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Exploratory Study of Serum Ubiquitin Carboxyl-Terminal Esterase L1 and Glial Fibrillary Acidic Protein for Outcome Prognostication after Pediatric Cardiac Arrest

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Abstract

Introduction—Brain injury is the leading cause of morbidity and death following pediatric cardiac arrest. Serum biomarkers of brain injury may assist in outcome prognostication. The objectives of this study were to evaluate the properties of serum ubiquitin carboxyl-terminal esterase-L1 (UCH-L1) and glial fibrillary acidic protein (GFAP) to classify outcome in pediatric cardiac arrest.

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Conflict of Interest Statement

The other co-authors have nothing to disclose.

Methods—Single center prospective study. Serum biomarkers were measured at 2 time points during the initial 72 h in children after cardiac arrest (n=19) and once in healthy children (controls, n=43). We recorded demographics and details of the cardiac arrest and resuscitation. We determined the associations between serum biomarker concentrations and Pediatric Cerebral Performance Category (PCPC) at 6 months (favorable (PCPC 1–3) or unfavorable (PCPC 4–6)).

Results—The initial assessment (time point 1) occurred at a median (IQR) of 10.5 (5.5–17.0) h and the second assessment (time point 2) at 59.0 (54.5–65.0) h post-cardiac arrest. Serum UCH-L1 was higher among children following cardiac arrest than among controls at both time points ($p<0.05$). Serum GFAP in subjects with unfavorable outcome was higher at time point 2 than in controls ($p<0.05$). Serum UCH-L1 at time point 1 (AUC 0.782) and both UCH-L1 and GFAP at time point 2 had good classification accuracy for outcome (AUC 0.822 and 0.796), $p<0.05$ for all.

Conclusion—Preliminary data suggest that serum UCH-L1 and GFAP may be of use to prognosticate outcome after pediatric cardiac arrest at clinically-relevant time points and should be validated prospectively.

Keywords

Biomarker; pediatric; brain injury; outcomes; cardiac arrest

Introduction

Neurologic injury is the leading cause of death in children with cardiac arrest^{1,2}. Children surviving cardiac arrest are at increased risk of neurologic morbidity, leading to emotional, cognitive, and functional disabilities³. A reliable test that could inform medical decision-making and/or provide families with meaningful information regarding prognosis would be extremely valuable. Challenges to early prognostication include the ambiguity of the early neurologic examination due to developmental stage, provision of analgesics, sedatives, and neuromuscular blocking agents, safety concerns using advanced technology to assess physiologically unstable patients (i.e., magnetic resonance imaging (MRI)), and that clinically, final outcome may not be conclusive for months or years^{4–6}. Although clinical variables have been associated with outcome after pediatric cardiac arrest, an individual child's risk of neurologic disability is not yet accurately ascertained early after resuscitation.

Serum biomarkers of brain injury can objectively estimate severity of brain injury. Data can inform specific disease pathophysiology and potentially identify new therapeutic targets, examine response to therapy, and assist in outcome prognostication^{7–9}. There are accumulating reports that concentrations of brain-specific biomarkers ubiquitin carboxyl-terminal esterase L1 (UCH-L1), a protein and component of the ubiquitin–proteasome system in neurons and glial fibrillary acidic protein (GFAP), a type III neurofilament protein present in astrocytes can be used to accurately classify outcome following an acute brain insult, but there are no data in pediatric cardiac arrest^{10–17}.

In this single center exploratory study, we examined serum concentrations of UCH-L1 and GFAP in children with cardiac arrest at two time points after return of spontaneous circulation (ROSC) and in a pediatric control group without cardiac arrest and tested their

ability to classify favorable vs. unfavorable outcome at 6 months. We hypothesized that children with unfavorable outcomes would have increased serum biomarker concentrations versus children with favorable outcome.

Methods

Design and Setting

The study was approved by the University of Pittsburgh Institutional Review Board and informed consent was obtained from the subject's parent or guardian. Between November 2009 and September 2011, 19 subjects with cardiac arrest were prospectively enrolled in an RCT (NCT00797680) at the Children's Hospital of Pittsburgh of UPMC for which results have not yet become unblinded (therefore data from both groups were pooled). Standard post-resuscitation care was provided to all children at the discretion of the treating clinicians. Banked serum samples from 43 healthy children without brain insults or other acute illness who received outpatient phlebotomy for routine laboratory testing were used as control group.

Inclusion and Exclusion Criteria

We studied children between the ages 1 week and 17 years who were admitted to the ICU with ROSC after in- or out-of-hospital cardiac arrest. Cardiac arrest 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 phlebotomy. Subjects were excluded if they had a do not resuscitate status, were pregnant, any contraindication for MRI, had other simultaneous acute brain disease (i.e., trauma), or were undergoing brain death evaluation. Subjects were included if Glasgow coma scale score ≥ 8 after ROSC and had therapeutic hypothermia initiated by their ICU attending. Subjects were also excluded from the RCT if they had active hemorrhage or a pre-existing anti-coagulation defect. Post-resuscitation care guidelines used at our institution are published elsewhere¹⁸.

Serum Biomarkers

Three milliliters of blood were collected twice daily (days 1–4) and once on day 7 after ROSC for the parent study. Samples were centrifuged, aliquoted, frozen at -70°C , and batched for analysis. Serum samples for this analysis were taken closest to but not after 24 and 72 hours post-ROSC. Serum UCH-L1 and GFAP were measured in the banked serum samples in duplicate using proprietary ELISAs as previously described (Banyan Biomarkers, Florida, USA)¹⁹. An experienced technician blinded to subject treatment and outcome performed all biomarker measurements. Clinical team members were unaware of the biomarker results. The limits of detection were 0.05 ng/ml for UCH-L1 and 0.1 ng/ml for GFAP.

Data Collection

Data were collected from medical charts using the Utstein template for cardiac arrest, including subject demographics, details about the cardiac arrest and resuscitation, and post-resuscitation care²⁰. We documented the subject's temperature at the time of the blood draw.

Outcome Measures

Subjects were followed until 6 months post-cardiac arrest. The primary objective of this study was to determine the accuracy of serum brain biomarker concentrations to predict favorable (Pediatric Cerebral Performance Category (PCPC) score 1–3) or unfavorable (PCPC 4–6) outcome (including death [PCPC=6])²¹. Pre-arrest PCPC was assigned based on medical records, and 6 month outcomes were obtained either the phone or in person.

Data analysis

Data are presented as median (interquartile range [IQR]) or mean + standard deviation (SD), as appropriate. The data were analyzed for outcome group differences with Fisher's exact tests for categorical variables. Median serum biomarkers were represented graphically by outcome group. Serum biomarker levels were correlated with each other and with subject age and temperature using the Spearman rho test. The Wilcoxon rank sum was used to compare serum biomarker concentration and outcome. Receiver operating characteristic (ROC) analyses were used to evaluate the sensitivity and specificity of the biomarkers. There were no missing biomarker data points. All tests were two-sided and a p-value of < 0.05 was considered to be significant. Data analysis was performed using SPSS version 18.

Results

Study subject and biomarker characteristics

We studied 19 subjects with cardiac arrest and 43 control subjects. Cardiac arrest subjects were older than control subjects (mean \pm SD 6.1 \pm 6.7 vs. 3.7 \pm 3.6 years) (Table 1). Most cardiac arrest events were due to asphyxia and most subjects presented with pulseless electrical activity or asystole as the initial cardiac rhythm. Ten (53%) of the cardiac arrest subjects had unfavorable outcome, including 5 who died. Subjects with unfavorable outcome were more likely to have had unwitnessed events than subjects with favorable outcome ($p=0.001$).

Serum was taken at median (IQR) 10.5 (5.5–17.0) hours post-ROSC for time point 1 and at 59.0 (54.5–65.0) hours post-ROSC for time point 2. Serum UCH-L1 and GFAP concentrations were not correlated with each other ($\rho=0.004$, $p=0.980$). Neither biomarker was associated with subject age ($p>0.05$). Neither biomarker was associated with age (UCH-L1: $\rho = -0.23$, $p = 0.22$; GFAP: $\rho = -0.30$, $p = 0.06$) or temperature (UCH-L1: $\rho = 0.25$, $p = 0.30$; GFAP: $\rho = -0.04$, $p = 0.86$) at the time of biomarker assessment.

Serum UCH-L1 performance

Serum UCH-L1 concentration was increased in subjects with cardiac arrest with favorable and unfavorable outcome versus controls at both time points (both $p < 0.05$) (Table 2 and Figure 1a).

Among subjects with cardiac arrest, serum UCH-L1 was not different among outcome groups at time point 1 (0.26 [0.15, 0.85] vs. 0.21 [0.11, 0.23] ng/ml, $p=0.315$). Serum UCH-L1 was increased in subjects with unfavorable versus favorable outcome at time point 2 (0.59 [0.27, 1.12] vs. 0.15 [0.12, 0.18] ng/ml, $p=0.006$).

Serum GFAP performance

Levels of serum GFAP at time point 1 were increased in controls vs. cardiac arrest subjects with favorable outcome (0.02 [0.00, 0.05] vs. 0.00 [0.00, 0.00] ng/ml, $p=0.016$) but concentrations were below the limits of detection for both groups (Table 2, Figure 1b). Serum GFAP in subjects with unfavorable outcome did not differ from controls at time point 1 (0.00 [0.00, 2.13] ng/ml, $p=0.632$) but was increased vs. controls at time point 2 (2.23 [0.73, 16.52] ng/ml, $p < 0.001$).

Serum GFAP was not different between outcome groups at time point 1, but subjects with unfavorable outcome had increased serum GFAP versus subjects with favorable outcome at time point 2 (2.23 [0.73, 16.52] vs. 0.00 [0.00, 0.49] ng/ml, $p = 0.001$).

Receiver operating characteristics of serum UCH-L1 and GFAP to prognosticate outcome

The area under the curve (AUC) [95% CI] for serum UCH-L1 to classify 6 month outcome at time points 1 and 2 were 0.782 [0.638–0.925] and 0.822 [0.702–0.942], respectively ($p<0.05$ for both) (Table 3). The AUC for serum GFAP was 0.408 [0.238–0.578] ($p>0.05$) and 0.796 [0.642–0.950] at time points 1 and 2 ($p<0.05$).

Discussion

This is the first study assessing the use of the neuron biomarker UCH-L1 and the astrocyte biomarker GFAP in children after cardiac arrest. We found that serum UCH-L1 in the first 3 days post-cardiac arrest distinguished children having cardiac arrest from healthy children while serum GFAP discerned cases versus controls only in children with unfavorable outcomes at the second time point. Similar to serum neuron specific enolase (NSE)'s time trajectory after pediatric cardiac arrest, both serum UCH-L1 and GFAP significantly discerned favorable versus unfavorable outcome groups at the later sampling time point.

Prior small clinical studies show that UCH-L1 and GFAP have promise in assessing the severity of illness and classifying outcome across various age groups and types of brain insults. In one of the earliest clinical studies to assess GFAP concentrations after birth asphyxia, CSF concentrations of GFAP as well as astrocyte marker S100b and neuronal marker NSE distinguished infants with hypoxic ischemic encephalopathy (HIE) ($n=22$) from healthy infants ($n=8$) and levels correlated significantly with outcome at one year²². In a study matching neonates with and without birth asphyxia, serum GFAP levels in the first four days were increased in neonates with HIE versus those without HIE and levels also correlated with brain MRI lesions consistent with HIE¹³. Similarly, in another pilot study of infants with HIE, umbilical cord GFAP and UCH-L1 concentrations discerned between babies with mild, moderate, and severe HIE²³. In this study, serum GFAP levels peaked between 78–96 hours while UCH-L1 was highest at birth in cord blood. Only serum GFAP significantly predicted neurological outcome in this study. Next, Douglas-Escobar, et al found that early (first 24 h) serum UCH-L1 levels were increased in infants with moderate-severe HIE compared to healthy infants and that levels correlated with the 10 minute Apgar score, a component of the Sarnat score that categorizes severity of encephalopathy, lending support to its use as stratification tool for prospective research studies¹⁶. There are no early

correlates to the Sarnat score that predict severity of encephalopathy for cardiac arrest outside of the newborn period, but a panel of serum biomarkers and clinical variables known to be associated with outcome would be useful to clinicians and researchers^{5,9}.

Serum GFAP has been investigated for prognostication qualities in adults with cardiac arrest. Serum GFAP distinguished subjects with favorable vs. unfavorable outcome in 44 adults with cardiac arrest, with best prediction accuracy occurring at 24 hours in adult kept normothermic and at 48 hours in adults who received hypothermia post-cardiac arrest¹⁴. In another pilot study (n=31) of adults with cardiac arrest, serum S100b had superior prognostication accuracy versus NSE and GFAP²⁴. Serum biomarker trajectories in this study were similar to our findings in pediatric cardiac arrest in that serum S100b peaked early (24 h) and NSE and GFAP performed better at later time points (48–72 h)¹⁸. Of note, Mortberg et al did not report the number of patient samples available at later time points, in which a smaller sample size due to drop out from early mortality may have contributed to its weaker performance since there was a trend of higher GFAP levels in subjects with worse outcomes. Similarly, in Hayashida et al, serum GFAP within first 24 hours of cardiac arrest was not predictive of outcome, but the sample size was small in addition to the relatively early time sampling time²⁵. A larger, more recent study of 125 adults with cardiac arrest showed that while serum GFAP predicted outcomes, it was not superior to NSE or S100b, and when placed in an inclusive model, addition of GFAP did not improve predictive accuracy²⁶. It should be noted that although neurologic injury is the leading cause of death after cardiac arrest, subjects also succumb from cardiovascular failure or multiorgan dysfunction, so a prognostication panel may benefit from inclusion of other organ biomarkers. In summary, serum GFAP on days 2–3 shows promise across various age groups with hypoxic-ischemic insult to prognosticate outcome. To our knowledge, there are no studies of serum UCH-L1 in adults with cardiac arrest.

For comparison, serum and cerebrospinal fluid (CSF) GFAP and UCH-L1 prognostication accuracy has been examined subjects with traumatic brain injury (TBI). In general, peak biomarker concentrations occur earlier after TBI versus hypoxia-ischemia. Serum and CSF UCH-L1 levels were predictive of outcome and concentrations highest at 6 hours post-TBI in adults^{27,28}. Serum GFAP levels in adults with severe TBI were associated with severity of illness as measured by Glasgow Coma Scale score, and increased levels were associated with the presence of brain CT lesions, mortality, and the need for neurosurgical intervention^{15,19}. Further illustrating differences between brain pathophysiology and biomarker trajectory, serum UCH-L1 and GFAP peaked earlier after pediatric TBI patients versus cardiac arrest²⁹. This suggests that the timing, ROC cutoffs, and/or specific biomarkers used may differ between hypoxic-ischemic and traumatic brain insults.

Serum UCH-L1 and GFAP performance characteristics at the two time points assessed in this study were similar to those seen for serum NSE in children with cardiac arrest in prior reports by our group and others, with best performance occurring between days 2 and 3 post-ROSC^{8,18,30}, paralleling the time period of early and delayed neuronal death and influx of and activation of microglia following hypoxic-ischemic insult^{31–34}. Interestingly, serum GFAP performed best on day 2–3 as compared to another astroglial protein S100b serum level, which peaks on day 1–2. These differences could relate to a number of factors such as

1) the initial concentration of each marker present in the tissue, 2) differences in biomarker location in the astroglia, 3) differences in biomarker half-life, 4) differential contributions of astrocyte death vs biomarker release, 4) a possible contribution of GFAP induction in injured glia to the ultimate levels observed, and 5) differential effects of tissue perfusion and/or glymphatic clearance on biomarker levels³⁵⁻³⁷.

Serum GFAP was undetectable in control subjects and cardiac arrest subjects with favorable outcome while increased in subjects with unfavorable outcome, demonstrating potential utility as an early biomarker for prognostication or for severity of illness stratification; its use as a theragnostic tool is unknown. A clinical research study is underway to validate a point of care test for serum UCH-L1 and GFAP to predict brain CT lesions in adult TBI (NCT01426919). Relating to our current report, positive findings would strongly support evaluation in a panel with clinical variables and tests for use in prognostication in children or adults after cardiac arrest.

Study limitations

This preliminary study used banked serum from a parent study. We did not assess the performance of the all time points collected, which could better inform on potential utility for either theragnostic use or to stratify subjects for clinical trials, but instead chose clinically relevant time points for practical and financial constraints. PCPC was assigned in a non-blinded fashion by the study PI and is a gross measure of function. Due to the limited sample size, were unable to compare the ability of serum biomarkers to classify outcome compared with clinical variables such as blood lactate or duration of resuscitation. Our study does not include data on long-term outcomes or detailed cognitive function. This study combined treatment groups that were not yet unblinded; therefore any treatment effect on biomarker levels cannot be excluded.

Conclusion

The data in this exploratory study suggest that serum UCH-L1 and GFAP measured at a clinically-relevant time point show promise in prognosticating outcome after pediatric cardiac arrest. Validation in a large, prospective trial and correlation with other clinical variables associated with outcome after pediatric cardiac arrest is needed.

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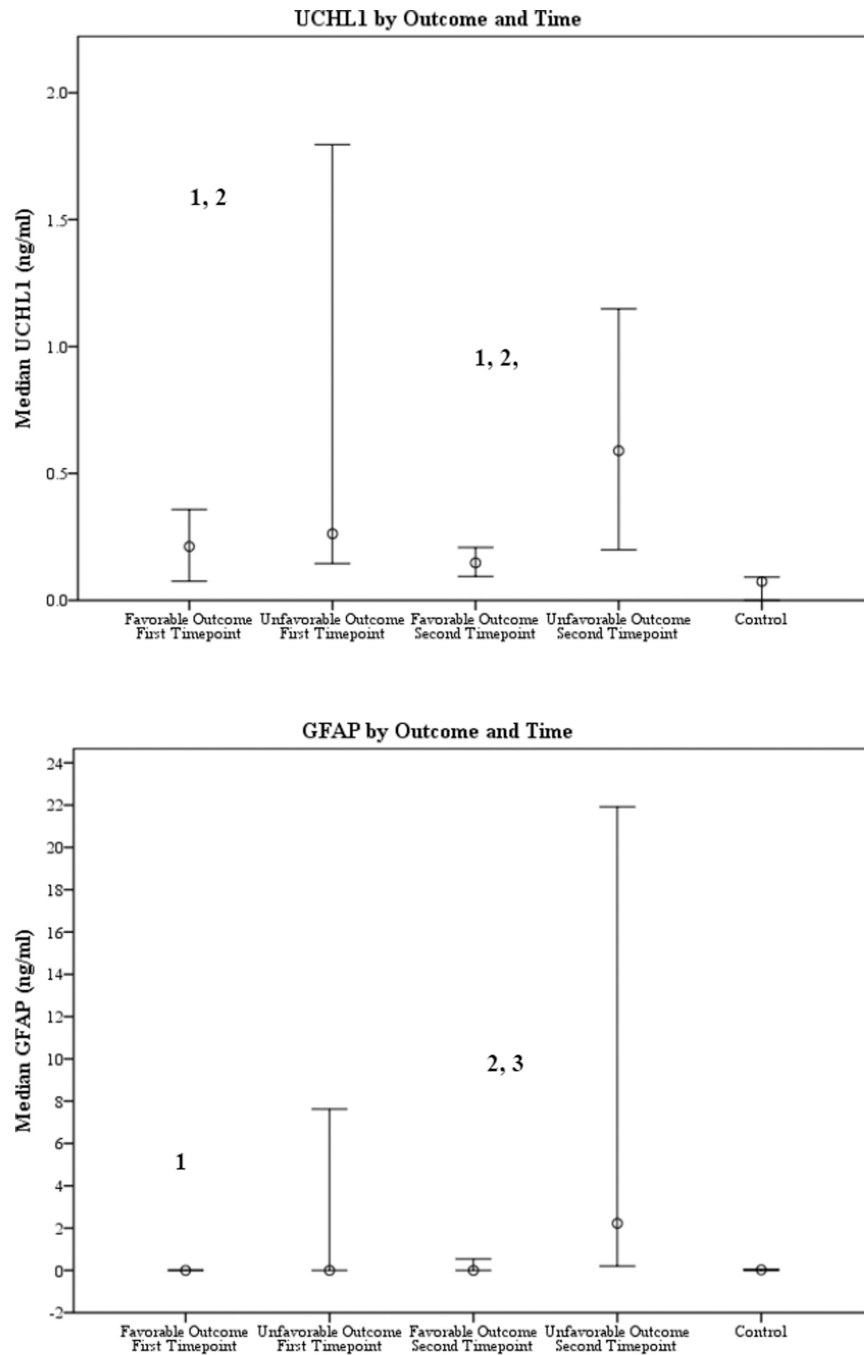


Figure 1.
 1a and 1b. Median serum biomarker concentrations (ng/ml) by outcome and time point. UCH-L1, ubiquitin carboxyl-terminal esterase L1; GFAP, glial fibrillary acidic protein
¹ Control vs. cardiac arrest with favorable outcome, $p < 0.05$
² Control vs. cardiac arrest with unfavorable outcome, $p < 0.05$
³ Cardiac arrest with favorable vs. unfavorable outcome, $p < 0.05$

Table 1

Subject demographics, cardiac arrest details, and outcomes at 6 months.

Mean±SD or n (%)	Controls (n=43)	Cardiac Arrest Overall (n=19)	Cardiac Arrest Favorable (n=9)	Cardiac Arrest Unfavorable (n=10)	p-value Favorable vs. Unfavorable
Age, y	3.7 ± 3.6	6.1 ± 6.7	7.0 ± 6.8	5.4 ± 6.8	0.905
Sex					
Female	18 (41.9)	6 (31.6)	3 (33.3)	3 (30.0)	
Male	25 (58.1)	13 (68.4)	6 (66.7)	7 (70.0)	0.630
Race					
White	25 (58.1)	12 (63.2)	5 (55.6)	7 (70.0)	
Nonwhite	18 (41.9)	7 (36.8)	4 (44.4)	3 (30.0)	0.430
Duration of CPR to ROSC (min)	n/a	16.5 ± 12.4	12.0 ± 6.4	20.0 ± 16.1	0.489
Doses of Epinephrine	n/a	3.0 ± 1.9	3.0 ± 2.4	2.0 ± 1.3	0.497
Hospital LOS, d	n/a	23.0 ± 15.7	16.0 ± 8.6	28.0 ± 20.0	0.549
ICU LOS, d	n/a	11.0 ± 13.9	11.0 ± 8.2	24.0 ± 16.2	0.243
First Rhythm					
PEA	n/a	9 (47.4)	5 (55.6)	4 (40.0)	
Asystole		8 (41.8)	2 (22.2)	6 (60.0)	
Sinus		0 (0.0)	0 (0.0)	0 (0.0)	0.057
VT/VF		1 (5.3)	1 (11.1)	0 (0.0)	
Other		1 (5.3)	1 (11.1)	0 (0.0)	
Etiology					
Asphyxia	n/a	16 (84.2)	6 (66.7)	10 (100.0)	0.087
Cardiac		3 (15.8)	3 (33.3)	0 (0.0)	
Defibrillated	n/a	3 (15.8)	6 (66.7)	0 (0.0)	0.087
Witnessed event	n/a	9 (47.4)	8 (88.9)	1 (10.0)	0.001
Bystander CPR	n/a	14 (73.7)	6 (66.7)	8 (80.0)	0.444
Died	n/a	5 (26.3)	0 (0.0)	5 (50.0)	n/a

SD, standard deviation; ROSC, return of spontaneous circulation; PEA, pulseless electrical activity; VT/VF, ventricular tachycardia or fibrillation; CPR, cardiopulmonary resuscitation; LOS, length of stay; ICU, intensive care unit; n/a, not applicable.

Table 2

Serum biomarker concentrations by outcome group and time point.

Median [IQR], ng/ml	Controls (n=43)	Cardiac arrest Overall (n=19)	Cardiac arrest Favorable (n=9)	Cardiac arrest Unfavorable (n=10)	p-values: Control vs. Fav Fav vs. Unfav
Time point 1 UCH-L1	0.07 [0.00,0.10]	0.21 [0.14,0.38]	0.21 [0.11,0.23]	0.26 [0.15,0.85]	0.002 <0.001 0.315
Time point 2 UCH-L1		0.21 [0.13,0.94]	0.15 [0.12,0.18]	0.59 [0.27,1.12]	0.001 <0.001 0.006
Time point 1 GFAP	0.02 [0.00,0.05]	0.00 [0.00,0.00]	0.00 [0.00,0.00]	0.00 [0.00,2.13]	0.016 [/] 0.632 0.156
Time point 2 GFAP		0.54 [0.00,3.11]	0.00 [0.00,0.49]	2.23 [0.73,16.52]	0.515 <0.001 0.001

UCH-L1, ubiquitin carboxyl-terminal esterase L1; GFAP, glial fibrillary acidic protein; IQR, interquartile range; Fav, favorable outcome 6 months after cardiac arrest; Unfav, unfavorable outcome 6 months after cardiac arrest

[/] Serum GFAP concentrations for subjects with cardiac arrest and favorable outcome and controls had levels below detection for the assay

Table 3

Receiver operator characteristic data for serum UCH-L1 and GFAP by time point.

	Area	P-value	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
Time point 1 UCH-L1	0.782	0.001	.638	.925
Time point 1 GFAP	0.408	0.256	.238	.578
Time point 2 UCH-L1	0.822	<0.001	.702	.942
Time point 2 GFAP	0.796	<0.001	.642	.950

UCH-L1, ubiquitin carboxyl-terminal esterase L1; GFAP, glial fibrillary acidic protein

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