Severe traumatic brain injury in children elevates glial fibrillary acidic protein in cerebrospinal fluid and serum*

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Objectives: **1) To determine the levels of glial fibrillary acidic protein (GFAP) in both cerebrospinal fluid and serum; 2) to determine whether serum GFAP levels correlate with functional outcome; and 3) to determine whether therapeutic hypothermia, as compared with normothermia, alters serum GFAP levels in children with severe traumatic brain injury (TBI).**

Design: **Laboratory-based analyses; postrandomized, controlled trial.**

Setting: **Four Canadian pediatric intensive care units and a university-affiliated laboratory.**

Patients: **Twenty-seven children, aged 2–17 yrs, with severe TBI (Glasgow Coma Scale score of ≤8).**

Interventions: **Hypothermia therapy (32.5°C) for 24 hrs with cooling started within 8 hrs of injury and rewarming at a rate of 0.5°C every 2 hrs or normothermia (37.0°C).**

Measurements and Main Results: **GFAP was measured in cerebrospinal fluid and serum, using enzyme-linked immunosorbent assay. Levels of GFAP were maximal on day 1 post-TBI, with cerebrospinal fluid GFAP (15.5 ± 6.1 ng/mL) 25-fold higher than serum GFAP (0.6 ± 0.2 ng/mL). Cerebrospinal fluid GFAP normalized by day 7, whereas serum GFAP decreased gradually to reach a steady state by day 10. Serum GFAP measured on day 1 correlated with Pediatric Cerebral Performance Category scores determined at 6 months post-TBI ($\rho = 0.527$; $p = .008$) but failed to correlate with the injury scoring on admission, physiologic variables, or indices of injury measured on computerized tomography imaging. The areas under the receiver operating characteristic curves for pediatric intensive care unit day 1 serum GFAP in determining good outcome were 0.80 (pediatric cerebral performance category, 1–2; normal-mild disability) and 0.91 (pediatric cerebral performance category, 1–3; normal-moderate disability). For a serum GFAP cutoff level of 0.6 ng/mL, sensitivity and specificity were 88% to 90% and 43% to 71%, respectively. Serum GFAP levels were similar among children randomized to either therapeutic hypothermia or normothermia.**

Conclusions: **GFAP was markedly elevated in cerebrospinal fluid and serum in children after severe TBI and serum GFAP measured on pediatric intensive care unit day 1 correlated with functional outcome at 6 months. Hypothermia therapy did not alter serum GFAP levels compared with normothermia after severe TBI in children. Serum GFAP concentration, together with other biomarkers, may have prognostic value after TBI in children. (Pediatr Crit Care Med 2011; 12:319–324)**

KEY WORDS: traumatic brain injury; glial fibrillary acidic protein; biomarker; cerebrospinal fluid; serum; therapeutic hypothermia

***See also p. 362.**

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Traumatic brain injury (TBI) is a leading cause of morbidity and mortality in children and adolescents (1, 2). The ability to determine the relationship between the severity of TBI and the long-term outcome in pediatric intensive care units (PICUs) would help pediatric critical care physicians determine the appropriate level of care and aid early prognostication during rehabilitation. Unfortunately, physiologic grading systems and most neuroimaging modalities offer only limited insight into TBI severity and prognostication. TBI severity is often classified according to the Glasgow Coma Scale, although the utility of this grading system can be compromised in infants and young children (3, 4). Although modern neuroimaging techniques have improved assessment of TBI, these techniques can be costly, require advanced expertise, and may not be available in many locations. In addition, advanced imaging techniques are typically not able to provide an accurate index of secondary cellular injury. These factors have led to considerable interest in biomarkers as prognostic indicators for TBI.

One possible biomarker for TBI is glial fibrillary acidic protein (GFAP), the major cytoskeletal protein of astrocytes (5, 6). GFAP is elevated in cerebrospinal fluid (CSF) and serum of adults with central nervous system (CNS) injury (7–12), and serum levels of GFAP correlate with outcome (11, 12). Serum GFAP seems to be specific for injury to the CNS, as non-CNS traumatic injuries are not associated with elevation of serum GFAP (11). The ability to extrapolate adult data to children is limited, however, since GFAP expression is developmentally and dynamically regulated. To our knowledge, GFAP has not been examined in either CSF or serum of children with TBI. We hypothesized that CSF and serum GFAP would be elevated in children with TBI, and that GFAP levels would correlate with brain injury severity. We therefore examined GFAP levels in relationship to injury severity scoring, physiologic variables, and functional outcome at 6 months postinjury.

Therapeutic hypothermia may provide potential neuroprotection post-TBI, as therapy with hypothermia improves neurologic outcome and survival in rodent models of TBI (13, 14). Although treatment of pediatric TBI with therapeutic hypothermia has been shown to be safe in some studies (15, 16), and meta-analyses of adult trials of hypothermia therapy in TBI have shown promise for use in pediatric TBI (17–19), our recent randomized controlled trial in 17 sites showed no benefit from early use of hypothermia therapy and a trend toward increased mortality (20). We therefore extended our analyses of serum GFAP levels between patients randomized to either therapeutic hypothermia or normothermia.

MATERIALS AND METHODS

Patients. Patients were recruited into the Hypothermia Pediatric Head Injury Trial, which was a randomized controlled trial of 24 hrs of hypothermia therapy in children with severe TBI, after informed consent by a parent or guardian was obtained (20). Serum and CSF samples for this study were gathered from patients at four of 17 study sites. Patients were eligible for study inclusion if they were aged 1–17 yrs, had TBI with a Glasgow Coma Scale score of ≤8 assessed at the a pediatric tertiary hospital, a computerized tomography scan showing acute brain injury, and a requirement for mechanical ventilation. Patients were excluded if they could not be enrolled within 8 hrs postinjury or if they had refractory shock, suspected brain death, intentional injury, prolonged cardiac arrest at the scene of the injury, high cervical spinal cord injury, severe neurodevelopmental disability before the injury, brain injury secondary to a gunshot wound, acute isolated epidural hematoma, or pregnancy.

The Institutional Review Boards at the Hospital for Sick Children (Toronto, ON, Canada), Children's Hospital of Eastern Ontario (Ottawa, ON, Canada), St. Justine's Hospital (Montreal, QC, Canada), Children's Hospital of Winnipeg (Winnipeg, MB, Canada), and Children's Hospital, London Health Sciences Centre (London, ON, Canada) approved sample procurement in this study.

After assessment and stabilization, study physicians randomly allocated patients, using a central telephone-based system that was available 24 hrs a day. The randomization was stratified by center, and patients were randomized to hypothermia therapy or normothermia. Patients in the hypothermia group were cooled to an esophageal temperature of 32.5 ± 0.5°C for 24 hrs and rewarmed at a rate of 0.5°C every 2 hrs. Patients in the normothermia group were maintained at an esophageal temperature of 37 ± 0.5°C for 24 hrs. All patients had placement of an external ventricular drain (21) or a parenchymal intracranial pressure (ICP) monitor and were managed using clinical care guidelines for ICP and cerebral perfusion pressure management (22). External ventricular drains were zeroed to the level of the tragus, and CSF was drained intermittently for 5 mins if ICP was >20 mm Hg. Failure of CSF drainage to reduce ICP resulted in intravenous administration of osmotic agents as per the study protocol (20). Baseline characteristics were documented, including demographic and injury data, Glasgow Coma Scale score, and pediatric trauma score (23). Computerized tomography scan results (hematomas, diffuse axonal injury, cerebral edema, and/or midline shift), highest daily ICPs, and lengths of stay were also recorded.

The primary outcome measure for the study was a blinded assessment of the six-point Pediatric Cerebral Performance Category (PCPC) score: 1 is normal; 2 is mild disability; 3 is moderate disability; 4 is severe disability; 5 is persistent vegetative state; and 6 is death (24, 25). A trained site psychologist assessed each patient, using a scripted telephone interview with a parent or guardian at 6 months postinjury.

Sample Procurement. Sample procurement at all sites followed the study protocol (20). We attempted to collect blood samples from the arterial catheter on a daily basis for as long as the arterial catheter was in place in the PICU. Blood samples were placed in a tube with no anticoagulant, allowed to clot at room temperature for 15 mins, centrifuged at 5000 rpm for 15 mins, divided into 100-μL aliquots, and stored in plastic tubes at −80°C. For patients with an external ventricular drain, CSF samples were taken at the same times as the serum samples, centrifuged, divided into aliquots of 100 μL, and stored in plastic tubes at −70°C. Samples were shipped on dry ice and stored centrally in a biobank at −70°C. Samples were thawed immediately before the GFAP enzyme-linked immunosorbent assays.

GFAP Measurements. All GFAP measurements were made in one laboratory (DDF, London, ON, Canada) by study investigators blinded to patient demographics, severity of injury scores, imaging results, physiologic variables, temperature randomization, and outcome. CSF and serum GFAP were measured using standard enzyme-linked immunosorbent assay technique via a commercially available test kit (Ridascreen Risk Material 10/5, R-Biopharm AG, Darmstadt, Germany). Standard GFAP concentration curves were completed with all sample GFAP measurements and yielded linear correlation coefficients greater than $r^2 = .96$. Sample GFAP measurements were optimized by diluting CSF 1:10 with kit dilution buffer and increasing the primary incubation times to 15 mins and 20 mins for CSF and serum, respectively. Sera from adult healthy controls were tested with patient samples and in all cases were <0.01 ng/mL (n = 5). Although pediatric controls were not tested, GFAP levels of normal children are much lower than those of normal adults (26). All GFAP measurements were carried out in duplicate.

Statistical Analysis. Data were described with mean ± SEM and compared between intervention groups with either Student's *t* test or Mann-Whitney *U* test. Correlations between continuous numerical values were determined with a Pearson product-moment correlation coefficient (*r*), and correlations between GFAP and rank values (i.e., PCPC score at 6 months) were

Table 1. Characteristics of 27 children admitted with severe traumatic brain injury

Age, yrs (range)	10.6 ± 0.9 (2.4–17)
Sex, male (%)	14 (52)
GCS (range)	4.8 ± 0.1 (3–7)
PRISM III (range)	14.9 ± 1.6 (2–31)
PTS (range)	2.7 ± 0.4 (0–6)
Mechanism of injury (%)	
MVC	17 (63)
Bicycle	4 (15)
Fall	2 (7)
Other	4 (15)
Other injuries (%)	
Thoracic	8 (30)
Cardiothoracic	1 (4)
Abdominal	4 (15)
Genitourinary	5 (19)
Fractures/dislocations	23 (85)
Initial CT (head) (%)	
Extradural hematoma	3 (11)
Intracerebral hematoma	20 (74)
Diffuse axonal injury	14 (52)
Cerebral edema	21 (78)
Midline shift	6 (22)
Highest ICP, mm Hg (range)	32.3 ± 3.0 (13–80)
Ventilation days (range)	10.1 ± 1.4 (1–30)
PICU days (range)	12.6 ± 1.5 (1–32)
PCPC score at 6 mos (range)	2.8 ± 0.3 (1–6)

GCS, Glasgow Coma Score; PRISM, Pediatric Risk of Mortality score; PTS, Pediatric Trauma Score; MVC, motor vehicle collision; CT, computerized tomography; ICP, intracranial pressure; PICU, intensive care unit; PCPC, Pediatric Cerebral Performance Category.

determined with a Spearman rank correlation (ρ). A p value of ≤.05 was considered statistically significant except for multiple comparisons using Student's t test with Bonferroni correction ($p \leq .01$; daily GFAP comparisons between interventions). Data were analyzed using SigmaStat version 3.5 for Windows (San Jose, CA).

Receiver operating characteristic curves were used to determine the optimal cutoff points for serum GFAP in predicting good outcome at 6 months postinjury (areas under the curve >0.7 are generally thought to be useful).

RESULTS

A total of 27 patients with severe TBI were included in this study. Patient characteristics and outcomes are shown in Table 1. GFAP was measured in CSF and sera of children who were admitted to the PICU with TBI. CSF GFAP levels were highest on days 1–2 (day 1 levels, 15.5 ± 6.1 ng/mL) and decreased gradually over 7 days (n = 6) (Fig. 1*A*). Serum GFAP levels were highest on day 1 (0.6 ± 0.2 ng/mL) and gradually decreased over 10 days (n = 27) (Fig. 1*B*).

The relationship between daily serum GFAP levels and PCPC score at 6 months after TBI was examined. Serum GFAP concentrations correlated with PCPC scores (ρ = 0.527, p = .008) (Fig. 2). With the exception of one outlier, low serum GFAP concentrations on PICU day 1 seemed to be associated with a good outcome (i.e., nine of ten patients with PCPC score of ≤2 [normal to mild disability]), despite a Glasgow Coma Scale of ≤8 and computerized tomography imaging evidence of acute brain injury. In contrast, there were no significant correlations between serum GFAP levels and scoring systems, physiologic variables, and the highest ICP. Furthermore, GFAP levels failed to correlate with computerized tomography imaging abnormalities, including hematomas (extradural or intracerebral), diffuse axonal injury, cerebral edema, and/or midline shifts. CSF GFAP levels from six patients failed to correlate with any TBI-related variables.

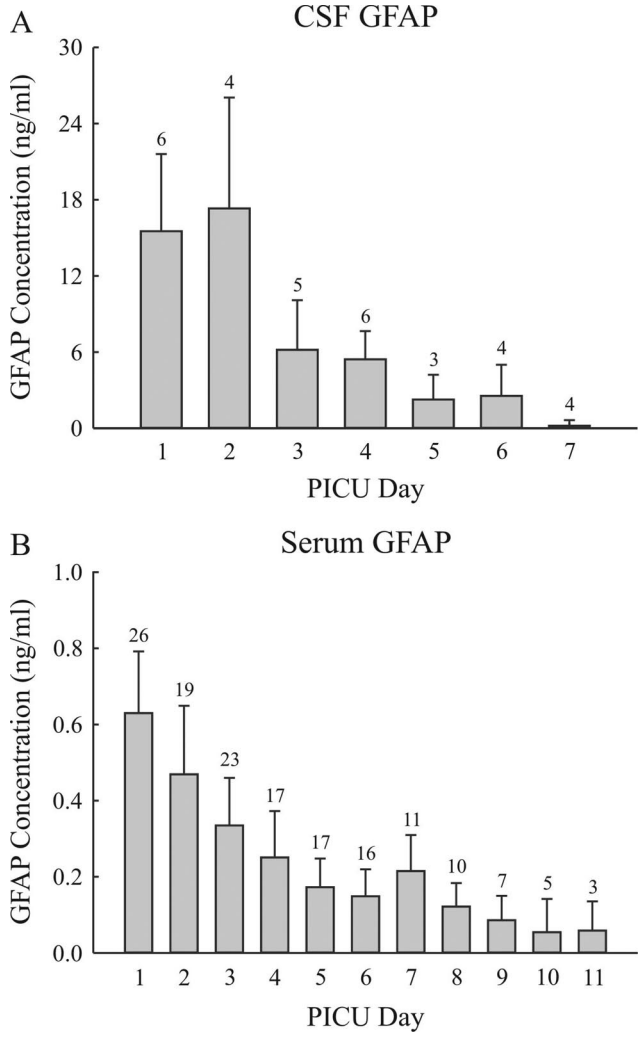

Figure 1. Glial fibrillary acidic protein (*GFAP*) levels measured in both cerebrospinal fluid (*CSF*) and serum after traumatic brain injury. *A*, A plot illustrating CSF GFAP over 7 pediatric intensive care unit (*PICU*) days (n = 6 patients; numbers above *error bar* indicate the number of samples). GFAP was greatest the first few days followed by a gradual decline to baseline over 1 wk. CSF was obtained by external ventricular drain. *B*, A plot demonstrating serum GFAP over 11 PICU days (n = 27 patients; numbers above *error bar* indicate the number of samples). GFAP was greatest on PICU day 1 and gradually declined to a steady state level by days 10–11.

The receiver operating characteristic curves were calculated for serum GFAP levels on PICU day 1 to predict good outcome at 6 months postinjury and yielded areas under the curve for PCPC scores of 1–2 (normal-mild disability) and PCPC scores of 1–3 (normal-moderate disability) of 0.80 and 0.91, respectively (Fig. 3). A serum GFAP level cutoff value of 0.6 ng/mL yielded 90% sensitivity and 43% specificity for PCPC scores of 1–2 and yielded 88% sensitivity and 71% specificity for PCPC scores of 1–3.

To determine whether therapeutic hypothermia alters serum GFAP levels in pediatric TBI patients, daily serum GFAP

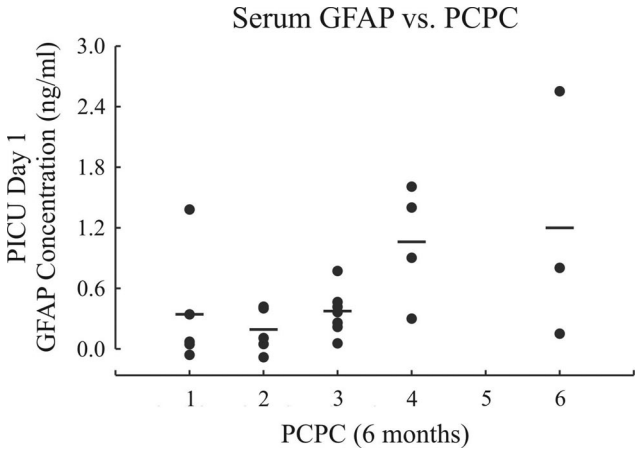

Figure 2. Serum glial fibrillary acidic protein (*GFAP*) levels on pediatric intensive care unit (*PICU*) day 1 correlate with Pediatric Cerebral Performance Category (*PCPC*) scores at 6 months postinjury. The *horizontal bar* represents the mean value for the vertically aligned data points. Data were compared with a Spearman rank correlation test (n = 24 patients; one patient did not have a day 1 serum sample drawn, and two patients were lost to follow-up).

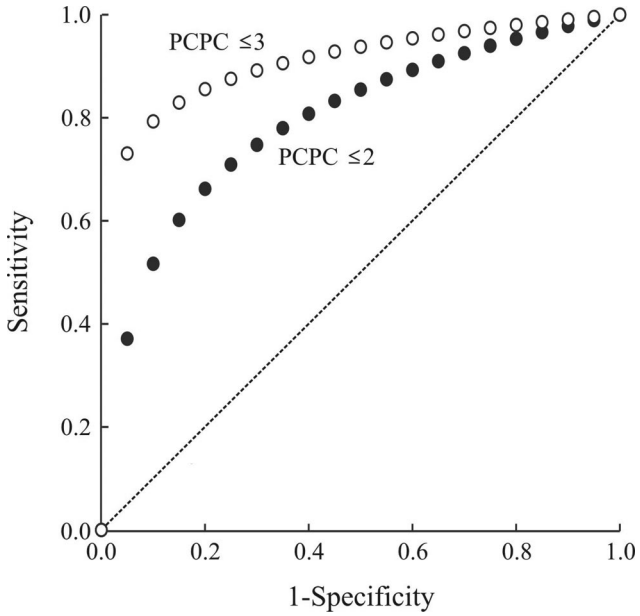

Figure 3. Receiver operating characteristic curves for pediatric intensive care unit day 1 serum GFAP levels to predict good outcome at 6 months postinjury. Data for two ranges of Pediatric Cerebral Performance Category (*PCPC*) scores, PCPC scores of 1–2 (normal-mild disability) or PCPC scores of 1–3 (normal-moderate disability) were fitted with a simple logarithmic curve ($y = 0.09\text{Ln}(x) + 1$).

levels were compared in patients who were randomized to therapeutic hypothermia or normothermia. Patients were well matched, with the single exception that those TBI patients who were treated with hypothermia had significantly greater maximal ICP (39.2 ± 5.1 mm Hg) than those in the normothermia group (25.9 ± 2.3 mm Hg) (Table 2). Daily serum GFAP levels were not significantly different between groups, although there were nonsignificant trends toward lower serum GFAP in hypothermic patients after rewarming and warming (i.e., day 6; $p = .07$) (Fig. 4).

DISCUSSION

In this study, we show elevated GFAP levels in CSF and serum in children with severe TBI within the first 24 hrs of admission to the PICU. Serum GFAP levels on PICU day 1 correlated with outcome as determined by 6-month PCPC scores. Furthermore, serum GFAP levels of <0.6 ng/mL had a sensitivity of 88% to 90% and a specificity of 43% to 71%, depending on the chosen outcome for determining good outcome (PCPC scores of 1–2 [normal-mild disability] or PCPC scores of 1–3 [normal-moderate disability]). Children randomized to therapeutic hypothermia had serum GFAP levels similar to those of children randomized to normothermia.

Table 2. Characteristics of patients with traumatic brain injury randomized to either normothermia or hypothermia

	Normothermia (n = 14)	Hypothermia (n = 13)	*p*
Age, yrs	11.2 ± 1.3	9.9 ± 1.2	.446
Sex, male (%)	8 (57)	6 (46)	.585
GCS	4.6 ± 0.4	5.0 ± 0.4	.517
PRISM III	12.4 ± 2.2	17.7 ± 2.1	.093
PTS	2.4 ± 0.4	3.1 ± 0.7	.415
Highest ICP (mm Hg)	25.9 ± 2.3	39.2 ± 5.1	.022
Ventilation days	10.6 ± 2.0	9.6 ± 1.9	.808
PICU days	13.1 ± 2.1	12.1 ± 2.3	.734
PCPC score at 6 mos	2.5 ± 0.3	3.3 ± 0.6	.449

GCS, Glasgow Coma Score; PRISM, Pediatric Risk of Mortality score; PTS, Pediatric Trauma Score; ICP, intracranial pressure; PICU, intensive care unit; PCPC, Pediatric Cerebral Performance Category.

Serum GFAP concentrations in children with TBI were much greater than those of previous studies in adults with TBI. For example, serum GFAP was six times lower on initial measurements from adults with head injury, with a rapid decrease in GFAP 24 hrs after injury (10). Others (27) reported initial serum GFAP concentrations roughly 100 times lower in adults with TBI. Higher serum GFAP levels in children post-TBI in this study may indicate more severe TBI or a tendency for increased GFAP expression and release from the developing CNS. Other possibilities include slower enzymatic degradation and/or clearance of GFAP from the bloodstream of children. Also, secondary injury was not accounted for in this study and could further increase GFAP levels.

Serum GFAP measured on PICU day 1 correlated with PCPC scores 6 months after TBI, suggesting that serum GFAP concentration may potentially have prognostic value in children with TBI. Furthermore, there seemed to be very good correlation between low serum GFAP and good outcome as determined by a PCPC score of ≤2 at 6 months. However, the serum GFAP concentrations at higher PCPC values seemed to vary considerably between patients with similar outcomes, suggesting that the prognostic utility of serum GFAP concentrations will likely be most valuable as part of a panel of biomarkers. The utility of a biomarker panel

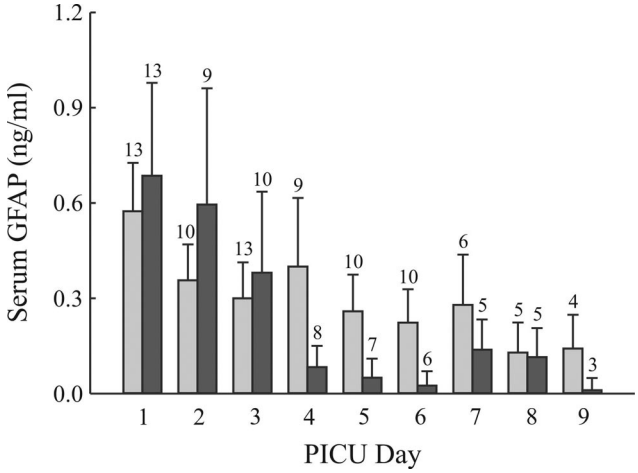

Figure 4. Analyses of serum glial fibrillary acidic protein (*GFAP*) levels measured on consecutive days showing patients randomized to either normothermia (n = 14 of 27 [*light gray bars*]); numbers above *error bar* indicate the number of samples) or therapeutic hypothermia (n = 13 of 27 [*dark gray bars*]; numbers above *error bar* indicate the number of samples). There were no statistically significant differences in GFAP levels between intervention groups. *PICU*, pediatric intensive care unit.

might be important both in gauging the initial injury, as we have done in this study, and in determining secondary injury, such as ischemia (28).

CSF and serum samples in this study were obtained as part of a larger randomized controlled trial of children with severe TBI who underwent therapeutic hypothermia started within 8 hrs of injury. Therapeutic hypothermia that was continued for 24 hrs did not result in improved neurologic outcome (20). Consistent with this functional neurologic outcome, we observed that serum GFAP was not significantly different between patients who received therapeutic hypothermia compared with normothermia. A lack of differences in serum GFAP between groups might have been influenced by the higher peak ICP recorded in the therapeutic hypothermia group; however, serum GFAP concentrations did not correlate with the highest ICP across all patients. It is possible that therapeutic hypothermia altered other temperature-related mechanisms that might influence serum GFAP levels, such as enzymatic degradation or clearance.

Multiple biomarkers of neurologic injury have been studied previously, by our group and others, including the astrocytic calcium binding protein S100β and neuron-specific enolase (29, 30). The utility of S100β and neuronal-specific enolase as reliable prognostic indicators after neurologic injury, however, has been challenged recently (31), particularly after pediatric TBI (32, 33). We therefore examined GFAP as a potential biomarker of neurologic injury after pediatric TBI, because serum GFAP is elevated after TBI in adults (34) but not after traumatic injury beyond the CNS (11). Furthermore, the sensitivity of serum GFAP for predicting neurologic injury after cardiac arrest was similar or better than either serum S100β or serum neuronal-specific enolase (35). A combination of all of these biomarkers, in addition to inflammatory biomarkers as a single panel, might eventually provide accurate neurologic prognostic information. Recently, combinations of serum concentrations of inflammatory and brain-specific biomarkers improved the sensitivity and specificity for prediction of outcome in children with TBI (36). Hence, GFAP is a promising biomarker of brain injury and should be further studied as part of a panel of brain and inflammatory biomarkers for prognostication and potentially to direct acute therapy.

The mechanisms by which GFAP is released into the blood after brain injury are unknown but may relate to absorption of CSF into the venous system or breakdown of the blood-brain barrier. Given the high concentrations of GFAP in CSF, measured in samples obtained from external ventricular drains (21), absorption of CSF likely results in slow accumulation of GFAP in blood. It is also possible that elevated serum GFAP results, at least in part, from traumatic disruption of the blood-brain barrier associated with GFAP leakage into the blood. GFAP is a relatively small astrocytic cytoskeletal intermediate filament with a molecular weight of 40–50 kDa. Astrocytic endfeet are rich in GFAP and directly interact with the endothelial cells of the cerebral blood vessels. Disruption of the blood-brain barrier might also stimulate GFAP expression, and eventual release, into surrounding tissues.

To our knowledge, this report is the first to evaluate GFAP levels after severe TBI in children and one of the few reports to measure GFAP simultaneously in both CSF and serum. CSF GFAP has been previously measured in children with autism, subacute sclerosing panencephalitis, and severe brain degenerative disease (35, 37). Serum GFAP was recently reported to be elevated in a subset of children after septic shock, suggesting that serum GFAP might also be a sensitive marker of septic encephalopathy (38). In this study, we did not measure GFAP levels from control children (we measured serum GFAP from five adult controls that in all cases were <0.01 ng/mL). Control levels of CSF GFAP in children, however, are lower than adults (<0.2 ng/mL) (26), and the levels of serum GFAP in normal children are undetectable with an assay limitation of 0.1 ng/mL (38).

Our study has several limitations. First, our sample size was limited and therefore might not detect small differences in GFAP concentrations between children who received therapeutic hypothermia and those who maintained normothermic. Second, the only outcome measure reported in this study is the PCPC score at 6 months post-TBI. It is possible that a longer period of rehabilitation or the use of more detailed neuromotor and neuropsychological testing might have yielded more robust results. Third, despite GFAP being proposed as one of the most specific of the neurologic injury biomarkers, GFAP has been detected in some peripheral nerves (39). Despite these caveats, our data suggest that low serum GFAP levels are associated with good outcome, a finding that deserves further investigation and validation.

CONCLUSIONS

In this study, we show that GFAP was elevated in serum and CSF of children with severe TBI and that serum GFAP measured on PICU day 1 correlated with functional outcome 6 months after injury. Serum GFAP levels measured in children with severe TBI were similar between those who received either therapeutic hypothermia or normothermia, a finding consistent with a lack of neuroprotective actions when therapeutic hypothermia was started in children within 8 hrs of TBI (20). Our data

suggest that serum GFAP levels may have prognostic value for neurologic injury in children with severe TBI; however, prognostic accuracy will likely be achieved once a panel of multiple biomarkers that includes GFAP is developed and subsequently validated.

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