ANTICOAGULATION AND NEUROMONITORING DURING EXTRACORPOREAL MEMBRANE OXYGENATION (ECMO)

by
Melania M. Bembea, MD, MPH

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ABSTRACT

Extracorporeal membrane oxygenation (ECMO) is a life-saving technique of cardiopulmonary bypass employed in intensive care units since 1976.1 ECMO is used in >1,000 children each year for cardiopulmonary failure refractory to maximal medical management, when chances of mortality exceed 80%.2 However, ECMO patients are at high risk for neurologic injury due to cannulation of large neck vessels, systemic anticoagulation and altered cerebrovascular dynamics. As many as 28%-52% of patients have abnormal neuroimaging findings during ECMO,3-8 and poor neurologic outcomes have been reported in 10% to 60% of survivors.7, 9, 10

The work presented in this thesis is organized around monitoring for coagulopathy, a risk factor for neurologic injury during ECMO, and monitoring for neurologic injury during ECMO by using plasma brain injury biomarkers. The overarching goal of our research program is to improve clinical care of ECMO patients to mitigate risk factors leading to acute neurologic injury and subsequent poor neurodevelopmental outcomes of these critically ill children.

Dissertation Advisors and Readers

Peter J. Pronovost, MD, PhD (Research Mentor)
Marie Diener-West, PhD (Academic Advisor)
Allen Everett, MD
Susan Furth, MD, PhD
James Tonascia, PhD
Elizabeth Colantuoni, PhD
PREFACE

Acknowledgements

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The projects described in this dissertation are a reflection of the multidisciplinary nature of the pediatric intensive care unit environment, where patient care can only take place with open collaboration among health care professionals that care first and utmost about improving outcomes of critically ill children. Hence, this work is equally collaborative, and I have many colleagues to thank for their contributions: Drs. Kenneth Brady and Blaine Easley from Texas Children’s Hospital, Houston, TX, Drs. Michael Johnston and Cynthia Salorio from the Kennedy Krieger Institute, Dr. Ryan Felling from the Division of Pediatric Neurology at Johns Hopkins University, Dr. Gregory Mueller from the Uniformed Services University in Bethesda, MD, Dr. Jamie Schwartz and Gary Oldenburg from the National Children’s Medical Center in Washington, DC, and many others in the pediatric intensive care unit, not least our patients and their families, who have generously participated in our studies.
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Chapter One

Introduction

Extracorporeal membrane oxygenation (ECMO) was first used in humans in 1971. The first neonatal case was reported in 1976. Since that time, ECMO has become a well-established method of support used in over 1,000 neonatal and pediatric patients annually who have severe cardiac and/or respiratory failure unresponsive to maximal medical therapy, when estimated chances of mortality exceed 80%. It involves direct cannulation of the heart or of one or two large vessels, typically the right carotid artery and the right internal jugular vein, to circulate blood through an extracorporeal circuit that includes a pump to maintain blood flow and an oxygenator to provide gas exchange. A continuous infusion of unfractionated heparin is used to prevent thrombus formation. Prolonged anticoagulation needed during ECMO is a major risk factor for intracranial hemorrhage.

Neurologic injury is the most severe complication in ECMO patients. ECMO survival varies by age and underlying diagnosis and ranges from 32%-42% for extracorporeal cardiopulmonary resuscitation (ECPR) to 50%-65% in the pediatric population excluding ECPR and as high as 75%-80% for selected neonatal populations. However, ECMO is associated with high risk for brain injury, including intracranial hemorrhage (7%) and stroke (7%). In children who develop an acute neurologic injury during ECMO, mortality increases by 89%. Neurologic complications during ECMO remain a significant problem and rates have stayed constant over the last three decades despite advances in extracorporeal technology.

Major risk factors for development of neurologic injury during ECMO include: (1) pre-ECMO factors: hypoxia, hypoperfusion and acidosis, encountered in most of these
children with refractory respiratory and/or cardiac failure; (2) ECMO risk factors: coagulopathy, circuit thrombosis, altered cerebrovascular dynamics, and cannulation and ligation of the right jugular vein ± carotid artery. Taken together, poor neurologic outcomes have been reported in 10% to as many as 60% of survivors.7, 9, 10

**Current methods for neuromonitoring of ECMO patients are inadequate.** The neurologic status of ECMO patients is monitored by serial neurologic examinations and daily head ultrasounds (HUS) in neonates and infants with an open anterior fontanel. Unfortunately, the diagnosis of an acute neurologic event is difficult. Neurologic examinations are sub-optimal because of ongoing sedation, movement restriction, the use of neuromuscular blocking agents and, for patients who cannot have bedside HUS studies, there are in-hospital transport risks for these heparinized, rigidly-cannulated patients.27 Furthermore, the daily HUS that are standard of care have low sensitivity for parenchymal and extraaxial lesions, and brain magnetic resonance imaging (MRI) cannot be conducted during ECMO as no MR-compatible circuits exist. ECMO patients require systemic anticoagulation with a continuous unfractionated heparin infusion, and, coupled with the imprecision of anticoagulation monitoring, are continuously at risk for intracranial hemorrhage vs circuit thrombosis. Timely diagnosis and intervention are critical, as intracranial hemorrhage can quickly increase and become life-threatening and an initially embolic/ischemic stroke can rapidly evolve into a hemorrhagic, devastating lesion.

**Existing ECMO outcome predictors have poor performance.** Several predictors of neurologic outcome post-ECMO have been proposed, including neuroimaging3, 26, 28, 29, seizure activity30, electroencephalogram changes,26, 31, 32 and somatosensory evoked potentials.26 Repeated measurements are required in ECMO patients, but these tests cannot be easily performed over many days of ECMO.
Neuroimaging is the only test that has entered routine clinical practice\(^3\),\(^{28}\), but recent data suggest that neither brain MRI nor HUS correlate with neurodevelopmental outcomes after neonatal ECMO.\(^{29}\)

In this dissertation, we present work related to management of anticoagulation and coagulopathy present during ECMO, and work related to neuromonitoring of ECMO patients, thus building the foundation for a research program that examines risk factors for neurologic injury and methods for detection of injury and prognostication of future development.

Chapter Two presents the results of a survey of international ECMO centers on anticoagulation management. The survey documents the variability in clinical practice across institutions and across countries and reveals that newer and more complex tests to assess the coagulation system are being used in the ECMO community. In Chapter Three, we present the results of a single center prospective observational study that compares some of these tests (e.g., anti-factor Xa, antithrombin III) with tests that have been used extensively over the last 40 years (e.g., activated clotting time).

Chapter Four is a review of the literature surrounding neuromonitoring methods during ECMO. Chapter Five introduces a candidate plasma brain injury biomarker as a potential tool for neuromonitoring during ECMO and as an indicator of neurocognitive outcomes post-ECMO and Chapter Six expands on the concept of using a plasma brain injury biomarker with the development of a panel of brain-specific proteins indicative of neuronal and/or glial injury, as well as neuroinflammation. Chapter Seven presents conclusions, ongoing studies and future directions.
Chapter Two

Variability in anticoagulation management of patients on extracorporeal membrane oxygenation: an international survey

Melania M. Bembea, MD, MPH\textsuperscript{1,2}

Gail Annich, MD\textsuperscript{3}

Peter Rycus, MPH\textsuperscript{4}

Gary Oldenburg\textsuperscript{1}

Ivor Berkowitz, MD\textsuperscript{1,2}

Peter Pronovost, MD, PhD\textsuperscript{1}

\textsuperscript{1}Department of Anesthesiology and Critical Care, Johns Hopkins University, Baltimore, MD

\textsuperscript{2}Department of Pediatrics, Johns Hopkins University, Baltimore, MD

\textsuperscript{3}Department of Pediatrics, University of Michigan, MI

\textsuperscript{4}Extracorporeal Life Support Organization, Ann Arbor, MI

Introduction

Management of anticoagulation and blood product administration in patients undergoing extracorporeal membrane oxygenation (ECMO) is controversial despite more than 30-years of ECMO experience. The rate of disorders of coagulation, including life-threatening hemorrhage and thrombosis, remains high, between 10%-33%. At present, there are no established evidence-based guidelines for anticoagulation during ECMO.

The Extracorporeal Life Support Organization (ELSO), an organization composed of international ECMO centers is spearheading an effort to create ECMO anticoagulation and blood product management guidelines for newborns, children and adults. As a starting point for this endeavor, we launched a survey with the overall goal of characterizing anticoagulation practices among ECMO centers in the U.S. and throughout the world.

We hypothesized that anticoagulation and blood product management practices vary widely among ECMO centers and that more specific methods to monitor the status of the coagulation system, such as anti-factor Xa, antithrombin III (ATIII) and thromboelastograms (TEG) are starting to be used broadly compared to traditional monitoring methods such as activated clotting time (ACT), prothrombin time (PT) and activated partial thromboplastin time (aPTT).

Methods

We conducted an anonymous, single cross-sectional survey of ECMO medical directors and ECMO program coordinators from all ELSO-reporting centers. The survey
was piloted by four ECMO medical directors and program coordinators for clarity of the questions. The sampling frame consisted of the publicly available list of ELSO-registered ECMO centers (http://www.elso.med.umich.edu/Member.asp) at the time the survey was launched in November 2010. The survey was then disseminated through the monthly ELSO newsletter (four postings) and on an internet page dedicated to ECMO professionals with ELSO affiliation (http://www.ecls-net.net/) (one posting). The survey was distributed using a commercial web-based instrument (SurveyMonkey.com), and was open from November 2010 to May 2011. The survey cover letter and the survey instrument are presented in Appendix A and Appendix B, respectively.

The survey was organized around the following four domains: 1) ECMO program characteristics, 2) patient population characteristics, 3) institution-specific anticoagulation protocols and blood product transfusion protocols and 4) methods for anticoagulation monitoring. Due to anonymity requirements by the Johns Hopkins Institutional Review Board (IRB), only the continent of origin was recorded. Only one response per institution was requested. A duplicate check was conducted for all variables, as well as for sets of variables within the four domains of the survey, for all respondents, and by continent. When two respondents were found to have more than two identical responses within one domain, a secondary check for duplicate answers was done focused on those respondents for the remainder of variables in the same domain as well as the other three domains. We found no evidence for answers originating from the same ECMO program. After the survey was closed, we performed exploratory data analysis and reported means, standard deviations and proportions of respondents, as appropriate. Respondents were divided by location, size of intensive care unit, ECMO capacity and primary patient population (i.e., neonatal, pediatric, adult or mixed). Differences between groups were analyzed using Wilcoxon rank sum test for non-normally distributed continuous variables, Student’s t test for normally distributed
continuous variables or $\chi^2$ test for binary variables. A $p$-value of 0.05 was considered significant. Statistical analysis was conducted using STATA 11.0 (StataCorp, College Station, TX, 2009).

Results

At the time the survey was open in November 2010, there were 187 ELSO-registered ECMO centers from 22 countries on five continents (132 in the USA, 7 in Canada, 31 in Europe, 9 in Australia and New Zealand, 5 in Asia and 3 in South America). The ELSO newsletter is only available to ELSO-registered ECMO medical director(s) and ECMO coordinator(s). There were 121 respondents who replied to at least one question of the survey, yielding an overall 65% response rate assuming no duplicates. There were 103 responses from North America, 12 responses from Europe and 6 responses from Australia and New Zealand. Twenty-four of 121 (20%) respondents were from ECMO programs that served a single intensive care unit (ICU) and 97 (80%) served more than one ICU. The programs were pediatric-only (neonatal ICU, pediatric ICU and/or pediatric cardiac ICU), 81 (67%), adult-only (adult cardiac ICU, adult surgical ICU and/or adult medical ICU), 4 (3%) or mixed pediatric and adult, 36 (30%). The size of the ICUs served was $\leq$10 beds, 5 (4%), 11-30 beds, 51 (42%), and >30 beds, 65 (54%). The ECMO capacity (i.e., maximum number of simultaneous ECMO runs the program can provide) was 1-4, 93 (77%) or 5-10, 28 (23%). In the six months prior, respondents have used ECMO at their respective centers for cardiac failure, 102/120 (85%), respiratory failure, 115/120 (96%), sepsis, 71/120 (59%), and extracorporeal cardiopulmonary resuscitation (ECPR), 65/120 (54%).

Institutional protocols and circuit characteristics

Most respondents reported having a written institutional ECMO protocol for both anticoagulation and blood product management at their institution, 84/117 (72%). Others had a written protocol for ECMO anticoagulation only, 15/117 (13%), for ECMO blood
product management only, 7/117 (6%), or neither, 11/117 (9%). Presence or absence of an institutional protocol was not associated with the size of the ICU(s) served nor with the maximum ECMO capacity or the population served \((p>0.05)\). Anticoagulation was managed mainly by the ICU staff on service making day-to-day decisions, 81/118 (69%) respondents. Forty-three respondents indicated that "Dedicated "expert" ICU staff makes/helps with decisions". With regards to the involvement of a Hematology/Thrombosis consulting service: the "Hematology/Thrombosis team is only consulted when the ICU team has difficulties" (32/118 respondents), "ICU staff and Hematology/Thrombosis team make decisions together" (16/118), "Hematology/Thrombosis team rounds with ICU team daily" (4/118), "Hematology/Thrombosis team is never consulted" (5/118), and "Hematology/Thrombosis team makes all decisions" (1/118).

**Equipment**

Forty-eight of 117 (41%) ECMO centers did not use heparin-bonded circuits, 38/117 (32%) reported use of tip-to-tip heparin-bonded circuits and 31/117 (27%) reported that certain components of the circuits were heparin bonded. Neonatal and pediatric centers use roller pumps only [43/81 (53%)], and centrifugal pumps or both centrifugal and roller pumps [38/81 (47%)], while the majority of adult ECMO centers use centrifugal pumps [36/40 (90%)].

**Circuit priming solutions**

The most common solution combination for ECMO circuit priming included packed red blood cells (PRBC), heparin, albumin, calcium chloride or calcium gluconate and sodium bicarbonate. (Table 1) Fresh frozen plasma (FFP) use was reported by 50% of respondents. Age differences were noted for PRBCs, heparin, sodium bicarbonate, which were all significantly more used in pediatric-only vs adult-only and mixed adult and pediatric centers \((p<0.05)\). There were significant differences in priming practices by
geographic location. Respondents from Australia/New Zealand reported no usage of normal saline and FFP, whereas both are commonly used in North America and Europe. All steroid and tromethamine use in circuit priming was reported from North America and none was reported from Europe and Australia/New Zealand. Use of PlasmaLyte and similar solutions was not reported by respondents from Europe, but was reported by half of the respondents from Australia/New Zealand, 3/6 (50%), and by 17/103 (17%) of North American respondents.

**Heparin and other coagulation-related medications**

All respondents indicated use of a continuous unfractionated heparin (UFH) infusion, with varying minimum and maximum heparin infusion rates as detailed in Table 1. Use of non-UFH anticoagulation remains rare: 10/117 respondents (8%) reported use of argatroban, lepirudin and/or bivalirudin in the 6 months prior to answering the survey. Fifty-seven of 107 (53%) respondents reported availability of argatroban, lepirudin and/or bivalirudin should an indication such as heparin-induced thrombocytopenia arise. There were no significant differences in usage of non-UFH products by patient population (children vs. adults), size and/or location of ECMO centers ($p>0.05$).

A variety of adjunct medications are used during ECMO to better control anticoagulation and/or hemorrhage or thrombosis, such as hemostatic agents (ε-aminocaproic acid, tranexamic acid, recombinant human factor VIIa, aprotinin and other serine protease inhibitors), antiplatelet agents (acetylsalicylic acid, prostacyclin) and vitamin K antagonists (warfarin). (Table 1) There were no differences in the use of these medications by type and size of ICU(s) served by the ECMO programs ($p>0.05$), but there were significant regional differences, with higher usage of ε-aminocaproic acid and recombinant human factor VIIa in North American ECMO centers, and higher usage of tranexamic acid in European and Australian/New Zealand centers.

**Anticoagulation monitoring and blood product administration**
Monitoring patterns for anticoagulation and thresholds for blood product administration are detailed in Table 2 and Table 3, respectively. The vast majority of respondents, 113/116 (97%), use ACT to monitor anticoagulation; the most commonly reported goal ACT range was 180-200 s by 43 of 113 (38%).

Ninety six of 117 respondents (82%) reported routine or occasional ATIII testing. (Table 2) The goal ATIII activity in a typical ECMO patient was: median 70% (range 30-100%), n=59/96 respondents. Ten of 96 respondents reported that their centers did not have a specified goal ATIII activity and 27 did not answer the question. To correct a patient’s lower-than-goal ATIII, 46/91 (51%) use either FFP or recombinant or pooled ATIII, 12/91 (13%) use only FFP, 31/91 (34%) use only recombinant or pooled ATIII and 2/91 (2%) use neither.

Seventy five of 115 respondents (65%) reported routine or occasional anti-factor Xa testing (Table 2) The most commonly reported goal anti-factor Xa range was 0.3-0.7 IU/ml, by 40 of 67 respondents (60%). Other ranges reported by 10 respondents (15%) varied from as low as 0-0.29 IU/ml to as high as 0.71-1 IU/ml. The remaining 17 of 67 respondents (25%) stated they “do not have a goal for anti-factor Xa levels”. If anti-factor Xa were lower-than-goal, respondents would increase heparin infusion rate or administer heparin bolus, 30/50 (60%), with or without FFP or ATIII administration to correct low ATIII if present; obtain more data on coagulation status via PT, aPTT, TEG, 9/50 (18%); and make individualized decisions or no changes at all, 11/50 (22%). If anti-factor Xa were higher-than-goal, respondents would decrease heparin rate, 31/46 (67%); adjust ACT range, 7/46 (15%); obtain more information on coagulation status, or consider interventions such as FFP administration or ATIII correction if low ATIII activity.

TEG use was reported as routine or occasional by 50 of 116 (43%) respondents. Of the 50 TEG users, 40 answered the follow-up question related to the type of TEG used at their center: 32/40 (80%) reported use of heparinase TEG alone or in
Combination with kaolin TEG or rotative TEG, 4/40 (10%) reported use of rotative TEG (thromboelastometry or ROTEM) only, 3/40 (8%) use kaolin TEG only, and 1/40 was not sure. Of the 27 respondents who further elaborated on TEG parameters used, most reported using some or all parameters (i.e., r, K, α, MA) to better define the status of the coagulation system (18/27), but no details on specific threshold were given; some only interpret and use the r time (2/27), and others do not have a protocol for TEG interpretation and/or do not use results to guide therapy (7/27).

**Discussion**

Our findings show that management of anticoagulation and blood product administration during ECMO is highly variable among international ECMO centers. This variability in practice patterns is likely due to a paucity of published studies to provide the groundwork for the development of evidence-based guidelines.

To our knowledge, this is the first comprehensive survey of ECMO practices of anticoagulation and blood product management. Previous ECMO program surveys focused on the use of ACT. This survey shows that the preferred method of point-of-care anticoagulation monitoring remains the serial measurement of ACT, as reported by 97% of respondents. However, ACT testing has several weaknesses: it can be prolonged in association with thrombocytopenia, platelet dysfunction, elevated d-dimers, low fibrinogen, other coagulation factor deficiencies, hypothermia or hemodilution and can be decreased in hypercoagulable states. Further, ACT devices may yield different results, potentially confusing clinicians on adequacy of anticoagulation. The use of ACT and common thresholds to guide therapy have not been prospectively analyzed to determine if they are associated with improved outcomes. Three respondents to this survey indicated that they do not use ACT to guide anticoagulation, but rather use aPTT and/or anti-factor IIa. Neither aPTT nor anti-factor IIa have been formally studied in ECMO. In healthy children and adults, both tests show age-related differences for a
given anti-factor Xa activity of heparin. Differences are more pronounced in young infants, with higher aPTT for the same anti-factor Xa. aPTT is also affected by hemodilution in infants on extracorporeal support. For these reasons, aPTT is likely to be used more in adult, rather than neonatal and pediatric centers.

For these reasons, many centers have moved to a more comprehensive panel of laboratory tests besides the classical ACT, PT, aPTT, fibrinogen or d-dimers. In this survey, 65% of respondents reported anti-factor Xa testing, 82% reported ATIII testing and 43% reported use of TEG during ECMO. Goal ranges for tests such as anti-factor Xa, ATIII or TEG and interventions triggered by values considered abnormal at a particular center were found to also be variable, indicating that clinical practice has evolved in the absence of adequate evidence from observational or experimental ECMO clinical studies.

Anti-factor Xa assays determine heparin activity in the plasma and could provide an alternative to ACT for monitoring anticoagulation. In two small single-center studies, using point-of-care heparin concentration tests to guide anticoagulation during cardiopulmonary by-pass led to more adequate anticoagulation, and helped reduce hemorrhage and need for transfusions when compared to ACTs. Heparin concentration and anti-factor Xa as monitoring tools during ECMO have only been reported in small studies. Anti-factor Xa levels were found to range between 0.2-0.6 IU/ml and up to 0.7 ± 0.2 IU/ml, and were found to have poor correlation with ACT values. Previously reported targets for anti-factor Xa during ECMO were 0.3-0.6 IU/ml or 0.3-0.7 IU/ml. In our survey, the most commonly reported goal for anti-factor Xa was similar: 0.3-0.7 IU/ml (60% of respondents). As many as a quarter of respondents indicated that their center does not have a clear target for anti-factor Xa. Anti-factor Xa levels are used as an adjunct test, and there are no published data on outcomes of ECMO patients monitored by anti-factor Xa compared to ACT.
ATIII is a plasma glycoprotein that forms an irreversible inactive complex with thrombin and activated factor X. This process is greatly accelerated by heparin. Adequate ATIII activity is therefore required for anticoagulation during continuous heparin infusion therapy. The target ATIII during ECMO is also not known. Respondents reported highly variable target ATIII ranges, from as low as 30% to as high as 120%. The appropriate ATIII activity during ECMO cannot be easily determined. In normal individuals, ATIII activity is lowest in neonates, and then is 10% higher compared to adult values for the rest of the childhood. Normal values in means and boundaries including 95% of the population are 76% (58%-90%) in neonates and 96% (66%-194%) in adults. In ECMO patients, published data are limited to a very small number of patients. In a series of 10 neonates on ECMO, median ATIII activity was found to be 19% (range: 3%-49%) immediately on ECMO, 28% (range: 17%-46%) at 6 h on ECMO and 33.5% (range: 15%-51%) at 24 h on ECMO. Some ECMO centers have previously reported replacing ATIII with plasma-derived or recombinant ATIII, but the target ATIII and cutoff for replacement in ECMO patients were not detailed. In six postcardiotomy patients, investigators administered a continuous ATIII infusion to achieve a goal ATIII activity of 100% and found this to be associated with a lower incidence of hemorrhage. In a different series, ATIII administration for ATIII activity <50% led to a decrease in markers of prothrombin activation and appeared to be beneficial. A note of caution for this practice comes from patients on cardiopulmonary by-pass, where thrombin formation was found to be significantly reduced by ATIII administration and could in fact pose a risk for hemorrhage. In addition, a randomized controlled trial in adults with septic shock who were treated with ATIII showed significantly higher rates of hemorrhage compared to placebo. These data suggest that significant thrombin suppression with high-dose or continuous ATIII infusions should be
approached with caution as it may be deleterious in ECMO patients. This practice will need additional investigation.

TEG use had previously been reported anecdotally by ECMO centers\textsuperscript{34, 51, 56} and may prove to be a useful tool to monitor anticoagulation, detect hypercoagulable states and aid in the management of ECMO-related bleeding complications, but future studies are needed. In this survey, heparinase, kaolin and rotative TEG are all being reported. Of note, at the time of this survey, rotative TEG (recently referred to as ROTEM) was only licensed in Europe. In contrast to ECMO, TEG is used extensively in clinical practice for guidance of anticoagulation and anti-platelet therapy in patients with ventricular assist devices.\textsuperscript{57} ECMO anticoagulation protocols will likely evolve combining both specific measures of anticoagulation (e.g., heparin concentration and functional assays of heparin activity such as anti-factor Xa) and global functional tests that measure overall clot reaction, such as TEG.\textsuperscript{34, 57}

\textit{Heparin and other coagulation-related medications.} All respondents indicated use of continuous UFH infusion as the main means of anticoagulation. Direct thrombin inhibitors (e.g., argatroban, lepirudin, bivalirudin) as alternatives to UFH were used by more than half of respondents, when clinically indicated (e.g., heparin-induced thrombocytopenia). A variety of hemostatic agents, antiplatelet agents and vitamin K antagonists were also used, with regional differences that may be related to clinical choice and/or country-specific availability.

Use of heparin-bonded circuits has been proposed as a method of reducing circuit clotting. Several studies have investigated the impact of these heparin-bonded circuits on the inflammatory response, platelet preservation, fibrinolysis, blood loss and amount of blood transfusion required, during ECMO and cardiopulmonary by-pass, with evidence suggesting reduced platelet activation, decreased leukocyte and complement activation and lower pro-inflammatory cytokine production, as well as decreased blood
transfusion requirements.\textsuperscript{58-61} It is not clear, though, how these findings translate for prolonged ECMO runs and in patients of different ages, co-morbidities and indications for ECMO. In this survey, the use of heparin-bonded circuits was reported by 61\% of respondents, either as tip-to-tip or for certain components. Different circuit configurations (roller vs centrifugal pump, membrane- vs hollow-fiber oxygenator, bridge vs bridgeless, type of biocompatible circuit surface coating) could also impact heparin requirements and coagulation profiles.\textsuperscript{58, 62}

\textit{Circuit priming solutions.} Many different combinations of priming solutions varying by age and geographical location were reported. FFP as part of the priming solution was reported by 50\% of respondents. Addition of plasma to the circuit can overcome the problem of hemodilution affecting ACT results. Further, in CPB patients, addition of plasma improved ACT to anti-factor Xa correlation.\textsuperscript{63, 64} This is another area that warrants further investigation.

This study has several limitations. Given the anonymous nature of this survey, we were not able to control for responses arriving from the same institution, although a duplicate check revealed no duplicates. There may also be selection bias introduced by potential systematic differences between ELSO-registered versus non-ELSO-registered ECMO centers. Non-response did not appear to be an issue for this survey, which showed relatively high response rates compared to most surveys published. The survey could not control for different practices for various age groups or for VA- vs VV-ECMO. Data on the storage age for PRBCs used for priming and for subsequent transfusion and data on ATIII and medication doses and titration were not collected. This study did not assess outcomes associated with different methods for anticoagulation employed by responding centers. This report offers a starting point for determining clinical practices and opinions among ECMO providers and provides the groundwork for ECMO anticoagulation and blood product administration guidelines and for future studies of the
association between anticoagulation and blood product management and ECMO complications and outcomes.

Conclusions

The results of this survey show that ECMO anticoagulation and blood product management policies among international ELSO centers vary widely. The vast majority of ECMO centers employ ACT as the preferred anticoagulation monitoring tool. The coagulation system is closely monitored using a variety of more specific markers such as anti-factor Xa, ATIII and TEG by a larger-than-expected number of centers. Thresholds used for blood product transfusions are equally variable, emphasizing the lack of studies in ECMO patients that could guide practice. Future studies are needed to improve standardization of these policies across institutions. While guidelines can help standardize practice, future, rigorous multicenter observational studies are needed to determine the association between anticoagulation and blood product management and ECMO complications and outcomes, followed by randomized controlled trials that will elucidate the optimal anticoagulation and blood product administration practice.
### Table 1. Circuit priming solutions and coagulation-related medications

<table>
<thead>
<tr>
<th>Question</th>
<th>Responses</th>
<th>n=119 respondents</th>
</tr>
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<tbody>
<tr>
<td>Your ECMO circuit prime contains which of the following (outside of an emergency)</td>
<td>Normal saline (0.9% NaCl)</td>
<td>29 (24%)</td>
</tr>
<tr>
<td></td>
<td>PRBC</td>
<td>109 (92%)</td>
</tr>
<tr>
<td></td>
<td>FFP</td>
<td>59 (50%)</td>
</tr>
<tr>
<td></td>
<td>Heparin</td>
<td>100 (84%)</td>
</tr>
<tr>
<td></td>
<td>Calcium chloride or calcium gluconate</td>
<td>97 (82%)</td>
</tr>
<tr>
<td></td>
<td>Albumin</td>
<td>98 (82%)</td>
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<tr>
<td></td>
<td>Sodium bicarbonate (NaHCO3)</td>
<td>90 (76%)</td>
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<tr>
<td></td>
<td>Steroids</td>
<td>6 (5%)</td>
</tr>
<tr>
<td></td>
<td>THAM</td>
<td>23 (19%)</td>
</tr>
<tr>
<td></td>
<td>PlasmaLyte</td>
<td>16 (13%)</td>
</tr>
<tr>
<td></td>
<td>Other †</td>
<td>12 (10%)</td>
</tr>
<tr>
<td>What is the minimum UFH infusion rate allowed by your protocol? (n=115 respondents)</td>
<td>0 U/kg/h a</td>
<td>35 (30%)</td>
</tr>
<tr>
<td></td>
<td>1-10 U/kg/h</td>
<td>62 (54%)</td>
</tr>
<tr>
<td></td>
<td>11-25 U/kg/h</td>
<td>18 (16%)</td>
</tr>
<tr>
<td></td>
<td>&gt;25 U/kg/h</td>
<td>0</td>
</tr>
<tr>
<td>What is the maximum UFH infusion rate allowed by your protocol? (n=115 respondents)</td>
<td>No upper limit</td>
<td>83 (72%)</td>
</tr>
<tr>
<td></td>
<td>50-75 U/kg/h</td>
<td>21 (18%)</td>
</tr>
<tr>
<td></td>
<td>76-100 U/kg/h</td>
<td>6 (7%)</td>
</tr>
<tr>
<td></td>
<td>101-125 U/kg/h</td>
<td>3 (3%)</td>
</tr>
<tr>
<td>In the last six months, have you used non-UFH anticoagulation? (n=117 respondents)</td>
<td>Yes</td>
<td>10 (8%)</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>107 (90%)</td>
</tr>
<tr>
<td>What non-UFH anticoagulation do/can you use in your ICU? (n=107 respondents)</td>
<td>Argatroban</td>
<td>48 (45%)</td>
</tr>
<tr>
<td></td>
<td>Bivalirudin</td>
<td>10 (9%)</td>
</tr>
<tr>
<td></td>
<td>Lepirudin</td>
<td>6 (6%)</td>
</tr>
<tr>
<td></td>
<td>We never use any other pharmacologic anticoagulation besides UFH</td>
<td>50 (47%)</td>
</tr>
<tr>
<td></td>
<td>Other</td>
<td>0</td>
</tr>
<tr>
<td>Do you ever use any of the products below for management of anticoagulation/hemorrhage/thrombosis in ECMO patients? (n=94 respondents)</td>
<td>ε-aminocaproic acid</td>
<td>63 (67%)</td>
</tr>
<tr>
<td></td>
<td>Recombinant human factor VIIa</td>
<td>63 (67%)</td>
</tr>
<tr>
<td></td>
<td>Tranexamic acid</td>
<td>21 (22%)</td>
</tr>
<tr>
<td></td>
<td>Aprotinin</td>
<td>13 (14%)</td>
</tr>
<tr>
<td></td>
<td>Other †</td>
<td>10 (11%)</td>
</tr>
</tbody>
</table>

UFH: unfractionated heparin; PRBC: packed red blood cells; FFP: fresh frozen plasma; THAM: tromethamine; †Other (number of respondents): fresh whole blood (1), platelets (1), Hartman’s solution (2), mannitol (1), lactated Ringer’s solution (3), Normosol (4); Other: acetylsalicylic acid (4), warfarin (2), prostacyclin (2), serine protease inhibitors (1), dipyridamole (1)

aNo in-depth data were collected on patient characteristics and duration of off-heparin ECMO, but respondents commented that this is mainly employed for brief periods of time in post-operative patients.
b6 of 8 respondents reporting maximum UFH infusion rate of 76-100 U/kg/h and 2 of 3 respondents reporting maximum UFH infusion rate of 101-125 U/kg/h were from ECMO programs caring solely for neonatal and pediatric patients.
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Responses</th>
<th>Minimum ACT goal, mean (SD)</th>
<th>Maximum ACT goal, mean (SD)</th>
<th>We do not follow ACT (n=3 respondents)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACT goal (sec) (n=116 respondents)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CBC monitoring frequency (n=119 respondents)</td>
<td></td>
<td>183 (13), range 140-220</td>
<td>210 (15), range 170-240</td>
<td></td>
</tr>
<tr>
<td>PT/aPTT monitoring frequency (n=116 respondents)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fibrinogen monitoring frequency (n=119 respondents)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D-dimer monitoring frequency (n=117 respondents)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Free hemoglobin monitoring frequency (n=117 respondents)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LDH monitoring frequency (n=112 respondents)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Factor VIII monitoring frequency (n=115 respondents)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ATIII measurements (n=117 respondents)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ATIII monitoring frequency (n=89 respondents)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-factor Xa measurements (n=115 respondents)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-factor Xa monitoring frequency (n=66 respondents)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TEG measurements (n=116 respondents)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
*All parameters refer to a typical ECMO patient at the respondent’s ECMO center; ACT: activated clotting time; SD: standard deviation; CBC: complete blood count; ECMO: extracorporeal membrane oxygenation; LDH: lactate dehydrogenase; AT: antithrombin; TEG: thromboelastogram

**Table 3. Transfusion thresholds in ECMO patients**

<table>
<thead>
<tr>
<th>Question</th>
<th>Responses</th>
<th>Pediatric-only programs (n=81)</th>
<th>Adult-only and mixed adult and pediatric programs (n=40)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>What is the low hematocrit threshold (%) you use for PRBC transfusion in a typical ECMO patient?, median (range)</td>
<td>35 (25 - 40)</td>
<td>30 (20 - 40)</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>What is your typical platelet threshold (cells/microL) for platelet transfusion in an otherwise uncomplicated ECMO run?, median (range)</td>
<td>100,000 (50,000 – 200,000)</td>
<td>100,000 (20,000 – 100,000)</td>
<td>0.034</td>
<td></td>
</tr>
<tr>
<td>What is your typical low fibrinogen threshold (mg/dL) for which you would administer FFP or cryoprecipitate?, median (range)</td>
<td>150 (60 – 200)</td>
<td>145 (50 – 200)</td>
<td>0.33</td>
<td></td>
</tr>
</tbody>
</table>
Chapter Three

Anticoagulation monitoring during pediatric extracorporeal membrane oxygenation

Melania M. Bembea, MD, MPH¹,²
Jamie M. Schwartz, MD¹,²
Nilay Shah, MD²
Elizabeth Colantuoni, PhD³
Christoph U. Lehmann, MD²
Thomas Kickler, MD⁴
Peter Pronovost, MD, PhD¹
John J. Strouse, MD, PhD²,⁵

¹Department of Anesthesiology and Critical Care and ²Department of Pediatrics, Johns Hopkins University, Baltimore, MD
³Department of Biostatistics, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD ⁴Department of Pathology and ⁵Department of Medicine, Johns Hopkins University, Baltimore, MD

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Introduction

Extracorporeal membrane oxygenation (ECMO) is a well-established method of support in pediatric and adult patients with severe cardiac and/or respiratory failure.\textsuperscript{2, 16} Despite ECMO having been used in pediatric patients for more than 30 years, appropriate measurement and intensity of anticoagulation of patient and circuit remain controversial. Although specific protocols vary from center to center, most ECMO programs use a continuous infusion of unfractionated heparin and monitor anticoagulation by point-of-care testing of the activated clotting time (ACT), in conjunction with blood cell counts and coagulation studies performed in a central laboratory.\textsuperscript{35} These results guide the intensity of anticoagulation therapy and help clinicians to balance the risk of bleeding complications with the need to maintain appropriate circuit flow and avoid thrombosis. However, disorders of coagulation, including life-threatening hemorrhage and thrombosis, still occur frequently.\textsuperscript{2, 16}

Only limited evaluations have been made of changes in the coagulation system occurring during ECMO. In addition, the methods used to control and monitor anticoagulation have been limited to global measures of coagulation, except within the confines of small studies.\textsuperscript{33, 40, 45, 47} Several measures may be used to monitor the state of the coagulation system including ACT, anti-factor Xa activity (heparin level), antithrombin (AT) activity, factor VIII activity, activated partial thromboplastin time (aPTT), and thromboelastography (TEG). The objective of this study was to compare global (ACT, aPTT) and specific (anti-factor Xa activity) measures of anticoagulation used in clinical practice and determine the agreement among them and potential confounding by low AT or high factor VIII activity.

Methods

Study Design. This is a prospective observational cohort study of children who initiated ECMO from April 2008 to September 2010 in a 26-bed pediatric intensive care
unit of an academic tertiary pediatric center. Patients younger than 18 years who
required ECMO for any indication were eligible for this study. Exclusion criteria were
history of heparin-induced thrombocytopenia and use of direct thrombin inhibitors for
anticoagulation during ECMO. No patient met these criteria. Informed consent from
parents or legal guardians was sought after patient stabilization within the first 6 h after
ECMO cannulation. If parents were not present in the intensive care unit, consent was
deferred. Demographic, clinical, laboratory, imaging, and survival data were collected
prospectively for each enrolled subject. The ECMO circuit consisted of custom-packed
1/4- or 3/8-inch flexible polyvinylchloride 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 U.S.A., Arvada, CO). The rate of heparin infusion was adjusted
based on the ACT, with a goal of 180 to 220 seconds, according to institutional
protocols. The ECMO specialists adjust the infusion rate by 0-5 U/kg/h no more
frequently than every two hours to maintain ACT within parameters. The institutional
transfusion protocol calls for transfusion of packed red blood cells (PRBC) to maintain
hematocrit ≥35%, platelet transfusion to maintain platelet count ≥100,000 cells/microL,
fresh frozen plasma (FFP) administration if aPTT > 100 seconds, even if ACT was within
goal range, and cryoprecipitate to maintain fibrinogen ≥100 mg/dL. During the study
period, pooled antithrombin was administered at clinician's discretion, based on
manufacturer's recommendations (ATIII dose in units [IU] = [desired – current] x weight
[kg] / 1.4). This study was approved by the Johns Hopkins Institutional Review Board.

Blood sampling and analysis. Venous blood samples (5 mL in sodium citrate
3.2%) were collected at 6 h, 12 h, 24 h, and then daily after initiation of ECMO until
ECMO discontinuation. All samples were collected from a designated circuit port (pre-
bladder and pre-pump) at the same time that regular clinical samples were collected,
with a 2–3 mL “waste”. After being separated by centrifugation within 1 h, platelet-poor plasma was processed for anti-factor Xa activity with supplementation of AT (Berichrom Heparin, Siemens Healthcare Diagnostics Products GmbH, Germany), AT (Berichrom Antithrombin III (A), Siemens Healthcare Diagnostics Products GmbH), fibrinogen and factor VIII activity (Siemens Healthcare Diagnostics Products GmbH). aPTT was measured using Dade® Actin® (Siemens Healthcare Diagnostics Products GmbH), d-dimers were measured using Innovance (Siemens Healthcare Diagnostics Products GmbH), and the ACT test was carried out using the Hemochron Signature (ITC Corporation, Edison, NJ). For factor VIII measurements, heparin was neutralized with Dade Hepzyme (Siemens Healthcare Diagnostics Products GmbH). All assays except the ACT (measured at point of care) were conducted in the Johns Hopkins Coagulation Laboratory in the Department of Pathology. Routine hematologic and coagulation assays, blood products administered, hourly ACT, and heparin infusion rates were recorded longitudinally.

Statistical Analysis. Descriptive data analysis was conducted to examine patient and ECMO course characteristics and to describe the distribution of heparin infusion rates, coagulation assays, and blood product use among subjects. The Mann-Whitney U test was used to compare these parameters between age groups (neonates ≤30 days and infants and children >30 days). Each patient’s ECMO course was divided into 1-h time periods, and each laboratory value or intervention was recorded within its corresponding time period(s). Data were analyzed using longitudinal linear regression to model association between coagulation markers. A p-value of 0.05 was considered significant. Statistical analysis was conducted using STATA 11.0 (StataCorp, College Station, TX, 2009).

Results
We screened 71 patients and enrolled 34 patients within the 6-h consent window from April 2008 to September 2010. One patient had two ECMO runs for different indications 2 years apart that were analyzed separately. Demographic and ECMO course characteristics are presented in Table 4.

Median heparin infusion rate was 34 U/kg/h (IQR: 22–48 U/kg/h), median ACT was 210 s (IQR: 195–227 s), and median aPTT was 91.5 s (IQR: 66.4–128.3 s). Median anti-factor Xa was 0.4 IU/mL (IQR: 0.2–0.6 IU/mL), median AT was 60% (IQR: 48–72%), and median factor VIII was 67% (IQR: 38–94%). Given differences in plasma proteins seen between newborns and infants and children, coagulation and blood transfusion requirement data are presented in Table 5 for the entire cohort as well as by age category. Significant differences were found between newborns vs. infants and children. Newborns had higher heparin infusion rates with higher ACTs, lower anti-factor Xa, lower AT levels, lower factor VIII levels, and higher weight-adjusted daily transfusion volumes of packed red blood cells, platelets, FFP, and cryoprecipitate.

**Heparin infusion rate, ACT, and aPTT.** On average, heparin infusion rate increased with each day on ECMO by 0.9 U/kg/h (95% CI: 0.7–1.1 U/kg/h, \( p < 0.001 \)), whereas ACT and aPTT decreased with each day on ECMO by 0.7 s (95% CI: 0.4–1.0 s, \( p = 0.001 \)) and 1.4 s (95% CI: 0.7–2.1 s, \( p < 0.001 \)), respectively, with an abrupt decrease in the first 48 h post-cannulation and a slower decrease thereafter. (Figure 1) By patient, simultaneously recorded heparin infusion rates and ACT showed that for each 10 U/kg/h increase in heparin infusion rate, the ACT was prolonged by 3 s (95% CI: 2.3–3.3 s, \( p = 0.005 \)). Within each patient, ACT correlated closely with heparin infusion rates over time (r=0.77).

**Anti-factor Xa.** Median anti-factor Xa was 0.4 IU/mL (IQR: 0.2–0.6 IU/mL). Of 352 anti-factor Xa measurements, 215 (61%) fell within the previously proposed target of
0.3–0.7 IU/mL during ECMO.\textsuperscript{33,47} Anti-factor Xa increased with ECMO duration by 0.01 IU/mL (95% CI: 0.005–0.014 IU/mL, \( p=0.001 \)) daily. (Figure 1) Anti-factor Xa was positively correlated with heparin dose when simultaneously measured values were compared (\( r=0.33 \)). For each 10 U/kg/h increase in heparin, the anti-factor Xa was higher by 0.07 IU/mL (95% CI: 0.06–0.09 IU/mL, \( p<0.001 \)). This association remained significant after adjustment for AT levels (\( p<0.001 \)).

We tested the agreement between target anti-factor Xa and target ACT in our population and found poor agreement (42%) for simultaneously measured anti-factor Xa and ACT. (Figure 2) For anti-factor Xa values between 0.3 and 0.7 IU/mL, corresponding median ACT was 214 s (IQR: 198–229 s, range: 160–349 s). ACT and anti-factor Xa remained poorly correlated when they were analyzed longitudinally and when time on ECMO and intra-patient correlation of serial measurements were taken into account (\( r=0.02 \)). The association between ACT and anti-factor Xa became significant only after adjusting for potential confounders (age, fibrinogen, d-dimer, AT and factor VIII deficiency, platelet count, and heparin dose; \( p<0.001 \)).

\( a\text{PTT} \) was also weakly correlated with anti-factor Xa, although somewhat better than ACT (\( r=0.17 \)). The \( a\text{PTT} \) values corresponding to anti-factor Xa between 0.3–0.7 IU/mL had a median of 89 s (IQR: 69–122 s, range: 37–200 s).

\textit{Antithrombin.} AT also increased with ECMO duration by an average of 1% (95% CI: 0.8–1.3%, \( p<0.001 \)) daily. (Figure 1) This increase was not related to daily volume of FFP transfusions (\( p=0.399 \)). Nine of 35 patients (26%) received 1 to 6 doses of plasma-derived AT concentrate, a median of 40 U/kg/dose (IQR: 29–45 U/kg/dose). Median AT activity in these 9 patients was 65% (IQR: 58–74%), a value that was significantly higher than that in the remainder of the cohort (56%; IQR: 46–69%, \( p<0.001 \)). Our ECMO
program does not have a set target for plasma AT, and all AT concentrate was administered at the clinicians’ discretion. The median baseline AT activity for which patients were treated was 43%, with a range of 38–64%.

AT was inversely correlated with ACT. For each 1% increase in AT, ACT was shorter by 0.6 s (95% CI: 0.4–0.7%, p<0.001, r=–0.33), even after adjusting for the heparin dose at the time of the ACT and AT measurements. AT showed a weak positive correlation with heparin infusion rate (r=0.15) and strong positive correlation with anti-factor Xa (r=0.57). For a 10% increase in AT activity, anti-factor Xa increased by 0.08 IU/mL (95% CI: 0.07–0.1 IU/mL, p<0.001). The shorter ACT and higher heparin infusion rates and higher anti-factor Xa in patients with higher AT levels may reflect less consumptive coagulopathy (a cause of low AT levels and prolongation of the ACT despite low rates of heparin infusion).

Factor VIII. On average, factor VIII activity increased by 14% in the first 48 h of ECMO (95% CI: 2–26%, p=0.024), after which it remained stable throughout the ECMO course (0.14% average daily increase, 95% CI: −0.5–1%, p=0.643). (Figure 1) The initial increase may have resulted from a higher volume of FFP transfusions given in the first 1–2 days post-ECMO cannulation. Thirty-one of 35 patients received FFP in the first 48 h of ECMO for a median of 26 mL/kg/day, whereas only 24 of 35 patients received FFP at some point after 48 h, for a median of 12 mL/kg/day. Another possibility is that consumptive coagulopathy decreased during the first 48 h.

The average heparin infusion rate, ACT, aPTT and anti-factor Xa were similar (p>0.05) for patients with high and low factor VIII activity, with cutoffs of 100% and 150%.

Thrombotic and hemorrhagic outcomes.
There were 14 patients (40%) who required a circuit change due to thrombus formation in the circuit that extended to the arterial side of the circuit, post-oxygenator, or was deemed by the clinical team to be too extensive to be tolerated. There were 6 oxygenator failures (17%) thought to be due to clotting. The percentage of discordant ACT to anti-factor Xa values did not differ between patients who required a circuit change vs those who did not: 60% (IQR: 53%–83%) vs 55% (IQR: 40%–90%), \( p = 0.82 \). The most discordant values that could potentially indicate inadequate anticoagulation (i.e., ACT>220 sec and concomitant anti-factor Xa <0.3 IU/mL) also showed no difference between patients who required a circuit change vs those who did not, \( p = 0.13 \).

There were 15 patients (33%) who experienced a hemorrhagic complication. The percentage of discordant ACT to anti-factor Xa values that could indicate excessive anticoagulation by high anti-factor Xa yet low ACTs (i.e., ACT<180 sec and concomitant anti-factor Xa >0.7 IU/mL) was significantly higher in patients who had a hemorrhagic complication compared to those who did not: 4% (IQR: 0%–14%, range 0%–40%) vs 0% (IQR: 0%–0%, range 0%–75%), \( p = 0.01 \).

**Discussion**

Newly available assays that may better describe the state of the coagulation system in patients on ECMO than traditional assays are being increasingly used in ECMO centers internationally and reported in the literature.\(^{34, 40, 45, 47, 50-52}\) Our study describes differences by age and by duration of ECMO as well as comparisons among these coagulation assays in a cohort of 34 pediatric patients on ECMO. We found significant differences by age: ACT and heparin doses were higher, whereas anti-factor Xa levels were lower in neonates than in infants/children. This finding may result from higher heparin needs in neonates secondary to faster heparin clearance\(^{66}\) or a relatively higher circuit-volume–to–patient-blood-volume ratio in smaller patients. AT activity was
also lower in neonates compared to older infants and children, mirroring trends previously described in large cohorts of healthy children.\textsuperscript{49, 50} Overall, however, median AT levels were higher in our newborn patients compared to values previously reported for neonatal ECMO.\textsuperscript{50}

ACT remains the preferred point-of-care measure of anticoagulation for most ECMO centers.\textsuperscript{35} In the absence of heparin, ACT is prolonged with elevated d-dimers, low platelet count or platelet dysfunction, low fibrinogen, other coagulation factor deficiency, hypothermia or hemodilution,\textsuperscript{37} and can be shortened with the opposite or in hypercoagulable states such as increased levels of fibrinogen or factor VIII. Further, different ACT devices may yield different results, potentially confusing clinicians on adequacy of anticoagulation.\textsuperscript{37-40} In our study, ACT showed poor correlation with anti-factor Xa and may not be an adequate measure of anticoagulation in patients on ECMO who have a high prevalence of thrombocytopenia, platelet dysfunction, elevated d-dimers, and/or coagulation factor deficiencies. Although it is not clear what the anti-factor Xa target should be in patients on ECMO, when using the most commonly reported target of 0.3–0.7 IU/mL,\textsuperscript{33, 34, 47} we found poor agreement between the most common ACT target of 180-220 s and anti-factor Xa. In our patients, the association between ACT and anti-factor Xa was confounded by factors that may affect the ACT (age, fibrinogen, d-dimer, AT and factor VIII deficiency, platelet count, and heparin dose) and became significant only after adjusting for these factors. Importantly, our results suggest that those patients who have low ACTs but high concomitant anti-factor Xa levels suggesting excessive anticoagulation could experience higher rates of hemorrhagic complications.

In this study, heparin dose, anti-factor Xa, and AT increased with time on ECMO, while ACT and aPTT decreased. Prior studies suggested that increasing anti-factor Xa
activity with time on ECMO may result from a decrease in AT, an increase in circulating heparin that is released from the circuit’s surface, and/or decreased clearance of heparin over time on ECMO.\textsuperscript{40} However, the anti-factor Xa assay used in this study was supplemented with additional AT to correct for potential AT deficiency. Although we could not test the latter hypothesis, we tested the former and found that in our patient population, AT levels increased, rather than decreased, with ECMO duration. One might expect this increase in the minority of patients who received plasma-derived AT concentrate, but the steady daily increase was also seen in patients without AT replacement therapy and may have been related to decreased consumptive coagulopathy after stabilization and resolution of inciting events leading to ECMO cannulation.

We found that AT was negatively correlated with ACT and remained so after adjusting for heparin infusion rate, suggesting that ACT values may be inaccurate in ECMO patients with AT deficiency. Some ECMO centers have begun replacing AT with plasma-derived or recombinant AT, but the target AT and cutoff for replacement in ECMO patients remain unclear.\textsuperscript{34, 45, 51, 52} In a single center case series, investigators attempted continuous AT infusion and found it to be associated with a lower incidence of hemorrhage in six postcardiotomy patients.\textsuperscript{51} However, significant suppression of thrombin with high-dose or continuous AT infusions may be deleterious in ECMO patients; therefore, this practice will need additional investigation. Routine daily AT monitoring was begun recently at our center, but AT replacement remains at the clinician’s discretion.

Factor VIII activity was remarkably stable throughout the ECMO course in our study group after an initial increase that may have been related to FFP transfusions in the first 48 h after cannulation or elevation secondary to the inflammation associated with acute illness.\textsuperscript{67} High factor VIII activity can be associated with apparent heparin
resistance, with shortened aPTT but stable antithrombotic effect of heparin as measured by anti-factor Xa.\textsuperscript{67,68} We tested for apparent factor VIII-induced heparin resistance in patients whose factor VIII was above 100% and 150%, but our patients did not show evidence for heparin resistance.

Our study was limited by a small sample size and heterogeneity of indications for ECMO. However, to our knowledge, this is the largest prospective study to compare coagulation monitoring assays that are increasingly used in clinical practice, such as anti-factor Xa, AT, and factor VIII. We also plan to evaluate the association between coagulation abnormalities and hemorrhagic and thrombotic outcomes in this study patient population. Future research is needed to 1) describe the role that other coagulation monitoring methods (e.g., TEG, anti-factor IIa) may play in the management of ECMO patients; 2) further describe the complicated relationships between anticoagulation and the risk of thrombosis and hemorrhage in critically ill children on ECMO, and 3) examine via rigorous prospective methodologies the safety and efficacy of interventions that are becoming popular, such as intermittent or continuous administration of recombinant or pooled AT.

**Conclusions**

This study describes the age differences as well as the variability over days of coagulation system monitoring tests such as ACT, anti-factor Xa or AT during ECMO. ACT remains the most popular method of anticoagulation monitoring during ECMO because it is a rapid assay available at the point of care. On the downside, ACT results are affected by multiple physiologic and equipment-related factors and therefore may not be an accurate measure of anticoagulation by heparin. Anti-factor Xa has recently been introduced in clinical practice as an alternative to anticoagulation monitoring during ECMO. However, processing of anti-factor Xa takes longer than processing for ACT, the
test is more expensive and not readily available at all centers. In our study, ACT was poorly correlated with anti-factor Xa, indicating that results should be interpreted with caution when managing anticoagulation on ECMO. Anti-factor Xa results had a direct relation to heparin dose and were not affected by coagulopathy or age. AT did not confound this association between anti-factor Xa and heparin dose but did confound the association between ACT and heparin dose. Based on our results, anti-factor Xa has potential as a marker of anticoagulation for ECMO patients, but additional validation studies are required.
Table 4. Patient and ECMO Characteristics

<table>
<thead>
<tr>
<th>Variable</th>
<th>All (n=35 ECMO runs)</th>
<th>Age ≤30 days (n=21 ECMO runs)</th>
<th>Age &gt;30 days (n=14 ECMO runs)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, median (IQR)</td>
<td>10d (2d - 10y)</td>
<td>3d (1d - 9d)</td>
<td>11y (5m – 15y)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Male, n(%)</td>
<td>16 (44)</td>
<td>9 (43)</td>
<td>10 (71)</td>
<td>0.096</td>
</tr>
<tr>
<td>Race</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caucasian, n(%)</td>
<td>16 (46)</td>
<td>9 (43)</td>
<td>7 (50)</td>
<td>0.538</td>
</tr>
<tr>
<td>African American, n(%)</td>
<td>14 (40)</td>
<td>9 (43)</td>
<td>5 (36)</td>
<td></td>
</tr>
<tr>
<td>Other, n(%)</td>
<td>5 (14)</td>
<td>3 (14)</td>
<td>2 (14)</td>
<td></td>
</tr>
<tr>
<td>ECMO indications, n(%)</td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Respiratory failure</td>
<td>18 (51)</td>
<td>17 (81)</td>
<td>1 (7)</td>
<td></td>
</tr>
<tr>
<td>Cardiac failure</td>
<td>9 (26)</td>
<td>3 (14)</td>
<td>6 (43)</td>
<td></td>
</tr>
<tr>
<td>ECPR</td>
<td>7 (20)</td>
<td>1 (5)</td>
<td>6 (43)</td>
<td></td>
</tr>
<tr>
<td>Sepsis</td>
<td>1 (3)</td>
<td>0</td>
<td>1 (7)</td>
<td></td>
</tr>
<tr>
<td>ECMO mode</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VA-ECMO</td>
<td>29 (83)</td>
<td>16 (76)</td>
<td>13 (93)</td>
<td>0.311</td>
</tr>
<tr>
<td>VV-ECMO</td>
<td>3 (9)</td>
<td>3 (14)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>VV- to VA-ECMO</td>
<td>3 (9)</td>
<td>2 (10)</td>
<td>1 (7)</td>
<td></td>
</tr>
<tr>
<td>ECMO duration, median(IQR)</td>
<td>7d (3d – 14d)</td>
<td>12d (6d – 15d)</td>
<td>3.5d (1d – 5d)</td>
<td>0.006</td>
</tr>
<tr>
<td>Neurologic injury during ECMO, n(%)*</td>
<td>12 (34)</td>
<td>7 (33)</td>
<td>5 (36)</td>
<td>0.884</td>
</tr>
<tr>
<td>Survival to discharge, n(%)</td>
<td>24 (69)</td>
<td>18 (86)</td>
<td>6 (43)</td>
<td>0.007</td>
</tr>
<tr>
<td>Circuit clotting requiring circuit</td>
<td>14 (40)</td>
<td>12 (57)</td>
<td>2 (14)</td>
<td>0.011</td>
</tr>
<tr>
<td>replacement, n(%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oxygenator failure, n(%)</td>
<td>6 (17)</td>
<td>5 (24)</td>
<td>1 (7)</td>
<td>0.200</td>
</tr>
<tr>
<td>ε-aminocaproic acid use, n(%)†</td>
<td>9 (26)</td>
<td>5 (24)</td>
<td>4 (29)</td>
<td>0.752</td>
</tr>
<tr>
<td>Hemorrhagic complications, n(%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ICH</td>
<td>7 (20)</td>
<td>5 (24)</td>
<td>2 (14)</td>
<td>0.490</td>
</tr>
<tr>
<td>Pulmonary hemorrhage</td>
<td>7 (20)</td>
<td>5 (24)</td>
<td>2 (14)</td>
<td>0.490</td>
</tr>
<tr>
<td>Other (hemorhorax, adrenal hemorrhage,</td>
<td>4 (11)</td>
<td>2 (10)</td>
<td>2 (14)</td>
<td>0.664</td>
</tr>
<tr>
<td>retroperitoneal hemorrhage)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Neurologic injury during ECMO is defined as: intracranial hemorrhage, ischemic stroke, cerebral edema or brain death. †No antifibrinolytic agents other than ε-aminocaproic acid were used in this cohort of patients.
<table>
<thead>
<tr>
<th>Variable</th>
<th>All (n=35 ECMO runs)</th>
<th>Age ≤30 days (n=21 ECMO runs)</th>
<th>Age &gt;30 days (n=14 ECMO runs)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heparin (U/kg/h)</td>
<td>34 (22-48)</td>
<td>35 (25-47)</td>
<td>25 (18-52)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ACT (s)</td>
<td>210 (195-227)</td>
<td>213 (198-228)</td>
<td>203 (190-221)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PT (s)</td>
<td>11.9 (11.1-13.6)</td>
<td>12 (11.3-13.6)</td>
<td>11.3 (10.7-13.7)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>aPTT (s)</td>
<td>91.5 (66.4-128.3)</td>
<td>92.7 (67.5-126.8)</td>
<td>87.1 (64.1-131.4)</td>
<td>0.604</td>
</tr>
<tr>
<td>Fibrinogen (mg/dL)</td>
<td>287 (214-365)</td>
<td>263 (211-345)</td>
<td>336 (218-472)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>d-dimer (mg/L)</td>
<td>6.7 (3.4-14.1)</td>
<td>7.1 (3.6-14.7)</td>
<td>6.2 (2.6-12.4)</td>
<td>0.013</td>
</tr>
<tr>
<td>Anti-factor Xa (IU/mL)</td>
<td>0.4 (0.2-0.6)</td>
<td>0.35 (0.2-0.5)</td>
<td>0.5 (0.35-0.7)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>AT (%)</td>
<td>60 (48-72)</td>
<td>57 (47-71)</td>
<td>64 (55-76)</td>
<td>0.007</td>
</tr>
<tr>
<td>Factor VIII (%)</td>
<td>67 (38-94)</td>
<td>58 (34-79)</td>
<td>114 (83-145)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Platelet count (K/mm³)</td>
<td>113 (96-131)</td>
<td>116 (102-134)</td>
<td>102 (83-121)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>12.5 (12.0-13.2)</td>
<td>12.6 (12.1-13.2)</td>
<td>12.3 (11.6-13.3)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PRBC (mL/kg/day)</td>
<td>28 (15-57)</td>
<td>29 (15-61)</td>
<td>24 (12-45)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Platelets (mL/kg/day)</td>
<td>29 (16-58)</td>
<td>33 (18-63)</td>
<td>18 (12-44)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>FFP (mL/kg/day)</td>
<td>0 (0-16)</td>
<td>0 (0-17)</td>
<td>0 (0-15)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Cryoprecipitate (mL/kg/day)</td>
<td>0 (0-1.4)</td>
<td>0 (range 0-30)</td>
<td>0 (range 0-12)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

ACT: activated clotting time; aPTT: activated partial thromboplastin time; AT: antithrombin III; ECMO: extracorporeal membrane oxygenation; FFP: fresh frozen plasma; PRBC: packed red blood cells; PT: prothrombin time. *Data are presented as median (interquartile range) for each group of patients after the median value for each variable was calculated longitudinally for each patient. *Data are presented as median (interquartile range) for each group of patients after the median value for each variable was calculated longitudinally for each patient.
Figure 1. Trends of coagulation markers by time in patients on ECMO.

ACT: activated clotting time; AT: antithrombin; aPTT: activated partial thromboplastin time
Agreement for clinically meaningful values was defined as: ACT <180 s corresponding to anti-factor Xa <0.3 IU/mL, ACT 180–220 s corresponding to anti-factor Xa 0.3–0.7 IU/mL and ACT >220 s corresponding to anti-factor Xa >0.7 IU/mL. Shaded areas represent agreement between ACT and anti-factor Xa, seen in only 42% of measurements. Longitudinal linear regression: $y = 218.5 + 7.2x$, $p = 0.251$, $r = -0.02$. 
Chapter Four

Neuromonitoring during ECMO – a review of the literature

Melania M. Bembea, MD, MPH\textsuperscript{1,2}

Ryan Felling, MD\textsuperscript{2}

\textsuperscript{1}Department of Anesthesiology and Critical Care Medicine and \textsuperscript{2}Department of Neurology, Johns Hopkins University, Baltimore, MD

Introduction

Neurologic complications during extracorporeal membrane oxygenation (ECMO) remain a significant problem and rates have stayed constant over the last three decades despite advances in extracorporeal technology. Several neuromonitoring methods have been proposed to diagnose acute neurologic injury during ECMO, including neuroimaging, electroencephalograms (EEG), somatosensory evoked potentials (SEP), plasma brain injury biomarkers and cerebral blood flow (CBF) velocity measurements by transcranial Doppler ultrasound (TCD). So far, the only method that entered routine clinical care consists of serial head ultrasounds (HUS) in neonates and young infants with open anterior fontanel (HUS cannot be performed though beyond infancy). While not very sensitive for detecting extra-axial lesions, HUS provide clinicians with an easy to use screening tool for intracranial hemorrhage, ischemic stroke and post-asphyxial changes.

In this chapter, we present the results of a literature review of neuromonitoring methods during ECMO.

Data sources

Electronic searches of PubMed, CINAHL, EMBASE, Web of Science, Cochrane and Scopus were conducted in July 2013, using a combination of medical subject heading terms and text words related to extracorporeal support, stroke, cerebral hemorrhage, brain edema, neurologic examination, neurologic outcomes, neurocognitive evaluation/neurodevelopment, neurologic monitoring, neuroimaging, seizures, electroencephalography, somatosensory evoked potentials, near infrared spectroscopy. The search was performed with the help of an experienced clinical informationist. Bibliographies of included studies and those of relevant review articles were hand
searched. Identified studies were imported into a reference manager and duplicates were removed. Two independent reviewers selected titles and abstract and one reviewer retrieved data from the full text articles.

**Study selection criteria**

Studies eligible for the present review met the following criteria: enrollment of patients on ECMO regardless of age; presence of a neuromonitoring method that was employed *during* the ECMO run, with the goal to detect acute neurologic injury during ECMO or to evaluate neurologic function. Studies that evaluated a neuromonitoring method that was applied before, during and after ECMO were included only if the data could be separated for during-ECMO monitoring.

**Data synthesis**

We constructed evidence tables by neuromonitoring method. Study quality was assessed by screening for clear reporting of methods and results. As we made no restrictions on number of subjects, and we anticipated that many studies would be conducted on a small number of patients and would be highly heterogeneous, we decided a priori to keep the review descriptive and not conduct aggregate analysis.

**Results**

**Study flow**

The search strategy yielded 2,982 citations, of which 113 abstracts and 77 full-text articles were reviewed. (Figure 3) Of these 77 articles, one was excluding due to overlapping population with a study from the same group, in 3 articles we were not able to separate the ECMO population from a more general population or separate studies done before, during and after ECMO, two were case reports, 3 were reviews and/or erratum, 6 were validation studies for a new technique, 14 did not meet criteria for
neuromonitoring, and we were not able to locate the full text of 8 articles. Ultimately, 39 articles met inclusion criteria.

**Description of included studies**

There were 16 retrospective reviews, 21 prospective observational studies, 2 case-control studies and no interventional studies. One study evaluated a neuromonitoring method retrospectively, including interventions based on recorded values as part of a clinical protocol, but those interventions were part of routine clinical care.\(^7\) The vast majority of studies evaluated newborns (27/39), 7 studies evaluated newborns and infants/children, two studies evaluated newborns, children and adults, one evaluated children and adults, one evaluated children only and one study evaluated adults only. ECMO neuromonitoring methods included: neuroimaging (serial HUS or brain computed tomography [CT]), EEG, amplitude-integrated EEG (aEEG), pulsed Doppler ultrasound/TCD, SEP, cerebral oximetry, CBF measurements using a radioactive tracer or combinations of the above. (Tables 6 and 7)

**Discussion**

**Head ultrasound**

Serial screening HUS have been used in the neonatal ECMO population since the early days of the technique. HUS probes are placed over an open anterior fontanel and are limited in the sense that they capture lesions occurring in the axial plane, but not in the periphery/extra-axial spaces. There is no gold standard for detection of neurologic injury during ECMO, therefore most studies compared HUS findings during the ECMO course with more confirmatory neuroimaging (brain CT or brain magnetic resonance imaging [MRI]). Rates of acute neurologic injury during ECMO captured by HUS ranged from zero to 46% in the articles included in this review.\(^7\)
The significance of abnormal findings by HUS is unknown. Data on the correlation of HUS findings with post-ECMO brain CT and/or MRI and with neurodevelopmental outcomes are inconclusive. Rollins et al in a series of 50 neonates with post-ECMO brain MRIs found that 50% of newborns with normal HUS during ECMO showed abnormalities on brain MRI. HUS was abnormal in 24% of neonates, and brain MRI was abnormal in 62% of neonates. Neither HUS nor brain MRIs, though, were predictive of neurodevelopmental outcomes. Glass et al examined 152 children at 5 years of age and correlated neurologic disability scores with severity of neuroimaging findings during and after ECMO in the neonatal period. A large proportion of children with mild, moderate and even severe lesions on neuroimaging had no neurologic disability detected (87%, 67% and 43%, respectively). Conversely, 10%-13.5% of neonates with normal HUS or post-ECMO brain CT or brain MRI did show neurodevelopmental delays. Similarly, in a series of 22 patients cannulated for ECMO at an average age of 1.18 years (range 0.1-7.8 years), Wagner et al reported a lack of correlation between neuroimaging findings and cognitive outcomes at follow up at 7.2 years of age (range 1.8-13.9 years of age). And in a group of 74 newborns who required ECMO for refractory respiratory failure, 13.5% had significant cognitive delay at serial follow up intervals up to 5 years post-ECMO, despite normal HUS and/or brain CT or MRI.

**Brain computed tomography**

Three studies that included populations of mixed ages reported retrospective data on the use of brain CT during ECMO, where brain CT was ordered by the clinical team in response to suspected neurologic complications, clinical neurologic symptoms (seizures, focal weakness, pathologic pupillary response, delay in awakening from sedation), difficult neurologic evaluation due to sedation, or as complementary study to
Findings that were abnormal and/or considered significant were present in 31%-57% of patients, and many of these helped with management decisions. There were no or only minor complications associated with transport to CT. Brain CT is, however, accompanied by the risk of radiation, and cannot be done serially over several day of ECMO even when portable devices are available.

**Electroencephalography**

EEG has been used for neuromonitoring during ECMO since the beginnings of the therapy, mostly in neonates. The grading methodology for EEG abnormalities used by most authors (see Table 7) are based on the Tharp and Laboyrie’s criteria for neonates and Tasker et al’s criteria for children. In the studies included in this review, the range of abnormal EEG studies during ECMO was 51%-95%. The proportion of electrographic seizures was much lower, around 8% when EEGs were conducted in consecutive patients, and up to 21% when EEGs were reviewed retrospectively and were obtained when clinicians had suspicion for clinical seizures or they requested EEG as an aid in management of neuroprotective measures in ECMO patients at high risk for brain hypoperfusion.

In most studies, abnormal EEGs did not correlate with abnormal neuroimaging findings by HUS during and after ECMO or by brain CT or MRI after ECMO. Gannon et al in a retrospective review of 160 newborns and infants showed that EEGs did not differ between patients with normal vs abnormal brain CT or MRI. The authors purported that EEG could aid when findings were added to abnormalities found by HUS to improve the positive predictive value of brain injury by brain CT or MRI from 56% (abnormal HUS only) to 69% (abnormal HUS and abnormal EEG). In a study of 46 neonates on ECMO, Graziani at al also attempted to correlate abnormalities on HUS to abnormalities...
on EEG recordings: while newborns with moderate to severe HUS abnormalities during or after ECMO also had at least one moderately to severely abnormal EEG, the opposite was not true (only 8/14 patients with markedly abnormal EEG during ECMO had HUS abnormalities after ECMO).93

Electrographic seizures and/or otherwise abnormal EEG were significantly associated with unfavorable long-term outcomes in two newborn studies (7 of 11 newborns with electrographic seizures during ECMO died before discharge or had developmental delay at one year89 and the most abnormal EEG during ECMO in a series of 19 newborns and children was associated with a negative neurologic outcome at one year,94 respectively.

EEGs are difficult to interpret in real time, require dedicated specialists, are expensive, especially when recorded continuously, and, during ECMO, can be associated with significant artifact due to interferences from the multiple bedside devices.95 An alternative in newborns are continuous recordings of aEEG, which are easier to interpret for significant abnormalities by personnel with far less training than pediatric epileptologists, and can be recorded continuously without a large investment on the part of hospitals.96, 97

A neonatal study that employed aEEG recordings continuously for the first 5 days on ECMO on 26 neonates showed that 16/26 (62%) recordings were normal throughout, while 7/26 (38%) recordings were moderately or severely abnormal, and that included subclinical seizures in two newborns.96 aEEG became abnormal 24h before HUS abnormality was detected in one newborn.96

Another study of aEEG that started before cannulation, continued up to 90m on ECMO, and was then repeated once for ≥30 minutes within the first 24h±8h on ECMO,
conducted in 20 newborns, showed that severe abnormalities on aEEG before and/or during ECMO predicted death or moderate to severe intracranial neuropathology by neuroimaging or autopsy, with a positive predictive value of 0.86. However, no data on temporal correlation between abnormal aEEG and the time of abnormal neuropathology findings was given, and aEEG abnormalities around the time of cannulation may have been due to pre-existing pathophysiology, and not necessarily to ECMO-related neurologic complications.

**Doppler ultrasound and measurements of cerebral blood flow velocities**

CBF velocity changes and disturbances of cerebrovascular autoregulation have been considered culprits in the development of neurologic injury during ECMO. Under normal conditions, autoregulation of the cerebral vasculature maintains CBF constant over a wide range of cerebral perfusion pressures. CBF is maintained by vasoconstriction in face of hypertension and by vasodilatation in face of low mean arterial blood pressures (ABP). Hypoxia, hypercarbia, hypotension/ hypoperfusion, and acidosis disrupt cerebrovascular autoregulatory mechanisms, resulting in CBF that is passive to fluctuations in ABP. These pathophysiologic processes (hypoxia, hypotension, acidosis) are hallmarks of pediatric patients with refractory cardiopulmonary failure in the immediate pre-ECMO period. If cerebrovascular autoregulatory mechanisms are overwhelmed, the dilating capacity of cerebral resistance vessels is exceeded, the CBF decreases, and the brain becomes vulnerable to injury. Animal studies conducted in newborn lambs showed that exposure to hypoxia leads to loss of autoregulation up to 6 hours after recovery from hypoxia. When animals were rescued from hypoxia via cannulation to ECMO and ligation of the carotid artery and jugular vein, autoregulation was lost for the duration of the follow-up period of
Even in the absence of hypoxia, exposure to ECMO was associated with loss of cerebrovascular autoregulation in normal neonatal lambs. Most Doppler ultrasound studies calculate the pulsatility index (PI) in one or more major cerebral vessels, depending on the windows used. The PI is calculated as systolic velocity – diastolic velocity/mean velocity. Decreased cerebral perfusion pressure and increased intracranial pressure lead to decreased diastolic velocity and increased PI.

Pulsatility in the pericallosal artery (part of the anterior cerebral circulation) was evaluated in two studies conducted in neonates in 1987 (n=13) and 1990 (n=21), respectively. The mean PI decreased at the initiation of ECMO and over time during ECMO, and the mean PI decreased at high ECMO flows and then increased gradually with lower flows. Authors inferred that the significant decreases in mean PI with initiation of ECMO and at high flows corresponded to increased CBF that could be involved in the pathogenesis of ICH. The two studies did not provide data on correlation of PI changes with clinical suspicion of neurologic injury or confirmed neurologic injury.

Other investigators conducted CBF velocity measurements in the internal, middle and/or anterior carotid arteries, as well as the intracranial venous system. Lohrer et al studied 25 newborns on VA-ECMO and described internal carotid artery flow velocities in patients with (n=18) vs without (n=7) neuroanatomic abnormalities detected by HUS or brain CT and found no difference in velocities between the two groups. Fukuda et al studied 33 newborns and found that patients with ICH had low CBF velocity associated with disturbed cardiac function in venoarterial vs venovenous ECMO. The authors inferred that the decrease in CBF due to myocardial failure could lead to ICH,
although there was no data on temporal correlation of abnormal CBF velocity measurements and onset of ICH and this hypothesis was not confirmed to our knowledge in animal studies or in other human studies. O’Brien and Hall studied 18 newborns and children using TCD to measure bilateral middle cerebral artery CBF velocities and found that, in 4 patients with cerebral hemorrhage, systolic flow velocity was supranormal for age and gender, significantly different from patients without clinically evident acute neurologic injury. In all 4 patients, elevated CBF velocities were sustained for 2-6 days before neurologic injury was recognized. The authors did not comment on differences between venoarterial vs venovenous ECMO.

**Plasma brain injury biomarkers**

There are two published reports on the use of plasma brain injury biomarkers for neuromonitoring during ECMO. One investigated S100b as a potential tool in early detection of ICH during ECMO, and found, in 16 newborns (8 cases with confirmed ICH and 8 controls without known ICH), that plasma S100b was significantly higher in all cases, 72h before any sign of ICH was detected by ultrasound. The authors also measured middle cerebral artery CBF velocity and found that, while the PI was significantly higher in cases compared to controls, this increase took place 48h-72h after S100b peaked, suggesting that S100b was an earlier and more sensitive marker of subclinical neurologic injury. The second published report is our study of GFAP in neonatal and pediatric patients (n=22) detailed in Chapter Five of this dissertation.

**Cerebral oximetry**

Cerebral oximetry has become widespread in the operating room and intensive care units and can be used to measure regional cerebral tissue oxygen saturation (rSO2), to measure changes in total hemoglobin as a surrogate for cerebral blood
volume or to dynamically measure the correlation between oxyhemoglobin and mean arterial blood pressure (ABP) as an indicator for intact cerebrovascular autoregulation. There are several reports on the use of cerebral oximetry as a tool for identification of neurologic injury.

Four of these reports evaluated events surrounding the cannulation of the right common carotid artery and found significant decrease in oxyhemoglobin and increase in deoxyhemoglobin concentration, and temporary decrease in rSO2 index and cerebral tissue oxygen saturation, respectively.\textsuperscript{112-115}

In a study published in 1992 that measured total hemoglobin using NIRS-based technology, Liem et al showed an increase in total hemoglobin in the brain tissue. This was interpreted as increased cerebral blood volume and thus increased CBF that was thought to contribute to the development of ICH during ECMO (n=5 newborns).\textsuperscript{116} The same group three years later described an increase in oxyhemoglobin, transcutaneous partial pressure of oxygen, arterial oxygen saturation and mean ABP shortly after initiation of ECMO, with deoxyhemoglobin concentrations being lower than precannulation values (n=24 newborns).\textsuperscript{112} The two Liem et al studies did not have clinical correlates. A similar study, but with clinical correlates, conducted by Van Heijst et al in 2004 in 10 newborns showed that three patients with asymmetric brain lesions had no differences in measurements of oxyhemoglobin, cerebral blood volume and CBF velocity between the two hemispheres.\textsuperscript{113}

Ejike et al studied 11 neonates and children to observe the effects of right common carotid artery cannulation and ECMO flow changes on the rSO2 index and found a 15%-20% decrease in rSO2 index in the right frontal region, no change in the left frontal region, and no change in rSO2 index during ECMO flow variations and
“trialing off” ECMO.\textsuperscript{114} There was no mention of any incident neurologic injury during or after the monitoring period.

In a more recent study of cerebral oximetry tracings in 20 adults on ECMO, Wong et al showed that 4 of 20 patients whose rSO2 did not correct with hemodynamic interventions such as increasing ABP, oxygenation and/or ECMO flow, and who underwent further diagnostic testing, were found to have a cerebrovascular accident.\textsuperscript{71}

Fenik et al evaluated the cerebral tissue oxygen saturation (SctO2) in 17 newborns before, during and up to 48h after ECMO cannulation and found SctO2 values <60\% in 12/17 patients pre-cannulation, with an increase >60\% and stabilization of SctO2 for all patients after ECMO started.\textsuperscript{115} This study did not have any clinical correlates either, and there was no mention of whether any patients developed acute neurologic injury during ECMO or not.

Lastly, the Papademetriou et al study published in 2012 took cerebral oximetry further and, in 6 newborns on VA-ECMO, showed that the wavelet cross-correlation between multisite oxyhemoglobin concentration and mean ABP increased with decreasing ECMO flows, indicating loss of cerebrovascular autoregulation with low extracorporeal support, but again, there were no clinical correlates, and no data given on potential association of the wavelet cross-correlation with abnormalities in neurologic exam or neuroimaging.\textsuperscript{117}

In summary, NIRS-based data show promise, but all studies investigating their utility were conducted in small numbers of patients, did not have adequate data collection to investigate association of abnormal tracings with onset or ongoing neurologic injury, and thus will require further validation. As part of our ongoing cohort,
we have been recording rSO2 continuously in neonatal and pediatric ECMO patients and plan analysis in the near future.

Conclusions

Several methods of neuromonitoring during ECMO have been reported. Most studies tend to have modest sample sizes, are either retrospective or prospective observational in nature and include patient populations that are of different ages and pathologies. Of note, we identified only a handful of studies of older children and adults, although at least one of these studies suggests that neurologic injury during non-neonatal ECMO is likely underdiagnosed and thus future data are direly needed. The main limitation of many of the methods described in this review is the intermittent nature of the monitoring (e.g., HUS, TCD, brain CT, plasma measurements for brain injury biomarkers) and, for those methods that can be continuously employed, limitations include high cost and need for continuous highly-trained staff availability (e.g., continuous EEG), and lack of validation in larger scale studies (e.g., NIRS-based cerebral oximetry). As presented in Chapter Seven, our research program aims to address two of these limitations by developing means of continuous monitoring for plasma brain-specific proteins and by utilizing a novel NIRS-based methodology for continuous evaluation of cerebrovascular autoregulation capacity in ECMO patients.
Figure 3. Study search flowchart

2981 unique titles identified for review

2868 excluded based on title review

113 included for abstract review

36 excluded based on abstract review
  - 3 case reports
  - 13 reviews
  - 20 abstract-only

77 included for full text review

38 excluded based on full text review
  - 1 overlapping study population
  - 1 Japanese only
  - 3 unable to separate ECMO population or studies done before and after ECMO
  - 2 case reports
  - 3 reviews/erratum
  - 6 technique validation studies
  - 8 unable to locate full text
  - 14 no neuromonitoring

39 included in study
Table 6. Study description

<table>
<thead>
<tr>
<th>Source/Year</th>
<th>Type of study</th>
<th>Number of centers</th>
<th>n</th>
<th>Age group/age at cannulation</th>
<th>Primary indication(s) for ECMO</th>
<th>ECMO mode</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Head ultrasound</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Slovis, 1988</td>
<td>Retrospective review</td>
<td>1</td>
<td>45</td>
<td>Newborns GA: 38w±2.3w</td>
<td>RF, CF, sepsis</td>
<td>NS</td>
</tr>
<tr>
<td>Taylor, 1989</td>
<td>Retrospective review</td>
<td>1</td>
<td>207</td>
<td>Newborns GA: 39.1w (range: 36w-43w) Age NS further</td>
<td>RF, CF</td>
<td>VA</td>
</tr>
<tr>
<td>Babcock, 1989</td>
<td>Retrospective cohort</td>
<td>1</td>
<td>50</td>
<td>Newborns Mean 68h (range: 12h-138h)</td>
<td>RF, CF, sepsis</td>
<td>VA</td>
</tr>
<tr>
<td>Griffin, 1992</td>
<td>Retrospective review</td>
<td>1</td>
<td>22</td>
<td>Newborns GA: mean 38w±2w Age NS further</td>
<td>RF, sepsis</td>
<td>VA</td>
</tr>
<tr>
<td>Radack, 1994</td>
<td>Retrospective review</td>
<td>1</td>
<td>212</td>
<td>Newborns GA: median 39w (range: 36w-43w)</td>
<td>RF, sepsis</td>
<td>NS</td>
</tr>
<tr>
<td>Lazar, 1994</td>
<td>Retrospective review</td>
<td>1</td>
<td>74</td>
<td>Newborns Age NS further</td>
<td>RF, sepsis</td>
<td>VA</td>
</tr>
<tr>
<td>Biehl, 1996</td>
<td>Retrospective review</td>
<td>1</td>
<td>343 neonatal charts reviewed, 30 newborns with ICH included in study</td>
<td>Newborns Mean GA: 38w</td>
<td>RF, CF, sepsis</td>
<td>VV, VA</td>
</tr>
<tr>
<td>Heard, 1997</td>
<td>Retrospective review</td>
<td>1</td>
<td>49</td>
<td>Newborns GA: median 39w (range: 32w-44w) Age NS further</td>
<td>RF, sepsis</td>
<td>VV/VA</td>
</tr>
<tr>
<td>Khan, 1998</td>
<td>Retrospective review</td>
<td>5</td>
<td>298</td>
<td>Newborns GA: mean 38w (range: 33w-43w)</td>
<td>RF, CF, sepsis</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Brain CT</strong></td>
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<tr>
<td>Lidegran, 2002</td>
<td>Retrospective review</td>
<td>1</td>
<td>112</td>
<td>Newborns, children and adults Age NS further</td>
<td>RF, CF, sepsis, ECPR</td>
<td>VV, VA</td>
</tr>
<tr>
<td>Lidegran, 2007</td>
<td>Retrospective review</td>
<td>1</td>
<td>123</td>
<td>Children and adults 54 children, age range 3m-17y 69 adults, age range 18y-62y</td>
<td>RF, CF, sepsis</td>
<td>VV, VA</td>
</tr>
<tr>
<td>Jepson, 2010</td>
<td>Retrospective review</td>
<td>1</td>
<td>14</td>
<td>Newborns (n=3), children (n=4 with 5 studies) and adults (n=7)</td>
<td>RF, CF</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Electroencephalography</strong></td>
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</tr>
<tr>
<td>Streletz, Prospective</td>
<td>1</td>
<td>145</td>
<td>Newborns</td>
<td>RF</td>
<td>VA</td>
<td></td>
</tr>
<tr>
<td>Source/Year</td>
<td>Type of study</td>
<td>Number of centers</td>
<td>n</td>
<td>Age group/age at cannulation</td>
<td>Primary indication(s) for ECMO</td>
<td>ECMO mode</td>
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</tr>
<tr>
<td>1992</td>
<td>observational</td>
<td></td>
<td></td>
<td>GA: 39.1w±2.2w Age NS further</td>
<td>RF</td>
<td>VA</td>
</tr>
<tr>
<td>Graziani, 1994</td>
<td>Prospective observational</td>
<td>1</td>
<td>119</td>
<td>Newborns</td>
<td>RF, CF, sepsis</td>
<td>VV, VA, VV-VA</td>
</tr>
<tr>
<td>Amigoni, 2005</td>
<td>Prospective observational</td>
<td>1</td>
<td>19</td>
<td>Newborns and children 12 newborns (age not given) 9 children (range: 4m-162m)</td>
<td>RF, CF, sepsis</td>
<td>VA</td>
</tr>
<tr>
<td>Trittenwein, 2006</td>
<td>Retrospective, case-control</td>
<td>1</td>
<td>7 cases (ECMO)/10 controls (critically ill neonates on mechanical ventilation and not on ECMO)</td>
<td>RF, CF</td>
<td>VA</td>
<td></td>
</tr>
<tr>
<td>Piantino, 2013</td>
<td>Retrospective review</td>
<td>1</td>
<td>19</td>
<td>Children Median 2.75y (IQR: 0.6y-6.8y)</td>
<td>RF, CF, ECPR</td>
<td>VV, VA</td>
</tr>
<tr>
<td>Gannon, 2001</td>
<td>Retrospective review</td>
<td>1</td>
<td>160</td>
<td>Newborns and infants 157 newborns 3 infants (40d, 55d, 134d)</td>
<td>RF, CF, sepsis, ECPR</td>
<td>VV, VA, VV-VA</td>
</tr>
<tr>
<td>Korinthenberg, 1993</td>
<td>Prospective observational</td>
<td>1</td>
<td>17</td>
<td>Newborns Median: 2.5d (range 1d-6d)</td>
<td>RF, sepsis</td>
<td>VA</td>
</tr>
<tr>
<td>Graziani, 1989</td>
<td>Prospective observational</td>
<td>1</td>
<td>46</td>
<td>Newborns GA≥36w Age NS further</td>
<td>RF</td>
<td>VA</td>
</tr>
<tr>
<td>Horan, 2007</td>
<td>Prospective observational</td>
<td>1</td>
<td>26</td>
<td>Newborns Age range 6h-432h</td>
<td>RF, CF, sepsis</td>
<td>NS</td>
</tr>
<tr>
<td>Pappas, 2006</td>
<td>Prospective observational</td>
<td>1</td>
<td>20</td>
<td>Newborns GA: mean 38.4w±3.0w Age NS further</td>
<td>RF, sepsis</td>
<td>VV, VA</td>
</tr>
<tr>
<td>Taylor, 1992</td>
<td>Prospective observational</td>
<td>1</td>
<td>13</td>
<td>Newborns Age NS</td>
<td>RF, sepsis</td>
<td>VA</td>
</tr>
<tr>
<td>Van de Bor, 1990</td>
<td>Prospective observational</td>
<td>1</td>
<td>21</td>
<td>Newborns 2.8d±1.5d</td>
<td>RF</td>
<td>VA</td>
</tr>
<tr>
<td>Taylor, 1992</td>
<td>Prospective observational</td>
<td>1</td>
<td>23</td>
<td>Newborns and infants&lt;1y Newborns (n=20): GA: mean 39.2w (range: 35w-42w)</td>
<td>RF, CF, sepsis</td>
<td>VA</td>
</tr>
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</table>

**Amplitude-integrated EEG (aEEG)**

<table>
<thead>
<tr>
<th>Source/Year</th>
<th>Type of study</th>
<th>Number of centers</th>
<th>n</th>
<th>Age group/age at cannulation</th>
<th>Primary indication(s) for ECMO</th>
<th>ECMO mode</th>
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<tbody>
<tr>
<td>Horan, 2007</td>
<td>Prospective observational</td>
<td>1</td>
<td>26</td>
<td>Newborns Age range 6h-432h</td>
<td>RF, CF, sepsis</td>
<td>NS</td>
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</table>

**Doppler ultrasound**

<table>
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<tr>
<th>Source/Year</th>
<th>Type of study</th>
<th>Number of centers</th>
<th>n</th>
<th>Age group/age at cannulation</th>
<th>Primary indication(s) for ECMO</th>
<th>ECMO mode</th>
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</thead>
<tbody>
<tr>
<td>Taylor, 1987</td>
<td>Prospective observational</td>
<td>1</td>
<td>13</td>
<td>Newborns Age NS</td>
<td>RF, sepsis</td>
<td>VA</td>
</tr>
<tr>
<td>Van de Bor, 1990</td>
<td>Prospective observational</td>
<td>1</td>
<td>21</td>
<td>Newborns 2.8d±1.5d</td>
<td>RF</td>
<td>VA</td>
</tr>
<tr>
<td>Source/Year</td>
<td>Type of study</td>
<td>Number of centers</td>
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<td>Age group/age at cannulation</td>
<td>Primary indication(s) for ECMO</td>
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<tr>
<td>Lohrer, 1992&lt;sup&gt;107&lt;/sup&gt;</td>
<td>Prospective observational</td>
<td>1</td>
<td>25 (and 15 normal newborn controls selected at random)</td>
<td>Newborns GA: 40w (range 36-42) Age NS further</td>
<td>RF</td>
<td>VA</td>
</tr>
<tr>
<td>Fukuda, 1999&lt;sup&gt;108&lt;/sup&gt;</td>
<td>Retrospective review</td>
<td>1</td>
<td>33</td>
<td>Newborns VA: 5d±15d VV: 4d±7d</td>
<td>RF, sepsis</td>
<td>VV, VA</td>
</tr>
<tr>
<td>O’Brien, 2013&lt;sup&gt;109&lt;/sup&gt;</td>
<td>Prospective observational</td>
<td>1</td>
<td>18</td>
<td>Newborns and children Median 3.8y (range: 2d-18y)</td>
<td>RF, CF, ECPR</td>
<td>VV, VA</td>
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<tr>
<td>Gazzolo, 2002&lt;sup&gt;110&lt;/sup&gt;</td>
<td>Prospective, case-control study</td>
<td>1</td>
<td>16 (8 cases, 8 controls)</td>
<td>Newborns Age NS further</td>
<td>CF</td>
<td>NS</td>
</tr>
<tr>
<td>Bembea, 2011&lt;sup&gt;111&lt;/sup&gt;</td>
<td>Prospective observational</td>
<td>1</td>
<td>22</td>
<td>Newborns and children Median 10d (range 2d-16y)</td>
<td>RF, CF, sepsis, ECPR</td>
<td>VV, VA, VV-VA</td>
</tr>
<tr>
<td>Carter, 1995&lt;sup&gt;112&lt;/sup&gt;</td>
<td>Prospective, convenience sample</td>
<td>1</td>
<td>11 (comparison with 99 children with brain injury and 17 neurologically normal controls)</td>
<td>Newborns and children Median 13d (range: 3d-12y)</td>
<td>RF, CF, sepsis</td>
<td>VA</td>
</tr>
<tr>
<td>Liem, 1992&lt;sup&gt;113&lt;/sup&gt;</td>
<td>Prospective observational</td>
<td>1</td>
<td>5</td>
<td>Newborns GA: 35w-40w Age NS further</td>
<td>NS</td>
<td>VA</td>
</tr>
<tr>
<td>Liem, 1995&lt;sup&gt;114&lt;/sup&gt;</td>
<td>Prospective observational</td>
<td>1</td>
<td>24</td>
<td>Newborns GA: 35.1w-40.9w Age NS further</td>
<td>RF, sepsis</td>
<td>VA</td>
</tr>
<tr>
<td>Van Heijst, 2004&lt;sup&gt;115&lt;/sup&gt;</td>
<td>Prospective observational</td>
<td>1</td>
<td>10</td>
<td>Newborns GA: range 37w-42w Age NS further</td>
<td>RF, sepsis</td>
<td>VA</td>
</tr>
<tr>
<td>Eijke, 2006&lt;sup&gt;116&lt;/sup&gt;</td>
<td>Prospective observational</td>
<td>1</td>
<td>11</td>
<td>Newborns and children Range: 1d-8m</td>
<td>RF, CF</td>
<td>VA</td>
</tr>
<tr>
<td>Fenik, 2009&lt;sup&gt;117&lt;/sup&gt;</td>
<td>Prospective observational</td>
<td>1</td>
<td>17</td>
<td>Newborns GA: 38-41w</td>
<td>RF, CF, sepsis</td>
<td>VV, VA</td>
</tr>
<tr>
<td>Papademetriou,</td>
<td>Prospective</td>
<td>1</td>
<td>12 enrolled, 6</td>
<td>Newborns</td>
<td>RF, CF</td>
<td>VA</td>
</tr>
<tr>
<td>Source/ Year</td>
<td>Type of study</td>
<td>Number of centers</td>
<td>n</td>
<td>Age group/ age at cannulation</td>
<td>Primary indication(s) for ECMO</td>
<td>ECMO mode</td>
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</tr>
<tr>
<td>2012</td>
<td>observational</td>
<td>analyzed (other 6 had recordings had poor signal-to-noise ratio or movement artifacts)</td>
<td>Age: 1d-25d</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wong, 2012</td>
<td>Retrospective review</td>
<td>1</td>
<td>20</td>
<td>Adults</td>
<td>RF, CF, ECPR</td>
<td></td>
</tr>
<tr>
<td>Stockwell, 1996</td>
<td>Prospective study</td>
<td>1</td>
<td>14</td>
<td>Newborns</td>
<td>RF</td>
<td></td>
</tr>
</tbody>
</table>

Cerebral blood flow measurement using radionuclide tracer

ECMO: extracorporeal membrane oxygenation; RF: respiratory failure; CF: cardiac failure; VV: venovenous ECMO; VA: venoarterial ECMO; VV-VA: venovenous with conversion to venoarterial ECMO; ECPR: extracorporeal cardiopulmonary resuscitation; ICH: intracranial hemorrhage; GA: gestational age; NS: not specified
<table>
<thead>
<tr>
<th>Source</th>
<th>Study goals</th>
<th>Neuromonitoring methods</th>
<th>Timing of studies</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slovis, 1988&lt;sup&gt;72&lt;/sup&gt;</td>
<td>Investigate central nervous system, (thoracic, peritoneal and visceral) abnormalities in neonates on ECMO</td>
<td>HUS</td>
<td>HUS before cannulation and daily during ECMO, with additional HUS for systemic hypertension, seizures, unexplained blood loss, or unexplained clinical change</td>
<td>9/45 had SEH before ECMO, of which 4/9 had no further bleeding on ECMO. Total of 17/45 (38%) patients had ICH diagnosed on ECMO. No data on timing of brain injury and no data on temporal correlation of abnormal clinical findings with HUS evidence for brain injury.</td>
</tr>
<tr>
<td>Taylor, 1989&lt;sup&gt;73&lt;/sup&gt;</td>
<td>Present single center experience with infants treated with ECMO and explore possible causes of cerebrovascular injury after ECMO</td>
<td>HUS</td>
<td>Before ECMO and daily during ECMO</td>
<td>Intracranial abnormalities identified in 95/207 (46%) newborns by HUS during ECMO and/or brain CT after ECMO. 37/66 (56%) lesions detected on brain CT were not shown on HUS (11 major and 26 minor lesions). Minor lesions were: SHE, subarachnoid hemorrhage, petechial parenchymal bleeding, focal widening of interhemispheric fissure, small areas of atrophy or focally lucent brain parenchyma, transient thrombosis of the sagittal sinus. All other injuries were classified as major.</td>
</tr>
<tr>
<td>Babcock, 1989&lt;sup&gt;118&lt;/sup&gt;</td>
<td>Determine the prevalence of ICH and other abnormalities in near-term infants treated with ECMO</td>
<td>HUS</td>
<td>Before ECMO, within 12h after starting ECMO, during ECMO as clinically indicated</td>
<td>Small SEH or cyst (n=6) before ECMO did not extend during ECMO. HUS during ECMO showed development of new abnormalities in 14 patients. Discrepancies between HUS and HCT (n=5).</td>
</tr>
<tr>
<td>Griffin, 1992&lt;sup&gt;74&lt;/sup&gt;</td>
<td>Determine the incidence of brain injury in neonates treated with ECMO, evaluate the use of MRI in detecting brain abnormalities after ECMO and correlate radiographic and clinical findings with neurodevelopmental outcomes</td>
<td>HUS</td>
<td>Before cannulation and every 1-2 days during ECMO</td>
<td>All HUS were normal except for one cystic lesion (incidental finding). Two brain MRIs post-ECMO showed global cerebral atrophy despite normal HUS. 20/22 survivors underwent neurodevelopmental evaluation at 3m-30m (BSID) and 3/20 were abnormal (one had normal HUS during ECMO and global cerebral atrophy on brain MRI post-ECMO and two had normal HUS and brain MRI).</td>
</tr>
<tr>
<td>Radack, 1994&lt;sup&gt;75&lt;/sup&gt;</td>
<td>Determine the frequency and the pattern of evolution of SEH in neonates on ECMO using HUS</td>
<td>HUS</td>
<td>Before cannulation and daily during ECMO</td>
<td>43/212 (20%) had a total of 65 SEH (22/43 patients had bilateral SEHs and 28/43 patients developed 38 SEHs during ECMO after pre-ECMO HUS showed no ICH, and 18/43 neonates had 22 SEHs on pre-ECMO HUS). 59/65 (91%) SEHs remained stable or resolved during</td>
</tr>
<tr>
<td>Source</td>
<td>Study goals</td>
<td>Neuromonitoring methods</td>
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<td>Results</td>
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<tr>
<td>Lazar, 1994</td>
<td>Determine if serial HUS could be correlated with more definitive diagnostic studies such as CT/MRI, autopsy data, or the long-term neurodevelopmental status, to discern the predictive value of daily HUS</td>
<td>HUS</td>
<td>Daily HUS</td>
<td>ECMO and 6/65 (9%) SEHs evolved to grade 2, 3 or 4 ICH. Brain CT/MRI post-ECMO and/or autopsy showed structural brain injury in 19 patients (16 ischemic infarction, 3 ICH). Serial HUS were normal in 9/19 and showed focal increase in echostructure evolving to diffuse effacement in 10/19. An additional 10 children had neurodevelopmental delay at 6w, 6m, then annually until 5y, but had normal HUS and brain CT/MRI. There were no difference between ischemic and hemorrhagic lesions diagnosed by post-ECMO brain CT/MRI/autopsy in those with normal vs abnormal HUS (p=0.08). No mention of timing of injury.</td>
</tr>
<tr>
<td>Biehl, 1996</td>
<td>Correlate clinical findings with HUS reports to determine the approximate timing of ICH occurrence in infants on ECLS to optimize the use of HUS</td>
<td>HUS</td>
<td>Daily during ECOMO</td>
<td>85% ICH occurred within the first 72h of bypass. Septic patients more likely to have an ICH on ECLS (p&lt;0.02). Late ICH (&gt;72h after initiation of ECLS) associated with neurologic changes or multiorgan failure.</td>
</tr>
<tr>
<td>Heard, 1997</td>
<td>Identify a patient population that does not require daily HUS while on ECMO to determine if overall cost of care would be reduced</td>
<td>HUS</td>
<td>HUS before cannulation then daily during ECOMO</td>
<td>HUS were classified as abnormal if they showed hemorrhage, cerebral edema or extraaxial fluid. Normal HUS pre-ECMO and first 24h on ECMO was seen in 41/49 patients and only 2/41 developed hemorrhage after day 1, vs 4/8 neonates with one early abnormal HUS (p=0.004).</td>
</tr>
<tr>
<td>Khan, 1998</td>
<td>Determine usefulness of performing daily HUS in infants on ECMO in detecting IVH of a magnitude sufficient to alter patient treatment</td>
<td>HUS</td>
<td>HUS prior to, then daily during ECOMO</td>
<td>52/298 (17.5%) diagnosed with ICH by HUS. 9/52 had an ICH on the pre-ECMO HUS, all grade I. 43/52 had a new ICH diagnosed during ECMO (15 grade I, 10 grade II, 10 grade III, 8 grade IV using the Papile Classification for ICH). 40/43 (93%) of ICH were diagnosed by HUS during the first 5d of ECMO. After 5d, there were 3/43 (7%) patients found to have new ICH by HUS. Authors conclude that cost effectiveness is poor for use of HUS after day 5. No mention of other types of brain injury.</td>
</tr>
</tbody>
</table>

Brain CT
<table>
<thead>
<tr>
<th>Source</th>
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</thead>
<tbody>
<tr>
<td>Lidegran, 2002&lt;sup&gt;22&lt;/sup&gt;</td>
<td>Review retrospectively the frequency, indications and findings on CT of patients with ARF on ECMO and to evaluate the risk of complications associated with transportation for CT examinations</td>
<td>Brain CT</td>
<td>Brain CT obtained once during ECMO for suspected complications of ECMO and/or the underlying disease or a delay in clinical improvement</td>
<td>Total of 92 brain CTs in 52/112 (46%) patients. Of the 92 brain CTs, there were 32 (31%) with abnormal findings (13 hemorrages/hemorrhagic infarction, 16 infarction/edema, 2 ceased intracranial circulation, 1 hydrocephalus). Unable to separate brain CT from chest and abdominal CT findings that affected treatment. No complications associated with transport to CT or the CT exam itself. No discussion on timing of CT/timing of injuries.</td>
</tr>
<tr>
<td>Lidegran, 2007&lt;sup&gt;23&lt;/sup&gt;</td>
<td>Evaluate the clinical utility of brain CT in pediatric and adult patients ongoing ECMO for acute respiratory failure and assess the frequency of ICH and infarction during treatment</td>
<td>Brain CT</td>
<td>Brain CT during ECMO as indicated for clinical neurologic symptoms (seizures, focal weakness, pathologic pupillary response, delay in awakening from sedation), 45/78 (58%), or difficult neurologic evaluation in sedated patients at risk for neurologic complications or brain CT complementary to thoracic or abdominal scans, 33/78 (42%)</td>
<td>ICH, infarction or cerebral edema was found in 45/123 (37%) patients (16/45: withdrawn from ECMO, 5/45: weaned off ECMO, 4/45: underwent neurosurgery based on findings, 20/45: continued ECMO with good survival). Authors conclude that intracranial complications are underreported in pediatric and adult ECMO because of low utilization of neuroimaging.</td>
</tr>
<tr>
<td>Jepson, 2010&lt;sup&gt;24&lt;/sup&gt;</td>
<td>Evaluate the benefits and logistical safety of CT imaging in patients undergoing ECMO therapy in a single institution</td>
<td>Brain CT</td>
<td>Retrospective review of brain CT done by the clinical team for new neurologic changes</td>
<td>4 significant findings in 7 adults, 1 in 4 children and 3 in 3 neonates. In all studies, information was provided that helped with management decisions. No major complications and 2 minor complications associated with transport to CT.</td>
</tr>
<tr>
<td>Streletz, 1992&lt;sup&gt;25&lt;/sup&gt;</td>
<td>Determine usefulness of EEG studies for monitoring cerebral function, for confirming seizure activity and for limited prediction of short-term outcome in neonates who required ECMO</td>
<td>EEG&lt;sup&gt;3&lt;/sup&gt;</td>
<td>At least once during ECMO, between 24h-72h after ECMO initiation and within 5 days after ECMO completion</td>
<td>Of 139 newborns with at least one EEG during ECMO, 119 (86%) were abnormal: 19 (14%) mild, 35 (25%) moderate, 65 (47%) marked abnormality. No lateralization found. 11 newborns had electrographic seizures during ECMO. Of these, 7 (64%) died before discharge or had developmental delay at 1y (BSID&lt;-2SD ± cerebral palsy) (p&lt;0.003) compared to patients without EEG seizure</td>
</tr>
<tr>
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<td>Results</td>
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<tr>
<td>Graziani, 1994</td>
<td>Determine the predictive value and the clinical correlates of EEG abnormalities recorded serially before and during VA ECMO</td>
<td>EEG&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Serial EEGs of ≥30m, every 2-4d while on ECMO</td>
<td>61/119 (51) had at least one EEG recording with burst suppression (n=37), electrographic seizure (n=10), or both burst suppression and electrographic seizure (n=14) during ECMO. OR for poor prognosis (death, developmental delay or cerebral palsy): 6.6 (95%CI: 2.2-20.2) when comparing newborns with ≥2 recordings of burst suppression or electrographic seizures vs newborns without EEG abnormalities. No difference in outcome if just a single burst suppression or electrographic seizure reported. No info on timing of abnormal EEGs with changes in neurologic status or with neuroimaging during ECMO.</td>
</tr>
<tr>
<td>Amigoni, 2005</td>
<td>Identify “before ECMO”, “during ECMO” and “after ECMO” predictors of negative long-term (12 months) neurologic outcome</td>
<td>EEG&lt;sup&gt;a&lt;/sup&gt;</td>
<td>NS</td>
<td>The most abnormal EEG during ECMO (p=0.017) was associated with a negative neurologic outcome at 12 months (GOS different from “good recovery” or neurodevelopmental score (BSID, SBS, WPPSI, WISC) &lt;70) (sensitivity=0.75, specificity=0.87)</td>
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<tr>
<td>Trittenwein, 2006</td>
<td>Investigate whether permanent ligation of the right common carotid artery in neonates after ECMO results in permanent changes in electrocortical function.</td>
<td>Quantitative EEG</td>
<td>Retrospective review of routinely recorded digital EEG traces of cases and controls; 20m per study; no data on timing and indication of EEGs.</td>
<td>Dominant frequency values showed no significant differences among ECMO patients during and after ECMO and controls. Power was significantly decreased in all frequency bands during ECMO. EEGs post-decannulation while neonates were still mechanically ventilated showed no differences compared to controls despite permanent right common carotid artery ligation. No clinical/neuroimaging correlates. HUS and EEG prior to discharge were normal in all subjects. Authors conclude that transient changes of quantitative EEG measures during VA ECMO are related to cerebral perfusion changes that seem to recover post-ECMO.</td>
</tr>
<tr>
<td>Piantino, 2013</td>
<td>Determine the frequency of nonconvulsive seizures or nonconvulsive status epilepticus in children on ECMO and to compare mortality and radiologic</td>
<td>Continuous EEG</td>
<td>Continuous EEG was requested by clinicians for clinical events suspicious for seizures (n=7), or for help with management of neuroprotective measures</td>
<td>Background EEG rhythm was abnormal in 18/19 (95%) patients. Seizures were detected in 4/19 (21%) patients, and were exclusively non-convulsive in 3 patients. Non-convulsive status epilepticus was detected in 2/4 patients.</td>
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<tr>
<td>Gannon, 2001&lt;sup&gt;92&lt;/sup&gt;</td>
<td>Determine if the combination of EEG and HUS during ECMO would be more highly correlated than either study alone with brain CT/MRI results obtained after completion of ECMO</td>
<td>HUS</td>
<td>Before-ECMO HUS and daily HUS during ECMO, EEG at least once during ECMO, for at least 30m per study</td>
<td>All patients with seizures had abnormal neuroimaging. 14/18 patients with abnormal EEG recordings had neuroimaging studies done (brain CT/MRI or HUS), of which 13/14 (92%) were abnormal. No data on temporal correlation of abnormal EEGs with brain injury diagnosis by neuroimaging.</td>
</tr>
<tr>
<td>Korinthenberg, 1993&lt;sup&gt;122&lt;/sup&gt;</td>
<td>Report the neurological and EEG findings of newborn infants before, during and after ECMO</td>
<td>HUS EG&lt;sup&gt;a&lt;/sup&gt;</td>
<td>HUS twice daily during ECMO with brain CT if abnormal HUS, EEG at least once during ECMO, for at least 30m per session</td>
<td>40/160 (25%) patients had brain injury by brain CT/MRI. Predictive value for HUS to detect subsequent abnormalities by CT/MRI was: PPV: 56%, NPV: 81%. EEG showed no difference between patients with normal vs abnormal brain CT/MRI. HUS combined with EEG improved PPV to 69% and NPV to 88%. No comment on when the EEGs were done and when HUS abnormalities were found and whether there was temporal correlation. No info on indications for EEG/timing of EEG.</td>
</tr>
<tr>
<td>Graziani, 1989&lt;sup&gt;93&lt;/sup&gt;</td>
<td>Determine whether focal EEG and/or localized HUS abnormalities follow cannulation and permanent ligation of the right common carotid artery and right internal jugular vein</td>
<td>HUS EG&lt;sup&gt;a&lt;/sup&gt;</td>
<td>HUS before, daily during ECMO and post-ECMO, EEG at least once during ECMO and at least once in the 5 days post-decannulation.</td>
<td>20 newborns had abnormal HUS pre-ECMO, 27 had abnormal HUS during ECMO, with increasing severity of lesions from pre- to during to post-ECMO. EEG was abnormal in 37 newborns during ECMO, of these, 3 had seizure activity. Infants with moderate/severe HUS abnormalities during or after ECMO also had at least one EEG with moderate/severe abnormalities. Only 8/14 infants with...</td>
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<tr>
<td>Horan, 2007</td>
<td>Observe aEEG in neonates receiving ECMO and determine whether mild hypothermia influenced the aEEG recordings</td>
<td>aEEG</td>
<td>Continuous recordings in the first 5d of ECMO</td>
<td>aEEG amplitude was stable across the temperature range. Of 26 recordings: 16 (62%) normal throughout, 6 (23%) intermittently moderately abnormal for median 54h (range: 24h-78h), 1 (14%) severely suppressed for 102h. Periods of frequent seizure activity seen in 3 (11%) recordings for median 48h (range: 24h-48h), not associated with clinical manifestations in 2 neonates. aEEG became abnormal 24h before HUS diagnosis of ICH in one newborn. aEEG amplitude decreased immediately after sedative boluses and returned to baseline within 1h. Amplitude not affected by continuous infusions of morphine and midazolam.</td>
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<tr>
<td>Pappas, 2006</td>
<td>Examine the utility and predictive value of aEEG for identifying infants at risk for death or severe neurologic complications (moderate to severe hydrocephaly, ischemia, infarction, or hemorrhage on neuroimaging or autopsy) in neonates on ECMO</td>
<td>aEEG</td>
<td>Recording started before cannulation in 19/20 newborns, and up to 90m on ECMO, then ≥30m of recording within the first 24h±8h on ECMO</td>
<td>No deterioration in background activity from pre-ECMO cannulation aEEG to on-ECMO aEEG (p=-.66). No acute changes in aEEG and no lateralizing effects noted during cannulation of right neck vessels. Severely abnormal aEEG before and/or during ECMO predicted death or moderate to severe intracranial neuropathology with sensitivity=1, specificity=0.75, PPV=0.86, NPV=1. No temporal correlation data between abnormal aEEG and time of abnormal neuroimaging findings.</td>
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<tr>
<td>Taylor, 1987</td>
<td>Monitor changes in intracranial hemodynamics in newborns on ECMO using transcutaneous Doppler US</td>
<td>Transcutaneous Doppler US, pericallosal portion of the ACA</td>
<td>Before ECMO then daily transcutaneous Doppler US during ECMO</td>
<td>Mean PI decreased significantly at the ECMO initiation and over time during ECMO. Marked increase in area under the velocity curve (as a measure of total CBF) at ECMO start was associated with increased partial pressure of CO2, increased MAP and increased ECMO flow rate). Area under the velocity curve tended to decrease during the ECMO course. No data on temporal correlation with clinical neurologic changes or with neuroimaging findings.</td>
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**Amplitude-integrated electroencephalography**

markedly abnormal EEG during ECMO had HUS abnormalities after ECMO (3 mild and 5 moderate/severe).
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<tr>
<td>Van de Bor, 1990</td>
<td>Study cerebral hemodynamics in the pericallosal artery of newborns on ECMO</td>
<td>Doppler US, pericallosal artery</td>
<td>Before ECMO, after every major change in ECMO flow, and post-ECMO</td>
<td>Mean PI decreased sharply at high ECMO flows and gradually increased again when lowering ECMO flows (authors interpret findings as indirect evidence for an increase in CBF that could be responsible for the occurrence of ICH). No data on correlation with clinical suspicion of acute neurologic injury. None of the screening HUS showed ICH.</td>
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<tr>
<td>Taylor, 1992</td>
<td>Describe experience with identification and monitoring of abnormal venous hemodynamics in newborns on ECMO</td>
<td>Doppler US of intracranial venous system</td>
<td>At least two studies during ECMO</td>
<td>The superior sagittal sinus was visualized in all 23 patients, the left and right transverse sinuses were seen in 14 and 8 patients, respectively. Persistently low superior sagittal sinus flow velocity was associated with a significantly higher risk for cerebrovascular injury (defined as parenchymal injury detected by HUS or by brain CT, of greater extent than SHE) (7/9 children with low velocity developed injury vs 2/14 children with normal velocity, p=0.002). Persistent reductions in venous blood flow velocity were seen at least 48h before the development of cerebrovascular injury.</td>
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<td>Lohrer, 1992</td>
<td>Assess ICA blood flow velocities before, during and after VA neonatal ECMO and association of ICA blood flow velocity values with HUS/brain CT reports of brain injury</td>
<td>Pulsed Doppler US of bilateral ICAs to measure blood flow velocities</td>
<td>ICA velocity measurements done before, 7/25, 2.3h±0.6h, during, 24/25, 26.7h±5.4h, and after ECMO, 15/25, 23.5h±8.1h</td>
<td>18/25 neonates had neuroanatomic abnormalities detected during or after ECMO by HUS/brain CT. 7/25 (28%) had major lesions (white matter and/or cortical infarctions (n=6), cerebellar infarction or hemorrhage (n=1)). Blood flow velocities in the ICAs were not associated with neuroanatomic abnormalities. There was no difference in incidence of ischemic and/or hemorrhagic parenchymal lesions (3/12 vs 4/12) between newborns with reverse flow vs forward flow in the right ICA.</td>
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<tr>
<td>Fukuda, 1999</td>
<td>Evaluate cerebral hemodynamics and cardiac function during VA and VV ECMO</td>
<td>HUS (Pulsations obtained as color flow for ACA, both ICAs, BA and both MCAs.)</td>
<td>Before cannulation and 1, 6, 12, and 24h and 2 and 3d after ECMO.</td>
<td>Decreased CBF velocity in ACA, right MCA, left MCA in VA vs VV ECMO. Increased CBF velocity in left ICA and BA in VA vs VV ECMO. In VA ECMO patients: no difference between 7 patients with ICH vs 7 without ICH in velocities in ACA, right ICA, left ICA, but velocity in BA, right MCA and left MCA were lower in 7/14 patients without ICH.</td>
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<td>O'Brien, 2013&lt;sup&gt;109&lt;/sup&gt;</td>
<td>Determine how ECMO affects CBF velocity and to determine whether specific changes in CBF velocity may be associated with neurologic injury</td>
<td>Transcranial Doppler US to measure bilateral MCA CBF velocities</td>
<td>Transcranial Doppler US daily during ECMO</td>
<td>CBF velocity in VA ECMO patients (particularly with ICH) decreased after onset of ECMO in association with poor cardiac function, whereas they were stable in VV ECMO patients (?) due to normal systemic hemodynamics and cardiac function. No mention of timing of ICH and how that was associated with timing of abnormal CBF velocity measurements. Authors inferred that hypoxia prior to ECMO was associated with loss of cerebral autoregulation especially in cases of ICH. Patients who developed ICH had low CBFV associated with disturbed cardiac function. Authors inferred that the decrease in CBF due to myocardial failure can lead to ICH and that mean CBF velocity in the MCA falling successively below 20 cm/s can place infants at high risk for ICH.</td>
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<tr>
<td>Gazzolo, 2002&lt;sup&gt;110&lt;/sup&gt;</td>
<td>Investigate if S100b could be a useful tool in the early detection of ICH during ECMO support</td>
<td>Plasma S100b HUS/Doppler US velocimetry waveform patterns obtained for the MCA as pulsatility index</td>
<td>Plasma S100b daily during ECMO HUS, MCA PI assessed before and daily during ECMO</td>
<td>First HUS ICH detection was on day 5 for 2 cases and day 6 for 6 cases. Plasma S100b was significantly higher in cases, 72h before any sign of ICH was detected by HUS (cases 2.91±0.91 microg/L vs controls 0.53±0.15 microg/L, p&lt;0.05) as well as when peaking at day 6, when HUS were suggestive of ICH (cases: 3.50±1.03 microg/L vs controls: 0.66±0.27 microg/L, p&lt;0.05). MCA PI in cases were significantly higher than controls.</td>
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**Plasma brain injury biomarkers**
## Source

**Bembea, 2011**<sup>11</sup>

Evaluate the association of plasma GFAP levels with acute neurologic injury, neurologic outcomes and survival in pediatric patients undergoing ECMO.

**Carter, 1995**<sup>124</sup>

Examine if cannulation of the rights carotid artery and internal jugular vein altered SEPs recorded from the right hemisphere in patients receiving ECMO therapy.

**Liem, 1992**<sup>116</sup>

Investigate the alteration of cerebral oxygenation and hemodynamics in relation to changes in physiological parameters during induction of ECMO in newborn infants.

**Liem, 1995**<sup>112</sup>

Investigate cerebral oxygenation and hemodynamics in relation to changes in relevant

## Study goals

- **Bembea, 2011**: Evaluate the association of plasma GFAP levels with acute neurologic injury, neurologic outcomes and survival in pediatric patients undergoing ECMO.
- **Carter, 1995**: Examine if cannulation of the rights carotid artery and internal jugular vein altered SEPs recorded from the right hemisphere in patients receiving ECMO therapy.
- **Liem, 1992**: Investigate the alteration of cerebral oxygenation and hemodynamics in relation to changes in physiological parameters during induction of ECMO in newborn infants.
- **Liem, 1995**: Investigate cerebral oxygenation and hemodynamics in relation to changes in relevant

## Neuromonitoring methods

- (PI)
- Plasma brain injury biomarker (GFAP)

## Timing of studies

- 6h, 12h, and every 24h after ECMO cannulation
- Once during ECMO in 9 patients and twice in 2 patients (range: 1d-11d on ECMO)
- NIRS measurements done 30m before cannulation and up to 30m after starting ECMO.

## Results

- but 48-72h after S100b peaked (cases: 2.31±0.22 vs controls: 1.81±0.24, p<0.05)
- There were no significant differences in cases vs controls during ECMO in serial neurologic exams with modified Amiel-Tison criteria. MCA PI increase would signify impaired cerebrovascular resistance.
- 7/22 (32%) patients developed acute neurologic injury. Peak GFAP levels were higher in children with brain injury than those without (median 5.9 vs 0.09 ng/mL, p=0.04). OR for brain injury for GFAP >0.436 ng/mL vs ≤0.436 ng/mL was 11.5 (95%CI: 1.3-98.3).
- 15% of tests showed disagreement between right and left hemispheres. SEPs were normal over the right hemisphere in 9/11 patients. Central conduction times were similar in ECMO patients and not significantly different from positive and negative controls. Neurologic outcome at least 8m after ECMO in 6 patients: 4 normal and had normal SEPs; 2 died and had bilateral absent responses. No data on correlation with neuroimaging findings.
- Immediately after ECMO start, cO2Hb increased (5/5) and chHb decreased (4/5). Total Hb increased despite decrease in Hb concentration due to hemodilution (authors interpreted as increased CBV and, given correlation between CBV and CBF, they infer increased CBF, too, which is speculated to contribute to development of ICH during ECMO).
- Significant decrease in oxyhemoglobin (cO2Hb) and increase in deoxyhemoglobin (chHb) concentration and MAP after carotid artery ligation. Increased MAP but no changes in cerebral oxygenation after jugular vein ligation.
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<td></td>
<td>physiologic variables during induction of ECMO in newborns</td>
<td>Pulsed Doppler US measurements of ICAs and MCAs, done at ~60m before cannulation and ~90m after ECMO initiated.</td>
<td>initiated</td>
<td>ligation. Immediately after ECMO started: increased arterial oxygen saturation (SaO2), transcutaneous partial pressure of oxygen (tcPO2), cO2Hb concentration and MAP, and decreased cHHb concentration, thought to be secondary to hemodilution. At 60m after ECMO initiated: cO2Hb, SaO2, tcPO2 and MAP were significantly higher, and cHHb was lower than precannulation values. At ~90m after ECMO initiated: increased CBF and cerebral mean blood flow velocity, thought to be secondary to reactive hyperperfusion, loss of autoregulation or compensation for hemodilution. Authors conclude that there is increased O2 extraction after carotid ligation, but not after jugular vein ligation. Increased cerebral O2 supply after cannulation is not accompanied by increased cellular O2 availability.</td>
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<tr>
<td>Van Heijst, 2004</td>
<td>Evaluate the effects of ligation of the right common carotid artery and the right internal jugular vein and the initiation of VA ECMO on the left and right cerebral hemisphere perfusion and oxygenation</td>
<td>Cerebral oximetry using two-channel NIRS (Radiometer, Copenhagen, Denmark), probes placed over the left and right parietotemporal regions to measure cO2Hb, cHHb as well as total Hb as surrogate for CBV</td>
<td>NIRS measurements done ~30m pre- and up to ~60min post-cannulation.</td>
<td>Right common carotid artery ligation led to a decrease in cO2Hb and an increase in cHHb. CBV was unchanged. No changes associated with right internal jugular vein ligation. At ~60m on ECMO, cO2Hb and CBV increased (accompanied by increased CBF velocity), and cHHb decreased. No differences noted between the hemispheres. Mean CBF velocity increased in the left ICA and increased equally in the MCAs bilaterally. Reversed flow seen in the right ICA. Three patients with asymmetric brain lesions showed no differences in measurements between the two hemispheres.</td>
</tr>
<tr>
<td>Ejike,</td>
<td>Observe the effects of ligation of the right common carotid artery and the right internal jugular vein and the initiation of VA ECMO on the left and right cerebral hemisphere perfusion and oxygenation</td>
<td>Cerebral oximetry</td>
<td>Right and left frontal NIRS</td>
<td>rSO2i in the right frontal region decreased by 12-25%</td>
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<tr>
<td>2006$^{14}$</td>
<td>right carotid artery ligation and variations in ECLS flow on rSO2i measured using NIRS</td>
<td>using NIRS (rSO2i) (Invos 5100 Cerebral Oximeter, Somanetics)</td>
<td>probes placed before cannulation or within 24h after cannulation, for 72h or until decannulation</td>
<td>from baseline for 17-45 min after ligation of the right carotid artery, then returned toward baseline. No change in left frontal rSO2i during cannulation. Initiation of ECLS: transient rSO2i increase above baseline bilaterally. ECLS flows and periods of “trialing off” ECLS not correlated with changes in rSO2i bilaterally. No mention of ANI during monitoring period or after.</td>
</tr>
<tr>
<td>Fenik, 2009$^{15}$</td>
<td>Determine the direct effects of ligation of the right internal jugular vein and right carotid artery on cerebral oxygenation</td>
<td>Cerebral oximetry using the FORE-SIGHT Cerebral Oximeter (CAS Medical Systems, Branford, CT) (SctO2)</td>
<td>Before, during and after cannulation (to at least 48h after cannulation)</td>
<td>12/17 had low (&lt;60%) SctO2 pre-cannulation, with the lowest SctO2 between cannulation to the onset of ECMO in most. SctO2 increased &gt;60% and remained stable for all after ECMO started. Low SctO2 defined as &lt;60% was based on approximation to SjvO2&lt;50% based on the estimation that SctO2 is 10% higher than SjvO2. No mention of cerebral oximetry and ANI during ECMO (either for diagnosis or for prediction). Not mentioned if any pt had ANI or not.</td>
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<tr>
<td>Papademetriou, 2012$^{17}$</td>
<td>Investigate the role of WCC as a method to study the concordance between multisite cerebral cO2Hb measures and MAP, to assess regional variations in cerebral autoregulation in newborns on ECMO</td>
<td>Cerebral oximetry using multichannel continuous NIRS system (ATG-100, Hitachi Medical Ltd., Japan) to measure changes in cO2Hb, cHHb, and total Hb concentrations</td>
<td>Measurements done during cannulation, decannulation, weaning from ECMO and alterations in ECMO flows</td>
<td>WCC increased with decreasing ECMO flows, indicating loss of cerebral autoregulation at low ECMO flows. WCC were higher on the right, suggesting more susceptibility to disruption of cerebral autoregulation in the right hemisphere. The article does not describe any clinical correlates, and no data given on association of high WCC with abnormalities in neurologic exam or neuroimaging.</td>
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<tr>
<td>Wong, 2012$^{11}$</td>
<td>Present single center experience with cerebral and lower limb NIRS in adults on ECMO</td>
<td>Cerebral oximetry using NIRS (Coviden Somatics, Manfield, MA)</td>
<td>Monitoring throughout the ECMO course</td>
<td>All patients had a significant drop (&lt;40 or &gt;25% from baseline) in bilateral cerebral oximetry tracings resulting in hemodynamic interventions (increasing pressure, oxygenation, and/or ECMO flow). In 16/20 (80%) patients, these interventions corrected underlying ischemia. 4/20 (20%) patients required further diagnostic testing and were found to have a cerebrovascular accident.</td>
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Cerebral blood flow measurement using radionuclide tracer

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<tr>
<td>Stockwell, 1996&lt;sup&gt;125&lt;/sup&gt;</td>
<td>Determine if CBF is symmetric after ICA and ipsilateral internal jugular vein ligation in infants during VA ECMO and determine the cerebral CO2 reactivity of neonates during VA ECMO and its correlation to neurodevelopmental outcomes</td>
<td>CBF (right and left hemispheric) measurements using Xe clearance at 3 different PaCO2 (&lt;34torr, 35-45torr, ≥45torr)</td>
<td>Early measurements (≤12h on ECMO, n=4), late (≥48h on ECMO, n=4) or both (n=6)</td>
<td>Right and left hemisphere CBF significantly correlated with each other during early and late measurements ($r^2=0.91$, $p=0.0001$, 47 studies). CO2 reactivity and CBF were highly variable during ECMO and were not predictive of short-term neurodevelopmental outcome at 3, 6, 12 and/or 24m. n=9 (BSID, standard neurologic exam).</td>
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ACA: anterior cerebral artery; aEEG: amplitude integrated electroencephalography; BA: basilar artery; BSID: Bayley Scales of Infant Development; CBF: cerebral blood flow; CBV: cerebral blood volume; CI: confidence interval; CT: computed tomography; ECMO: extracorporeal membrane oxygenation; EEG: electroencephalography; GFAP: glial fibrillary acidic protein; GOS: Glasgow Outcome Scale; Hb: hemoglobin; HCT: head computed tomography; HUS: head ultrasound; ICA: internal carotid artery; ICH: intracranial hemorrhage; IVH: intraventricular hemorrhage; MAP: mean arterial blood pressure; MCA: middle cerebral artery; MRI: magnetic resonance imaging; NIRS: near infrared spectroscopy; NPV: negative predictive value; NS: not specified; PPV: positive predictive value; PI: pulsatility index; OR: odds ratio; rSO2i: regional cerebral oxygenation index; SctO2: cerebral tissue oxygen saturation; SjvO2: brain venous oxygen saturation measured as a cephalad catheter internal jugular oxygen saturation; SEH: subependymal hemorrhage; SEP: somatosensory evoked potentials; SBS: Stanford-Binet Scale; WCC: wavelet cross-correlation; WPSSI: Wechsler Preschool and Primary Scale of Intelligence; WISCR: Wechsler Intelligence Scale for Children-Revised; <sup>a</sup>aEEGs interpreted as normal, mildly, moderately, or markedly abnormal based on the Tharp and Laboyrie’s criteria for neonatal patients and Tasker et al’s criteria for children.<sup>87, 88</sup>
Chapter Five

Glial fibrillary acidic protein as a brain injury biomarker in children undergoing extracorporeal membrane oxygenation

Melania M. Bembea, MD, MPH\textsuperscript{1,2}
William Savage, MD, PhD\textsuperscript{2,3}
John J. Strouse, MD, PhD\textsuperscript{2}
Jamie McElrath Schwartz, MD\textsuperscript{1,2}
Ernest Graham, MD\textsuperscript{4}
Carol B. Thompson, MBA, MS\textsuperscript{5}
Allen Everett, MD\textsuperscript{2}

\textsuperscript{1}Department of Anesthesiology and Critical Care, \textsuperscript{2}Department of Pediatrics, \\
\textsuperscript{3}Department of Pathology, and \textsuperscript{4}Department of Gynecology and Obstetrics, Johns \\
Hopkins University, Baltimore, MD \\
\textsuperscript{5}Department of Biostatistics, Johns Hopkins Bloomberg School of Public Health, \\
Baltimore, MD

Adapted from: Bembea MM, Savage W, Strouse JJ, Schwartz JM, Graham E, 
Thompson CB, Everett A. Glial fibrillary acidic protein as a brain injury biomarker in 
Introduction

Extracorporeal membrane oxygenation (ECMO) is used in over 1,000 children each year for severe cardiopulmonary failure.² Survival varies by age and underlying diagnosis and ranges from 32%-42% for extracorporeal cardiopulmonary resuscitation (ECPR)², 14, 15 to 50%-65% in children excluding ECPR,², 16-20 and as high as 75%-80% for selected neonatal populations.², 16, 21 ECMO is associated with high risk for neurologic injury, including intracranial hemorrhage, brain infarction and brain death.², 3, 126 Major causes for neurologic injury are thought to be related to disturbances of cerebral blood flow (CBF) at the time of right neck vessel cannulation,¹²⁷, ¹²⁸ altered cerebral autoregulation during the recovery phase from hypoxia¹⁰¹ and during venoarterial (VA) ECMO non-pulsatile flow,¹²⁹ profound hypoxia and shock encountered by the critically-ill patient around the time of cannulation and exposure to systemic heparinization. Poor neurologic outcomes have been reported in 10% to as many as 60% of survivors,⁷, ⁹, ¹⁰, ¹⁸, ²⁶, ¹³⁰-¹³⁶ and brain death is declared in 1%-11% of newborns and children who undergo ECMO for a variety of indications.², ¹⁶, ²², ²³ Several predictors of neurologic outcome post-ECMO have been proposed, including neuroimaging findings,³, ²⁶, ²⁸ seizure activity during the ECMO course,³⁰ abnormal electroencephalograms during ECMO,²⁶, ³¹, ³² and somatosensory evoked potentials.²⁶ To our knowledge, there is a single prior study that examined the association between a biochemical marker of brain injury, S100-B, and development of intracranial hemorrhage in infants who required ECMO after open-heart surgery.¹³⁷ S100-B, though, is not specific to the brain and has been shown to be elevated in shock and after cardiopulmonary by-pass due to extracerebral sources.¹³⁸-¹⁴⁰ We hypothesized that circulating glial fibrillary acidic protein (GFAP), a brain-specific biochemical marker, is associated with brain injury and neurologic and survival outcomes of children post-ECMO.
GFAP is a class III intermediate filament protein that is specific to astrocytes and is upregulated in reactive gliosis after central nervous system injury. As a blood biomarker, GFAP was first evaluated to diagnose acute stroke in adults, and it has since been applied to other clinical scenarios that carry a high risk for brain injury. Alone or in combination with other brain injury biomarkers, GFAP has been shown to be a predictor for neurologic injury and outcomes after acute stroke, cardiac arrest, and traumatic brain injury.

As the quality of neurologic exams is limited during pediatric ECMO by sedation, and serial brain imaging is only practical for those children who are candidates for head ultrasound, a simple, fast, blood biomarker of brain injury would be immediately useful to clinicians for monitoring neurologic status in children on ECMO. In this study, we evaluated the association of plasma GFAP levels with acute neurologic injury, neurologic outcome, and survival in pediatric patients undergoing ECMO.

Methods

Study Design. This is a prospective observational cohort study of children who underwent ECMO in a 26-bed Pediatric Intensive Care Unit (PICU) of a single tertiary care, academic pediatric center, from April 2008 to August 2009. Patients <18 years who required ECMO for any indication were eligible for this study. This cohort was initiated for the study of coagulation-related risk factors for neurologic injury during ECMO, with a secondary aim to investigate brain injury biomarkers in this population. Exclusion criteria were: history of heparin induced thrombocytopenia and use of direct thrombin inhibitors for anticoagulation during ECMO. Parents or legal guardians were approached for consent after patient stabilization, within the first 6 hours after ECMO cannulation, only when present in the PICU. No consent was conducted over the phone. Demographic,
clinical, laboratory, imaging and survival data were collected for each enrolled subject. The ECMO circuit consisted of: custom-packed 1/4 or 3/8-inch flexible polyvinylchloride tubing (Medtronic, Minneapolis, MN) with a silicone reservoir, a bladderbox (Johns Hopkins Hospital, Baltimore, MD), a 0.8 m² – 4.5 m² membrane oxygenator (Medtronic, Minneapolis, MN), a heat exchanger (Medtronic, Minneapolis, MN) and a roller pump (Sorin Cardiovascular U.S.A., Arvada, CO). This study was approved by the Johns Hopkins Institutional Review Board.

**Biomarker sampling and analysis.** Venous blood samples (5mL in sodium citrate 3.2%) were collected at 6, 12, and 24 hours after initiation of ECMO and then daily until ECMO discontinuation. After separation by centrifugation within one hour, platelet-poor plasma was stored at -80°C. Fifty microliters were used for GFAP measurements in undiluted duplicate plasma samples using an electrochemiluminescent sandwich immunoassay developed on the MesoScale Discovery platform (MesoScale Discovery, Gaithersburg, MD) at Johns Hopkins University and based on the assay of Petzold et al. The monoclonal anti-GFAP blend SMI-26 (Covance, Princeton, NJ) was used at 100 ng per well as capture antibody in standard bind plates (MesoScale Discovery) coated either by the manufacturer or in our laboratory with overnight incubation in phosphate buffered saline (PBS). Polyclonal anti-GFAP (Dako, Carpinteria, CA) that was directly conjugated with Sulfo-Tag (MesoScale Discovery) was used for detection at 1 µg/mL in PBS. Plates were read with a Sector Imager 2400 (MesoScale Discovery). Standard curves were constructed with purified GFAP (Calbiochem, La Jolla, CA) in 1% bovine serum albumin (BSA) (SeraCare Life Sciences, Milford, MA). After experiments to determine the optimal antibody concentrations, plate type, and blocking material, the final assay for GFAP values had a standard curve with a linear range of quantification from 0.040-40.0 ng/mL. Our GFAP assay had a lower limit of detection of 0.011 ng/mL.
as defined by two standard deviations above the background of blank wells (n= 19 experiments). Values <0.040 ng/mL were reported as 0. The signal to noise ratio was 1.17 at 0.01 ng/mL (n= 19 experiments). The lower limit of quantification, defined as the lowest dilution with a calculated concentration ±20% of a known concentration, was 0.040 ng/mL (n= 3 experiments). Inter-assay precision was 2.4% at 10 ng/mL and 3.4% at 0.156 ng/mL (n= 21 experiments). Plasma spiked with GFAP shows 49.8%±22.9% recovery at 10 ng/mL, when compared to a standard curve generated in BSA. Validation of the GFAP assay was conducted using discarded diagnostic specimens from normal and positive controls. This GFAP assay validation study was approved in a separate application by the Johns Hopkins Institutional Review Board with a waiver of consent.

**Outcome Measures.** The primary independent variable was plasma GFAP elevation above the 95th percentile of normal values in children. The primary outcome was development of acute neurologic injury during ECMO, defined as intracranial hemorrhage, brain infarction or cerebral edema diagnosed by brain imaging and/or neurologic examination by a pediatric neurologist consistent with brain death, while the patient was on ECMO support. It is our institutional protocol to obtain daily head ultrasounds for newborns and infants with open fontanelles; older children had brain computed tomography or magnetic resonance imaging (MRI) studies obtained at the discretion of their physicians. All imaging studies were reviewed by pediatric radiologists as part of routine clinical care. The secondary outcomes were neurologic outcome and survival to discharge from hospital. Neurologic outcome was measured using the pediatric cerebral performance category (PCPC) (48, 49). The PCPC is a six-point scale developed from the Glasgow Outcome Scale to assess changes in cognitive abilities in pediatric intensive care. The six PCPC categories are 1: normal, age-appropriate neurodevelopmental functioning; 2: mild cerebral disability, 3: moderate cerebral
disability; 4: severe cerebral disability; 5: coma or vegetative state and 6: brain death.\textsuperscript{153, 154} A trained pediatric critical care physician assigned PCPC retrospectively by conducting a chart review of the patient condition at admission to the hospital and at discharge from hospital. Good neurologic outcome was defined \textit{a priori} as PCPC of 1 or 2 at discharge from hospital, or no change from PCPC at hospital admission.

**Statistical Analysis**

Exploratory descriptive data analysis was conducted to examine patient and ECMO course characteristics, to describe the distribution of GFAP values among subjects and to determine the proportion of subjects in whom GFAP levels were above the 95\textsuperscript{th} percentile. The Kruskal-Wallis test was used to compare GFAP concentrations across age categories in normal controls.

Patients were divided into two categories for each outcome: those with and without acute neurologic injury, good versus poor neurologic outcomes at hospital discharge, and survivors versus non-survivors at hospital discharge. The Mann-Whitney U test was used to compare the median peak GFAP between these groups. Fisher's exact test was used to compare percent of cases above and below 95\textsuperscript{th} percentile of peak GFAP between the groups.

Logistic regression with clustering by patient was used to estimate odds of brain injury and death using all serial GFAP data points. For the clustered analysis, subjects were coded as having no acute neurologic injury until brain injury by imaging or a first neurologic exam consistent with brain death was observed. Subsequent observations were coded as having acute neurologic injury. The odds ratio (OR) and 95\% confidence intervals (CIs) are provided. A p-value of 0.05 was considered significant. Statistical analysis was conducted using STATA 10.0 (StataCorp, College Station, TX, 2007).
Results

Patient Characteristics. Twenty two of 46 eligible patients were enrolled; 18 patients’ parents were not present in the PICU or we were not able to conduct a full consent discussion during the 6-hour consent window, 5 parents declined consent and one patient had no samples available for testing. Demographic and clinical characteristics of the 22 evaluated patients are presented in Table 8.

Individual patient characteristics and outcomes are presented in Table 9. Seven of 22 patients (31.8%) suffered an acute neurologic injury during ECMO based on our a priori definition. Using baseline and discharge PCPC, 14/22 (63.6%) patients had good neurologic outcome and 8/22 (36.4%) patients had poor neurologic outcome: one had a PCPC >2 and 7 died. Causes of death included: brain death (1 patient), large intracranial hemorrhage (2 patients), and withdrawal of mechanical support for medical futility in face of irreversible multisystem organ failure (4 patients).

The median duration of ECMO was 12 days (IQR: 5 days-17 days). The median number of GFAP measurements per patient was 4.5 (range: 1-16). Seventeen patients had ≥3 measurements, three patients had two measurements and two patients had only one measurement.

GFAP Results: Controls. In normal pediatric controls with no known neurologic injury, GFAP levels had a median of 0.055 ng/mL (IQR: 0-0.092 ng/mL). GFAP levels were similar across age categories (newborns: median 0.041 ng/mL [IQR: 0-0.096 ng/mL], 6 months – 4 years: median 0.046 ng/mL [IQR: 0-0.117 ng/mL], 5 years – 16 years: median 0.057 ng/mL [IQR: 0-0.088 ng/mL], p-value=0.7). A 95th percentile cutoff for normal values in children (plasma GFAP ≤0.436 ng/mL) was determined using samples from 158 infants and children: 59 newborns <4 days of life in the neonatal
intensive care unit without known genetic disorders or intracranial pathology and 99 healthy children 6 months to 16 years of age who presented to the Johns Hopkins pediatric outpatient clinic for well-child visits. We further validated our assay for the detection of neurologic injury in patients with brain tumor resection (n=13), brain biopsy (n=3) and stroke (n=12). GFAP levels in positive controls samples were overall 1-55 fold higher than for normal controls.

**GFAP Results: ECMO Patients.** The median initial GFAP level within 12 hours after starting ECMO was 0.07 ng/mL (IQR: 0 - 0.155 ng/mL). There were 3/22 patients with abnormal GFAP concentrations in the first 24 hours after cannulation – two were patients who suffered cardiac arrest and underwent ECPR with VA cannulation of right neck vessels and one was a patient without known neurologic injury who was placed on venovenous (VV) ECMO via a double-lumen right jugular vein cannula. All other 19/22 patients had low GFAP levels in the first 24 hours after cannulation. The median GFAP level on the last ECMO day for those children with ≥3 samples was 0.07 ng/mL (IQR: 0.053 - 0.795 ng/mL; n=17). Peak GFAP concentrations were similar comparing newborns with children and infants >30 days (0.155 ng/mL vs 0.162 ng/mL, respectively, p-value=0.48).

Median peak GFAP levels were significantly higher in children with acute neurologic injury diagnosed during the ECMO course than those without (5.9 vs 0.09ng/mL, p=0.04) (Figure 4), in children with poor vs good neurologic outcome (3.6 ng/mL vs 0.09 ng/mL, p<0.01), and in non-survivors compared to survivors to hospital discharge (5.9 ng/mL vs 0.09 ng/mL, p=0.04). Serial GFAP concentrations in patients with and without acute neurologic injury are displayed in Figure 5a and Figure 5b.
Peak plasma GFAP concentrations >95th percentile for normal controls (i.e., >0.436 ng/mL) were noted in 6/22 (27.3%) patients. The proportion of patients with acute neurologic injury was higher in patients with peak GFAP >0.436 ng/mL than in those with peak GFAP ≤0.436 ng/mL (4/6, 66.7% vs 3/16, 18.8%; p=0.054). Poor neurologic outcome was seen more frequently in patients with peak GFAP>0.436 ng/mL than in those with normal GFAP measurements (5/6, 83.3% vs 3/16, 18.8%; p<0.01). Similarly, hospital mortality was higher in patients with peak GFAP>0.436 ng/mL than in those with normal GFAP measurements (4/6, 66.7% vs 3/16, 18.8%, p=0.054).

To account for repeated measures per patient, we evaluated the association of all 126 serial GFAP levels with the outcomes using logistic regression clustered by patient. The odds of acute neurologic injury given elevated GFAP (>0.436ng/ml) were 11.5 (95%CI: 1.3-98.3). Similar statistically significant results were found for poor neurologic outcome (OR: 25.7, 95%CI: 2.2-298.5) and hospital mortality (OR: 13.6, 95%CI: 1.7-108.5).

After adjusting for neonatal status (≤30 days), the odds of acute neurologic injury remained significantly higher in patients with abnormally elevated plasma GFAP compared to patients with normal GFAP (adjusted OR, 15.7, 95% CI, 1.8-139.9). In the subgroup of 17 newborns and infants who had daily head ultrasounds performed throughout the duration of ECMO, the unadjusted OR for acute neurologic injury was 22.3 (95%CI: 2.0-245.9).

Although exploratory, this analysis yields an area under receiver operating characteristic curve (AUC) in an acceptable range for acute neurologic injury (AUC, 0.72, 95%CI, 0.50-0.94), poor neurologic outcome (AUC, 0.78, 95%CI, 0.58-0.97), and death (AUC, 0.72, 95%CI, 0.50-0.94).
Elevations in GFAP correlated temporally with the imaging diagnosis of brain injury during ECMO. Abnormal GFAP levels (>0.436 ng/mL) were observed 1-2 days prior to the imaging diagnosis of severe acute neurologic injury or brain death in 2/4 patients. GFAP levels remained normal in three patients with acute neurologic injury diagnosed by head ultrasound during ECMO, including a patient with a small subdural hematoma and good neurologic function at discharge (PCPC=1), a patient with grade I intraventricular hemorrhage and good neurologic function at discharge (PCPC=1) and a patient with a small right cerebellar hemorrhage who developed multisystem organ failure and ultimately expired. (Table 9) While we can speculate that in the first two patients we found no elevations in GFAP as the lesions were minor and extraparenchymal in location, we do not have a good explanation for a lack in GFAP “response” to a cerebellar intraparenchymal hemorrhage in the third patient. Of note, cause of death in this latter patient was not related to neurologic injury but rather to multisystem organ failure and withdrawal of support due to medical futility.

There were two patients without a diagnosis of acute neurologic injury during ECMO but with peak GFAP >95th percentile on the first and the 10th day of ECMO, respectively (Table 9). These were newborns with normal daily head ultrasounds throughout the ECMO course. However, at 6 weeks and 2 weeks after ECMO decannulation, respectively, one patient was found to have small old intraventricular and intraparenchymal hemorrhagic foci and the other had findings of unilateral unilobar focal encephalomalacia consistent with prior ischemic event on brain MRI.

This initial cohort of ECMO patients included three patients who underwent extracorporeal cardiopulmonary resuscitation (ECPR): one survived with good neurologic outcome and had normal GFAP levels throughout the ECMO course (median GFAP, 0.07 ng/mL, IQR: 0.05-0.09 ng/mL); two patients had poor outcomes: one
suffered a hypoxic pulseless electrical activity cardiac arrest due to status asthmaticus and evolved to brain death (median GFAP, 27.2 ng/mL, IQR: 9.5-44.9 ng/mL) and one patient developed severe cerebral edema, was successfully decannulated from ECMO, but eventually support was withdrawn for multisystem organ failure and medical futility (median GFAP: 5.8 ng/mL, IQR, 2.8-10.5 ng/mL).

Discussion

ECMO is a procedure with high risk for brain injury, including intracranial hemorrhage, brain infarction and brain death. The means for timely assessment of such injuries in patients on ECMO are often lacking. While acute neurologic insult is of great concern in critically-ill patients, no brain injury biomarker is available yet for routine clinical practice, although many coordinated efforts are ongoing. The plasma GFAP biomarker used in this study has many advantages, such as: high specificity to brain, easy to obtain samples for small blood volumes, fast processing, precise quantification, low cost and minimal technical expertise required for the assay. Serial GFAP measurements could thus be used to monitor neurologic status and response to potential neuroprotective interventions, aid in the prompt diagnosis of acute brain injury and predict outcomes.

This study demonstrates that plasma concentrations of GFAP are associated with brain injury in children on ECMO. Serial GFAP concentrations appeared stable over time in the absence of neurologic insults and were significantly elevated in patients who were diagnosed with brain injury during ECMO. The majority of patients (19/22) had normal GFAP levels in the first 24 hours after ECMO cannulation, suggesting that cannulation of right jugular vein +/- the right carotid artery is not accompanied by injury leading to reactive gliosis and GFAP elevations. GFAP concentrations were elevated in
four patients prior to a diagnosis of brain injury: two patients were diagnosed with acute neurologic injury while on ECMO and two patients had imaging evidence of prior brain ischemia or hemorrhage after ECMO decannulation. This may be particularly important for infants with intraparenchymal lesions that cannot be detected by transfontanellar sonography, thus providing false reassurance to clinicians. While there is evidence that serum GFAP concentrations can discriminate between ischemic and hemorrhagic stroke in adults within specific time windows after onset of symptoms\textsuperscript{144, 156, 157} and volume of lesions,\textsuperscript{143} in our small cohort, we could not determine if plasma GFAP could discriminate among different types of brain injury (e.g., hemorrhagic vs ischemic, local vs global). Future, larger studies of patients undergoing ECMO with standardized imaging protocols and serial blood biomarker measurements are needed to address these important questions.

Acute neurologic injury is found more frequently in ECPR patients compared to ECMO for other indications.\textsuperscript{2, 23} Recent studies report 73\% survival to hospital discharge in pediatric ECPR patients,\textsuperscript{158} with 75\%-78\% of survivors having favorable neurologic outcomes.\textsuperscript{23, 158} Acute neurologic injury occurs in 22\% of pediatric ECPR patients; of these, 89\% die prior to hospital discharge.\textsuperscript{23} In our study, we found normal serial GFAP levels in one patient who underwent ECPR and survived with good neurologic outcome. In contrast, two children who underwent ECPR and subsequently developed severe hypoxic brain injury, had plasma GFAP levels 20 to 100 times higher than the 95\textsuperscript{th} percentile for normal children. To our knowledge, this is the first report of plasma GFAP as a potential predictive biomarker for ECPR: two prior studies of GFAP after cardiac arrest excluded patients who underwent ECPR.\textsuperscript{145, 146} However, these data are very preliminary and no further inferences can be made at this time.
This study has several limitations. Neurologic assessment and brain imaging were not standardized. According to our *a priori* definitions, for purposes of analysis, we assumed that no child developed brain injury until demonstrated by routine brain imaging or a first neurologic examination consistent with brain death. Thus, it is possible that neurologic injury is reflected in GFAP levels prior to imaging or clinical diagnosis and that the sensitivity of GFAP is underestimated. Also, the variable timing of GFAP measurements and diagnostic studies may have limited the ability to assess sensitivity. We used as cutoff plasma GFAP concentration of 0.436 ng/mL, the 95th percentile for plasma GFAP measured in newborns, infants and children free of neurologic injury. This is higher than the cutoff used in most adult studies 0.1-0.3 ng/mL,145, 148-150 which may have led to underestimation of sensitivity. Further, it is not known if the dilution of endogenous protein concentrations that occurs by adding the volume of the ECMO circuit to the patient’s blood volume may underestimate the sensitivity of this biomarker. Finally, this preliminary analysis shows that GFAP may be a predictor of mortality or poor neurologic outcome defined by PCPC; however, only limited adjustment for potential confounding factors was possible with this small cohort and OR estimates are imprecise as reflected by the wide CIs. Missing data points for GFAP levels may make our analysis less accurate.

More detailed study is needed to assess the performance of GFAP as a biomarker of acute neurologic injury during ECMO. Standardized imaging and neurologic exams would need to be coordinated with sample collection to more precisely establish the temporal relationship between elevations of GFAP and brain injury. The rigorous demonstration of this temporal relationship would advance GFAP as an adjunct to brain imaging for the surveillance of new or ongoing acute neurologic injury in ECMO patients.
In situations where brain imaging is difficult, serial GFAP testing could be used as a screening test to triage patients with elevated values to imaging.

Conclusions

This series of 22 pediatric patients who underwent ECMO for various indications in a single institution provides preliminary data to support the use of plasma GFAP, a brain-specific protein, to detect acute neurologic injury in this high risk population. Studies specifically designed to assess the diagnostic performance of single or multi-panel blood biomarkers are needed to further investigate the utility of brain-specific biomarkers for acute and ongoing brain injury in children and also as a monitoring tool for neuroprotective therapies employed after occurrence of a neurologic insult.
<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Patient</th>
<th>ECMO Course</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, median (range)</td>
<td>10d (2d-16y)</td>
<td>Primary indication for ECMO, n(%)</td>
</tr>
<tr>
<td>Male, n(%)</td>
<td>12 (54.5)</td>
<td>Cardiac failure</td>
</tr>
<tr>
<td>Race, n(%)</td>
<td></td>
<td>Respiratory failure</td>
</tr>
<tr>
<td>African American</td>
<td>12 (54.5)</td>
<td>Cardiac arrest/ECPR</td>
</tr>
<tr>
<td>Caucasian</td>
<td>7 (31.8)</td>
<td>Sepsis</td>
</tr>
<tr>
<td>Other</td>
<td>3 (13.6)</td>
<td>Mode of extracorporeal support, n(%)a</td>
</tr>
<tr>
<td>Hispanic, n(%)</td>
<td>2 (9.1)</td>
<td>VA-ECMO</td>
</tr>
<tr>
<td>Weight (kg), median (IQR)</td>
<td>3.8 (3.1, 6.9)</td>
<td>VV-ECMO</td>
</tr>
<tr>
<td>Illness category, n(%)</td>
<td></td>
<td>VV to VA-ECMO</td>
</tr>
<tr>
<td>Medical cardiac</td>
<td>5 (22.7)</td>
<td>Complications on ECMO, n(%)</td>
</tr>
<tr>
<td>Medical noncardiac</td>
<td>14 (63.6)</td>
<td>Acute neurologic injury</td>
</tr>
<tr>
<td>Surgical cardiac</td>
<td>1 (4.6)</td>
<td>Intracranial hemorrhage</td>
</tr>
<tr>
<td>Surgical noncardiac</td>
<td>2 (9.1)</td>
<td>Severe cerebral edema</td>
</tr>
<tr>
<td>PICU length of stay, median (IQR)</td>
<td>15d (8d-23d)</td>
<td>Brain death</td>
</tr>
<tr>
<td>Hospital length of stay, median (IQR)</td>
<td>30d (19d-53d)</td>
<td>Pulmonary hemorrhage</td>
</tr>
<tr>
<td>Mortality prior to PICU discharge, n(%)</td>
<td>7 (31.8)</td>
<td>Renal replacement therapy, n(%)</td>
</tr>
<tr>
<td>ECMO to ANI diagnosis, median (IQR)</td>
<td>82h (69h-104h)</td>
<td>ECMO duration, median (IQR)</td>
</tr>
</tbody>
</table>

ECMO: extracorporeal membrane oxygenation; IQR: interquartile range; VA: veno-arterial; VV: veno-venous; PICU: pediatric intensive care unit; ANI: acute neurologic injury; d: days; h: hours; y: years. aAll ECMO cannulations were performed via the right jugular vein and right carotid artery for VA-ECMO and via the right jugular vein using a double lumen cannula for VV-ECMO. None of the patients enrolled in this study underwent direct intracardiac cannulation.
Table 9. Patient, ECMO and neurologic outcomes characteristics

<table>
<thead>
<tr>
<th>Patient</th>
<th>Primary Diagnosis</th>
<th>ECMO Indication</th>
<th>ECMO Duration</th>
<th>#GFAP Measurements</th>
<th>Peak GFAP</th>
<th>ANI Diagnosed during ECMO</th>
<th>ANI Diagnostic Modality</th>
<th>ECMO to ANI Diagnosis</th>
<th>ECMO to GFAP &gt;95th percentile</th>
<th>Baseline PCPC</th>
<th>Discharge PCPC</th>
<th>Neurologic Outcome</th>
<th>PICU Survival</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>Liver failure, CPA post-transplant</td>
<td>Cardiac disease</td>
<td>4d</td>
<td>5</td>
<td>5.86</td>
<td>Unilateral IVH, intraparenchymal (basal ganglia, right temporal and occipital) hemorrhage, 2 cm midline shift, edema</td>
<td>HCT</td>
<td>104h</td>
<td>24h</td>
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<td>6</td>
<td>Poor</td>
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<td>Dilated cardiomyopathy</td>
<td>Cardiac disease</td>
<td>3d</td>
<td>4</td>
<td>20.5</td>
<td>Frontoparietal 4x5x4cm intraparenchymal hemorrhage, 2.5 cm midline shift, unilateral SDH, SAH, IVH</td>
<td>HUS</td>
<td>11h</td>
<td>19h</td>
<td>2</td>
<td>6</td>
<td>Poor</td>
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<td>Cardiac disease</td>
<td>5d</td>
<td>3</td>
<td>0.053</td>
<td>No</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>1</td>
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<tr>
<td>4</td>
<td>PPHN</td>
<td>Respiratory disease</td>
<td>6d</td>
<td>4</td>
<td>0.164</td>
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<td>-</td>
<td>-</td>
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<tr>
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<td>Septic shock</td>
<td>Sepsis</td>
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<td>0°</td>
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<td>-</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>6</td>
<td>Poor</td>
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<tr>
<td>6</td>
<td>PPHN</td>
<td>Respiratory disease</td>
<td>5d</td>
<td>8</td>
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<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>7</td>
<td>PPHN</td>
<td>Respiratory disease</td>
<td>4d</td>
<td>5</td>
<td>0.081</td>
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<td>-</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>1</td>
<td>Good</td>
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<tr>
<td>8</td>
<td>Dilated cardiomyopathy</td>
<td>Cardiac disease</td>
<td>1d</td>
<td>2</td>
<td>0.162</td>
<td>No</td>
<td>-</td>
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<tr>
<td>9</td>
<td>PPHN</td>
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<td>-</td>
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<tr>
<td>10</td>
<td>Critical aortic stenosis</td>
<td>Cardiac disease</td>
<td>12d</td>
<td>6</td>
<td>0.194</td>
<td>No</td>
<td>-</td>
<td>-</td>
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<td>1</td>
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<td>11</td>
<td>PPHN</td>
<td>Respiratory disease</td>
<td>30d</td>
<td>16</td>
<td>1.43</td>
<td>No</td>
<td>-</td>
<td>-</td>
<td>12h$^c$</td>
<td>1</td>
<td>3</td>
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</tr>
<tr>
<td>12</td>
<td>Diaphragmatic eventration</td>
<td>Respiratory disease</td>
<td>14d</td>
<td>11</td>
<td>0.398</td>
<td>Unilateral cerebellar hemisphere, 1.5x 2cm intraparenchymal hemorrhage</td>
<td>HUS</td>
<td>82h</td>
<td>-</td>
<td>1</td>
<td>6</td>
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<td>ECPR</td>
<td>4d</td>
<td>3</td>
<td>0.090</td>
<td>No</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>2</td>
<td>Good</td>
<td>Yes</td>
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<tr>
<td>14</td>
<td>Status asthmaticus</td>
<td>ECPR</td>
<td>4d</td>
<td>2</td>
<td>44.9</td>
<td>Rapid progression to brain death</td>
<td>BD exam</td>
<td>64h</td>
<td>12h</td>
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<td>2</td>
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<tr>
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<td>12d</td>
<td>3</td>
<td>0.795</td>
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<td>15d</td>
<td>11</td>
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<td>Small SDH along the falx cerebri</td>
<td>HUS</td>
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<td>4d</td>
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<td>Patient</td>
<td>Primary Diagnosis</td>
<td>ECMO Indication</td>
<td>ECMO Duration</td>
<td>#GFAP Measurements</td>
<td>Peak GFAP</td>
<td>ANI Diagnosed during ECMO</td>
<td>ANI Diagnostic Modality</td>
<td>ECMO to ANI Diagnosis</td>
<td>ECMO to GFAP &gt;95th percentile</td>
<td>Baseline PCPC</td>
<td>Discharge PCPC</td>
<td>Neurologic Outcome</td>
<td>PICU Survival</td>
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<tr>
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<td>Respiratory disease</td>
<td>18d</td>
<td>13</td>
<td>0.400</td>
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<td>-</td>
<td>-</td>
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<tr>
<td>19 Truncus arteriosus</td>
<td>Cardiac disease</td>
<td>4d</td>
<td>2</td>
<td>0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>No</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>1</td>
<td>Good</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>20 PPHN</td>
<td>Respiratory disease</td>
<td>3d</td>
<td>1</td>
<td>0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Unilateral subependymal grade I IVH</td>
<td>HUS</td>
<td>69h</td>
<td>-</td>
<td>1</td>
<td>1</td>
<td>Good</td>
<td>Yes</td>
<td></td>
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<tr>
<td>21 CDH</td>
<td>Respiratory disease</td>
<td>17d</td>
<td>8</td>
<td>0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>No</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>1</td>
<td>Good</td>
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<td></td>
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<tr>
<td>22 Neonatal enterovirus sepsis</td>
<td>ECPR</td>
<td>8d</td>
<td>8</td>
<td>14.6</td>
<td>Severe diffuse cerebral edema</td>
<td>HUS</td>
<td>2h</td>
<td>6h</td>
<td>3</td>
<td>6</td>
<td>Poor</td>
<td>No</td>
<td></td>
</tr>
</tbody>
</table>

ECMO: extracorporeal membrane oxygenation, ANI: acute neurologic injury, GFAP: glial fibrillary acidic protein, PCPC: Pediatric Cerebral Performance Category, PICU: pediatric intensive care unit, CPA: cardiopulmonary arrest, IVH: intraventricular hemorrhage, SDH: subdural hemorrhage, SAH: subarachnoid hemorrhage, HCT: head computed tomography, HUS: head ultrasound, PPHN: persistent pulmonary hypertension of the newborn, TOF: tetralogy of Fallot, ECPR: extracorporeal cardiopulmonary resuscitation, CDH: congenital diaphragmatic hernia. <sup>a</sup>Denotes values below the lower limit of quantification (LLQ=0.040 ng/mL); <sup>b</sup>Normal serial HUS during ECMO. Brain MRI 6 weeks post-ECMO decannulation: few old intraventricular and intraparenchymal hemorrhagic foci; <sup>c</sup>Normal serial HUS during ECMO. Brain MRI 2 weeks post-ECMO decannulation: unilateral unilobar focal encephalomalacia consistent with prior ischemic event.
Figure 4. Peak plasma GFAP concentrations in children on ECMO with and without acute neurologic injury (n=22)

GFAP: glial fibrillary acidic protein; ECMO: extracorporeal membrane oxygenation. Please note logarithmic scale on Y axis.
Figure 5A. Serial plasma GFAP concentrations in children on ECMO with acute neurologic injury (n=7).

X represents death in the pediatric intensive care unit; open squares represent the time of diagnosis of acute neurologic injury closest in time (within 24 hours) to the last GFAP measurement; dashed line marks the 95th percentile of normal controls. Note only 6 open squares – one patient had a diagnostic head ultrasound 48 hours after the only GFAP measurement (GFAP measurement 24 hours prior to diagnosis is missing). Please note different scales in Figures 5A and 5B.
Figure 5B. Serial plasma GFAP concentrations in children on ECMO without acute neurologic injury (n=15).

No Acute Neurologic Injury

X represents death in the pediatric intensive care unit; dashed line marks the 95th percentile of normal controls. Please note different scales in Figures 5A and 5B.
Chapter Six

Use of a multiplex plasma brain-specific protein assay for diagnosis of acute brain injury during ECMO

Melania M. Bembea, MD, MPH
Nicole Rizkalla, MD
Allen Everett, MD
James Freedy
Gregory Mueller, PhD

Department of Anesthesiology and Critical Care Medicine and Department of Pediatrics, Johns Hopkins University, Baltimore, MD
Department of Physiology, Uniformed Services University of the Health Sciences, Bethesda, MD
Introduction

Extracorporeal life support has seen a dramatic increase in use in clinical practice with improved reported survival in severe neonatal, pediatric and adult respiratory and cardiac disease.\textsuperscript{159-162} Advances in technology and additional design innovations have improved ease of use and augmented safety profiles, resulting in widespread use in patients with significant illness and co-morbidities.\textsuperscript{163, 164} However, despite improvements in circuit design, anti-coagulation strategies, and approaches to sedation limitation and rehabilitation on extracorporeal membrane oxygenation (ECMO), there have been few advances in neurologic monitoring of the patient requiring extracorporeal support.

Neurologic injury of the patient supported with ECMO remains a significant problem impacting long-term functional outcome, and is associated with increased risk of mortality.\textsuperscript{130} Ischemic stroke occurs in 7.4\% of neonates and 4.0\% of children, while intracranial hemorrhage (ICH) occurs in 7.0\% of neonates and 6.0\% of children,\textsuperscript{25} and these rates remain the same despite four decades of experience.

At 5-year follow-up, severe neurologic deficits in the neonatal population requiring ECMO have been described in 6-13\% of patients.\textsuperscript{135, 165} Kambekar et al report an incidence of 8\% of neonatal ECMO survivors with some neurologic impairment, 4\% of who have severe disability.\textsuperscript{166} In a multicenter 3-year follow-up of pediatric respiratory ECMO survivors, 16\% had neurologic deficits described as seizures and/or developmental delay.\textsuperscript{167}

Neurologic injury in the patient managed with ECLS is likely multifactorial. Pre-ECMO hypoxemia, extreme acidemia or alkalemia, and hypoperfusion may alter cerebral physiologic processes, resulting in compromise of cerebral autoregulation.\textsuperscript{101, 168} Injury is compounded by immediate anticoagulation, alterations in pulsatile flow that may occur with venoarterial support, microthrombi associated with the ECMO circuit,
alternations in intrinsic coagulation, and changes in cerebral blood flow and drainage that may be exacerbated by cannulation of the jugular and carotid vessels. Pre-ECMO physiologic perturbations resulting in compromised autoregulation may leave the cerebral microcirculation particularly vulnerable to fluctuations in blood pressure, with resulting hyperemia and/or ischemia.

Elimination of pre-ECMO physiologic derangements in critically ill patients is often not possible. As such, additional focus must center on early detection and prevention of neurologic injury to achieve improvements in long-term neurologic outcome in critically ill patients treated with ECMO. Neurologic monitoring on ECMO varies between institutions and practitioners. Historically, it has included a combination of neuroradiographic studies, cerebral oximetry, transcranial Doppler, electroencephalography (EEG), serial physical examinations, and somatosensory evoked potentials. Each of these modes of monitoring has limitations in early detection of brain injury, and often reveals abnormalities after catastrophic injury has occurred. Furthermore, few have correlated with clinical outcome.

Plasma brain biomarkers may be important monitoring tools to aid in outcome prediction and neurologic monitoring of patients on ECMO at high risk for neurologic injury. Biomarkers have been utilized in a wide spectrum of neurologic disease, including stroke, traumatic brain injury, neurodegenerative processes, hypoxic ischemic injury and spontaneous intracranial hemorrhage. Biomarkers can be used to establish the occurrence of brain damage, quantitatively monitor for additional neurologic insult, and/or predict clinical changes and future neurologic function. In our prior work, we showed a significant association between high plasma glial fibrillary acidic protein (GFAP) and acute brain injury and death in pediatric patients managed with ECMO. Elevated S100B protein has been described as an early indicatory of intracranial hemorrhage in infants requiring ECMO by others.
Continued identification and understanding of factors associated with neurologic morbidity should impact neuroprotective strategies employed during the use of ECMO, especially during periods of vulnerability, with the goal to limit both primary and secondary brain injury. To this effort, this study describes the use of a multiplex assay of six brain injury biomarkers as a tool in a multimodal monitoring approach to the ECMO patient at risk for neurologic insult. We hypothesized that elevations in brain injury biomarkers occur prior to clinical or neuroimaging diagnosis of brain injury and are predictive of neurologic disability at hospital discharge. The goal of this study was to examine the association of a multiplex brain-specific protein assay with acute neurologic injury during ECMO and neurologic outcome.

**Methods**

**Study design**

This is an Institutional Review Board-approved prospective observational study of children <18 years who underwent ECMO for any indication in a 40-bed pediatric intensive care unit in an academic, tertiary-care center. Electronic medical records were reviewed to abstract clinical information related to demographics, pre-ECMO hospital course, ECMO characteristics and mortality prior to discharge. Neuroimaging studies during ECMO and up to six weeks post-ECMO were interpreted by pediatric neuroradiologists and reviewed for detection of abnormal structural findings. Abnormal neuroimaging was defined as any structural abnormality outside of grade I intraventricular hemorrhage and/or increased extra-axial spaces detected by head ultrasound (HUS), brain computed tomography (CT) or brain magnetic resonance imaging (MRI). Good neurologic outcome at hospital discharge was defined as a Pediatric Cerebral Performance Category (PCPC) score of 1, 2, or no change from baseline.¹⁵³, ¹⁵⁴

**Specimens and multiplex assay**

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89
Specimens were collected for measurement of brain-specific proteins from remaining fractions of plasma from daily complete blood cell count tests (EDTA-treated tubes) and 3.2% sodium citrate samples obtained for study purposes. Specimens were collected daily for the duration of ECMO, and, when available, a sample prior to ECMO cannulation was also obtained. Platelet-poor plasma was separated by centrifugation and stored at -80°C. A multiplex assay platform for six biomarkers was established on SECTOR® Imager 6000 reader plates (Meso Scale Discovery, Gaithersburg, MD). Each assay consisted of a capture and primary antibody, each recognizing separate epitopes on their respective target protein. Primary antibodies were derivatized with Sulfo-tag NHS-ester R91AN-1 for electrochemiluminescent detection (manufacture instructions; Meso Scale Discovery). Assays were optimized for specificity and sensitivity by the identification of optimal antibody pairs, protein standards and assay conditions including: buffer compositions, incubation times, blocking reagents and matrix controls. Limits of detection and quantitation were determined and the detection of known amounts of calibrant protein spiked into plasma was shown to parallel standard curves for each assay. Samples were assayed in duplicate in a total volume of 100 uL consisting of 25 microL pediatric plasma and 75 microL phosphate buffered saline containing 5% fetal bovine serum. Assays were processed in two batches, in July 2012 and November 2012. Laboratory personnel was blinded to clinical data, and medical record data abstractors, neuroradiologists and study team members assigning PCPC were blinded to multiplex assay results.

There were six proteins measured in the multiplex assay, selected for their potential to reflect glial injury (GFAP, S100b), neuronal injury (neuron specific enolase [NSE], intercellular adhesion molecule-5 [ICAM5], brain derived neurotrophic factor
[BDNF]) and neuroinflammation (ICAM5, monocyte chemoattractant protein 1 / chemokine (C-C motif) ligand 2 [MCP1/CCL2]).

**GFAP** is a cytoskeletal class III intermediate filament protein found in mature astrocytes of the central nervous system. GFAP expression is increased in the brain during reactive astrogliosis following brain injury. Accumulating evidence suggests that after ischemia, astrocytic demise may precede neuronal loss. As plasma GFAP originates solely from the central nervous system, it is an ideal marker for early brain injury. Alone or in combination with other brain injury biomarkers, GFAP has been shown by us to be a predictor for neurologic injury and outcomes in neonates and children after ECMO, in children with sickle cell disease, after whole body cooling for neonatal hypoxic ischemic encephalopathy, and by others in adults after acute stroke, cardiac arrest, and traumatic brain injury.

**Intercellular adhesion molecule-5 (ICAM-5)** is a synaptic adhesion molecule with a molecular weight of 96kD, localized solely in the telencephalon, in soma and dendrites of neurons, and highly expressed in newborns. ICAM5 plays roles in regulation of brain immunological activity and in the development of neuronal synapses.

**Brain-derived neurotrophic factor (BDNF)** is part of the neurotrophins family, is produced by neurons, glial cells and cells of the immune system (macrophages, leucocytes) and plays roles in synaptic plasticity, neurogenesis, higher cognitive function and neuronal survival in the brain.

**S100B** is a calcium-binding protein with a molecular mass of 21 kD and serum half-life of 1-2 hours. It is localized predominantly in astroglia but is also present in skeletal muscle, adipocytes, bone marrow, and melanocytes. Plasma S100B can be elevated in shock states, cardiac arrest and cardiopulmonary bypass.
has been shown to be sensitive or specific as a diagnostic marker in traumatic brain injury, acute stroke, cardiac arrest, and subarachnoid hemorrhage as well as in a case-control study of 16 ECMO patients.\textsuperscript{137, 149, 172, 194-196} In the case series of infants during ECMO, S100B performed well as an early indicator of intracranial hemorrhage; plasma concentrations were elevated up to 72 hours before imaging diagnosis or clinical suspicion of intracranial hemorrhage.\textsuperscript{137}

\textbf{Neuron specific enolase (NSE)} is a glycolytic enzyme with a molecular mass of 78 kD and serum half-life of >20 hours. It is localized mostly in neuronal cytoplasm.\textsuperscript{171} NSE has previously been found to be sensitive and specific as a diagnostic marker for traumatic brain injury, stroke, and cardiac arrest in children and adults.\textsuperscript{172, 180, 193, 196-198} Like S100B, NSE had been shown to be elevated in shock states.\textsuperscript{199, 200} NSE is also present in red blood cells, platelets, smooth muscle, and adipocytes.\textsuperscript{201}

\textbf{Monocyte chemoattractant protein 1 (MCP1) / Chemokine (C-C motif) ligand 2 (CCL2)} is produced in the CNS by astrocytes, microglia, infiltrating macrophages and neurons and participates in acute and chronic neuroinflammation.\textsuperscript{202} Plasma MCP1/CCL2 has been shown to increase in both acute injury such as stroke as well as in chronic conditions that have a neuroinflammatory component, such as multiple sclerosis or Alzheimer’s disease.\textsuperscript{202}

\textbf{Statistical analysis}

The groups of children with abnormal vs normal neuroimaging and children with good vs poor neurologic outcome were compared using the Wilcoxon rank-sum test for non-normally distributed continuous variables, Student’s \textit{t} test for normally distributed continuous variables, or chi-square test for binary variables. Receiver operator characteristic (ROC) curves were constructed for each protein to determine the optimal cutoff (i.e., value providing the maximal area under the ROC curve) for plasma protein concentrations during the ECMO course to identify children with brain injury by
neuroimaging and children with poor neurologic outcome at discharge. The area under the ROC curve was then calculated for combinations of proteins that were individually significantly associated with the outcomes. A $p$ value of 0.05 was considered significant. Statistical analysis was conducted using STATA 11.0 (StataCorp, College Station, TX).

**Results**

There were 56 patients who underwent ECMO at our institution between June 2010 – October 2012. One patient was on ECMO <15h and did not have samples available for analysis. Patient characteristics are presented in Table 10. ECMO course characteristics, neuroimaging and neurologic complications during ECMO are presented in Table 11. Overall survival was 36/55 (65.5%) and poor neurologic outcome at hospital discharge was seen in 21/55 (38%) patients. Fifty of 55 patients had neuroimaging done during or after ECMO, in the form of daily HUS in neonates and young infants with open fontanel, brain computed tomography (CT) during and/or after ECMO in older children and brain magnetic resonance imaging (MRI) after ECMO decannulation in all neonates per institutional protocol and in older children if clinically indicated. There were 5/55 patients who did not have any neuroimaging done: 3/5 died on ECMO and 2/5 were older children who had normal neurologic exams after decannulation and thus the clinical team deferred obtaining a brain MRI. The rate of neurologic injury by neuroimaging was 7/50 (14%) cerebral infarction, 9/50 (18%) ICH, and 3/55 (5.5%) clinical seizures. Six patients showed evidence of areas of infarction as well as areas of ICH, yielding an overall rate of abnormal neuroimaging findings of 10/50 (20%). EEGs were abnormal in 10 (77%) of 13 patients who underwent EEG recordings due to concerns on the part of the clinical team for clinical or subclinical seizures.

Plasma measurements of brain-specific proteins were done on 512 daily, serial blood samples during ECMO. Peak biomarker concentrations were significantly higher in patients with poor vs good neurologic outcome for GFAP (median: 0.64ng/mL, IQR:
0.4ng/mL-1.01ng/mL vs 0.37ng/mL, IQR: 0.19ng/mL-9.54ng/mL, p=0.010), S100b (median: 1.96ng/mL, IQR: 0.8ng/mL-3.7ng/mL vs median 0.89ng/mL, IQR: 0.51ng/mL-1.96ng/mL, p=0.048) and MCP1/CCL2 (median: 2.42ng/mL, IQR: 1.12ng/mL-3.06ng/mL vs 0.98ng/mL, IQR: 0.5ng/mL-2.85ng/mL, p=0.034). There were no significant differences between the two groups in plasma ICAM-5, BDNF and NSE concentrations. (Figure 6)

Among the 50 patients who had neuroimaging studies done during and after ECMO, patients with vs those without newly diagnosed neurologic injury had significantly elevated plasma levels of GFAP (median: 0.78ng/mL, IQR: 0.38ng/mL-1.32ng/mL vs 0.38ng/mL, IQR: 0.19ng/mL-0.64ng/mL, p=0.026), ICAM-5 (median: 0.78ng/mL, IQR: 0.37ng/mL-1.12ng/mL vs 0.49ng/mL, IQR: 0.26ng/mL-0.68ng/mL, p=0.026) and MCP1/CCL2 (median: 2.44ng/mL, IQR: 1.03ng/mL-3.74ng/mL vs 1.15ng/mL, IQR: 0.56ng/mL-2.63ng/mL, p=0.049). (Figure 7) No significant differences were found for BDNF, S100b and NSE. Individually, the area under the receiver operating characteristic (ROC) curve for GFAP, ICAM-5 and MCP1/CCL2 was 0.64, 0.62 and 0.64, respectively. When the three proteins were analyzed as a group, the area under the ROC curve improved to 0.73. Eight of 11 patients with a neuroimaging diagnosis of acute neurologic injury attributed to ECMO had neuroimaging studies while cannulated. Out of these 8 patients, plasma GFAP and plasma ICAM-5 peaked before the imaging diagnosis was made in 7 patients, by a median of 3.5d (IQR: 2.1d-8.8d) and median of 3.5d (IQR: 1.3d-4.8d), respectively. Plasma MCP1/CCL2 peaked before the imaging diagnosis of cerebral infarction and/or ICH was made in 6 patients, by a median of 3.8d (IQR: 2.6d-6.1d). The one patient who showed no significant increase in plasma GFAP, ICAM-5 or MCP1/CCL2, suffered a subependymal hemorrhage (grade I intraventricular hemorrhage), which generally is considered minor and inconsequential.

Discussion
This prospective, observational study of neonates and children on ECMO included 55 patients in a single academic center, most of them undergoing VA-ECMO (91%) for primarily cardiac failure and ECPR (60%), with similar survival (65.5%) and average rate of acute neurologic injury on ECMO (20%) compared to other published studies.\textsuperscript{25} We found that, using a multiplex assay for six brain-specific proteins with high expression in neurons and glial cells and/or with roles in neuroinflammation, acute neurologic injury on ECMO can be detected during its subclinical phase, before a neuroimaging diagnosis is made. The time interval between peaks in plasma concentrations of brain injury biomarkers and neuroimaging diagnosis could be leveraged for obtaining more definitive studies (such as brain CT) and for initiation of neuroprotective interventions (e.g., decreased heparin infusion rate to avoid extension of an ICH, improved blood pressure support, optimization of hydration status and oxygenation to avoid extension and secondary injury in case of ischemic changes, initiation of antiepileptic prophylaxis, etc). A continued refinement of brain injury proteins will need to take place to improve diagnostic abilities. Current area under the ROC curve is 0.72 with a three-protein combination for diagnosis of acute neurologic injury, but this is still suboptimal. The brain is extremely complex and a diagnostic test will need to have high sensitivity for detection of both hemorrhagic and ischemic injury, and, ideally, for detection of injury at the cellular level that precedes cellular death or apoptosis. In this respect, we plan future studies in collaboration with Dr. Jennifer Van Eyk’s laboratory to conduct biomarker discovery using mass spectrometry, with the long term goal of discovering high-sensitivity proteins for development of ECMO specific multiplex assays.

This study has several limitations. First, the study has a modest sample size and heterogeneity of ages and diagnoses. This limitation will be overcome by the additional analysis of data from 30 additional patients enrolled between November 2012 and December 2013 that will bring the sample size to 85 patients. Second, there is no gold
standard for diagnosis of acute neurologic injury, so we used neuroimaging diagnosis by
daily HUS during ECMO, brain CT during or post-ECMO or brain MRI post-ECMO as a
surrogate. HUS are known to have poor sensitivity for detection of injury outside of the
axial space, but have the advantage of serial daily examinations. Brain CTs during
ECMO were obtained by the clinical team when a significant concern for neurologic
injury occurred (e.g., pupillary changes), and were not done serially. Brain CT and brain
MRI post-ECMO were done at variable times after decannulation, and neuroradiologists
could only estimate the age of injury as having occurred during ECMO. Given this
limitation, we used neurologic outcome at hospital discharge as a secondary outcome to
evaluate its association with elevated plasma biomarker levels. In a study for which we
are actively enrolling patients, we are also evaluating long-term neurologic outcomes at
6 months and 12 months post-ECMO using a standardized neurologic exam, the
Pediatric Stroke Outcomes Measure, as well as a battery of neurocognitive tests.
Lastly, the selected brain specific proteins are markers of subclinical disease but do not
provide potential mechanism or pathophysiologic therapeutic pathways.

Conclusions

Elevated plasma brain injury biomarker concentration during the ECMO course is
associated with acute neurologic injury. Combining results from multiple brain-specific
proteins increases the sensitivity and specificity for detection of neurologic injury.
Circulating brain injury biomarkers may also provide a triage mechanism for more
advanced neurologic imaging for earlier therapeutic intervention. Ongoing development
of a commercial-grade panel of highly-specific brain injury biomarkers with rapid turnover
of assay results could lead to accurate neuromonitoring and outcome prediction in
ECMO patients.
Table 10. Patient characteristics

<table>
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<th>Results</th>
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<td>Age, median (IQR), n=55</td>
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<td>Male, no (%), n=55</td>
<td>29 (53)</td>
</tr>
<tr>
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<tr>
<td>White</td>
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</tr>
<tr>
<td>Black</td>
<td>15 (27)</td>
</tr>
<tr>
<td>Other</td>
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</tr>
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<td>Weight, kg, median (IQR), n=55</td>
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<td>Pre-ECMO inotrope use, n=54&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>Gestational age, median (IQR) n=34 newborns</td>
<td>39w (37w-39w)</td>
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<td>Apgar score at 1 minute, median (IQR), n=33 newborns</td>
<td>6 (2-8)</td>
</tr>
<tr>
<td>Apgar score at 5 minutes, median (IQR), n=33 newborns</td>
<td>8 (6-8)</td>
</tr>
<tr>
<td>Survival to hospital discharge, no (%)</td>
<td>36 (65.5)</td>
</tr>
<tr>
<td>Unfavorable neurologic outcome at hospital discharge by PCPC, no (%)</td>
<td>21 (38)</td>
</tr>
</tbody>
</table>

IQR: interquartile range; ECMO: extracorporeal membrane oxygenation; PCPC: Pediatric Cerebral Performance Category. <sup>a</sup> One patient was cannulated at a different hospital and transported on ECMO, unknown if inotropes were used prior to cannulation.
<table>
<thead>
<tr>
<th>Variable</th>
<th>Results</th>
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<tbody>
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<td>Mode of ECMO, no (%), n=55</td>
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<td>VA-ECMO</td>
<td>50 (91)</td>
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<td>VV-ECMO</td>
<td>4 (7)</td>
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<td>VV- to VA-ECMO</td>
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<td>Duration of ECMO support, median (IQR), n=55</td>
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<td>Neuroimaging during or post-ECMO, n=55</td>
<td>50 (91)</td>
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<tr>
<td>Post-ECMO brain MRI, no (%), n=36 survivors</td>
<td>22 (61)</td>
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<tr>
<td>Time from ECMO decannulation to brain MRI, median (IQR)</td>
<td>21d (9d-41d)</td>
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<td>Neurologic complications, no (%)</td>
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<td>Cerebral infarction by neuroimaging, n=50</td>
<td>7 (14)</td>
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<tr>
<td>ICH by neuroimaging, n=50</td>
<td>9 (18)</td>
</tr>
<tr>
<td>Clinical seizures, n=55</td>
<td>3 (5.5)</td>
</tr>
<tr>
<td>EEG obtained for concern for clinical or subclinical seizures, no (%), n=55</td>
<td>13 (24)</td>
</tr>
<tr>
<td>Abnormal EEG, no (%), n=13</td>
<td>10 (77)</td>
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ECMO: extracorporeal membrane oxygenation; VA: venoarterial; VV: venovenous; IQR: interquartile range; MRI: magnetic resonance imaging; ICH: intracranial hemorrhage; EEG: electroencephalography.

*Neuroimaging included daily head ultrasound during ECMO, brain CT during or post-ECMO or brain MRI post-ECMO.*
Figure 6. Brain biomarker multiplex results in ECMO patients with and without poor neurologic outcome by Pediatric Cerebral Performance Category at hospital discharge (n=55)

GFAP: glial fibrillary acidic protein; ICAM5: intercellular adhesion molecule-5; BDNF: brain derived neurotrophic factor; NSE: neuron specific enolase; MCP1/CCL2: monocyte chemoattractant protein 1 / chemokine (C-C motif) ligand 2
Figure 7. Brain biomarker multiplex results in ECMO patients with and without acute neurologic injury by neuroimaging (n=50)

GFAP: glial fibrillary acidic protein; ICAM5: intercellular adhesion molecule-5; BDNF: brain derived neurotrophic factor; NSE: neuron specific enolase; MCP1/CCL2: monocyte chemoattractant protein 1 / chemokine (C-C motif) ligand 2
Chapter Seven
Conclusions and future plans

This work examined the variability in management of anticoagulation and blood product administration in ECMO patients using a survey instrument administered to ECMO program directors and coordinators from international ECMO centers affiliated with the Extracorporeal Life Support Organization. The survey revealed the prevalent use of novel and more specific assays to monitor anticoagulation that is not supported by published, evidence-based, guidelines. In a single center, prospective observational study, we then demonstrated that these new assays have poor correlation with the anticoagulation monitoring assays still used in the majority of ECMO centers.

Inadequate monitoring of anticoagulation can lead to either over anticoagulation or, conversely, to inadequate anticoagulation, both states placing patients at risk for neurologic injury manifested as intracranial hemorrhage or embolic stroke, respectively.

Neurologic complications that develop during ECMO lead to prolonged, costly hospitalizations, long-term neurologic disability and decreased quality of life. The long-term goal of this research is to improve the risk margin of anticoagulation management during ECMO, develop sensitive, real-time diagnostic and therapeutic strategies to reduce the incidence of brain injury during ECMO, institute timely neuroprotective therapies and identify patients who would benefit the most from early neurodevelopmental interventions post-hospitalization. Our preliminary studies suggest a significant association between abnormally high concentrations of candidate brain-specific biomarkers, plasma GFAP, ICAM-5, MCP1/CCL2, and onset of acute neurologic injury during ECMO. Brady et al have also shown that non-invasive measures of cerebrovascular autoregulation derived from near-infrared spectroscopy (NIRS), are significantly associated with perioperative stroke in patients undergoing
cardiopulmonary bypass, a different type of extracorporeal support. We hypothesize that loss of cerebrovascular autoregulation is a risk factor for the development of neurologic injuries during ECMO and correlates with plasma elevations of brain injury biomarkers. We further hypothesize that significant increases in plasma brain-specific markers and loss of cerebrovascular autoregulation can predict poor neurologic outcomes and mortality.

To test our hypotheses, we have started a prospective study of children requiring ECMO, organized around the following specific aims:

**Aim 1.** Determine if periods of lost cerebrovascular autoregulation during ECMO in children are correlated with periods of elevations in plasma biomarkers for brain injury (e.g. GFAP).

Hypothesis: Periods of loss of cerebrovascular autoregulation occur frequently during ECMO in children, and these periods expose the brain to ischemia which leads to the release of protein biomarkers into the systemic circulation.

**Aim 2.** Determine if periods of loss of autoregulation and release of protein biomarkers for brain injury into the systemic circulation during ECMO are correlated with conventional neuroimaging markers for injury including cranial ultrasound and magnetic resonance imaging (MRI).

Hypothesis: The length and severity of periods of loss of cerebrovascular autoregulation and the release of protein biomarkers from brain injury into the systemic circulation will correlate with elevations in neuroimaging markers for injury and will occur before changes in neuroimaging.

**Aim 3.** Determine if elevated plasma brain injury biomarker concentrations and loss of autoregulation during ECMO as well as neuroimaging markers of brain injury are independent predictors of survival and neurologic outcomes at hospital discharge, and at 6 months and 1 year post-ECMO.
Hypothesis: Elevations in brain injury biomarkers and periods of loss of cerebrovascular autoregulation as well as neuroimaging markers for brain injury (head ultrasound and brain MRI) will predict later neurological disability, but changes in biomarkers and autoregulation will provide an earlier correlate of injury.

The prospective observational study with the three specific aims detailed above is ongoing. We have enrolled 53 patients thus far, with the goal of enrolling 120 neonates and children through June 2015, and complete 6-month and 1-year follow up on all patients by June 2016. We are extending this study to other centers. We started enrolling patients at the Children’s National Medical Center, Washington, DC (site PI: Dr. Jamie Schwartz) and plan to start enrollment at All Children’s Hospital, St Petersburg, FL (site PI: Dr. Arabella Stock), in 2014.

Our work seeks to create a paradigm shift in the practice of neuromonitoring during ECMO, from a passive and reactive approach, to a proactive approach with real-time monitoring guiding mid-course correction. To this end, we are using physiologic markers of loss of cerebrovascular autoregulation coupled with biochemical markers of brain injury as novel tools for early, prompt diagnosis of neurologic risk and insult. Similarly, circulating brain injury biomarkers could be used as a surrogate for monitoring therapeutic intervention efficacy. Presently, it is impossible to improve ECMO neurocognitive outcomes unless brain injury can be diagnosed as it is occurring. We will couple the use of a noninvasive measure to detect disruption of cerebral blood flow - blood pressure autoregulation as an indicator of brain vulnerability to injury, with biomarkers of end organ brain injury. Early detection of injury risk will enable rapid intervention such as restoring cerebral blood flow autoregulation by tight control of vasoreactive infusions and ECMO circuit flows to target blood pressure to a level above an individual’s lower cerebral blood flow autoregulatory threshold during ECMO.
We are also extending this work to include brain injury biomarker discovery using mass spectrometry technology at the Johns Hopkins NHLBI Proteomic Center, in collaboration with Dr. Jennifer Van Eyk. To accelerate biomarker results for more rapid interventions, in collaboration with the Department of Materials Sciences and Engineering (Dr. Howard Katz), we plan to work towards the development of a real time monitoring device for clotting factors and brain injury biomarkers in plasma of ECMO patients, using organic field-effect transistors (OFET) attached to the ECMO circuit.

Lastly, in collaboration with the Institute for Computational Medicine (Dr. Raimond Winslow), we plan to automate physiologic data monitoring in ECMO patients (including electrocardiography, heart rate, respiratory rate and blood pressure waveforms, mixed venous saturation, central venous pressure, cerebral oximetry, etc). We plan to integrate continuous waveform signals from the cardiac, respiratory and central nervous systems to identify patterns of instability that can predict complications and to assess physiologic responses to currently employed medical interventions.
Appendix A

Copy of Extracorporeal Life Support Organization (ELSO) cover letter for:

Variability in anticoagulation management of patients on extracorporeal membrane oxygenation (ECMO): an international survey

Dear ELSO member,

We are fortunate to be working with the Extracorporeal Life Support Organization on a project to survey anticoagulation practices during extracorporeal membrane oxygenation (ECMO).

The link to the anonymous, online survey is provided below. It is voluntary, and takes less than 15 minutes to complete. Your completing this survey will serve as your consent to be in this research study.

https://www.surveymonkey.com/s/YPPL5NK

Please limit the respondents to one per ECMO center.

Thank you in advance for your time. Please do not hesitate to contact us with any questions.

Sincerely,

Melania M. Bembea, MD, MPH    Gail Annich, MD, MS
Assistant Professor     Associate Professor,
Johns Hopkins University    University of Michigan
Department of Anesthesiology & Critical Care Pediatric Critical Care Medicine
600 N Wolfe St, Blalock 904 F-6890 Mott/0243, 1500 E Medical Center Dr.
Baltimore, MD 21287     Ann Arbor, MI 48109
Tel: 443-844-8030     Tel: 734-764-5302
Fax: 410-502-5312     Fax: 734-647-5624
E-mail: mbembea1@jhmi.edu     E-mail: gannich@med.umich.edu
Appendix B

Survey instrument for:

Variability in anticoagulation management of patients on extracorporeal membrane oxygenation (ECMO): an international survey
ECMO anticoagulation and blood product management survey

1. Please select the location of your hospital.
   - Europe
   - North America
   - South America
   - Asia
   - Australia/New Zealand
   - Africa

2. What kind of ICU is your ECMO program serving? (choose all that apply)
   - Neonatal ICU
   - Pediatric ICU
   - Cardiac ICU (pediatric)
   - Cardiac ICU (adult)
   - Surgical ICU (adult)
   - Medical ICU (adult)
   - Other (please specify): ________________

3. How large is/are the ICU(s) served by your ECMO program?
   - ≤10 beds
   - 11-20 beds
   - 21-30 beds
   - >30 beds
   - Other (please specify): ________________
4. What is the maximum ECMO capacity (i.e., maximum number of simultaneous ECMO runs you can provide)?
   - 1-4
   - 5-10
   - >10
   Other (please specify):

5. In the last 6 (six) months, have you used ECMO for (choose all that apply):
   - Cardiac failure
   - Respiratory failure
   - Sepsis
   - Extracorporeal cardiopulmonary resuscitation (ECPR)
   Other (please specify):

6. What type of ECMO pump do you use for your ECMO circuits? (choose all that apply)
   - Roller pump
   - Centrifugal pump
   Other (please specify):

7. What ECMO circuit manufacturer(s) provide(s) your ECMO circuits?

8. What is the minimal ECMO flow used in any patient at your institution?

9. Do you use Heparin-bonded circuits?
   - Yes, tip to tip
   - Yes, only certain components
   - No
   Please add any comments
ECMO anticoagulation and blood product management survey

10. How is anticoagulation managed at your institution? (choose all that apply)
   - [ ] ICU staff on service make day-to-day decisions
   - [ ] Hematology/Thrombosis team makes all decisions
   - [ ] ICU staff and Hematology/Thrombosis team make decisions together
   - [ ] Dedicated “expert” ICU staff makes/helps with decisions
   - [ ] Hematology/Thrombosis team rounds with ICU team daily
   - [ ] Hematology/Thrombosis team is only consulted when the ICU team has difficulties
   - [ ] Hematology/Thrombosis team is never consulted

Other (please specify):

11. Do you have a written institutional ECMO anticoagulation and blood product management protocol?
   - [ ] Yes - anticoagulation
   - [ ] Yes - blood product management
   - [ ] Yes - both
   - [ ] No

Please add any comments:

12. Does your protocol differ among age groups?

If your protocol differs among age groups, for all following questions, please refer only to the protocol for your predominant patient population.

   - [ ] Yes
   - [ ] No
   - [ ] Not applicable

Please add any comments:
ECMO anticoagulation and blood product management survey

13. Your ECMO circuit prime contains which of the following (outside of an emergency): (choose all that apply)
   - Normal saline (0.9% NaCl)
   - Packed red blood cells (PRBC)
   - Fresh frozen plasma (FFP)
   - Heparin
   - Calcium chloride or calcium gluconate
   - Albumin
   - Sodium bicarbonate (NaHCO3)
   - Steroids

   Other solutions/additives (please add any comments)

14. What is the minimum Heparin infusion rate allowed by your protocol?
   - 0 units/kg/hour
   - 1-10 units/kg/hour
   - 11-25 units/kg/hour
   - 26-35 units/kg/hour
   - >35 units/kg/hour

   Please add any comments

15. What is the maximum Heparin infusion rate allowed by your protocol?
   - No upper limit
   - 60-75 units/kg/hour
   - 76-100 units/kg/hour
   - 101-125 units/kg/hour

   Please add any comments
# ECMO anticoagulation and blood product management survey

16. What are you typical activated clotting time (ACT) goals (choose all that apply):

- [ ] 180-200 sec
- [ ] 180-210 sec
- [ ] 200-220 sec
- [ ] 220-240 sec
- [ ] We do not follow ACT

Please add any comments:

---

17. In the last 6 (six) months, have you used non-unfractionated Heparin anticoagulation (e.g., direct thrombin inhibitors)?

- [ ] Yes
- [ ] No
- [ ] We never use any other pharmacologic anticoagulation besides unfractionated Heparin
1. What non-unfractionated Heparin anticoagulation do/can you use in your ICU? (choose all that apply)

- [ ] Argatroban
- [ ] Lepirudin
- [ ] Bivalirudin
- [ ] We never use any other pharmacological anticoagulation besides unfractionated Heparin

Other (please specify):

2. How frequently do you monitor complete blood cell counts (CBC) in a typical ECMO patient according to your institutional protocol?

- [ ] Every 1-3 hours
- [ ] Every 4-5 hours
- [ ] Every 6-8 hours
- [ ] Every 9-12 hours
- [ ] >12 hours apart

Please add any comments:

3. What is the hematocrit threshold you use for PRBC transfusion?

- [ ] <20%
- [ ] 20%-30%
- [ ] 31%-35%
- [ ] 36%-40%
- [ ] 41%-45%
- [ ] >45%

Other (please specify):

## ECMO anticoagulation and blood product management survey

### 4. What is your typical platelet threshold for platelet transfusion in an otherwise uncomplicated ECMO run?

- [ ] 50,000-60,000 cells/μL
- [ ] 61,000-70,000 cells/μL
- [ ] 71,000-80,000 cells/μL
- [ ] 81,000-90,000 cells/μL
- [ ] 91,000-100,000 cells/μL

Other (please specify): 

### 5. How frequently do you monitor prothrombin time (PT) and activated partial thromboplastin time (PTT) in a typical ECMO patient?

- [ ] Every 1-3 hours
- [ ] Every 4-5 hours
- [ ] Every 6-8 hours
- [ ] Every 9-12 hours
- [ ] >12 hours apart

Please add any comments:

### 6. How frequently do you measure fibrinogen in a typical ECMO patient?

- [ ] Never
- [ ] Every 1-3 hours
- [ ] Every 4-5 hours
- [ ] Every 6-8 hours
- [ ] Every 9-12 hours
- [ ] >12 hours apart
- [ ] Only as needed

Other (please specify): 

---

Page 7
ECMO anticoagulation and blood product management survey

1. What is your typical fibrinogen threshold for administration of FFP or cryoprecipitate?
   - [ ] 80-99 mg/dL
   - [ ] 100-119 mg/dL
   - [ ] 120-139 mg/dL
   - [ ] ≥140 mg/dL
   - [ ] Other (please specify)

2. How frequently do you measure d-dimers in a typical ECMO patient?
   - [ ] Never
   - [ ] Every 1-3 hours
   - [ ] Every 4-5 hours
   - [ ] Every 6-8 hours
   - [ ] Every 9-12 hours
   - [ ] >12 hours apart
   - [ ] Only as needed
   - [ ] Other (please specify)

3. How frequently do you measure free hemoglobin?
   - [ ] Never
   - [ ] Every 1-6 hours
   - [ ] Every 7-12 hours
   - [ ] >12 hours apart
   - [ ] Only as needed
   - [ ] Other (please specify)
4. How frequently do you measure lactate dehydrogenase (LDH)?

- Never
- Every 1-6 hours
- Every 7-12 hours
- >12 hours apart
- Only as needed
- Other (please specify)

5. How frequently do you measure factor VIII?

- Never
- Every 1-6 hours
- Every 7-12 hours
- >12 hours apart
- Only as needed
- Other (please specify)

6. Do you measure antithrombin III (ATIII)?

- Yes, routinely
- Yes, occasionally
- Never
- Other (please specify)
ECMO anticoagulation and blood product management survey

1. How frequently do you measure ATIII?
   - Every 1-8 hours
   - Every 9-12 hours
   - Every 13-24 hours
   - Other (please specify):

2. What is the goal ATIII in a typical ECMO patient according to your protocol?

3. Do you use FFP or recombinant ATIII to correct the patient’s lower-than-goal ATIII level?
   - No
   - Yes, we use either FFP or recombinant ATIII
   - Yes, but we only use FFP for this indication
   - Yes, but we only use recombinant ATIII for this indication

   Please add any comments:

4. What is the ATIII threshold for which you would administer FFP and/or recombinant ATIII?
   - <40%
   - 41%-60%
   - 61%-80%
   - 81%-100%
   - >101%
   - Other (please specify):

Page 10
ECMO anticoagulation and blood product management survey

5. Do you measure anti-factor Xa levels?
   - Yes, routinely
   - Yes, occasionally
   - Never

   Please add any comments
ECMO anticoagulation and blood product management survey

1. How frequently do you measure anti-factor Xa?
   - Every 1-8 hours
   - Every 9-12 hours
   - Every 13-24 hours
   - Other (please specify):

2. What are the goal anti-factor Xa levels in a typical ECMO patient according to your protocol?
   - 0-0.29 units/mL
   - 0.3-0.7 units/mL
   - 0.71-1 units/mL
   - >1 units/mL
   - We do not have a goal for anti-factor Xa levels
   - Other (please specify):

3. What interventions (if any) do you do for lower-than-goal anti-factor Xa levels in a typical ECMO patient?

4. What interventions (if any) do you do for higher-than-goal anti-factor Xa levels in a typical ECMO patient?

5. Do you use thromboelastograms (TEG) in your ECMO patients?
   - Yes, routinely
   - Yes, occasionally
   - Never
   - Other (please specify):
ECMO anticoagulation and blood product management survey

1. What type of TEG do you use? (choose all that apply)
   - Kaolin TEG (kTEG)
   - Heparinase TEG (hTEG)
   - Rotational TEG (RoTEG)
   Other (please specify):

2. What TEG parameters (e.g., r, K, α, MA) do you mainly use for interpretation and how? Please describe.

3. Do you ever use any of the products below for management of (anti) coagulation/hemorrhage/thrombosis in ECMO patients? (choose all that apply)
   - E-aminocaproic acid
   - Tranexamic acid
   - Activated Factor VII
   - Aspirin
   - Warfarin
   - Prostacyclin
   - Serine protease inhibitors
   - Aprotinin
   Other (please specify):
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Curriculum Vitae

Melania M. Bembea, MD, MPH

Current Position

Assistant Professor
Department of Anesthesiology and Critical Care Medicine
Department of Pediatrics
Johns Hopkins University School of Medicine, Baltimore, MD

Contact Information

Office:
Johns Hopkins University School of Medicine
Department of Anesthesiology and Critical Care Medicine
Division of Pediatric Anesthesiology and Critical Care Medicine
Charlotte R. Bloomberg Children’s Center
1800 Orleans Street, Suite 6321
Baltimore, MD 21287
Tel: 410-955-6412
Pager: 410-434-2659
Email: mbembea1@jhmi.edu

Education/ Training

07/2009- PhD candidate, the Graduate Training Program in Clinical Investigation (GTPCI), Johns Hopkins Bloomberg School of Public Health, Baltimore, MD. Thesis: “Anticoagulation and neuromonitoring during extracorporeal membrane oxygenation (ECMO)”. Primary research mentor: Peter Pronovost, MD, PhD
07/2006-06/2009 Fellow, Pediatric Critical Care, Johns Hopkins University School of Medicine, Baltimore, MD
07/2007-06/2008 Master of Public Health, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD
06/2003-06/2006 Pediatric Resident, The Children’s Hospital at the Cleveland Clinic Foundation, Cleveland, OH
04/2002-05/2003 Research Assistant, Division of Human Cancer Genetics, The Ohio State University, Columbus, OH. Received intensive training in molecular biology techniques investigating the role of DNA methylation in leukemia
01/2001-12/2001 Pediatric Intern, Children’s Clinical Hospital, Oradea, Romania
10/1994-09/2000 M.D., Medical School of the University of Medicine and Pharmacy “Iuliu Hatieganu”, Cluj-Napoca, Romania
1998 Research Assistant, Department of Genetics of the Faculty of Medicine, Oradea, Romania.

Positions

2009- Assistant Professor, Departments of Anesthesiology and Critical Care Medicine and Pediatrics, Johns Hopkins University, School of Medicine, Baltimore, MD
2007-2009 Clinical Instructor, Pediatric Emergency Department, Johns Hopkins Hospital, Baltimore, MD
2008- Pediatric Advanced Life Support Instructor, Johns Hopkins Hospital Outreach for Pediatric Education Program
Leadership Activities

2012-  Director, Johns Hopkins Pediatric Critical Care Medicine Clinical Research Program
2012-  Associate Director, Johns Hopkins Pediatric ECMO Program
2012-  Member, Extracorporeal Life Support Organization (ELSO) ECMO Anticoagulation Task Force
2011-  Member, Pediatric Research Task Force, Get With The Guidelines-Resuscitation, American Heart Association
2008-  Member, Johns Hopkins Hospital Pediatric Cardiopulmonary Resuscitation Committee
2008-2011  Volunteer, Operation Smile, pediatric team leader and team physician

Professional Certifications

11/2010  American Board of Pediatrics, Pediatric Critical Care Medicine
2007-  State of Maryland Medical Licensure, Number D0066628
10/2006  American Board of Pediatrics, General Pediatrics

Professional Memberships

2009-  Extracorporeal Life Support Organization
2006-  Society of Critical Care Medicine
2007-2009  Society for Simulation in Health Care
2003-2009  American Academy of Pediatrics

Honors and Awards

2013-2015  Clinician Scientist Career Development Award, Johns Hopkins University School of Medicine (relinquished after NIH/NINDS K23 notice of award)
2009-2013  Clinical Research Scholar Award, Johns Hopkins Institute for Clinical and Translational Research
2010  The Robert H. Bartlett Award for Best Oral Abstract Presentation, 21st Annual ELSO Conference, St. Petersburg, FL, October 2, 2010
2005-2006  Excellence in Teaching Award, the Children’s Hospital at the Cleveland Clinic
2006  Resident of the Year, Department of Pediatric Critical Care Medicine, the Children’s Hospital at the Cleveland Clinic
1999  Erasmus Grant, Faculty of Medicine of the University “Joseph Fourier”, Grenoble, France
1995  International Federation of Medical Students’ Associations study grant at the School of Medicine of the University of Marilia, Sao Paolo, Brazil

Research Support

Ongoing Research Support

1K23NS076674-01A1  Bembea (PI)  July 1, 2013 – June 30, 2016
NIH/NINDS
“Novel methods for brain injury detection and outcome prediction in pediatric ECMO”
This is a prospective observational study of the use of plasma brain injury biomarkers and of non-invasive monitoring for cerebrovascular autoregulation disturbances as early indicators of acute neurologic injury and as predictors of neurologic deficits during and after extracorporeal membrane oxygenation in critically ill neonates.
Role: PI
Extracorporeal Life Support Organization
"Neurologic outcomes of extracorporeal cardiopulmonary resuscitation (ECPR): Linking the
Extracorporeal Life Support Organization (ELSO) and the American Heart Association Get With
The Guidelines - Resuscitation (AHA GWTG-R) databases"
Role: PI

1R01AI084011-01  Randolph (PI)  November 10, 2009 – August 31, 2013
NIH/NIAID
"Genetic Epidemiology of Life-Threatening Influenza Infection in Children"
The major goal of this study is to evaluate how the host innate immune response is associated
with disease susceptibility, severity and outcome among children admitted to the pediatric
intensive care unit.
Role: PI for subcontract

Completed Research Support

200-2010-F-33396  Shay, Randolph, Klein Walker (Co-PIs)  Sep 22, 2010 – Nov 30, 2012
CDC
"Evaluation of Novel H1N1 Influenza A Virus Vaccine Effectiveness among Two High Risk
Populations at Priority for Early Receipt of Vaccine"
The goal of this study is evaluate the effectiveness of seasonal and 2009 H1N1 vaccine in
preventing influenza infection associated with admission to an intensive care unit among children
during the 2010-2011 influenza season.
Role: PI for subcontract

–  Fink (PI)  October 6, 2011 – September 24, 2012
The Laerdal Foundation for Acute Medicine
"Prevalence of Acute critical Neurological disease in children: a Global Epidemiological
Assessment (PANGEA)"
The objective of this study is to describe the epidemiology and gross outcomes of acute critical
neurologic disease in children.
JHU site PI: Bembea

U01HL094345  Moler, Frank (PI)  March 1, 2012 – October 2, 2012
NIH/NHLBI
"Therapeutic Hypothermia after Pediatric Cardiac Arrest “THAPCA” Trial"
The goal of this randomized controlled trial is to investigate therapeutic hypothermia after cardiac
arrest in children.
Role: PI for subcontract

1KL2RR025006-03  Bembea (PI for project)  July 1, 2009 – June 30, 2012
NIH/NCRR Ford (PI)
"Activation of Coagulation in Pediatric Patients Undergoing Extracorporeal Membrane
Oxygenation"
This was a prospective observational study of the relationship between markers of coagulation
and heparin concentration levels and the development of complications such as life-threatening
hemorrhage and ECMO circuit thrombosis.
Role: PI for project

1R21DA029295-01  Gauda (PI)  April 1, 2010- March 30, 2012
NIH/NIDA
"Efficacy of clonidine in reducing opioid dependence in critically ill infants"
The goal of this randomized controlled trial is to investigate the use of clonidine as adjunct therapy for the treatment of iatrogenic neonatal abstinence syndrome in critically-ill, intubated and sedated newborns.

Role: Co-investigator

UL1 RR 025005 Bembea (PI) September 10, 2008 – January 6, 2011
National Center for Research Resources (NCRR)
“Activation of Coagulation in Pediatric Patients Undergoing Extracorporeal Membrane Oxygenation”
Role: PI

HHSN268200536179C Randolph (PI) November 9, 2009 – July 31, 2010
NIH/NHLBI
“Clinical Coordinating Center for a Clinical Research Network for the Treatment of Acute Lung Injury (ALI) and the Acute Respiratory Distress Syndrome (ARDS)”
The goal of this prospective surveillance registry is to characterize the demographics, clinical features, outcomes, and resource utilization of patients with H1N1 influenza infection who require intensive care.
Role: PI for subcontract

Peer-Reviewed Publications


Abstracts


Pediatric Academic Societies (PAS) Annual Meeting, Boston, MA, April 2012 (abstract and poster)


Schwartz J, Bembea M, Savage W, Easley RB, Everett A. Ability of GFAP to detect neurologic injury in Pediatric Patients with cardiac disease on ECMO: A preliminary study. SPA/AAP Pediatric Anesthesiology 2010 - Winter Meeting, San Antonio, TX, April 2010 (abstract and poster)


Jurca A, Jurca C, Bembea M. Particular aspects of osteochondrodysplasias during puberty. 12th European Students' Conference at Charite, Berlin, for Medical Students and Young Doctors, November 2001 (abstract and poster)

Lectures and Presentations

International

**National/Regional**

ECMO medical simulation training. Invited lecture and workshop at the East Coast Upper Level PCCM Fellow Bootcamp, Baltimore, MD, December 2013, November 2012, October 2011, October 2010

Neuromonitoring during ECMO and neurologic outcomes post-ECMO. Pediatric Neurocritical Care Research Group Meeting, Boston, MA, November 2013

Neuromonitoring of pediatric extracorporeal membrane oxygenation (ECMO) patients. Visiting Professor, Children’s National Medical Center, Washington, DC, October 2013

Use of a multiplex plasma brain-specific protein assay for diagnosis of acute brain injury during ECMO. Invited oral presentation at the 24th Annual ELSO Conference, Philadelphia, PA, September 2013


Anticoagulation and blood product management during ECMO: research opportunities. BloodNet PALISI meeting, Snowbird, UT, March 2012


Utilization of brain injury biomarkers as diagnostic tools in ECMO patients. Pediatric Neurocritical Care Research Group Meeting, Pittsburgh, PA, October 2011

Brain Injury Biomarkers as Indicators for Acute Neurologic Injury and Predictors of Neurologic Outcomes after ECMO. Pediatric Neurocritical Care Research Group Meeting, Chicago, IL, November 2010

Anticoagulation and blood product management during ECMO; ECMO physiology; and ECMO hematology and chemistry laboratory monitoring. Invited lectures at the Johns Hopkins ECMO Training Course for ECMO programs in Boston and New Orleans, Baltimore, MD, August 2009 and July 2011

Practical Use of Simulation to Address the Council of Medical Student Education in Pediatrics (COMSEP) Curriculum; Pediatric Academic Societies (PAS) Annual Meeting, Baltimore, MD, May 2009

Lower Airway Obstruction; Pre-Operative Screening; Team Concepts. Simulation facilitator and lecturer at Operation Smile’s Third Annual Peri-Operative Pediatric Intensivist Course, Children’s Hospital of Philadelphia, May 2008

Simulation and Team Training to Improve Quality of In-Hospital Resuscitation and Time to ECMO Cannulation in Pediatric Cardiopulmonary Arrest. Invited lecture and workshop at the 17th Southeastern ECMO Conference, Baltimore, MD, May 2007

**Institutional**

Clinical Research Core lecture series for the Johns Hopkins pediatric critical care fellowship program, Johns Hopkins School of Medicine, October 2010 – present

ECMO simulation training sessions for 1st year PCCM fellows, Johns Hopkins School of Medicine, July 2009 – present (annual)
Anticoagulation during ECMO and ECMO Physiology lecture series for the Johns Hopkins pediatric critical care fellowship program, Johns Hopkins School of Medicine, 2009 – present

Anticoagulation and blood product management during ECMO; ECMO physiology; and ECMO hematology and chemistry laboratory monitoring. Lectures at new ECMO specialist institutional training. Johns Hopkins School of Medicine, July 2009 – present (semiannual)

ECMO transport simulation workshops and competencies, Johns Hopkins School of Medicine, July 2009 – present (annual)

Utilizing Simulation as an Adjunctive Educational Methodology for Interdisciplinary Team Training and Curriculum Implementation; The Art and Science of Education Symposium, Johns Hopkins School of Medicine, September 2008

Handling Pediatric Office Emergencies. Monthly pediatric resident lecture series, Johns Hopkins Hospital, 2007-2008

Approaches to Anticoagulation Therapy during Pediatric Extracorporeal Membrane Oxygenation Support. Institutional ECMO team meeting, Johns Hopkins Hospital, April 2008

Stabilization of Post-Arrest Pediatric Patients during Transport to a Tertiary Care Facility. Pediatric Critical Transport Team meeting, Johns Hopkins Hospital, March 2008

The Johns Hopkins Hospital Experience: Persistent Pulmonary Hypertension of the Newborn and Extracorporeal Membrane Oxygenation, 2001-2006. NICU/PICU Forum on PPHN, Johns Hopkins Hospital, November 2006

Hydrocephalus and Seizures. Pediatric Neurology Grand Rounds, the Children’s Hospital at the Cleveland Clinic, August 2003

Doctoral Thesis for Doctorate in Medicine: Genetic Analysis of the Cases of Cystic Fibrosis in Bihor County, Department of Cellular and Molecular Biology, University of Medicine and Pharmacy Cluj-Napoca, Romania, June 2000

**Academic Conference Leadership Positions**

2012 Program Planning Committee Member, 23rd Annual ELSO Pre-conference symposium: "State of the Art" ECMO Simulation: A "How To" Workshop to promote excellence in ECMO Training, Practice and Performance for Multidisciplinary Teams

**Reviewer**

2010- *Pediatric Critical Care Medicine*
2011- Protocol Review Committee of the Pediatric Research Task Force of the GWTG-R, American Heart Association
2011- *Cardiology in the Young*
2011- *Pediatric Blood and Cancer*