Original Article

# Serum and CSF Biomarkers of Traumatic Brain Injury: Correlation with Radiological Severity

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## Abstract

**Background:** Traumatic brain injury (TBI) remained one of the major causes of neurological disability and death across all age groups. Although computed tomography (CT) scanning served as the gold standard for initial assessment, it often failed to reveal early biochemical alterations occurring within the brain tissue. Serum and cerebrospinal fluid (CSF) biomarkers such as neuron-specific enolase (NSE), S-100β, and glial fibrillary acidic protein (GFAP) were studied as potential indicators of neuronal and astrocytic damage, offering a biochemical correlate to radiological severity. The objective is to estimate serum and CSF levels of NSE, S-100β, and GFAP in patients with traumatic brain injury and to correlate these biomarker concentrations with CT-based severity and clinical grading. **Material and Methods:** This prospective observational study was carried out on 60 patients diagnosed with acute TBI admitted to a tertiary-care hospital. Serum and CSF samples were collected within 24 hours of injury and analysed using ELISA. Radiological grading was done using the Marshall CT classification, and neurological status was recorded with the Glasgow Coma Scale (GCS). Data were analysed using ANOVA and Pearson correlation tests. **Results:** The mean serum NSE levels were 18.4 ± 4.2 ng/mL in mild, 31.6 ± 6.7 ng/mL in moderate, and 52.3 ± 8.5 ng/mL in severe TBI cases ($p < 0.001$). Similarly, serum S-100β concentrations increased from 0.09 ± 0.03 µg/L in mild to 0.26 ± 0.05 µg/L in severe injuries ($p = 0.002$). CSF GFAP levels showed the strongest correlation with CT severity ($r = 0.84$, $p < 0.001$), with mean values of 0.38 ± 0.06 µg/L in mild and 1.12 ± 0.14 µg/L in severe TBI. Biomarker levels were inversely correlated with GCS scores ($r = –0.71$ for NSE, –0.68 for S-100β, and –0.79 for GFAP). Receiver operating characteristic (ROC) analysis revealed GFAP had the highest diagnostic accuracy (AUC = 0.92) in differentiating severe from mild–moderate cases. **Conclusion:** Serum and CSF concentrations of NSE, S-100β, and GFAP significantly reflected the degree of neuronal and glial injury and correlated strongly with radiological and clinical severity. The findings suggest that early biomarker assessment, when combined with CT imaging and GCS scoring, could improve prognostic evaluation and guide clinical decision-making in traumatic brain injury.

**Keywords:** Traumatic brain injury, cerebrospinal fluid, neuron-specific enolase, S-100β, GFAP, CT grading, biomarkers, prognosis.

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## Introduction

Traumatic brain injury (TBI) represented a global public-health challenge, accounting for substantial numbers of hospital admissions, long-term neurological sequelae, and socioeconomic burden.[1,2] The initial mechanical insult to the brain triggered a cascade of biochemical, cellular and vascular events that extended beyond the primary injury. This secondary injury phase included blood–brain barrier disruption, excitotoxicity, oxidative stress, axonal shearing, and glial activation.[3,4] Conventional neuroimaging and clinical scales (such as the Glasgow Coma Scale) provided essential assessment of gross structural damage and neurological status but lacked sensitivity for detecting the full extent of microstructural and molecular injury.[5]

In recent years, research shifted toward the measurement of biomarkers in peripheral body fluids (serum, plasma, cerebrospinal fluid) as adjuncts to imaging and clinical evaluation. Biomarkers of interest included those released from injured astrocytes (e.g., glial fibrillary acidic protein, GFAP; S100 calcium-binding protein B, S100B) and from injured neurons (e.g., neuron-specific enolase, NSE; ubiquitin carboxy-terminal hydrolase L1, UCH-L1).[6,7] The rationale was that astrocytic injury and blood–brain barrier permeability permitted release of these proteins into circulation, thereby offering temporal and quantitative insight into injury severity.[8] S100B was among the earliest widely studied markers and was proposed to assist in decisions regarding neuroimaging in mild TBI.[9] GFAP emerged more recently and appeared to have stronger associations with intracranial lesions detectable on CT

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and MRI.[10]

Studies had shown that elevated serum GFAP and S100B levels correlated with worse outcomes in TBI patients. For example, one observational cohort found that patients who died following TBI had median serum GFAP levels that were 33-fold higher and S100B levels 2-fold higher than survivors.[11] In addition, biomarker kinetics mattered: GFAP tended to peak later than some neuronal markers and remained elevated for longer, suggesting potential utility for monitoring evolving injury.[7] Despite this, significant heterogeneity existed in assay protocols, sampling times, and cut-off values, which impeded standardisation and clinical adoption.[12]

Within the context of neurosurgical and pathological evaluation, linking fluid biomarkers to radiological severity (e.g., CT classification) and to clinical grading offered promise for improved stratification of patients, guiding management and prognosis. The potential to correlate serum and CSF biomarkers with radiological grade—specifically CT evidence of intracranial haemorrhage, contusion, diffuse axonal injury—and correlate these with neurological status, had begun to gather evidence.[13] Such correlative studies were especially relevant to multidisciplinary teams, including pathology faculty analysing biomarker expression, as well as neurosurgeons and neuro-intensivists integrating imaging and biochemical data.

Nevertheless, gaps remained: many studies focused on mild TBI and blood biomarkers, rather than CSF; fewer studies examined the full spectrum of TBI severity; and fewer still correlated serum and CSF biomarkers simultaneously with standardized radiological grading and clinical scales. Given these considerations, the current investigation sought to measure selected biomarkers in serum and CSF of patients with acute TBI and correlate the levels with radiological severity (CT grading) and with clinical neurological assessment.

## Materials and Methods

**Study Design and Setting:** This prospective observational study was carried out in the Department of Neurology in collaboration with the Department of Neurosurgery at Kakatiya Medical College-Superspeciality Hospital (PMSSY), Hanumakonda, Telangana. The study duration extended over 18 months. Ethical approval was obtained from the Institutional Ethics Committee prior to patient recruitment. Written informed consent was obtained from all participants or their legally authorized representatives.

**Study Population**

**Inclusion criteria**

A total of sixty consecutive patients who presented to the emergency department with a clinical diagnosis of acute TBI were included in the study. All participants were selected according to predefined eligibility criteria to maintain uniformity and minimize bias. Only individuals between 18 and 65 years of age were recruited, as this age range represents the adult population most commonly affected by head trauma while excluding extremes of age where physiological variations could influence biomarker levels. Patients who reported to the hospital within 24 hours of sustaining the head injury were considered eligible, ensuring that biological samples reflected the acute phase of brain injury. Furthermore, inclusion was limited to cases in which both serum and CSF samples could be safely collected for biomarker analysis, allowing simultaneous evaluation of systemic and central biochemical responses. These selection parameters facilitated the formation of a well-defined and homogeneous study group suitable for correlating biochemical markers with the severity of traumatic brain injury.

**Exclusion criteria**

Patients with certain medical conditions or circumstances were excluded from the study to prevent confounding factors that could influence biomarker levels or clinical interpretation. Individuals with a known history of chronic neurological or psychiatric disorders were not considered, as pre-existing brain or mental health conditions might alter baseline neurochemical profiles. Those with significant extracranial injuries or polytrauma were also excluded to avoid systemic inflammatory responses that could interfere with biomarker specificity. Patients who had previously undergone neurosurgical procedures were omitted because prior surgical manipulation could affect cerebrospinal fluid composition and brain tissue integrity. Similarly, individuals suffering from hepatic, renal, or endocrine disorders known to modify protein metabolism or clearance were excluded to maintain the biochemical reliability of serum and CSF analyses. Finally, patients who were unwilling or unable to provide informed consent, or whose legal representatives could not consent on their behalf, were not enrolled in the study. These exclusion criteria ensured the selection of a homogenous study population in which the observed biomarker variations could be attributed primarily to traumatic brain injury.

**Clinical Assessment:** All patients underwent a thorough clinical assessment at the time of admission, which included a comprehensive neurological examination and evaluation of their level of consciousness using the GCS. Based on the GCS scores, patients were grouped into three categories representing the severity of head injury. Those with scores between 13 and 15 were classified as having mild traumatic brain injury, while scores ranging from 9 to 12 were considered moderate, and scores of 8 or below indicated severe TBI. In addition to neurological assessment, detailed documentation of vital parameters such as blood pressure, pulse rate, and respiratory rate was performed. Pupil size and reactivity to light were carefully observed as important indicators of neurological function. Other systemic parameters, including oxygen saturation and body temperature, were also recorded at presentation to obtain a complete baseline profile of each patient's physiological status before initiating further diagnostic and therapeutic measures.

**Radiological Evaluation:** Neuroimaging evaluation was carried out for all patients using non-contrast computed tomography (CT) of the head, performed within six hours of hospital admission to ensure early detection of intracranial abnormalities. The CT scans were independently reviewed by two experienced radiologists who were blinded to the patients' clinical status and biomarker findings to eliminate observer bias. The extent and pattern of brain injury were classified according to the Marshall CT Classification System, which stratifies lesions into categories ranging from diffuse injury types I to IV, evacuated mass lesions,

and non-evacuated mass lesions. This standardized classification allowed uniform assessment of radiological severity across the study population. In instances where there was a difference in interpretation between the two observers, a joint review was conducted, and the final grading was assigned through mutual agreement. For the purpose of statistical analysis, the radiological findings were further consolidated into three groups—mild, moderate, and severe—based on the extent of structural damage and intracranial pathology observed on imaging. This approach ensured consistency and objectivity in correlating radiological grades with biochemical and clinical parameters.

**Sample Collection and Processing:** Peripheral venous blood samples of approximately 5 mL were collected from each patient under strict aseptic precautions within 24 hours of sustaining the injury. The blood was drawn into plain, sterile tubes to obtain serum for subsequent biochemical analysis. CSF samples, ranging from 2 to 3 mL, were obtained from patients who underwent lumbar puncture or ventriculostomy as part of their diagnostic or therapeutic management, after obtaining necessary approval from the attending neurosurgeon. All samples were processed promptly to maintain specimen integrity. Blood and CSF were centrifuged at 3000 revolutions per minute for 10 minutes to separate clear serum and supernatant fractions. The resulting aliquots were carefully transferred into pre-labeled sterile cryovials and preserved at –80 °C until further analysis. Samples exhibiting hemolysis, turbidity, or any form of contamination were discarded to avoid analytical errors. This standardized collection and storage protocol ensured the reliability and reproducibility of biomarker estimation across all study participants.

**Biochemical Estimation of Biomarkers:** The quantitative estimation of neuron-specific enolase (NSE), S-100β, and glial fibrillary acidic protein (GFAP) was carried out in both serum and CSF samples using commercially available ELISA kits (Elabscience®, USA). All analyses were conducted in duplicate to ensure accuracy and reproducibility, strictly adhering to the manufacturer's standard operating instructions. Following sample preparation, the optical density of each well was measured at a wavelength of 450 nm using a calibrated microplate reader. For every biomarker, a calibration curve was generated by plotting known standard concentrations against their corresponding absorbance values, which allowed precise determination of biomarker concentrations in the test samples. The analytical sensitivity of the assays was 0.05 ng/mL for S-100β, 0.1 ng/mL for GFAP, and 0.2 ng/mL for NSE, ensuring reliable detection even at low concentrations. To maintain analytical consistency, both inter-assay and intra-assay coefficients of variation were kept below 8%, indicating high reproducibility and precision of the experimental measurements. This standardized biochemical estimation procedure enabled accurate quantification of neuronal and glial injury markers essential for subsequent statistical analysis and correlation with clinical severity.

**Statistical Analysis:** All collected data were systematically entered into Microsoft Excel 2021 and subsequently analysed using the SPSS version 26.0 (IBM Corp., USA). Continuous variables, such as biomarker concentrations and clinical scores, were expressed as mean ± SD, while categorical data were summarized as frequencies and percentages. Comparisons of biomarker levels among the mild, moderate, and severe traumatic brain injury groups were carried out using ANOVA. When a significant difference was identified, Tukey's post-hoc test was applied to determine pairwise differences between the groups. The relationship between serum and cerebrospinal fluid biomarker concentrations, GCS scores, and CT severity grades was analysed using the Pearson correlation coefficient (r) to assess the strength and direction of associations. To evaluate the diagnostic performance of each biomarker in distinguishing between varying degrees of TBI severity, receiver operating characteristic (ROC) curves were constructed, and the area under the curve (AUC) was calculated. A p-value less than 0.05 was considered statistically significant for all comparisons, ensuring the reliability of the observed associations.

**Ethical Considerations:** Confidentiality of patient information was maintained throughout the study. All procedures adhered to the ethical principles outlined in the Declaration of Helsinki (2013 revision). Samples were used solely for research purposes, and no additional invasive procedures were performed exclusively for study participation.

## Results

**Demographic and Clinical Profile:** A total of sixty patients who had sustained acute traumatic brain injury were enrolled in the present study. Among them, forty-two were males and eighteen were females, reflecting a clear male predominance. This gender distribution is consistent with global trends, as men are more frequently exposed to occupational and vehicular hazards that increase their risk of head trauma. The participants' ages ranged from nineteen to sixty-four years, with an average age of 39.2 years and a standard deviation of 11.8. Most of the affected individuals were adults in their most active years, representing the working-age group commonly involved in outdoor activities, driving, and other high-risk environments.

An analysis of the causes of injury showed that road traffic accidents were the leading contributor, accounting for roughly sixty-three percent of all cases. This pattern underscores the ongoing challenges related to road safety, especially in developing regions where compliance with traffic rules and helmet use remains suboptimal. Falls were the second most common cause, responsible for about twenty-seven percent of cases, occurring predominantly among older adults and construction workers. Physical assaults made up the remaining ten percent, often resulting in focal lesions such as contusions or subdural hematomas.

Neurological evaluation at admission was performed using the Glasgow Coma Scale. Based on the score at presentation, eighteen patients (30%) had mild head injury, twenty patients (33.3%) had moderate injury, and twenty-two patients (36.7%) presented with severe traumatic brain injury. The overall mean GCS score across the study population was 9.8 with a standard deviation of 3.6, indicating that a considerable proportion of patients arrived in a state of significant neurological impairment. When the GCS findings were compared with computed tomography grading using the Marshall classification, a strong

inverse relationship was observed, with a p-value less than 0.001. Patients who exhibited higher CT grades, showing diffuse brain swelling, midline shift, or mass lesions, had proportionately lower GCS scores. This finding confirms that the severity of clinical neurological dysfunction correlated closely with the extent of radiological abnormalities. Together, these observations outline a representative clinical profile of acute traumatic brain injury in a tertiary hospital setting—characterized by young to middle-aged male predominance, predominance of road traffic accidents as the causative factor, and a clear association between worsening GCS scores and increasing radiological severity.

**Serum Biomarker Levels and TBI Severity:** The analysis of serum biomarker concentrations revealed a clear and progressive rise in the levels of NSE, S-100β, and GFAP as the severity of TBI increased. Patients with mild head injury exhibited the lowest concentrations of all three markers, whereas those with moderate and severe injuries showed proportionately higher values, reflecting the extent of underlying neuronal and astrocytic damage. This pattern indicates that the biochemical response in the bloodstream parallels the clinical and radiological severity of trauma.

Statistical evaluation using ANOVA confirmed that the differences observed among the three severity groups were highly significant for all markers, with p-values less than 0.001. This strong statistical association suggests that the elevation of these proteins is not random but directly related to the magnitude of cellular disruption within the brain. The progressive rise in NSE levels corresponds to increased neuronal membrane injury and cytoplasmic enzyme release following mechanical insult. Similarly, elevated S-100β levels are indicative of astroglial activation and blood–brain barrier permeability, while the marked increase in GFAP reflects the extent of astrocytic filament breakdown and glial damage in more severe cases.

Together, these findings demonstrate that serum NSE, S-100β, and GFAP act as sensitive biochemical indicators of brain injury. Their graded increase across mild, moderate, and severe categories highlights their potential utility in quantifying neuronal and glial damage and in supplementing conventional clinical assessment tools such as the Glasgow Coma Scale and CT grading systems [Table 1].

**Table 1: Mean Serum Biomarker Levels Across TBI Severity Groups**

| Biomarker | Mild (n = 18) | Moderate (n = 20) | Severe (n = 22) | p-value |
|---|---|---|---|---|
| NSE (ng/mL) | 18.4 ± 4.2 | 31.6 ± 6.7 | 52.3 ± 8.5 | < 0.001 |
| S-100β (µg/L) | 0.09 ± 0.03 | 0.17 ± 0.04 | 0.26 ± 0.05 | 0.002 |
| GFAP (µg/L) | 0.22 ± 0.05 | 0.54 ± 0.08 | 0.98 ± 0.12 | < 0.001 |

Post-hoc Tukey analysis confirmed significant pairwise differences ($p < 0.05$) between all severity categories for each biomarker, confirming progressive elevation of neuronal and astrocytic injury markers with increasing TBI severity.

**CSF Biomarker Levels and Radiological Severity:** CSF analysis revealed a pattern similar to that observed in serum, with all three biomarkers—NSE, S-100β, and GFAP—showing progressive elevation in concentration corresponding to the increasing severity of traumatic brain injury. However, the absolute values of these markers in CSF were notably higher than their serum counterparts, which is expected given the close proximity of CSF to the site of primary neuronal and glial damage. Since CSF directly bathes the central nervous system, it reflects biochemical alterations in the brain more accurately and earlier than peripheral blood.

Among the measured biomarkers, GFAP exhibited the most prominent and statistically significant rise across the mild, moderate, and severe TBI groups ($p < 0.001$). This strong association suggests that GFAP, an intermediate filament protein specific to astrocytes, serves as a highly sensitive indicator of astroglial injury and structural disruption within the brain parenchyma. Patients classified as having severe TBI demonstrated markedly elevated CSF GFAP concentrations, corresponding to radiological findings of diffuse edema, contusions, and mass lesions on computed tomography scans [Table 2].

The correlation between biomarker levels and CT-based grading further emphasized the diagnostic value of CSF markers. The strong statistical relationship implies that CSF biomarker concentrations not only mirror the clinical severity reflected by the Glasgow Coma Scale but also align closely with objective radiological evidence of tissue damage. In particular, the steep gradient observed in GFAP levels across severity groups highlights its potential as a robust predictor of radiological severity and as a complementary tool for early assessment of brain injury beyond traditional imaging parameters.

**Table 2: CSF Biomarker Concentrations in Relation to Radiological Severity**

| Biomarker | Mild (n = 18) | Moderate (n = 20) | Severe (n = 22) | p-value |
|---|---|---|---|---|
| NSE (ng/mL) | 26.2 ± 5.1 | 44.7 ± 7.4 | 63.1 ± 9.8 | < 0.001 |
| S-100β (µg/L) | 0.12 ± 0.04 | 0.21 ± 0.06 | 0.31 ± 0.07 | 0.004 |
| GFAP (µg/L) | 0.38 ± 0.06 | 0.78 ± 0.09 | 1.12 ± 0.14 | < 0.001 |

**Correlation Between Biomarkers, GCS, and CT Severity:** Correlation analysis showed a clear and statistically significant association between the biochemical markers and both clinical as well as radiological indices of traumatic brain injury severity. The levels of serum and CSF biomarkers were found to be inversely related to the GCS scores and positively related to CT severity grades. This pattern indicates that patients with more severe neurological impairment, reflected by lower GCS scores, exhibited higher biomarker concentrations, whereas those with greater structural brain damage on CT imaging showed proportionately elevated levels.

The inverse relationship with GCS suggests that as the degree of consciousness and neurological responsiveness decreased, the release of neuronal and glial proteins into the bloodstream and CSF increased, corresponding to more extensive tissue injury. Similarly, the positive correlation with CT grading demonstrates that biochemical alterations parallel the anatomical damage visualized on neuroimaging, including diffuse axonal injury, contusions, or cerebral edema.

Among all measured parameters, CSF GFAP showed the strongest negative correlation with GCS ($r = –0.79$, $p < 0.001$), highlighting its close link with the extent of clinical neurological deterioration. This strong association indicates that GFAP, which is released during astrocytic injury, accurately reflects the severity of central nervous system damage. In contrast, serum NSE exhibited the highest positive correlation with CT severity ($r = 0.76$, $p < 0.001$), suggesting that neuronal cytoplasmic leakage contributes significantly to the observed radiological abnormalities [Table 3].

Taken together, these results confirm that both serum and CSF biomarkers can serve as reliable biochemical indicators of traumatic brain injury severity. Their close association with GCS and CT findings reinforces their usefulness as complementary tools for assessing clinical status, predicting outcome, and supporting decision-making in acute neurotrauma management.

**Table 3: Correlation of Biomarkers with Clinical and Radiological Parameters**

| Biomarker | Correlation with GCS (r) | Correlation with CT Severity (r) | p-value |
|---|---|---|---|
| Serum NSE | –0.71 | 0.74 | < 0.001 |
| Serum S-100β | –0.68 | 0.69 | < 0.001 |
| Serum GFAP | –0.75 | 0.78 | < 0.001 |
| CSF NSE | –0.73 | 0.77 | < 0.001 |
| CSF S-100β | –0.70 | 0.72 | < 0.001 |
| CSF GFAP | –0.79 | 0.84 | < 0.001 |

**Diagnostic Accuracy of Biomarkers:** ROC curve analysis was performed to evaluate the diagnostic accuracy of the measured biomarkers in distinguishing severe traumatic brain injury from mild and moderate cases. The results demonstrated that all three biomarkers—NSE, S-100β, and GFAP—showed good discriminatory capacity, as reflected by high AUC values. This indicates that their serum and CSF concentrations can effectively differentiate patients based on the severity of brain injury.

Among the analyzed parameters, CSF GFAP exhibited the best diagnostic performance, with an AUC of 0.92, indicating excellent sensitivity and specificity for identifying severe cases. Serum GFAP ranked next with an AUC of 0.88, followed closely by serum NSE, which showed an AUC of 0.85. These findings emphasize that both astrocytic and neuronal markers contribute valuable diagnostic information, with GFAP emerging as the most reliable single biomarker for assessing the extent of brain tissue damage.

The superior performance of CSF GFAP can be attributed to its direct release into the cerebrospinal fluid following astrocytic injury, resulting in a more immediate and pronounced rise compared to serum levels. The close alignment between biomarker elevation and radiological findings further supports the potential of CSF GFAP as an early and sensitive indicator of structural damage. Overall, the ROC analysis confirms that these biomarkers, particularly GFAP, can serve as effective biochemical tools for stratifying the severity of traumatic brain injury and enhancing clinical decision-making alongside imaging and neurological evaluation [Table 4].

**Table 4: ROC Analysis of Biomarkers for Prediction of Severe TBI**

| Biomarker | Sample Type | AUC | Sensitivity (%) | Specificity (%) |
|---|---|---|---|---|
| NSE | Serum | 0.85 | 82 | 80 |
| S-100β | Serum | 0.81 | 78 | 76 |
| GFAP | Serum | 0.88 | 86 | 82 |
| NSE | CSF | 0.87 | 85 | 83 |
| S-100β | CSF | 0.84 | 80 | 78 |
| GFAP | CSF | 0.92 | 90 | 85 |

## Discussion

This study demonstrates that biochemical indicators of neuronal and astroglial injury measured during the acute window after TBI track closely with clinical impairment and radiological severity. Serum and CSF concentrations of NSE, S-100β, and GFAP rose in parallel with increasing severity categories, while higher levels were associated with lower GCS scores and higher Marshall CT grades. These observations align with contemporary evidence that blood- and CSF-based markers provide complementary information to imaging and clinical scales in the early stratification of TBI.[14,15]

Among the analytes tested, GFAP emerged as the most informative—particularly in CSF—where it showed the strongest inverse association with GCS and a robust positive relationship with CT severity. This is biologically plausible because GFAP is an intermediate filament protein enriched in astrocytes; mechanical and secondary insults precipitate astroglial disruption and blood–brain barrier dysfunction, facilitating GFAP release into CSF and, subsequently, the bloodstream.[14] Recent multicentre programmes have repeatedly highlighted GFAP's diagnostic value. CENTER-TBI analyses reported that GFAP outperforms several legacy markers in predicting CT abnormalities within 24 hours of injury,[20] while TRACK-TBI and allied cohorts have shown high discriminative

accuracy of GFAP for acute lesions and outcomes, including when sampled very early—within minutes to the first hour after trauma.[16,19] Our ROC data, with CSF GFAP achieving an AUC of 0.92 and serum GFAP 0.88, accord with these reports and place GFAP at the forefront of clinically actionable biomarkers for acute assessment.

NSE and S-100β provided additive context. NSE reflects neuronal cytoplasmic disruption; in our cohort, serum NSE correlated most strongly with CT grade, suggesting a close link between neuronal injury burden and structural lesions visible on imaging. While multiple studies and meta-analyses have questioned the stand-alone diagnostic precision of NSE and S-100β—especially for detecting scan-positive mild TBI,[3,17] —they still contribute to risk stratification when interpreted with timing and matrix (blood vs CSF) in mind.[8,18] In particular, S-100β's negative predictive utility has been used to reduce head CTs in low-risk mild TBI in some health systems, though performance can vary in anticoagulated populations and older adults.[25] Our pattern—graded rises for all three markers but the steepest gradient and best ROC performance for GFAP—is consistent with meta-analytic rankings where GFAP typically edges out S-100β and NSE for acute lesion detection and short-term prognostication.[8,12,18]

Timing of sampling remains critical. Kinetic studies suggest that GFAP rises within hours, often remaining elevated longer than S-100β, which tends to peak earlier and decline more rapidly; NSE shows variable trajectories depending on injury type and sampling schedule.[22,25] Very early sampling can still be informative: in a 2024 cohort, GFAP measured as early as 30 minutes post-injury retained high diagnostic accuracy for acute outcomes.[16] Serial trajectories may offer incremental value for monitoring secondary injury and predicting recovery, as suggested by repeated-measure work in moderate–severe TBI.[23] Our single-timepoint approach (≤24 h) captured the major between-group differences; future work in this setting could examine whether combining admission values with short-interval resampling improves discrimination and prognostication.

The clinical adoption landscape is evolving. The US FDA's 2018 de novo authorization of the combined GFAP/UCH-L1 assay (Brain Trauma Indicator) for evaluation of adults with suspected TBI established a regulatory precedent for blood tests aiding CT decision-making within 12 hours of injury.[17,29] Subsequent studies have explored integration of GFAP and UCH-L1 with clinical decision rules, with evidence that biomarker-augmented strategies can match or exceed established rules and potentially reduce unnecessary CT utilisation in mild TBI pathways.[19,21] Head-to-head comparisons suggest that panels such as GFAP/UCH-L1 may be comparable to, or an alternative for, S-100B-based strategies, depending on local logistics and assay availability.[24] Our findings—particularly the excellent AUC for CSF GFAP and strong serum performance—support the broader proposition that GFAP-anchored algorithms could enhance early triage in mixed-severity cohorts, not only for deciding on imaging but also for flagging patients who merit closer observation.

Beyond acute diagnosis, biomarker levels may carry long-term prognostic information. Elevated acute GFAP and neurofilament light (NfL) in CSF have been linked to poorer functional outcomes up to 1 and even 10–15 years after injury, underscoring their relevance to neurodegeneration and chronic disability trajectories.[4] Conversely, single blood draws within 24 hours in mild TBI may not predict persistent post-concussive symptoms, reminding clinicians that biochemical severity is only one component of complex, multifactorial outcomes.[16] In our dataset, the strong cross-sectional associations with GCS and CT suggest utility for early severity stratification; assessing longer-term endpoints (e.g., 6–12-month GOSE) would clarify prognostic calibration in this population.

Several methodological considerations deserve attention. First, matrix choice matters: CSF, being closer to the injury milieu, yielded higher absolute concentrations and tighter associations with CT grade in our study, mirroring prior reports that CSF assays often outperform blood for pathophysiologic sensitivity.[14] Second, pre-analytical and analytical variability—sampling time, haemolysis, assay platform, and cut-offs—can influence effect sizes and thresholds across studies, complicating universal adoption.[12,20] We mitigated variability with standardized collection and low coefficients of variation, yet we recognize that harmonization efforts and platform-specific reference ranges are still needed for widespread clinical use.[12,21] Third, while our cohort represents real-world neurosurgical practice in a tertiary centre, larger multicentre validation would improve generalizability, enable robust cut-point optimization (e.g., for ruling-out scan-positive lesions), and facilitate health-economic analyses of biomarker-guided pathways.

Finally, the convergence of our results with contemporary literature supports a pragmatic pathway: incorporate GFAP (preferably alongside a neuronal marker such as UCH-L1 or NSE) early in evaluation, interpret values in the context of time since injury and clinical risk, and use combined clinical–biochemical–radiological algorithms to prioritize imaging, observation, and escalation. In centres with ready CSF access (e.g., ventriculostomy or diagnostic LP in selected cases), CSF GFAP may provide additional fidelity for severity stratification. As automated platforms mature and rapid-turnaround assays become commonplace, biomarker-enhanced triage could reduce unnecessary CTs, identify high-risk patients sooner, and standardize early prognostication across diverse care settings.[21,25]

In summary, the graded increases of GFAP, NSE, and S-100β observed here—and the superior diagnostic performance of GFAP, especially in CSF—are concordant with current evidence that astroglial injury markers are central to modern acute TBI evaluation. These data support integrating GFAP-anchored biomarker testing into multimodal assessment frameworks to complement GCS and CT, with the aim of improving early decisions and downstream outcomes.[14,15,19–22,25]

## Conclusion

The present study demonstrates that neuronal and glial biomarkers—NSE, S-100β, and GFAP—are closely related to the clinical and radiological severity of traumatic brain injury. Both serum and CSF concentrations increased proportionally with worsening injury grades, indicating that biochemical

alterations reflect the extent of brain tissue damage.

Among the markers tested, CSF GFAP showed the strongest association with GCS scores and CT findings, confirming its usefulness as a sensitive indicator of astroglial injury. ROC analysis further revealed that GFAP had the highest diagnostic accuracy for distinguishing severe from non-severe TBI cases.

In practical terms, early estimation of these biomarkers, especially GFAP, could complement neurological and radiological assessments, improving triage and prognosis in emergency settings. Broader studies are still required to establish standardized thresholds and to evaluate the role of serial measurements in predicting long-term neurological recovery.

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## Conflicts of interest

There are no conflicts of interest.

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