Correlation of UCH-L1 and GFAP in predicting severity of traumatic brain injury in an Asian population

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Key words: traumatic brain injury, biomarkers, GFA-protein, UCH-L1 protein, Asian

Contributions: KP, conceptualisation (support); methodology (support); formal analysis (equal); resources (equal), writing – original draft (lead); writing – review and editing (equal); visualization (equal); supervision (support); project administration (equal); funding acquisition (support). RP, conceptualisation (lead); methodology (lead); software (support); validation (equal); investigation (equal); writing – review and editing (equal); supervision (lead); project administration (equal); funding acquisition (equal). SMN, conceptualisation (support); methodology (equal); software (equal); validation (equal); investigation (lead); writing – review and editing (equal); funding acquisition (equal). AB, writing – original draft (support); writing – review and editing (equal); visualization (equal). AN, software (equal); validation (lead); investigation (support); data curation (equal); writing – review and editing (equal); funding acquisition (support). AZ, formal analysis (support); writing – original draft (equal); writing – review and editing (equal). MNA, formal analysis (lead); writing – original draft (equal); writing – review and editing (equal); visualization (equal).

Conflict of interest: the authors have no conflict of interest to disclose.

Funding: this research received funding from the Universiti Malaya Faculty Research Grant (GPF010C-2018).

Ethical statement: ethics approval was granted by the UMMC Medical Ethics Committee (MREC ID NO: 201510-1766) in accordance with Declaration of Helsinki

Availability of data and materials: data will be provided upon request from corresponding author

Received: 1 July 2024.
Accepted: 7 November 2024.
Early view: 20 December 2024.

Emergency Care Journal 2025; 21:12764
doi:10.4081/ecj.2024.12764

Abstract

The application of biomarkers in TBI management remains underutilised with paucity of data in Asian populations. This study investigated the correlation between UCH-L1 and GFAP with TBI severity and patient outcomes in a Malaysian tertiary centre. The study was conducted at Universiti Malaya Medical Centre in Kuala Lumpur, Malaysia, from February 1, 2017, to November 30, 2019. GFAP and UCH-L1 were measured in 61 TBI cases and 19 controls. Correlations between biomarkers and TBI severity, as well as patient outcomes, were assessed using Spearman's rank correlation coefficient. GFAP/UCHL1 showed significant correlation with Marshall CT classification ($r=0.437$, $p<0.001$), Glasgow Coma Scale on arrival ($r=-0.444$, $p<0.001$), and Acute Physiology and Chronic Health Evaluation II (APACHEII) score ($r=0.501$, $p<0.001$). GFAP demonstrated fair-to-good accuracy in predicting TBI severity and outcomes. A consistent cut-off value of 0.01845 ng/mL for GFAP and 0.01960 for GFAP/UCHL1 predicted TBI severity, with high sensitivity (72.2-100%) and acceptable specificity (38.8-80.0%). GFAP and GFAP/UCHL1 showed promising utility in predicting TBI severity and patient outcomes in the Asian population. The findings underscore the potential clinical significance of biomarker assessment in TBI management, though further validation in larger cohorts is warranted.

Introduction

In 2019, there were 27.16 million new cases of Traumatic Brain Injury (TBI) worldwide, with 48.99 million prevalent cases and 7.08 million years lived with disability.[1] Tools such as Glasgow Coma Scale (GCS) and Injury Severity Scale (ISS), have varying reliability to predict and prognosticate TBI severity.[2-4] CT scans are widely used in clinical decision-making, but their use should be carefully considered due to factors such as the time required for the procedure, the exposure to radiation, and the associated costs.

Biomarkers offer the potential for more repeatable and objective assessments in Traumatic Brain Injury (TBI). While research suggests they may aid in determining the urgency of CT scans, improve patient monitoring, and potentially identify Diffuse Axonal Injury (DAI) not visible on CT scans,[5] further validation is needed. Ubiquitin C-terminal hydrolase L1 (UCH-L1) and Glial Fibrillary Acidic Protein (GFAP) are among the most studied biomarkers for mild TBI,[6] with varying degrees of success in studies. Although some studies have shown promising results for the sensitivity and specificity of these biomarkers in diagnosing TBI,[7,8] predicting outcomes,[7] and indicating the need for neurosurgical intervention,[8] more robust evidence is required before they can be widely incorporated into clinical guidelines.

The application of biomarkers in TBI management remains underutilised with paucity of data in Asian populations. This study investigated the correlation between UCH-L1 and GFAP with TBI severity and patient outcomes in a Malaysian tertiary centre.

Materials and Methods

Study design and setting

This was a prospective observational cohort study conducted at Universiti Malaya Medical Centre, a university hospital with neurosurgical and general Intensive Care Units (ICU), in Kuala Lumpur, Malaysia from 1 February 2017 to 30 November 2019. Ethics approval was granted by the UMMC Medical Ethics Committee (MREC ID NO: 201510-1766). 'Opt-out consent' was obtained from the legally authorised representative within 72 hours of recruitment to ensure sample collection in a timely manner.

Patient recruitment

During the study period, all adult patients with TBI presenting to the Emergency Department (ED) were evaluated for eligibility. Inclusion criteria were individuals aged 18 to 65 with non-penetrating TBI requiring admission. Non-penetrating TBI was defined as an acute insult to the brain caused by blunt trauma and no clinical evidence of a pierced skull. Patients aged >65 were excluded due to the more conservative treatment approach typically employed in this age group, which might influence patient outcomes more than biomarker levels. Patients were excluded if they had concurrent thoracic injuries, with an Abbreviated Injury Scale (AIS)>4, and abdominal injuries with AIS>3;[9] developed cardiac arrest or presented with a GCS=3 and fixed dilated pupils; no active resuscitation, pregnant, identified 12 hours after presentation, and transferred to or from another facility. Follow-up assessments were conducted up to 24 hours for biochemical and clinical parameters, throughout the hospital stay for mortality and the development of morbidities, and at 6 months for the Extended Glasgow Outcome Scale (GOS-E).

Control recruitment

Age-matched healthy subjects with no history of TBI or neurodegenerative disease were recruited via convenience sampling on a voluntary basis. Written consent was obtained prior to blood sampling.

Measurements

Baseline clinical characteristics documented were demographic data, mechanism of injury and vital signs. Laboratory tests measured were full blood count, renal profile, and liver function test. An additional 10 ml of blood was collected within 12 hours of the event into serum separator tubes. Samples were centrifuged within 30 minutes of clotting and stored at -80°C until further analysis. The samples were then analysed batch-by-batch in duplicate using commercially available sandwich enzyme-linked immunosorbent assay kits according to manufacturer's instructions.

UCH-L1 and GFAP levels were measured using purified monoclonal capture antibodies. The specifications are: i) anti-UCH-L1 (Cloud Clone Corp./USCN, Houston, TX) detection limit 0.270 ng/ml, intra-assay precision coefficient of variation (CV)<10%, and inter-assay precision CV<12%, and ii) anti-GFAP (Biovendor, Candor, NC) detection limit 0.045 ng/ml, accuracy (recovery) 102.9%, intra-assay precision CV=5.1%, and inter-assay precision CV=5.7%. The assays were measured at 450 nm using a 96-well microplate reader. Additionally, for GFAP, the reference wavelength was set to 630 nm, and readings at 630 nm were subtracted from the readings at 450 nm to be calculated for GFAP concentration. The intensity of colour development was proportional to the amount of target protein bound.

All patients underwent standard CT scan of the head. Board-certified radiologists masked to the study protocol interpreted the CT scans. The Marshall CT Classification (MC) was assigned as 'Normal' for controls, 'Diffuse Injury I' for no visible intracranial pathology, 'Diffuse Injury II' for cisterns present with midline shift <5 mm, no high or mixed density lesion >25 mm^3, 'Diffuse Injury III' for cisterns compressed or absent with midline shift <5 mm, no high or mixed density lesion >25 mm^3, 'Diffuse Injury IV' for midline shift >5 mm, no high or mixed-density lesion >25 mm^3, 'Evacuated Mass Lesion V' for any lesion surgically evacuated.[10] Depending on the clinical presentation, additional radiographs or CT scans were performed to identify AIS.[9] Injury Severity Score (ISS) was calculated as the sum of the squared AIS for the three most severe injuries, categorized based on body regions. The Acute Physiology and Chronic Health Evaluation (APACHE) II score was calculated from the physiological, clinical and laboratory characteristics.[11]

Outcome measures

Primary outcome was the correlation between biomarker levels and TBI severity based on MC. Secondary outcomes were the correlation between biomarker levels and GCS, ISS, AIS and APACHEII scores on arrival, level of neurosurgical intervention, SOFA score, length of ICU stay, in-hospital mortality, ventilator-free days, and 6-month GOS-E. AIS score was according to the Abbreviated Injury Scale: 2015 Revision.[9] Level of neurosurgical intervention was categorised as 'ICP insertion', 'ICP insertion and craniotomy', 'ICP insertion and craniectomy'. GOS-E categorises outcomes into 'dead', 'vegetative state', 'lower severe disability', 'upper severe disability', 'lower moderate disability', 'upper moderate disability', 'lower good recovery', and 'upper good recovery'.

Sample size

The a priori sample size for the primary outcome of this study was determined using G*Power 3.1 for MacOS, incorporating the following parameters: a statistical test of bivariate normal mode, a significance level (α) of 0.05, and a desired statistical power of 80%. Target correlation coefficient was set at 0.28.[12] The calculated sample size required for the study was 77 participants.

Statistical analysis

Data were analysed using IBM SPSS statistics version 29 for MacOS. All continuous variables were tested for normality with Shapiro-Wilk test. Demographic data, injury severity, management, and outcome of the patients were analysed using descriptive statistics. Parametric variables were reported in mean and standard deviation, while non-parametric variables were reported in median and Interquartile Range (IQR).

For correlation between injury severity and outcomes with biomarkers, Spearman's rank correlation coefficient was used for continuous data and binary logistic was used for mortality outcome. The reliability of the biomarkers was tested using Intraclass Correlation Coefficient (ICC). The prognostic accuracy of each biomarker was evaluated using Receiver Operating Characteristic (ROC) curves and Area Under the Curve (AUC). The cut-off value, sensitivity, and specificity for each biomarker were determined by analysing the Youden index of each ROC coordinate point.[13] AUC was categorised as 'excellent' for values between 0.9 – 1.0, 'good' for 0.8 – 0.9, 'fair' for 0.7 – 0.8, 'poor' for 0.6 – 0.7, and 'failed' for 0.5 – 0.6(14). The cut-off value, sensitivity, and specificity for each biomarker were determined by analysing the

Youden index of each ROC coordinate point.[13] AUC was categorised as 'excellent' for values between 0.9 – 1.0, 'good' for 0.8 – 0.9, 'fair' for 0.7 – 0.8, 'poor' for 0.6 – 0.7, and 'failed' for 0.5 – 0.6(14). A p-value < 0.05 was considered statistically significant.

Results

A total of 65 patients met the inclusion criteria. Nineteen healthy age-matched individuals were recruited into the study as controls (Figure 1). For the primary outcome, all 61 patients were included in the analysis. For the 6-month GOS-E outcome, 32 patients were lost to follow up.

Baseline characteristics and biomarker levels of patients and controls are summarised in Table 1. Median levels of GFAP and UCH-L1 were significantly different between the patient and control groups, 0.599 ng/ml (0.097-2.854) versus 0.000 ng/ml (0.000-0.538), p=0.011 and 1.412 ng/ml (0.928-2.326) versus 2.676 ng/ml (1.305-3.178), p=0.018 respectively. Additionally, the GFAP/UCHL1 was 0.287 (0.000-0.992) in the patient group and 0.000 (0.000-0.110) in the control group, p=0.007. The ICC among the biomarkers, including GFAP/UCHL1, was 0.582 (95% CI 0.283, 0.769), p<0.001.

GFAP had significant correlations with GCS on arrival (r=-0.381, p<0.001), critical AIS head and neck (r=0.309, p<0.010), APACHEII score (r=0.384, p=0.001), surgical intervention (r=0.300, p=0.031), length of ICU stay (r=0.275, p=0.024), cerebral protection duration (r=0.261, p=0.035), and day 1 SOFA (r=0.342, p=0.006). UCH-L1 had significant correlations with Marshall CT classification (r=-0.402, p=0.005), GCS on arrival (r=0.290, p=0.046), critical AIS head and neck (r=-0.344, p=0.017), APACHEII score (r=-0.338, p=0.020), surgical intervention (r=-0.404, p=0.010), Day 1 SOFA (r=-0.339, p=0.023) and GOS-E (r=0.432, p=0.019). GFAP/UCHL1 ratio showed significant correlations with Marshall CT classification (r=0.437, p=0.002), GCS on arrival (r=-0.444, p=0.002), critical AIS head and neck (r=0.394, p=0.006), total ISS (r=0.338, p=0.019), APACHEII score (r=0.501, p<0.001), surgical intervention (r=0.457, p=0.003), ventilator free days(r=-0.419, p=0.004), length of ICU stay (r=0.377, p=0.009), cerebral protection duration r=0.413, p=0.004), and day 1 SOFA (r=0.502, p<0.001); Table 2.

Table 3 summarises the predictive accuracy and cut-off values of GFAP and UCH-L1 for patient outcomes. GFAP, UCH-L1, and GFAP/UCHL1 predicted MC of <3 *versus* ≥3, AUC >0.70, sensitivity >0.85, and specificity between 0.38-0.63.

Discussion

To our knowledge, this is the first study to assess the correlation between GFAP and UCH-L1 with TBI severity and patient outcome in an Asian population. The strengths of our study were the correlation of these biomarkers with clinical parameters, and the use of GFAP/UCHL1.

Our findings highlighted a significant correlation between GFAP/UCHL1 and MC. This supports the elevated glial-neuronal ratio indicated by GFAP/UCHL1 in MC-V injuries and GFAP's known association with mass lesions.[15,16] Conversely, UCH-L1, a neuronal biomarker involved in protein regulation, is linked to DAI.[15,17] In our study, UCH-L1 exhibited a significant negative correlation with MC, whereas GFAP showed no significant association. These results contradict previous studies that reported positive correlations between GFAP and UCH-L1 with CT severity.[8,18-20] To ensure reproducibility, the GFAP ELISA in our study was independently repeated, yielding consistent inter-assay variability with CV within the acceptable range. The contradictory results in our study may be attributed to the exclusion of patients with a GCS of 3 and fixed dilated pupils, who were deemed ineligible for surgical intervention. This exclusion could have led to an underrepresentation of patients with severe neuronal damage compared to glial injury.

Secondary analysis revealed fair-to-good accuracy for GFAP, UCH-L1, and GFAP/UCHL1 in predicting markers of TBI severity, including MC≥3, GCS>8, APACHEII score≥23, ISS≥16, and

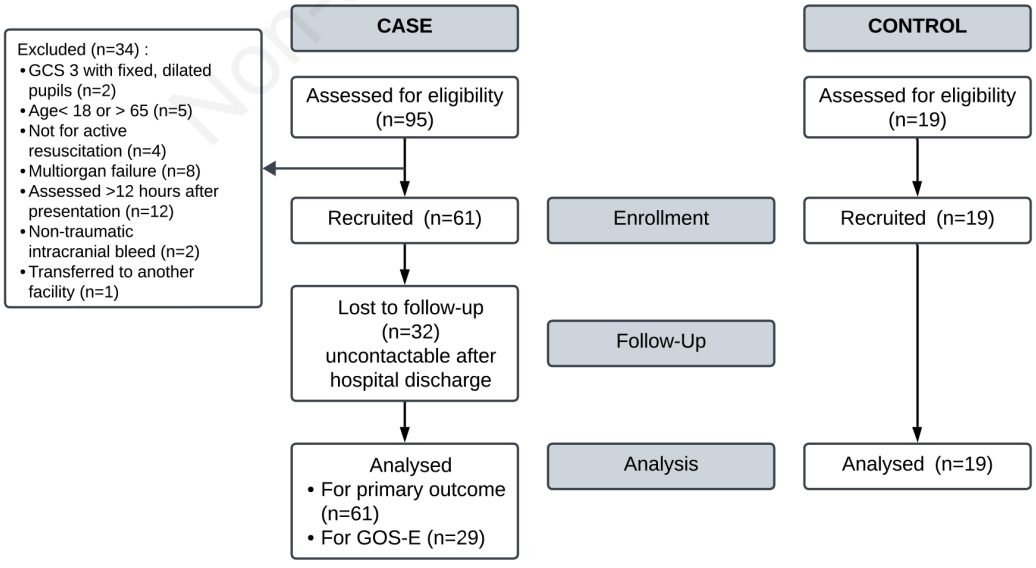

Figure 1. Flow of patient and control enrolment, follow up and analysis. GCS, Glasgow coma scale, GOS-E, extended Glasgow outcome scale.

critical head and neck AIS score. These biomarkers demonstrated 'fair' accuracy in predicting the need for surgical intervention and intubation. Notably, cut-off values of 0.01845 ng/mL for GFAP and 0.01960 for GFAP/UCHL1 were identified across measures of TBI severity, achieving high sensitivity (72.2-100%) and acceptable specificity (38.8-80.0%). Previous studies reported higher GFAP and GFAP/UCH-L1 cut-off values for predicting CT scan abnormalities and predicting unfavourable Glasgow outcome scale(15, 21, 22). These discrepancies may be attributed to variations in patient populations, TBI severity and type of injuries, and outcome measures of each study.

This study demonstrates that the utility of biomarkers in mea-

Table 1. Characteristics of patients and controls.

Characteristics	Case (n=61)	Control (n=19)	p
Median age, year (IQR)†	29.0 (24.0 – 42.0)	25.0 (21.0-31.25)	0.078
Male sex, n (%)‡	57 (93.4)	9 (47.4)	<0.001
Ethnicity, n (%)‡			
Malay	26 (42.6)	9 (47.4)	<0.001
Indian	20 (32.8)	0 (0)	
Chinese	6 (9.8)	10 (52.6)	
Others	9 (14.8)	0 (0)	
GCS on arrival, n (%)†			
3-8	35 (57.4)	0 (0)	<0.001
9-12	22 (36.1)	0 (0)	
13-15	4 (6.6)	19 (100)	
Marshall CT classification, n (%)			
Diffuse injury I	0 (0.0)	NA	NA
Diffuse injury II	31 (50.8)	NA	NA
Diffuse injury III	9 (14.8)	NA	NA
Diffuse injury IV	8 (13.1)	NA	NA
Evacuated mass lesion V	13 (21.3)	NA	NA
ISS score, median (IQR)†	38.0 (29.0 – 46.0)	0 (0)	<0.001
Critical AIS head and neck, n (%)†	61 (100)	0 (0)	<0.001
Median biomarker (IQR) †			
GFAP, ng/ml	0.599 (0.097-2.854)	0.000 (0.000-0.538)	0.011
UCH-L1, ng/ml	1.412 (0.928-2.326)	2.676 (1.305-3.178)	0.018
GFAP/UCHL1	0.287 (0.000-0.992)	0.000 (0.000-0.110)	0.007
APACHEII score on arrival, mean (SD)	13.52 (4.88)	NA	NA
Mechanism of injury			
MVC, n (%)	44 (72.1)	NA	NA
Fall from height, n (%)	9 (14.8)	NA	NA
Unknown, n (%)	8 (13.1)	NA	NA

†Mann-Whitney U, ‡Fisher exact, NA, not applicable, IQR, interquartile range, SD, standard deviation, GCS, Glasgow coma scale, CT, computed tomography, ISS, Injury Severity Scale, AIS, Abbreviated Injury Scale, APACHE, Acute Physiology and Chronic Health Evaluation, GFAP, Glial Fibrillary Acidic Protein, UCH-L1, Ubiquitin C-Terminal Hydrolase L1, MVC, motor vehicle collision. Critical AIS head and neck is according to the Abbreviated Injury Scale: 2015 Revision.[9]

Table 2. Correlation between injury severity, and outcome with biomarkers.

Parameter	Correlation coefficient					
	GFAP		UCH-L1		GFAP/UCHL1	
Marshall CT classification†	0.216		-0.402	**	0.437	*
GCS on arrival†	-0.381	**	0.290	*	-0.444	*
Critical AIS head and neck‡	0.309	*	-0.344	*	0.394	*
Total ISS†	0.182		-0.226		0.338	*
APACHEII score†	0.384	*	-0.338	*	0.501	**
Surgical intervention†	0.300	*	-0.404	*	0.457	*
Mortality‡	1.048		7.634		0.924	
Ventilator-free days†	-0.235		0.251		-0.419	*
Length of ICU stay†	0.275	*	-0.174		0.377	*
Cerebral protection duration†	0.261	*	-0.119		0.413	*
Day 1 SOFA†	0.342	*	-0.339	*	0.502	**
GOS-E†	-0.295		0.432	*	-0.361	

†Spearman's rank correlation coefficient, ‡binary logistic, *p<0.05, **p<0.001. CT, computed tomography, AIS, abbreviated injury scale, GCS, Glasgow Coma Scale, AIS, Abbreviated Injury Scale, ISS, Injury Severity Score, APACHEII, Acute Physiology and Chronic Health Evaluation II, ICU, intensive care unit, SOFA, Sequential Organ Failure Assessment, GOS-E, Extended Glasgow Outcome Scale, GFAP, Glial Fibrillary Acidic Protein, UCH-L1, Ubiquitin C-Terminal Hydrolase L1.

suring TBI severity is enhanced when analysed as ratios rather than individually. To our knowledge, this is the first study to incorporate various measures of TBI severity, such as the Marshall classification, APACHE II score, head and neck AIS, and ISS, alongside biomarkers. While consistent cut-off points were identified across various measures of TBI severity, these levels differ from those reported in other studies, likely due to variations in patient populations. Including a more diverse patient population could help address this discrepancy and lead to the identification of more robust cut-off values.

Limitations

Firstly, this study was conducted in a single tertiary centre with neurosurgical and general ICU services, thus, our findings may not be generalised to settings with different resources. Secondly, the biomarkers were sampled within 12 hours from ED presentation. Although peak serum levels of biomarkers are typically observed between 6 to 12 hours following TBI, the timing of biomarker sampling was not protocolised. Thirdly, the sample size was relatively small with cases lost to follow up. Although the study was powered to measure the primary outcome, the study lacked sufficient power for secondary outcomes. Furthermore, the control group was recruited through convenience sampling and were only age-matched to cases, potentially limiting comparability. Finally, the observed biomarker levels in our study differed from those reported in other studies. This discrepancy is likely due to variations in patient populations, injury severity, and other factors. Future studies with larger sample sizes and more standardized methodologies may be needed to further elucidate the relationship between biomarkers and TBI outcomes.

Conclusions

This study adds to existing literature for potential associations between GFAP and UCH-L1, with TBI severity. GFAP/UCHL1 demonstrated a significant correlation with MC, suggesting its potential role in identifying patients with significant lesions. GFAP and GFAP/UCHL1 demonstrated fair to good accuracy in predicting various measures of TBI severity and outcomes, with consistent cut-off values. Multicentre studies with larger and diverse populations encompassing a wider spectrum of TBI severity and injuries are necessary to validate the generalisability of the cut-off values for each biomarker.

Transparency, rigor, and reproducibility statement

The study design and analytic plan were preregistered at the Universiti Malaya Medical Centre (MREC ID NO: 201510-1766). A sample size of 77 participants was determined for the primary statistical analysis, assessing the bivariate correlation between

Table 3. Predictive accuracy of biomarkers.

Parameter	Biomarker	AUC (95% CI)	p	Cut-off value	Sens (95% CI)	Spec (95% CI)
Marshall CT classification <3 *vs* ≥3	GFAP	0.715 (0.561,0.869)	0.006	0.01845†	0.854 (0.077)	0.625 (0.106)
	UCH-L1‡	0.710 (0.562,0.857)	0.005	2.76300†	0.955 (0.045)	0.384 (0.107)
	GFAP/UCHL1	0.738 (0.590,0.885)	0.002	0.01960	0.864 (0.075)	0.625 (0.106)
GCS ≤8 vs > 8 on arrival	GFAP	0.698 (0.545,0.851)	0.011	0.01845†	0.792 (0.089)	0.591 (0.108)
	UCH-L1‡	0.657 (0.501,0.813)	0.048	0.76770†	0.950 (0.048)	0.071 (0.056)
	GFAP/UCHL1	0.709 (0.559,0.860)	0.006	0.01960	0.792 (0.089)	0.591 (0.108)
APACHEII <23 *vs* ≥23	GFAP	0.841 (0.733, 0.949)	<0.001	0.01845†	1 (0.0)	0.386 (0.107)
	UCH-L1‡	0.870 (0.772, 0.967)	<0.001	0.84960†	1 (0.0)	0.870 (0.074)
	GFAP/UCHL1	0.886 (0.793, 0.980)	<0.001	0.01960	1 (0.0)	0.386 (0.107)
Total ISS <16 *vs* ≥ 16	GFAP	0.767 (0.609,0.925)	0.001	0.01845†	0.722 (0.098)	0.800 (0.088)
	UCH-L1‡	0.718 (0.555,0.882)	0.009	3.38500†	0.971 (0.037)	0.214 (0.090)
	GFAP/UCHL1	0.775 (0.626,0.924)	<0.001	0.01960	0.722 (0.098)	0.800 (0.088)
Critical head and neck AIS score	GFAP	0.767 (0.609, 0.925)	0.001	0.01845†	0.722 (0.098)	0.800 (0.088)
	UCH-L1‡	0.718 (0.555, 0.882)	0.009	3.38500†	0.971 (0.037)	0.214 (0.090)
	GFAP/UCHL1	0.775 (0.626, 0.924)	<0.001	0.01960	0.722 (0.098)	0.800 (0.088)
Surgical intervention	GFAP	0.739 (0.578,0.901)	0.004	0.01845†	0.821 (0.084)	0.722 (0.098)
	UCH-L1‡	0.642 (0.485,0.799)	0.077	-	-	-
	GFAP/UCHL1	0.749 (0.594,0.904)	0.002	0.01960	0.821 (0.084)	0.722 (0.098)
Need for intubation, yes *vs* no	GFAP	0.784 (0.537,1.032)	0.024	0.01845†	0.828 (0.083)	0.833 (0.082)
	UCH-L1‡	0.525 (0.288,0.762)	0.838	-	-	-
	GFAP/UCHL1	0.709 (0.559,0.860)	0.006	-	-	-
Mortality	GFAP	0.578 (0.325,0.831)	0.545	-	-	-
	UCH-L1‡	0.790 (0.636,0.943)	<0.001	1.48950†	1 (0.0)	0.591 (0.108)
	GFAP/UCHL1	0.602 (0.340,0.865)	0.444	-	-	-
Day 1 SOFA <2 *vs* ≥2	GFAP	0.795 (0.645,0.945)	<0.001	0.01845†	0.815 (0.085)	0.800 (0.088)
	UCH-L1‡	0.683 (0.525,0.841)	0.023	3.38500†	0.964 (0.041)	0.176 (0.083)
	GFAP/UCHL1	0.805 (0.663,0.947)	<0.001	0.01960	0.815 (0.085)	0.800 (0.088)
GOS-E ≥5 *vs* <5	GFAP	0.758 (0.546,0.971)	0.017	0.01845†	1 (0.0)	0.462 (0.906)
	UCH-L1‡	0.779 (0.614,0.944)*	0.001	0.78120†	1 (0.0)	0.00077 (0.002)
	GFAP/UCHL1	0.802 (0.600,1.004)	0.003	0.01960	1 (0.0)	0.462 (0.906)

†ng/ml, ‡1-value, Sens, sensitivity, Spec, specificity, AUC, area under ROC Curve, CT, computed tomography, AIS, abbreviated injury scale, GCS=Glasgow Coma Scale, AIS, Abbreviated Injury Scale, ISS, Injury Severity Score, APACHEII, Acute Physiology and Chronic Health Evaluation II Score, SOFA, Sequential Organ Failure Assessment, GOS-E, Extended Glasgow Outcome Scale, GFAP, Glial Fibrillary Acidic Protein, UCH-L1, Ubiquitin C-Terminal Hydrolase L1.

biomarker levels and Marshall CT classification. This determination was based on a target correlation coefficient of 0.28,[12] a power of 80%, and a significance level of $p<0.05$. Out of 95 eligible patients, 61 were included in the study, alongside 19 age-matched healthy individuals recruited as controls. Thirty-two patients were lost to follow-up for the 6-month GOS-E outcome assessment. To maintain impartiality, investigators measuring biomarkers were blinded to patients' severity and outcomes, and were not involved in patient management. Additionally, board-certified radiologists, unaware of the study protocol, interpreted the CT scans. All biomarkers were analyzed according to the manufacturer's recommendations, ensuring consistency in inter-assay variability, with Coefficients of Variation (CV) within acceptable ranges. Selected biomarkers were independently repeated to ensure reproducibility.

The normal distribution of primary outcome data was confirmed through scatter plots and Shapiro-Wilk tests.

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