Serum Biomarkers of Brain Injury to Classify Outcome After Pediatric Cardiac Arrest*

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Objectives: Morbidity and mortality in children with cardiac arrest largely result from neurologic injury. Serum biomarkers of brain injury can potentially measure injury to neurons (neuron-specific enolase), astrocytes (S100b), and axons (myelin basic protein). We hypothesized that serum biomarkers can be used to classify outcome from pediatric cardiac arrest.

Design: Prospective observational study.

Setting: Single tertiary pediatric hospital.

Patients: Forty-three children with cardiac arrest.

Interventions: None.

Measurements and Main Results: We measured serum neuron-specific enolase, S100b, and myelin basic protein on days 1–4 and 7 after cardiac arrest. We recorded demographics, details of the cardiac arrest and resuscitation, and Pediatric Cerebral Performance Category at hospital discharge and 6 months. We analyzed the association of biomarker levels at 24, 48, and 72 hours with favorable (Pediatric Cerebral Performance Category 1–3) or unfavorable (Pediatric Cerebral Performance Category 4–6) outcome and mortality. Forty-three children (49% female; mean age of 5.9 ± 6.3) were enrolled and 17 (40%) died. Serum S100b concentrations peaked earliest, followed by neuron-specific enolase and finally myelin basic protein. Serum neuron-specific enolase and S100b concentrations were increased in the unfavorable versus favorable outcome group and in subjects who died at all time points (all $p < 0.05$). Serum myelin basic protein at 24 and 72 hours correctly classified survival but not good versus poor outcome. Using best specificity, serum S100b and neuron-specific enolase had optimal positive and negative predictive values at 24 hours to classify both favorable versus

unfavorable outcome and survival, whereas serum myelin basic protein's best accuracy occurred at 48 hours. Receiver operator curves for serum S100b and neuron-specific enolase to classify favorable versus unfavorable outcome at 6 months were superior to clinical variables.

Conclusions: Preliminary data show that serum S100b, neuron-specific enolase, and myelin basic protein may aid in outcome classification of children surviving cardiac arrest. (*Crit Care Med* 2014; 42:664–674)

Key Words: biomarker; brain; child; heart arrest; hypoxia-ischemia; outcome assessment (healthcare); resuscitation

Children with cardiac arrest (CA) have mortality rates of about 50% for in-hospital CA and 80% for out-of-hospital CA, with many survivors having neurological disability (1–8). Unlike adult CA or neonatal asphyxia, there are no novel treatment strategies proven to be effective in improving outcomes in pediatric CA.

Clinical and laboratory tests, such as motor and pupillary examination and serum lactate, when performed early in the hospital course in pediatric CA are not robustly associated with long-term outcomes when used alone (9, 10). Early recognition of brain injury on physical examination can be obscured by medications and developmental stage, and underrecognized brain injury may prevent implementation of neuroprotective therapies and monitoring.

Serum brain-specific biomarkers may help classify outcome after various pediatric brain insults. Serum biomarkers of brain injury, such as neuron-specific enolase (NSE), S100b, and myelin basic protein (MBP) from neurons, astrocytes, and axons, respectively, have the potential to assist in the early detection and quantification of the severity of brain injury, response to therapeutic interventions, and classification of outcome after CA (11–14). Brain biomarkers have different patterns of release and limitations to their use (15, 16). As a result, clinical applicability may be limited by type of brain injury (i.e., trauma, stroke, hypoxia-ischemia, and cardiopulmonary bypass) (17–21). A prospective pilot study involving pediatric subjects who received 24 hours of therapeutic hypothermia found that serum NSE and S100b had excellent specificity and fair sensitivity at 48 hours or later to classify outcome at hospital discharge (11). White matter injury seen after hypoxia-ischemia suggests a potential role for MBP but clinical studies have been limited (22, 23).

In this single-center prospective study, we examined the time course of serum NSE, S100b, and MBP levels in children during the first week after CA and tested the accuracy of serum and clinical biomarkers to classify favorable versus unfavorable outcome at 6 months. We hypothesized that serum biomarkers of brain injury targeting various cell types (neurons, astrocytes, and axons) would classify subject outcome (survival and favorable vs unfavorable) at clinically relevant time points.

MATERIALS AND METHODS

Design and Setting

Between November 2009 and September 2011, 43 subjects with CA were enrolled in one of two studies at the Children's Hospital of Pittsburgh. Twenty-five subjects were enrolled in an RCT (NCT00797680) and were randomized to either 24 or 72 hours of therapeutic hypothermia (target temperature 33°C ± 1°C). Eighteen subjects who did not meet the criteria for the RCT were enrolled in an observational study, and the PICU physician made decisions about postresuscitation temperature management. Duration of hypothermia is not disclosed in this article because both studies remain open to enrollment. Both studies were approved by the University of Pittsburgh Institutional Review Board and informed consent was obtained from the subject's parent or guardian.

Inclusion and Exclusion Criteria

We studied children between the ages 1 week and 17 years who were admitted to the ICU with return of spontaneous circulation (ROSC) after in- or out-of-hospital CA. CA was defined as receipt of chest compressions for pulselessness by a healthcare worker. Subjects were included if they had an indwelling arterial or venous catheter for blood draws. Subjects were excluded if they had a do-not-resuscitate status, were pregnant, had any contraindication for MRI, had another simultaneous acute brain disease (including traumatic brain injury), were undergoing brain death evaluation, or had a metabolic disease affecting the brain. Subjects were also excluded from the RCT if they had active hemorrhage or a preexisting coagulation defect or if they had a long bone fracture (because of the potential for false-positive S100b concentrations). Additionally, specifically for the RCT, subjects were included if Glasgow Coma Scale (GCS) score was less than or equal to 8 after ROSC and if they had therapeutic hypothermia initiated by their ICU attending.

Postresuscitation Care

Postresuscitation care in the PICU consisted of prevention of secondary neurologic insults, treatment of organ dysfunction, and investigation of cause of CA if unknown (24). Standards of care for most children post-CA included endotracheal intubation and mechanical ventilation and placement of central venous and arterial catheters. Targets for Pa_{O_2} and Pa_{CO_2} were 100–150 and 35–45 mm Hg, respectively, as well as maintenance of normal mean arterial blood pressure for age. Pain and sedation medications were used at the discretion of the attending physician and were often withheld initially to obtain an accurate neurological examination. Electroencephalography (EEG) and brain CT were commonly obtained in the first 24–48 hours after ROSC, but continuous EEG was not routinely used. Prophylactic antiepileptic medications were not used as standard of care. Rectal and/or esophageal temperature probes were used for continuous temperature reading. Fever (> 38°C) was treated aggressively in all subjects with methods similar to those used for cooling induction as well as with antipyretics (25).

Data Collection

Data were collected from medical charts using the Utstein template for CA, including subject demographics, details about the CA and resuscitation, postresuscitation care, and outcomes (26). Inotrope score was calculated using the highest concentrations within the first 24 hours postarrest (27). Initial and subsequent GCS score, GCS motor score, and pupillary reactivity were recorded from nurse and physician charting when the patient was thought to be free of sedation and neuromuscular blockade. The initial GCS was the first GCS performed after resuscitation and without neuromuscular blockade and sedation. It may have been documented by our transport team, emergency department, or ICU nurse or physician. GCS on day 3 was the first GCS on that day or if necessary the subsequent day when neuromuscular blockade was discontinued. The use of sedation and neuromuscular blockade medications are not protocolized and are prescribed by the treating physician. Standard of care is to minimize their use to allow for neurological examination unless clinically necessary. EEG result was designated as "good" if it was read by the attending neurologist as a continuous background without diffuse slowing or "poor" if the background was discontinuous, demonstrated diffuse slowing, burst suppression, or was markedly attenuated (28). Brain CT scan was recorded as "acute brain lesion" if the attending neuroradiologist identified brain edema, loss of gray-white matter differentiation, or herniation was present or "no acute lesion" in the absence of those findings.

Serum Biomarkers

Three milliliters of blood was collected twice daily (days 1–4) and once on day 7 after ROSC. For the first 4 subjects, the initial blood sample was obtained only after informed consent was acquired. Subsequently, the institutional review board granted permission for the initial sample to be drawn prior to consent. If consent was not obtained, the sample was discarded. Samples were centrifuged, aliquoted, frozen at –70°C, and analyzed in batches. Serum NSE, S100b, and MBP were measured in duplicate using commercially available enzyme-linked immunosorbent assays (International Point of Care, Toronto, ON, Canada). NSE concentration was corrected for hemolysis (15). The sensitivity of the assays was 0.1 ng/mL for NSE and MBP and 0.01 ng/mL for S100b. The coefficient of variation for each assay was less than 10%. An experienced technician blinded to subject treatment and outcome performed all biomarker measurements. Clinical team members were unaware of the biomarker results. Serum biomarker time points were measured from time of ROSC as documented in the chart. Samples closest to but not greater than 24, 48, 72, 96, and 120 hours after ROSC were used in the analysis. No sample time points were missed from living subjects, but 10 subjects did not survive to day 7.

Outcome Measures

Subjects were followed until 6 months post-CA. The primary outcome was the accuracy of serum brain biomarker concentrations to classify favorable (Pediatric Cerebral Performance Category [PCPC] score 1–3) or unfavorable (PCPC 4–6) outcome (29). Unfavorable outcome therefore includes death (PCPC = 6). The pediatric intensivist (PI), who assigned the prearrest, hospital discharge, and 6-month PCPC scores, was blinded to biomarker results but not to clinical course. Six-month outcomes were performed in surviving children either over the telephone (almost always) or during in-person interview with the parent or guardian during a scheduled outpatient visit. Secondary outcomes included mortality at hospital discharge and 6 months. Additional analyses included clinical variable assessment for classification of primary and secondary outcomes.

Data Analysis

Biomarker data are presented as median (interquartile range) since data were skewed. Other continuous variables are presented as mean ± SD. The data were analyzed for outcome group differences with Fisher exact tests for categorical variables. Median serum biomarkers were represented graphically by outcome group. Serum samples closest to but not after 24 hours, 48 hours, and so on were chosen to represent that time point. The Wilcoxon rank sum was used to compare serum biomarker concentration and outcome. Spearman's rank correlation was used to test correlation between serum biomarker concentration and age. Mann-Whitney *U* test was then used to determine where differences existed between groups. Receiver operating characteristic (ROC) curves were used to evaluate the probability that a serum or clinical biomarker would correctly classify an outcome. Clinical biomarkers chosen for ROC curves were continuous variables that classified outcome with p value less than 0.05 in univariate analysis. ROC curves were generated using raw data from all time points. Sensitivity analysis was performed for serum biomarkers at clinically relevant time points. Missing values were replaced for NSE at limited and later time points for four subjects who had other data upon which to estimate scores using linear interpolation. All p values were two-sided. Data analysis was performed using SPSS version 18 (IBM, New York, NY).

RESULTS

Forty-three subjects were enrolled and ranged in age from infancy to adolescence (**Table 1**). The sample was evenly split by sex and a strong majority of the participants had asphyxia or shock as the etiology of CA. Seventeen subjects (40%) died and 26 (60%) overall had unfavorable outcome at 6 months post-CA. Subjects with unfavorable outcome were more likely to have had an unwitnessed CA and asystole as the first documented rhythm. Motor and pupillary examination findings, first lactate and blood pH, and EEG early post-CA were associated with favorable versus unfavorable outcome (**Supplemental Table 1**, Supplemental Digital Content 1, http://links.lww.com/CCM/A762). Thirty-five subjects (81%) received therapeutic hypothermia for less than or equal to 72 hours while the remaining eight subjects were maintained normothermic.

TABLE 1. Subject Demographics and Details of Cardiac Arrest Overall and by 6-Month Outcome Postarrest

Mean ± SD (25th, 75th %) or *n* (%)	All (*n* = 43)	Favorable Outcome (*n* = 17)	Unfavorable Outcome (*n* = 26)	*p*
Age (yr)	5.87 ± 6.30 [0.33, 11.52]	6.53 ± 6.25 [0.32, 13.14]	5.43 ± 6.42 [0.48, 10.21]	0.785
Sex, *n* (% male)	22 (51)	9 (53)	13 (50)	0.549
Race/ethnicity				
White	34 (79)	13 (77)	21 (81)	0.929
Black	7 (16)	3 (18)	4 (15)	
Other	2 (5)	1 (6)	1 (4)	
History of chronic illness	18 (42)	8 (47)	10 (39)	0.403
Primary etiology				
Asphyxia/shock	37 (86)	13 (76)	24 (92)	0.155
Cardiac	6 (14)	4 (24)	2 (8)	
Location				
Out-of-hospital	32 (74)	11 (65)	21 (81)	0.205
In-hospital	11 (26)	6 (35)	5 (19)	
Interval of CPR to return of spontaneous circulation (min)	26.6 ± 28.3 [9, 30]	18.0 ± 16.3 [7.5, 25]	32.4 ± 33.2 [11, 40]	0.120
Epinephrine boluses	2.9 ± 2.9 [1, 3]	2.8 ± 2.8 [0, 4.5]	2.9 ± 3.0 [1, 3]	0.811
Defibrillated	7 (16)	5 (29)	2 (8)	0.073
First rhythm				
Pulseless electrical activity	21 (49)	10 (59)	11 (42)	0.066
Asystole	16 (37)	3 (18)	13 (50)	
Sinus	3 (7)	3 (18)	1 (4)	
Ventricular tachycardia/ ventricular fibrillation	1 (2)	1 (6)	1 (4)	
Witnessed event	20 (47)	14 (82)	6 (23)	< 0.001
Bystander CPR	35 (81)	13 (77)	22 (85)	0.388

CPR = cardiopulmonary resuscitation.

Serum Biomarker Patterns After Pediatric CA

Serum S100b concentration peaked the earliest after ROSC at a median of 19 hours followed by NSE at 37.5 hours and lastly MBP at 57.5 hours (**Fig. 1**, ***A*** and ***B***). NSE peaked earlier in subjects with favorable outcome versus unfavorable outcome (30 vs 47.3 hr, $p = 0.043$), but there were no differences in time to peak by mortality.

Serum biomarker concentrations measured between 0 and 120 hours post-ROSC (median [± 95% CI]) were plotted by both favorable versus unfavorable outcome and mortality (**Fig. 2*A–C***; **Supplemental Fig. 1*A–C***, Supplemental Digital Content 2, http://links.lww.com/CCM/A763, which describes serum NSE, S100, and MBP concentrations over the study period by mortality at 6 mo. *$p < 0.05$, favorable vs unfavorable outcome or alive vs dead. Data presented as median [95% CI].). Subjects with favorable outcome had values within the normal range for all three biomarkers when compared with historical controls (13). Serum S100b increased and decreased rapidly in subjects with unfavorable outcome or who died. Serum NSE remained increased at 120 hours in most subjects with unfavorable outcome or who died. Serum MBP showed a delayed, sustained increase in subjects with unfavorable outcome or who died.

Serum NSE, S100b, and MBP Concentrations and Outcome

Participants who died had significantly higher 24-hour NSE, S100b, and MBP levels when compared with survivors (**Supplemental Table 2**, Supplemental Digital Content 1, http://links.lww.com/CCM/A762). Serum NSE and

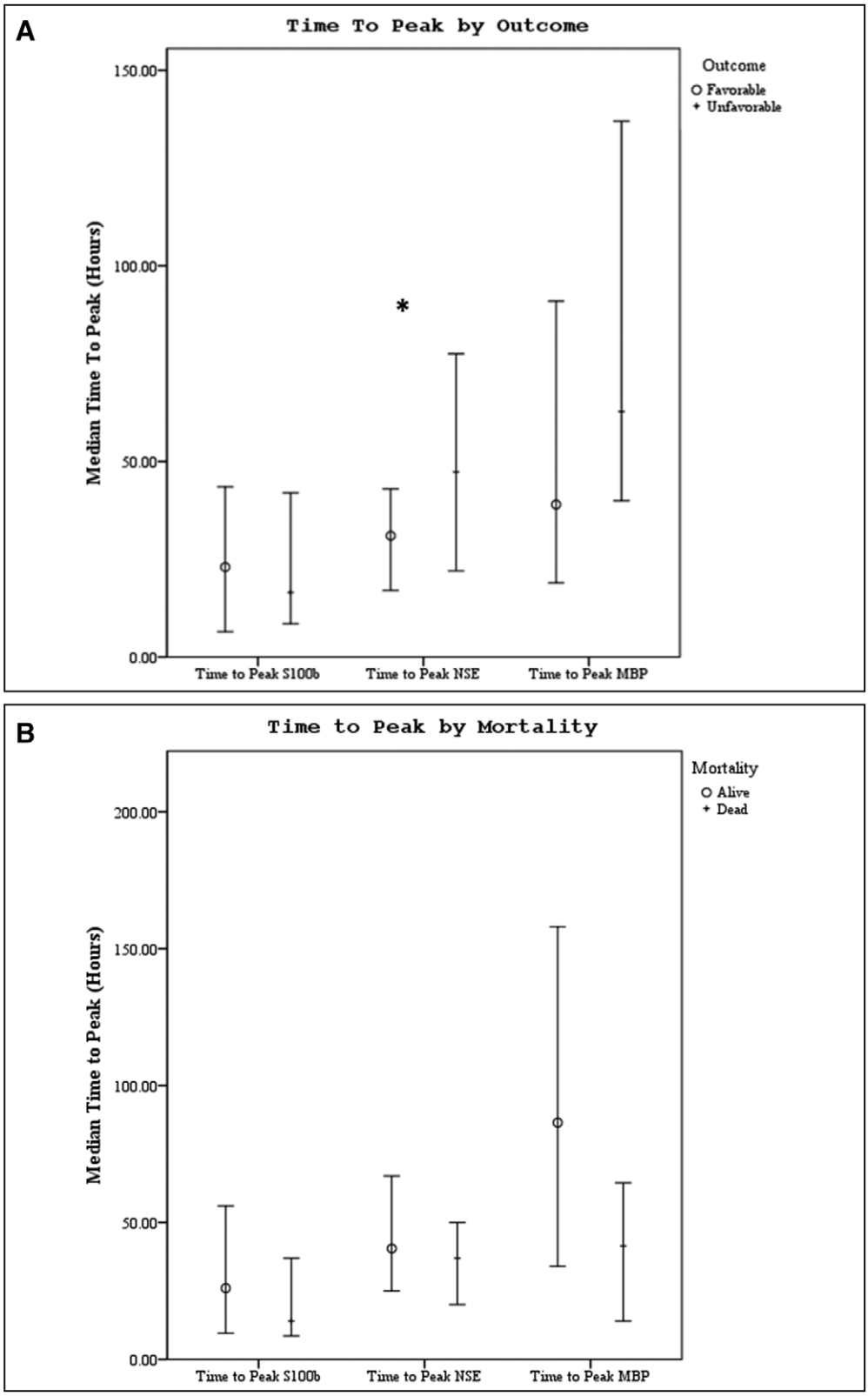

Figure 1. A and **B**, Time to peak serum neuron-specific enolase (NSE), S100b, and myelin basic protein (MBP) concentration by favorable versus unfavorable outcome and mortality at 6 mo. *$p < 0.05$, favorable versus unfavorable outcome or alive versus dead. Data presented as median (95% CI).

S100b concentrations were increased in the unfavorable versus favorable outcome group and in subjects who died versus survived at 48 and 72 hours post-ROSC, all p values less than 0.05. Mean, median, and peak serum MBP concentrations were increased in subjects with unfavorable versus favorable outcome. Additionally, serum MBP significantly classified favorable versus unfavorable outcome and mortality at 72 hours.

Age was inversely correlated with initial S100b (r = –0.40, p = 0.008), peak S100b (r = –0.31, p = 0.042), and at the 24-hour S100b (r = –0.30, p = 0.05). Initial NSE was inversely correlated with age (r = –0.41, p = 0.006), whereas MBP concentration was not correlated with age.

Serum Biomarker ROCs and Sensitivity Analysis

The area under the curve (AUC) (95% CI) for serum S100b to classify unfavorable 6 months outcome and mortality were 0.955 (0.922–0.987) and 0.908 (0.866–0.950), respectively (**Supplemental Fig. 2*A–F***, Supplemental Digital Content 3, http://links.lww.com/CCM/A764, which describes receiver operating characteristic curves for serum and clinical variables by favorable vs unfavorable outcome and mortality at 6 mo.). Similarly, serum NSE AUC was 0.859 (0.796–0.922) and 0.787 (0.721–0.852) and serum MBP was 0.732 (0.647–0.817) and 0.727 (0.654–0.799) (all $p < 0.05$). AUC for cardiopulmonary resuscitation (CPR)-ROSC time, first lactate, and blood pH are shown in **Supplemental Figure 2*G–L*** (Supplemental Digital Content 3, http://links.lww.com/CCM/A764). AUC for S100b and NSE for both outcomes were superior to all clinical variables tested (**Table 2**).

Tables 3 and **4** feature biomarker concentration thresholds for best sensitivity and specificity and corresponding positive and negative predictive value at 24- and 48-hour time points. For example, a cutoff point of 0.128 ng/mL for S100b at

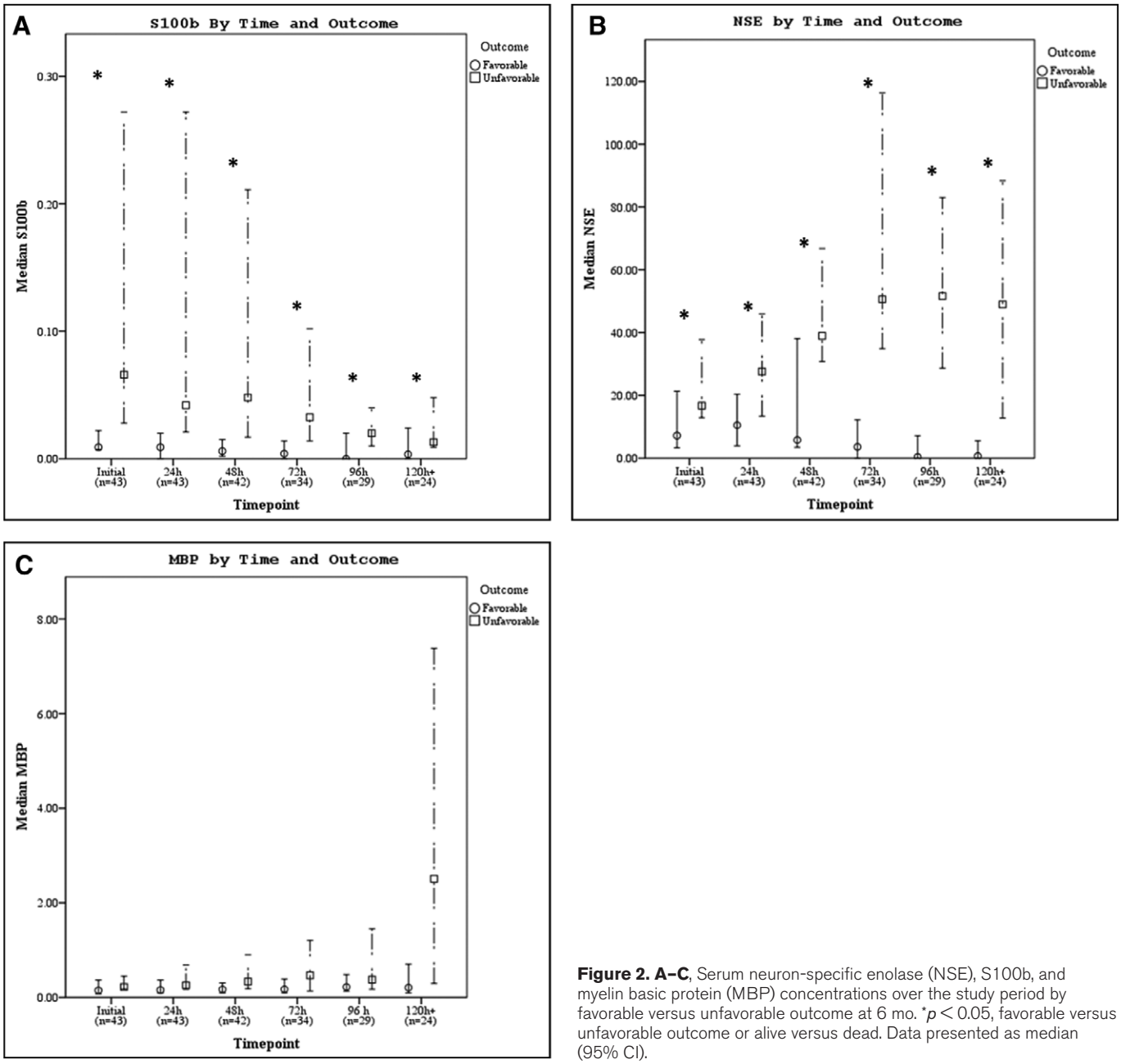

Figure 2. A–C, Serum neuron-specific enolase (NSE), S100b, and myelin basic protein (MBP) concentrations over the study period by favorable versus unfavorable outcome at 6 mo. $^{*}p < 0.05$, favorable versus unfavorable outcome or alive versus dead. Data presented as median (95% CI).

24 hour yielded a positive predictive value of 100% and negative predictive value of 53% for favorable versus unfavorable outcome. Using mortality, a cutoff point of 53 ng/mL for NSE at 24 hours resulted in a positive predictive value of 100% and negative predictive value of 73%.

DISCUSSION

In summary, we found that serum biomarkers of brain injury, S100b, NSE, and MBP, have unique patterns of release after pediatric CA. Furthermore, despite a relatively small sample size, biomarker concentrations at multiple time points classified favorable or unfavorable outcome and mortality at 6 months with remarkable accuracy using AUC. While positive predictive values calculated at the best specificity to predict outcome were robust for each biomarker, negative predictive values were less dependable in identifying subjects with good outcomes. Importantly, the initial and 24-hour biomarker time points have potential value to clinicians in helping to decide whether to initiate a neuroprotective therapy and assisting in counseling families. Although not the focus of this study, serum biomarker concentrations can be used as entry criteria or for stratification by severity of injury in randomized controlled trials (RCTs) in order to obtain a more selective subject sample (30). Subjects who died made up the majority of those with unfavorable outcome. However, one third of the subjects in the unfavorable outcome group did survive, but there were too few subjects with PCPC of 4 or 5 to determine whether serum biomarkers can detect differences between each of the individual unfavorable PCPC assignments (data not shown).

Serum S100b can detect early brain injury (11, 31), change in response to secondary brain insults (32), and classify outcome after various adult and pediatric CNS injuries (20, 31, 33–36). Inflammatory mediators released acutely after ischemia-reperfusion by the blood-brain barrier's epithelial layer may provoke astrocytes to release cytokines and S100b (37, 38). Berger et al (21) found that initial and peak serum S100b concentrations were increased in children with hypoxicischemic injury and traumatic brain injury (TBI), but children with TBI peaked earlier (6 vs 9 hr). In our study, S100b was the most robust biomarker to classify outcome at all time points measured with sufficient sample size. S100b was also found to be superior to NSE in classifying outcome in a study in adults with CA (39). In children with CA, Topjian et al (11) found that serum S100b concentrations classified survival but not favorable versus unfavorable outcome when measured at 48 and 72 hours, whereas our study found all time points to be associated with neurological outcome and survival, but the reasons for this are unclear. S100b consistently peaked prior to the neuronal and myelin biomarkers. It is possible that the use of different enzyme-linked immunosorbent assay kits to measure serum biomarkers contributed to these different results.

Peak serum NSE concentrations occurred between days 1 and 2 after CA and tended to remain increased for up to a week or longer in children who fared poorly. The later appearance and often prolonged release of NSE in serum may reflect neuronal cell death from the instigating event as well as ongoing secondary cell death from apoptosis (40). This finding of delayed neuronal death has been reported in classic studies of global brain ischemia (41). Serum NSE has been evaluated in neonates, children, and adults with hypoxic-ischemic injury and correlates with severity of injury and classifies outcome (22, 42–47). Notably, serum NSE was endorsed by the American Academy of Neurology as an early clinical marker to classify outcome (48). However, this recommendation was prior to hypothermia becoming standard of care in adults with CA due to ventricular arrhythmia, inviting reevaluation. Additionally, serum NSE is responsive to therapy. In adults surviving CA, NSE concentrations were decreased in subjects randomized to hypothermia compared with subjects in the normothermic group. Lower NSE concentrations were associated with improved gross outcome at 6 months in the hypothermia group and trended toward improved cognitive and neurophysiological scores in the authors' follow-up study (15, 49).

MBP, accounting for 30% of protein in the myelin sheath, peaked late in serum after CA in this study, similar to previous studies in TBI where it remained increased for up to 2 weeks in subjects with unfavorable outcome (14, 50). MBP has been previously documented in the cerebrospinal fluid in children after severe hypoxic insult (51). MBP's relatively late peak after pediatric CA is consistent with white matter being more resistant to ischemia when compared with gray matter (52, 53). The pathophysiology of myelin injury after CA is unknown, but the presence of white matter injury in the splenium of the corpus callosum suggests a role for Wallerian degeneration after adult CA (54). Focal and diffuse white matter injury also occurs after neonatal asphyxia (55).

Initial serum S100b and NSE concentrations were inversely correlated with age in this study. Notably, similar to previous reports, S100b concentrations at later time points also displayed this trend, but NSE did not (56). However, population norms have been established for S100b that may allow for its use in all age groups (57). Unlike pediatric TBI, younger age was not inversely correlated with MBP concentrations

TABLE 2. Area Under the Curve for Serum and Clinical Biomarkers to Classify Favorable Versus Unfavorable Outcome and Mortality at 6 Months

				95% CI	
Biomarker (Outcome)	Area	SE	*p*	Lower	Upper
NSE (favorable vs unfavorable)	0.859	0.032	**< 0.001**	0.796	0.922
NSE (mortality)	0.787	0.033	**< 0.001**	0.721	0.852
S100b (favorable vs unfavorable)	0.955	0.017	**< 0.001**	0.922	0.987
S100b (mortality)	0.908	0.021	**< 0.001**	0.866	0.950
MBP (favorable vs unfavorable)	0.732	0.043	**< 0.001**	0.647	0.817
MBP (mortality)	0.727	0.037	**< 0.001**	0.654	0.799
CPR-ROSC (favorable vs unfavorable)	0.642	0.086	0.121	0.474	0.811
CPR-ROSC (mortality)	0.696	0.086	**0.032**	0.528	0.865
First lactate (favorable vs unfavorable)	0.749	0.074	**0.006**	0.604	0.893
First lactate (mortality)	0.809	0.079	**0.001**	0.654	0.964
First blood pH (favorable vs unfavorable)	0.752	0.074	**0.006**	0.608	0.896
First blood pH (mortality)	0.736	0.091	**0.009**	0.558	0.915

NSE = neuron-specific enolase, MBP = myelin basic protein, CPR = cardiopulmonary resuscitation, ROSC = return of spontaneous circulation.
Bolded *p* values < 0.05.

TABLE 3. Best Sensitivity and Specificity for Serum Biomarkers to Classify Favorable Versus Unfavorable Outcome at 6 Months

Biomarker	Concentration (ng/mL) Favorable/Unfavorable Outcome	Sensitivity	Specificity	Positive Predictive Value	Negative Predictive Value
S100b					
24 hr (best sensitivity)	0.001	0.96	0.29	67.6	83.3
24 hr (best specificity)	0.128	0.40	1.00	100.0	53.1
48 hr (best sensitivity)	0.008	0.96	0.59	77.4	90.9
48 hr (best specificity)	0.283	0.20	1.00	100.0	45.9
NSE					
24 hr (best sensitivity)	2.15	0.96	0.12	62.5	66.7
24 hr (best specificity)	53.10	0.20	1.00	100.0	45.9
48 hr (best sensitivity)	0.48	0.96	0.18	63.2	75.0
48 hr (best specificity)	76.71	0.24	1.00	100.0	47.2
MBP					
24 hr (best sensitivity)	0.08	1.00	0.18	65.0	100.0
24 hr (best specificity)	5.83	0.04	1.00	100.0	40.5
48 hr (best sensitivity)	0.05	1.00	0.06	61.0	100.0
48 hr (best specificity)	5.43	0.12	1.00	100.0	43.6

NSE = neuron-specific enolase, MBP = myelin basic protein.

at any time point despite having less brain myelination developmentally (58).

Clinical variables associated with outcome after pediatric CA include duration of pulselessness, first blood gas pH upon ROSC, motor and pupillary examination at 24 hours after ROSC, and EEG (10, 59–62). Remarkably, serum NSE and S100b performed better than clinical variables in discriminating both mortality and favorable versus unfavorable outcome. All three biomarkers and first blood pH performed slightly better using favorable versus unfavorable outcome while CPR-ROSC duration and first lactate had superior AUC for mortality.

Evidence suggests that each brain disease requires separate studies to determine their accuracy and cutoff values with and without treatments such as hypothermia to optimize their use. Our findings, in particular the robust ROC results, strongly suggest the need for validation in a larger sample of subjects and consideration for a biomarker panel to maximize accuracy of outcome classification. The ultimate objective would be the development of a point of care test that can be used for rapid results at the bedside for clinical and research purposes.

Study Limitations

There were several limitations to the design of this study. Laboratory, imaging, and EEG studies were not mandated in this study. The relatively limited sample size precluded multivariate analysis to see if the highly accurate classification rate could be improved on by modeling a panel of serum and clinical biomarkers or by stratifying by important variables such as location or etiology of arrest. PCPC was assigned in a nonblinded fashion by the study PI and obtained mostly by interviewing the parent or guardian over the phone. There were no assessments performed by a pediatric neurologist in this study. It was only necessary to contact the families of children who survived to 6 months, and the PI was sometimes also part of the clinical team treating study subjects. Finally, PCPC is a gross measure of function and may not accurately depict outcome in infants and young children (63). Despite the use of multiple comparisons, results were consistent across those comparisons, thereby providing evidence that this is not a limitation. Serum biomarker test kits are expensive and not yet readily available as a rapid diagnostic bedside test in the United States. Finally, we recognize that subject temperature may be an important factor in biomarker concentration, but this was not the focus of this report.

CONCLUSIONS

Our preliminary data show that serum biomarkers S100b, NSE, and MBP have potential to aid in guiding treatment decisions and outcome classification of children surviving pediatric CA. Modeling and validation of serum and clinical biomarkers may

TABLE 4. Best Sensitivity and Specificity for Serum Biomarkers to Classify Mortality at 6 Months

Timepoint	Concentration (ng/mL) Favorable/Unfavorable Outcome	Sensitivity	Specificity	Positive Predictive Value	Negative Predictive Value
S100b					
24 hr (best sensitivity)	0.001	0.96	0.29	45.9	100.0
24 hr (best specificity)	0.128	0.40	1.00	100.0	81.3
48 hr (best sensitivity)	0.008	0.96	0.59	81.8	77.4
48 hr (best specificity)	0.283	0.20	1.00	100.0	70.3
NSE					
24 hr (best sensitivity)	2.15	0.96	0.12	48.5	90.0
24 hr (best specificity)	53.10	0.20	1.00	100.0	73.3
48 hr (best sensitivity)	0.48	0.96	0.18	53.6	92.9
48 hr (best specificity)	76.71	0.24	1.00	80.0	67.6
MBP					
24 hr (best sensitivity)	0.08	1.00	0.18	42.5	100.0
24 hr (best specificity)	5.83	0.04	1.00	39.5	60.5
48 hr (best sensitivity)	0.05	1.00	0.06	70.0	71.9
48 hr (best specificity)	5.43	0.12	1.00	100.0	63.4

NSE = neuron-specific enolase, MBP = myelin basic protein.

strengthen early prognostication and assist with risk stratification in future clinical studies.

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