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Evaluating Glial and Neuronal Blood Biomarkers GFAP and UCH-L1 as Gradients of Brain Injury in Concussive, Subconcussive and Nonconcussive Trauma in a Large Cohort of Pediatric and Adult Trauma Patients Over Time

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ABSTRACT

OBJECTIVES: To evaluate the ability of GFAP and UCH-L1 to detect concussion in children and adult trauma patients with a normal mental status and assess biomarker concentrations over time as gradients of injury in concussive and nonconcussive head and body trauma.

DESIGN: Large prospective cohort study.

SETTING: Three Level One Trauma Centers in the United States.

PARTICIPANTS: Pediatric and adult trauma patients of all ages, with and without head trauma, presenting with a normal mental status (GCS 15) within 4 hours of injury. Rigorous screening for concussive symptoms was conducted. Of 3462 trauma patients screened, 751 were enrolled and 712 had biomarker data. Repeated blood sampling was conducted at 4, 8, 12, 16, 24, 36, 48, 60, 72, 84, 96, 108, 120, 132, 144, 156, 168 and 180 hours post-injury in adults.

MAIN OUTCOMES: Detection of concussion and gradients of injury in children versus adults by comparing three groups of patients: 1) those with concussion; 2) those with head trauma without overt signs of concussion (nonconcussive head trauma controls); and 3) those with peripheral (body) trauma without head trauma or concussion (nonconcussive body trauma controls).

RESULTS: A total of 1904 samples from 712 trauma patients were analyzed. Within 4 hours of injury, there were incremental increases in levels of both GFAP and UCH-L1 from nonconcussive body trauma (lowest), to mild elevations in nonconcussive head trauma, to highest levels in patients with concussion. In concussion patients, GFAP concentrations were significantly higher compared to body trauma controls ($p<0.001$) and

to head trauma controls (p<0.001) in both children and adults, after controlling for multiple comparisons. However, for UCH-L1 there were no significant differences between concussion patients and head trauma controls (p=0.894) and between body trauma and head trauma controls in children. The AUC for initial GFAP levels to detect concussion was 0.80 (0.73-0.87) in children and 0.76 (0.71-0.80) in adults. This differed significantly from UCH-L1 with AUCs of 0.62 (0.53-0.72) in children and 0.69 (0.64-0.74) in adults.

CONCLUSIONS: In a cohort of trauma patients with normal mental status, GFAP out-performed UCH-L1 in detecting concussion in both children and adults. Blood levels of GFAP and UCH-L1 showed incremental elevations across three injury groups, from nonconcussive body trauma, to nonconcussive head trauma, to concussion. However, UCH-L1 was expressed at much higher levels than GFAP in those with nonconcussive trauma, particularly in children. Elevations in both biomarkers in patients with nonconcussive head trauma may be reflective of a subconcussive brain injury. This will require further study.

Key Words:

Biomarkers; Concussion; Mild Traumatic Brain Injury; Subconcussive, Head Trauma, Trauma, Children; Pediatric; Glial fibrillary acidic protein (GFAP), Ubiquitin C-terminal hydrolase (UCH-L1); Blood test

INTRODUCTION

Glial Fibrillary Acidic Protein (GFAP) and Ubiquitin C-terminal hydrolase (UCH-L1) have been evaluated in several studies to determine the need for CT scan and neurosurgical intervention in mild to moderate TBI patients (mmTBI) in adults[1-7] and more recently in children with mmTBI.[8-10] In early 2018, GFAP and UCH-L1 were FDA-approved for clinical use in adult patients with mmTBI to help determine need for CT scan within 12 hours of injury.[11] The approval was based on the ability to find lesions on CT scan but was not approved to diagnose a concussion or a mild TBI. Moreover, it was not approved for use in children.

Following trauma, patients often have a constellation of injuries and it is important that TBI biomarkers indicate brain-specific injury in order to be clinically useful. A number of articles have described how GFAP and UCH-L1 were able to distinguish mmTBI patients from orthopedic controls and motor vehicle crash controls as well as in those TBI patients with negative CT's.[1, 3, 4] In these studies, many trauma control patients were exposed to significant trauma including the acceleration-deceleration vectors of MVCs and substantial falls and both GFAP and UCH-L1 showed a graded response to severity of injury from normal controls to trauma controls, to mild and moderate TBI. Moreover, GFAP has consistently shown very good specificity to brain injury in cases of polytrauma.[3, 12] GFAP has demonstrated the ability to detect intracranial lesions in victims of multiple trauma with mild TBI who had substantial extracranial injuries and fractures.[3]

Importantly, there is a group of individuals with head trauma who have been significantly understudied, and in whom biomarkers are rarely, if at all, examined. These

are people who experience head trauma without symptoms of concussion. They may be classified as having "no injury" or they may represent milder forms of concussion that do not elicit the typical signs or symptoms associated with concussion and are referred to as subconcussive injuries. To date, there is a paucity of studies addressing the effects of subconcussive head impacts following head trauma. The issue of subconcussive trauma has been a particular concern in military personnel[13] and in athletes, as repetitive subconcussive impacts have the potential for long-term deleterious effects.[14-16] Therefore, studying these biomarkers in patients with head trauma without symptoms could provide unique insights into how neuronal and glial biomarkers behave in subconcussive trauma.[17]

There is insufficient data on the diagnostic accuracy of GFAP and UCH-L1 in children and adults in determining which trauma patients with normal mental status have a concussion and how well they perform over time following different degrees of mild head trauma. This study evaluated the diagnostic accuracy of serum glial and neuronal serum biomarkers GFAP and UCH-L1, both individually and in combination, in detecting the presence of a concussion and grading potential subconcussive and nonconcussive brain injury in pediatric and adult trauma patients presenting to the emergency department with a normal mental status (GCS score of 15). Gradients of brain injury were defined by comparing serum biomarker concentrations in three groups of patients: 1) those with concussion; 2) those with blunt head trauma without overt signs or symptoms of concussion (head trauma controls); and 3) those with peripheral (body) trauma without head trauma or concussion (body trauma controls). Additionally, the

temporal profile of GFAP and UCH-L1 were measured over seven days in these three groups in adults.

METHODS

Study Population

This prospective cohort study enrolled a convenience sample of adult and pediatric trauma patients presenting to the emergency departments of three Level I Trauma Centers: a Pediatric Level 1 Trauma Center in Philadelphia, Pennsylvania, a Pediatric Level 1 Trauma Center in Orlando, Florida, and an affiliated Adult Level 1 trauma center in Orlando, Florida. This study was approved by the respective Institutional Review Boards (IRB) of each institution. Informed consent was obtained from patients and/or their legal authorized representatives prior to enrollment and assent was obtained for children between the ages of 7 to 18 years.

Eligibility for concussion patients was determined by the treating physician based on the history of blunt head trauma followed by either loss of consciousness, amnesia, or disorientation (or change in behavior in children) and presenting to the emergency department within 4 hours of injury with a Glasgow Coma Scale (GCS) Score of 15. Eligibility was also prospectively verified by the research team prior to enrollment. Head CT Scans were performed at the discretion of the treating physician. Patients were excluded if they: 1) had no history of trauma as their primary event (e.g. syncope or seizure); 2) had known dementia, chronic psychosis or active CNS pathology; 3) were pregnant; or 4) were incarcerated or 5) had hemodynamic instability.

Both nonconcussive trauma groups, the body trauma control patients (no head trauma and no concussion) and the head trauma control patients (head trauma and no concussion) had a GCS score of 15 presenting to the emergency department with a traumatic mechanism of injury but without concussion. They experienced similar mechanisms of injury as the concussion group and all had a normal mental status since injury (as verified by the research team prospectively by at least two different sources) and had no evidence of acute brain injury or hemodynamic instability. Peripheral (body) trauma controls were primarily composed of orthopedic and soft tissue injuries These patients were carefully screened to ensure they had no loss of consciousness, no amnesia and no alteration in sensorium at any time after injury. The purpose of enrolling nonconcussive body trauma controls and nonconcussive head trauma controls was to have appropriate comparison groups to compare the accuracy of the biomarkers in detecting concussion and simulate the real world challenges faced by clinicians. The head trauma controls provided an opportunity to assess biomarker release in the setting of head trauma without symptoms and the potential for subconcussive brain injury.

Study Procedures

All initial patient assessments were made by board certified adult and pediatric emergency medicine physicians trained by a formal one-hour session on evaluating patient eligibility for the study. Following the initial screening, a meticulous secondary assessment was conducted by the research team. All prehospital and emergency department records were reviewed, patients, families and witnesses (if available) were carefully questioned and the final determination was made by the emergency physician together with the research team. Patient classification was performed prospectively.

Blood samples were obtained within 4 hours of time of injury. Repeated blood sampling was conducted for as long as the patient remained in hospital at 4, 8, 12, 16, 24, 36, 48, 60, 72, 84, 96, 108, 120, 132, 144, 156, 168 and 180 hours after injury and discontinued when discharged. Patient management was not altered by the study. For each blood draw a single vial of approximately 5mL of blood was collected and placed in a serum separator tube and allowed to clot at room temperature. The blood was centrifuged within 30 minutes and the serum was placed in bar-coded aliquot containers and stored in a freezer at -70 degrees Celsius until it was transported to a central laboratory (Banyan Biomarkers Inc, Alachua Florida USA). There, the samples were analyzed in batches using sandwich enzyme-linked immunosorbent assays (ELISA) to GFAP and UCH-L1. Lab personnel running the samples were blinded to the clinical data.

Outcome measures

Performance of GFAP and UCH-L1 was evaluated within 4 hours of injury in both adults and children and over a 7-day period in hospitalized adults. The main outcome measures included the performance of the biomarkers in: 1) detecting the presence of concussion compared to trauma patients without concussion in children and adults (separately and as a whole); 2) assessing gradients of injury defined by comparing three groups of patients a) those with concussion, b) those with head trauma without overt signs of concussion (nonconcussive head trauma controls), and c) those with peripheral (body) trauma without head trauma or concussion (nonconcussive body trauma controls); 3) determining the time course of GFAP and UCH-L1 over 7 days after injury in these three groups in adults.

Statistical Analysis

Descriptive statistics with means medians and proportions were used to describe the data. For statistical analysis, biomarker levels were treated as continuous data, measured in ng/ml and expressed as medians with interquartile range. Data were assessed for equality of variance and distribution. Logarithmic transformations were conducted on non-normally distributed data. Group comparisons for different trauma groups were performed using analysis of variance with multiple comparisons using Games-Howell post-hoc test. Receiver Operating Characteristics (ROC) curves were created to explore the ability of the biomarkers to identify the presence of a concussion. Estimates of the area under these curves (AUC) were obtained (AUC=0.5 indicates no discrimination and an AUC=1.0 indicates a perfect diagnostic test).

A power analysis yielded a sample of 281 cases of concussion and 141 cases without concussion achieves an 80% power to detect a difference of 0.06 between the area under the ROC curve (AUC) under the null hypothesis of 0.81 and an AUC under the alternative hypothesis of 0.75 using a two-sided z-test at a significance level of 0.05. All analyses were performed using the statistical software package SPSS 22.0 (IBM Corporation®, Somers NY).

Biomarker Analysis

Serum GFAP and UCH-L1 levels were measured in duplicate for each sample using a validated ELISA platform (Banyan Biomakers Inc., Alachua Florida USA). For the GFAP assay, the lower limit of quantification (LLOQ) is 0.030ng/ml and upper limit of quantification (ULOQ) is 50ng/ml. The limit of detection (LoD) is 0.008ng/mL. For the UCH-L1 assay, the lower limit of quantification (LLOQ) is 0.100ng/ml and upper limit of quantification (ULOQ) is 9ng/ml. The limit of detection (LoD) is 0.045ng/mL.

Any samples yielding a signal over the quantification or calibrator range were diluted and re-assayed.

Patients and the public were not involved in the design, recruitment or conduct of the study.

RESULTS

Over the study period, 3462 pediatric and adult trauma patients were screened, 1385 met eligibility criteria, 751 with a GCS score of 15 were enrolled and 712 had biomarker data available for analysis (Figure 1). Of those enrolled, 371 (52%) had a concussion, 149 (21%) had nonconcussive head trauma (head trauma controls), and 192 (27%) had nonconcussive body trauma without head trauma (body trauma controls). The flow diagram in Figure 1 describes the distribution of enrolled patients. There were 176 (25%) children and 537 (75%) adults. The distribution of clinical characteristics of all enrolled patients is presented in Table 1. The injury severity score was consistent across groups with median scores of 4.

There were a total of 1904 samples drawn in 712 patients. Patients had serum samples drawn within 4 hours of injury (16 children had samples drawn between 4 and 8 hours) with the average time from injury to serum sample collection of 3.1 hours (SD 0.9). Seven hundred and twelve patients had initial samples drawn at enrollment, 567 patients had samples at 4-hours post-injury, 109 at 8-hours post-injury, 80 at 12 hours post-injury, 73 at 16-hours post-injury; 70 at 20-hours post-injury, 67 at 24-hours post-injury, 46 at 36-hours post-injury, 40 at 48-hours post-injury, 33 at 60-hours post-injury, 32 at 72-hours post-injury, 20 at 84-hours post-injury, 17 at 96-hours post-injury, 8 at

108-hours post-injury, 8 at 120-hours post-injury, 4 at 132-hours post-injury, 6 at 144-hours post-injury, 6 at 156-hours post-injury, and 5 at 168-hours post-injury.

Among body trauma control patients, 229 (60%) samples were below the LLOD and 90 (24%) below the LLOQ for GFAP. In head trauma control patients 144 (38%) samples were below the LLOD and 88 (24%) below the LLOQ for GFAP. In concussion patients, 276 (24%) samples were below the LLOD and 172 (15%) below the LLOQ for GFAP. Among body trauma control patients, 61 (16%) samples were below the LLOD and 70 (19%) below the LLOQ for UCH-L1. In head trauma control patients 34 (9%) samples were below the LLOD and 39 (10%) below the LLOQ for UCH-L1. In concussion patients, 128 (11%) samples were below the LLOD and 103 (11%) below the LLOQ for UCH-L1.

The time course of GFAP and UCH-L1 over a week post trauma is depicted in Figure 2 and contrasted in three groups of patients (concussion, head trauma controls, and body trauma controls). In the concussion patients the serum concentration of GFAP was detectible within 1 hour of injury and reached a peak at 20 hours post-injury and decreased over 72 hours. GFAP concentrations exhibited a slower decline thereafter, but were still detectable at 168 hours (7 days) post-injury. In contrast, UCH-L1 rose very rapidly after injury, reached a peak at 8 hours, decreased quickly to 12 hours and was followed by a slower decline to 60 hours post-injury. Subsequently, UCH-L1, like GFAP, exhibited some smaller peaks and toughs over 7 days and was also detectable at 168 hours post-injury.

In head trauma controls GFAP levels were remarkably lower than in the concussion patients with very slight elevations until 48 hours. The peak appeared at 20

hours (as in the concussion patients) and after 48 hours GFAP levels remained almost undetectable. Interestingly, in head trauma controls UCH-L1 levels peaked within 4 hours and remained lower than concussion patients over 12 hours. Thereafter, UCH-L1 concentrations become quite variable with levels either slightly lower, at par, or slightly higher than concussion patients.

In body trauma control patients, concentrations of GFAP were negligible over time without any appreciable elevations. Initial UCH-L1 levels were slightly elevated but significantly lower than either head trauma or concussion patients. UCH-L1 levels decreased quickly over 12 hours (as it did in the head trauma controls) and levels remained noticeably elevated over the next several days.

The ability of GFAP and UCH-L1, individually and in combination, to distinguish concussion patients from body trauma controls (Table 2) and head trauma controls (Table 3) over time was assessed by calculating the area under the ROC Curve (AUC) at each time-point post-injury . A comparison between concussion and both trauma and head trauma controls can be found in Table 4. When comparing concussion patients to body trauma controls, GFAP demonstrated a range of AUC's between 0.75 (0.39-1.00) to 0.89 (0.69-1.00) and UCH-L1 demonstrated AUC's between 0.50 (0.05-0.23) to 0.78 (0.56-1.00). When GFAP and UCH-L1 were combined, the AUC ranged from 0.75 (0.49-1.00) to 0.90 (0.76-1.00) and closely mimicked the pattern of GFAP. GFAP out-performed UCH-L1 at all time-points. The combination of GFAP and UCH-L1 marginally out-performed GFAP alone at some time-points, however, the differences were not statistically significant.

When comparing concussion patients to head trauma controls, GFAP demonstrated a range of AUC's between 0.62 (0.57-0.68) and 0.86 (0.73-1.00) and UCH-L1 demonstrated AUC's between 0.13 (0-1.00) to 0.61 (0.49-0.73). When GFAP and UCH-L1 were combined, the AUC ranged from 0.33 (0-0.71) to 0.86 (0.73-1.00). GFAP out-performed UCH-L1 at all time-points and out-performed the combination of the two biomarkers at all time-points.

A comparison of the performance of GFAP and UCH-L1 measured within 4 hours of injury in children and adults is shown in Figure 3. There are incremental increases in levels of GFAP and UCH-L1 from nonconcussive body trauma controls to nonconcussive head trauma controls to patients with concussion. In concussion patients, GFAP concentrations were significantly higher compared to body trauma controls ($p<0.001$) and to head trauma controls ($p<0.001$) in both children and adults, after controlling for multiple comparisons. There were also significantly higher levels of GFAP in head trauma controls compared to body trauma controls in children ($p<0.001$) and adults ($p<0.001$). In adults, concentrations of UCH-L1 measured within 4 hours of injury were also significantly higher in concussion patients than body trauma controls ($p<0.001$) and head trauma controls ($p=0.002$). There were also significantly higher levels of UCH-L1in head trauma controls compared to body trauma controls ($P=0.017$). Similarly, in children, concentrations of UCH-L1 were significantly higher in concussion patients than body trauma controls ($p=0.045$). However, there were no significant differences between concussion patients and head trauma controls ($p=0.894$) and between body trauma and head trauma controls in children.

When ROC Curves were compared in children and adults, the AUC's demonstrated that initial GFAP levels were able to distinguish concussion patients from body trauma controls with an AUC of 0.80 (0.73-0.87) in children and 0.76 (0.71-0.80) in adults (Table 2). In contrast, initial UCH-L1 levels distinguished concussion from body trauma controls with a significantly lower AUC of 0.62 (0.53-0.72) in children (p=0.003) and 0.69 (0.64-0.74) in adults (p=0.04) (Table 2). The AUC's for GFAP for distinguishing concussion patients from head trauma controls was 0.64 (0.54-0.73) in children and 0.66 (0.61-0.71) in adults. AUC's were lower for UCH-L1 with an AUC of 0.54 (0.43-0.66) in children (p=0.23) and 0.58 (0.51-0.64) in adults (p=0.04) (Table 3). Overall, when concussion was compared to all (head and body) trauma control cases without concussion, the AUC for GFAP was 0.73 (0.66-0.81) in children and 0.71 (0.67-0.76) in adults. AUC's were significantly lower for UCH-L1 with an AUC of 0.59 (0.51-0.67) in children (p=0.013) and 0.64 (0.59-0.69) in adults (p=0.030)(Table 4). Additionally, we excluded the 35 (11%) patients with intracranial lesions on CT scan and found similar results (Figure 4).

DISCUSSION

This prospective study assessed the diagnostic accuracy of glial and neuronal biomarkers, GFAP and UCH-L1, for detecting concussion in a very large cohort of children and adult trauma patients presenting to three Level I trauma centers. The study investigated the pattern of biomarker release in trauma patients with and without concussion at twenty distinct time-points, making it is among the first and largest studies to assess the temporal profile of these two biomarkers in three groups of trauma patients

with a normal mental status. In both children and adults, GFAP and UCH-L1 concentrations increased incrementally from those with nonconcussive body trauma to those with nonconcussive head trauma with highest levels in patients with concussion. GFAP showed very distinct patterns of release in all three groups, whereas UCH-L1 demonstrated similar patterns of release in all three groups but at much higher concentrations in both nonconcussive trauma groups. There were significant differences between the three groups controlling for multiple comparisons for both biomarkers in adults, however, UCH-L1 could not distinguish concussive from nonconcussive head trauma in children.

When the time course of GFAP and UCH-L1 was contrasted in three groups of patients (concussion, head trauma controls, and body trauma controls), GFAP showed a clear increase over the first 20 hours post-injury and decline from 20 to 72 hours in concussion patients and was still detectable at 7 days post-injury making it potentially useful over a week from injury. Although GFAP was mildly elevated in head trauma without concussion, the expression was very low and very early compared to concussion. In body trauma control patients, concentrations of GFAP were negligible over all time points suggesting very good specificity for concussion. These results are consistent with previous studies showing how robust it is in multiple trauma.[3,4] In contrast, UCH-L1 rose more rapidly after concussion than GFAP, peaking within 8 hours and steadily decreasing from 12 to 60 hours. Unexpectedly, UCH-L1 was much higher in the head trauma control group compared to GFAP at all time-points from injury over seven days. Even more surprising, was that UCH-L1 was elevated in body trauma too and showed a similar pattern of release as head trauma control patients. Possible explanations for UCH-L1

elevations in control patients include that 1) UCH-L1 may not be completely brain specific and is released from other organ or tissue trauma, or 2) UCH-L1 is an ultrasensitive marker of any neuronal disruption that may occur from impacts to the body that jostle the brain.

Given that GFAP appears to be so brain specific and that it also showed low level elevations in the first 48 hours following head trauma without concussion symptoms (head trauma controls), these elevations may represent milder forms of concussion that do not elicit typical signs or symptoms associated with concussion. These injuries may be irrelevant or they may represent important trauma that is just below the level of clinical detection and referred to as subconcussive trauma. To date, there is a paucity of studies addressing the effects of subconcussive head impacts following head trauma. Studies in athletes have documented that both clinically diagnosed concussion and subconcussive traumas can induce similar changes in brain structure and functions on brain imaging.[18, 19] These changes include alterations in white matter and cerebrovascular integrity, blood flow, brain activation during working memory tasks, resting-state functional connectivity, and brain chemistry as measured by various forms of magnetic resonance imaging (MRI).[18, 20, 21] The effect of thousands of subconcussive impacts has the potential for long-term deleterious effects on brain function and neurodegeneration in select individuals.[14, 15] In athletes UCH-L1 has shown elevations in both concussive[22] and subconcussive trauma.[23]

Concussion is a clinical diagnosis that could benefit from a relatively noninvasive complementary tool such as a blood test.[8, 9, 24-26] Based on these results, the potential utility of GFAP to distinguish concussion from body trauma controls over 7 days post-

injury was fair to excellent with AUC's of 0.75 to 0.89, and UCH-L1's ability was guarded and variable with AUC's from poor to good depending on timing of samples (AUC's of 0.54 to 0.78) with earlier samples being better. The combination of the both biomarkers did not significantly improve concussion detection among trauma control patients. The distinction between head trauma patients with and without concussion was not as robust as with body trauma controls. GFAP performed with fair to very good AUC's (0.62 to 0.86) over the week post-injury, with optimal performance between 24 to 96 hours. The ability of UCH-L1 to distinguish concussion from head trauma control patients was very poor with AUC's of 0.13 -0.61 and did not contribute to or improve the performance of GFAP alone.

Since it is not uncommon for patients who have suffered a concussion or mild TBI not to seek immediate medical attention, understanding when to use the biomarkers for detection of injury is critical. In the context of developing a point-of-care test, UCH-L1's early and rapid rise could be useful in the early post injury setting such as in the ambulance, on the playing field or on the battle field. The longer half-life of GFAP makes it a very favorable marker to use in both the acute and subacute phases of injury as it is able to detect concussion for up to 7 days after injury.

The authors recognize that there are limitations to this study. This study addressed diagnosis of concussion in the acute care setting and did not describe long-term outcome in these patients. The main outcomes used in this study reflect current standards of practice and accepted definitions of concussion. However, future studies to better define the severity of concussion and mild TBI need to be pursued, particularly when neuroimaging is negative. Accordingly, we performed an analyses of patients with

negative neuroimaging acutely and found no significant differences in the results whether we included those with positive scans or not. The number of samples available for analysis decreased over the course of the study. This reflects the challenge of obtaining samples over time in patients with less severe injuries because they are not hospitalized as long. However, there were a large number of patients without TBI and patients with mild TBI who were captured in our longitudinal sample because they were admitted for other injuries. Important next steps will be to capture samples within minutes of injury. Uninjured controls were not included in this analysis as the concentrations of these two biomarkers have already been well characterized in uninjured normal control patients.[1, 2]

CONCLUSION

In a cohort of trauma patients with normal mental status, GFAP out-performed UCH-L1 in detecting concussion in both children and adults. Blood levels of GFAP and UCH-L1 showed incremental elevations across three injury groups, from nonconcussive body trauma, to nonconcussive head trauma, to concussion. However, UCH-L1 was expressed at much higher levels than GFAP in those with nonconcussive trauma, particularly in children, suggesting that UCH-L1 is either not completely brain specific or ultrasensitive to subtle impacts. Each biomarker exhibited a distinct temporal profile in each trauma group over seven days with earlier elevations in UCH-L1 and more consistent and sustained elevations in GFAP. Furthermore, elevations in both biomarkers in patients with nonconcussive head trauma may be reflective of a subconcussive brain injury. The stage is set for future studies to verify these findings.

KEY MESSAGES

What is already known on this topic

In 2018 serum biomarkers Glial Fibrillary Acidic Protein (GFAP) and Ubiquitin C-terminal hydrolase (UCH-L1) were FDA-approved in adults to guide CT scan ordering in mild to moderate traumatic brain injury. However, their ability to detect concussion in either children or adults has not been determined and there currently exists no objective measure to diagnose concussion acutely after injury. The challenge for clinicians is to detect concussion in the setting of head and/or peripheral trauma when patients have a normal mental status. Having an objective measure of concussion would be very helpful in managing trauma patients.

What this study adds

GFAP out-performed UCH-L1 in detecting concussion in both children and adults within 4 hours of injury. Blood levels of GFAP and UCH-L1 showed incremental elevations across three injury groups, from nonconcussive body trauma, to nonconcussive head trauma, to concussion. UCH-L1 was expressed at much higher levels than GFAP in those with nonconcussive trauma, particularly in children, suggesting that UCH-L1 is either not completely brain specific or ultrasensitive to subtle impacts. Elevations of these biomarkers in nonconcussive head trauma suggests possible subconcussive brain injury. GFAP could be potentially useful to detect concussion for up to a week post-injury.

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Contributors: LP had full access to the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. LP conceived and designed the study. Data was acquired by LP, JR, PG, CFB, CNT, NJA, MAL, CAH, DMG, MRZ, and MM. All authors were involved in the analysis and interpretation of the data. LP drafted the manuscript and all authors were involved in critical revision of the manuscript for important intellectual content. Statistical analysis was conducted by LP. Funding was obtained by LP. Administrative, technical, or material support was provided by LP, PG, MRZ, and MM. The study was supervised by LP, JR, PG, CFB, CNT, NJA, MAL, CAH, DMG, MRZ, and MM. The corresponding author attests that all listed authors meet authorship criteria and that no others meeting the criteria have been omitted.

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Data sharing: Data are not available for sharing at this time.

Transparency: The lead author (LP) affirms that this manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned (and, if relevant, registered) have been explained.

Dissemination declaration: Dissemination of results to participants is not possible/applicable.

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FIGURE LEGEND

Figure 1. Flow diagram of screened and enrolled patients.

Figure 2. Temporal Profile of GFAP and UCH-L1 in three groups of trauma patients

Figure 2a. Temporal Profile of GFAP and UCH-L1 in body trauma control patients. Means with error bars representing standard error of the mean (SEM).

Figure 2b. Temporal Profile of GFAP and UCH-L1 in head trauma control patients. Means with error bars representing standard error of the mean (SEM).

Figure 2c. Temporal Profile of GFAP and UCH-L1 in trauma patients with concussion. Temporal Profile of GFAP and UCH-L1 in trauma patients with concussion. Means with error bars representing standard error of the mean (SEM).

Figure 3. Boxplot comparing initial levels of GFAP and UCH-L1 in three groups of trauma patients: 1) trauma controls without head trauma (controls), 2) head trauma controls without concussion (no concussion); and 3) head trauma with concussion (concussion).

There were 47, 34, and 94 children in each group respectively and 145, 115, and 277 adults patients respectively. Medians with bars representing interquartile range (IQR).

Figure 4. ROC Curves comparing initial levels of GFAP and UCH-L1 (individually and in combination) for predicting a concussion in trauma patients excluding patients with traumatic intracranial lesions on CT.

TABLES

Table 1. Characteristics of Enrolled Patients

Characteristics	*Trauma Patients without Head Trauma and Without Concussion (Trauma Controls)*	*Head Trauma Patients without Concussion (Head Trauma Controls)*	*Head Trauma Patients with Concussion (Concussion)*	*Total*	*Children*	*Adults*
	N=192	*N=149*	*N=371*	*N=712*	*N=175*	*N=537*
Mean age (yrs±SD) Range	33 (±20) (0-83)	32 (±20) (1-79)	32 (±19) (0-78)	32 (±19) (0-83)	9 (±5) (0-17)	40 (±16) (18-83)
Gender (%) Male	115 (60)	91 (61)	248 (67)	454 (64)	122 (70)	332 (62)
Race (%)						
Asian	2 (1)	1 (1)	7 (2)	10 (1)	2 (1)	8 (2)
Black	60 (31)	37 (25)	85 (23)	182 (26)	55 (31)	127 (24)
Hispanic	46 (24)	33 (22)	70 (20)	149 (21)	37 (21)	112 (21)
Native American	3 (2)	0 (0)	0 (0)	3 (<1)	0 (0)	3 (1)
White	76 (40)	75 (50)	196 (53)	347 (49)	76 (43)	271 (51)
Other	5 (3)	3 (2)	13 (3)	21 (3)	5 (3)	16 (3)
Mechanism of Injury (%)						
Motor Vehicle Crash	97 (51)	54 (36)	138 (37)	289 (41)	8 (5)	281 (52)
Fall	53 (28)	29 (20)	100 (27)	182 (26)	92 (53)	90 (17)
Motorcycle	0 (0)	17 (11)	29 (8)	46 (7)	0 (0)	46 (9)
Pedestrian Struck	5 (3)	4 (3)	18 (5)	27 (4)	8 (5)	19 (4)
Bicycle Struck by Vehicle	3 (2)	9 (6)	14 (4)	26 (4)	5 (3)	21 (4)
Fall off Bicycle	1 (1)	1 (1)	8 (2)	10 (1)	4 (2)	6 (1)
Assault	0 (0)	6 (4)	15 (4)	21 (3)	5 (3)	16 (3)
Sports Injury	16 (8)	7 (5)	24 (7)	47 (7)	37 (21)	10 (2)
Other Motorized Vehicle	0 (0)	5 (3)	5 (1)	10 (1)	5 (3)	5 (1)
Other	17 (9)	17 (11)	20 (5)	54 (8)	11 (6)	43 (8)
Loss of Consciousness (%)						
No	192 (100)	149 (100)	89 (24)	430 (60)	115 (66)	315 (59)
Yes	0 (0)	0 (0)	259 (70)	259 (36)	37 (21)	222 (41)
Unknown	0 (0)	0 (0)	23 (6)	23 (3)	23 (13)	0 (0)
Amnesia (%)						
No	192 (100)	149 (100)	207 (56)	548 (77)	117 (67)	431 (80)
Yes	0 (0)	0 (0)	122 (33)	122 (17)	17 (10)	105 (20)
Unknown	0 (0)	0 (0)	42 (11)	42 (6)	41 (23)	1 (<1)
Injury Severity Score (n=629)						
Median (IQR)	4.0 (1.0-4.8)	4.0 (1.0-6.0)	4.0 (2.0-8.0)	4.0 (2.0-8.0)	4.0 (1.0-5.0)	4.0 (2.0-8.0)

Means (95%CI)	4.3 (3.7-5.0)	4.3 (3.7-4.9)	5.8 (5.3-6.3)	5.1 (4.7-5.5)	4.3 (3.6-5.0)	5.3 (4.9-5.7)
Admitted to Hospital (%)	54 (28)	25 (17)	130 (35)	209 (29)	49 (28)	160 (30)
CT Head performed (%)	18 (9)	91 (61)	341 (92)	450 (63)	86 (49)	365 (68)
Intracranial Lesions on CT (%)	0 (0)	0 (0)	36 (11)	36 (8)	14 (17)	22 (6)

Note: Due to rounding, percentages may not add up to 100

Table 2. Area Under the Curve for distinguishing between concussion and **body trauma controls** (no head trauma and no concussion symptoms). Shown is the performance of GFAP and UCH-L1 alone and in combination.

HOURS POST-INJURY	GFAP	UCH-L1	COMBINATION GFAP AND UCH-L1
Enrollment (Children) (n=141)	0.80 (0.73-0.87)	0.62 (0.53-0.72)	0.80 (0.73-0.87)
Enrollment (Adults) (n=422)	0.76 (0.71-0.80)	0.69 (0.64-0.74)	0.78 (0.73-0.82)
Enrollment (Both) (n= 563)	0.76 (0.72-0.80)	0.67 (0.63-0.72)	0.78 (0.74-0.81)
4 hours (n= 452)	0.76 (0.72-0.80)	0.65 (0.60-0.70)	0.77 (0.73-0.81)
8 hours (n=92)	0.82 (0.72-0.91)	0.72 (0.56-0.87)	0.86 (0.75-0.96)
12 hours (n=68)	0.83 (0.73-0.93)	0.74 (0.58-0.90)	0.85 (0.74-0.96)
16 hours (n=66)	0.84 (0.73-0.95)	0.77 (0.62-0.91)	0.86 (0.75-0.97)
20 hours (n=61)	0.82 (0.71-0.94)	0.63 (0.43-0.84)	0.82 (0.70-0.94)
24 hours (n=56)	0.87 (0.76-0.98)	0.74 (0.58-0.90)	0.87 (0.74-1.00)
36 hours (n=35)	0.86 (0.72-1.00)	0.76 (0.51-1.00)	0.90 (0.76-1.00)
48 hours (n=30)	0.85 (0.68-1.00)	0.71 (0.53-0.90)	0.85 (0.65-1.00)
60 hours (n=27)	0.79 (0.57-1.00)	0.70 (0.50-0.90)	0.83 (0.61-1.00)
72 hours (n=25)	0.87 (0.71-1.00)	0.78 (0.56-1.00)	0.94 (0.82-1.00)
84 hours (n=15)	0.89 (0.69-1.00)	0.75 (0.40-1.00)	0.89 (0.69-1.00)
96 hours (n=13)	0.75 (0.39-1.00)	0.54 (0.18-0.90)	0.75 (0.49-1.00)
108 hours (n=6)	n/a	n/a	n/a
120 hours (n=6)	0.80 (0.40-1.00)	0.50 (0.05-0.23)	0.90 (0.62-1.00)
132 hours (n=2)	n/a	n/a	n/a
144 hours (n=4)	n/a	n/a	n/a
156 hours (n=4)	n/a	n/a	n/a
168 hours (n=2)	n/a	n/a	n/a

n/a – data not available

Table 3. Area Under the Curve for distinguishing between concussion and **head trauma controls** (no concussion symptoms) in patients with head trauma. Shown is the performance of GFAP and UCH-L1 alone and in combination.

HOURS POST-INJURY	GFAP	UCH-L1	COMBINATION GFAP AND UCH-L1
Enrollment (Children) (n=128)	0.64 (0.54-0.73)	0.54 (0.43-0.66)	0.63 (0.53-0.72)
Enrollment (Adults) (n=392)	0.66 (0.61-0.71)	0.58 (0.51-0.64)	0.66 (0.61-0.72)
Enrollment (Both) (n= 520)	0.65 (0.60-0.70)	0.56 (0.51-0.62)	0.65 (0.60-0.70)
4 hours (n= 421)	0.62 (0.57-0.68)	0.55 (0.49-0.61)	0.62 (0.57-0.88)
8 hours (n=99)	0.72 (0.61-0.82)	0.61 (0.49-0.73)	0.72 (0.61-0.82)
12 hours (n=74)	0.69 (0.55-0.82)	0.51 (0.37-0.66)	0.67 (0.54-0.80)
16 hours (n=69)	0.76 (0.64-0.88)	0.58 (0.39-0.76)	0.74 (0.62-0.85)
20 hours (n=65)	0.68 (0.53-0.83)	0.41 (0.23-0.59)	0.66 (0.52-0.81)
24 hours (n=64)	0.74 (0.60-0.88)	0.40 (0.24-0.56)	0.72 (0.59-0.86)
36 hours (n=44)	0.76 (0.61-0.91)	0.56 (0.37-0.75)	0.76 (0.61-0.92)
48 hours (n=38)	0.81 (0.67-0.95)	0.52 (0.32-0.73)	0.79 (0.64-0.93)
60 hours (n=31)	0.86 (0.73-1.00)	0.54 (0.34-0.74)	0.86 (0.73-1.00)
72 hours (n=30)	0.78 (0.60-0.95)	0.43 (0.20-0.66)	0.71 (0.51-0.91)
84 hours (n=19)	0.84 (0.65-1.00)	0.37 (0.13-0.62)	0.74 (0.52-0.97)
96 hours (n=16)	0.75 (0.51-0.99)	0.28 (0.02-0.55)	0.65 (0.38-0.91)
108 hours (n=8)	0.67 (0.26-1.00)	0.17 (0-0.47)	0.33 (0-0.71)
120 hours (n=7)	0.80 (0.46-1.00)	0.20 (0-0.58)	0.70 (0.30-1.00)
132 hours (n=4)	0.75 (0.20-1.00)	0 (0-0)	0.50 (0-1.00)
144 hours (n=6)	0.75 (0.20-1.00)	0 (0-0)	0.50 (0.01-0.99)
156 hours (n=6)	0.75 (0.34-1.00)	0 (0-0)	0.50 (0.01-0.99)
168 hours (n=4)	0.75 (0.20-1.00)	0.13 (0-1.00)	0.63 (0-1.00)

Table 4. Area Under the Curve for distinguishing between concussion and **all trauma controls** (head and body controls with no concussion). Shown is the performance of GFAP and UCH-L1 alone and in combination.

HOURS POST-INJURY trauma	GFAP	UCH-L1	COMBINATION GFAP AND UCH-L1
Enrollment (Children) (n=175)	0.73 (0.66-0.81)	0.59 (0.51-0.67)	0.73 (0.65-0.80)
Enrollment (Adults) (n=537)	0.71 (0.67-0.76)	0.64 (0.59-0.69)	0.73 (0.68-0.77)
Enrollment (Both) (n= 712)	0.71 (0.67-0.75)	0.63 (0.59-0.67)	0.72 (0.68-0.76)
4 hours (n= 567)	0.70 (0.66-0.74)	0.61 (0.56-0.65)	0.70 (0.66-0.75)
8 hours (n=109)	0.75 (0.66-0.84)	0.65 (0.54-0.75)	0.77 (0.68-0.86)
12 hours (n=80)	0.73 (0.62-0.85)	0.59 (0.46-0.72)	0.73 (0.62-0.85)
16 hours (n=73)	0.79 (0.69-0.90)	0.64 (0.49-0.80)	0.78 (0.68-0.89)
20 hours (n=70)	0.73 (0.61-0.86)	0.49 (0.33-0.65)	0.72 (0.59-0.84)
24 hours (n=67)	0.77 (0.64-0.89)	0.47 (0.31-0.63)	0.75 (0.63-0.88)
36 hours (n=46)	0.77 (0.63-0.91)	0.59 (0.41-0.77)	0.78 (0.64-0.92)
48 hours (n=40)	0.81 (0.68-0.95)	0.55 (0.37-0.74)	0.80 (0.66-0.93)
60 hours (n=33)	0.85 (0.71-0.98)	0.58 (0.39-0.77)	0.86 (0.73-0.98)
72 hours (n=32)	0.80 (0.64-0.95)	0.51 (0.29-0.73)	0.76 (0.59-0.94)
84 hours (n=20)	0.85 (0.67-1.00)	0.44 (0.18-0.69)	0.77 (0.56-0.98)
96 hours (n=17)	0.75 (0.51-0.98)	0.33 (0.07-0.60)	0.67 (0.41-0.92)
108 hours (n=8)	0.67 (0.26-1.00)	0.17 (0-0.47)	0.33 (0-0.71)
120 hours (n=8)	0.80 (0.48-1.00)	0.30 (0-0.69)	0.77 (0.42-1.00)
132 hours (n=4)	0.75 (0.20-1.00)	0 (0-0)	0.50 (0-1.00)
144 hours (n=6)	0.75 (0.34-1.00)	0 (0-0)	0.50 (0.01-0.99)
156 hours (n=6)	0.75 (0.34-1.00)	0.38 (0-0.85)	0.63 (0.15-1.00)
168 hours (n=4)	0.75 (0.20-1.00)	0.13 (0-1.00)	0.63 (0-1.00)

Figure 1. Flow diagram of screened and enrolled patients

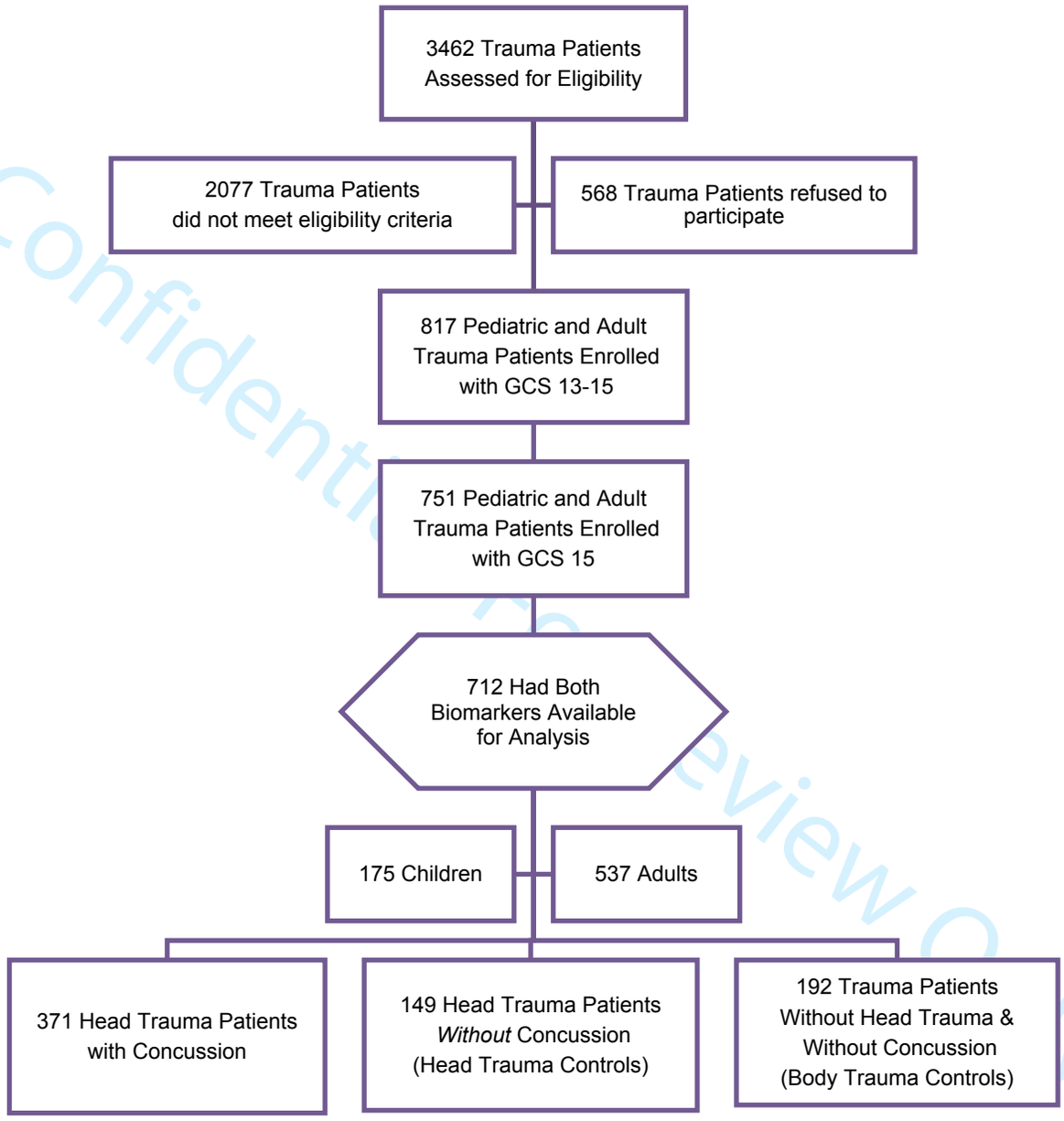


Figure 2. Temporal Profile of GFAP and UCH-L1 in three groups of trauma patients

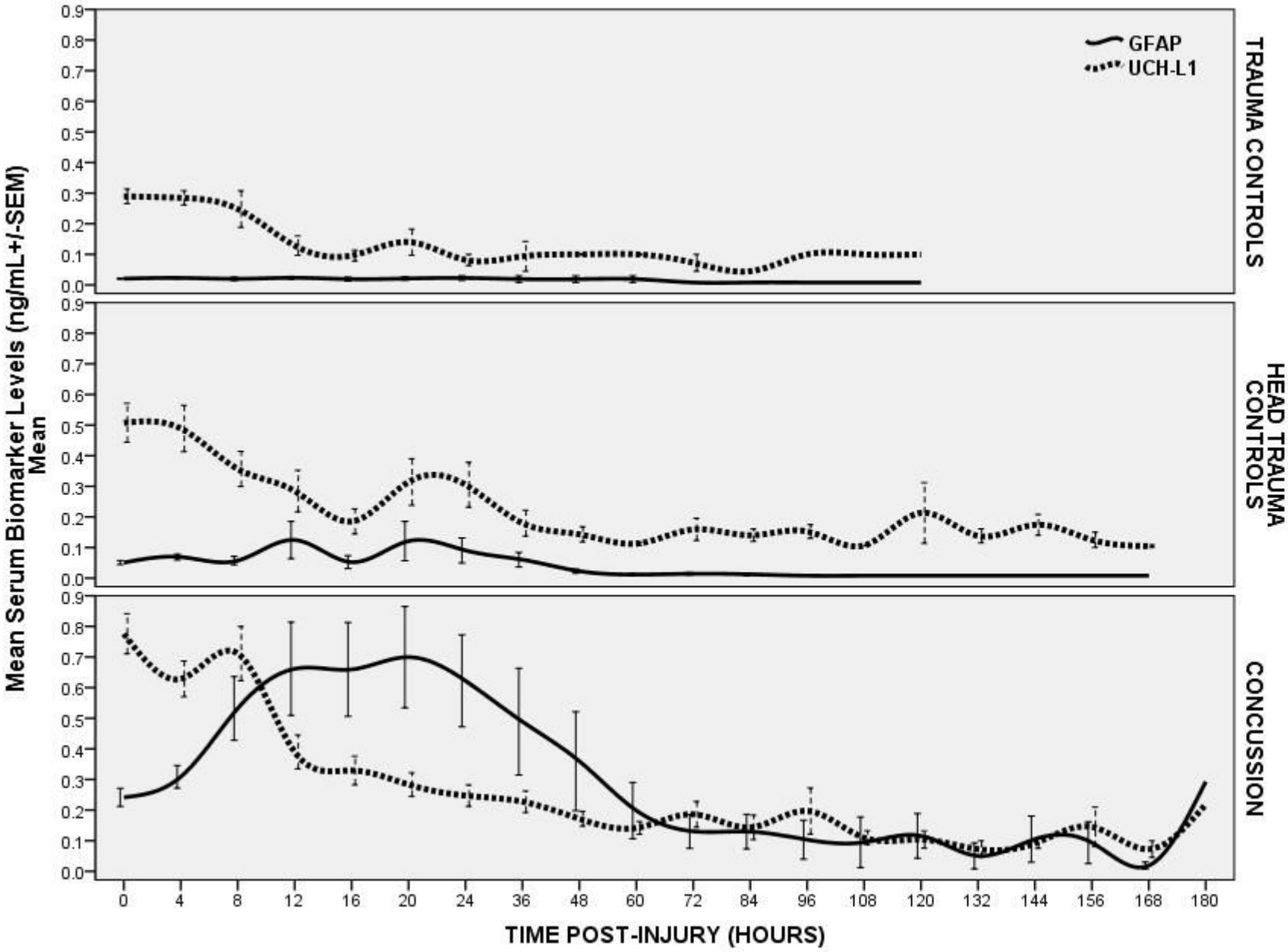
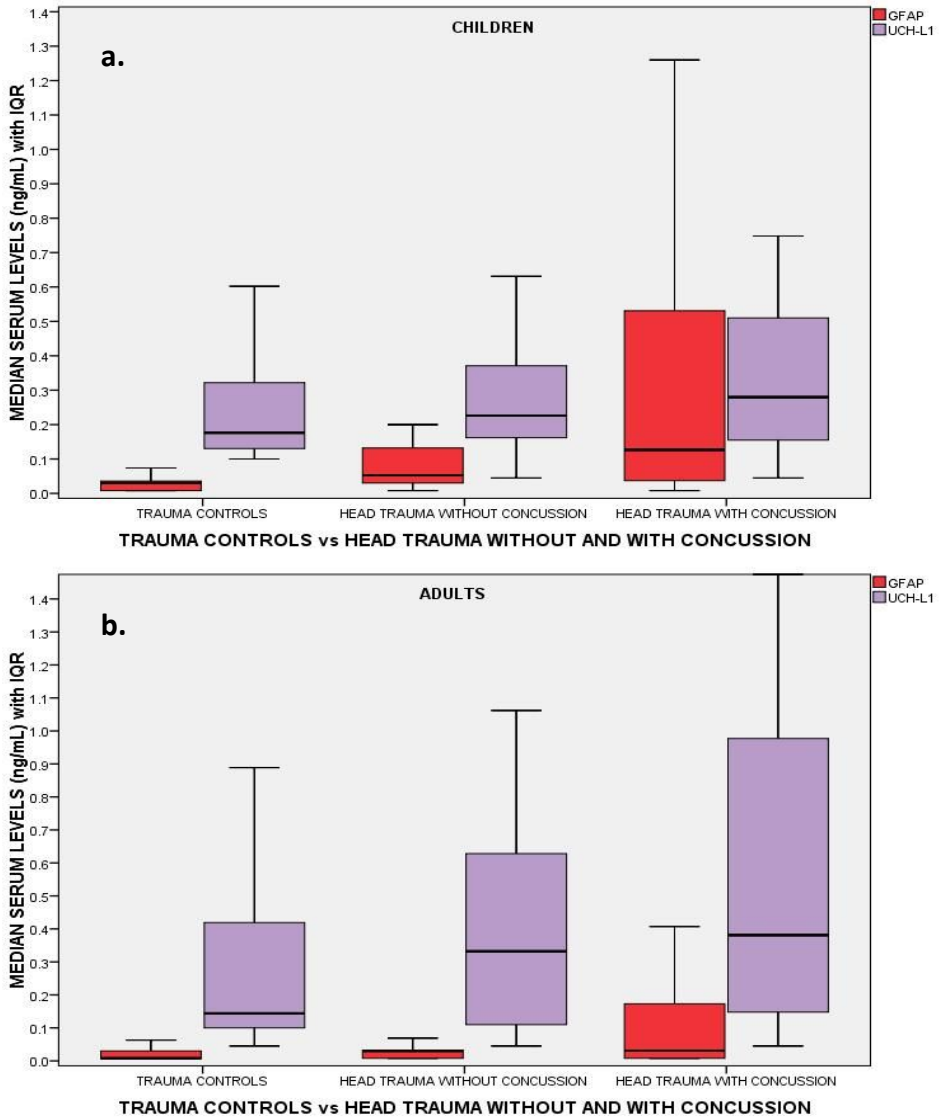


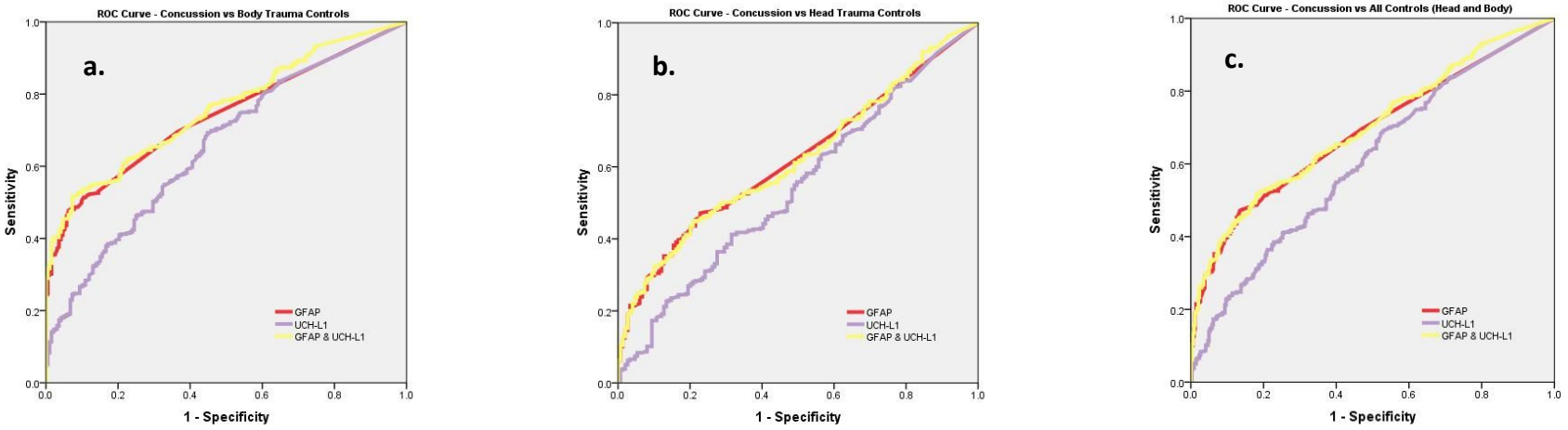
Figure 3. Boxplot comparing initial levels of GFAP and UCH-L1 in three groups of trauma patients: 1) trauma controls without head trauma (controls), 2) head trauma controls without concussion (no concussion); and 3) head trauma with concussion (concussion).



MULTIPLE COMPARISONS	CHILDREN N=176	ADULTS N=537
GFAP		
Trauma Control **vs** Head Trauma Control	<0.001	<0.001
Trauma Control **vs** Concussion	<0.001	<0.001
Head Trauma Control **vs** Concussion	<0.001	<0.001
UCH-L1		
Trauma Control **vs** Head Trauma Control	0.410	0.002
Trauma Control **vs** Concussion	0.045	<0.001
Head Trauma Control **vs** Concussion	0.894	0.017

**Controlled for multiple comparisons*

Figure 4. ROC Curves comparing initial levels of GFAP and UCH-L1 (individually and in combination) for predicting a concussion in trauma patients excluding patients with traumatic intracranial lesions on CT.



CONCUSSION VS NO CONCUSSION (EXCLUDING PATIENTS WITH POSITIVE CT)	CHILDREN N=175	ADULTS N=537	*ALL PATIENTS N=712
Concussion **vs** Body Trauma (No Concussion)	n=80 & n=47	n=255 & n=145	n=335 & n=149
GFAP	**0.77** (0.68-0.85)	**0.74** (0.69-0.79)	**0.74** (0.70-0.78)
UCH-L1	**0.58** (0.48-0.69)	**0.68** (0.62-0.73)	**0.66** (0.61-0.70)
GFAP & UCH-L1	**0.76** (0.68-0.84)	**0.76** (0.72-0.81)	**0.76** (0.72-0.80)
Concussion **vs** Head Trauma (No Concussion)	n=80 & n=34	n=255 & n=115	n=335 & n=192
GFAP	0.58 (0.47-0.68)	0.64 (0.58-0.70)	0.62 (0.57-0.67)
UCH-L1	0.50 (0.39-0.62)	0.56 (0.50-0.62)	0.54 (0.49-0.60)
GFAP & UCH-L1	0.57 (0.46-0.67)	0.64 (0.58-0.70)	0.62 (0.57-0.67)
Concussion **vs** Head and Body Trauma Controls	n=80 & n=81	n=255 & n=260	n=335 & n=341
GFAP	0.69 (0.60-0.77)	0.70 (0.65-0.74)	0.69 (0.65-0.73)
UCH-L1	0.55 (0.46-0.64)	0.63 (0.58-0.67)	0.61 (0.56-0.65)
GFAP & UCH-L1	0.68 (0.60-0.76)	0.71 (0.66-0.75)	0.70 (0.66-0.74)

*Depicted in the ROC curve graphs

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Evaluating Glial and Neuronal Blood Biomarkers GFAP and UCH-L1 as Gradients of Brain Injury in Concussive, Subconcussive and Nonconcussive Trauma: a Prospective Cohort Study

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Evaluating Glial and Neuronal Blood Biomarkers GFAP and UCH-L1 as Gradients of Brain Injury in Concussive, Subconcussive and Nonconcussive Trauma: a Prospective Cohort Study

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ABSTRACT

OBJECTIVES: To evaluate the ability of GFAP and UCH-L1 to detect concussion in children and adult trauma patients with a normal mental status and assess biomarker concentrations over time as gradients of injury in concussive and nonconcussive head and body trauma.

DESIGN: Large prospective cohort study.

SETTING: Three Level One Trauma Centers in the United States.

PARTICIPANTS: Pediatric and adult trauma patients of all ages, with and without head trauma, presenting with a normal mental status (GCS 15) within 4 hours of injury. Rigorous screening for concussive symptoms was conducted. Of 3462 trauma patients screened, 751 were enrolled and 712 had biomarker data. Repeated blood sampling was conducted at 4, 8, 12, 16, 24, 36, 48, 60, 72, 84, 96, 108, 120, 132, 144, 156, 168, and 180 hours post-injury in adults.

MAIN OUTCOMES: Detection of concussion and gradients of injury in children versus adults by comparing three groups of patients: 1) those with concussion; 2) those with head trauma without overt signs of concussion (nonconcussive head trauma controls); and 3) those with peripheral (body) trauma without head trauma or concussion (nonconcussive body trauma controls).

RESULTS: A total of 1904 samples from 712 trauma patients were analyzed. Within 4 hours of injury, there were incremental increases in levels of both GFAP and UCH-L1 from nonconcussive body trauma (lowest), to mild elevations in nonconcussive head trauma, to highest levels in patients with concussion. In concussion patients, GFAP concentrations were significantly higher compared to body trauma controls ($p<0.001$) and

to head trauma controls (p<0.001) in both children and adults, after controlling for multiple comparisons. However, for UCH-L1 there were no significant differences between concussion patients and head trauma controls (p=0.894) and between body trauma and head trauma controls in children. The AUC for initial GFAP levels to detect concussion was 0.80 (0.73-0.87) in children and 0.76 (0.71-0.80) in adults. This differed significantly from UCH-L1 with AUCs of 0.62 (0.53-0.72) in children and 0.69 (0.64-0.74) in adults.

CONCLUSIONS: In a cohort of trauma patients with normal mental status, GFAP out-performed UCH-L1 in detecting concussion in both children and adults. Blood levels of GFAP and UCH-L1 showed incremental elevations across three injury groups, from nonconcussive body trauma, to nonconcussive head trauma, to concussion. However, UCH-L1 was expressed at much higher levels than GFAP in those with nonconcussive trauma, particularly in children. Elevations in both biomarkers in patients with nonconcussive head trauma may be reflective of a subconcussive brain injury. This will require further study.

Key Words:

Biomarkers; Concussion; Mild Traumatic Brain Injury; Subconcussive, Head Trauma, Trauma, Children; Pediatric; Glial fibrillary acidic protein (GFAP), Ubiquitin C-terminal hydrolase (UCH-L1); Blood test

INTRODUCTION

Glial Fibrillary Acidic Protein (GFAP) and Ubiquitin C-terminal hydrolase (UCH-L1) have been evaluated in several studies to determine the need for CT scan and neurosurgical intervention in mild to moderate TBI patients (mmTBI) in adults[1-7] and more recently in children with mmTBI.[8-10] In early 2018, GFAP and UCH-L1 were FDA-approved for clinical use in adult patients with mmTBI to help determine need for CT scan within 12 hours of injury.[11] The approval was based on the ability to find lesions on CT scan but was not approved to diagnose a concussion or a mild TBI. Moreover, it was not approved for use in children.

Following trauma, patients often have a constellation of injuries and it is important that TBI biomarkers indicate brain-specific injury in order to be clinically useful. A number of articles have described how GFAP and UCH-L1 were able to distinguish mmTBI patients from orthopedic controls and motor vehicle crash controls as well as in those TBI patients with negative CT's.[1, 3, 4] In these studies, many trauma control patients were exposed to significant trauma including the acceleration-deceleration vectors of MVCs and substantial falls and both GFAP and UCH-L1 showed a graded response to severity of injury from normal controls to trauma controls, to mild and moderate TBI. Moreover, GFAP has consistently shown very good specificity to brain injury in cases of polytrauma.[3, 12] GFAP has demonstrated the ability to detect intracranial lesions in victims of multiple trauma with mild TBI who had substantial extracranial injuries and fractures.[3]

Importantly, there is a group of individuals with head trauma who have been significantly understudied, and in whom biomarkers are rarely, if at all, examined. These

are people who experience head trauma without symptoms of concussion. They may be classified as having "no injury" or they may represent milder forms of concussion that do not elicit the typical signs or symptoms associated with concussion and are referred to as subconcussive injuries. To date, there is a paucity of studies addressing the effects of subconcussive head impacts following head trauma. The issue of subconcussive trauma has been a particular concern in military personnel[13] and in athletes, as repetitive subconcussive impacts have the potential for long-term deleterious effects.[14-16] Therefore, studying these biomarkers in patients with head trauma without symptoms could provide unique insights into how neuronal and glial biomarkers behave in subconcussive trauma.[17]

There is insufficient data on the diagnostic accuracy of GFAP and UCH-L1 in children and adults in determining which trauma patients with normal mental status have a concussion and how well they perform over time following different degrees of mild head trauma. This study evaluated the diagnostic accuracy of serum glial and neuronal serum biomarkers GFAP and UCH-L1, both individually and in combination, in detecting the presence of a concussion and grading potential subconcussive and nonconcussive brain injury in pediatric and adult trauma patients presenting to the emergency department with a normal mental status (GCS score of 15). Gradients of brain injury were defined by comparing serum biomarker concentrations in three groups of patients: 1) those with concussion; 2) those with blunt head trauma without overt signs or symptoms of concussion (head trauma controls); and 3) those with peripheral (body) trauma without head trauma or concussion (body trauma controls). Additionally, the

temporal profile of GFAP and UCH-L1 were measured over seven days in these three groups in adults.

METHODS

Study Population

This prospective cohort study enrolled a convenience sample of adult and pediatric trauma patients presenting to the emergency departments of three Level I Trauma Centers: a Pediatric Level 1 Trauma Center in Philadelphia, Pennsylvania, a Pediatric Level 1 Trauma Center in Orlando, Florida, and an affiliated Adult Level 1 trauma center in Orlando, Florida. This study was approved by the respective Institutional Review Boards (IRB) of each institution. Informed consent was obtained from patients and/or their legal authorized representatives prior to enrollment and assent was obtained for children between the ages of 7 to 18 years.

Eligibility for concussion patients was determined by the treating physician based on the history of blunt head trauma followed by either loss of consciousness, amnesia, or disorientation (or change in behavior in children) and presenting to the emergency department within 4 hours of injury with a Glasgow Coma Scale (GCS) Score of 15. Eligibility was also prospectively verified by the research team prior to enrollment. Head CT Scans were performed at the discretion of the treating physician. Patients were excluded if they: 1) had no history of trauma as their primary event (e.g. syncope or seizure); 2) had known dementia, chronic psychosis or active CNS pathology; 3) were pregnant; or 4) were incarcerated or 5) had hemodynamic instability.

Both nonconcussive trauma groups, the body trauma control patients (no head trauma and no concussion) and the head trauma control patients (head trauma and no concussion) had a GCS score of 15 presenting to the emergency department with a traumatic mechanism of injury but without concussion. They experienced similar mechanisms of injury as the concussion group and all had a normal mental status since injury (as verified by the research team prospectively by at least two different sources) and had no evidence of acute brain injury or hemodynamic instability. Peripheral (body) trauma controls were primarily composed of orthopedic and soft tissue injuries These patients were carefully screened to ensure they had no loss of consciousness, no amnesia and no alteration in sensorium at any time after injury. The purpose of enrolling nonconcussive body trauma controls and nonconcussive head trauma controls was to have appropriate comparison groups to compare the accuracy of the biomarkers in detecting concussion and simulate the realworld challenges faced by clinicians. The head trauma controls provided an opportunity to assess biomarker release in the setting of head trauma without symptoms and the potential for subconcussive brain injury.

Study Procedures

All initial patient assessments were made by board certified adult and pediatric emergency medicine physicians trained by a formal one-hour session on evaluating patient eligibility for the study. Following the initial screening, a meticulous secondary assessment was conducted by the research team. All prehospital and emergency department records were reviewed, patients, families and witnesses (if available) were carefully questioned and the final determination was made by the emergency physician together with the research team. Patient classification was performed prospectively.

Blood samples were obtained within 4 hours of time of injury. Repeated blood sampling was conducted for as long as the patient remained in hospital at 4, 8, 12, 16, 24, 36, 48, 60, 72, 84, 96, 108, 120, 132, 144, 156, 168 and 180 hours after injury and discontinued when discharged. Patient management was not altered by the study. For each blood draw a single vial of approximately 5mL of blood was collected and placed in a serum separator tube and allowed to clot at room temperature. The blood was centrifuged within 30 minutes and the serum was placed in bar-coded aliquot containers and stored in a freezer at -70 degrees Celsius until it was transported to a central laboratory (Banyan Biomarkers Inc, Alachua Florida USA). There, the samples were analyzed in batches using sandwich enzyme-linked immunosorbent assays (ELISA) to GFAP and UCH-L1. Lab personnel running the samples were blinded to the clinical data.

Outcome measures

Performance of GFAP and UCH-L1 was evaluated within 4 hours of injury in both adults and children and over a 7-day period in hospitalized adults. The main outcome measures included the performance of the biomarkers in: 1) detecting the presence of concussion compared to trauma patients without concussion in children and adults (separately and as a whole); 2) assessing gradients of injury defined by comparing three groups of patients a) those with concussion, b) those with head trauma without overt signs of concussion (nonconcussive head trauma controls), and c) those with peripheral (body) trauma without head trauma or concussion (nonconcussive body trauma controls); 3) determining the time course of GFAP and UCH-L1 over 7 days after injury in these three groups in adults.

Statistical Analysis

Descriptive statistics with means medians and proportions were used to describe the data. For statistical analysis, biomarker levels were treated as continuous data, measured in ng/ml and expressed as medians with interquartile range. Data were assessed for equality of variance and distribution. Logarithmic transformations were conducted on non-normally distributed data. Group comparisons for different trauma groups were performed using analysis of variance with multiple comparisons using Games-Howell post-hoc test. Receiver Operating Characteristics (ROC) curves were created to explore the ability of the biomarkers to identify the presence of a concussion. Estimates of the area under these curves (AUC) were obtained (AUC=0.5 indicates no discrimination and an AUC=1.0 indicates a perfect diagnostic test).

A power analysis yielded a sample of 281 cases of concussion and 141 cases without concussion achieves an 80% power to detect a difference of 0.06 between the area under the ROC curve (AUC) under the null hypothesis of 0.81 and an AUC under the alternative hypothesis of 0.75 using a two-sided z-test at a significance level of 0.05. All analyses were performed using the statistical software package SPSS 22.0 (IBM Corporation®, Somers NY).

Biomarker Analysis

Serum GFAP and UCH-L1 levels were measured in duplicate for each sample using a validated ELISA platform (Banyan Biomarkers Inc., Alachua Florida USA). For the GFAP assay, the lower limit of quantification (LLOQ) is 0.030ng/ml and upper limit of quantification (ULOQ) is 50ng/ml. The limit of detection (LoD) is 0.008ng/mL. For the UCH-L1 assay, the lower limit of quantification (LLOQ) is 0.100ng/ml and upper limit of quantification (ULOQ) is 9ng/ml. The limit of detection (LoD) is 0.045ng/mL.

Any samples yielding a signal over the quantification or calibrator range were diluted and re-assayed.

Patients and the public were not involved in the design, recruitment or conduct of the study.

RESULTS

Over the study period, 3462 pediatric and adult trauma patients were screened, 1385 met eligibility criteria, 751 with a GCS score of 15 were enrolled and 712 had biomarker data available for analysis (Figure 1). Of those enrolled, 371 (52%) had a concussion, 149 (21%) had nonconcussive head trauma (head trauma controls), and 192 (27%) had nonconcussive body trauma without head trauma (body trauma controls). The flow diagram in Figure 1 describes the distribution of enrolled patients. There were 175 (25%) children and 537 (75%) adults. The distribution of clinical characteristics of all enrolled patients is presented in Table 1. The overall injury severity score in children and adults was consistent with median scores of 4.

There were a total of 1904 samples drawn in 712 patients. Patients had serum samples drawn within 4 hours of injury (16 children had samples drawn between 4 and 8 hours) with the average time from injury to serum sample collection of 3.1 hours (SD 0.9). Seven hundred and twelve patients had initial samples drawn at enrollment, 567 patients had samples at 4-hours post-injury, 109 at 8-hours post-injury, 80 at 12 hours post-injury, 73 at 16-hours post-injury; 70 at 20-hours post-injury, 67 at 24-hours post-injury, 46 at 36-hours post-injury, 40 at 48-hours post-injury, 33 at 60-hours post-injury, 32 at 72-hours post-injury, 20 at 84-hours post-injury, 17 at 96-hours post-injury, 8 at

108-hours post-injury, 8 at 120-hours post-injury, 4 at 132-hours post-injury, 6 at 144-hours post-injury, 6 at 156-hours post-injury, and 4 at 168-hours post-injury.

Among body trauma control patients, 229 (60%) samples were below the LLOD and 90 (24%) below the LLOQ for GFAP. In head trauma control patients 144 (38%) samples were below the LLOD and 88 (24%) below the LLOQ for GFAP. In concussion patients, 276 (24%) samples were below the LLOD and 172 (15%) below the LLOQ for GFAP. Among body trauma control patients, 61 (16%) samples were below the LLOD and 70 (19%) below the LLOQ for UCH-L1. In head trauma control patients 34 (9%) samples were below the LLOD and 39 (10%) below the LLOQ for UCH-L1. In concussion patients, 128 (11%) samples were below the LLOD and 103 (11%) below the LLOQ for UCH-L1.

The time course of GFAP and UCH-L1 over a week post trauma is depicted in Figure 2 and contrasted in three groups of patients (concussion, head trauma controls, and body trauma controls). In the concussion patients the serum concentration of GFAP was detectible within 1 hour of injury and reached a peak at 20 hours post-injury and decreased over 72 hours. GFAP concentrations exhibited a slower decline thereafter but were still detectable at 168 hours (7 days) post-injury. In contrast, UCH-L1 rose very rapidly after injury, reached a peak at 8 hours, decreased quickly to 12 hours and was followed by a slower decline to 60 hours post-injury. Subsequently, UCH-L1, like GFAP, exhibited some smaller peaks and toughs over 7 days and was also detectable at 168 hours post-injury.

In head trauma controls GFAP levels were remarkably lower than in the concussion patients with very slight elevations until 48 hours. The peak appeared at 20

hours (as in the concussion patients) and after 48 hours GFAP levels remained almost undetectable. Interestingly, in head trauma controls UCH-L1 levels peaked within 4 hours and remained lower than concussion patients over 12 hours. Thereafter, UCH-L1 concentrations become quite variable with levels either slightly lower, at par, or slightly higher than concussion patients.

In body trauma control patients, concentrations of GFAP were negligible over time without any appreciable elevations. Initial UCH-L1 levels were slightly elevated but significantly lower than either head trauma or concussion patients. UCH-L1 levels decreased quickly over 12 hours (as it did in the head trauma controls) and levels remained noticeably elevated over the next several days.

The ability of GFAP and UCH-L1, individually and in combination, to distinguish concussion patients from body trauma controls (Table 2) and head trauma controls (Table 3) over time was assessed by calculating the area under the ROC Curve (AUC) at each time-point post-injury. A comparison between concussion and both trauma and head trauma controls can be found in Table 4. When comparing concussion patients to body trauma controls, GFAP demonstrated a range of AUC's between 0.75 (0.39-1.00) to 0.89 (0.69-1.00) and UCH-L1 demonstrated AUC's between 0.50 (0.05-0.23) to 0.78 (0.56-1.00). When GFAP and UCH-L1 were combined, the AUC ranged from 0.75 (0.49-1.00) to 0.90 (0.76-1.00) and closely mimicked the pattern of GFAP. GFAP out-performed UCH-L1 at all time-points. The combination of GFAP and UCH-L1 marginally out-performed GFAP alone at some time-points, however, the differences were not statistically significant.

When comparing concussion patients to head trauma controls, GFAP demonstrated a range of AUC's between 0.62 (0.57-0.68) and 0.86 (0.73-1.00) and UCH-L1 demonstrated AUC's between 0.13 (0-1.00) to 0.61 (0.49-0.73). When GFAP and UCH-L1 were combined, the AUC ranged from 0.33 (0-0.71) to 0.86 (0.73-1.00). GFAP out-performed UCH-L1 at all time-points and out-performed the combination of the two biomarkers at all time-points.

A comparison of the performance of GFAP and UCH-L1 measured within 4 hours of injury in children and adults is shown in Figure 3. There are incremental increases in levels of GFAP and UCH-L1 from nonconcussive body trauma controls to nonconcussive head trauma controls to patients with concussion. In concussion patients, GFAP concentrations were significantly higher compared to body trauma controls ($p<0.001$) and to head trauma controls ($p<0.001$) in both children and adults, after controlling for multiple comparisons. There were also significantly higher levels of GFAP in head trauma controls compared to body trauma controls in children ($p<0.001$) and adults ($p<0.001$). In adults, concentrations of UCH-L1 measured within 4 hours of injury were also significantly higher in concussion patients than body trauma controls ($p<0.001$) and head trauma controls ($p=0.002$). There were also significantly higher levels of UCH-L1in head trauma controls compared to body trauma controls ($P=0.017$). Similarly, in children, concentrations of UCH-L1 were significantly higher in concussion patients than body trauma controls ($p=0.045$). However, there were no significant differences between concussion patients and head trauma controls ($p=0.894$) and between body trauma and head trauma controls in children.

When ROC Curves were compared in children and adults, the AUC's demonstrated that initial GFAP levels were able to distinguish concussion patients from body trauma controls with an AUC of 0.80 (0.73-0.87) in children and 0.76 (0.71-0.80) in adults (Table 2). In contrast, initial UCH-L1 levels distinguished concussion from body trauma controls with a significantly lower AUC of 0.62 (0.53-0.72) in children (p=0.003) and 0.69 (0.64-0.74) in adults (p=0.04) (Table 2). The AUC's for GFAP for distinguishing concussion patients from head trauma controls was 0.64 (0.54-0.73) in children and 0.66 (0.61-0.71) in adults. AUC's were lower for UCH-L1 with an AUC of 0.54 (0.43-0.66) in children (p=0.23) and 0.58 (0.51-0.64) in adults (p=0.04) (Table 3). Overall, when concussion was compared to all (head and body) trauma control cases without concussion, the AUC for GFAP was 0.73 (0.66-0.81) in children and 0.71 (0.67-0.76) in adults. AUC's were significantly lower for UCH-L1 with an AUC of 0.59 (0.51-0.67) in children (p=0.013) and 0.64 (0.59-0.69) in adults (p=0.030)(Table 4). There were significantly higher levels of GFAP and UCH-L1 in those with intracranial lesions on CT, therefore, we excluded the 36 (8%) patients with CT lesions and found similar results (Figure 4).

DISCUSSION

This prospective study assessed the diagnostic accuracy of glial and neuronal biomarkers, GFAP and UCH-L1, for detecting concussion in a very large cohort of children and adult trauma patients presenting to three Level I trauma centers. The study investigated the pattern of biomarker release in trauma patients with and without concussion at twenty distinct time-points, making it is among the first and largest studies

to assess the temporal profile of these two biomarkers in three groups of trauma patients with a normal mental status. In both children and adults, GFAP and UCH-L1 concentrations increased incrementally from those with nonconcussive body trauma to those with nonconcussive head trauma with highest levels in patients with concussion. GFAP showed very distinct patterns of release in all three groups, whereas UCH-L1 demonstrated similar patterns of release in all three groups but at much higher concentrations in both nonconcussive trauma groups. There were significant differences between the three groups controlling for multiple comparisons for both biomarkers in adults, however, UCH-L1 could not distinguish concussive from nonconcussive head trauma in children.

When the time course of GFAP and UCH-L1 was contrasted in three groups of patients (concussion, head trauma controls, and body trauma controls), GFAP showed a clear increase over the first 20 hours post-injury and decline from 20 to 72 hours in concussion patients and was still detectable at 7 days post-injury making it potentially useful over a week from injury. Although GFAP was mildly elevated in head trauma without concussion, the expression was very low and very early compared to concussion. In body trauma control patients, concentrations of GFAP were negligible over all time points suggesting very good specificity for concussion. These results are consistent with previous studies showing how robust it is in multiple trauma.[3,4] In contrast, UCH-L1 rose more rapidly after concussion than GFAP, peaking within 8 hours and steadily decreasing from 12 to 60 hours. Unexpectedly, UCH-L1 was much higher in the head trauma control group compared to GFAP at all time-points from injury over seven days. Even more surprising, was that UCH-L1 was elevated in body trauma too and showed a similar

pattern of release as head trauma control patients. Possible explanations for UCH-L1 elevations in control patients include that 1) UCH-L1 may not be completely brain specific and is released from other organ or tissue trauma, or 2) UCH-L1 is an ultrasensitive marker of any neuronal disruption that may occur from impacts to the body that jostle the brain.

Given that GFAP appears to be so brain specific and that it also showed low level elevations in the first 48 hours following head trauma without concussion symptoms (head trauma controls), these elevations may represent milder forms of concussion that do not elicit typical signs or symptoms associated with concussion. These injuries may be irrelevant, or they may represent important trauma that is just below the level of clinical detection and referred to as subconcussive trauma. Emerging data have demonstrated that significant alterations in brain function can occur in the absence of clinically obvious symptoms following even a single head trauma.[15, 18, 19] Given the lack of concussive symptoms acutely, biomarkers (such as GFAP and UCH-L1) could provide a more objective measure of injury and potentially identify those at risk for neurocognitive problems. Studies in athletes have documented that both clinically diagnosed concussion and subconcussive traumas can induce similar changes in brain structure and functions on brain imaging.[15, 19-21] These changes include alterations in white matter and cerebrovascular integrity, blood flow, neuroinflammation, brain activation during working memory tasks, resting-state functional connectivity, and brain chemistry as measured by various forms of magnetic resonance imaging (MRI).[20, 22, 23] The effect of thousands of subconcussive impacts has the potential for long-term deleterious effects on

brain function and neurodegeneration in select individuals.[14, 15, 19] In athletes, UCH-L1 has shown elevations in both concussive[24] and subconcussive trauma.[25]

To date, there is a lack of research addressing the effects of subconcussive head impacts following head trauma in an emergency department population. Acute biomarkers may have a role in assessing these patients if the markers can be shown to correlate with long-term neurocognitive dysfunction. Most recently, microRNA biomarkers measured pre and post-season in collegiate football players were associated with worsening neurocognitive functioning over the course of a season in those with no concussions.[26]

Concussion is a clinical diagnosis that could benefit from a relatively noninvasive complementary tool such as a blood test.[8, 9, 27-29] Based on these results, the potential utility of GFAP to distinguish concussion from body trauma controls over 7 days post-injury was fair to excellent with AUC's of 0.75 to 0.89, and UCH-L1's ability was guarded and variable with AUC's from poor to good depending on timing of samples (AUC's of 0.54 to 0.78) with earlier samples being better. The combination of the both biomarkers did not significantly improve concussion detection among trauma control patients. The distinction between head trauma patients with and without concussion was not as robust as with body trauma controls. GFAP performed with fair to very good AUC's (0.62 to 0.86) over the week post-injury, with optimal performance between 24 to 96 hours. The ability of UCH-L1 to distinguish concussion from head trauma control patients was very poor with AUC's of 0.13 -0.61 and did not contribute to or improve the performance of GFAP alone.

Since it is not uncommon for patients who have suffered a concussion or mild TBI not to seek immediate medical attention, understanding when to use the biomarkers for detection of injury is critical. In the context of developing a point-of-care test, UCH-L1's early and rapid rise could be useful in the early post injury setting such as in the ambulance, on the playing field or on the battlefield. The longer half-life of GFAP makes it a very favorable marker to use in both the acute and subacute phases of injury as it can detect concussion for up to 7 days after injury.

The authors recognize that there are limitations to this study. This study addressed diagnosis of concussion in the acute care setting and did not describe long-term outcome in these patients. The main outcomes used in this study reflect current standards of practice and accepted definitions of concussion. However, future studies to better define the severity of concussion and mild TBI need to be pursued, particularly when neuroimaging is negative. Accordingly, we performed an analysis of patients with negative neuroimaging acutely and found no significant differences in the results whether we included those with positive scans or not. The number of samples available for analysis decreased over the course of the study. This reflects the challenge of obtaining samples over time in patients with less severe injuries because they are not hospitalized as long. However, there were many patients without TBI and patients with mild TBI who were captured in our longitudinal sample because they were admitted for other injuries. Important next steps will be to capture samples within minutes of injury. Uninjured controls were not included in this analysis as the concentrations of these two biomarkers have already been well characterized in uninjured normal control patients.[1, 2]

CONCLUSION

In a cohort of trauma patients with normal mental status, GFAP out-performed UCH-L1 in detecting concussion in both children and adults. Blood levels of GFAP and UCH-L1 showed incremental elevations across three injury groups, from nonconcussive body trauma, to nonconcussive head trauma, to concussion. However, UCH-L1 was expressed at much higher levels than GFAP in those with nonconcussive trauma, particularly in children, suggesting that UCH-L1 is either not completely brain specific or ultrasensitive to subtle impacts. Each biomarker exhibited a distinct temporal profile in each trauma group over seven days with earlier elevations in UCH-L1 and more consistent and sustained elevations in GFAP. Furthermore, elevations in both biomarkers in patients with nonconcussive head trauma may be reflective of a subconcussive brain injury. The stage is set for future studies to verify these findings.

KEY MESSAGES

What is already known on this topic

In 2018 serum biomarkers Glial Fibrillary Acidic Protein (GFAP) and Ubiquitin C-terminal hydrolase (UCH-L1) were FDA-approved in adults to guide CT scan ordering in mild to moderate traumatic brain injury. However, their ability to detect concussion in either children or adults has not been determined and there currently exists no objective measure to diagnose concussion acutely after injury. The challenge for clinicians is to detect concussion in the setting of head and/or peripheral trauma when patients have a normal mental status. Having an objective measure of concussion would be very helpful in managing trauma patients.

What this study adds

GFAP out-performed UCH-L1 in detecting concussion in both children and adults within 4 hours of injury. Blood levels of GFAP and UCH-L1 showed incremental elevations across three injury groups, from nonconcussive body trauma, to nonconcussive head trauma, to concussion. UCH-L1 was expressed at much higher levels than GFAP in those with nonconcussive trauma, particularly in children, suggesting that UCH-L1 is either not completely brain specific or ultrasensitive to subtle impacts. Elevations of these biomarkers in nonconcussive head trauma suggests possible subconcussive brain injury. GFAP could be potentially useful to detect concussion for up to a week post-injury.

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Contributors: LP had full access to the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. LP conceived and designed the study. Data was acquired by LP, JR, PG, CFB, CNT, NJA, MAL, CAH, DMG, MRZ, and MM. All authors were involved in the analysis and interpretation of the data. LP drafted the manuscript and all authors were involved in critical revision of the manuscript for important intellectual content. Statistical analysis was conducted by LP. Funding was obtained by LP. Administrative, technical, or material support was provided by LP, PG, MRZ, and MM. The study was supervised by LP, JR, PG, CFB, CNT, NJA, MAL, CAH, DMG, MRZ, and MM. The corresponding author attests that all listed authors meet authorship criteria and that no others meeting the criteria have been omitted.

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Data sharing: Data are not available for sharing at this time.

Transparency: The lead author (LP) affirms that this manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned (and, if relevant, registered) have been explained.

Dissemination declaration: Dissemination of results to participants is not possible/applicable.

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FIGURE LEGEND

Figure 1. Flow diagram of screened and enrolled patients.

Figure 2. Temporal Profile of GFAP and UCH-L1 in three groups of trauma patients

Figure 2a. Temporal Profile of GFAP and UCH-L1 in body trauma control patients. Means with error bars representing standard error of the mean (SEM).

Figure 2b. Temporal Profile of GFAP and UCH-L1 in head trauma control patients. Means with error bars representing standard error of the mean (SEM).

Figure 2c. Temporal Profile of GFAP and UCH-L1 in trauma patients with concussion. Temporal Profile of GFAP and UCH-L1 in trauma patients with concussion. Means with error bars representing standard error of the mean (SEM).

Figure 3. Boxplot comparing initial levels of GFAP and UCH-L1 in three groups of trauma patients: 1) trauma controls without head trauma (controls), 2) head trauma controls without concussion (no concussion); and 3) head trauma with concussion (concussion).

There were 47, 34, and 94 children in each group respectively and 145, 115, and 277 adult patients respectively. Medians with bars representing interquartile range (IQR).

Figure 4. ROC Curves comparing initial levels of GFAP and UCH-L1 (individually and in combination) for predicting a concussion in trauma patients excluding patients with traumatic intracranial lesions on CT.

TABLES

Table 1. Characteristics of Enrolled Patients

Characteristics	*PEDIATIRC Trauma Patients without Head Trauma and Without Concussion (Trauma Controls)*	*PEDIATRIC Head Trauma Patients without Concussion (Head Trauma Controls)*	*PEDIATRIC Head Trauma Patients with Concussion (Concussion)*	*ADULT Trauma Patients without Head Trauma and Without Concussion (Trauma Controls)*	*ADULT Head Trauma Patients without Concussion (Head Trauma Controls)*	*ADULT Head Trauma Patients with Concussion (Concussion)*
	N=47	*N=34*	*N=94*	*N=145*	*N=115*	*N=277*
Mean age (yrs±SD)	9 (±4)	6 (±5)	9 (±6)	41 (±16)	40 (±16)	39 (±15)
Range	(0-17)	(1-16)	(1-17)	(18-83)	(18-79)	(18-78)
Gender (%)						
Male	33 (27)	23 (19)	66 (54)	82 (25)	68 (21)	182 (55)
Female	14 (26)	11 (21)	28 (53)	63 (31)	47 (23)	95 (46)
Race (%)						
Asian	0 (0)	1 (3)	1 (1)	2 (1)	0 (0)	6 (2)
Black	19 (40)	5 (15)	31 (33)	41 (28)	32 (28)	54 (20)
Hispanic	7 (15)	13 (38)	17 (18)	39 (27)	20 (17)	53 (19)
Native American	0 (0)	0 (0)	0 (0)	3 (2)	0 (0)	0 (0)
White	19 (40)	15 (44)	42 (45)	57 (39)	60 (52)	154 (56)
Other	2 (4)	0 (0)	3 (3)	3 (2)	3 (3)	10 (4)
Mechanism of Injury (%)						
Motor Vehicle Crash	3 (6)	1 (3)	4 (4)	94 (65)	53 (46)	134 (48)
Motorcycle Crash	0 (0)	0 (0)	0 (0)	0 (0)	17 (15)	29 (11)
Other Motorized Vehicle	0 (0)	3 (9)	2 (2)	0 (0)	2 (2)	3 (1)
Bicycle Struck by Vehicle	0 (0)	2 (6)	3 (3)	3 (2)	7 (6)	11 (4)
Fall off Bicycle	0 (0)	1 (3)	3 (3)	1 (1)	0 (0)	5 (2)
Pedestrian Struck	1 (2)	1 (3)	6 (6)	4 (3)	3 (3)	12 (4)
Fall	33 (70)	17 (50)	42 (45)	20 (14)	12 (10)	58 (21)
Sports Injury	9 (19)	5 (15)	23 (25)	7 (5)	2 (2)	1 (<1)
Assault	0 (0)	0 (0)	5 (5)	0 (0)	6 (5)	10 (4)
Other	1 (2)	4 (12)	6 (6)	16 (11)	13 (11)	14 (5)
Loss of Consciousness (%)						
No	47 (100)	34 (100)	34 (36)	145 (100)	115 (100)	55 (20)
Yes	0 (0)	0 (0)	37 (39)	0 (0)	0 (0)	222 (80)
Unknown	0 (0)	0 (0)	23 (24)	0 (0)	0 (0)	0 (0)
Amnesia (%)						
No	47 (100)	34 (100)	36 (38)	145 (100)	115 (100)	171 (62)
Yes	0 (0)	0 (0)	17 (18)	0 (0)	0 (0)	105 (20)
Unknown	0 (0)	0 (0)	41 (44)	0 (0)	0 (0)	1 (<1)

Injury Severity Score (n=629) Median (IQR) Means (95%CI)	4.0 (4.0-10) 6.0 (3.0-9.1)	2.0 (1.0-4.0) 3.3 (2.1-4.6)	4.0 (1.0-7.0) 4.5 (3.6-5.4)	4.0 (1.0-4.0) 4.2 (3.5-4.9)	4.0 (2.0-6.5) 4.6 (4.0-5.3)	5.0 (2.0-8.8) 6.1 (5.5-6.8)
Admitted to Hospital (%)	12 (26)	8 (24)	29 (31)	42 (29)	17 (15)	101 (37)
CT Head performed (%)	0 (0)	11 (32)	74 (79)	18 (12)	80 (70)	267 (96)
Intracranial Lesions on CT (%)	0 (0)	0 (0)	14 (16)	0 (0)	0 (0)	22 (6)

Note: Due to rounding, percentages may not add up to 100

Table 2. Area Under the Curve for distinguishing between concussion and **body trauma controls** (no head trauma and no concussion symptoms). Shown is the performance of GFAP and UCH-L1 alone and in combination.

HOURS POST-INJURY	GFAP	UCH-L1	COMBINATION GFAP AND UCH-L1
Enrollment (Children) (n=141)	0.80 (0.73-0.87)	0.62 (0.53-0.72)	0.80 (0.73-0.87)
Enrollment (Adults) (n=422)	0.76 (0.71-0.80)	0.69 (0.64-0.74)	0.78 (0.73-0.82)
Enrollment (Both) (n= 563)	0.76 (0.72-0.80)	0.67 (0.63-0.72)	0.78 (0.74-0.81)
4 hours (n= 452)	0.76 (0.72-0.80)	0.65 (0.60-0.70)	0.77 (0.73-0.81)
8 hours (n=92)	0.82 (0.72-0.91)	0.72 (0.56-0.87)	0.86 (0.75-0.96)
12 hours (n=68)	0.83 (0.73-0.93)	0.74 (0.58-0.90)	0.85 (0.74-0.96)
16 hours (n=66)	0.84 (0.73-0.95)	0.77 (0.62-0.91)	0.86 (0.75-0.97)
20 hours (n=61)	0.82 (0.71-0.94)	0.63 (0.43-0.84)	0.82 (0.70-0.94)
24 hours (n=56)	0.87 (0.76-0.98)	0.74 (0.58-0.90)	0.87 (0.74-1.00)
36 hours (n=35)	0.86 (0.72-1.00)	0.76 (0.51-1.00)	0.90 (0.76-1.00)
48 hours (n=30)	0.85 (0.68-1.00)	0.71 (0.53-0.90)	0.85 (0.65-1.00)
60 hours (n=27)	0.79 (0.57-1.00)	0.70 (0.50-0.90)	0.83 (0.61-1.00)
72 hours (n=25)	0.87 (0.71-1.00)	0.78 (0.56-1.00)	0.94 (0.82-1.00)
84 hours (n=15)	0.89 (0.69-1.00)	0.75 (0.40-1.00)	0.89 (0.69-1.00)
96 hours (n=13)	0.75 (0.39-1.00)	0.54 (0.18-0.90)	0.75 (0.49-1.00)
108 hours (n=6)	n/a	n/a	n/a
120 hours (n=6)	0.80 (0.40-1.00)	0.50 (0.05-0.23)	0.90 (0.62-1.00)
132 hours (n=2)	n/a	n/a	n/a
144 hours (n=4)	n/a	n/a	n/a
156 hours (n=4)	n/a	n/a	n/a
168 hours (n=2)	n/a	n/a	n/a

n/a – data not available

Table 3. Area Under the Curve for distinguishing between concussion and **head trauma controls** (no concussion symptoms) in patients with head trauma. Shown is the performance of GFAP and UCH-L1 alone and in combination.

HOURS POST-INJURY	GFAP	UCH-L1	COMBINATION GFAP AND UCH-L1
Enrollment (Children) (n=128)	0.64 (0.54-0.73)	0.54 (0.43-0.66)	0.63 (0.53-0.72)
Enrollment (Adults) (n=392)	0.66 (0.61-0.71)	0.58 (0.51-0.64)	0.66 (0.61-0.72)
Enrollment (Both) (n= 520)	0.65 (0.60-0.70)	0.56 (0.51-0.62)	0.65 (0.60-0.70)
4 hours (n= 421)	0.62 (0.57-0.68)	0.55 (0.49-0.61)	0.62 (0.57-0.88)
8 hours (n=99)	0.72 (0.61-0.82)	0.61 (0.49-0.73)	0.72 (0.61-0.82)
12 hours (n=74)	0.69 (0.55-0.82)	0.51 (0.37-0.66)	0.67 (0.54-0.80)
16 hours (n=69)	0.76 (0.64-0.88)	0.58 (0.39-0.76)	0.74 (0.62-0.85)
20 hours (n=65)	0.68 (0.53-0.83)	0.41 (0.23-0.59)	0.66 (0.52-0.81)
24 hours (n=64)	0.74 (0.60-0.88)	0.40 (0.24-0.56)	0.72 (0.59-0.86)
36 hours (n=44)	0.76 (0.61-0.91)	0.56 (0.37-0.75)	0.76 (0.61-0.92)
48 hours (n=38)	0.81 (0.67-0.95)	0.52 (0.32-0.73)	0.79 (0.64-0.93)
60 hours (n=31)	0.86 (0.73-1.00)	0.54 (0.34-0.74)	0.86 (0.73-1.00)
72 hours (n=30)	0.78 (0.60-0.95)	0.43 (0.20-0.66)	0.71 (0.51-0.91)
84 hours (n=19)	0.84 (0.65-1.00)	0.37 (0.13-0.62)	0.74 (0.52-0.97)
96 hours (n=16)	0.75 (0.51-0.99)	0.28 (0.02-0.55)	0.65 (0.38-0.91)
108 hours (n=8)	0.67 (0.26-1.00)	0.17 (0-0.47)	0.33 (0-0.71)
120 hours (n=7)	0.80 (0.46-1.00)	0.20 (0-0.58)	0.70 (0.30-1.00)
132 hours (n=4)	0.75 (0.20-1.00)	0 (0-0)	0.50 (0-1.00)
144 hours (n=6)	0.75 (0.20-1.00)	0 (0-0)	0.50 (0.01-0.99)
156 hours (n=6)	0.75 (0.34-1.00)	0 (0-0)	0.50 (0.01-0.99)
168 hours (n=4)	0.75 (0.20-1.00)	0.13 (0-1.00)	0.63 (0-1.00)

Table 4. Area Under the Curve for distinguishing between concussion and **all trauma controls** (head and body controls with no concussion). Shown is the performance of GFAP and UCH-L1 alone and in combination.

HOURS POST-INJURY trauma	GFAP	UCH-L1	COMBINATION GFAP AND UCH-L1
Enrollment (Children) (n=175)	0.73 (0.66-0.81)	0.59 (0.51-0.67)	0.73 (0.65-0.80)
Enrollment (Adults) (n=537)	0.71 (0.67-0.76)	0.64 (0.59-0.69)	0.73 (0.68-0.77)
Enrollment (Both) (n= 712)	0.71 (0.67-0.75)	0.63 (0.59-0.67)	0.72 (0.68-0.76)
4 hours (n= 567)	0.70 (0.66-0.74)	0.61 (0.56-0.65)	0.70 (0.66-0.75)
8 hours (n=109)	0.75 (0.66-0.84)	0.65 (0.54-0.75)	0.77 (0.68-0.86)
12 hours (n=80)	0.73 (0.62-0.85)	0.59 (0.46-0.72)	0.73 (0.62-0.85)
16 hours (n=73)	0.79 (0.69-0.90)	0.64 (0.49-0.80)	0.78 (0.68-0.89)
20 hours (n=70)	0.73 (0.61-0.86)	0.49 (0.33-0.65)	0.72 (0.59-0.84)
24 hours (n=67)	0.77 (0.64-0.89)	0.47 (0.31-0.63)	0.75 (0.63-0.88)
36 hours (n=46)	0.77 (0.63-0.91)	0.59 (0.41-0.77)	0.78 (0.64-0.92)
48 hours (n=40)	0.81 (0.68-0.95)	0.55 (0.37-0.74)	0.80 (0.66-0.93)
60 hours (n=33)	0.85 (0.71-0.98)	0.58 (0.39-0.77)	0.86 (0.73-0.98)
72 hours (n=32)	0.80 (0.64-0.95)	0.51 (0.29-0.73)	0.76 (0.59-0.94)
84 hours (n=20)	0.85 (0.67-1.00)	0.44 (0.18-0.69)	0.77 (0.56-0.98)
96 hours (n=17)	0.75 (0.51-0.98)	0.33 (0.07-0.60)	0.67 (0.41-0.92)
108 hours (n=8)	0.67 (0.26-1.00)	0.17 (0-0.47)	0.33 (0-0.71)
120 hours (n=8)	0.80 (0.48-1.00)	0.30 (0-0.69)	0.77 (0.42-1.00)
132 hours (n=4)	0.75 (0.20-1.00)	0 (0-0)	0.50 (0-1.00)
144 hours (n=6)	0.75 (0.34-1.00)	0 (0-0)	0.50 (0.01-0.99)
156 hours (n=6)	0.75 (0.34-1.00)	0.38 (0-0.85)	0.63 (0.15-1.00)
168 hours (n=4)	0.75 (0.20-1.00)	0.13 (0-1.00)	0.63 (0-1.00)

Figure 1. Flow diagram of screened and enrolled patients

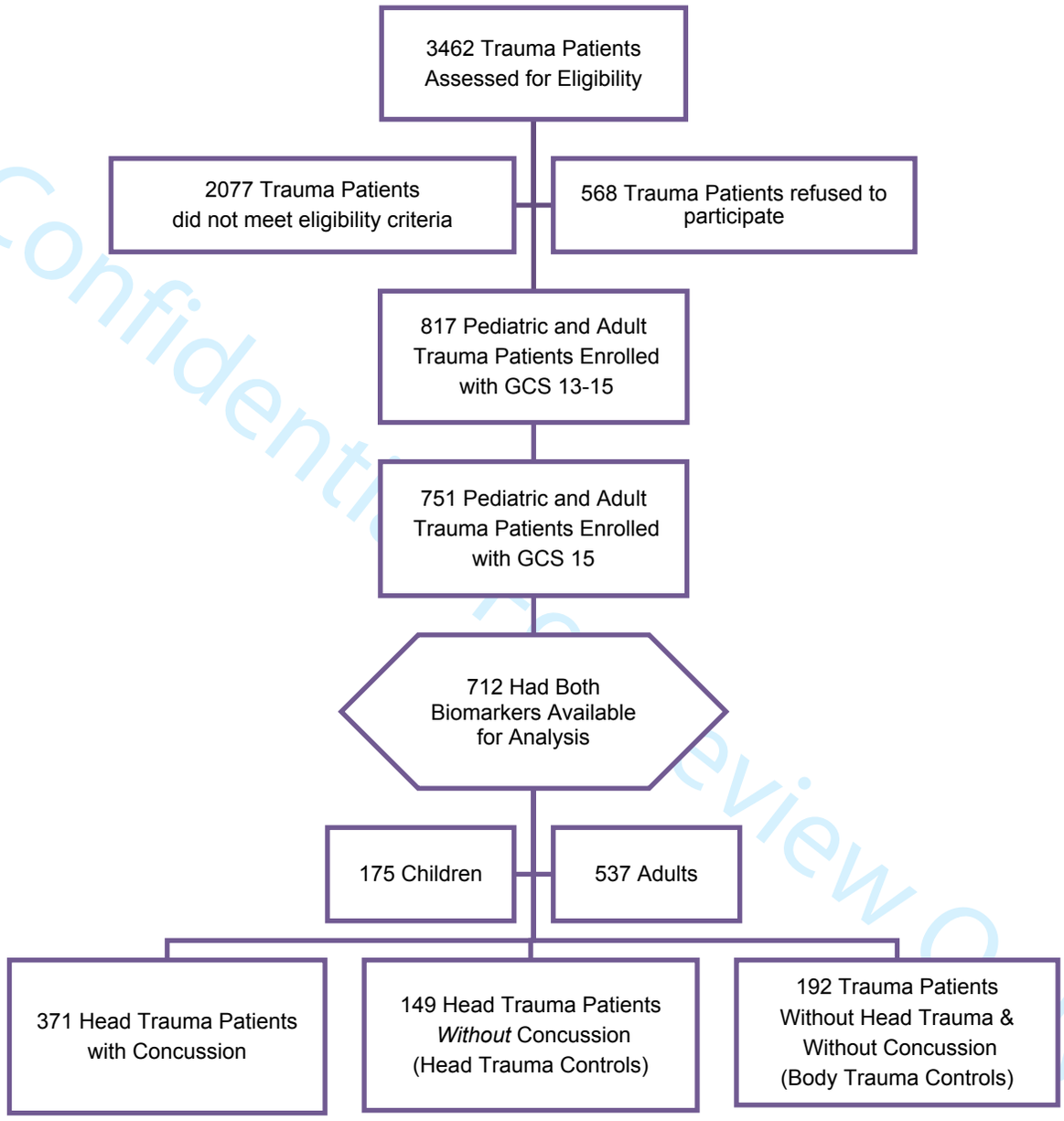


Figure 2. Temporal Profile of GFAP and UCH-L1 in three groups of trauma patients

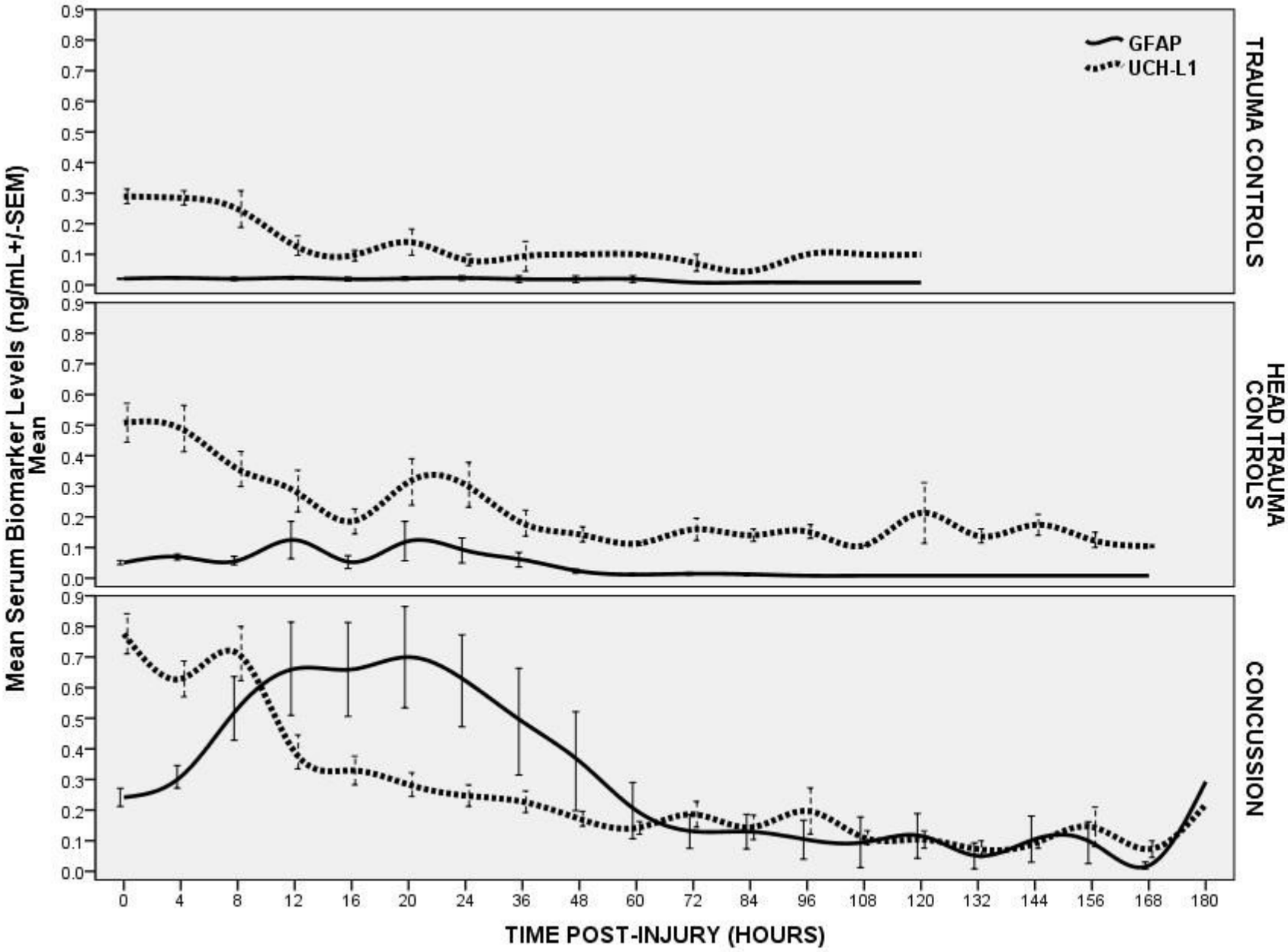
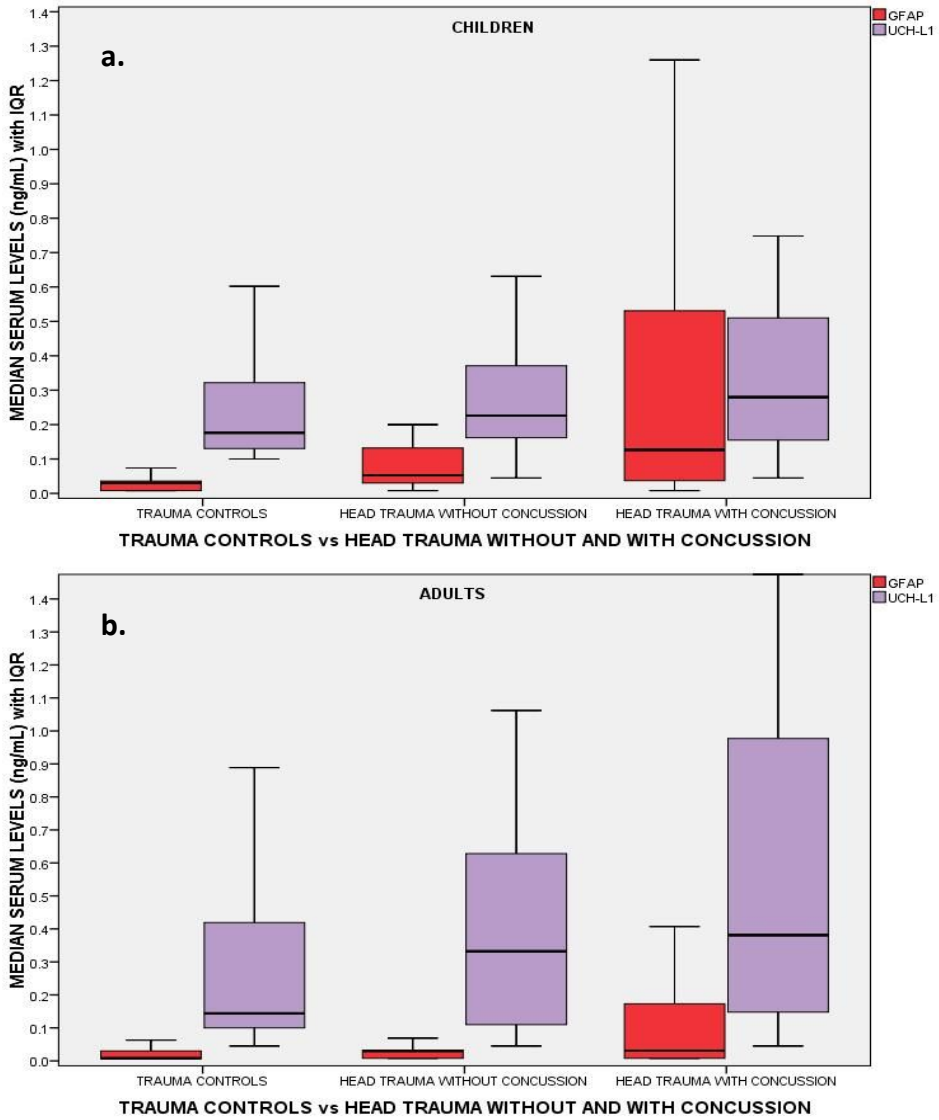


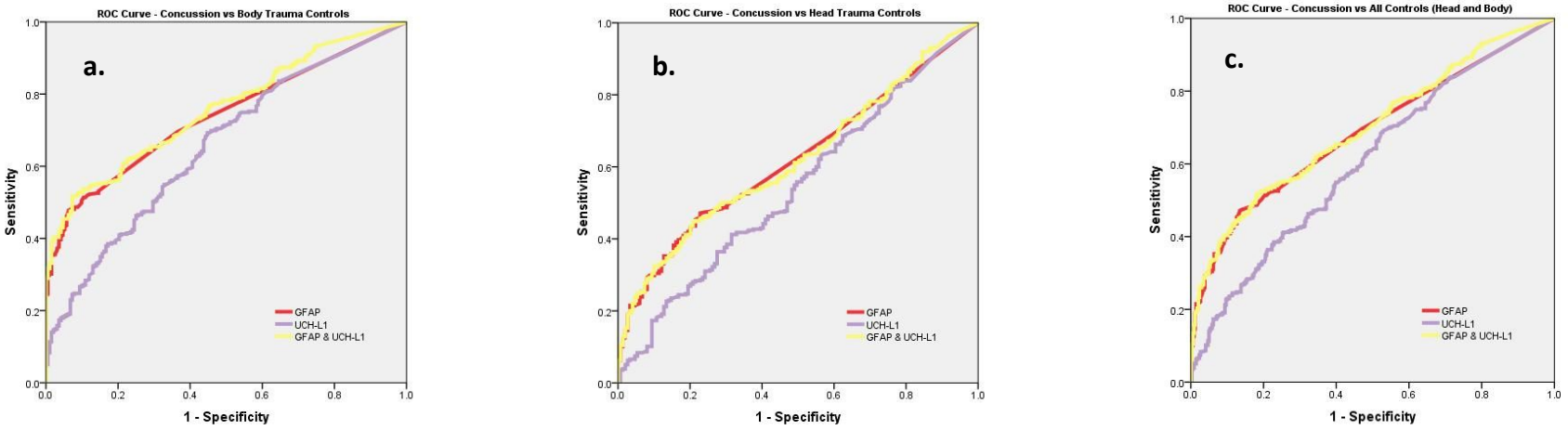
Figure 3. Boxplot comparing initial levels of GFAP and UCH-L1 in three groups of trauma patients: 1) trauma controls without head trauma (controls), 2) head trauma controls without concussion (no concussion); and 3) head trauma with concussion (concussion).



MULTIPLE COMPARISONS	CHILDREN N=175	ADULTS N=537
GFAP		
Trauma Control **vs** Head Trauma Control	<0.001	<0.001
Trauma Control **vs** Concussion	<0.001	<0.001
Head Trauma Control **vs** Concussion	<0.001	<0.001
UCH-L1		
Trauma Control **vs** Head Trauma Control	0.410	0.002
Trauma Control **vs** Concussion	0.045	<0.001
Head Trauma Control **vs** Concussion	0.894	0.017

**Controlled for multiple comparisons*

Figure 4. ROC Curves comparing initial levels of GFAP and UCH-L1 (individually and in combination) for predicting a concussion in trauma patients excluding patients with traumatic intracranial lesions on CT.



CONCUSSION VS NO CONCUSSION (EXCLUDING PATIENTS WITH POSITIVE CT)	CHILDREN N=175	ADULTS N=537	*ALL PATIENTS N=712
Concussion **vs** Body Trauma (No Concussion)	n=80 & n=47	n=255 & n=145	n=335 & n=149
GFAP	**0.77** (0.68-0.85)	**0.74** (0.69-0.79)	**0.74** (0.70-0.78)
UCH-L1	**0.58** (0.48-0.69)	**0.68** (0.62-0.73)	**0.66** (0.61-0.70)
GFAP & UCH-L1	**0.76** (0.68-0.84)	**0.76** (0.72-0.81)	**0.76** (0.72-0.80)
Concussion **vs** Head Trauma (No Concussion)	n=80 & n=34	n=255 & n=115	n=335 & n=192
GFAP	0.58 (0.47-0.68)	0.64 (0.58-0.70)	0.62 (0.57-0.67)
UCH-L1	0.50 (0.39-0.62)	0.56 (0.50-0.62)	0.54 (0.49-0.60)
GFAP & UCH-L1	0.57 (0.46-0.67)	0.64 (0.58-0.70)	0.62 (0.57-0.67)
Concussion **vs** Head and Body Trauma Controls	n=80 & n=81	n=255 & n=260	n=335 & n=341
GFAP	0.69 (0.60-0.77)	0.70 (0.65-0.74)	0.69 (0.65-0.73)
UCH-L1	0.55 (0.46-0.64)	0.63 (0.58-0.67)	0.61 (0.56-0.65)
GFAP & UCH-L1	0.68 (0.60-0.76)	0.71 (0.66-0.75)	0.70 (0.66-0.74)

*Depicted in the ROC curve graphs