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Pediatric ECMO: Unfavorable Outcomes are Associated with Inflammation and Endothelial Activation

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Abstract

Background: Inflammatory and endothelial activation responses during extracorporeal membrane oxygenation (ECMO) support in children are poorly understood. In this study, we aimed to determine if circulating inflammatory, endothelial activation and fibrinolytic markers are associated with mortality and with neurologic outcomes in children on ECMO.

Methods: We conducted a secondary analysis of a two-center prospective observational study of 99 neonatal and pediatric ECMO patients. Inflammatory (interferon gamma [IFNγ, interleukin-6 [IL-6], IL-1β, tumor necrosis factor alpha [TNFα]), endothelial activation (E-selectin, P-selectin, intercellular adhesion molecule-3 [ICAM-3], thrombomodulin [TM]), and fibrinolytic markers (tissue plasminogen activator [tPA], plasminogen activator inhibitor-1 [PAI-1]) were measured in plasma on days 1, 2, 3, 5, 7, and every third day thereafter during the ECMO course.

Results: All ECMO day 1 inflammatory biomarkers were significantly elevated in children with abnormal vs normal neuroimaging. ECMO day 1 and peak levels of IL-6 and PAI-1 were significantly elevated in children who died compared to those who survived to hospital discharge.

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Consent Statement: Informed consent was obtained from parents or legal guardians within 24 hours of ECMO initiation.

Tested biomarkers showed no significant association with long-term neurobehavioral outcomes measured using the Vineland Adaptive Behavioral Scales, Second Edition.

Conclusions: High levels of circulating inflammatory, endothelial activation and fibrinolytic markers are associated with mortality and abnormal neuroimaging in children on ECMO.

Introduction

Extracorporeal membrane oxygenation (ECMO), initially employed in the 1970s, is a well-established form of mechanical support for severe cardiopulmonary failure. While ECMO is used increasingly in intensive care units in the U.S. and internationally,[1,2] survival has remained relatively unchanged at around 55% of all ECMO cases,[1,3] and neurologic injury remains a significant challenge impacting both survival and long-term outcomes.[4-6]

Neurologic injury manifested as hypoxic ischemic injury, intracranial hemorrhage, thromboembolic stroke or brain death has been reported in as many as 15-36% of ECMO patients.[7-12] The pathophysiology of neurologic injury in patients on ECMO is multifactorial. Pre-ECMO profound cardiopulmonary failure with its corollary of hypoxia, hypotension and acidosis can lead to hypoxic-ischemic injury, rendering the brain vulnerable.[11,13-15] The profound inflammatory state associated with critical illness is then compounded by exposure to the foreign surfaces of the extracorporeal circuit, which triggers a global innate immune response by activating the contact and complement systems,[12,13,16] and provokes perturbations in proinflammatory and pro-thrombotic pathways implicated in the pathogenesis of ECMO-associated neurologic injury.[10,12,17] The device-induced proinflammatory state and endothelial cell dysfunction increase thrombin generation and platelet activation.[18,19] Within the patient, the device contributes to coagulation factor consumption, platelet exhaustion, and reduced platelet aggregation potential.[18,19] Added to that is the need for systemic anticoagulation to prevent circuit thrombus formation, creating the common paradox of thrombotic events in the device with simultaneous bleeding in the patient.[20] Thus, patients are at risk for hypoxic brain injury as well as thromboembolic and hemorrhagic intracerebral complications.

In this secondary analysis of a two-center prospective observational cohort of critically ill children requiring ECMO support,[7] we examined circulating inflammatory, endothelial activation and fibrinolytic markers at ECMO initiation and serially during the ECMO course. We hypothesized that children on ECMO who develop neurological complications or have worse outcomes (in-hospital mortality or unfavorable neurobehavioral outcomes among survivors) would have increased concentrations of pro-inflammatory, endothelial activation and fibrinolytic markers in plasma compared to those without neurological complications and with survivors with favorable neurobehavioral outcomes up to 1 year post-ECMO.

Materials and Methods

Study Design and Patient Selection

We conducted a retrospective analysis of an observational cohort study. Patients were prospectively enrolled within 24 hours of ECMO initiation at two academic, urban, pediatric intensive care units. Inclusion and exclusion criteria as well as enrollment details have been

described previously.[7] All patients enrolled in the parent cohort were eligible for this study. This study was approved by the Institutional Review Boards at both participating sites.

Demographic, clinical, and neuroimaging data were collected prospectively during the hospitalizations that included the ECMO course. The primary outcome was neurological complications (diffuse hypoxic injury, intracranial hemorrhage, and ischemic stroke) determined by neuroimaging. Neuroimaging studies obtained by the clinical team as part of institutional clinical protocols or for clinical indications, included head ultrasound (obtained pre-ECMO and daily during the ECMO course at both sites), brain computed tomography (CT) and brain magnetic resonance imaging (MRI). The date, time, and type of new neuroimaging abnormality were recorded for all neuroimaging studies available during ECMO and up to 6 weeks after decannulation. Secondary outcomes included in-hospital mortality, and, among survivors to hospital discharge, the Vineland Adaptive Behavior Scales-II (VABS-II) score dichotomized at 85 (i.e., −1 SD below the reference population mean).

ECMO Circuit Components

The ECMO circuits during the study period consisted of custom-packed 1/4 or 3/8 inch flexible PVC tubing (Medtronic, Minneapolis, MN) with a silicone reservoir, a bladderbox (Johns Hopkins Hospital, Baltimore, MD), a 0.8–4.5 m2 membrane oxygenator (Medtronic), a heat exchanger (Medtronic), and a roller pump (Sorin Cardiovascular, Arvada, CO) up to January 2011, and the Better Bladder (Coastal Life Systems Inc, San Antonio, TX), the Quadrox-ID oxygenator (Maquet Cardiopulmonary, Rastatt, Germany), and a roller or centrifugal pump (Sorin), thereafter, at one site. At the other site, the ECMO circuit consisted of custom-packed 1/4 or 3/8 inch flexible PVC tubing (Medtronic, Minneapolis, MN), the Better Bladder (Circulatory Technology, Inc), the Quadrox-ID oxygenator (Getinge Cardiopulmonary, Rastatt, Germany), and a centrifugal pump (Getinge Cardiopulmonary, Rastatt, Germany). Catheters used at both institutions include Bio-Medicus One-Piece Femoral Arterial Cannula (Medtronic), Bio-Medicus One-Piece Femoral Venous Cannula (Medtronic), OriGen reinforced dual lumen catheter (OriGen), and Avalon Elite Bi-Caval Dual Lumen Catheter (Getinge). One site also used Percutaneous Sheath Introducer Kit (reperfusion cannula) (Arrow), while the other institution also used DLP Pediatric One-Piece Arterial Cannula (Medtronic) and DLP Malleable Single Stage Venous Cannula (Medtronic).

Long-term Outcome Assessment

VABS-II assessment was conducted in person by trained study personnel at each site at 6 months and 1 year post-ECMO. The VABS-II instrument measures adaptive behavior skills and provides age-corrected standard scores from birth to 18 years of age for overall adaptive behavior composite (mean=100, SD = 15) and four domains (motor skills, socialization, daily living, and communication). Higher scores are reflective of higher adaptive behavior function. Study participants who survived to discharge were stratified by status at 6-month and 1-year follow-up and those who did not complete a follow-up visit.

Biomarker Sampling and Analysis

Blood samples were obtained daily during ECMO from the ECMO circuit or existing indwelling catheters, at the same time as routine clinical blood draws. Plasma was separated by centrifugation and stored at −80°C. Plasma concentrations for interferon gamma (IFNγ), interleukin-6 (IL-6), interleukin 1 beta (IL-1β), tumor necrosis factor alpha (TNFα), E-selectin, P-selectin, intercellular adhesion molecule-3 (ICAM-3), thrombomodulin (TM), tissue plasminogen activator (tPA), and plasminogen activator inhibitor-1 (PAI-1), were measured for ECMO days 1, 2, 3 and 5. Measurements for ECMO day 7 and every third day thereafter until ECMO decannulation were conducted if ECMO duration was longer than 5 days.

All assays were conducted in the Johns Hopkins Institute for Clinical and Translational Research (ICTR) Clinical Research Unit: Core Laboratory, using the Meso Scale Discovery (Rockville, MD) V-PLEX Human Proinflammatory Panel (for IFNγ, IL-6, IL-1β, TNFα), the Human Vascular Injury I Kit (for E-selectin, P-selectin, ICAM-3, TM), and single plex assays for tPA and PAI-1. Dynamic ranges for each analyte have been published.[21-23] The inter-assay coefficients of variation for the two multiplex assays used were: IFNγ, 7.96; IL-6, 15.93; IL-1β, 0.02; TNFα, 5.21; E-selectin, 6.45; P-selectin, 14.39; ICAM-3, 11.78; TM, 8.6. Laboratory personnel were blinded to clinical data.

Statistical Analysis

For each biomarker, we characterized the distributions of the first measured sample (within 24 hours of ECMO cannulation). The ECMO day 1 biomarker level may be most clinically relevant as an initial level collected prospectively prior to specific ECMO complications and outcomes. Distributions were stratified by ECMO indication, neurological complications, in-hospital mortality status, and VABS-II score ≥85 up to one year among those who survived to discharge. Distributions were graphically depicted by percentile boxplots and were compared using the Wilcoxon rank sum test.

To estimate the association of differences in ECMO day 1 and longitudinal biomarker concentrations and the risk of in-hospital mortality, separate Cox proportional hazards models were fit for each biomarker to provide time-constant hazard ratios (HRs). Those who survived to hospital discharge were treated as censored, and the HRs are therefore interpreted as cause-specific HRs. This modeling strategy circumvented tethering inherent in estimating subdistribution HRs and was better supported by this smaller dataset.[24,25] In this model, the independent variables were the biomarker on ECMO day 1, and the difference of the biomarker from this first day at each subsequent longitudinal measurement. All biomarkers were log2 transformed. The HRs are interpreted as the between-individual risk of in-hospital mortality associated with a 2-fold higher level from ECMO day 1 (adjusting for longitudinal changes), and the risk associated with a subsequent doubling of the biomarker from ECMO day 1 (adjusting for ECMO day 1 level). For consistency across biomarkers, we estimated risk associated with a 2-fold between- and within-person difference, which was very close to a standard deviation of the biomarker distributions. The models were additionally adjusted for age (neonate vs non-neonate) and primary ECMO indication (respiratory vs non-respiratory).

To measure the risk of abnormal neuroimaging during or following the ECMO course, we used Cox proportional hazards models similar to those described above, with time to abnormal neuroimaging as the outcome. These models describe the risk associated with higher ECMO day 1 and longitudinal biomarker levels, adjusted for age and primary ECMO indication. Those who did not have neuroimaging (n = 11) or whose imaging was abnormal prior to biomarker sample collection (n = 10) were excluded from this part of the analysis.

As a supplementary analysis, we characterized the distributions of peak levels, which could have occurred at any time point during ECMO, and are assumed to reflect how biomarkers were maximally modified by disease course while on ECMO. These distributions were stratified by the same variables described above.

Statistical significance was assessed at $p<0.05$. All analyses were conducted in R 3.6.0 (R Core Team, Vienna, Austria).

Results

Full demographic and clinical characteristics have been described previously[7] and are also summarized in Table 1. Briefly, we enrolled 99 patients at two centers. Fifty-one (52%) study participants were neonates (<30 days), one third (33%) underwent ECMO support for primary respiratory failure, and 66 (67%) underwent ECMO for non-respiratory primary indications, including cardiac failure (39%), extracorporeal cardiopulmonary resuscitation (ECPR) (19%), and sepsis (8%). Eighty-eight (89%) children had neuroimaging during ECMO or within 6 weeks from ECMO decannulation. Forty-four of 88 (50%) children with neuroimaging showed evidence of embolic infarction, intracranial hemorrhage, or postasphyxial brain injury. Forty-two (42%) children died prior to hospital discharge. Plasma collected for research purposes was available for this study in 97/99 (98%) of study participants. Plasma biomarker concentrations on ECMO day 1 and the highest concetration observed during the ECMO course (peak levels) are presented in Supplemental Table 1.

ECMO Day 1 and Peak Biomarkers by ECMO Indication

Day 1 biomarker levels in children on ECMO for primary respiratory failure were compared to children on ECMO for non-respiratory indications (Fig. 1). IL-6 (median 65.0 vs 17.5 pg/mL), thrombomodulin (median 5.4 vs 4.4 ng/mL), tPA (median 7.4 vs 4.2 ng/mL) and PAI-1 (median 200.3 vs 30.0 ng/mL) were significantly elevated in children on ECMO for non-respiratory indications compared to those with respiratory failure. Median peak biomarker levels all increased from day 1 regardless of diagnosis, with the exception of P-selectin for which peak and day 1 were similar. Peak levels were significantly elevated in children supported on ECMO for non-respiratory indications compared to those on ECMO for respiratory failure for IL-6 (median 135.6 vs 19.1 pg/mL), TNFα (median 6.6 vs 4.9 pg/mL), thrombomodulin (median 7.6 vs 6.5 ng/mL), tPA (median 10.2 vs 6.0 ng/mL) and PAI-1 (median 284.3 vs 54.6 ng/mL) (Fig. 1).

ECMO Day 1 and Peak Biomarkers by Neurological Complications

Forty-three of 44 patients who had abnormal findings on neuroimaging had biomarker concentrations evaluated. Evaluation of biomarkers in children with abnormal vs normal

neuroimaging revealed ECMO day 1 levels to be significantly elevated for all inflammatory biomarkers (IFNγ, median 17.5 vs 9.0 pg/mL; IL-6, median 57.9 vs 26.5 pg/mL; IL-1β; 0.4 vs 0.3 pg/mL; TNFα, 4.7 vs 3.2 pg/mL) (Fig. 2). Peak levels remained significantly elevated only for TNFα (median 6.5 vs 5.1 pg/mL) in children with abnormal neuroimaging compared to normal neuroimaging (Fig. 2).

ECMO Day 1 and Peak Biomarkers by Mortality and Long-term Outcomes

Of the 42 children who died prior to hospital discharge, 41 had plasma samples available. ECMO day 1 concentrations were significantly elevated in children who died vs those who survived for IL-6 (median 98.5 vs 23.1 pg/mL) and PAI-1 (median 200.3 vs 94.6 ng/mL) and significantly decreased for E-selectin (13.9 vs 18.0 ng/mL) (Fig. 3). Peak biomarker concentrations remained significantly elevated for IL-6 (median 102.8 vs 32.6 pg/mL) and PAI-1 (330.3 vs 99.5 ng/mL) in those who died compared to those who survived. Peak concentrations of tPA (median 9.8 vs 8.3 ng/ml) were also significantly higher in children who died compared to those who survived (Fig. 3).

Among survivors to hospital discharge, day 1 and peak biomarker concentrations during the ECMO course were similar for children who at 6 and/or 12-month follow-up had a VABS-II composite score of ≥85, <85 or post-discharge mortality, or no follow-up, respectively.

Multivariable Models of ECMO Day 1 and Longitudinal Biomarker Trajectory and Outcomes

To explore the relationship between biomarker levels on ECMO day 1 and longitudinally, cause-specific hazard ratios were estimated from Cox proportional hazards models, with adjustment for age and primary indication for ECMO.

In these multivariate models, between-person 2-fold higher biomarker levels on ECMO day 1 and within-person doubling of biomarker levels over time, respectively, were not significantly associated with abnormal neuroimaging during or following the ECMO course.

In multivariable Cox proportional hazards models, higher inflammatory markers on ECMO day 1 were associated with in-hospital mortality, with the relationships for IL-6, IL-1β and TNFα being statistically significant (Fig. 4). Between-person 2-fold higher ECMO day 1 IL-6, IL-1β, and TNFα concentrations were associated with higher hazard for mortality: adjusted HR (aHR), 1.22 (95% CI: 1.07, 1.39), 1.38 (95% CI: 1.13, 1.70), and 1.45 (95% CI: 1.07%, 1.96), respectively. Within-person 2-fold increase from day 1 of IL-1β and TNFα concentrations during the ECMO course was associated with higher hazard for mortality: aHR, 1.24 (95% CI: 1.05, 1.46) and 1.36 (95% CI: 1.02, 1.83), respectively.

Markers of endothelial activation were less consistent. For E-selectin, higher ECMO day 1 and longitudinal levels were associated in protective and harmful directions, respectively, and neither was significant. While higher day 1 P-selectin concentration was not strongly associated with mortality, higher longitudinal changes from day 1 were significantly associated with increased risk, although the confidence interval was wide (aHR: 1.82, 95% CI: 1.02, 3.24). ECMO day 1 and longitudinal increases of TM and ICAM-3 were near null.

Between-person 2-fold higher ECMO day 1 tPA was not associated with increased hazard for mortality, but a within-person 2-fold increase of tPA from day 1 was significantly associated with a higher hazard for mortality, aHR 1.69 (95% CI: 1.02, 2.82). For PAI-1, 2-fold higher day 1 levels between patients, and a within-person 2-fold increase from day 1, were strongly associated with in-hospital mortality, aHR 1.62 (95% CI: 1.26, 2.10) and aHR 1.73 (95% CI: 1.36, 2.21), respectively (Fig. 4).

PAI-1/tPA Molar Ratios

ECMO day 1 and peak PAI-1/tPA molar ratios were calculated and were significantly elevated in children on ECMO for non-respiratory vs respiratory indications (ECMO day 1 and peak $p < 0.001$) as well as in those who died compared to those who survived to hospital discharge (ECMO day 1 $p = 0.005$; peak $p < 0.001$) (Supplemental Fig. 1). Two-fold higher ECMO day 1 PAI-1/tPA was associated with increased hazard for mortality, aHR: 1.71 (95%CI: 1.33, 2.20) and a 2-fold within-person increase from ECMO day 1 was also significantly associated with mortality (aHR: 1.60, 95%CI: 1.28, 2.00).

Discussion

ECMO is regularly used as a longer term mode of cardiopulmonary support in heterogeneous populations including patients with cardiac disease, respiratory disease, and sepsis with a high risk of neurologic injury and death.[2,4-9] Patients supported on ECMO often display profound inflammation, endothelial activation and coagulation abnormalities due to their underlying disease.[16,26,27] In addition, ECMO initiation triggers further inflammation and coagulation system activation.[12-16,28] Inflammation, endothelial activation and coagulation abnormalities are intertwining and complex and their roles in mortality, neurologic injury, and neurologic outcomes for children on ECMO are not well understood.[10,12,18,19,29,30]

In this study of critically ill children on ECMO support, nonsurvivors and those with new abnormal neuroimaging findings displayed biomarker profiles indicative of more profound proinflammatory and fibrinolytic pathway activation compared to survivors and those with normal neuroimaging, respectively. After adjusting for age and primary ECMO indication, higher ECMO day 1 levels between patients and/or longitudinal increases of pro-inflammatory cytokines IL-6, IL-1β and TNFα, and of fibrinolytic markers tPA and PAI-1, were associated with increased hazard for mortality but not with abnormal neuroimaging during or following the ECMO course. There were no significant biomarker differences in survivors with VABS-II scores above vs below 85 (1 SD below the population mean) up to one year post-ECMO.

Significant changes in inflammatory, endothelial and fibrinolytic activation biomarkers have been described as occurring within minutes to hours of ECMO initiation regardless of indication.[12-16] In this cohort, pre-ECMO plasma samples were not available. ECMO day 1 and peak levels of inflammatory (IL-1β, IL-6, TNFα), endothelial (TM) and fibrinolytic activation (tPA and PAI-1) biomarkers were higher in patients with a non-respiratory vs respiratory indication for ECMO. These findings differ from those of a prior study that evaluated patterns of rise and fall of IL-1β, IL-6, IL-8 and IL-10 during ECMO, and

that found no difference in these patterns among patients on ECMO with cardiac versus respiratory etiology.[27]

Similar to other studies that have demonstrated increases in pro-inflammatory cytokines IL-1β, IL-6 and TNFα during ECMO,[15,27,30,31] we observed an increase in levels during the ECMO course for all the markers evaluated, compared to day one. When evaluating longitudinal changes in biomarkers during the ECMO course, though, it is difficult to determine if and how exposure to the ECMO circuit modulates the inflammatory and fibrinolytic responses above and beyond what would have been observed in the context of the underlying critical illness, should the patient not be on ECMO support.

Inflammatory responses in ECMO patients reflect pathophysiologic processes preceding ECMO, as well as some promoted by the ECMO circuit or care. Cytokines are often elevated in critical illness for which ECMO is used, including congenital diaphragmatic hernia, sepsis,[32-34] and trauma,[35] regardless of ECMO status. Different types of oxygenators[36] and PaO_2 levels attained during ECMO support[37] also promote inflammatory responses. Pro-inflammatory cytokines in turn are implicated in the development of multiple organ dysfunction and are associated with worse outcomes when the cytokine cascade is amplified, leading to a hyperinflammatory phenotype.[38]

IL-6 induces the production of acute phase reactants including C-reactive protein and fibrinogen, decreases albumin production, and promotes platelet activation, differentiation of CD4 and CD8 T-cells, and differentiation of active B-cells.[39,40] Although, elevations in plasma IL-6 concentrations are associated with mortality in several critically ill populations,[20,35,41] data from ECMO studies are minimal. In a study conducted in 22 patients of all ages on ECMO support, Risnes et al. reported no significant overall differences in plasma IL-6 concentrations between survivors and non-survivors, although those who survived had an early peak followed by a rapid decrease in IL-6 levels within 2 days from cannulation, while those who died had persistently elevated IL-6 levels during the ECMO course.[27]

Similar to IL-6, TNFα is a pro-inflammatory cytokine with many effects linking the innate and adaptive immune system. TNFα activates and promotes differentiation of macrophages, induces cytokine and prostaglandin production, increases leukocyte and endothelial activation, promotes neutrophil activation and promotes thrombosis.[12,42,43] In a pediatric ECMO series (n=16) TNFα was noted to increase significantly during ECMO in nonsurvivors compared to survivors,[30] a finding not seen in our cohort.

In a study aiming to evaluate whether extracorporeal life support results in immune dysregulation, Beshish et al. reported serially measured plasma cytokine levels from 19 patients of all ages stratified by presence vs absence of immunoparalysis prior to ECMO cannulation.[44] Plasma TNFα levels were significantly elevated in subjects with vs those without immunoparalysis and did not change significantly over time during the ECMO course in either group. Plasma IL-6 levels showed no significant difference between subjects with vs those without immunoparalysis. During the ECMO course, IL-6 levels remained high over time within subjects with immunoparalysis, but decreased significantly from

pre-ECMO to day 3 within subjects without immunoparalysis.[44] No mortality outcomes were reported in this study.[44] A study by Risnes et al. that did not examine immune function during ECMO reported a significant decrease in IL-6 levels by day 2 in patients who survived ECMO compared to those who died.[27] We did not evaluate the immune function status in our cohort, but similarly found that higher ECMO day one and higher peak plasma IL-6 levels during the ECMO course were associated with mortality. Whether persistent plasma IL-6 elevation during the ECMO course is associated with mortality or immunoparalysis remains to be determined.

Cytokines, including IL-6, IL1β and TNFα, also play a role in coagulation activation by increasing the expression of leukocyte-adhesion molecules, activating platelets, inducing tissue factor and increasing PAI-1 and thrombosis.[18,40,45,46] In turn, cytokines can be suppressed by fibrinolytic factors such as activated protein C.[47] Bleeding and thrombotic events have been associated with increased mortality among patients on ECMO.[17] PAI-1 is a significant inhibitor of tPA and fibrinolysis.[32,48] Together, PAI-1 and tPA form a complex that is mutually inhibitory.[48] In this study, higher ECMO day 1 PAI-1 and longitudinal increase in both tPA and PAI-1 levels during the ECMO course were associated with mortality after adjusting for age and ECMO indication, although, due to the nature of the data collected in the primary study, we were not able to evaluate whether this association was mediated by bleeding and thrombotic events. PAI-1/tPA molar ratios were calculated and noted to be significantly associated with increased hazard for mortality in multivariable models. There was no significant difference in PAI-1/tPA molar ratios between those with abnormal vs normal neuroimaging. In this study, we did not investigate the association of a shift in balance between PAI-1 and tPA and specific types of neurologic injuries that could be evaluated via brain MRI (e.g., white matter injury),[49,50] however we will plan to do so in the future studies.

IL-6, IL-1β, IFNγ and TNFα, all pro-inflammatory cytokines and all noted to be elevated in patients with abnormal neuroimaging in this cohort, have been implicated in the development of brain injury.[19,29,51,52] Studies have demonstrated relationships between elevated proinflammatory cytokines in cerebrospinal fluid (CSF) and serum with neurologic injury. IL-6 was shown to be elevated in the CSF of neonates with hypoxic ischemic injury,[20] in umbilical cord blood of neonates with periventricular leukomalacia,[53] and in CSF and serum in adults after a stroke.[51,54,55] IL-1β has also been noted to be significantly increased in CSF and serum of adults after stroke.[54,55] In addition, the concentration of IL-6 in CSF was associated with the degree of neurologic injury.[20,51,54] TNFα has been shown to be elevated in the amniotic fluid of neonates with periventricular leukomalacia[56] but not in the CSF of neonates with hypoxic ischemic injury.[20] Despite not being significantly elevated in the CSF of subjects with stroke compared to controls, TNFα has been shown to be elevated in the CSF of adults with white matter lesions three months after stroke.[51,55]

Elevated cytokine levels have not only been associated with neurologic injury on neuroimaging but have been associated with clinical findings as well. In adults with stroke, elevations in IL-6 and TNFα were significantly elevated in those who experience clinical deterioration, described as a worsening Canadian Stroke Scale during the first 48 hours post stroke.[51] In term neonates who had hypoxic ischemic injury, IL-6 was

significantly increased in patients with an abnormal outcome including those who had seizures, abnormalities in tone or reflexes, or blindness.[20] Neonates with white matter lesions were noted to have increased levels of TNFα, IL-1b and IL-6 in amniotic fluid and these were correlated with the development of cerebral palsy in those who survived.[56] To our knowledge no other study has evaluated the association of inflammatory, endothelial or coagulation biomarkers with neurologic outcomes in critically ill children on ECMO. Though we saw no association between these biomarkers and long-term neurobehavioral outcomes, further studies are warranted.

There were several limitations to this study. First, this was a relatively small cohort (n=99) but it was well-characterized by several repeated samples obtained during the ECMO course. In this cohort, it was not feasible, nor was the intent to derive or validate any prediction models for risk stratification. This work is investigational in nature and thus each biomarker was evaluated individually with no adjustment done for multiple comparisons. Future models in an ongoing multicenter prospective observational cohort of neonatal and pediatric ECMO patients will be informed by the key biomarkers identified here. Secondly, we were not able to characterize disease severity and trajectory prior to ECMO initiation. The indications for ECMO were heterogeneous. Biomarker levels associated with unmeasured disease severity prior to ECMO that cause in-hospital mortality may bias the results. However, we note that biomarkers measured at the time of ECMO initiation and over the treatment course may be most clinically relevant.

In conclusion, the inflammatory and fibrinolytic profile of children on the first day of ECMO support differs by primary ECMO indication, with significantly higher IL-6, TM, tPA and PAI-1 plasma concentrations seen in children with primary cardiac, ECPR, and sepsis indications for ECMO compared to those with primary respiratory indications for ECMO. After adjusting for age, ECMO indication, and ECMO day 1 plasma concentrations for each biomarker, a within-subject doubling of IL-1β, TNFα, P-Selectin, tPA and PAI-1 during the ECMO course was significantly associated with increased hazard of in-hospital mortality, while only ECMO day 1 elevations of IL-6 (regardless of subsequent longitudinal trajectory) were associated with mortality. Children who eventually developed neurologic injury confirmed by neuroimaging displayed significantly elevated ECMO day 1 plasma levels of pro-inflammatory cytokines IL-6, IL-1β, TNFα and IFNγ, compared to children with normal neuroimaging throughout the ECMO course and up to 6 weeks post-decannulation. However this association became non-significant after adjusting for age and ECMO indication. Lastly, while there are limitations related to the smaller sample size of survivors to 6-month and 1-year follow-up, none of the tested biomarkers was associated with long-term neurobehavioral outcomes. Further studies are needed to investigate the association of markers of inflammation, endothelial activation, and fibrinolysis with mortality through potential mediation by bleeding and thrombosis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Impact:

- The inflammatory, endothelial activation and fibrinolytic profile of children on ECMO differs by primary indication for extracorporeal support
- Proinflammatory biomarkers on ECMO day one are associated with abnormal neurologic imaging in children on ECMO in univariable but not multivariable models
- In multivariable models, a pronounced proinflammatory and prothrombotic biomarker profile on ECMO day one and longitudinally was significantly associated with mortality
- Further studies are needed to identify inflammatory, endothelial and fibrinolytic profiles associated with increased risk for neurologic injury and mortality through potential mediation of bleeding and thrombosis

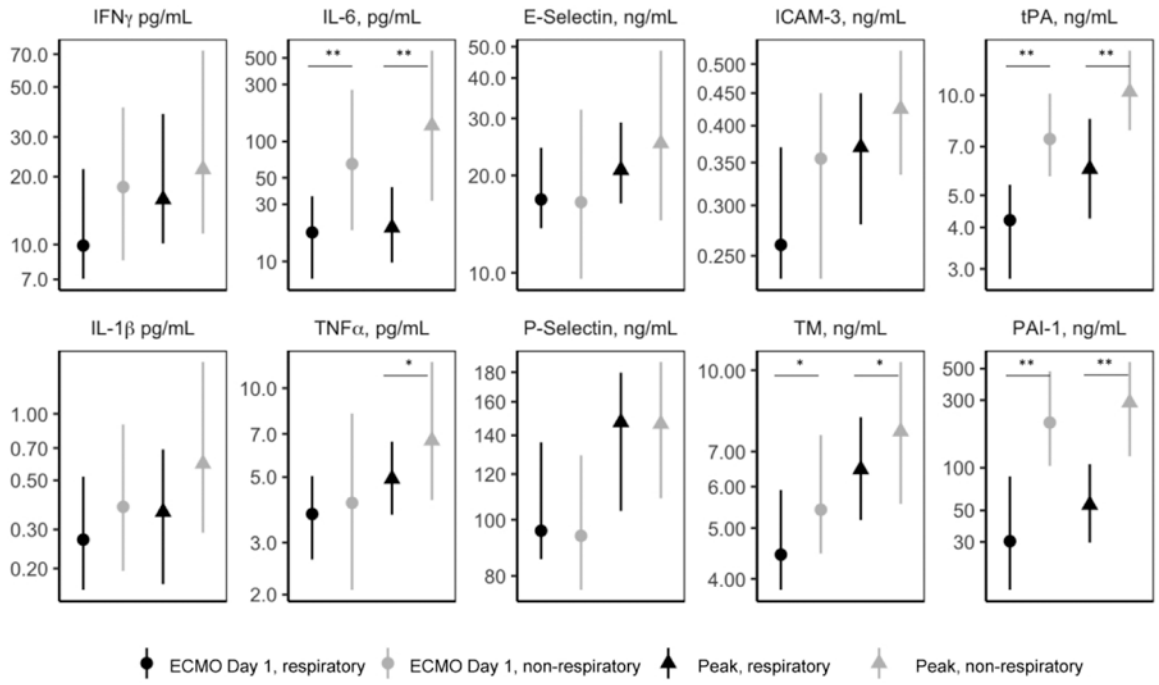

Fig. 1.

Distributions of ECMO day 1 and peak (highest level observed during ECMO course) biomarker levels, stratified by ECMO indication (primary respiratory vs. non-respiratory indications).

ECMO day 1 biomarker level for primary respiratory indication (black ●) vs non-respiratory indication (gray ●), Peak biomarker level for primary respiratory indication (black ▲) vs non-respiratory indication (gray ▲).

Biomarkers represent underlying inflammatory, endothelial activation and fibrinolytic processes. Wilcoxon rank sum *p*-values (* indicates $p < 0.05$, ** indicates $p < 0.001$) are presented contrasting differences in location between respiratory failure and non-respiratory indications for ECMO.

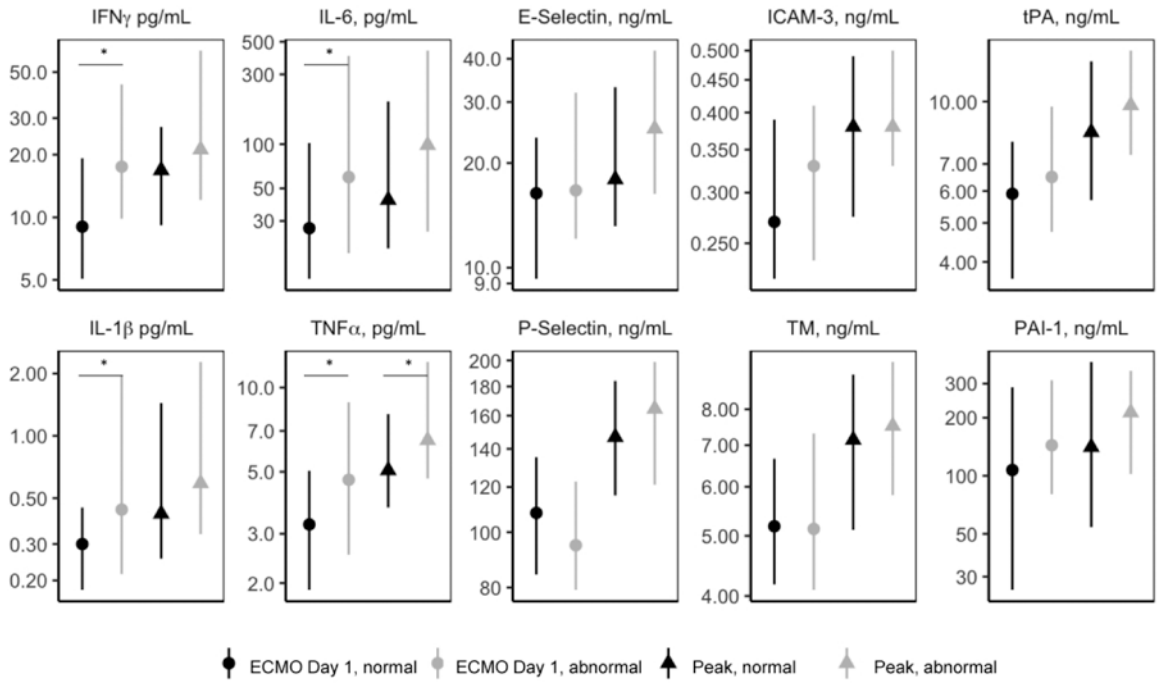

Fig. 2.

Distributions of ECMO day 1 and peak (highest level observed during ECMO course) biomarker levels, stratified by abnormal neuroimaging, among children who underwent neuroimaging during the ECMO course or within 6 weeks post-decannulation.

ECMO day 1 biomarker level with normal neuroimaging (black ●) vs abnormal neuroimaging (gray ●), Peak biomarker level with normal neuroimaging (black ▲) vs abnormal neuroimaging (gray ▲).

Wilcoxon rank sum *p*-values (* indicates $p < 0.05$) are presented contrasting differences in location between those with abnormal vs normal neuroimaging.

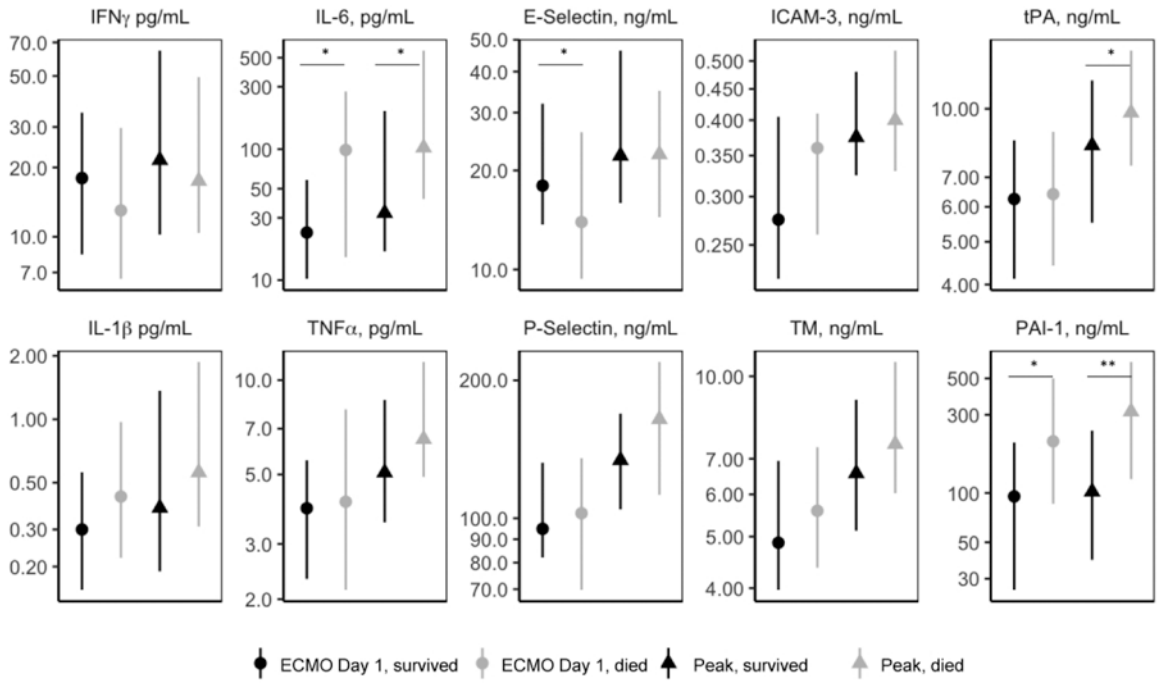

Fig. 3.

Distributions of ECMO day 1 and peak (highest level observed during ECMO course) biomarker levels stratified by survival to hospital discharge.

ECMO day 1 biomarker level in those who survived (black ●) vs those who died (gray ●), Peak biomarker level in those who survived (black ▲) vs those who died (gray ▲).

Wilcoxon rank sum *p*-values (* indicates $p < 0.05$, ** indicates $p < 0.001$) are presented contrasting differences in location between those who survived and those who died in-hospital.

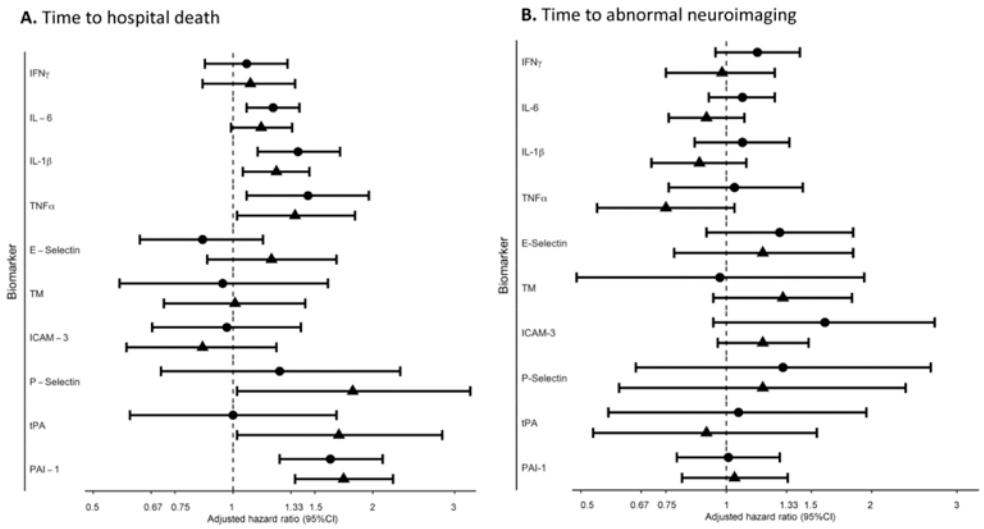

Fig. 4.

Risk of in-hospital mortality (A) and abnormal neuroimaging (B) associated with a 2-fold higher biomarker level on ECMO day 1 between patients (●) and a within-patient 2-fold increase longitudinally (▲), both expressed as adjusted hazard ratios from a multivariate Cox proportional hazards model.

Models were adjusted for ECMO day 1 biomarker level, age category (neonate, infant, child and adolescent) and ECMO indication (primary respiratory vs. non-respiratory indications).

Table 1.

Clinical Characteristics and Distribution of Plasma Samples among 99 Children on ECMO Support[a].

Variable	All (n = 99)
Age group	
Neonate (<30 days)	51 (52)
Infant (30 days to <1 year)	21 (21)
Child (1 to <12 years)	19 (19)
Adolescent (≥12 years)	8 (8)
Male	54 (55)
Race	
Caucasian	51 (52)
African American	33 (33)
Other race	15 (15)
Hispanic	9 (9)
Weight (kg)	4.0 [3.1, 11.8]
Primary indication for ECMO	
Respiratory failure	33 (33)
Non-respiratory indication(cardiac, ECPR, or sepsis)	66 (67)
Hospital length of stay, days	47 [23, 81]
PICU length of stay, days	28 [12, 50]
Duration of ECMO support, days	4.8 [3.2, 10.5]
Abnormal neuroimaging	44 (50)[b]
Died in-hospital	42 (42)
Number of plasma samples	
0	2 (2)
1 to 2	42 (42)
3 to 4	55 (56)
5 to 7	24 (24)

ECMO, extracorporeal membrane oxygenation; ECPR, extracorporeal cardiopulmonary resuscitation; PICU, pediatric intensive care unit

[a]Median [interquartile range] or n (%).

[b]Neuroimaging during or within 6 weeks post-ECMO decannulation: daily head ultrasound (HUS) during and/or post-ECMO, 35 (35%); daily HUS during ECMO, brain computed tomography (CT) during or post-ECMO, and/or brain magnetic resonance imaging (MRI) post-ECMO, 34 (34%); at least one brain CT during or post-ECMO and/or brain MRI post-ECMO, without HUS, 19 (19%); no neuroimaging, 11 (11%).