Title: Neurofilament light as a biomarker in traumatic brain injury

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AUTHOR CONTRIBUTION

Dr. Shahim had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study concept and design: Shahim, Chan.
Acquisition of data: Shahim, van der Merwe, Gill, Chan.
Statistical analysis: Shahim.
Drafting of the manuscript: Shahim.
Analysis and interpretation of data: All authors.
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ROLE OF THE FUNDER/SPONSOR

The funding organization had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

CONFLICTS OF INTEREST

Dr. Shahim reports no conflicts of interest. Dr. Zetterberg has served at scientific advisory boards for CogRx, Samumed, Roche Diagnostics and Wave, has given a scientific presentations in symposia sponsored by Biogen and Alzecure, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB, a GU Ventures-based platform company at the University of Gothenburg (all unrelated to the work presented in this paper). Dr. Blennow has served as a consultant or at advisory boards for Alector, Alzheon, CogRx, Biogen, Lilly, Novartis and Roche Diagnostics, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB, a GU Ventures-based platform company at the University of Gothenburg, all unrelated to the work presented in this paper.

ABSTRACT:

Objective To determine whether serum neurofilament light (NfL) correlates with CSF NfL, traumatic brain injury (TBI) diagnosis, injury severity, brain volume, and diffusion tensor imaging (DTI) estimates of traumatic axonal injury (TAI).

Methods Participants were prospectively enrolled in Sweden and the United States between 2011 and 2019. The Swedish cohort included 45 hockey players with acute concussion sampled at 6 days, 31 with repetitive concussion with persistent postconcussive symptoms (PCS) assessed with paired CSF and serum (median 1.3 years after concussion), 28 preseason controls, and 14 nonathletic controls. Our second cohort included 230 clinic-based participants (162 with TBI and 68 controls). Patients with TBI also underwent serum, functional outcome, and imaging assessments at 30 (n = 30), 90 (n = 48), and 180 (n = 59) days and 1 (n = 84), 2 (n = 57), 3 (n = 46), 4 (n = 38), and 5 (n = 29) years after injury.

Results In athletes with paired specimens, CSF NfL and serum NfL were correlated ($r = 0.71$, $p < 0.0001$). CSF and serum NfL distinguished players with PCS >1 year from PCS ≤1 year (area under the receiver operating characteristic curve [AUROC] 0.81 and 0.80). The AUROC for PCS >1 year vs preseason controls was 0.97. In the clinic-based cohort, NfL at enrollment distinguished patients with mild from those with moderate and severe TBI ($p < 0.001$ and $p = 0.048$). Serum NfL decreased over the course of 5 years ($ß = −0.09$ log pg/mL, $p < 0.0001$) but remained significantly elevated compared to controls. Serum NfL correlated with measures of functional outcome, MRI brain atrophy, and DTI estimates of TAI.

Conclusions Serum NfL shows promise as a biomarker for acute and repetitive sports-related concussion and patients with subacute and chronic TBI.

Classification of evidence This study provides Class III evidence that increased concentrations of NfL distinguish patients with TBI from controls.

INTRODUCTION

Traumatic brain injury (TBI) is a growing concern in civilians, athletes, and military service members.[1-3] The majority of TBIs are classified as mild TBI (mTBI), yet a substantial subset of individuals exhibit persistent behavioral, cognitive, and physical symptoms.[4] Existing studies suggest that TBI may trigger progressive neurodegeneration, especially in moderate or severe cases.[5-8] Also, data from military and sports-related TBI suggest that this risk is increased when multiple mTBIs are sustained.[9-14] Thus, biomarkers that can objectively measure severity of brain injury and predict prognosis of patients with TBI have become an important research focus.

Axonal injury is a key pathophysiologic process following TBI.[15,16] Neurofilament light (NfL) and tau are both axonal proteins, which have been reported to be elevated in cerebrospinal fluid (CSF) of individuals with TBI and other neurodegenerative diseases.[17-19] Recently, glial fibrillary acidic protein (GFAp), expressed in astroglial cells and ubiquitin C-terminal hydrolase-L1 (UCH-L1), a cytosolic neuronal protein (REF) , have shown the ability to distinguish patients with or without intracranial lesion on CT when measured acutely.[20-24] Although several recent studies have demonstrated the utility of these biomarkers in the acute post-injury period, the time course beyond months to years after TBI remains unknown. Understanding the temporal change of these biomarkers in the months to years following TBI is crucial for improving the utility of these biomarkers in the clinical setting.

In this study, we examined NfL, GFAp, tau and UCH-L1 in patients with mild, moderate and severe TBI for up to five years following injury. We hypothesized that: (*1*) patients with TBI would have increased serum concentrations of axonal and glial proteins compared to controls, with higher concentrations in moderate to severe cases, and (*2*) patients with TBI who are symptomatic or those with moderate to severe cases would have elevated concentrations of axonal and glial proteins even up to five years after injury. Finally, we sought to determine the relationship between serum biomarkers, clinical outcome, and brain volume changes on MRI.

METHODS

Standard protocol approvals, registrations, and patient consents

The institutional review board at the National Institutes of Health (NIH), Bethesda, MD, USA approved the study. Written and informed consent was obtained from all participants.

Study population

This is a prospective study of patients with mild, moderate, or severe TBI enrolled between January 2009 to July 2018 at the NIH, Bethesda, MD, USA. The inclusion criteria are described in detail in **Supplement 1**.

Outcome measures

The primary outcome measures were changes in serum concentrations of NfL, GFAp, tau, and UCH-L1 from 30 days to 5 years after injury in relation to diagnosis of TBI, clinical outcome, and brain MRI volumes. Clinical outcome was assessed with the the Glasgow Outcome Scale-Extended (GOS-E).[25] Associations were also tested between blood biomarkers and gray matter (GM), white matter (WM), and corpus callosum (CC) volumes.

Biochemical assessment

Serum NfL, GFAp, tau, and UCH-L1 concentrations were measured simultaneously using the Neurology 4-plex assay kit (Quanterix Corporation, Lexington, MA, USA) on a Single molecule array HD-1 Analyzer (Quanterix Corporation, Lexington, MA, USA). The average coefficient of variation (CV) of measurement of tau, NfL, UCH-L1, and GFAp included in the final analyses was < 20 %, 4 %, < 20 %, and 3 %, respectively. Serum tau and UCH-L1 samples with CVs > 20 % (n = 156 and n = 121, respectively) were excluded from the analyses. Therefore, tau and UCH-L1 data presented in the paper should be interpreted with caution and were included in the analyses solely for comparison purposes. The sample processing procedure is described in detail in **Supplement 1**.

Imaging acquisition

Structural MR images were acquired on a 3 Tesla MR scanner (Siemens Biograph) with a 16-channel head coil in the Neuroimaging Department at the NIH, Bethesda, MD, USA. The image acquisition protocol and post-processing are detailed in **Supplement 1**.

Statistical analyses

The diagnostic utility of serum biomarkers was determined by calculating the area under the receiver-operating characteristic curve (AUROC). The association between blood biomarkers and outcome was tested using linear models, adjusted for age, education and gender. All tests were two-sided and statistical significance was determined at $P < .05$. All statistical calculations were performed using R (v. 3.0.3, The R Foundation for Statistical Computing). The statistical tests are summarized in **Supplement 1**.

Data availability

The data supporting the findings in this study are available upon reasonable request from the corresponding author.

RESULTS

Demographics and clinical characteristics

A total of 613 individuals were screened between 2009 and 2018 of whom 243 (175 with TBI, and 68 healthy controls) were enrolled. Thirteen patients with TBI were excluded due to screening failure. Of 162 patients with TBI, 106 underwent repeated blood, MRI, and outcome assessment at 30 (n = 30), 90 (n = 48), and 180 (n = 59) days, and 1 (n = 82), 2 (n = 57), 3 (n = 46), 4 (n = 38), and 5 (n = 29) years after injury. The demographic and clinical characteristics of the participants at enrollment (median time since injury, 7 months, interquartile range [3-17]) are shown in **Table 1**. Of 162 patients with TBI, 89 were classified as mTBI and had no abnormalities on conventional MRI, 48 were moderate, and 25 were severe (**Table 1**). Age and education distributions were essentially the same between the TBI and control group (P = .78 [effect size estimate, es = 0.02], P = .20 [es = 0.10], respectively; **Table 1**). However, there were significant differences in gender and race between the groups (P = .040 [es = 0.17], $P < .01$ [es = 0.32], respectively; **Table 1**).

Biomarker concentrations at enrollment

At enrollment, patients with TBI had increased concentrations of NfL (P = .0001, *es* = 0.52) and GFAp (P < .001 [*es* = 0.38]) compared with controls (**Table 1**). The most pronounced increase was found for NfL (203% of controls) and GFAp (167% of controls), while changes in tau and UCH-L1 were minor and not statistically different from controls (P = .16 [*es* = 0.10], P < .12 [*es* = 0.12], respectively; **Table 1**).

Serum NfL was elevated in patients with severe TBI vs. moderate, moderate vs. mild, and mild vs. controls ($P_{adjusted}$ = .05, $P_{adjusted}$ = .0001, and $P_{adjusted}$ = .0001, respectively; **Figure 1A**). Also, serum GFAp was elevated in mTBI vs. controls, as well as severe vs. moderate cases, but not moderate vs. mild ($P_{adjusted}$ < .0001, $P_{adjusted}$ =.005, and $P_{adjusted}$ =.17, respectively; **Figure 1B**). Serum concentrations of tau and UCHL-1 were significantly higher in patient with severe TBI compared with moderate but did not significantly distinguish mild, moderate, and controls (**Figure 1C** and **D**).

Time course of blood-based biomarkers

Figure 2A and **eFigure 1A** show the time course of serum NfL. Serum NfL was increased at the 30-day time point, with levels decreasing over course of 5 years (ß = – 0.09, P <.0001). Next, we conducted pairwise group comparisons across TBI severity and controls at different sampling time points. The significant results are summarized in **eTable 1**. In summary, serum NfL was increased in patients with mild, moderate, and severe TBI compared with controls even at 5-year time point after injury (**eTable 1**).

Figure 2B and **eFigure 1B** show the time course of serum GFAp. The longitudinal changes in GFAp concentrations from 30 days to 5 years were not statistically significant (ß = 0.003, P =.60). There was no difference in serum GFAp between mTBI and controls at any sampling time points after correcting for multiple comparisons; however, those with severe TBI had higher serum GFAp concentrations at all measured time points compared with controls (**eTable 1**).

Figure 2C and **eFigure 1C** show the time course of serum tau. Serum tau concentrations were variable over the course of the 5-year period, and there was no difference in tau concentrations over time (ß = – 0.02, *P* =.23). Serum tau concentrations were elevated in severe cases compared with controls at all measured time points, except the 5-year time point where the number of participants was low (**eTable 1**).

Figure 2D and **eFigure 1D** show the time course of serum UCH-L1. The time course for serum UCH-L1 over the 5-year period was variable, and there was no effect of time on UCH-L1 concentrations (ß = – 0.025, *P* =.08). Also, there was no significant differences in UCH-L1 concentrations either across TBI severity or compared with controls at 30, 90, and 180-day time points (**eTable 1**). However, at 1, 2, 3, and 4-year time points higher concentrations of serum UCH-L1 were observed in the severe cases vs. controls (**eTable 1**).

Diagnostic utility of the biomarkers

Serum NfL could separate mTBI cases from controls at the 30-day time point with an AUROC of 0.84 (**Figure 3**). The AUROC for serum NfL distinguishing mTBI from controls declined over time, with the highest AUROCs measured at the 1, 2, 3, 4, and 5-year time points (0.78, 0.72, 0.81,0.79, and 0.81, respectively; **Figure 3)**. The highest AUROCs for serum NfL were measured for moderate to severe cases at the 30, 90 and 180-day time points (ARUOCs, 0.84-0.98; **Figure 3**).

The AUROCs for serum GFAp over the course of five years ranged from 0.60-0.89, irrespective of TBI severity (**Figure 3**). The AUROC for serum GFAp distinguishing mTBI from controls at the 30-day time point was 0.71, while for the moderate and severe cases 0.89, and 0.89, respectively (**Figure 3**).

The AUROC for serum tau distinguishing TBI cases from controls over the course of 5 years ranged from 0.51-0.76, with the highest AUROC measured at 3 years, distinguishing mTBI from controls (ARUOC, 0.76, CI 0.51-1.0; **Figure 3**).

With regard to serum UCH-L1, the highest AUROCs for distinguishing mTBI from controls were measured at the 30-day time point (0.78, CI 0.88-1.0); however, there were only 4 mTBI cases at that time point due to exclusion of values with higher CVs (**Figure 3**). The AUROCs for serum UCH-L1 were also low for moderate and severe cases, ranging from 0.5-0.77 over the course of 5-year (**Figure 4**).

Associations between blood biomarkers and outcome

At enrollment, we observed a relationship between serum NfL concentrations and GOS-E scores (ß = – 0.28, *P* = .00187; **Figure 4A**). No associations were observed with GOS-E scores for GFAp, tau, and UCH-L1 (**Figure 4B-D**).

Serum concentrations of NfL and GFAp at the 30-day time point could predict a change in GOS-E at 90-day (ß = 0.64, *P* = .0002, ß = 1.14, *P* = .032; **eFigure 2** ***row A and B***). There was no association between change in GOS-E scores and serum biomarker concentration of NfL and GFAp beyond the 30-day time point (**eFigure 2** ***row A and B***). No associations were observed for tau and UCH-L1 and change in GOS-E at any of the time points (**eFigure 2** ***row C and D***).

Associations between blood biomarkers and brain volumetry

Serum NfL showed inverse relationships with GM, WM, CC mid anterior, CC central, and CC mid posterior volumes (**eFigure 3** ***column A***). No associations were observed between NfL and CC anterior and CC posterior volumes (**eFigure 3** ***column A***). Serum GFAp showed inverse relationships between GM, WM, CC, anterior, mid anterior, CC central, and CC posterior volumes (**eFigure 3** ***column B***). No associations were observed between GFAp and CC posterior volume (**eFigure 3** ***column B***). Serum tau showed an inverse relationship with GM volume, while no relationships were observed with either WM volume or CC volumes (**eFigure 3** ***column C***). Serum UCH-L1 showed inverse relationships with GM and WM volumes. There was a relationship between serum UCH-L1 and CC anterior, which was mainly driven by outliers (**eFigure 3** ***column D***). There were no relationships between UCH-L1 and other segments of CC (**eFigure 3** ***column D***).

The summary of serum biomarkers predicting future change in brain MR volumes are presented in **eTable 2**. Serum NfL at the 180-day time point could predict WM volume loss at 1-year (ß = – 3881, *P* = .001; **eTable 2**). Serum NfL and GFAp at 1-year could predict loss of CC mid anterior and central volumes at 2-year timepoint (**eTable 2**), however, after inspecting the regression plots and excluding the outliers, only the association for serum NfL remained significant (**eFigure 4A-D**). Also, serum NfL at 3-year time point was inversely associated with change in CC central at 4-year time point (**eTable 2**).

No associations were observed between tau, UCH-L1 and changes in GM, WM, and CC volumes over time after correcting for multiple comparisons (**eTable 2)**.

DISCUSSION

The main findings of this study were: (*1*) at median time of 7 months after injury, patients with TBI had increased concentrations of NfL and GFAp compared with controls with higher concentrations in those with moderate to severe cases; serum tau and UCHL-1 were increased in the moderate to severe cases compared with controls; (*2*) In longitudinal samples, serum NfL was elevated at 30 days after injury and the levels decreased in a linear fashion over five years; Serum concentrations of GFAp, tau, and UCH-L1 did not significantly change over time; (*3*) Serum NfL distinguished patients with TBI from controls at 30, 90, and 180-days with good to excellent accuracy; The diagnostic accuracies of GFAp, tau, and UCH-L1 were lower, especially for the mild TBI cases; and (*4*) serum NfL was the only biomarker that showed relationships with clinical outcome, cerebral WM and CC volume changes.

Neurofilament light is predominantly expressed in large-caliber myelinated axons that extend subcortically.[26] CSF NfL is a sensitive biomarker of axonal degeneration and has been assessed extensively in neurodegenerative disorders.[27,28] Recently, with the development of high-sensitive immunoassay technology, NfL could be readily and reliably quantified in serum samples from both disease and healthy.[29] In the context of civilian TBI, serum NfL measured within 48 hours of injury have been shown to distinguish patients with CT findings from those without CT findings.[24,30,31] In contrast to the existing studies, we herein found that serum NfL can distinguish patients with mid, moderate and severe TBI from each other as well compared to

controls up to 5 year after TBI. Serum NfL also showed modest associations with GOS-E score. In direct comparison to these findings, we previously observed increased concentrations of serum NfL up to 1-year after injury in patients with severe TBI, with initial levels associated with GOS-E score.[29] Additionally, serum NfL showed inverse relationships with brain MRI WM and CC volumes, meaning higher concentrations of serum NfL are associated with reduced brain volumes. The fact that these two methodologies yielded results in a similar direction is a principal proof of independent cross-validation of these methods and increases the confidence of interpretation of the two methods. Together, these findings suggest that a single TBI may cause long-term axonal degeneration that could be detectable in serum months to years after injury.

Furthermore, we found increased serum concentrations of GFAp between patients and controls with the highest concentrations measured at the 30-day time point. However, the AUROCs for GFAp ranged between 0.52-0.77 across TBI severities over 5 years, which is not considered clinically useful. Also, there was no association between GFAp and outcome, despite an association between serum GFAp and brain WM volume. Therefore, unlike previous studies in acute TBI, [17,32] these results indicate that GFAp may not perform well as a diagnostic biomarker in chronic TBI.

Increased concentrations of CSF tau have previously been found in patients with moderate to severe TBI.[19,32] Serum tau increases within hours after concussion in athletes compared with preseason baseline.[33-35] In the present study, serum tau concentrations were elevated in patients with TBI, with higher concentrations in moderate to severe cases. However, the diagnostic utility of serum tau was low, and we found no relationship between tau and outcome or brain MRI volumes. These findings are consistent with previous reports showing elevated concentrations of tau following mTBI, however, with limited prognostic utility.[37] Similar findings have also been observed in CSF of athletes with a history of repetitive concussive TBI, where there was no association between CSF tau and outcome.[11,12]

The fourth neuronal biomarker measured was serum UCH-L1. Although showing potential in discriminating moderate to severe TBI patients from controls, the CV for serum UCH-L1 was high and variable, casting doubt on the robustness of UCH-L1 as a biomarker for

TBI, especially if used in multiplex assays. A plausible explanation for the higher CVs could be the presence of heterophilic antibodies in the blood which may interfere in immunoassay.[36] We have also observed high CVs when measuring UCH-L1 in serum previously.[30] Although serum UCH-L1 was elevated in moderate to severe TBI patients, we found no association with outcome and only weak associations with brain volumetric analyses. These finding are consistent with previous studies showing higher concentrations of blood UCH-L1 in TBI patients, with limited added prognostic power to the clinical variables.[37]

Comparing the 4 serum biomarkers measured herein, the performance of NfL was robust in distinguishing patients with different TBI severities at enrollment and over time, as well as showing better associations with both outcome and brain MRI WM and CC volumes compared to the other biomarkers. Additionally, serum NfL was elevated in mild, moderate and severe cases compared with controls at 5 years after injury. The levels of GFAp, tau, and UCH-L1 were variable and showed only diagnostic utility in moderate to severe TBI cases. These findings indicate that axonal injury and astrogliosis may persist for years after TBI, more evident in the moderate or severe cases, which is consistent with existing animal model and human histopathological studies.[38,39]

Limitations

There are limitations to this study. First, the number of participants at day 30, and 4 and 5 years after injury were low, especially when assessing the biomarker concentrations longitudinally across TBI severities. Second, the number of blood samples were lower for tau and UCH-L1 due to exclusion of participants with high CVs. Third, the present study was designed to include participants at 30 days and onwards after injury, precluding comparison to their acute concentrations as well as direct comparison to the existing studies of these biomarkers. Fourth, the controls used herein were from healthy individuals; more appropriate controls would have been orthopedic trauma patients without head injury. Finally, serum is not the optimal sample for measurement of tau, as tau concentrations in serum are lower than in plasma, possibly explaining the higher analytical variation for this biomarker.

Conclusion and clinical relevance

The findings in the present study suggest that TBI is associated with axonal and astroglial injury, which can be measured using serum NfL and GFAp months to years after injury, with serum NfL showing greater diagnostic utility and associations with outcome and brain MRI volumes.

FIGURE LEGENDS

Figure 1. Biomarker concentrations across TBI severities at enrollment.
Plots **A**-**D** show the serum concentrations of NfL, GFAp, tau, and UCH-L1 across different TBI severities at median 7 months after TBI. The boxplots show the median and interquartile range. The *P* values are adjusted for multiple comparisons using Holm-Bonferroni method. *Abbreviations*: TBI, traumatic brain injury; NfL, neurofilament light; UCH-L1, ubiquitin C-terminal hydrolase-L1; GFAp, glial fibrillary acidic protein.

Figure 2. Time course of the blood biomarkers.
Plots **A**-**D** show the time course of serum NfL, GFAp, tau, and UCH-L1 across TBI severities. The error bars indicate the standard error of mean. The y-axes are long-transformed (log 10) for better visual clarity. The x-axes show the sampling time points after TBI. *Abbreviations*: TBI, traumatic brain injury; NfL, neurofilament light; GFAp, glial fibrillary acidic protein; UCH-L1, ubiquitin C-terminal hydrolase-L1.

Figure 3. Diagnostic utility of blood biomarkers over time.
The plots show the diagnostic utility (AUROC) of serum NfL, GFAp, tau, and UCH-L1 in distinguishing TBI patients from controls. The error bars indicate the 95 % confidence interval. *Abbreviations*: TBI, traumatic brain injury; AUROC, area under the receiver operating characteristics curve; NfL, neurofilament light; GFAp, glial fibrillary acidic protein; UCH-L1, ubiquitin C-terminal hydrolase-L1.

Figure 4. Association between blood biomarkers and outcome.
Plots **A**-**D** show the association of GOS-E with serum concentrations of NfL, GFAp, tau, and UCH-L1. The *x*-axes show the log-transformed values for visual clarity. The ß estimates and *P* values are from linear regression models, covaried for age, education, and gender. The fitted

lines including the standard error are from the regression models. *Abbreviations*: GOS-E, Glasgow Outcome Scale Extended; NfL, neurofilament light; GFAp, glial fibrillary acidic protein; UCH-L1, ubiquitin C-terminal hydrolase-L1.

Table 1. Demographic and clinical characteristics of TBI patients and controls at enrollment				
Variables	**TBI (*n* = 162)**	**Controls (*n* = 68)**	***P* Value**	**Effect Size Estimate**
Age, years, median (IQR)	43 (30-56)	42 (27-54)	.38	0.09
Gender, female: male	55:107	21:55	.040	0.17
Race			.01	0.32
White	120	23		
African American	24	19		
Asian	4	1		
Multiple races	12	1		
Other	2	0		
Years of education, median (IQR)	16 (14-18)	16 (16-17)	.20	0.10
Time since recent TBI, months	7 (3-17)	0	NA	NA
Alteration of consciousness, no. (%)	99 (71)	0	NA	NA
Injury severity				
Mild, no. (%)	89 (55)	0	NA	NA
Moderate, no. (%)	48 (30)	0	NA	NA
Severe, no. (%)	25 (15)	0	NA	NA
Causes of injury				
Acceleration/deceleration, no. (%)	47 (28)	0	NA	NA
Blast, no. (%)	8 (5)	0	NA	NA
Direct impact-blow to the head, no. (%)	46 (28)	0	NA	NA
Fall, no. (%)	50 (30)	0	NA	NA
Other, no. (%)	11 (9)	0	NA	NA
GOS-E, no. (%)	146 (90)	0	NA	NA
1-Dead	0 (0)	0	NA	NA
2-Vegetative state	0 (0)	0	NA	NA
3-Low severe disability	8 (5)	0	NA	NA
4-Upper severe disability	14 (10)	0	NA	NA
5-Low moderate disability	34 (23)	0	NA	NA
6-Upper moderate disability	45 (31)	0	NA	NA
7-Low good recovery	25 (17)	0	NA	NA
8-Upper good recovery	20 (14)	0	NA	NA
Serum biomarkers, median (IQR)				
NfL, pg/mL	12.8 (7.2-33.0)	6.3 (3.6-9.2)	<.0001	0.52
GFAp, pg/mL	100.8 (64.0-143.5)	60.2 (46.4-80.1)	<.001	0.38
Tau, pg/mL	0.21 (0.13-0.42)	0.15 (0.07-10.27)	.16	0.10
UCH-L1, pg/mL	9.6 (6.4-15.5)	8.3 (5.5-11.7)	<.12	0.12

Abbreviations: Glasgow outcome scale extended; NA, not applicable; NfL, neurofilament light; GFAp, glial fibrillary acidic protein; UCH-L1, Ubiquitin C-terminal hydrolase-L1. Data are presented as median (interquartile range). Categorical variables were calculated using Chi ^{2} test. Continuous variables were calculated using Mann-Whitney *U* test. The effect size for the Mann-Whitney *U* test was calculated using the formula $Z/\sqrt{N}$, where *Z* represents the transformed distribution of the test statistics and *N* represents the pooled samples size. The effect size estimate for contingency data was calculated using Cramer's V or φ_c ($\sqrt{\chi 2/N(k-1)}$), where $\chi 2$ is derived from the Pearson's chi^2 test, *N* is the pooled samples size, and *k* being the number of columns.

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