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# Evaluation of GFAP/UCH-L1 biomarkers for computed tomography exclusion in mild traumatic brain injury (mTBI)

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## Abstract

**Introduction** Mild traumatic brain injury (mTBI) represents a major public health concern and affects millions of people worldwide every year. Diagnosis mainly relies on clinical criteria and computed tomography (CT) scans. GFAP (glial fibrillary acidic protein) and UCH-L1 (ubiquitin carboxyl-terminal hydrolase-L1) have been recently studied as potential biomarkers of mTBI. This study retrospectively evaluated the possible use of these combined biomarkers as negative predictors for excluding brain injuries in patients with suspected mTBI in the emergency department.

**Methods** Adult patients ($n=130$) enrolled at Tor Vergata University Hospital (Rome, Italy), consecutively registered at the triage of the emergency department between October 2022 and January 2023, with non-penetrating TBI and Glasgow Coma Scale (GCS) score of 13–15, were considered. All eligible patients underwent intracranial CT scans and blood tests, within 12 h after trauma, for GFAP and UCH-L1 serum concentrations.

**Results** Intracranial CT detected injuries in only seven patients (5%); GFAP and UCH-L1 tested positive in 96 patients and negative in 34 patients (74% vs. 26%). Combined biomarkers had a sensitivity equal to 1.00 (95% CI 0.64-1.00) and a negative predictive value (NPV) of 1.00 (0.99-1.00) in mTBI diagnosis with a negative CT.

**Conclusions** Combined laboratory tests for GFAP and UCH-L1 biomarkers might play a potential clinical role in avoiding unnecessary head CT scans after mTBI in emergency departments.

**Keywords** Mild traumatic brain injury, Biomarkers, Head CT scan, Patient treatment turnaround time, Diagnostic accuracy

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## Background

Mild traumatic brain injury (mTBI) is a significant health concern with immediate and long-term consequences [1]. CT scans are routinely used to evaluate TBI cases, particularly in hospital emergencies. However, the overuse of CT scans, even for mild TBI cases, has become a relevant issue for healthcare systems. The excessive use of CT scans exposes patients to unnecessary ionizing radiation, which could carry inherent risks [2].

Recently, the role of blood biomarkers has become critical in addressing the challenges associated with mTBI [3]. Among biomarkers, GFAP (glial fibrillary acidic protein) and UCH-L1 (ubiquitin carboxyl-terminal hydrolase-L1) have emerged as promising tools in TBI assessment [4]. GFAP is an intermediate filament protein in astrocytes, providing structural and functional support to glial cells and neurons. UCH-L1 is abundant in neurons and involved in the regulation of brain proteins. Due to their brain specificity, very low concentrations of these proteins are released into the bloodstream in a normal condition. Instead, higher levels of circulating GFAP and UCH-L1 can be found after a brain injury, so potentially providing insights into the extent of the neural damage, even when CT scans appear negative [5]. The two proteins are expressed in different cell types and, after the concussion with a complementary pattern, they are released at different times within the first minutes (UCH-L1) or after a few hours (GFAP) from the trauma [6]. Indeed, although their increase is still under evaluation for complementing CT imaging in mTBI management, some studies have shown that GFAP and UCH-L1, when used in combination, can offer high sensitivity and negative predictive value (NPV), thus excluding intracranial lesions when measured within 12 h after a mild traumatic injury, and consequently reducing the need for redundant CT scans [7].

This paper reports our experience in evaluating commercial test compliance with this expected use. In particular, this retrospective study aims to assess the possible use of these combined biomarkers as negative predictors for excluding brain injuries in patients with suspected mTBI in the emergency department.

## Materials and methods

Patients included in the study had suffered mild cranial trauma and attended the emergency department of Tor Vergata University Hospital (Rome, Italy) from October 2022 to January 2023. The local Ethics Committee approved the study protocol (approval number 15/22) that was performed with ethical standards laid down in the 1964 Declaration of Helsinki. Consent declarations were not applicable as in this retrospective study residual sample volumes from routine blood tests were used following good clinical practice rules and all clinical data were retrieved from clinical reports.The inclusion criteria were age > 18 years, GCS score 13–15, and head CT scan. Exclusion criteria included: GCS score < 13; C-reactive protein (CRP) level > 5 mg/L; current anticoagulant therapy; venous sampling > 12 h after the traumatic event. The demographic, epidemiological, and clinical data were obtained from electronic clinical records.

Peripheral blood samples were collected within 12 h after injury, centrifuged for 7 min at 2000 xg, and serum was frozen at −80 °C until use.

GFAP and UCH-L1 serum levels were then measured by Abbott CMIA assays on Alinity i platform following the manufacturer's instructions. The cut-offs for UCH-L1 and GFAP were 400 pg/mL and 35 pg/mL, respectively.

The Alinity TBI test was defined as positive if GFAP or UCH-L1 or both concentrations were above their respective cut-offs. The combined GFAP and UCHL1 test sensitivity and specificity were assessed to investigate the test performance concerning CT positivity/negativity prediction.

Demographic information (age and gender), medical history, GCS scores, CT requests, result delivery time, hematochemical test results, and patient outcomes, including admission and discharge, were considered.

Times from mTBI and head CT requests and result delivery to physicians have been calculated directly or indirectly from retrieved medical reports and reported as histograms for the medians and whiskers for the third quartile of the distribution.

Statistical analysis results are presented as mean ± standard deviation (SD) for continuous variables and median with the first and third quartiles (Q1 and Q3) for non-normally distributed variables. Percentages (%) are used for categorical variables. Pearson's chi-squared and Fisher's exact tests were employed to assess percentual differences. Tables for 2 × 2 contingency were built to measure specificity/sensitivity and predictive values comparing TBI test results vs. CT scan positivity. To better stratify age and TBI biomarkers expression, wide age clusters were created. The distribution data were represented in box-and-whiskers format, where the band within the box represents the median, the box lower and upper border, and the 25th and 75th percentiles of the distribution, respectively. The lower and upper fences were calculated as per the Tukey formula (Q1 − 1.5 × IQR; Q3 + 1.5IQR).

Multiple comparisons in non-normally distributed data were conducted using the non-parametric pairwise Steel-Dwass-Critchlow-Fligner ranking method.

A p value < 0.05 was considered significant and it was applied to all experiments. The sample size was determined to achieve a statistical power of 0.8. Data are reported with a 95% confidence interval. The dataset was created in Microsoft Excel, and statistical analysis was

**Table 1** Population characteristics vs. mTBI serum assay test

| | | mTBI Serum Assay Test | | |
|---|---|---|---|---|
| **Factor** | **Overall** | **Negative** | **Positive** | ***p-value*** |
| n (%) | 130 | 34 (26.2) | 96 (73.8) | 0.01 |
| Age (average ± SD) | 54 ± 23 | 42.7 ± 13.9 | 57.7 ± 24.2 | < 0.001 |
| Cardiopathy n (%) | 12 (9.2) | 2 (5.9) | 10 (10.4) | 0.73 |
| Diabetes n (%) | 10 (7.7) | 1 (2.9) | 9 (9.4) | 0.45 |
| Hypertension n (%) | 34 (26.2) | 3 (8.8) | 31 (32.3) | 0.007 |
| Neurological Disease n (%) | 11 (8.5) | 2 (5.9) | 9 (9.4) | 0.72 |
| Positive Head CT Scan | 7 (5.4) | 0 (0.0) | 7 (7.3) | < 0.001 |
| Antiaggregant Therapy n (%) | 15 (11.5) | 3 (8.8) | 12 (12.5) | 0.75 |
| GFAP Median [IQR] pg/mL | 35.65 [19.1–84.0] | 17.02 [14–23] | 54 [28–105] | < 0.001 |
| UCH-L1 Median [IQR] pg/mL | 452.7 [275–901] | 206 [164–323] | 677 [383–956] | < 0.001 |
| mTBI Assay Sensitivity % (95% CI) | 100 (64.5–100) | - | - | - |
| mTBI Assay Specificity % (95% CI) | 27.6 (20.0-36.4) | - | - | - |
| mTBI Assay NPV % (95% CI) | 100 (88–100) | - | - | - |

n = Number of patients; [IQR] = Interquartile Range; (95%CI) = 95% Confidence Interval; NPV = Negative Predictive Value

conducted using Analyze-it for Microsoft Excel 4.92.4 and R software version 3.6.0.

## Results

A cohort of 130 patients was enrolled. Among these, 72 males (55.4%) and 58 females (44.6%). Although the adopted criteria included patients with GCS scores of 13–15, all the patients reported a GCS score of 14 or 15, and no patient with a score of 13 was recorded in the study period.

Table 1 shows population characteristics of serum mTBI assay results, including comorbidities. No significant differences between the mTBI test positive and negative groups were retrieved considering the presence of other comorbidities, such as cardiac conditions ($p=0.73$), diabetes ($p=0.45$), neoplasms, trauma-independent neurological problems ($p=0.72$), and antiplatelet therapy ($p=0.75$) except for hypertension ($p=0.007$) that resulted more frequent in subjects with GFAP and UCH-L1 positive tests (91%) even without a positive CT scan. Although the mTBI test apparent prevalence was 73.8%, with a 95% confidence interval (CI) ranging from 65.4 to 81.2%, only 5.4% was positive for a CT scan. The diagnostic sensitivity among all individuals with mTBI was measured at 100%, with a 95% CI between 64.5% and 100%. Conversely, specificity was estimated at 27.6%, with a 95% CI ranging from 20 to 36.4%. Due to the high sensitivity of the combined test, NPV was 100%, with a 95% CI between 85.1% and 100%.

Reported mild brain traumatic injuries causes are shown in Fig. 1. The highest percentage (47%) was related to road accidents (motor vehicle accidents), followed by generic falls (39%). The remaining 14% was represented by physical aggression (8%) and syncope (6%). The population average age was 54 (range 31–77 years), with the mTBI test-positive population significantly ($p<0.001$) older (on average 57.66 vs. the negative 42.73, respectively). To further investigate this finding, we stratified the population for GFAP and UCH-L1 levels of expression in wide age clusters. Data in Fig. 2A show no significant variation for UCH-L1 among clusters ramping from

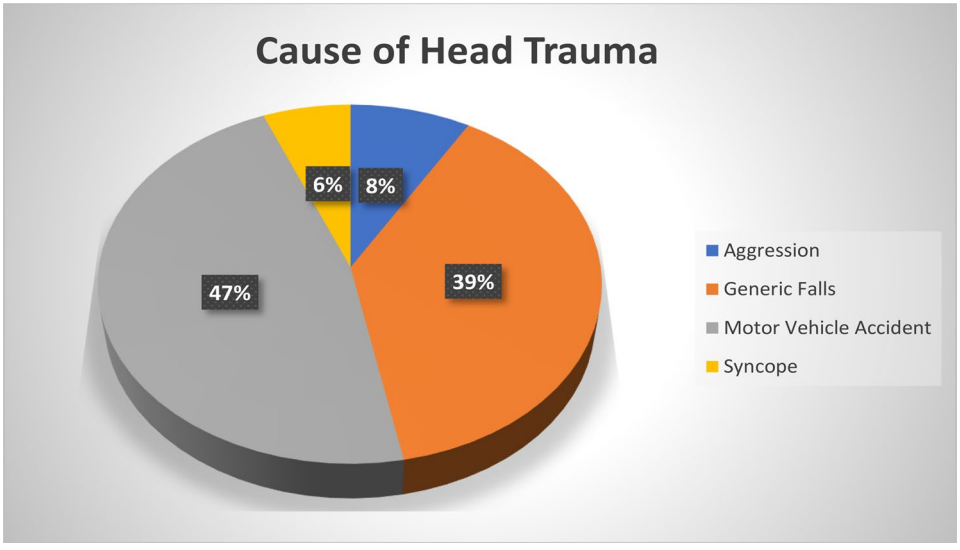


**Fig. 1** The pie chart shows the relative proportion (%) of causes of brain injuries in the analyzed population. Retrieved categories are reported in picture legend

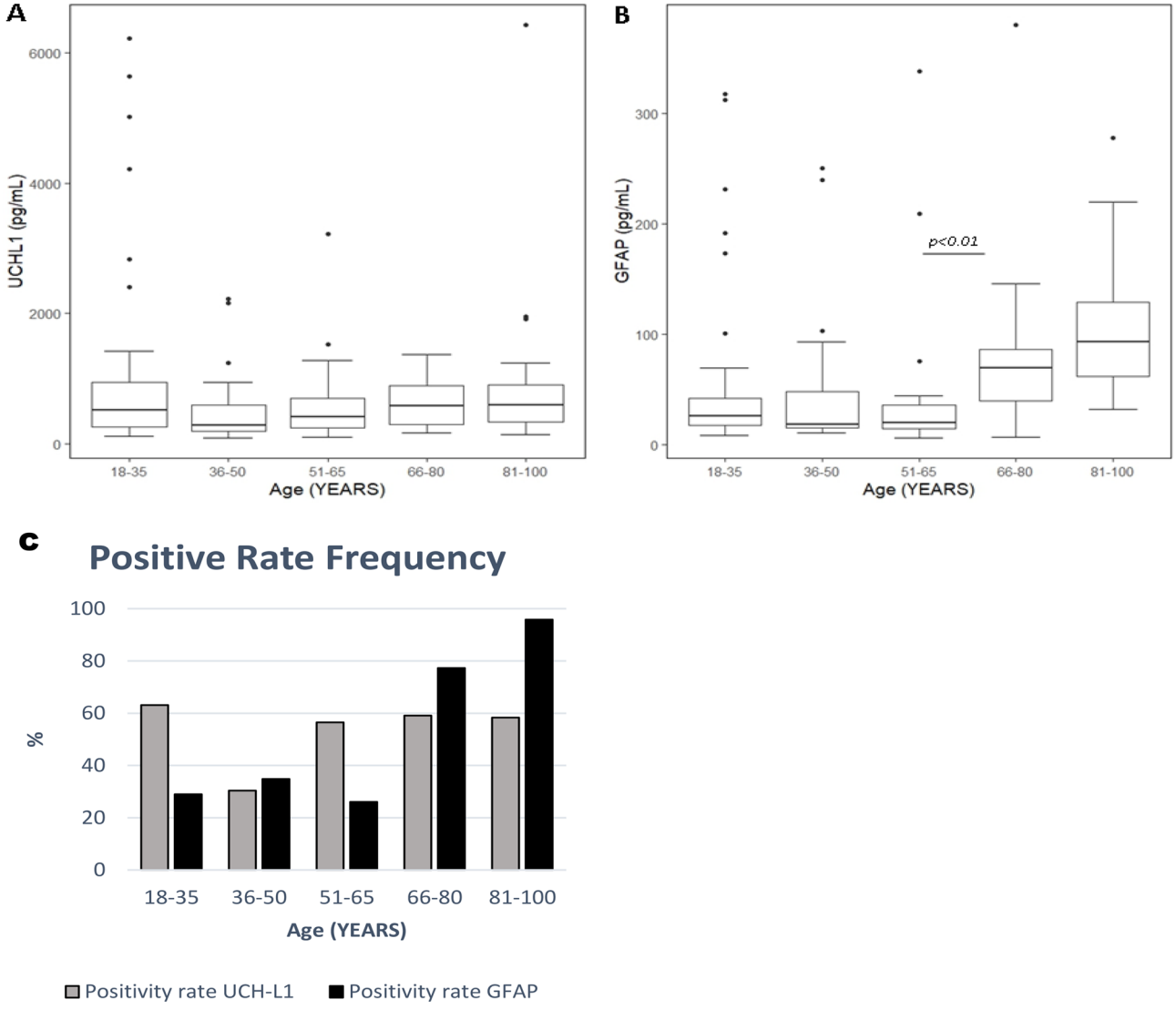


**Fig. 2** Age and biomarkers expression. Box-and-whiskers plot of UCH-L1 expressed in pg/mL (**A**), and GFAP expressed in pg/mL (**B**) at different age clusters as shown in picture. Box plots indicate the median and interquartile range (IQR); the whiskers represent 1.5 times the IQR. Significant p-values are indicated over the brackets. Histogram represents percentual positive rate in indicated age clusters (**C**)

18 to 100 years. On the contrary, a significant shift up was recorded to GFAP in association with older age clusters, particularly starting from 65 years of age ($p<0.01$), (Fig. 2B), where most of the population (87%) was far over the positivity cut-off (Fig. 2C).

We also examined the characteristics of positive CT scan patients, which are reported in Table 2. All seven patients were aged from 26 to 85 years, and all had GCS 15; furthermore, 57% were males, and 43% females and reported different injuries such as subarachnoid hemorrhage (SAH), comminuted skull fractures (CSF), subdural hemorrhage (SDH), Intracerebral hemorrhage (ICH), and subgaleal hemorrhage (SGH). All these patients were positive for the mTBI serum test, and 71% were overexpressing both biomarkers. In particular, the highest UCH-L1 levels were more than two times the cut-off (991 pg/mL) in a female patient with intracranial hemorrhage, and the highest GFAP level was measured more than ten times the cut-off in a male patient (380,3 pg/mL) who had a subdural hemorrhage. Interestingly, this patient reported the highest biomarker positivity combination with UCH-L1, measuring 514,3 pg/mL.

We investigated mTBI assay and CT availability time-gaps from clinical request to laboratory results. Reported data (Fig. 3) show that serological results are expected to be released within 60 min from the clinical request compared to two hours for CT execution and medical report release.

**Table 2** Positive CT scan patient characteristics

| Gender | Age (years) | Trauma Cause | GCS Score | NEURO | CANCER | CKD | CARDIO | HBP | DIAB | APT | UCH-L1 (pg/mL) | GFAP (pg/mL) | Head CT Outcome |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| M | 26 | MVA | 15 | NO | NO | NO | NO | NO | NO | NO | 901,9 | 20,5 | SAH |
| M | 32 | Generic/Fall | 15 | NO | NO | NO | NO | NO | NO | NO | 435,2 | 28,1 | CFS |
| M | 48 | Aggression | 15 | NO | NO | NO | NO | NO | NO | NO | 227,8 | 239,8 | CFS |
| F | 73 | Syncope | 15 | NO | NO | NO | NO | NO | NO | NO | 514,3 | 380,3 | SDH |
| M | 75 | MVA | 15 | NO | NO | NO | NO | NO | NO | NO | 991 | 43 | ICH |
| F | 83 | Generic/Fall | 15 | NO | NO | NO | NO | NO | YES | YES | 461,8 | 128,4 | SGH |
| F | 85 | MVA | 15 | NO | NO | NO | YES | YES | YES | YES | 726,7 | 98,9 | ICH |

NEURO = Neurological disease; CANCER = Cancer disease; CKD = Chronic kidney disease; CARDIO = Cardiac disease; HBP = High Blood Pressure; DIAB = Diabetes; APT = Antiplatelet Therapy; MVA = Motor Vehicle Accident; SAH = Subarachnoid Hemorrhage; CSF = Comminuted Skull Fractures; SDH = Subdural Hemorrhage; ICH = Intracerebral Hemorrhage; SGH = Subgaleal Hemorrhage

## Discussion

In this evaluation, we focused on GFAP and UCHL1 combined use evaluation for CT exclusion in a cohort of 130 patients with mTBI.

The reference paper about the combined use of GFAP and UCHL1 biomarkers in mild TBI does not consider the presence of comorbidities that can often occur in exposed patients presenting to emergency departments [8]. Previous neurological issues in our study do not appear significantly associated with mTBI outcomes, in contrast to the literature suggesting patients with pre-existing neurological problems may have an increased risk of brain injuries following cranial trauma [9]. Notably, we observed that 81.8% of all patients with previous neurological problems, who tested negative CT outcomes, reported positivity for GFAP, and 50% also for UCH-L1, with values at least double the cutoff (data not shown). Despite the lack of statistical significance, probably due to the limited sample size, considering that GFAP and UCH-L1 are indicators of brain injuries, this observation raises questions about the suitability of using these biomarkers in patients with pre-existing neurological issues after cranial trauma. Indeed, very recent papers indicate GFAP as a promising progression marker in Alzheimer's disease [10].

Among the considered comorbidities, we observed only a higher rate in subjects with hypertension. Previous reports showed that hypertension may increase the risk of complications following cranial trauma, such as cerebral edema or intracranial bleeding [11].

The use of antiplatelet agents has been a subject of debate concerning this status as a risk factor for intracranial hemorrhage (ICH) and positive anomalies in CT scans. Indeed, some recent systematic meta-analyses reported that antiplatelet therapy was associated with a very low risk of related delayed intracranial bleeding [12] We did not observe any statistically significant differences in biomarker levels in antiplatelet therapy patients. Moreover, in line with our observation, more recent studies suggest that prior use of antiplatelet and anticoagulant medications seems not to relate to GFAP and UCHL1 levels and mild trauma complications [13].

Analyzing the overexpression of biomarkers among patients who reported a negative CT scan for brain injuries, we observed that both biomarkers were modulated in most patients As expected, the average concentrations of the single biomarkers GFAP and UCH-L1 were higher with combined test positivity. In the study population, the average value of GFAP in patients with overexpression was 83.75 pg/mL, more than double the suggested threshold. For UCH-L1, the level was about three times the indicated cut-off (1015.70 pg/mL), in line with other studies suggesting that UCH-L1 would have more significant overexpression in the early stages acting as an

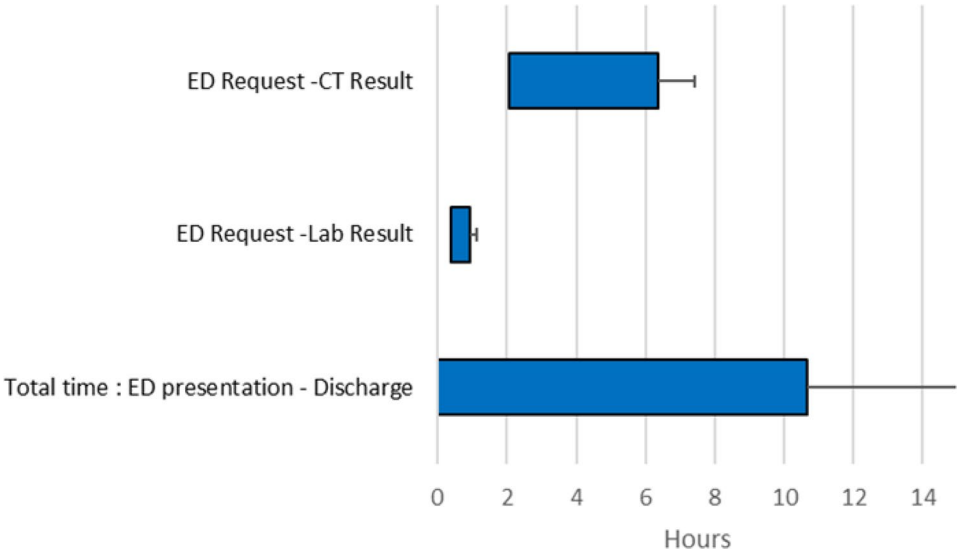


**Fig. 3** Time gap analysis. The lower end of histograms represents the minimal time difference (in hours) from patient ED presentation time (0 h). The higher end of the histograms represents the median time. The upper whiskers represent the third quartile of the distribution

acute-phase biomarker [6]. We found a relative specificity of 27,6%. Among recent evidence, Yue et al. [14]. showed that following a mild traumatic brain injury, a good percentage of TBI test positives correlated with magnetic resonance imaging (MRI) positive neuroaxonal damage, which was not revealed by a CT scan. This suggests that GFAP and UCH-L1 biomarkers are more sensitive to possible subclinical damage unrelated to CT scan positivity. Consequently, we might hypothesize that test specificity could have been higher using MRI instead of a CT scan for the study.

On the other hand, in line with Bazarian et al. [7], we found a remarkably high sensitivity (and related NPV of 100%), thus suggesting the combined use for CT exclusion.

While no significant differences were found ($p>0.05$) when comparing mTBI biomarker expression by gender (data not shown) in line with a previous report [15] we found that the mTBI test-positive population was significantly older than the negative one. Unfortunately, due to the low number of patients with positive CT outcomes, we cannot conclusively assert whether age represents a risk factor for CT outcomes. On the other hand, we found a positive correlation only between GFAP levels and age after 65 years, resulting in an increased ratio of positive TBI test results measured between 75% and 95% in age clusters 65–80 and 81–100, respectively. This result is in line with the recent literature indicating age as a possible confounding factor for GFAP and UCHL1 [16] Even though we cannot explain if it is due to physiological pathways or geriatric age-associated neurological diseases, we do not believe that this finding is impacting our aim since the Canadian CT Head Rule (CCHR)- based guidelines [17] states that being 65 years old or older is a decision criterion for avoiding the exclusion of CT scans in these patients.

We also investigated patients with positive CT scans, looking for correlations between injury and TBI test expression. As expected, all the patients were found positive for the combined test and mostly for both biomarkers. Nevertheless, assessing a common threshold for CT positivity was impossible, and different ranges of values were associated with different injuries and even with the same kind of injury. A recent systematic review suggested an optimal threshold for GFAP at 626 pg/mL in this regard [18]. In our case, GFAP levels in correspondence to subarachnoid hemorrhage, comminuted skull fractures, subdural hemorrhage, intracerebral hemorrhage, and subgaleal hematoma reached a maximum value of 380,3 pg/mL, suggesting that such a cutoff can vary and that further specific studies are required.

In the absence of dedicated studies on turnaround time, our simulation of triage for mild traumatic brain injury has revealed the potential to obtain biomarker results before the execution of a computed tomography scan. This insight contributes to the broader possibility of refining triage protocols for mild traumatic brain injury, enhancing efficiency, especially in terms of excluding patients with negative biomarker values, as demonstrated by the high NPV. Indeed, having the test results quickly and before the actual CT scan request is in line with our purpose of using this test as decision support for CT scan exclusion.

Even though our pilot study suggests interesting results, it is also **burdened by some limits**. Indeed, the number of patients is low and needs to be increased, possibly through multicentric studies. In addition, the small number of patients with positive CT scans and the

differences in found injuries do not allow definitive conclusions about the overexpression of the biomarkers and the CT scan positive outcome, limiting evaluating extensions to the routine clinical use of these combined biomarkers as positive CT scan predictors.

However, in conclusion, our results affirm the functional efficacy of the tool for the intended purpose; in fact, these combined biomarkers show actual effectiveness in predicting the lack of brain injuries in patients affected by mTBI in the emergency department, as confirmed by their high NPV, which together with the execution speed, represent two optimal features in a clinical setting where the quick patients rule-out is a key-point in the daily challenge to the ED overcrowding.

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**Author contributions**
J.M.L and M.M. conceptualized the study and edited the manuscript; M.B, A.G., A.A., D.B., L.B. and A.M.DeA. performed experiments and analyzed results; A.T. and M.P. analyzed data; E.N. and M.C. , V.N.DiL. and C.P. visioned and edited the manuscript; G.F. and S.L. visioned the manuscript; S.B. supervisioned the study and the manuscript.

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No funds were obtained for this study.

**Data availability**
No datasets were generated or analysed during the current study.

## Declarations

**Ethical approval**
The study protocol was approved (approval number 15/22) by the local Ethics Committee of the Tor Vergata University Hospital (Rome, Italy). The authors certify that the study was performed with ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards. Consent declarations were not applicable since in this retrospective study residual sample volumes from routine blood tests were used and all clinical data were retrieved from clinical reports.

**Consent to participate**
Not applicable. See "Ethical approval".

**Consent for publication**
Not applicable.

**Competing interests**
The authors declare no competing interests.

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## Publisher's note