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Clinical paper

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.
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.

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 disabilities.[3] A reliable test that could inform medical decision-making and/or provide families with meaningful information regarding prognosis would be extremely valuable.

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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 $\leq$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 elsewhere.[18]

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 h 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^{-1} for UCH-L1 and 0.1 ng ml^{-1} 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 care.[20] 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 ± SD 6.1 ± 6.7 vs. 3.7 ± 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) h post-ROSC for time point 1 and at 59.0 (54.5–65.0) h 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