THE LEVELS OF GFAP AND UCH-L1 DURING THE FIRST WEEK AFTER A TRAUMATIC BRAIN INJURY - CORRELATIONS WITH CLINICAL AND IMAGING FINDINGS

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ABSTRACT

Objective: Astrocyte specific glial fibrillary acidic protein (GFAP) and neuron specific ubiquitin C-terminal hydrolase-L1 (UCH-L1) are promising biomarkers of traumatic brain injury (TBI). We investigated the relation of the GFAP and UCH-L1 levels to the severity of TBI during the first week after the injury.

Methods: Plasma UCH-L1 and GFAP were measured from 389 consecutive patients with acute TBI and 81 controls enrolled in a multicentre prospective study. The baseline measures included initial Glasgow Coma Scale (GCS), head CT scan on admission and blood samples for protein biomarkers that were collected on admission and on days 1, 2, 3 and 7 after the injury.

Results: Plasma levels of GFAP and UCH-L1 on admission and during the first two days after the injury strongly correlated with the initial severity of TBI as assessed with GCS. Additionally, levels of UCH-L1 on the seventh day after the injury were significantly related with the admission GCS scores. On admission, both biomarkers were capable of distinguishing mass lesions from diffuse injuries in computed tomography (CT) and AUC for prediction of any pathological finding in CT was 0.739 (0.636-0.815, 95% CI) and 0.621 (0.517-0.713, 95% CI), for GFAP and UCH-L1, respectively.

Conclusions: These results support the prior findings of potential role of GFAP and UCH-L1 in acute phase diagnostics of TBI. The novel finding in the current study is that levels of GFAP and UCH-L1 correlated with the initial severity of TBI during the first two days after the injury, thus enabling a window for TBI diagnostics with latency.

INTRODUCTION

Acute phase traumatic brain injury (TBI) diagnostics is still based on neurological examination and brain imaging. Reliable determination of the initial severity of TBI is of paramount importance for appropriate triage at emergency departments (ED), decision about admission into ward or intensive care unit, planning of follow-up and rehabilitation, and often also for medico-legal issues.

Given the complexity and heterogeneity of TBI, a peripheral blood-based brain sensitive and specific panel of biomarkers would greatly improve TBI diagnostics and treatment. Glial fibrillary acidic protein (GFAP) is a specific blood biomarker of astroglial injury. GFAP is a monomeric intermediate filament protein, a major constituent of the astroglial cytoskeleton. [1, 2] In earlier reports, the admission blood levels of GFAP have been increased in TBI patients, and have correlated with both initial GCS scores and brain imaging findings. [3, 4]

Ubiquitin C-terminal hydrolase-L1 (UCH-L1) is involved in either adding or removing ubiquitin from proteins targeted for metabolism, abnormal proteins, and proteins damaged by oxidation. [5] The ubiquitous expression of UCH-L1 in the brain and its specificity to neurons represent a promising complement for the GFAP. [6] Increased UCH-L1 levels in blood have been reported in TBI patients and they have correlated with the injury severity, imaging findings, need of neurosurgical interventions, and outcome. [7, 8]

The purpose of this study was to evaluate the utility of GFAP and UCH-L1 in the diagnostics of mild to severe TBI using blood biomarker sampling at five distinct time points during the first week after the injury.

METHODS

STUDY POPULATION

Patient recruitment of this prospective multicenter study was part of the EU funded TBIcare (Evidence-based Diagnostic and Treatment Planning Solution for Traumatic Brain Injuries) project. A total of 389 adult patients with acute TBI and 81 patients with acute orthopedic trauma without acute or previous brain disorders were prospectively recruited at Turku University Hospital, Finland and at Addenbrooke's Hospital Cambridge, United Kingdom.

Inclusion criteria were as follows: age ≥ 18 years (≥ 16 years in UK), clinical diagnosis of TBI and indications for acute head CT according to NICE criteria (http://www.nice.org.uk/guidance/cg176). Exclusion criteria were: blast-induced or penetrating injury, chronic subdural hematoma, inability to live independently due to pre-existing brain disease, TBI or suspected TBI not needing head CT, more than two weeks from the injury, not speaking native language, and no consent obtained. The control group consisted of patients with acute orthopedic non-trivial trauma without any signs of acute central nervous system involvement, previous central nervous system disease, or previous non-concussional TBI. If a patient sustained a TBI and was admitted to hospital e.g. on the second day after the injury, the biomarkers were labelled accordingly so that the first sample was a day 2 sample. South-West Finland Hospital District Research Ethics Committee, the Cambridgeshire 2 Research Ethics Committee, and the Norfolk Research Ethics Committee approved the protocol. All patients or their next of kin were given both oral and written information about the study and a written informed consent was obtained. All patients were treated according to standard local guidelines based on the current international guidelines and recommendations. [9]

ANALYSIS OF GFAP AND UCH-L1

Blood samples for protein biomarkers were collected on admission and on days 1, 2, 3 and 7. The samples were centrifuged for 10 minutes at 10 000 rpm at 4 ˚C and the plasma was immediately frozen in -70 ˚C for further analysis.

Proteomic analyses were conducted at Randox Laboratories Ltd (Crumlin, County Antrim, UK) using Randox Biochip technology that is a solid-state device containing an array of discrete test regions of immobilized antibodies specific to different cerebral immunoassays. Increased levels in a specimen lead to increased binding of antibody labeled with horseradish peroxidase and thus an increase in the chemiluminescent signal emitted. The light signal generated from each of the test regions on a biochip is detected using digital imaging technology and compared to that from a stored calibration curve. The concentration of analyst present in the sample was calculated from the calibration curve. The Evidence Investigator™ Cerebral Custom Array IV was employed to quantitatively test for UCH-L1 and GFAP simultaneously.

TBI SEVERITY AND CT SCAN GRADING

GCS scores in the scene of accident assessed by paramedics or an emergency physician were retrieved from the ambulance sheets. GCS scores at emergency departments (ED) were assessed on admission. Lowest recorded GCS before intubation from the scene of accident or ED was used in the statistical analysis. GCS 13-15 was considered mild, 9-12 moderate and 3-8 severe. The mechanism of injury was recorded upon arrival. Most of the patients underwent CT imaging of the head at the time of initial presentation to the ED (supplemental table). During the hospital stay repeated CT scans were performed ad hoc after neurosurgical interventions, decline in the clinical course, and refractory intracranial pressure problems. CT scans were analyzed according to descriptive system proposed by Marshall and colleagues [10], which assesses the presence or absence of a mass lesions and differentiates diffuse injuries by signs of increased intracranial pressure.

STATISTICAL ANALYSES

Demographics of the subjects are presented as mean ± SD. The Marshall classification was treated as an ordered variable, and the GCS was treated as a continuous variable. Normality of biomarkers was assessed using the Kolmogorov-Smirnov test and histograms. As UCH-L1 and GFAP were not normally distributed, non-parametric tests were used. Biomarker levels between two groups were compared using the Mann-Whitney U test and between three groups using the Kruskal-Wallis test. Since the Kruskal-Wallis test does not reveal the pairs which differ significantly among the three or more groups, Mann-Whitney U test was used to find out the significantly different pairs. To account for multiple comparisons in these analyses, Bonferroni-adjusted significance level was set to 0.017. Correlations between the GCS scores and levels of GFAP and UCH-L1 were assessed with Spearman's rank correlation coefficient. Receiver operating characteristic curve (ROC) and areas under the ROC curve (AUC) were used to assess discrimination of patients with and without lesions seen on CT on different days. AUC of 0.8-1.0 was considered very good, AUC of 0.7-0.8 adequate and AUC of 0.5- 0.7 poor. A p value below 0.05 was considered statistically significant. Control patients are not included in the statistical analyses, but their GFAP and UCH-L1 values are presented. Statistical analysis was done using IBM SPSS Statistics 22 (IBM Corporation, Armonk, NY, USA) and Matlab R2012b (MathWorks, Natick, MA, USA).

RESULTS

DEMOGRAPHICS, INJURY SEVERITIES, CT IMAGING

Altogether 389 patients with TBI (Turku, Finland 201 and Cambridge, UK 188) and 81 control patients (40 Turku and 41 Cambridge) were recruited representing all classes of TBI severity with mean age of 45.3 ± 19.2 and most being male (72%). The majority of patients had mild TBI (59%) and one-third had severe TBI (30%). Demographics and causes of injury are shown in Table 1.

Supplemental table shows number of patients with available UCH-L1 and GFAP samples, injury severities, and CT scan characteristics. Of those patients that had GFAP and UCH-L1 available on admission, 56 % had abnormal CT findings.

GFAP AND UCH-L1 AND SEVERITY OF INJURY

Levels of GFAP and UCH-L1 at admission significantly correlated with the GCS scores (Spearman rho -0.426, $p < 0.001$ and -0.294, $p < 0.001$, respectively). Similarly, the levels of these biomarkers at day 1 (Spearman rho -0.408, $p < 0.001$ and -0.315, $p < 0.001$, respectively), and day 2 (Spearman rho -0.160, $p = 0.029$ and -0.183, $p = 0.012$, respectively), correlated significantly with the injury severity. Furthermore, UCH-L1 levels on day 7 reached statistically significant, although weaker, correlation with the initial GCS score (Spearman rho -0.183, $p = 0.046$) (Table 2). In patients with TBI, median plasma GFAP levels (upper and lower quartiles) on admission, the first day, and the second day were 0.23 (0.83, 0.00), 0.25 (0.61, 0.17), 0.23 (0.37, 0.00) ng/ml, respectively, and UCH-L1 levels 0.50 (0.70, 0.40), 0.40 (0.60, 0.30), 0.40 (0.60, 0.30) ng/ml. GFAP levels significantly correlated with UCH-L1 levels on admission and day 1 (Spearman's rho 0.744, $p < 0.001$ and 0.544, $p = 0.004$), but during the following days, no correlation was observed. A graphical box-plot presentation of the distribution of GFAP and UCH-L1 levels in all TBI severity classes and controls at different time points are illustrated in Figure 1.

GFAP AND UCH-L1 AND HEAD CT FINDINGS

Levels of GFAP were found to adequately predict any CT scan pathology for all injury severity classes as measured with Marshall grading (Marshall I vs. II-V), while levels of UCH-L1 reached only poor prediction capability. On admission, AUC was 0.739 (0.636-0.815, 95% CI) and 0.621 (0.517-0.713, 95% CI), for GFAP and UCH-L1, respectively. On the day 1, AUC was 0.657 (0.551-0.735, 95% CI) and 0.597 (0.468-0.706, 95% CI), for GFAP and UCH-L1, respectively. Figure 2

presents ROC curves of UCH-L1 and GFAP for discrimination of patients with and without lesions on CT on different days.

Tables 4A and 4B shows biomarker levels in different categorizations of Marshall grading. When no visual pathology (Marshall I), diffuse injury (II-IV), and mass lesions (V) groups were compared, differences in UCH-L1 and GFAP levels were statistically significant. A more detailed analysis showed that the UCH-L1 levels were significantly higher in Marshall V than in Marshall I (p value = 0.002). The levels of GFAP were significantly higher in Marshall V than in Marshall I (p value < 0.0005) and Marshall II-IV (p value = 0.015) (Table 3A). When mass lesions (Marshall V) were compared to other groups, UCH-L1 and GFAP levels were significantly higher in mass lesions groups (Table 3B).

DISCUSSION

This prospective, observational, multicenter study of patients with TBI explored the diagnostic performance of blood protein biomarkers GFAP and UCH-L1 for the early diagnosis of TBI during the first week after the injury. The plasma levels of GFAP and UCH-L1 substantially correlated with initial severity of TBI as assessed with the lowest pre-intubation/admission GCS, sampled both at admission and during the first two days after the injury. There were no correlations with UCH-L1 levels and severity on the third day samples after the injury, but a weak significant relationship was found later on the seventh day. These are the main new findings in our study, suggesting that these biomarkers may be used in the clinical diagnostics and assessment of TBI also beyond the first 24 hours.

Both biomarkers were associated with the visual CT pathology. UCH-L1 levels were significantly higher in Marshall V than in Marshall I group and GFAP levels were significantly higher in Marshall V than in Marshall I and Marshall II-IV groups. In ROC analysis when all severity classes

were included, the levels of GFAP and UCH-L1 showed some value for discriminating Marshall I from Marshall II-V grades.

There are many previous studies that have investigated the relationship of GFAP and UCH-L1 levels with the severity of TBI. Recently, Papa et al. [7, 11] and TRACK-TBI investigators have published results confirming that both GFAP and UCH-L1 are promising biomarkers of TBI [12-14]. Protocols of these studies have included blood samples obtained only on admission or a few hours later, while in the current study, samples were obtained at five different time points during the first week after admission. Mondello et al. conducted a study in patients with severe TBI with multiple serum and cerebrospinal fluid (CSF) samples up to maximum of seven days. [8] The cohorts in the aforementioned studies differ in proportion of TBI severity classes. While cohorts studied by Papa et al. and TRACK-TBI investigators involved 90% and 83% patients with mild TBI, respectively, our cohort had only 59% of mild cases. Furthermore, the previous cohorts predominantly consist of patients with normal CT scans (70% and 57%, respectively), whereas in our study population only 29% of patients did not exhibit visual pathology in CT scans.

The results of the current analysis are principally concordant with the aforementioned studies, but some differences exist. GFAP and UCH-L1 statistically correlated with GCS score, which is similar finding as in admission analysis results published by Papa [7, 11] and TRACK-TBI investigators [12, 13]. Similarly in line with the earlier study, there was also a statistical correlation between the UCH-L1 and GFAP levels [13]. On the other hand, our ROC analysis did not provide as optimistic results for GFAP and UCH-L1 in detecting any intracranial CT scan pathology as in the previous studies. Papa et al. have published UCH-L1 performance ROC AUC of 0.73 (0.62-0.83, 95% CI) [7] and GFAP performance ROC AUCs of 0.79 (0.69-0.89, 95% CI) [11] and 0.84 (0.73-0.95, 95% CI) [4]. TRACK-TBI investigators published GFAP and UCH-LI ROC AUCs of 0.88 (0.84-0.93, 95% CI) and 0.71 (0.64-0.78, 95% CI). [12, 13] Discrepancy to our results may stem from different proteomic analysis

methodology and that our study population included clearly more patients with moderate and severe TBI. Also in our study, both GFAP and UCH-L1 levels were able to distinguish patients with mass lesions from other CT groups.

Marmarou and coworkers reported that admission GCS score is the most accurate measure of TBI outcome, compared to earlier GCS assessments. [15] However, the result was based on the NIH IMPACT database [16] that was gathered over the period 1984-1997. During the last 20 years, pre-hospital treatment has become more aggressive including early sedation, intubation, and muscle relaxation, thus often precluding reliable assessment of GCS at admission. In a recent report in moderate to severe TBI population enrolled in 2009-2012, prognostic performance regarding mortality did not differ substantially between GSC assessment at scene of accident or at admission. [17] In our study the majority of patients with severe TBI (80 out of 116) were sedated and intubated at the scene of accident because of deteriorating consciousness level or they were already comatose. Since all patients had GCS score either from the field and / or ED, the worst total GCS score before intubation was used as a measure of the initial severity of TBI. We recognize that this may have caused both over- and underestimations of severity compared to other methods of severity assessment, but currently there is no clinical method to assess initial severity that would be without potential sources of error.

There are also other limitations in our study. Another frailty is that albeit 186 patients had biomarker samples available at admission, only 22 patients had samples taken at all five time points. This is mainly because most patients, except the severest ones, were discharged during the first post-injury week. We did not recruit patients during the night and this is why some of the first samples are taken on day 1. Those patients, who underwent surgery, were not analysed separately, but it has been reported GFAP levels are not affected by surgical interventions [18]. However, the most significant strength of the current study is that samples were collected thus far from the largest

population of patients with acute TBI and at five distinct time points during the first week after admission.

This study sheds light on the potential use of GFAP and UCH-L1 as acute clinical biomarkers of TBI. The half-lives of GFAP and UCH-L1 are relatively short, less than 48 hours [13] and less than 10 hours [19], respectively, but they are long enough to enable diagnostic use in the great majority of cases with acute TBI. They might be used also to diagnose secondary insults, e.g. cerebral hypoxia, cerebral hypoperfusion, and expanding lesions, but we have not yet analysed our results in this respect. The current study extends prior findings in one essential way. The levels of GFAP and UCH-L1 substantially correlated with the initial severity of TBI not only at admission but also during the first two days after the injury. This suggests that these biomarkers may have diagnostic value also beyond the first 24 hours of injury. Our further analyses try to find out in more detail the clinical correlates of these prolonged elevations, which is necessary in evaluating the true clinical value of these novel biomarker candidates.

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TABLES

Table 1. Patient characteristics

Characteristic	TBI patients	Controls
Age	45.3 ± 19.2	44.9 ± 18.8
Sex		
Male	281 (72.2%)	35 (43.2%)
Female	108 (27.8%)	46 (56.8%)
Severity		
Mild (GCS 13-15)	230 (59.1)	
Moderate (GCS 9-12)	43 (11.1)	
Severe (GCS 3-8)	116 (29.8)	
Cause of injury		
Incidental fall	174 (44.7)	
Road traffic accident	136 (35.0)	
Violence/assault	37 (9.5)	
Other non-intentional injury	28 (7.2)	
Suicide attempt	3 (0.8)	
Other/not known	11 (2.8)	
CT findings (Marshall grade)		
No visual pathology	114 (29.3)	
Diffuse injury	68 (17.5)	
Diffuse injury with swelling	6 (1.5)	
Diffuse injury with shift	4 (1.0)	
Mass lesions	148 (38)	
Missing	49 (12.6)	

Data are presented as mean ± SD for continuous variables and as number of patients (percentage of patients) for categorical variables. Injury severity was based on the worst total pre-hospital/admission Glasgow Coma Score (GCS) score before intubation.

Table 2. Correlation coefficients between the worst pre-hospital/admission GCS score before intubation and UCH-L1 and GFAP levels

	UCH-L1			GFAP		
	Spearman rho	p value	N	Spearman rho	p value	N
Admission	-0.294	**<0.0001**	141	-0.426	**<0.0001**	141
Day 1	-0.315	**<0.0001**	186	-0.408	**<0.0001**	186
Day 2	-0.183	**0.012**	188	-0.160	**0.029**	188
Day 3	-0.056	0.486	155	0.028	0.728	155
Day 7	-0.183	**0.046**	119	-0.112	0.223	119

Table 3. A) Levels of UCH-L1 and GFAP at admission in patients with no pathology (Marshall I), diffuse injury (II-IV) or mass lesions (V); B) Levels of UCH-L1 and GFAP at admission in patients with no visual pathology and diffuse injury (Marshall I-IV) or mass lesions (V);

A	UCH-L1				GFAP				
	Median	Lower quartile	Upper quartile	p value	Median	Lower quartile	Upper quartile	p value	N
Marshall I	0.40	0.30	0.50	**0.007**	0.19	0.00	0.26	**<0.0005**	55
Marshall II-IV	0.40	0.33	0.60		0.23	0.00	1.13		28
Marshall V	0.60	0.40	1.10		0.73	0.21	4.21		43

p value from Kruskal-Wallis test

B	UCH-L1				GFAP				
	Median	Lower quartile	Upper quartile	p value	Median	Lower quartile	Upper quartile	p value	N
Marshall I-IV	0.40	0.30	0.60	**0.002**	0.20	0.00	0.29	**<0.0005**	83
Marshall V	0.60	0.40	1.10		0.73	0.21	4.21		43

p value from Mann-Whitney U test

FIGURES

Figure 1. Levels of UCH-L1 and GFAP in different injury severity groups, Y-axis is zoomed. UCH-L1 and GFAP levels are shown for severe, moderate, and mild TBI patients and controls.

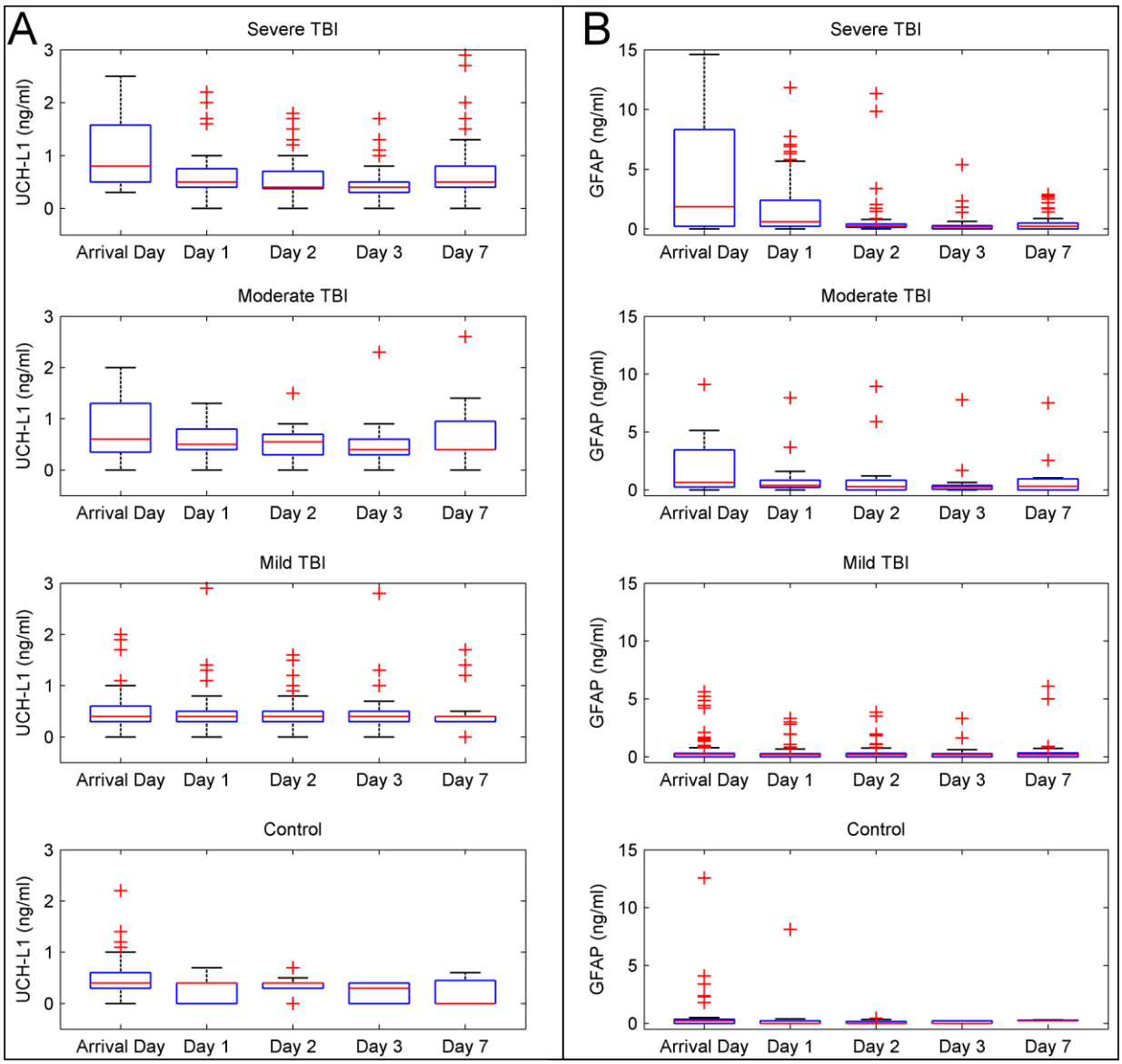
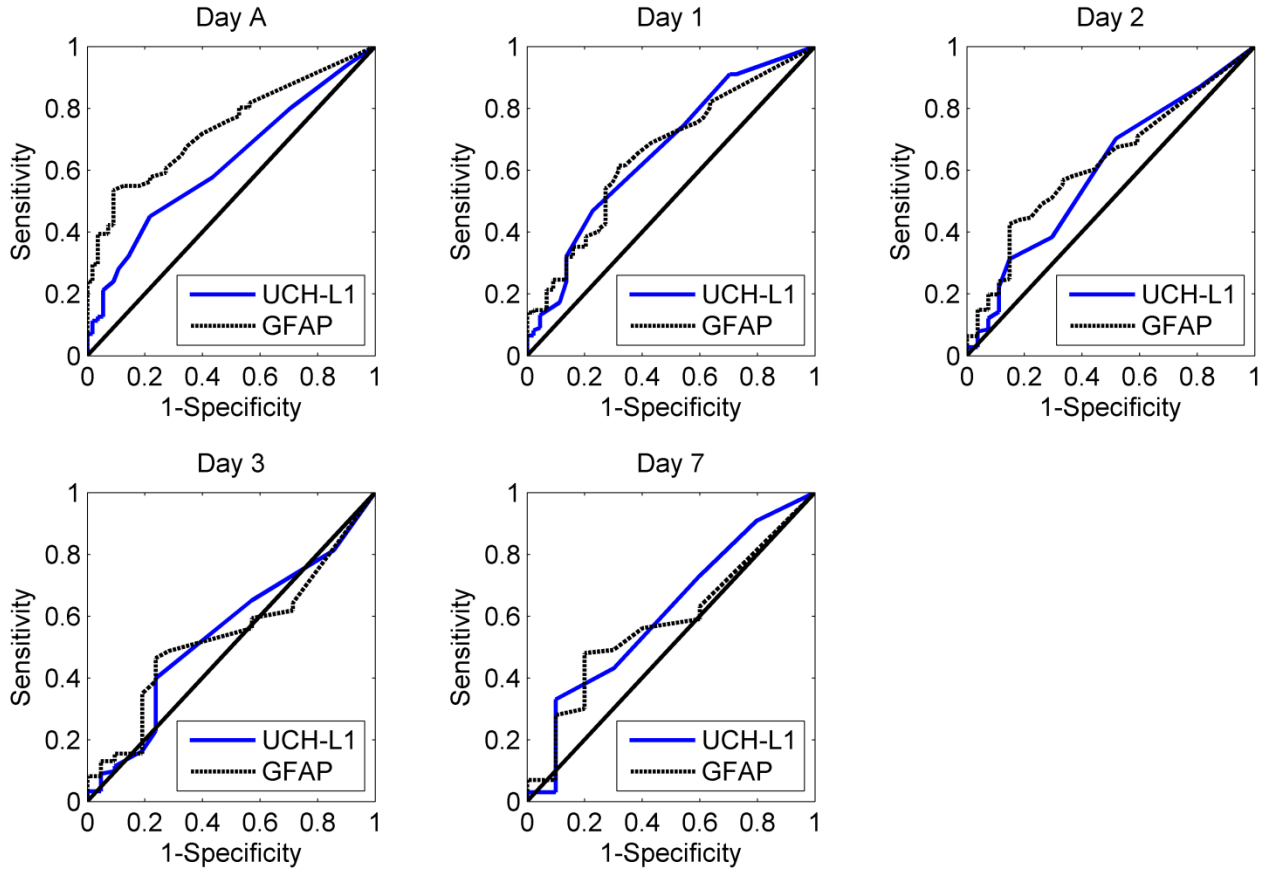


Figure 2. Receiver operating characteristics curves of UCH-L1 and GFAP for discrimination of patients with and without lesions (Marshall I vs. Marshall II-V) on CT on different days



	GFAP			UCH-L1				
	AUC	95% CI		AUC	95% CI		N1	N2
Day A	0.739	0.636	0.815	0.621	0.517	0.713	55	71
Day 1	0.657	0.551	0.735	0.660	0.565	0.749	44	122
Day 2	0.624	0.496	0.721	0.597	0.468	0.706	27	141
Day 3	0.536	0.421	0.660	0.542	0.407	0.666	21	123
Day 7	0.578	0.380	0.731	0.608	0.372	0.777	10	100

Day A: admission; AUC: area under the receiver operating characteristic curve; CI: confidence interval; N1: number of patients without lesions; N2: number of patients with lesions.

SUPPLEMENTAL DATA

Supplemental table. Number of patients with available UCH-L1 and GFAP samples, injury severities, and CT scan characteristics

Characteristic	Admission	Day 1	Day 2	Day 3	Day 7
Group					
TBI	141	186	188	155	119
Control	45	26	15	9	3
Total	186	212	203	164	122
TBI Injury Severity					
Severe (GCS 3-8)	23	52	69	70	64
Moderate (GCS 9-12)	16	29	26	27	21
Mild (GCS 13-15)	102	105	93	58	34
Total	141	186	188	155	119
CT findings (Marshall grade)					
No visual pathology	55	44	27	21	10
Diffuse injury	24	41	41	38	20
Diffuse injury with swelling	2	3	3	3	3
Diffuse injury with shift	2	3	2	1	0
Mass lesions	43	75	95	81	77
Total	126	166	168	144	110

All subjects did not have both admission CT scan and biomarkers available on all time points. Of those patients that had GFAP and UCH-L1 available on admission, 56 % had abnormal CT findings. The corresponding proportion on the first day was 73 %, on the second day 84 %, and on the third day 42 %.