay (days)⁹</td>
<td>9 (5–19)</td>
</tr>
<tr>
<td>PICU length of stay (days)¹</td>
<td>6.5 (4–9)</td>
</tr>
<tr>
<td>Mechanical ventilation</td>
<td>31 (62%)</td>
</tr>
<tr>
<td>28-Day Mortality</td>
<td>3 (6%)</td>
</tr>
<tr>
<td>Use of advanced technologies</td>
<td></td>
</tr>
<tr>
<td>Extracorporeal membrane oxygenation</td>
<td>45 (90%)</td>
</tr>
<tr>
<td>Continuous venovenous hemodialysis</td>
<td>42 (84%)</td>
</tr>
<tr>
<td>Plasma exchange</td>
<td>47 (94%)</td>
</tr>
<tr>
<td>Plasma albumin (g/dL)⁹²</td>
<td>3.2 (2.4–3.5)</td>
</tr>
<tr>
<td>Plasma 25(OH)D (ng/mL)⁹</td>
<td>18.5 (11.3–32.9)</td>
</tr>
<tr>
<td>Plasma 25(OH)D &lt; 20 ng/mL</td>
<td>29 (58%)</td>
</tr>
<tr>
<td>Plasma 25(OH)D &lt; 30 ng/mL</td>
<td>37 (74%)</td>
</tr>
</tbody>
</table>

Abbreviations: PICU, pediatric intensive care unit; 25(OH)D, 25-hydroxyvitamin D; PRISM III, Pediatric Risk of Mortality version 3; PELOD, Pediatric Logistic Organ Dysfunction.

⁹Reported as median (IQR).

²N = 40.
### Table 2

Plasma biomarkers of aminothiol redox and immunity by plasma 25(OH)D cut-off levels.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>N</th>
<th>25(OH)D &lt; 20 ng/mL</th>
<th>N</th>
<th>25(OH)D 20–30 ng/mL</th>
<th>N</th>
<th>25(OH)D ≥ 30 ng/mL</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cys (µM)</td>
<td>29</td>
<td>0.42 (0.28 – 0.65)</td>
<td>8</td>
<td>0.67 (0.29 – 1.50)</td>
<td>13</td>
<td>0.89 (0.47 – 1.68)</td>
<td>0.160</td>
</tr>
<tr>
<td>CySS (µM)</td>
<td>29</td>
<td>0.58 (0.34 – 0.99)</td>
<td>8</td>
<td>0.68 (0.24 – 1.87)</td>
<td>13</td>
<td>0.47 (0.21 – 1.05)</td>
<td>0.855</td>
</tr>
<tr>
<td>Total Cys (µM)</td>
<td>29</td>
<td>1.75 (1.11 – 2.76)</td>
<td>8</td>
<td>2.34 (0.99 – 5.55)</td>
<td>13</td>
<td>1.92 (0.98 – 3.78)</td>
<td>0.845</td>
</tr>
<tr>
<td>Cys:CySS</td>
<td>29</td>
<td>0.73 (0.51 – 1.04)</td>
<td>8</td>
<td>0.98 (0.50 – 1.92)</td>
<td>13</td>
<td>1.87 (1.10 – 3.16)</td>
<td>0.020</td>
</tr>
<tr>
<td>E_{GSS}CySS (mV)</td>
<td>29</td>
<td>−54.64 (−62.11 – (−47.17))</td>
<td>8</td>
<td>−64.49 (−78.71 – (−50.26))</td>
<td>13</td>
<td>−76.54 (−87.70 – (−65.38))</td>
<td>0.009</td>
</tr>
<tr>
<td>GSH (µM)</td>
<td>29</td>
<td>0.63 (0.32 – 1.25)</td>
<td>8</td>
<td>0.51 (0.14 – 1.89)</td>
<td>13</td>
<td>0.13 (0.05 – 0.37)</td>
<td>0.055</td>
</tr>
<tr>
<td>GSSG (µM)</td>
<td>29</td>
<td>0.27 (0.17 – 0.42)</td>
<td>8</td>
<td>0.35 (0.15 – 0.81)</td>
<td>13</td>
<td>0.10 (0.05 – 0.19)</td>
<td>0.034</td>
</tr>
<tr>
<td>GSH:GSSG</td>
<td>29</td>
<td>2.34 (1.27 – 4.32)</td>
<td>8</td>
<td>1.47 (0.46 – 4.70)</td>
<td>13</td>
<td>1.34 (0.54 – 3.35)</td>
<td>0.557</td>
</tr>
<tr>
<td>Total GSH (µM)</td>
<td>29</td>
<td>1.65 (0.99 – 2.75)</td>
<td>8</td>
<td>1.48 (0.56 – 3.91)</td>
<td>13</td>
<td>0.40 (0.19 – 0.86)</td>
<td>0.013</td>
</tr>
<tr>
<td>E_{GSS}GSSG (mV)</td>
<td>29</td>
<td>−89.03 (−104.96 – (−73.11))</td>
<td>8</td>
<td>−80.23 (−110.55 – (−49.92))</td>
<td>13</td>
<td>−61.68 (−85.46 – (−37.90))</td>
<td>0.183</td>
</tr>
<tr>
<td>Glutamine (mmol/L)</td>
<td>25</td>
<td>0.64 (0.55 – 0.75)</td>
<td>7</td>
<td>0.87 (0.65 – 1.17)</td>
<td>11</td>
<td>0.45 (0.36 – 0.57)</td>
<td>0.004</td>
</tr>
<tr>
<td>LL-37 (ng/mL)</td>
<td>28</td>
<td>70.51 (50.27 – 98.90)</td>
<td>8</td>
<td>68.10 (36.16 – 128.25)</td>
<td>12</td>
<td>140.85 (84.00 – 236.18)</td>
<td>0.081</td>
</tr>
</tbody>
</table>

Data are presented as back-transformed, model-based mean estimates and 95% CI (redox potential data were not transformed).

Abbreviations: 25(OH)D, 25-hydroxyvitamin D; Cys, cysteine; CySS, cystine; E_{GSS}, redox potential; GSH, glutathione; GSSG, glutathione disulfide. Total Cys was calculated as [Cys] + 2[CySS], and total GSH as [GSH] + 2[GSSG]. Bold values indicate statistical significance; values not connected by same superscript are significantly different.