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Serum GFAP and S100B in Older Adults With Mild Traumatic Brain Injury: A Diagnostic Accuracy Study

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Research article

Title: Serum GFAP and S100B in Older Adults With Mild Traumatic Brain Injury: A Diagnostic Accuracy Study

Running title: GFAP and S100B for Intracranial Injury Detection

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Abstract

Purpose: Blood biomarkers may reduce unnecessary computed tomography (CT) in mild traumatic brain injury (mTBI), but their value in older patients remains uncertain. We evaluated glial fibrillary acidic protein (GFAP) and S100B for detecting traumatic intracranial lesions (TILs) in a predominantly elderly cohort with frequent antithrombotic therapy.

Methods: This prospective observational diagnostic accuracy study included 98 adults with mTBI (Glasgow Coma Scale score 14–15; all included patients scored 15) presenting to a tertiary care emergency department between June and December 2025. Head CT served as the reference standard. GFAP was measured within 12 hours of injury (n=92) and S100B within 6 hours (n=89). Sensitivity, specificity and ROC analysis were calculated.

Results: The mean age was 78.9 years, 51.0% of patients received antiplatelet or anticoagulant therapy, and 9.2% had TILs. At the prespecified thresholds, GFAP >0.15 ng/mL showed a sensitivity of 62.5%, specificity of 60.7% and negative predictive value (NPV) of 94.4%, whereas S100B >0.105 µg/L showed 77.8%, 28.8% and 92.0%. The area under the curve was 0.670 for GFAP and 0.556 for S100B; both 95% confidence intervals included 0.5. An exploratory GFAP threshold of 0.10 ng/mL, derived from the same cohort, identified all eight lesions with available GFAP (sensitivity 100%, 95% CI 63.1–100; specificity 33.3%) and could have avoided 30.4% of CT scans, but requires external validation.

Conclusion: Neither biomarker was accurate enough to safely rule out intracranial injury at the prespecified thresholds. The exploratory GFAP threshold is hypothesis-generating and warrants validation in larger multicenter studies.

Keywords: Mild traumatic brain injury; GFAP; S100B; Head CT; Older adults; Biomarkers

Introduction

Mild traumatic brain injury (mTBI) is one of the leading causes of emergency department (ED) visits, accounting for approximately 90% of all traumatic brain injuries managed in this setting [1, 2]. Although most patients have a favorable clinical course, a proportion may develop traumatic intracranial lesions (TILs), necessitating a diagnostic strategy capable of safely identifying patients who require neuroimaging. Conventional management has historically

relied on head computed tomography (CT) to rule out TILs [3, 4]. However, current evidence indicates that fewer than 10% of CT scans performed according to standard protocols reveal acute pathological findings [3]. This high number of negative scans not only results in unnecessary exposure to ionizing radiation and a substantial increase in healthcare costs but also contributes significantly to overcrowding and resource utilization in EDs [2, 3].

In this context, blood biomarkers of brain injury have emerged as a promising strategy to improve diagnosis. Among these, S100 calcium-binding protein B (S100B) and glial fibrillary acidic protein (GFAP) are two of the most extensively studied biomarkers in mTBI.

S100B is expressed mainly by astrocytes and other glial cells in the central nervous system (CNS). However, it is also found in extracranial tissues such as bone and adipose tissue, which may limit its specificity for brain injury [5]. Its rapid release and clearance (<6 hours) allow early detection after injury making it useful for ruling out TILs when measured within an appropriate time window [5, 6]. Nevertheless, its limited specificity constitutes one of its main restrictions for clinical application, particularly in patients with characteristics that may affect baseline S100B concentrations.

By contrast, GFAP may represent a more specific alternative for CNS injury. It is a cytoskeletal filament protein of CNS astroglial cells, which may confer greater specificity for CNS injury and provide a wider diagnostic window of up to 12 hours after trauma [7, 8].

The combination of GFAP and ubiquitin C-terminal hydrolase L1 (UCH-L1) has been evaluated in a large international multicenter cohort [8], and S100B is incorporated into Scandinavian guidelines [9]. However, the applicability of these findings to populations with heterogeneous demographic and clinical characteristics remains less studied. This study aimed to evaluate these biomarkers in our population, which is characterized by advanced age and a high prevalence of antithrombotic therapy, factors that complicate clinical decision-making and could affect biomarker performance. Despite the availability of robust evidence, uncertainty remains regarding their diagnostic performance in older patients. Age may influence the concentrations of certain biomarkers, and a progressive decline in the specificity of S100B with increasing age has been reported [5, 6]. Moreover, the high prevalence of antithrombotic therapy in this population adds further complexity because of its association with an increased risk of hemorrhagic complications.

The aim of this study was to evaluate the diagnostic performance of S100B and GFAP for the detection of TILs in patients with mTBI presenting to our ED, which serves a population with a high proportion of older patients and a high prevalence of antithrombotic therapy.

Materials and Methods

Study Design and Population

A prospective observational cohort study was conducted in the ED of Hospital Universitari Parc Taulí in Sabadell, Spain. Between June and December 2025, we consecutively included adult patients aged ≥18 years with a Glasgow Coma Scale (GCS) score of 14–15 (all included patients ultimately scored 15) who were treated within the first 12 hours after trauma and underwent a cranial CT scan according to the institutional protocol.

The sample size was calculated to detect a difference of at least 5 units, assuming a common standard deviation of 20, a two-sided alpha of 0.05, a statistical power above 0.80 and a 10%

rate of losses, which yielded 153 patients. This calculation was not based on diagnostic accuracy parameters such as the expected sensitivity or the prevalence of intracranial lesions. The study should therefore be considered exploratory, and the precision of the estimates is limited by the small number of intracranial lesions. Recruitment was stopped when the study reagents expired, before the target sample size could be reached (98 of 153 planned patients, 64%). Recruitment took place between June and December 2025, and 98 patients with an available CT scan were ultimately included in the study. GFAP and S100B measurements were available for 92 and 89 patients, respectively, resulting in different denominators across analyses.

The exclusion criteria were age less than 18 years, presentation to the ED more than 12 hours after the mTBI, moderate-to-severe TBI or need for immediate surgical intervention. The number of patients assessed for eligibility and of those excluded was not recorded.

Biomarker Measurements and Cutoffs

Biomarker concentrations were measured using electrochemiluminescence immunoassays (ECLIA) with the S100 assay (CE-marked in vitro diagnostic [CE-IVD]) and GFAP assay (Research Use Only [RUO]) from Roche Diagnostics, processed on a cobas pro e 801 analyzer. Blood samples were centrifuged on the day of collection, and the serum was frozen at −20 °C and analyzed in weekly batches. Biomarker results were not made available to the treating clinicians at any time. S100B concentrations were expressed in µg/L and GFAP concentrations in ng/mL. Throughout the entire study period, the different assays and analyzers were subjected to strict quality evaluation criteria to guarantee the analytical quality of the results. Internal quality controls were processed on a daily basis.

S100B was measured within 6 hours of injury in accordance with the Scandinavian Neurotrauma Committee guidelines [9]. The cutoff for a positive result was 0.105 µg/L, as specified by the manufacturer. Patients who presented more than 6 hours after injury were not eligible for S100B measurement (n = 9).

GFAP was measured within 12 hours of injury, and two thresholds were evaluated. The primary analysis used the prespecified laboratory threshold of 0.15 ng/mL. An additional proposed threshold of 0.10 ng/mL was evaluated in an exploratory analysis. This threshold was obtained by rounding the optimal cutoff derived from the receiver operating characteristic (ROC) curve for the study cohort (0.1002 ng/mL). In both cases, any value strictly above the cutoff was considered positive. GFAP could not be measured in six patients because no serum sample was available within the predefined post-injury time window.

The reference standard was the presence or absence of a traumatic intracranial lesion confirmed by head CT. A traumatic intracranial lesion was defined as the presence on head CT of any of the following: subarachnoid, subdural, epidural or intraparenchymal hemorrhage, cerebral contusion, or a fracture with intracranial involvement. Head CT scans were interpreted by radiologists who were blinded to the biomarker results.

The study is reported in accordance with the Standards for Reporting Diagnostic Accuracy Studies (STARD) guidelines [10]. The protocol was designed in accordance with the International Council for Harmonisation Good Clinical Practice standards [11].

Statistical Analysis

Continuous variables were summarized as means and standard deviations and as medians and interquartile ranges, as appropriate, whereas categorical variables were summarized as absolute frequencies and percentages. Normality of S100B and GFAP concentrations was assessed using the Shapiro–Wilk test when fewer than 30 complete observations were available and the Lilliefors test when 30 or more complete observations were available. Biomarker concentrations were compared between patients with negative and positive computed tomography (CT) findings using the independent-samples t-test when the data were normally distributed and the Mann–Whitney U test otherwise.

For the diagnostic accuracy analyses, biomarker results were classified as true positives (TP), false positives (FP), false negatives (FN), or true negatives (TN), using CT findings as the reference standard. The diagnostic performance of the dichotomized biomarkers was evaluated in terms of sensitivity, specificity, positive predictive value, negative predictive value, and accuracy. These measures were reported with exact 95% binomial confidence intervals calculated using the Clopper–Pearson method.

For GFAP, receiver operating characteristic (ROC) curve analysis was performed. The area under the ROC curve (AUC) was estimated with its 95% confidence interval calculated using DeLong's method, and the optimal cutoff value was defined as the value that maximized the Youden index, calculated as sensitivity + specificity − 1.

The number of CT scans that could potentially have been avoided based on the biomarker results was also estimated. Potentially avoidable CT scans were defined as true-negative cases, namely patients with a negative biomarker result and no pathological findings on CT. The proportion of potentially avoidable CT scans was calculated as the number of true-negative cases divided by the total number of patients with an available measurement for the corresponding biomarker (TN/n). Diagnostic performance and potentially avoidable CT scans were evaluated among all patients with an available biomarker measurement: 92 patients for GFAP and 89 patients for S100B.

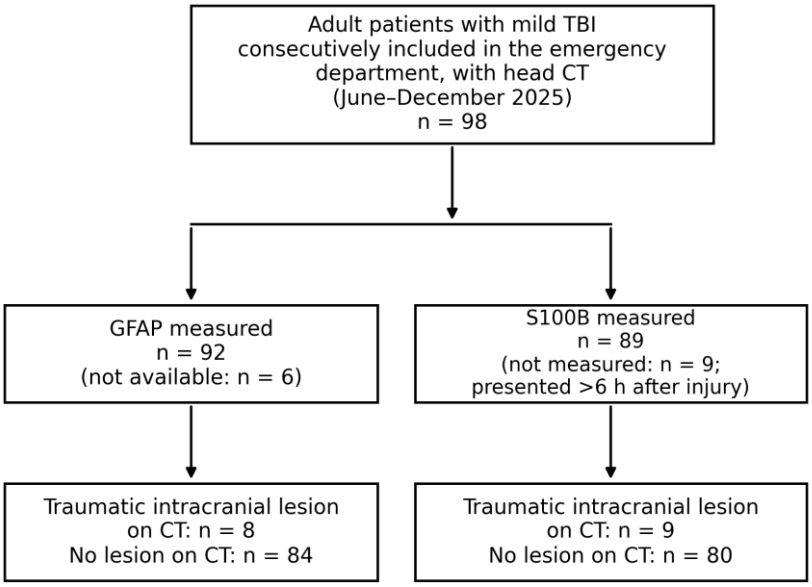
Analyses were conducted using complete cases, with no imputation of missing data. The number of observations included in each analysis was therefore determined by the availability of the variables required for that analysis. All tests were two-sided, and a p-value ≤ 0.05 was considered statistically significant. No adjustment was made for multiple comparisons. All statistical analyses were performed using R software, version 4.5.0.

Results

Cohort Characteristics

A total of 98 patients were analyzed (Fig. 1). The mean age was 78.9 years (standard deviation [SD], 13.4), and the median age was 83 years (interquartile range [IQR], 74.3–87.0; range, 33–100), showing a left-skewed distribution. Women accounted for 64.3% (n=63) and men for 35.7% (n=35). A total of 50 patients (51.0%) were receiving antiplatelet or anticoagulant therapy:

acetylsalicylic acid (n = 20), apixaban (n = 13), edoxaban (n = 5), rivaroxaban (n = 5), clopidogrel (n = 3), acenocoumarol (n = 3), or dabigatran (n = 1). Loss of consciousness was recorded in 12 patients (12.2%), and all patients had a GCS score of 15. Eighty-nine patients (90.8%) were evaluated within 6 hours of injury and nine (9.2%) between 6 and 12 hours. Cranial CT scan showed no traumatic intracranial lesions in 89 patients (90.8%), and traumatic intracranial lesions were detected in 9 patients (9.2%).



TBI, traumatic brain injury; CT, computed tomography; GFAP, glial fibrillary acidic protein.

Fig. 1 Flow diagram of the study participants and the biomarker measurements available (n = 98)

Biomarker Distributions

Both biomarkers showed markedly asymmetrical distributions. The median GFAP concentration was 0.13 ng/mL (IQR, 0.09–0.19; mean, 0.166; range, 0.015–0.948; skewness, 2.81; n = 92). For S100B the median concentration was 0.15 µg/L (IQR, 0.10–0.30; mean, 0.239; range, 0.032–1.490; skewness, 2.55; n = 89).

Using the prespecified thresholds, S100B concentrations were positive (>0.105 µg/L) in 64 of 89 patients (71.9%), whereas GFAP concentrations were positive (>0.15 ng/mL) in 38 of 92 patients (41.3%).

Diagnostic Performance at the Prespecified Thresholds

The diagnostic performance of both biomarkers, using head CT as the reference standard and the prespecified thresholds, is summarized in Table 1.

Table 1. Diagnostic performance of GFAP and S100B at the prespecified thresholds (95% confidence intervals)

Parameter	GFAP >0.15 ng/mL	S100B >0.105 µg/L
Patients analyzed, n	92	89
Patients with traumatic intracranial lesions on CT, n	8	9
Sensitivity	62.5% (24.5–91.5)	77.8% (40.0–97.2)
Specificity	60.7% (49.5–71.2)	28.8% (19.2–40.0)
PPV	13.2% (4.4–28.1)	10.9% (4.5–21.3)
NPV	94.4% (84.6–98.8)	92.0% (74.0–99.0)
Accuracy	60.9% (50.1–70.9)	33.7% (24.0–44.5)
Potentially avoidable CT scans (TN/n)	51/92 (55.4%)	23/89 (25.8%)
p value, Mann–Whitney U test	0.1158	0.5909

GFAP, glial fibrillary acidic protein; S100B, S100 calcium-binding protein B; NPV, negative predictive value; PPV, positive predictive value; CT, computed tomography; TN, true negative. Potentially avoidable CT scans were defined as true-negative cases, that is, patients with a negative biomarker result and no traumatic intracranial lesions on CT, divided by the total number of patients with an available biomarker measurement.

ROC Curve Analysis and the Proposed Threshold for Older Patients

ROC curve analysis yielded an area under the curve (AUC) of 0.670 for GFAP (95% CI, 0.483–0.856). The ROC curve is shown in Fig. 2. Because this confidence interval included 0.5, the absence of discriminatory ability could not be ruled out. For comparison, the AUC for S100B was 0.556 (95% CI, 0.364–0.747), with a confidence interval that also included 0.5.

The optimal GFAP cutoff identified in the study cohort was 0.1002 ng/mL. This value was rounded to 0.10 ng/mL as the proposed threshold. Table 2 compares the diagnostic performance of the prespecified and proposed thresholds.

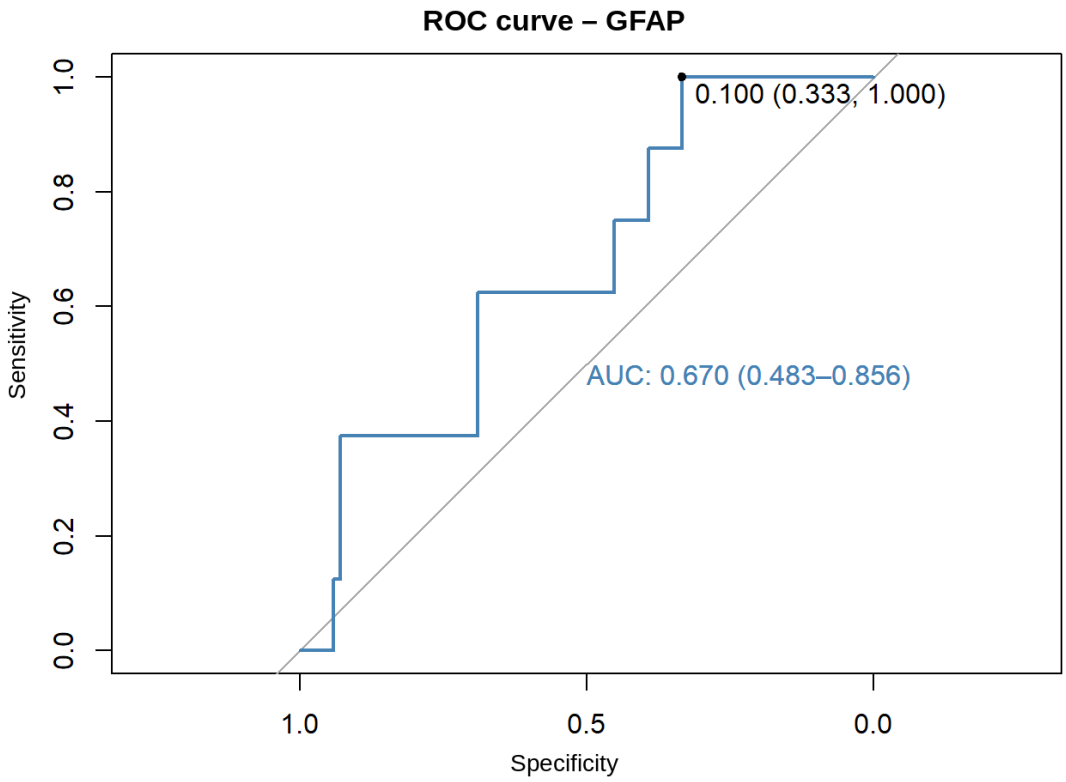


Fig. 2 Receiver operating characteristic (ROC) curve of serum GFAP for detecting traumatic intracranial lesions on head CT (n = 92; 8 lesions). The area under the curve was 0.670 (95% CI, 0.483–0.856). The dot marks the optimal cut-off in the study cohort (0.10 ng/mL; specificity 0.333, sensitivity 1.000)

Table 2. Comparison of the prespecified and proposed GFAP thresholds (n = 92; 95% confidence intervals)

Parameter	GFAP >0.15 ng/mL	GFAP >0.10 ng/mL
Sensitivity	62.5% (24.5–91.5)	100% (63.1–100)
Specificity	60.7% (49.5–71.2)	33.3% (23.4–44.5)
PPV	13.2% (4.4–28.1)	12.5% (5.6–23.2)
NPV	94.4% (84.6–98.8)	100% (87.7–100)
Accuracy	60.9% (50.1–70.9)	39.1% (29.1–49.9)
Missed lesions	3/8	0/8
Potentially avoidable CT scans (TN/n)	51/92 (55.4%)	28/92 (30.4%)

GFAP, glial fibrillary acidic protein; NPV, negative predictive value; PPV, positive predictive value; TN, true negative. All metrics, including potentially avoidable CT scans, were calculated for the 92 patients with available GFAP measurements. The threshold of 0.10 ng/mL was derived from

the same cohort; therefore, its diagnostic performance should be considered exploratory and does not constitute independent validation.

Lowering the GFAP threshold from 0.15 to 0.10 ng/mL increased sensitivity from 62.5% to 100% and the NPV from 94.4% to 100%, while reducing specificity from 60.7% to 33.3%. This increase in sensitivity was accompanied by a substantial reduction in the proportion of potentially avoidable CT scans, from 51 to 28 scans, corresponding to a decrease from 55.4% to 30.4% among the 92 patients with available GFAP measurements. If all biomarker-negative patients, including false negatives, were counted, the proportions would be 54/92 (58.7%) for GFAP >0.15 ng/mL, 28/92 (30.4%) for GFAP >0.10 ng/mL and 25/89 (28.1%) for S100B >0.105 µg/L.

Importantly, applying the 0.15 ng/mL threshold would have classified 51 cases as true negatives but would also have missed three patients with traumatic intracranial lesions. Therefore, the estimated 55.4% reduction in CT use would not represent a clinically acceptable imaging-reduction strategy.

False-Negative Results

At the prespecified threshold of 0.15 ng/mL, GFAP failed to identify 3 of the 8 traumatic intracranial lesions among patients with an available measurement. GFAP concentrations in all three patients were above 0.10 ng/mL; therefore, the proposed threshold would have correctly identified these cases.

At the 0.105 µg/L threshold, S100B failed to identify 2 of the 9 lesions. One was a thin right tentorial and posterior interhemispheric subdural hematoma in a 76-year-old woman anticoagulated with apixaban, whose S100B concentration was 0.063 µg/L. The other consisted of foci of subarachnoid hemorrhage in the left anterior basal temporal region in an 87-year-old woman, whose S100B concentration was 0.081 µg/L. Both lesions were small, had no mass effect, and remained stable or showed regression on follow-up CT.

The three lesions missed by GFAP consisted of thin subdural collections or foci of subarachnoid hemorrhage. Both patients with false-negative S100B results were also among these three cases; thus, the same lesions were missed by both biomarkers. Table 3 shows the cross-tabulation of each biomarker result against the reference standard.

Table 3. Cross-tabulation of biomarker results against head CT findings (reference standard)

Biomarker result	**TIL on CT, n**	**No TIL on CT, n**	**Total, n**
GFAP >0.15 ng/mL (prespecified threshold; n = 92)			
Positive	5	33	38
Negative	3	51	54

Total	*8*	*84*	*92*
GFAP >0.10 ng/mL (exploratory threshold; n = 92)			
Positive	8	56	64
Negative	0	28	28
Total	*8*	*84*	*92*
S100B >0.105 µg/L (manufacturer threshold; n = 89)			
Positive	7	57	64
Negative	2	23	25
Total	*9*	*80*	*89*

CT, computed tomography; GFAP, glial fibrillary acidic protein; S100B, S100 calcium-binding protein B; TIL, traumatic intracranial lesion. A result was considered positive when the biomarker concentration was strictly above the threshold. In each block, the four cells correspond to true positives (positive, TIL on CT), false positives (positive, no TIL on CT), false negatives (negative, TIL on CT) and true negatives (negative, no TIL on CT). The 0.10 ng/mL GFAP threshold was derived from the study cohort and is exploratory. GFAP was unavailable for 6 patients (1 with a TIL on CT) and S100B for 9 patients (none with a TIL on CT).

Subgroup Receiving Antithrombotic Therapy

Among patients receiving anticoagulant or antiplatelet therapy (n = 50), GFAP measurements were available for 48 patients and S100B measurements for 45. Traumatic intracranial lesions were identified on CT in 3 patients.

At the proposed threshold of 0.10 ng/mL, all three patients with traumatic intracranial lesions had GFAP concentrations above the cutoff (0.112, 0.165, and 0.426 ng/mL), corresponding to an NPV of 100% in this subgroup. At the threshold of 0.15 ng/mL, one of the three lesions would have been missed. The small number of events precluded firm conclusions from this subgroup analysis.

Discussion

The diagnostic performance observed in our cohort was lower than that reported in the ALERT-TBI multicenter study [8], which evaluated the combination of GFAP and UCH-L1 with different assays and in a different population. Neither biomarker was significantly associated with head CT findings in our cohort, and the confidence intervals for sensitivity were wide for both biomarkers, a direct consequence of the small number of events (9 patients with traumatic intracranial lesions on CT).

The prevalence of traumatic intracranial lesions in our cohort was 9.2%, which is consistent with previous reports. In the meta-analysis by Yang et al. [14], CT-confirmed traumatic intracranial hemorrhage was identified in 7.9% of the 26,040 patients analyzed. Nevertheless, differences in population characteristics, definitions of traumatic intracranial injury, and imaging criteria should be considered when comparing prevalence estimates across studies.

GFAP showed a numerically higher AUC than S100B, although this difference was not formally tested: the AUC was 0.670 (95% CI, 0.483–0.856) compared with 0.556 (95% CI, 0.364–0.747) for S100B. No formal statistical comparison between the two AUCs was performed, and the analyses had different denominators (92 and 89 patients) and different sampling windows (12 and 6 hours). This direction is consistent with previous studies reporting better diagnostic performance for GFAP than for S100B [15]. However, the confidence intervals for both AUC estimates included 0.5, and differences between the populations studied limit direct comparisons.

An important finding of this study was that the prespecified laboratory GFAP threshold of 0.15 ng/mL did not provide adequate safety in our population. At this threshold, GFAP failed to identify 3 of the 8 assessable traumatic intracranial lesions and yielded an NPV of 94.4%, rather than 100%. Clinically, a protocol based on this threshold would have incorrectly classified three patients with traumatic intracranial lesions as biomarker-negative and, therefore, as potential candidates for avoiding CT. Any reduction in imaging use must be balanced against the risk of missed intracranial lesions. On the basis of our results, the laboratory threshold of 0.15 ng/mL cannot be recommended as a stand-alone rule-out criterion in this setting.

When measured within 6 hours of injury, S100B showed a sensitivity of 77.8% and an NPV of 92.0%. These findings do not support the use of S100B as a stand-alone rule-out test. However, it is within the range reported in cohorts of older patients with a high prevalence of anticoagulant therapy, in whom S100B specificity is known to be low. The wide 95% CI for sensitivity (from 40.0% to 97.2%) reflects the small number of patients with traumatic intracranial lesions in the S100B cohort (n = 9).

The large number of false-positive S100B results (57 cases) limits the number of potentially avoidable CT scans to 23 of 89 (25.8%). This finding is consistent with previous evidence showing particularly low S100B specificity in older patients and in those with extracranial comorbidities or injuries that may increase baseline protein concentrations.

Lowering the GFAP threshold to 0.10 ng/mL resulted in a sensitivity and NPV of 100% in this cohort and would have allowed 28 of the 92 patients with available GFAP measurements to avoid CT, corresponding to 30.4%, without missing any traumatic intracranial lesions.

The potential reduction in imaging observed in our cohort falls within the range reported in the literature, which estimates that incorporating biomarkers into the management of mTBI may

reduce CT use by approximately 20–30% [16–18]. Our estimates of 25.8% for S100B and 30.4% for GFAP at the threshold of 0.10 ng/mL are consistent with this range. Nevertheless, the S100B-based figure was obtained with a strategy that missed 2 of 9 lesions and should not be interpreted as a safe reduction in imaging. However, this comparison requires an important qualification. The published figures come from S100B-based algorithms that have already been validated and implemented in clinical practice, whereas our estimate of 30.4% was obtained from an exploratory GFAP threshold obtained from the study sample itself.

The false-negative cases may also provide insight into the lower-than-expected performance of the biomarkers. The three lesions missed by GFAP at the threshold of 0.15 ng/mL consisted of thin subdural collections or small foci of subarachnoid hemorrhage. These were low-volume lesions without substantial mass effect and might plausibly result in less release of astroglial proteins into the bloodstream than larger or more destructive injuries. The two lesions missed by S100B were also among these three cases, indicating that the same low-volume lesions were simultaneously missed by both biomarkers. These findings suggest the possibility that lesion burden and injury pattern may influence circulating biomarker concentrations; our data cannot separate this effect from that of age. However, given the small number of false-negative cases, this biological interpretation should be regarded as hypothesis-generating.

Age-Adjusted Thresholds in Light of Our Findings

Current evidence generally supports increasing the GFAP threshold in older patients. Ladang et al. [19] reported that, when the manufacturer's cutoffs were used, the specificity of the combined GFAP/UCH-L1 test decreased from 40.6% overall to 14.9% in patients older than 65 years. They proposed a GFAP threshold of 115 ng/L (equivalent to 0.115 ng/mL) for this subgroup, which doubled the number of potentially avoidable CT scans without reducing sensitivity. Lapić et al. [20] similarly proposed age-adjusted cutoffs, with progressively higher thresholds from the age of 50 years onward.

Both studies used Abbott plasma immunoassays, which are not standardized against the Roche assay used in our study. Direct comparisons between their thresholds and ours should therefore be interpreted with caution.

The rationale for age-adjusted thresholds is supported by a well-documented phenomenon: baseline GFAP concentrations increase with age in healthy individuals. Tybirk et al. [21] established reference intervals for serum GFAP in 371 healthy adults aged 21–90 years and observed a progressive increase in both GFAP concentrations and their variability with advancing age. Lapić et al. [20] reported a similar pattern in their healthy controls, with an apparent inflection point at approximately 50 years of age.

In our cohort, however, higher thresholds missed lesions; this observation is based on only eight events, and our study was not designed to test age dependence. The lowest GFAP concentration among patients with traumatic intracranial lesions on CT was approximately 0.102 ng/mL. Applying the threshold of 0.115 ng/mL proposed for older patients would have missed two of the eight assessable lesions in our cohort, while a third patient had a GFAP concentration close to that threshold, at 0.120 ng/mL.

One possible explanation is that the proposed age-adjusted thresholds were derived from cohorts with different injury profiles. The three lesions missed in our cohort consisted of thin subdural collections and small foci of subarachnoid hemorrhage. These types of low-volume lesions are commonly observed in older patients receiving anticoagulant therapy after low-

energy falls and may be associated with relatively limited release of astroglial proteins. In cohorts containing a greater proportion of high-energy trauma or larger-volume lesions, increasing the GFAP threshold may have less effect on sensitivity.

If this hypothesis is confirmed, the optimal GFAP threshold may depend not only on age but also on the predominant injury mechanism, lesion type, and injury burden in the population being assessed. This interpretation remains speculative and requires evaluation in larger cohorts with sufficient representation of different lesion patterns.

Limitations

This study has several important limitations that should be considered when interpreting the findings.

First, the proposed GFAP threshold of 0.10 ng/mL was derived and evaluated in the same cohort, without internal or external validation. Its apparent diagnostic performance is therefore likely to be optimistic. Moreover, the safety margin was minimal: the lowest GFAP concentration among patients with traumatic intracranial lesions was approximately 0.102 ng/mL, only slightly above the proposed threshold. The estimated sensitivity of 100% was based on only eight events, and its 95% CI ranged from 63.1% to 100%. A single additional lesion in a patient with a lower GFAP concentration could substantially alter the estimated performance. Consequently, this finding should be regarded as a hypothesis-generating result, not a threshold validated for clinical use.

Second, the Roche Diagnostics GFAP assay used in this study was labeled for Research Use Only and did not have CE-IVD status or manufacturer-validated reference values for clinical diagnostic use. Any translation of these findings into clinical practice would require analytical validation of the assay by the clinical laboratory in accordance with ISO 15189:2022 and Regulation (EU) 2017/746 on in vitro diagnostic medical devices. Such validation should include assessments of precision, linearity, trueness, and analytical interference. Implementation would also require oversight by the clinical laboratory and ED leads, together with a standard operating procedure and appropriate staff training. In addition, samples were frozen and analyzed in weekly batches, so our results do not reflect the turnaround times or preanalytical conditions of real-time clinical use. The results are therefore not directly extrapolable to routine clinical practice, nor necessarily to other analytical platforms.

Third, the sample size of 98 patients was smaller than the 153 participants required according to the initial statistical power calculation. With only nine patients presenting traumatic intracranial lesions on CT, the confidence intervals were wide and the point estimates were unstable. In addition, the sample size calculation was not based on diagnostic accuracy parameters, so the study was not powered to estimate sensitivity or specificity precisely.

Fourth, biomarker data were missing for a relevant proportion of patients. GFAP could not be measured in 6 patients, and S100B could not be measured in 9, resulting in different denominators across analyses. This may have introduced bias if biomarker availability was not random. Notably, GFAP results were unavailable for one of the nine patients with a traumatic intracranial lesion on CT.

Fifth, the S100B and GFAP cohorts were not equivalent. S100B was measured in 89 patients within a maximum post-injury interval of 6 hours, whereas GFAP was measured in 92 patients within 12 hours. Direct comparisons of diagnostic performance between the biomarkers should

therefore be interpreted cautiously because their measurement windows, included patients, and denominators differed.

Sixth, this was a single-center study, and the cohort had a distinctive clinical profile, with a median age of 83 years and 51.0% of patients receiving antithrombotic therapy. This limits the generalizability of the results and may have contributed to the lower specificity compared with that reported in younger cohorts [19].

Seventh, all included patients had a GCS score of 15. The cohort did not include patients with a GCS score of 14; therefore, the findings cannot be extrapolated to the lower end of the mTBI severity spectrum.

Finally, head CT was used as the sole reference standard. This approach may fail to detect microstructural injuries that are visible only on magnetic resonance imaging. Such injuries could potentially account for a proportion of the apparently false-positive biomarker results and contribute to the observed limited specificity [22].

Larger multicenter prospective studies are needed to validate these findings and refine the optimal GFAP threshold for older patients in the Spanish population. The selective use of magnetic resonance imaging as an additional reference standard could also help determine whether some apparently false-positive biomarker results reflect brain injuries not visible on CT. Further research should additionally establish whether S100B retains a clinically useful role in this population.

Conclusions

In this prospective study of predominantly older patients with mild traumatic brain injury and a high prevalence of antithrombotic therapy, neither GFAP (>0.15 ng/mL) nor S100B (>0.105 µg/L) demonstrated sufficient diagnostic performance at prespecified thresholds to safely rule out traumatic intracranial lesions without head CT. The S100B results were inconclusive regarding sensitivity (77.8%; 95% CI, 40.0–97.2%; 9 events) and showed low specificity in this elderly setting. Conversely, although an exploratory GFAP threshold of 0.10 ng/mL achieved 100% sensitivity and negative predictive value—potentially avoiding 30.4% of CT scans—this cutoff was derived from a small number of events within the same cohort and should be considered strictly hypothesis-generating. In our cohort, GFAP thresholds above approximately 0.10 ng/mL missed low-volume hemorrhagic lesions, but this observation rests on only eight events; therefore, larger multicenter studies are warranted to establish and externally validate safe, clinically applicable biomarker cutoffs for this vulnerable population.

Statements and Declarations

Funding: Roche Diagnostics provided the S100B and GFAP reagents free of charge (in-kind support). No other funding was received for this study.

Ethics approval and consent to participate: The study was conducted in accordance with the principles of the Declaration of Helsinki and was approved by the Clinical Research Ethics Committee of the Institut d'Investigació i Innovació Parc Taulí (I3PT; CEIm [Research Ethics

Committee for Medicinal Products] approval code 20255011). All patients or their legal representatives provided written informed consent after receiving detailed information about the study. Data processing was conducted in strict accordance with the General Data Protection Regulation (GDPR) and the applicable Spanish Organic Law on Personal Data Protection and Guarantee of Digital Rights (LOPDGDD), ensuring the anonymization and confidentiality of clinical and biological data. The composition and procedures of the committee comply with Good Clinical Practice standards (CPMP/ICH/135/95) and current legislation.

Competing interests: Roche Diagnostics supplied the study reagents, as detailed under Funding. Roche Diagnostics had no role in the study design, data collection, analysis, interpretation of the results, manuscript preparation, or the decision to submit the manuscript for publication. The authors declare no other competing interests.

Data availability: the de-identified dataset generated and analysed during the study is available from the corresponding author on reasonable request.

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