Elevaed Levels of Serum Glial FibrillaryAcidic Protein Breakdown Products in Mild and Moderate Traumatic Brain Injury are Associated With Intracranial Lesions and Neurosurgical Intervention

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Abstract

Objective—This study examined whether serum levels of GFAP breakdown products (GFAP-BDP) were elevated in mild and moderate TBI compared to controls and if they were associated with traumatic intracranial lesions on CT scan (+CT) and having a neurosurgical intervention (NSI).

Methods—This prospective cohort study enrolled adult patients presenting to three Level 1 Trauma Centers following blunt head trauma with loss of consciousness, amnesia, or disorientation and a GCS 9–15. Control groups included normal uninjured controls and trauma controls presenting to the ED with orthopedic injuries or an MVC without TBI. Blood samples were obtained in all patients within 4 hours of injury and measured by ELISA for GFAP-BDP (ng/ml).

Results—Of the 307 patients enrolled, 108 were TBI patients (97 with GCS 13–15, and 11 with GCS 9–12) and 199 were controls (176 normal controls and 16 MVC controls and 7 orthopedic controls). ROC curves demonstrated that early GFAP-BDP levels were able to distinguish TBI from uninjured controls with an AUC of 0.90 (95%CI 0.86–0.94) and differentiated TBI with a GCS 15 with an AUC 0.88 (95%CI 0.82–0.93). Thirty two TBI patients (30%) had lesions on CT. The AUC for discriminating those patients with CT lesions versus those without CT lesions was 0.79 (95%CI 0.69–0.89). Moreover, the ROC curve for distinguishing NSI from no NSI yielded an AUC of 0.87 (95%CI 0.77–0.96).

Conclusions—GFAP-BDP is detectable in serum within an hour of injury and is associated with measures of injury severity including the GCS score, CT lesions and neurosurgical intervention. Further study is required to validate these findings before clinical application.

Keywords

Traumatic Brain Injury; head injury; trauma; human; biomarkers; proteomics; diagnostic; Serum; Computed Tomography; neurosurgical intervention; sensitivity; specificity

INTRODUCTION

Background

Traumatic brain injury (TBI) is a significant cause of death and disability in the United States[1] with 52,000 annual deaths and 5.3 million impaired by its effects. Each year in the United States there are at least 1.7 million people who sustain a TBI with 1.4 million of these treated and released from emergency departments across the country.[2] Traumatic brain injury is a leading cause of combat casualty with an estimated 15–25% of all injuries sustained in 20th century conflicts being to the head.[3–5] Tools to diagnose and triage brain injury victims would be useful in both civilian and military settings. Accurate diagnosis in acute care environments is critical to patient outcome. Such decisions include performing Computed Tomography (CT) scans of the brain, seeking neurosurgical consultation, admitting or transferring to a higher level of care, returning to play or duty, and averting the

consequences of "second impact syndrome,"[6, 7] when repeated concussions in a short period become potentially debilitating or fatal.

Importance

Conventionally, early risk stratification of brain injury is based on CT scanning.[8–10] According to recent estimates, 62 million CT scans are performed annually in the US.[11] While increasing CT use has improved diagnostic capabilities and reduced hospital admissions,[12] it has also raised concern over unnecessary exposure to ionizing radiation.[13–17] Some studies suggest that CT scans of the head may be among the largest contributors to radiation exposure due to the frequency with which they are performed.[18, 19] Moreover, in the United States there is a very high rate of ordering CT scans for mild TBI (also known as concussion) that is fostered by the nature of ED practice: high case volumes, brief physician-patient contact, uncertain follow-up, and fear of medicolegal repercussions.[20] Yet, emergency departments with a high ordering rate of head CT scans can still miss intracranial injuries.[21, 22] Furthermore, more subtle lesions or diffuse injury may not be evident on CT acutely.[23–26]

Unlike other organ-based diseases, such as myocardial ischemia, where rapid serum-based biomarkers prove invaluable to guide diagnosis and treatment, there are no such rapid, definitive diagnostic neurochemical markers for TBI that quantify the gravity of the injury. There have been a number of biomarkers assessed in TBI.[27] The most extensively studied among these include glial protein S-100 beta( )[28–32], neuron-specific enolase (NSE)[33–36], and myelin basic protein (MBP)[37–40] Although some of these published studies suggest that these biomarkers correlate with degree of injury; there are conflicting results.[41–46]

Glial Fibrillary Acidic Protein (GFAP) is a monomeric intermediate protein found in astroglial skeleton that was first isolated by Eng et al. in 1971.[47] GFAP is found in white and gray brain matter and is strongly upregulated during astrogliosis.[48] Current evidence indicates that serum GFAP might be a useful marker for various types of brain damage from neurodegenerative disorders[49, 50] and stroke[51] to severe traumatic brain injury.[52–57] Recently, Vos et al. described serum GFAP profile in severe and moderate TBI (GCS <12)[31] but there are no published studies on profiling serum GFAP levels following mild TBI.

Goals of This investigation

Recently we became aware that GFAP appears highly vulnerable to proteolytic modifications in vitro and in vivo and confirmed that the form of GFAP in biofluids is likely to be breakdown product (BDP) of GFAP.[58–60] This study examined whether GFAP-BDP was significantly elevated in the serum of mild and moderate TBI patients compared to controls. Additionally, this study examined the relationship between GFAP-BDP levels and measures of acute injury severity such as GCS, traumatic intracranial lesions on CT scan and neurosurgical intervention.

MATERIALS AND METHODS

Study Design

This prospective controlled cohort study enrolled a convenience sample of adult patients with suspected TBI following blunt head trauma presenting to the emergency department within 4 hours of injury with a GCS of 9 to 15. Trauma control patients were enrolled simultaneously.

Setting

Study sites included the Emergency Departments (ED) of three Level I Trauma Centers; Shands at University of Florida in Gainesville, Florida; Orlando Regional Medical Center in Orlando, Florida; and Washington University in St. Louis, Missouri. This study was approved by the respective Institutional Review Boards (IRB) of each institution. Written informed consent was obtained from patients and/or legal authorized representatives prior to enrollment. In the event the patient was unable to consent for themselves and a legal authorized representative was not present or reachable, the IRB granted permission to collect initial samples using delayed 24-hour consent. Consent was required within 24 hours of being deemed eligible. Delayed consent was rarely required.

Selection of Participants

Eligibility for suspected mild TBI was determined by the treating physician based on the history of blunt head trauma followed by either loss of consciousness, amnesia, or disorientation[61] and presenting to the emergency department within 4 hours of injury with a GCS of 9 to 15. Head CT Scans were performed at the discretion of the treating physician. Patients were excluded if: 1) they were less than 18 years old; 2) there was no history of trauma as their primary event (e.g. syncope or seizure); 3) they had known dementia, chronic psychosis or active CNS pathology; or 4) were pregnant.

There were two control groups: 1) normal adult volunteers without any acute injuries who responded to advertisements in a local flyer; 2) non-head injured patients presenting to the emergency department with either a single limb orthopedic injury or following a motor vehicle collision without blunt head trauma. The mechanisms of injury for the orthopedic controls included falls, crush injuries, or direct impact to a limb resulting in a single limb fracture and/or dislocation. There could be no other concomitant injuries. Orthopedic controls could be exposed to significant forces but could not be in a motorized vehicle.

Trauma control patients had a normal mental status at the time of enrollment and had no evidence of acute brain injury or hemodynamic instability and were enrolled during the same period as TBI patients were enrolled. These controls were carefully screened to ensure they had no blunt head trauma and no symptoms of brain injury, including no loss of consciousness, no amnesia and no alteration in sensorium at any time after injury. The purpose of including non-head injured trauma controls was to examine biomarker levels in patients who were exposed to the acceleration and deceleration forces without blunt trauma.

Data Collection and Processing

All initial patient assessments were made by board certified emergency medicine physicians trained by a formal one-hour session on evaluating patient eligibility. At the time of enrollment, the study team carefully reassessed every patient to ensure each patient met inclusion criteria and verified any exclusions. Any finding of loss of consciousness, amnesia or disorientation had to be documented in the medical record for the patient to be enrolled. Blood samples were obtained from each TBI and non-head injured trauma control shortly after arrival to the ED and within 4 hours of the reported time of injury. There was only one serum GFAP-BDP biomarker level analyzed per patient in the 4-hour post-injury period. A single vial of approximately 5mL of blood was collected and placed in clot tubes with a serum separator 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.). There, the samples were analyzed in batches using a sandwich enzyme-linked immunosorbent assay (ELISA) to GFAP-BDP. Reports of the assay results were sent after the lab had received a given quota. After assessment and treatment in the emergency

department, patients were either discharged home or admitted to hospital based on severity of their injuries and patient management was not altered by the study. Blood sampling and handling in uninjured normal controls was conducted in the same manner.

Patients underwent standard CT scan of the head according to the judgment of the treating physician. The CT scan ordering pattern at the participating Level I trauma centers is such that most patients with blunt head injury with subsequent symptoms have a head CT scan performed as part of usual care. In some instances, physicians ordered CT scans of the head on trauma controls based on mechanism or clinical circumstances. CT examinations of each TBI patient were interpreted by board-certified radiologists who recorded location, extent and type of brain injury. Radiologists were blinded to the study protocol but had the usual clinical information. Lab personnel running the samples were blinded to the clinical data.

Biomarker Analysis

When a full length purified recombinant human GFAP is subjected to in vitro treatment with calpain, it is processed to mainly 42kD and 38kD breakdown products (BDPs).[59, 60] To construct a GFAP-BDP specific sandwich ELISA, many mouse monoclonal antibodies (Mab) raised against GFAP and it BDP's were actively screened. Full length GFAP was almost undetected, as determined by probing with human GFAP in cell lysate with or without calpain digestion in vitro. However, one identified Mab IgG (clone 2H12) detected GFAP breakdown products (BDP's) and this antibody was used as the capture antibody for the ELISA. In contrast, a rabbit polyclonal antibody (Pab) raised against the midsection of full length GFAP detected both full length human GFAP (50K) and its major BDP's (42K, 38K) with high affinity. Since the capture Mab 2H12 provided the required specificity for GFAP-BDP, the Pab served as the detection antibody, Using this antibody pair and denatured recombinant GFAP as BDP-mimic, a GFAP-BDP specific ELSIA was constructed. The intra-assay Coefficient of Variance (CV) was 4.3% to 7.8% while the inter-assay CV was 7.8% to 14.3% from high to low concentration. The limit of detection (LOD) within the linear dynamic range was determined to be 0.020 ng/mL. Samples with undetectable (ND) levels of GFAP-BDP were assigned a value of 50% of the lower limit of detection (i.e. ND=0.010 ng/mL). Any samples yielding a signal over the quantification range was diluted and re-assayed.

Outcome measures

The primary outcome measure tested the ability of GFAP-BDP to distinguish patients with mild and moderate TBI from those without TBI (normal uninjured controls) and assessed the relationship to non-head injured trauma controls. The secondary outcome measures tested the ability of GFAP-BDP to distinguish between different levels of injury severity. These severity measures included: 1) Glascow Coma Score (GCS) scores [62, 63] obtained at presentation to the emergency department; 2) the presence of intracranial lesions on initial CT scan; and 3) having a neurosurgical intervention. Intracranial lesions on CT included any acute traumatic intracranial lesions visualized on CT scan. Neurosurgical intervention was defined as either death within 7 days secondary to head injury or the need for any of the following procedures within 7 days: craniotomy, elevation of skull fracture, intracranial pressure monitoring, or intubation for head injury.[64, 65]

Primary Data Analysis

Descriptive statistics with means 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 means (±95%CI). Data were assessed for equality of variance and distribution. Logarithmic transformations were conducted on non-normally distributed data. Group comparisons for different GCS Scores 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 biomarker to distinguish between controls and TBI patients within 4 hours of injury, as well as for intracranial lesions on CT scan and neurosurgical intervention. 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). GFAP-BDP cutpoints were selected to maximize the sensitivity and correctly identify as many patients with CT lesions and neurosurgical intervention as possible. Classification performance was assessed by sensitivity, specificity, positive and negative predictive values with 95% confidence intervals. All analyses were performed using the statistical software package PASW 17.0 (IBM Corporation®, Somers NY).

A pilot study provided preliminary data to calculate a sample size for distinguishing TBI patients with a positive CT versus a negative CT. A sample of 28 from the positive CT group and 28 from the negative CT group achieve 80% power to detect a difference of 0.10 between the area under the ROC curve AUC0 (for GFAP) under the null hypothesis of 0.89 and an AUC1 (for standard CT) under the alternative hypothesis of 0.99 using a two-sided z-test at a significance level of 0.05.

RESULTS

A total of 307 patients were enrolled in the study and had serum samples drawn within 4 hours of injury for analysis. There were 108 TBI patients: 97 with GCS 13–15 and 11 with GCS 9–12; and 199 controls: 176 normal controls with no injuries and 23 trauma controls who had peripheral injuries without TBI (16 MVC controls and 7 orthopedic controls). The flow diagram in Figure 1 describes the distribution of enrolled patients. CT scan of the head was performed in all TBI patients and traumatic intracranial lesions on CT scan were evident in 32 (30%): 24 (75%) of patients presented with a GCS 13–15 and 8 (25%) with GCS 9–12. A CT scan was also performed in 9 trauma control patients despite the patients' lack of signs or symptoms of TBI – no blunt trauma, no loss of consciousness, no amnesia and no alteration in sensorium at any time after injury. These CT's were performed at the discretion of the treating physician based on mechanism or clinical circumstances and none of them showed any signs of traumatic intracranial lesions. Neurosurgical intervention was performed on 14 patients (13%), 6 (43%) presented with a GCS 13–15 and 8 (57%) with GCS 9–12.

There were 25 TBI subjects enrolled from the University of Florida, 53 from Orlando Regional Medical Center and 30 from Washington University. The distribution of clinical characteristics for each site is presented in Table 1. There were no statistically significant differences in the demographic characteristics of the controls (normal and trauma) and TBI patients for age or gender (Table 1).

Both the TBI and trauma controls had serum samples drawn within 4 hours of injury with the average time from injury to serum sample collection at 2.7 hours (95%CI 2.5–2.9). The average time to serum collection for TBI patients was 2.6 hours (95%CI 2.4–2.9); for orthopedic controls it was 2.5 hours (95%CI 1.9–3.2); and for MVC controls it was 3.2 (95%CI 2.7–3.7). The temporal profile of GFAP-BDP in TBI patients within 4 hours post-injury is shown in Figure 2. GFAP-BDP demonstrated a rapid appearance in serum post-injury with levels detectible within an hour of injury. Some of the higher levels were seen starting at approximately 2 hours post-injury. There was a significant difference in the levels of GFAP-BDP between all TBI patients and all controls. A comparison of mean, median and log transformed serum levels of GFAP-BDP between various control groups and TBI groups are presented in Table 2.

In Figure 3a levels of GFAP-BDP in normal and trauma controls are shown relative to 3 groups of GCS score divided as GCS 15, GCS 13–14, and GCS 9–12. There were statistically significant differences between each of the groups relative to the uninjured controls (as noted by the asterisks). In particular, when patients with an ED GCS score of 15 were isolated from the TBI group, early serum GFAP-BDP levels were significantly different between patients with a GCS 15 versus trauma controls (Figure 3b). When we dichotomized the GCS score into the traditional GCS 13–15 versus GCS 9–12 there were significant differences between the groups (Figure 3c). Additionally, when we dichotomized the GCS score into GCS 14–15 versus GCS 9–13 there were also statistically important differences between the groups (Figure 3d).

Area under the curve (AUC) was calculated from the ROC curves constructed to assess the performance of early GFAP-BDP levels in TBI versus control patients. ROC curves demonstrated that early GFAP-BDP levels were able to distinguish TBI from uninjured controls with an AUC of 0.90 (95%CI 0.86–0.94)(Figure 4a). More specifically, GFAP-BDP was able to differentiate TBI patients with a GCS 15 from normal controls with an AUC 0.88 (95%CI 0.82–0.93)(Figure 4b).

When serum levels of GFAP-BDP were compared in patients with traumatic intracranial lesions on CT scan (CT positive) to those without CT lesions (CT negative), levels were significantly higher in those with lesions on CT scan (Figure 5a). Patients with GCS 15 were assessed independently and serum GFAP-BDP levels were significantly more elevated in those with CT scan lesions than those without (Figure 5b). The area under the curve for discriminating between CT scan positive and CT scan negative intracranial lesions was 0.79 (95%CI 0.69–0.89)(Figure 5c). Figure 5d shows GFAP-BDP levels in the 16 trauma controls having no CT performed versus the 9 trauma control patients who had CT scans of the head ordered by their treating physician despite lack of TBI symptoms. There was no difference in GFAP-BDP levels between the trauma controls who did or did not have CT scans performed. TBI patients with a negative CT had significantly higher levels of GFAP-BDP than trauma controls with a negative CT. Most notable was that GFAP-BDP levels were significantly elevated in patients with traumatic intracranial lesions on CT (CT positive) than those without CT lesions (CT negative) (significantly different from each group) regardless of whether they were trauma controls or TBI.

Additionally, we compared serum levels of GFAP-BDP in patients who had a neurosurgical intervention versus those who received no such intervention. Substantially higher serum levels were detected in those who had a neurosurgical intervention (Figure 6a). The ROC curve for discriminating between those having and not having a neurosurgical intervention yielded an AUC of 0.87 (95%CI 0.77–0.96)(Figure 6b).

Cutoff points for GFAP-BDP were derived from the ROC Curves for detecting intracranial lesions on CT scan and having a neurosurgical intervention. The aim of this exploratory analysis was to maximize the sensitivity and correctly classify all CT positive lesions and all those with a neurosurgical intervention. Classification performance for detecting intracranial lesions on CT at a GFAP-BDP cutoff level of 0.035 ng/ml yielded a sensitivity of 97% (95%CI 82–100), a specificity of 18% (95%CI 11–28) and a negative predictive value of 94% (95%CI 68–100)(Table 3a). Classification performance for predicting neurosurgical intervention at a GFAP-BDP cutoff level of 0.17 ng/ml yielded a sensitivity of 100% (95%CI 73–100), a specificity of 42% (95%CI 32–52) and a negative predictive value of 100% (95%CI 90–100)(Table 3b).

LIMITATIONS

While these data are encouraging, the authors recognize there are limitations to this study. The current study was performed in a limited cohort of patients with mild and moderate TBI, a disease that tends to be heterogeneous in nature. Patients were enrolled as a convenience sample because research team members could not be on duty 24/7. Despite this, patients were recruited consecutively when research assistants were on duty including on weekends and nights so a representative sample could be enrolled.

We used very strict criteria for defining suspected TBI subjects and control subjects. This may have limited the clinical applicability of our findings in trauma patients. Our definition specified that the patient had to have blunt head trauma followed by either loss of consciousness, amnesia, or disorientation. However, there were nine control patients who physicians felt sufficiently concerned about to order a head CT on, despite the lack of clinical signs and symptoms of TBI. We attempted to adjust for this by adding these nine trauma controls to our analysis of CT and neurosurgical intervention in order to reflect actual practice and improve the clinical applicability of our findings. Future studies should consider testing the biomarker in its intended setting which includes any patient with suspected TBI following trauma based on the judgment of the treating physician.

At this time we cannot confirm that GFAP-BDP is entirely CNS specific and not released from other organs. However, its ability to distinguish between TBI and controls is encouraging. Injured controls were exposed to significant non-trivial trauma and they served as robust comparators for the TBI patients. Current studies of patients with multi-trauma will further address the effect of extracranial injuries on GFAP-BDP values. Additionally, analysis on the biokinetics and temporal profile of GFAP-BDP are being conducted that will give insight into pattern of release of GFAP-BDP over time and during different clinical circumstances. This will also help characterize the optimal timing of sampling after injury.

This study addressed severity of injury in the acute care setting and did not describe long-term outcome in these patients. Outcome data will be assessed as these data become available in our ongoing studies.

DISCUSSION

Although there are studies assessing GFAP in serum, these studies have looked primarily at patients with severe TBI.[53–56] This clinical study is among the first to systematically assess early levels of GFAP-BDP in human serum in TBI patients with GCS 9–15. We elected to study both mild and moderate injury because initial GCS scores in the ED in this population can be surprisingly deceptive. The classification of a TBI as a mild or a moderate can change based on neuroimaging results and the presence of factors altering mental status such as intoxication, medications and other injuries. A patient with a GCS of 15 who has an acute bleed on CT scan can be classified as moderate. Conversely, a patient with a GCS of 11 who has no evidence of intracranial injury on CT scan can be classified as a mild. Although we studied TBI patients from GCS 9–15 we included focused analyses of those with a GCS score of 15.

Our group recently discovered that GFAP appears highly vulnerable to proteolytic modifications in vitro and in vivo and confirmed that the form of GFAP in biofluids is likely to be breakdown product (BDP) of GFAP.[59, 60] GFAP has previously been studied rather limitedly as a possible protease substrate. Following TBI GFAP-BDP is likely a product of calpain over-activation, leading to its release from injured or degenerative glia cells. Therefore, it appears that GFAP-BDP's are not only released during gliosis subacutely but appears within hours after glial damage from TBI. Thus, in addition to the clinical

correlations this new biochemical finding adds to the significance of the interpretation of these biomarker levels after brain injury.

Much of the previous work on biomarkers in mild TBI has been limited by factors such as wide variations in sample collection times and inadequate control groups. When we designed the study we carefully considered these limitations. To overcome the sample schedule shortfall we restricted sample collection to within 4 hours of injury to reflect actual clinical practice and measure GFAP-BDP as soon after injury as possible. Additionally, we incorporated three different control groups in this study. Uninjured controls represented the general population and non-head injured trauma controls had either orthopedic injuries or exposure to the forces of motor vehicle crashes. This allowed for a robust comparison of the biomarker levels between TBI and various controls and reflects how it would be used in a real-time clinical setting for trauma patients. Many trauma controls were exposed to significant trauma including the acceleration-deceleration vectors of motor vehicle crashes and falls from heights over 5 feet. Their injuries paralleled TBI patients except for their lack of both blunt head trauma and TBI symptoms. The fact that trauma controls had significantly higher levels of GFAP-BDP's than uninjured controls may reflect the force of the mechanism. To our advantage, in nine trauma patients the mechanism was so significant that physicians actually ordered head CT's as part of their clinical care despite the lack of blunt head injury and the lack of signs and symptoms of brain injury. This allowed us to assess levels of GFAP-BDP in CT negative trauma controls against trauma controls without CT and CT negative TBI patients. TBI patients with a negative CT had higher levels than trauma controls, regardless of having a CT done. More importantly, the largest elevation in GFAP-BDP occurred in those with traumatic intracranial lesions on CT, regardless of GCS or the type of trauma control.

This provides a unique glimpse of GFAP-BDP levels in clinically apparent TBI from non-TBI trauma or, at least, non-clinically apparent TBI. Ongoing studies by our group are assessing long-term outcomes in these patients which will ultimately determine the true impact of the TBI on patient outcome. A large number of normal controls were included to facilitate the development of appropriate normative data.

Also unique to this study is that the majority of serum samples were obtained very early post-injury when patients first arrived in the emergency department. GFAP-BDP demonstrated a rapid appearance in serum post-injury with levels detectable in less than an hour of injury. Levels rose incrementally with severity of injury. They were either undetectable or in very low levels in normal controls, slightly higher in trauma controls, significantly elevated in TBI patients with a GCS 15 and were seen in its highest levels in TBI subjects with a positive CT.

Such acute elevations of this biomarker can make it a potentially useful clinical tool for determining injury severity early in the course after injury. Potential applications could include influencing the decision to image patients who are intoxicated or sedated, determining the degree brain injury in a multiple trauma victim, seeking neurosurgical consultation or making transport decisions about transfer to a neurosurgical facility.

Concerns over exposure to ionizing radiation from CT scans[13–17] has led to calls for judicious use of such tests.[66, 67] Evidently, high GFAP-BDP levels were associated with traumatic intracranial lesions on CT scan (CT positive), a finding which held true for patients with a GCS of 15 as well. If these findings can be validated, GFAP-BDP's association with the presence of intracranial lesions on CT scans could help emergency and trauma physicians reduce the number CT's they perform. Accordingly, GFAP-BDP could be

incorporated into guidelines for neuroimaging decisions and decisions to transfer patients to neurosurgical facilities.

CONCLUSION

This study is among the first to systematically assess GFAP-BDP in human serum following mild and moderate TBI. We confirmed that the GFAP-BDP is present in human serum and that its levels are significantly elevated in this population using ELISA analysis, including those presenting with a GCS 15. GFAP-BDP is detectable in serum within a few hours of injury and is associated with measures of injury severity including the GCS score, CT lesions and neurosurgical intervention. This present work extends findings from studies in severe TBI. Further study is required to validate these findings before clinical application.

Acknowledgments

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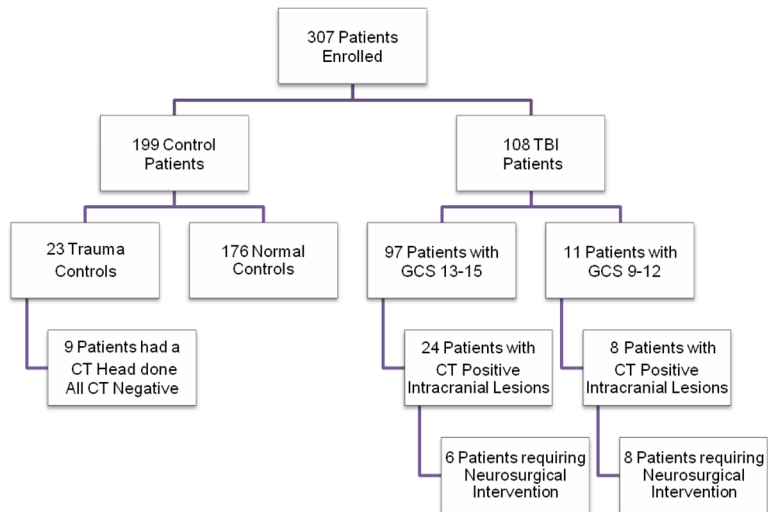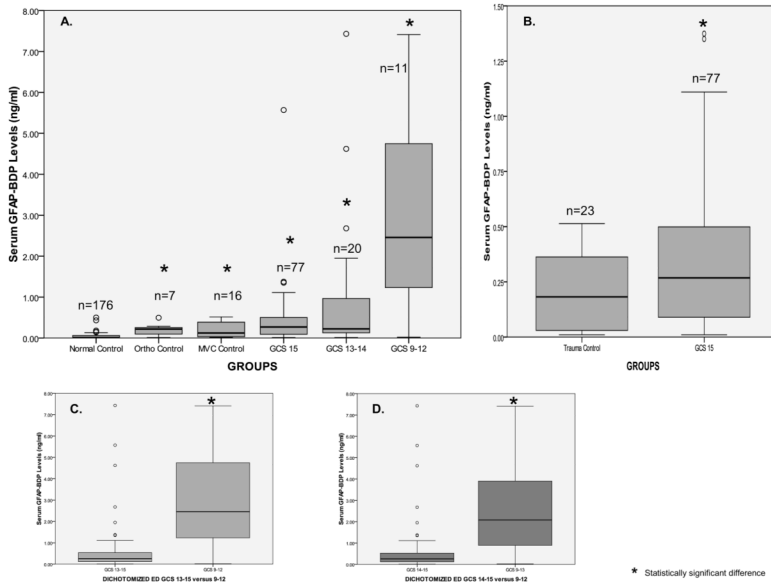**Figure 1. Flow diagram of enrolled patients**
Flow diagram showing the number of all TBI and control patients enrolled.

Figure 2. Temporal profile of GFAP-BDP in TBI patients within 4 hours of injury
The dots represent levels of GFAP-BDP (ng/ml) at different times post-injury. GFAP-BDP
demonstrated a rapid appearance in serum post-injury with levels detectible within an hour
of injury.

Figures 3a, 3b, 3c, & 3d.
Figure 3a. A comparison of serum levels of GFAP-BDP drawn within 4 hours of injury in TBI patients with different GCS scores versus normal and trauma controls. GCS scores were divided as GCS 15, GCS 13–14, and GCS 9–12. There are 3 control groups: i-non-injured, ii- non-head injured orthopedic controls, and iii- non-head injured controls from motor vehicle collisions. There are statistically significant differences between the uninjured controls and each of the groups (indicated by the asterisk "*") and serum levels of GFAP-BDP increased incrementally with worsening GCS scores. Boxplots represent medians in ng/ml and interquartile ranges.
Figure 3b. A comparison of serum levels of GFAP-BDP drawn within 4 hours of injury in TBI patients with GCS 15 versus trauma controls. When TBI patients with an ED GCS score of 15 are isolated from the TBI group early GFAP-BDP levels demonstrate significant differences between patients with a GCS 15 versus trauma controls. Boxplots represent medians in ng/ml and interquartile ranges.
Figure 3c. A comparison of serum levels of GFAP-BDP in TBI patients with GCS dichotomized into GCS 13–15 versus GCS 9–12. When TBI patients are dichotomized into the traditional GCS 13–15 versus GCS 9–12, early GFAP-BDP levels are significantly different between the 2 groups. Boxplots represent medians in ng/ml and interquartile ranges.
**Figure 3d. A comparison of serum levels of GFAP-BDP in TBI patients with GCS dichotomized into GCS 14–15 versus GCS 9–13.** When TBI patients are dichotomized into the more recently suggested dichotomy of GCS 14–15 versus GCS 9–13, early GFAP-BDP levels are significantly different between the 2 groups. Boxplots represent medians in ng/ml and interquartile ranges.
* Statistically significant difference
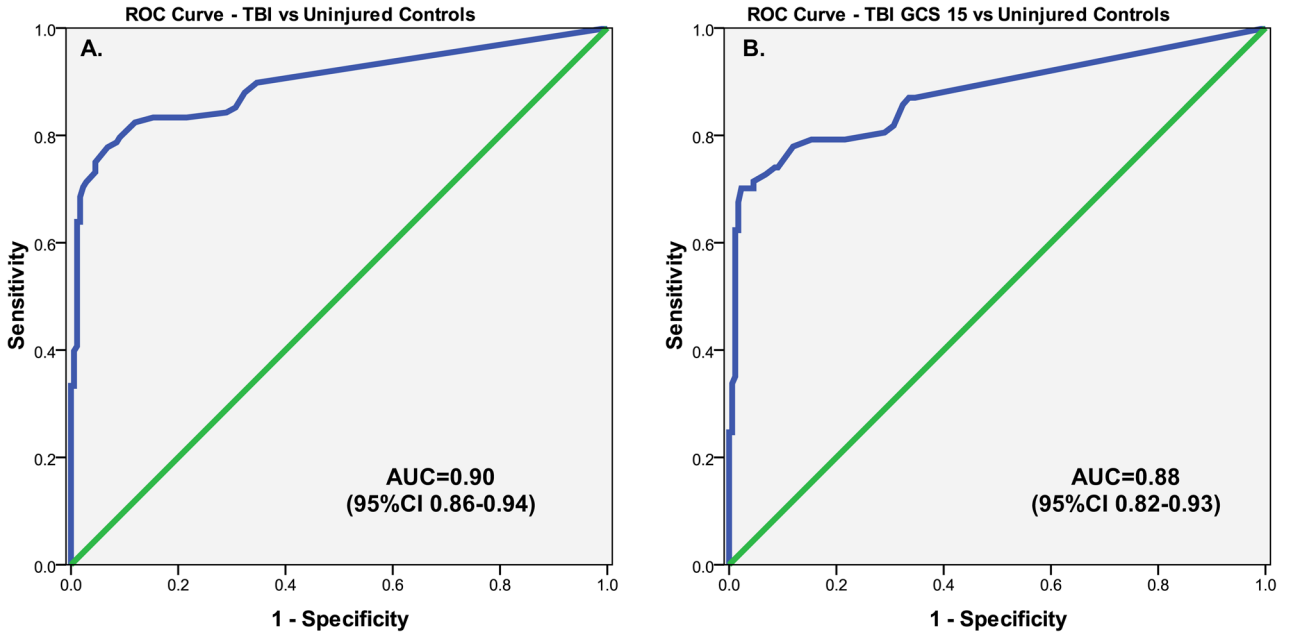
Figures 4a & 4b.
Figure 4a. ROC Curve for distinguishing TBI versus uninjured controls. The area under the ROC curve (AUC) demonstrates that early GFAP-BDP levels are able discriminate between patients without injuries from patients with TBI with an AUC 0.90 (95%CI 0.86–0.94).
Figure 4b. ROC Curve for distinguishing TBI patients with a GCS 15 versus uninjured controls. TBI Patients with a GCS 15 are isolated from the group and compared to uninjured controls. Early GFAP-BDP levels are able discriminate between patients without injuries from TBI patients with a GCS 15 with an AUC 0.88 (95%CI 0.82–0.93).
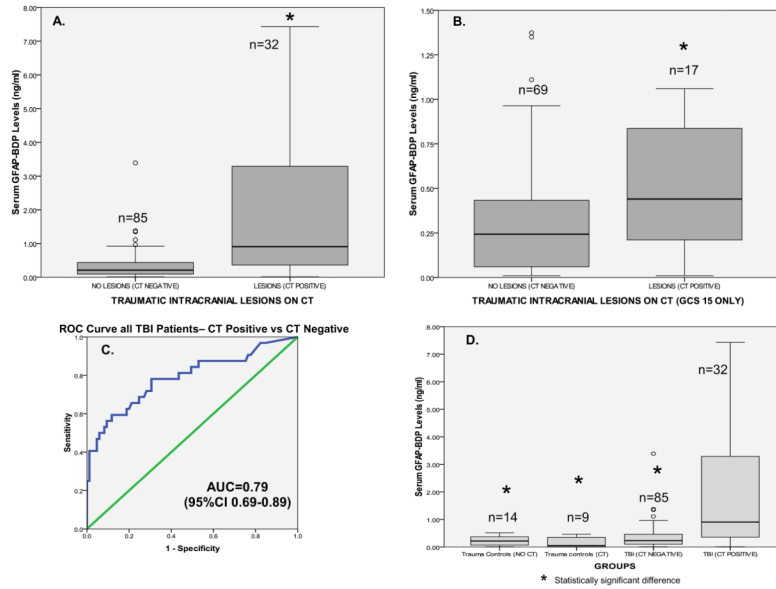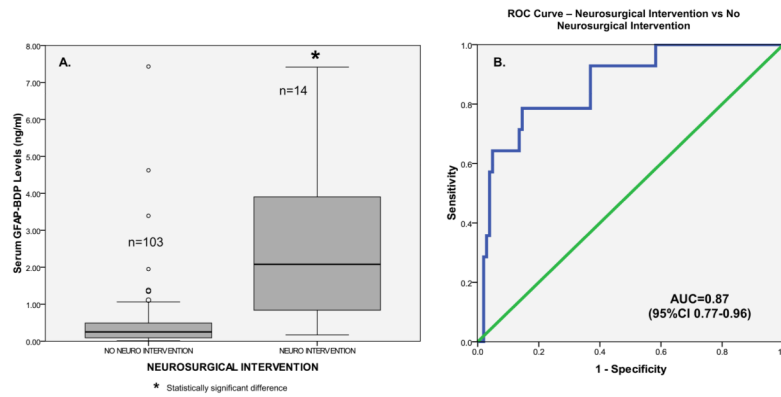
Figures 5a, 5b & 5c.

Figure 5a. Boxlplot comparing serum GFAP-BDP levels drawn within 4 hours of injury in patients with and without traumatic intracranial lesions on CT. Levels of serum GFAP-BDP are significantly higher in patients with traumatic intracranial lesions on CT (CT positive) than those without CT lesions (CT negative). Bars represent medians in ng/ml and interquartile ranges.

Figure 5b. Boxplot comparing serum GFAP-BDP levels drawn within 4 hours of injury in patients with and without traumatic intracranial lesions on CT in TBI patients with GCS 15. In a subset of TBI patients with a GCS 15 levels of serum GFAP-BDP are significantly higher in those with traumatic intracranial lesions on CT (CT positive) than those without CT lesions (CT negative). Bars represent medians in ng/ml and interquartile ranges.

Figure 5c. ROC Curve for distinguishing CT positive versus CT negative patients. The area under the ROC curve (AUC) demonstrates that early GFAP-BDP levels are able discriminate between patients with and without traumatic intracranial lesions on CT (AUC 0.79; 95%CI 0.69–0.89).

Figure 5d. Boxplot comparing serum GFAP-BDP levels drawn within 4 hours of injury in patients with and without traumatic intracranial lesions on CT in both TBI and trauma controls. GFAP-BDP levels are significantly higher in patients with traumatic intracranial lesions on CT (CT positive) than those without CT lesions (CT negative) regardless of whether they are trauma controls or TBI. Bars represent medians in ng/ml and interquartile ranges.

* Statistically significant difference

Figures 6a, 6b.

Figure 6a. Boxplot comparing serum GFAP-BDP levels drawn within 4 hours of injury in patients with neurosurgical intervention versus those without intervention. Levels of serum GFAP-BDP are significantly higher in patients with who had neurosurgical intervention versus those who did not. Bars represent medians in ng/ml and interquartile ranges.

Figure 6b. ROC Curve for distinguishing those who had a neurosurgical intervention versus those who did not. The area under the ROC curve (AUC) demonstrates that early GFAP-BDP levels are able discriminate between patients with who required neurosurgical intervention versus those who did not (AUC 0.87; 95%CI 0.77–0.96).

* Statistically significant difference

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Table 1

Demographic and clinical data for all subjects included in the study

The table describes data from patients enrolled overall and at each center.

Characteristics	Total N=108	University of Florida N=25	Orlando Regional Medical Center N=53	Washington University N=30	Normal Controls N=176	Trauma Controls N=23
		TBI			CONTROL	
Mean age (yrs±SD)	39 (±15)	35 (±15)	39 (±15)	44 (±16)	37 (±14)	44 (±17)
Range	(18–89)	(18–61)	(19–70)	(19–89)	(18–65)	(19–83)
Gender (%) Male	70 (65)	16 (64)	34 (64)	20 (67)	93 (53)	16 (70)
GCS Score in ED (%)						
GCS 9–12	11 (10)	5 (20)	3 (6)	3 (10)	0 (0)	0 (0)
GCS 13–15	97 (90)	20 (80)	50 (94)	27 (90)	176 (100)	23 (100)
Mechanism of Injury						
Motor Vehicle Crash	45 (42)	15 (60)	25 (47)	5 (17)	0 (0)	16 (70)
Fall	24 (22)	3 (12)	7 (13)	14 (46)	0 (0)	2 (9)
Motorcycle	14 (14)	2 (8)	11 (21)	1 (3)	0 (0)	0 (0)
Pedestrian Struck	7 (7)	2 (8)	3 (6)	2 (7)	0 (0)	1 (4)
Bicycle	6 (6)	0 (0)	4 (7)	2 (7)	0 (0)	0 (0)
Hit with blunt object	4 (3)	0 (0)	1 (2)	3 (10)	0 (0)	0 (0)
Assault	4 (3)	0 (0)	2 (4)	2 (7)	0 (0)	0 (0)
Sports/Other	4 (3)	3 (12)	0 (0)	1 (3)	0 (0)	4 (17)
Loss of Consciousness (%)						
Yes	77 (71)	16 (64)	43 (81)	18 (60)	0 (0)	0 (0)
No	25 (23)	6 (24)	10 (19)	9 (30)	176 (100)	23 (100)
Unknown	6 (6)	3 (12)	0 (0)	3 (10)	0 (0)	0 (0)
Amnesia (%)	43 (40)	9 (36)	23 (43)	11 (37)	0 (0)	0 (0)
Admitted to Hospital (%)	66 (61)	23 (92)	27 (51)	16 (53)	0 (0)	10 (43)

Characteristics	Total N=108	University of Florida N=25	TBI Orlando Regional Medical Center N=53	Washington University N=30	CONTROL Normal Controls N=176	Trauma Controls N=23
Intoxicated (Alcohol or Drugs)	13 (12)	7 (28)	4 (8)	2 (7)	-	1 (4)
Neurosurgical Intervention	14 (13)	5 (20)	8 (15)	1 (3)	-	0 (0)
Traumatic Intracranial Lesions on CT Scan of Head	32 (30)	10 (40)	12 (23)	10 (33)	-	0 (0)
Lesion Types on CT Head						
Epidural hematoma	3 (9)	1 (10)	1 (8)	1 (10)		0 (0)
Subdural hematoma	7 (22)	0 (0)	1 (8)	6 (60)		0 (0)
Subarachnoid hemorrhage	8 (25)	3 (30)	3 (25)	2 (20)	-	0 (0)
Contusion	3 (9)	3 (30)	0 (0)	0 (0)		0 (0)
Intracerebral hemorrhage	1 (3)	0 (0)	1 (8)	0 (0)		0 (0)
Pneumocephalus	2 (7)	0 (0)	2 (2)	0 (0)		0 (0)
Combination of lesions	8 (25)	3 (10)	4 (33)	1 (10)		0 (0)

Table 2

Description of GFAP-BDP levels in the different groups

Description of median, mean, and log transformed mean of serum GFAP-BDP levels in the different groups.

	Median [IQR] (ng/ml)	Mean (95%CI) (ng/ml)	SD (ng/ml)	Log transformed Mean (95%CI)
All TBI	0.316 [0.600]	0.893 (0.573, 1.213)	1.677	−1.300 (−1.618, −0.982)
All Controls	0.010 [0.060]	0.057 (0.044, 0.071)	0.098	−3.72 (−3.889, −3.554)
TBI				
GCS 15 (n=77)	0.268 [0.420]	0.5331 (0.259, 0.807)	1.206	−1.614 (−1.961, −1.266)
GCS 14 (n=18)	0.223 [1.132]	1.130 (0.147, 2.113)	1.976	−1.080 (−1.887, −0.273)
GCS 13 (n=2)	0.510	0.510 (0, 5.465)	0.552	−1.113 (−13.91, 11.69)
GCS 12 (n=2)	0.455	0.455 (0, 5.982)	0.615	−2.014 (−26.13, 22.10)
GCS 11 (n=1)	-	7.41	-	2.003
GCS 10 (n=0)	-	-	-	-
GCS 9 (n=8)	2.923 [3.467]	3.208 (1.586, 4.830)	1.940	0.948 (0.291, 1.606)
Uninjured Controls	0.010 [0.050]	0.038 (0.029, 0.047)	0.059	−3.902 (−4.054, −3.750)
Ortho Controls	0.216 [0.275]	0.203 (0.048, 0.357)	0.167	−2.274 (−3.776, −0.771)
MVC Controls	0.122 [0.373]	0.204 (0.102, 0.306)	0.191	−2.371 (−3.196, −1.546)

Table 3a. & 3b

Contingency table and Classification Performance of Serum GFAP-BDP in detecting Intracranial Lesions on CT and Contingency table and Classification Performance of Serum GFAP-BDP in detecting Need for Neurosurgical Intervention

	CT positive	CT negative	Sensitivity	**97% (82–100)**
GFAP-BDP positive 0.035 ng/ml	31	70	Specificity	18% (11–28)
GFAP-BDP negative <0.035 ng/ml	1	15	NPV	94% (68–100)
			PPV	31% (22–41)

	Neurosurgical Intervention	No Neurosurgical Intervention	Sensitivity	**100% (73–100)**
GFAP-BDP positive 0.17 ng/ml	14	60	Specificity	42% (32–52)
GFAP-BDP negative <0.17 ng/ml	0	43	NPV	100% (90–100)
			PPV	19% (11–30)