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Prognostic value of GFAP and UCHL-1 biomarkers in high-risk mild traumatic brain injury: A prospective longitudinal study of short- and long-term outcomes

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ABSTRACT

Background: Blood biomarkers such as glial fibrillary acidic protein (GFAP) and ubiquitin carboxy-terminal hydrolase L1 (UCH-L1) have been shown to rise after mild traumatic brain injury, improving early detection of intracranial lesions. However, evidence on their role in detecting delayed intracranial hemorrhage, especially in patients on anticoagulants, and on their ability to predict long-term post-concussive symptoms is still limited and remains largely unexplored. Our study is the first to address this gap, evaluating these biomarkers for early detection of delayed bleeding and their association with symptom persistence at 3 and 6 months.
Objective: To investigate the diagnostic and prognostic utility of serum biomarkers GFAP and UCH-L1 in adult patients with mild traumatic brain injury (mTBI), focusing on their association with delayed intracranial hemorrhage and post-concussive symptoms at 3 and 6 months.
Methods: This prospective, single-center study enrolled adult patients (≥18 years) with mTBI (Glasgow Coma Scale ≥13) presenting within 24 h of injury. All patients were considered at high risk for intracranial bleeding due to blood thinners. Initial head CT and serum biomarker sampling were performed upon emergency department (ED) admission, followed by repeated CT imaging at 24 h. Serum GFAP and UCH-L1 levels were analyzed for their sensitivity and negative predictive value (NPV) in detecting acute and delayed intracranial injury, and for their association with post-concussive symptoms (PCS) assessed at 3 and 6 months using the Rivermead Post-Concussion Symptoms Questionnaire (RPQ). Symptomatic status was defined by clinically significant worsening relative to the pre-injury baseline.
Results: Overall, 441 patients fulfilled the inclusion criteria and were enrolled. Seventy-five patients (17 %) had positive findings on initial CT. These individuals were significantly older and more frequently hypertensive, while other clinical and laboratory parameters showed no significant differences. GFAP levels >30 pg/ml, and UCH-L1 > 360 pg/ml, and combined GFAP/UCH-L1 elevation were strongly associated with CT abnormalities, yielding high sensitivity (96 %; 95 % CI: 88.8–99.2) and NPV (96 %; 95 % CI: 90.6–98.9), though specificity was limited (24 %; 95 % CI: 20–29). Among 366 patients with negative baseline CTs, delayed intracranial hemorrhage occurred in only 3 cases (0.82 %). None of the patients with negative biomarker results at admission developed delayed intracranial hemorrhage. At follow-up, 15–22 % of patients reported persistent mild PCS, with no significant predictive value from baseline biomarkers, clinical features, or imaging findings.
Conclusion: GFAP and UCH-L1 demonstrate excellent sensitivity for detecting acute intracranial lesions in mTBI and may support safer, more selective use of CT imaging in the ED. The low incidence of delayed hemorrhage

Abbreviations: mTBI, mild Traumatic Brain Injury; TBI, Traumatic Brain Injury; GFAP, glial fibrillary acidic protein; UCH-L1, ubiquitin carboxy-terminal hydrolase L1; ED, Emergency Department; RPQ, Rivermead Post-Concussion Symptoms Questionnaire; GCS, Glasgow Coma Scale; NPV, Negative Predictive Value; PPV, Positive Predictive Value; CI, Confidence Interval; PCS, Post-Concussive Symptoms.

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following a negative initial CT suggests that routine repeat imaging may be unnecessary in mTBI patients, particularly in the case of negative biomarkers at admission. However, persistent post-concussive symptoms remain common and unpredictable, underscoring the need for improved prognostic tools beyond current biomarkers.
Trial registration: ClinicalTrials.gov NCT06069674.

1. Introduction

Traumatic brain injury (TBI) represents a significant global health burden, with an estimated 69 million new cases occurring annually worldwide [1]. Incidence rates are rising across all age groups and in countries of every income level, from high- to low-resource settings [1]. In Europe, approximately 2.5 million cases of TBI are reported each year, while in the United States, the estimated annual incidence reaches 3.5 million cases [2]. Mild traumatic brain injury (mTBI) accounts for nearly 90 % of all TBI cases presenting to hospitals [2].

These epidemiological trends underscore not only the widespread impact of TBI but also the urgent need for improved diagnostic strategies, particularly in mild cases where clinical assessment alone may be insufficient. In the absence of universally accepted diagnostic criteria for mTBI, diagnosis relies on the integration of commonly used definitions [3]. This includes establishing a plausible mechanism of injury, identifying acute alterations in mental status—such as loss of consciousness, amnesia, or confusion—and excluding alternative causes such as intoxication, psychological stress, or metabolic disturbances [3].

Moreover, mTBI can result in persistent symptoms, with up to 50 % of adult patients failing to fully recover to their pre-injury health status within six months [2]. Computed tomography (CT) of the head remains the standard imaging modality for evaluating traumatic intracranial injury in patients with mTBI. However, current evidence suggests that intracranial abnormalities are detected on head CT in approximately one out of six individuals with mTBI [4], and only about 1 % require neurosurgical intervention [5]. Growing awareness of the risks associated with radiation exposure has further emphasized the importance of minimizing unnecessary neuroimaging in patients at low risk [6,7].

In the context of mild traumatic brain injury (mTBI), which accounts for approximately 90 % of all TBI cases presenting to the hospital, Glial Fibrillary Acidic Protein (GFAP) and Ubiquitin C-Terminal Hydrolase L1 (UCH-L1) have demonstrated clinical utility in the acute setting [8-11]. Multiple studies have shown that serum levels of GFAP and UCH-L1 rise rapidly following mTBI and can effectively differentiate patients with intracranial lesions detectable on computed tomography (CT) from those without [12–17].

Although current evidence, limited by small sample sizes, potential selection bias, and underrepresentation of patients receiving aspirin or non-vitamin K oral anticoagulants, suggests that delayed intracranial hemorrhage is uncommon in anticoagulated or antiplatelet-treated patients with mTBI [18,19], clear discharge instructions with explicit return precautions remain essential [19]. Nevertheless, the potential role of serum biomarkers in identifying patients at risk for delayed hemorrhage has not been adequately investigated and warrants further study.

The primary aim of this study is to evaluate the relationship between biomarker concentrations and the occurrence of acute and delayed intracranial hemorrhage in patients undergoing clinical observation and repeat CT imaging in the Emergency Department (ED). As a secondary endpoint, the study explores the prognostic value of these biomarkers by examining their association with the development of post-concussive symptoms at 3 and 6 months post-injury.

2. Materials and methods

2.1. Population sample

This single-center, prospective observational study was conducted between September 2022 and November 2024, enrolling non-pregnant adult patients (≥18 years) who presented to the Emergency Department with mild traumatic brain injury, defined as a Glasgow Coma Scale (GCS) score of ≥13 within 24 h of trauma. Patients with moderate or severe TBI were excluded. Additionally, individuals not meeting criteria for 24-h observation and repeat computed tomography (CT) scanning were excluded. Furthermore, patients who refused the first CT scan were excluded.

This study was conducted in the ED of a tertiary academic hospital, serving a catchment area of approximately 1.8 million inhabitants. The ED manages an annual volume of nearly 80,000 emergency admissions and hosts a trauma center, which is the referral facility for surrounding minor hospitals. The trauma center receives approximately 2000 major trauma cases per year.

All eligible patients underwent venous blood sampling and head CT upon ED presentation. In cases of mTBI (GCS 13–15), CT scans were considered positive if acute intracranial hemorrhage was identified; isolated skull fractures without intracranial involvement were not classified as positive findings. In patients with an initial negative CT, a second CT scan was performed according to the local institutional protocol. In accordance with the protocol, a second CT scan was required for patients receiving anticoagulant therapy, either direct oral anticoagulants or vitamin K antagonists, as well as for those undergoing antiplatelet treatment. Additionally, all patients presenting with persistent neurological symptoms at 6 h after the initial assessment were considered candidates for a follow-up CT scan at 24 h.

Our definition of “loss of consciousness” includes any patient who reports uncertainty regarding whether a true loss of consciousness occurred and its timing, as well as all patients for whom this information is unknown or unreported.

Post-concussive symptoms were assessed using the Rivermead Post-Concussion Symptoms Questionnaire (RPQ), administered via structured telephone follow-up after hospital discharge. The RPQ evaluates 16 physical, cognitive, and emotional symptoms, with severity rated from 0 (absent) to 4 (severe), relative to the patient's pre-injury baseline. Patients with pre-existing conditions causing similar symptoms were excluded unless a clear post-traumatic exacerbation was documented. For analytical purposes, asymptomatic patients (score 0) and those reporting mild, non-disruptive symptoms (score 1) were grouped as having no clinically relevant symptoms. Patients who were unavailable for follow-up or declined participation were excluded from the final dataset.

See Fig. 1 for a detailed overview of the algorithm used for selecting patients with mTBI.

2.2. TBi test

Serum biomarker measurements were performed using the TBi test, a panel of chemiluminescent microparticle immunoassays (CMIA) designed to quantify GFAP and UCH-L1 in human plasma and serum. The assays were processed on the Alinity® i platform

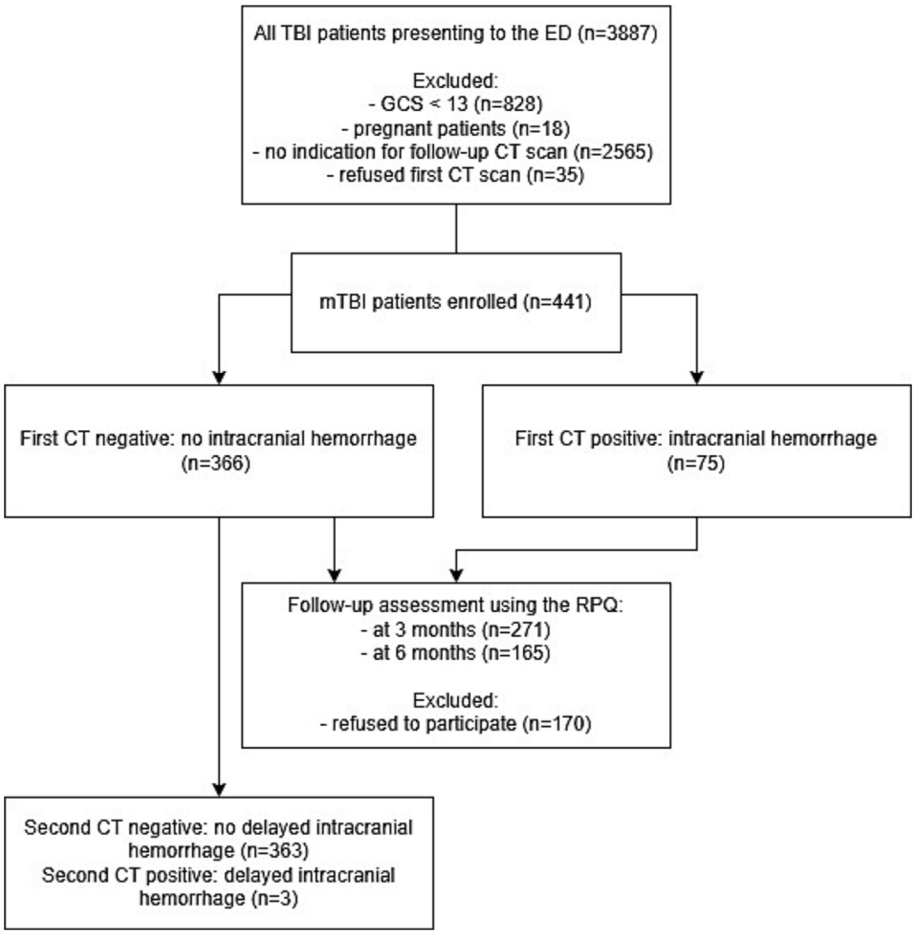

Fig. 1. Algorithm applied for the selection of patients with mTBI where "n" refers to the number of patients included or excluded at each step.

(Abbott Laboratories, Abbott Park, IL, USA), which provides a semi-quantitative interpretation of biomarker levels. The chemiluminescent signal was measured in relative light units (RLU), with values directly proportional to the concentrations of GFAP and UCH-L1. For data processing, both assays applied a four-parameter logistic curve fitting model (4PLC, weighted Y) to generate calibration curves and compute the final results. According to the manufacturer, GFAP was considered positive for values >30 pg/ml, and UCH-L1 was considered positive for values >360 pg/ml).

2.3. Statistical analysis

Continuous variables were described using medians and interquartile ranges (IQR), as this approach provides a robust measure of central tendency and variability for data that are not normally distributed. Categorical variables were reported as absolute frequencies and corresponding percentages, allowing for a clear comparison of proportions between groups. For inferential analysis, the Mann–Whitney *U* test was applied to evaluate differences in continuous variables across groups, given its suitability for non-parametric data. Associations between categorical variables were examined using the chi-square test; when the assumptions for this test were not met, particularly in the presence of small expected cell counts, Fisher's exact test was employed to ensure the accuracy of the statistical inference. A two-tailed *p*-value of <0.05 was considered indicative of statistical significance for all analyses. Statistical analysis was performed by SPSS® v26 (IBM, Armonk, NY, USA).

3. Results

3.1. Population characteristics

A total of 441 patients met the inclusion criteria and were enrolled in the study cohort. A follow-up CT scan was performed in 377 cases (85.5 %) due to concomitant therapy with blood-thinning agents, and in 64 cases (14.5 %) because of persistent neurological symptoms at 6 h after admission. Among patients receiving blood thinners, 269 (61 %) were on single-agent therapy (28 on vitamin K antagonists, 147 on direct oral anticoagulants, 86 on aspirin, and 8 on clopidogrel), while 108 patients (24.5 %) were on combined therapy, including 22 on vitamin K antagonists plus an antiplatelet agent, 52 on DOACs plus an antiplatelet agent, and 34 on dual antiplatelet therapy.

Table 1 presents clinical, demographic, and outcome measures stratified by admission Glasgow Coma Scale (GCS) score. Lower GCS scores were associated with older age, a higher burden of comorbidities, and longer hospital stays. Domestic accidents represented the leading cause of injury, and triage categories reflected initial clinical severity. Laboratory parameters, including prothrombin time, activated partial thromboplastin time, international normalized ratio, and platelet count, were comparable across groups. However, loss of consciousness and amnesia were more frequently observed in patients with lower GCS scores. Overall, patients with a GCS of 13 exhibited greater clinical complexity and slower recovery compared to those with higher scores.

Biomarker analysis revealed distinct trends. GFAP positivity (>30 pg/ml) was observed in all patients with GCS 13 (100 %), while

Table 1
Description of Study Population.

Category	Variable	GCS 13 (n[a] = 14)	GCS 14 (*n* = 108)	GCS 15 (*n* = 319)
Demographics	Age, median (IQR[b])	79.5 (73.8–83.8)	76.0 (58.3–83.0)	68.0 (51.0–81.0)
	Female sex (%)	6 (42.9 %)	52 (48.1 %)	160 (50.2 %)
	Male sex (%)	8 (57.1 %)	56 (51.9 %)	159 (49.8 %)
	Charlson Comorbidity Index, median (IQR)	3.5 (2.8–6.0)	0 (0–2.0)	0 (0–1.0)
	Hospital stay, days, median (IQR)	10.9 (4.0–13.0)	0 (0–2.5)	0 (0–1.0)
Trauma location	Home	9 (64.3 %)	80 (74.1 %)	188 (58.9 %)
	Motor vehicle accidents	4 (28.6 %)	22 (20.4 %)	120 (37.6 %)
	Workplace	1 (7.1 %)	1 (0.9 %)	7 (2.2 %)
	Sports	0 (0.0 %)	4 (3.7 %)	3 (0.9 %)
	Violence	0 (0.0 %)	1 (0.9 %)	1 (0.3 %)
Triage category	Emergency	9 (64.3 %)	4 (3.7 %)	87 (27.3 %)
	Urgency	3 (21.4 %)	101 (93.5 %)	100 (31.3 %)
	Minor Urgency	2 (14.3 %)	3 (2.8 %)	132 (41.4 %)
Clinical findings[c]	Loss of consciousness (%)	35.7 %	79.6 %	62.1 %
	Amnesia (%)	21.4 %	35.2 %	33.5 %
	Severe headache (%)	7.1 %	7.4 %	5.6 %
	Vertigo (%)	7.1 %	3.7 %	5.0 %
	Nausea/vomiting (%)	7.1 %	13.9 %	7.5 %
	Facial bone fracture (%)	14.3 %	6.5 %	9.1 %
	Extremity bone fracture (%)	28.6 %	12.0 %	21.9 %
Timing	Time from trauma <12h[d] (%)	78.6 % (11)	54.6 % (59)	56.4 % (180)
	Time from trauma 12–24 h (%)	21.4 % (3)	45.4 % (49)	43.6 % (139)
Biomarkers	GFAP >30 pg/ml (%)	100 % (14)	69.4 % (75)	72.4 % (231)
	UCH-L1 > 360 pg/ml (%)	64.3 % (9)	41.7 % (45)	42.3 % (135)
Radiology	1st CT positive (%)	78.6 % (11)	13.0 % (14)	15.7 % (50)
	2nd CT positive[e] (%)	0 %	1.9 % (2)	0.3 % (1)
RPQ	Any symptoms at 3 months (%)	33.3 % (4/12)	32.3 % (20/62)	37.6 % (74/197)
	Any symptoms at 6 months (%)	50.0 % (3/6)	39.5 % (15/38)	42.1 % (51/121)

[a] n: number of patients; [b]IQR: Interquartile Range; [c]Patient-reported symptoms at ED evaluation; [d]: hours; [e]This refers to patients who presented with a negative initial CT scan and subsequently had a positive follow-up CT scan.

it was lower among those with GCS 14 (69.4 %) and GCS 15 (72.4 %). Similarly, UCH-L1 positivity (>360 pg/ml) was more prevalent in the GCS 13 group (64.3 %) compared to GCS 14 (41.7 %) and GCS 15 (42.3 %). Radiological findings supported these differences: 78.6 % of patients with GCS 13 had a positive initial CT scan, versus 13.0 % and 15.7 % in the GCS 14 and 15 groups, respectively. On repeat CT, positivity persisted in 71.4 % of GCS 13 patients, while rates were lower in the other groups (14.8 % and 12.5 %). Delayed hemorrhage was rare across all categories.

At follow-up, persistent symptoms were common in all GCS groups. At 3 months, approximately one-third of patients remained symptomatic (33.3 %, 32.3 %, and 37.6 % for GCS 13, 14, and 15, respectively). At 6 months, symptom persistence was noted in 50.0 % of GCS 13 patients, 39.5 % of GCS 14 patients, and 42.1 % of GCS 15 patients.

Further details on the study population are provided in Table 1.

3.2. Sensitivity and specificity of GFAP and UCH-L1 in identifying intracranial Hemorrhage in mTBI

Stratification by initial CT results showed that patients with positive scans were older and more often hypertensive, while sex, coagulation parameters, and comorbidities were similar across groups. Clinical symptoms were more frequent in the positive CT group but not statistically significant. Elevated GFAP (>30 pg/ml), UCH-L1 (>360 pg/ml), and combined biomarker positivity were strongly associated with CT abnormalities. GFAP and combined biomarkers demonstrated high sensitivity and negative predictive value for detecting acute lesions, supporting their utility for ruling out injury, though specificity remained low. Persistent post-concussive symptoms at 3 and 6 months were comparable between groups.

GFAP and combined serum biomarkers demonstrated high sensitivity and negative predictive value for detecting acute CT-positive lesions, supporting their use to rule out injury, whereas low specificity limits their ability to confirm lesions. For a comprehensive overview of the diagnostic accuracy of GFAP and UCH-L1, see Table 2.

3.3. Evaluation of GFAP and UCH-L1 in detecting delayed intracranial Hemorrhage

Among the 366 patients with an initially negative CT scan who underwent follow-up imaging, delayed intracranial hemorrhage was uncommon, occurring in only three cases. Demographic characteristics, laboratory parameters, and clinical presentation did not differ significantly between patients with positive and negative second CT scans. Interestingly, all patients with delayed hemorrhage exhibited a positive combined biomarker panel at admission, with GFAP elevated in all three cases and UCH-L1 elevated in two of the three cases. Additionally, vertigo showed a non-significant trend toward association with delayed bleeding, while Parkinson's disease was more prevalent among patients with positive follow-up CT findings (33.3 % vs. 3.3 %, $p = 0.020$). Follow-up assessments at 3 and 6 months revealed no significant differences in persistent symptoms between groups, indicating a low

Table 2
GFAP and UCH-L1 accuracy for detecting intracranial hemorrhage in mTBI.

Marker	Sensitivity % (95 % CI[a])	Specificity % (95 % CI)	+LR[b]	−LR	PPV[c] % (95 % CI)	NPV[d] % (95 % CI)
GFAP >30	94.7 (86.9–98.5)	32.0 (27.2–37.0)	1.39	0.17	22.2 (20.7–23.8)	96.7 (91.8–98.7)
UCHL-1 > 360	53.3 (41.4–64.9)	59.3 (54.1–64.4)	1.31	0.79	21.2 (17.4–25.5)	86.1 (82.8–88.9)
Combined	96.0 (88.8–99.2)	24.3 (20.0–29.0)	1.27	0.16	20.6 (19.4–21.9)	96.7 (90.6–98.9)

[a] CI: Confidence Interval; [b]LR: Likelihood Ratio; [c]PPV: Positive Predictive Value; [d]NPV: Negative Predictive Value.

Table 3
Evaluation of GFAP and UCH-L1 as biomarkers for delayed intracranial hemorrhage.

Category	Variable	CT2[a] negative	CT2 positive	p-value
Demographics	Age, median (IQR[b])	69 (51–82)	85 (75–85)	0.382
	Sex, female	175 (48.2 %)	1 (33.3 %)	0.353
	Sex, male	188 (51.8 %)	2 (66.6 %)	0.353
Time from trauma	<12h[c]	208 (57.5 %)	1 (33.3 %)	0.764
	12–24 h	154 (42.5 %)	2 (66.6 %)	0.764
Biomarkers	GFAP >30 pg/ml	246 (68.0 %)	3 (100 %)	0.764
	UCH-L1 > 360 pg/ml	147 (40.6 %)	2 (66.6 %)	0.704
	Combined markers positive	274 (75.7 %)	3 (100 %)	0.974

[a] CT2: follow-up CT scan; [b]IQR: InterQuartile Range; [c]: hours.

incidence and limited clinical impact of delayed hemorrhage in this population.

For further details regarding the assessment of GFAP and UCH-L1 as predictive biomarkers for delayed intracranial hemorrhage, see Table 3 and Table 4.

3.4. Association of Clinical, radiological, and biomarker variables with 3 and 6-month symptom persistence

Overall, 271 patients were available for evaluation at 3 months, and 165 at 6 months. The analysis at 6 months included only those patients who continued in follow-up to avoid the introduction of a selection bias.

At 3- and 6-month follow-up, 22.2 % and 15.6 % of patients, respectively, reported persistent post-traumatic symptoms. Symptom persistence was not significantly associated with initial GCS scores, clinical presentation, comorbidities, skeletal injuries, radiological findings (including initial or follow-up CT positivity), or biomarker levels (GFAP, UCH-L1, or combined panel). Female sex showed a non-significant trend toward fewer symptoms. The 6-month analysis was limited to patients who remained in follow-up, introducing potential selection bias. Overall, no baseline clinical, radiological, or biomarker variable reliably predicted mid-term symptom persistence.

Detailed data regarding patient-reported symptoms are presented in Fig. 2.

At three months post-injury, most patients reported no or only mild post-traumatic symptoms. The most frequently reported complaints included headache, dizziness, fatigue, and sleep disturbances. Cognitive and affective symptoms, such as memory impairment, concentration difficulties, irritability, and depressive mood, were less common and generally mild. Chi-square analysis revealed no significant association between initial GCS scores and the severity of most symptoms at six months, with the exception of fatigue ($p = 0.021$). Photophobia, diplopia, and other nonspecific symptoms were rare and showed no correlation with GCS scores. Overall, residual symptoms were typically mild and self-limiting, with minimal dependence on initial injury severity.

At six months post-injury, persistent symptoms were not significantly associated with baseline clinical characteristics, comorbidities, presence of fractures, radiological findings, or biomarker levels. Female

Table 4
Accuracy of GFAP and UCH-L1 in detecting delayed intracranial hemorrhage in patients with mTBI. Values were calculated for the 366 out of 441 patients who had negative findings on the initial imaging assessment.

Marker	Sensitivity % (95 % CI[a])	Specificity % (95 % CI)	+LR[b]	−LR	PPV[c] % (95 % CI)	NPV[d] % (95 % CI)
GFAP >30	100 (29.2–100)	32.2 (27.4–37.3)	1.48	0.00	1.2 (1.1–1.3)	100 (100−100)
UCHL-1 > 360	66.7 (9.4–99.2)	59.5 (54.3–64.6)	1.65	0.56	1.3 (0.6–3.0)	99.5 (97.8–99.9)
Combined	100 (29.2–100)	24.5 (20.2–29.3)	1.32	0.00	1.1 (1.0–1.1)	100 (100–100)

[a] CI: Confidence Interval; [b]LR: Likelihood Ratio; [c]PPV: Positive Predictive Value; [d]NPV: Negative Predictive Value.

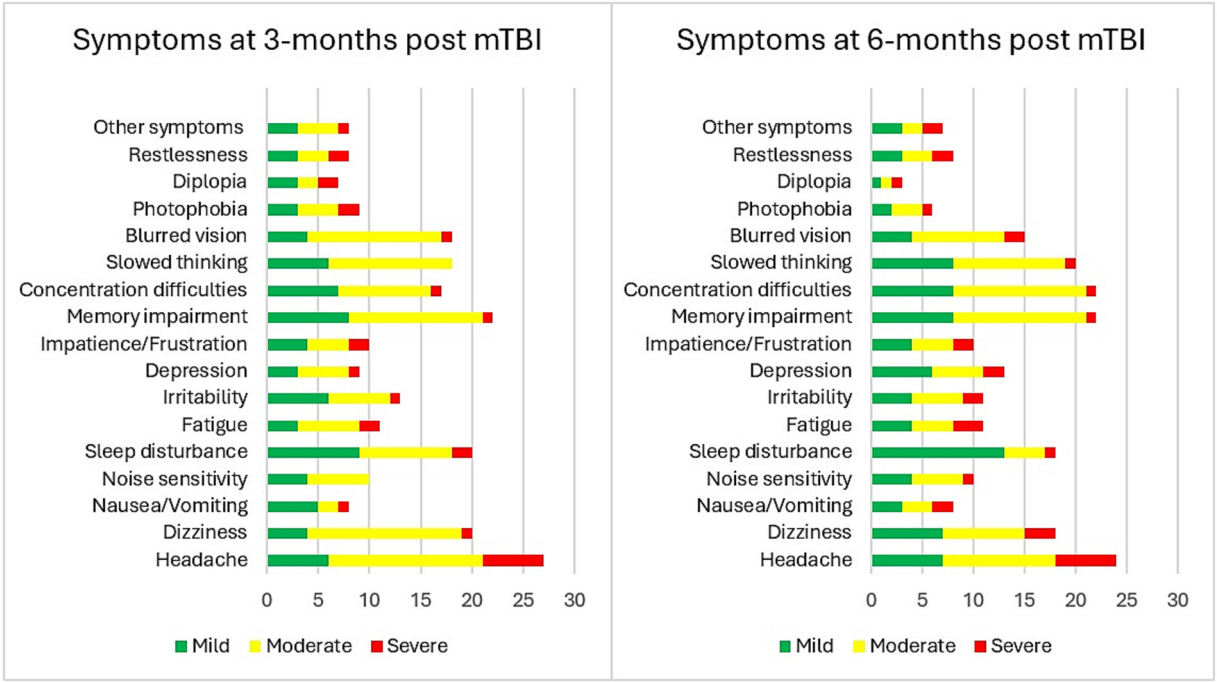

Fig. 2. PCS prevalence at 3 and 6 months. Left: distribution of symptoms at 3 months; right: distribution at 6 months. Bars represent the number of patients reporting each symptom (x-axis). Colour coding indicates symptom severity according to the Rivermead Post-Concussion Questionnaire (y-axis): green for mild (score 2), yellow for moderate (score 3), and red for severe (score 4).

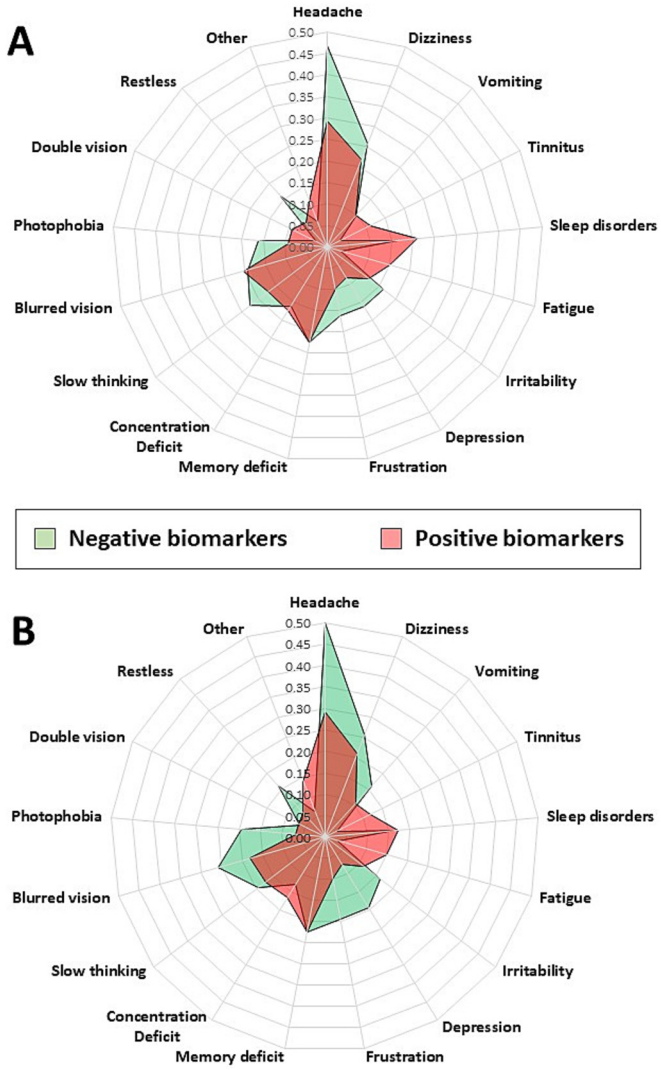

Fig. 3. Post concussive symptoms reported at 3 months (A) and 6 months (B) in patients with negative (Green) and positive (Red) combined biomarkers panel at ED admission.

sex, loss of consciousness, amnesia, acute symptoms, and biomarker positivity (GFAP, UCH-L1, or combined panel) did not demonstrate predictive value for symptom persistence (Fig. 3). No baseline variable was found to reliably predict mid-term post-traumatic outcomes.

4. Discussion

Our study's findings are consistent with existing literature regarding the diagnostic utility of blood biomarkers in mild traumatic brain injury. Specifically, we observed that elevated levels of GFAP and UCH-L1 were significantly associated with the presence of acute intracranial lesions on initial head CT scans. These results corroborate previous studies that have demonstrated the sensitivity and specificity of these biomarkers in detecting acute brain injury [12,13].

In our cohort of patients with mTBI, the incidence of delayed intracranial hemorrhage was very low, aligning with prior reports that estimate rates below 1 % in patients with initially negative head CT scans [20,21]. None of the delayed events required neurosurgical intervention, reinforcing their limited clinical significance. The predictive utility of serum biomarkers GFAP and UCH-L1 for delayed intracranial hemorrhage could not be fully assessed due to the rarity of such events, consistent with previous findings that highlight how low incidence limits the evaluation of prognostic markers [18,22]. Moreover, the timing of biomarker sampling, within 24 h post-injury, may have influenced sensitivity, as peak levels may have already occurred or begun to decline, reducing their ability to detect hemorrhagic complications that manifest later [23]. Overall, evidence from the literature consistently indicates that delayed intracranial hemorrhage following blunt head trauma is an infrequent occurrence in neurologically intact patients receiving anticoagulant or antiplatelet therapy [19].

Notably, none of the patients with negative biomarker results at admission developed delayed intracranial hemorrhage in our cohort. Consequently, if patients with negative biomarker results at admission had not undergone a second CT scan, both clinical observation and repeat imaging could have been avoided in 89 cases (24.3 %).

However, our analysis also suggests that these biomarkers have limited prognostic value in predicting the development of persistent post-concussive symptoms at 3 and 6 months post-injury (Fig. 3). This finding indicates that while GFAP and UCH-L1 are useful for acute diagnostic purposes, they may not serve as reliable predictors of long-term outcomes [24]. These results differ from those reported by Lange et al. (2022) [25], who found that elevated GFAP and UCH-L1 levels within the first 12 months post-injury were predictive of chronic neurobehavioral symptom deterioration. Differences in biomarker timing, follow-up duration, and analytical methodology may account for these discrepancies. Nonetheless, our findings underscore the need for further research to clarify the prognostic utility of serum biomarkers in mTBI.

Additionally, our study found no significant association between initial Glasgow Coma Scale (GCS) scores and the persistence of symptoms at 3 and 6 months post-injury. This finding aligns with previous research indicating that the GCS score, although widely used to assess injury severity, does not reliably predict mid-term outcomes in patients with mTBI [26]. As supported by existing evidence [27], early indicators of emotional distress and coping style assessed at two weeks post-injury were more predictive of six-month outcomes than initial GCS scores.

Headache, dizziness, fatigue, and sleep disturbances were commonly reported at both follow-up intervals. Notably, cognitive and emotional symptoms—such as memory impairment, concentration difficulties, irritability, and depressive mood—were less frequent but tended to persist, suggesting a slower recovery trajectory for higher-order mental functions. These findings are consistent with those of Røe et al. (2009) [26], who observed that cognitive symptoms were more prominent than physical or behavioral symptoms at three months post-injury. Regarding symptom progression, our data showed that somatic complaints such as headache and dizziness generally declined over time, whereas cognitive and emotional symptoms often persisted. This pattern underscores the importance of ongoing monitoring and individualized rehabilitation strategies to address the evolving nature of post-concussive symptoms [28].

5. Conclusion

Our study confirms the diagnostic accuracy of GFAP and UCH-L1 in detecting acute intracranial lesions in patients with mild TBI. The low prevalence of events in our cohort does not allow for definitive conclusions regarding their utility in identifying delayed intracranial hemorrhage. Nonetheless, a negative biomarker profile at admission may suggest that further clinical observation and repeat neuroimaging could be redundant in patients with mild TBI. However, it also demonstrates their limited utility in predicting long-term post-concussive symptoms. These findings should be considered when integrating serum biomarkers into clinical decision-making pathways for the management of mTBI.

Availability of data and materials

All data generated or analyzed during this study are described in this article and its supplementary materials. The datasets include raw patient data, aggregated survey responses, and the scripts used for statistical

analyses. These data are available upon request from the corresponding author. Access will be granted following review of the proposed use and approval by the relevant ethics committee. Any limitations on sharing, such as sensitive or confidential information, are clearly indicated to ensure compliance with ethical and legal requirements.

CRediT authorship contribution statement

Giacomo Spaziani: Writing – review & editing, Writing – original draft, Validation, Resources, Methodology, Investigation, Data curation, Conceptualization. **Gloria Rozzi:** Writing – review & editing, Validation, Methodology, Investigation, Data curation. **Silvia Baroni:** Project administration. **Giulia Napoli:** Formal analysis. **Grazia De Ninno:** Formal analysis. **Davide Della Polla:** Formal analysis. **Nicola Bonadia:** Project administration. **Giuseppe De Matteis:** Project administration. **Andrea Piccioni:** Project administration. **Giuseppe Maria Della Pepa:** Project administration. **Andrea Urbani:** Project administration. **Antonio Gasbarrini:** Project administration. **Francesco Franceschi:** Project administration. **Marcello Covino:** Writing – review & editing, Validation, Supervision, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

Consent for publication

Not applicable.

Ethics approval and consent to participate

The study was conducted in accordance with the principles outlined in the Declaration of Helsinki and its subsequent amendments. The protocol was approved by the Institutional Review Board of Fondazione Policlinico Universitario A. Gemelli, Rome, and was registered under protocol number 001243/23 on April 19, 2023. The trial is also registered at ClinicalTrials.gov (NCT06069674). All participants provided written informed consent prior to enrollment.

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Declaration of competing interest

The authors declare that they have no competing interests.

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