



Paediatric Traumatic Brain Injury: The Evolving Role of Blood and Salivary Biomarkers

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Abstract

Traumatic brain injury is an important priority in intensive care, particularly in paediatrics. Many brain biomarkers, particularly serum glial fibrillary acidic protein, S100 calcium-binding protein B and ubiquitin C-terminal hydrolase L1, have been proposed to improve sensitivity and specificity of diagnosis and management. This is particularly important for identifying clinically significant mild traumatic brain injury in paediatric patients, as it could potentially reduce unnecessary hospitalisations and neuroimaging scans. This manuscript focuses on recent clinical guidelines and research on clinical chemistry tests for various biological fluids, particularly saliva. The text discusses biomarkers in adults and children, highlighting their application in blood and saliva, focused on studies published between January 2021 and June 2025. Firstly, we report on the characteristics of brain biomarkers and the relevance of serum biomarkers of mild traumatic brain injury in paediatric population, as well as the its epidemiology in paediatric and adult populations. Then, we focuses on six important areas: a) Diagnostic guidelines and the rationale for biomarkers: a) Neuroanatomical and functional vulnerabilities in paediatric traumatic brain injury; b) Molecular mechanisms of injury and inflammation in paediatric traumatic brain injury; c) Saliva as an emerging matrix for traumatic brain injury biomarkers; e) Analytical, biological and clinical challenges in biomarker use; f) Experimental biomarkers: exosomes and non coding RNAs.

Research and their potential clinical applications is promising. However, many challenges remain in controlling for biological variability and potential pre- and analytical confounding factors in order to obtain reference values and cut-offs, particularly for salivary biomarkers, and to implement them in paediatric clinical practice.

Keywords: Biomarker; GFAP; S100; UCHL-1; Saliva; Pediatrics; Traumatic brain injury (TBI); Guideline; Exosomes; Reference values; Cut-offs

Abbreviations

AAMR: Age-adjusted mortality rates

Alb: albumin

AD: Alzheimer's Disease

aTBI: adult TBI

APOE: Apolipoprotein E

AQP: Aquaporin

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BBB: Blood-Brain Barrier
BDNF: Brain-derived neurotrophic factor
CBF: Cerebral Blood Flow
CDSS: Clinical Decision Support System
CE-IVD: in vitro diagnostic CE marked products
CGRP: calcitonin gene-related peptide
circRNA: Circular RNA
GCF: gingival crevicular fluid
CLIA: Chemiluminescence Immunoassay
CMIA: Chemiluminescent Microparticle Immunoassay
CN: Cranial Nerve
CNI: Cranial Nerve Injury
CRP: C-reactive protein
CRP/Abl: C-reactive protein to albumin ratio
CT: Computed Tomography
CV: Coefficient of Variation
ECLIA: Electrochemiluminescence Immunoassay
ED: Emergency Department
ELISA: Enzyme-Linked Immunosorbent Assay
EIA: Enzyme Immunoassay
EV: extracellular vesicles
Hb: Hemoglobin
HPA: hypothalamic-pituitary-adrenal
FDA: Food and Drug Administration, USA
FGFR: Fibroblast Growth Factor Receptor
GCS: Glasgow Coma Scale
GFAP: Glial Fibrillary Acidic Protein
LDH: lactate dehydrogenase
lncRNA: Long non-coding RNA
miRNA: microRNA
MRI: Magnetic Resonance Imaging
mTBI: mild Traumatic Brain Injury
mTBIS: mild Traumatic Brain Injury Symptoms
MVBs: multivesicular bodies
MW: Molecular Weight
ncRNA: non coding RNA
NI: neuroimaging

NSE: Neuron-specific enolase
NT-proBNP: N-terminal pro-B-type natriuretic peptide
PACAP-38: pituitary adenylate cyclase-activating polypeptide-38
PCS: post-concussion syndrome
PICO: Population, Intervention, Comparison, Outcome
PPCS: Persistent Post-concussive Symptoms
PTT: partial thromboplastin time
RAGE: Receptor for Advanced Glycation Endproducts
sAA: salivary α -Amylase
SG: salivary gland
S100B: S100 calcium-binding protein B
SP: substance P
SS: Sjögren's Syndrome
TBI: Traumatic Brain Injury
TJ: Tight Junction
PPCS: Persistent Post-concussive Symptoms
pH: hydrogen ion concentration index
pTBI: paediatric Traumatic Brain Injury
UCH-L1: Ubiquitin c-terminal Hydrolase L1
uSFR: unstimulated salivary flow rate
VIP: vasoactive intestinal peptide
WHO: World Health Organization
YLL: Years of Life Lost

Introduction

In Italy, current national guidelines lack specific recommendations on good laboratory, clinical, and healthcare practices for the diagnosis and monitoring of traumatic brain injury (TBI), particularly with regard to the use of biological biomarkers [1]. This research is based on a critical synthesis of recent systematic reviews [2-16], original studies [17-30], and commentaries [31,32], as well as the latest guidelines [33-38] on TBI diagnosis and prognosis in the paediatric and adult populations, with a particular focus on mild TBI (mTBI), concussion [35], and the use of diagnostic biomarkers [3,4,6,10,11,15,21,23,25-30]. TBI represents an important priority in intensive care due to the associated health problems and economic costs (estimated at \$400 billion annually in the USA). It is projected to become a leading cause of death and disability by 2030 [2,39], primarily resulting from acute trauma such as road accidents, falls, sports-related injuries, and violence [35]. In children, over half (55.5%) of TBI cases are related to sports or recreational activities, and 62.4%

of these patients receive medical evaluation, underscoring the need for rapid and accessible diagnostic strategies in paediatric settings [40]. Globally, the incidence rate of moderate-to-severe TBI was 182.7 per 100,000 population in 2019, accounting for over half of all TBI cases, with falls and road traffic accidents as leading causes. The Italian age-standardized incidence was notably higher, at 377 (95% UI: 319-451) per 100,000 [41]. In comparison, recent data from the United States indicate that 3.0% of the population reported a TBI in the past year, including 2.2% of children aged ≤ 17 years (40). Epidemiological data from Huang et al. highlight age- and sex-related differences in TBI patterns, potentially relevant to diagnostic approaches [41]. The Centers for Disease Control and Prevention (CDC) report that children aged 0-17 years account for approximately 4.1% of all TBI-related deaths [35]. This is particularly relevant given that paediatric TBI (pTBI) is the leading cause of death in this age group [42]. Furthermore, pTBI has been shown to impact brain development and cognitive abilities [35,43,44].

To date, neither a single objective laboratory test [31,45] nor harmonised neuroimaging (NI) protocols [46,47] are considered a gold standard for TBI management [48]. In fact, inconsistent findings have been reported when different biomarkers are associated with specific imaging phenotypes including diffuse axonal injury, cerebral oedema, and intracranial hemorrhage. These findings suggest a low level of diagnostic specificity and may reflect the complex and heterogeneous nature of TBI, underestimated biological variability and pre-analytical and analytical issues [6,9-11,24,26-30]. Diagnosing and predicting the outcome of pTBI remains particularly challenging, especially in cases of mild TBI (mTBI) and post-concussion syndrome (PCS) [49,50]. PCS is characterized by the persistence of symptoms following mTBI [51,52]. A recent review explored the diagnostic challenges of PCS and proposed strategies to improve both research design and clinical practice, including the use of salivary biomarkers [15]. In addition, several studies and reviews have investigated brain function biomarkers in both paediatric and adult populations [3,4,6,10,11,15,17,21,23,25-30,45,46,49,51,52]. Recent guidelines highlight the potential of TBI biomarkers to limit NI, particularly in the diagnostic workup of pTBI using serum and saliva samples [5,12,15,17-22,25,28,30,31,36-38,53,54]. In a recent multicentre observational study, 84.6% of children presented with mild neurotrauma, while 14.2% and 1.2% displayed moderate and severe injuries, respectively [55]. Overall, the literature suggests that the diagnostic performances of biomarkers for TBI, particularly in terms of sensitivity and specificity, varies according to the type of biological fluid used (plasma, cerebrospinal fluid (CSF) or saliva) and across populations including adults, pediatric patients and athletes [3,12, 56-70].

Many biomarkers have been proposed for evaluating TBI. These are categorized based on their characteristics as

follows: brain cell proteins (mainly S100 calcium-binding protein B (S100B), glial fibrillary acidic protein (GFAP) and ubiquitin c-terminal hydrolase L1(UCH-L1)); stress marker (cortisol) [17]; nerve proteins (lectin-binding glycan molecules) [23]; enzymes (α -amylase, lactate dehydrogenase (LDH) [20,71]; messengers within the immune system (cytokines); cellular integrity proteins (protein tau and amyloid β) [72]; neuropeptides (plasma calcitonin gene-related peptide (CGRP), pituitary adenylate cyclase-activating polypeptide-38 (PACAP-38), vasoactive intestinal peptide (VIP), and substance P (SP)); natriuretic peptide (N-terminal pro-B-type natriuretic peptide (NT-proBNP); acute phase proteins (C-reactive protein to albumin ratio (CRP/Alb) [71]; transport proteins (hemoglobin (Hb) and serum albumin (Alb); blood clotting (PTT test) [73,74]; functional and non-coding (nc) RNA (i.e. miRNAs, circRNAs, lncRNAs)); nano-sized, membrane-bound particles that act as messengers in cell-to-cell communication (extracellular vesicles (EV)) [5,15,22,25]; lipid and lipid transport (apoE genotype) [75,76]; epigenetic mechanism (BDNF gene expression) [77]; and acid/base balance (pH index) [73]. Cerebrospinal fluid (CSF) is the biofluid that most accurately reflects brain functions [70], but its use is discouraged due to several limitations, including the difficulty, pain and safety concerns related to sample collection, the complexity of CSF biomarker interpretation in paediatric neurological disorders, challenges in laboratory standardization, and the limited feasibility of CSF use in large-scale or emergency settings [11,78]. As a result, blood-derived biofluids and saliva are considered more suitable matrices for paediatric biomarker analysis. The presence of brain-derived proteins in peripheral fluids (blood, saliva, urine) relies on their ability to cross the blood-brain barrier (BBB) and vascular endothelium, particularly when injured [79,80]. These proteins can subsequently be detected in gingival crevicular fluid (GCF) and saliva, through both exocrine as well as non-exocrine pathways [81]. The high vascularisation of salivary glands enables the transfer of blood-derived components, including brain proteins, into saliva, an exchange largely influenced by the molecular size of these proteins [79].

Saliva is expected to play a key role in the future diagnosis of pTBI, giving several operational advantages, including easy collection, transport, and storage, and is suitable for use in emergency settings and point-of-care testing [11,12,82]. Saliva testing is non-invasive and well accepted in pediatric population, particularly when repeated sampling is needed. Several devices are available for whole saliva collection, including those specifically designed for infants. More recently, wireless biosensors for analytes like glucose and electrolytes have been integrated into saliva collection devices (e.g. glucose-tracking wireless pacifiers) [83,84]. The diagnostic potential of using saliva is partly explained by the close physiological relationship between saliva

production and central nervous system regulation [81]. The production and composition of saliva are subject to constant and variable influences by peripheral sensory inputs and circadian rhythms. The fine regulation of secretion depends on both the parasympathetic and orthosympathetic systems. Finally, a growing body of evidences supports the idea that neurodevelopmental disorders may be linked to the oral–brain axis, partly through mechanisms mediated by the oral microbiome [81,85,86]. Consistently, proteomic data indicate that proteins associated with neurodegenerative disorders are equally abundant in saliva (n°38; 2.5%) and plasma (n°37; 2.6%) (87). However, S100B, GFAP and UCH-L1, three well established TBI biomarkers, were absent from the top 1,000 blood-derived salivary proteins identified using the manifold ranking method [88,89], suggesting limited detectability in saliva.

Our work first outlines the key features of brain biomarkers and the clinical relevance of serum biomarkers in pediatric mild TBI (pmTBI), as well as the epidemiology of this condition in adult and paediatric populations. Then, we explore six key thematic areas:

- a) Diagnostic guidelines and the rationale for biomarkers
- b) Neuroanatomical and functional vulnerabilities in pTBI
- c) Molecular mechanisms of injury and inflammation in pTBI
- d) Saliva as an emerging matrix for TBI biomarkers
- e) Analytical, biological and clinical challenges in biomarker use
- f) Experimental biomarkers: exosomes and nc RNAs

This work aims to support the planning of well-designed clinical trials to validate current biomarker-based best practices in the paediatric population. Establishing reliable, evidence-based reference ranges and diagnostic cut-off values could reduce the reliance on NI in cases of pmTBI[15,31].

Materials and Methods

Focused Question

What is the diagnostic value of serum and salivary biomarkers in paediatric patients with mild traumatic brain injury (mTBI)?

Search Strategy

Given the novelty and evolving nature of the topic, we adopted a narrative review approach, with a particular focus on the translational potential of salivary biomarkers. Although narrative synthesis is less common in this domain, we considered it more appropriate than conducting an additional systematic review, given the number of recent high quality systematic and umbrella reviews already published [3-7,10-16,36,43-46,48,52,52,57-59,61,63-65,66,68,87].

Nevertheless, to ensure methodological rigor, we structured the research according to the PICO model (Table 1) (Population, Intervention, Comparison, and Outcome) and conducted a literature search of the PubMed (MEDLINE) and Scopus databases, based on the following three aspects: population, concept, and context.

The following keywords and MeSH terms were used in various combinations with Boolean operators (OR, AND): "traumatic brain injury", "neuroimaging", "neurodegenerative diseases", "children", "adolescent", "adult", "patient safety", "disease prediction", "biomarkers", "astrocyte injury", "neuronal cell injury", "salivary gland tumors", "GFAP", "S100B", "UCH-L1", biological functions, "serum", "saliva", "blood", "brain derived extracellular vesicles", "blood biomarkers", "saliva biomarkers", "laboratory parameters", "diagnostic indicators", "sensitivity", "specificity", "diagnostic accuracy", "reference values", "salivary gland", "salivary flow", "salivary flux", "pre-analytical phase", "molecular weight", "half-life/kinetics", "isoelectric point", "salivary gland filtration", "blood-brain barrier", "systematic and narrative review", "sport", "brain anatomy".

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Inclusion and Exclusion Criteria

The following inclusion criteria guided our analysis: (I) English language; (II) full text available; (III) published on-line firsts between January 2021 and June 2025; (IV) studies conducted in high- and middle-income countries (to limit salivary variability due to malnutrition); (V) studies on COVID-19 patients (when relevant to salivary biomarker analysis). References were excluded for: (I) absence of a described methodology; (II) duplications; (III) irrelevant scope; (IV) content redundancy; (V) studies without freely accessible full text; (VI) abstract-only publications; (VII) Outdated reviews when updated or more recent versions are available.

Research

This paper represents an original translational research, focusing on the evaluation of TBI biomarkers (particularly GFAP, S100B and UCH-L1) among different biological fluids (serum, plasma, saliva). The literature search was conducted via PubMed (MEDLINE), Scopus, and Google Scholar for studies published between January 2021 to June 2025. The final search was conducted on June 30, 2025, and data extraction spanned approximately six weeks. Subsequently, bibliographic material from the papers has been used in order to find other or older appropriate sources in relation to specific topics and operative problems. Cochrane sources (reviews, protocols, clinical answers) were reviewed to identify pharmacological interventions in TBI management. Two independent reviewers (L.B. and A.B.) screened titles and

abstracts in a blinded fashion. Discrepancies were resolved by discussion or, if needed, by consultation with a third reviewer (M.V.). A total of 354 articles were initially identified. After applying inclusion and exclusion criteria, the final number on included references was 202. The heterogeneity of study designs and outcome measures prevented quantitative meta-analysis, especially for salivary biomarkers and pre-analytical variables.

Results and Discussion

TBI represents a major cause of disability and mortality in the paediatric population, with clinical presentation, injury mechanisms, and outcomes differing substantially from those observed in adults [51]. While neurological examination and neuroimaging (primarily head CT) remain the cornerstone of diagnosis, their limited specificity [90], particularly in cases of mTBI [91], often leads to overuse of imaging procedures with associated risks, especially in children. This scenario has prompted increasing interest in the use of circulating brain-derived biomarkers to improve risk stratification and guide decision-making [30,35-38,92].

Biomarkers released into blood or other bodily fluids, following brain cell injury, may offer additional diagnostic and prognostic value. However, their integration with clinical and radiological data remains an evolving field [4,39,54,61-66,71,73,78,93]. Table 2 summarizes the key characteristics of the most studied brain biomarkers in TBI, including their biological properties and limitations [4,16,30,57,62,65,79,94-105]. Recently, some reviews and papers have been published, reporting interesting findings on biomarker application from relevant paediatric case studies [3,6,9-11,24,26-30,71,78,106-111] (Table 3) [6,9-11,24,26-30,71,110-111]. Additional epidemiological and comparative data between adult and pediatric TBI are provided in Supplementary Material S1-S2.

Diagnostic Guidelines and the Rationale for Biomarkers

Recent international guidelines have refined the diagnostic criteria for mTBI, placing increasing emphasis on clinical evaluation and selective use of NI [2,30,32,33,35,37-39]. The World Health Organization (WHO) defines mTBI by the presence of transient neurological dysfunction and a

Glasgow Coma Scale (GCS) score between 13 and 15 at least 30 minutes after trauma. Conditions such as intoxication, pre-existing neurological disorders, or co-morbid injuries must be excluded [50,112]. The CDC recommends combining clinical signs and risk factors [35] to determine the necessity of NI, though it acknowledges the limitations of CT scans: up to 95% of children with suspected mTBI undergoing CT have no detectable intracranial injury [39]. Importantly, CT does not exclude the presence of structural brain injury, as demonstrated by MRI findings in up to 30% of children with normal CT results. Moreover, 20–40% of patients with normal CT scan may experience long-lasting post-concussive symptoms [113]. In addition, CT is often poorly accepted in paediatric populations due to the need for sedation, radiation exposure, limited accessibility in peripheral hospitals, and associated healthcare costs. The recently modified Brain Injury Guidelines (mBIG) [114,115], while reaffirming the relevance of CT scan in pediatric TBI, advise against routine repeat imaging in low-risk patients (mBIG 1 and 2), a recommendation supported by recent studies [90,116]. In parallel, recent evidence highlights the clinical value of measuring plasma levels of GFAP and UCH-L1 in the early management of both adult and paediatric mTBI (pmTBI). Early sampling, within 3 to 12 hours post-injury, has been associated with increased diagnostic power. Combining GFAP and UCH-L1 has been shown to improve sensitivity in detecting clinically significant TBI in children aged 16 years or younger [30]. Moreover, biomarker-based risk stratification models, including the use of S100B as a screening tool, have been proposed to reduce unnecessary CT scans and adopted by the Scandinavian guidelines; while French guidelines adopted the use of GFAP and UCH-L1 [14,36,37,96,117,118] (Table 4) [36,37,96].

The development of biomarker-based Clinical Decision Support Systems (CDSS) represents a significant advancement. Some models propose scoring systems based on panels including S100B, GFAP, UCH-L1, NfL, and tau, stratifying patients into low, moderate, and high risk categories. While promising, these models require validation in children under five due to physiological differences in biomarker expression. Summary of the main recommendations are provided in table 4. Other recommendations are provided from evidences by meta-analysis and modeling [14,117,118], and further technical details are included in table 3 and Supplementary Material S3. The integration of biomarkers into TBI classification systems remains challenging. Reference ranges must account for age, sex, and developmental stage, and must correlate with advanced NI and clinical outcomes [9,24,28,30,94,107-109]. Studies on mTBI in animal models will enhance translational research and deepen our understanding of neurodevelopmental changes in humans (Supplementary Material S4).

Moreover, TBI pathophysiology involves both primary

Table 1: Summary of the PICO framework applied.

Population	Children, adolescents, pediatric patients, adults with TBI
Intervention/exposure	Role and limitations of serum and salivary biomarkers (particularly, GFAP, S100B and UCH-L1)
Comparison/control	Use of NI or reference to normal brain function
Outcomes	Role and limitations of biomarkers in biological fluids to reduce NI use

Table 2: Main characteristics of selected biomarkers for TBI [16,30,57,62,65,79,94-105].

Biomarker acronym and characteristics (name; molecular weight; pHi)	Function in brain / Biological role	Expression and detection	Kinetics	Diagnostic value & limitations
GFAP (Glial Fibrillary Acidic Protein; ~49.8 kDa; pHi 5.4–5.8)	Major intermediate filament protein of mature astrocytes, structural component of the cytoskeleton, contributing to cell shape and stability. Supports bidirectional fluid exchange across CNS barriers and serves as a marker of astrocyte damage and astrogliosis	mRNA: ~10-fold higher in brain vs salivary gland. Protein: brain ~4 Log ₁₀ ppm; saliva ~3 Log ₁₀ ppm. Ratio saliva/blood: 0	Half-life 24–48 h	Specific for astrocyte injury, but also elevated in elderly and orthopedic patients. Interference from oral health status (see “Saliva in pTBI”).
UCH-L1 (Ubiquitin CarboxylTerminal Hydrolase Isozyme L1; 26–28 kDa; pHi ~5.3)	Neuronal protein degradation enzyme; maintains axonal stability, regulates synaptic function; marker of neuronal injury	mRNA: ~10-fold higher in brain vs salivary gland. Protein: brain 3–4 Log ₁₀ ppm; plasma and salivary gland ~2.2 Log ₁₀ ppm; saliva unknown. Ratio saliva/blood: 0.1	Half-life in patients with TBI: CSF=7 (0.1–55) h Serum=9 (2–55) h	Reflects neuronal damage; unstable marker (levels fluctuate with BBB integrity). Also expressed in PNS, endocrine and cancer cells; mutations linked to Parkinson disease.
S100B (S100 Calcium Binding Protein B; 10.5 kDa; pHi 4.1–4.5)	Astrocytic protein with neurotrophic and cytokine functions; regulates cell cycle, differentiation, Ca ²⁺ fluxes; marker of astrocyte activation and BBB disruption	mRNA: ~10-fold higher in brain vs salivary gland. Protein: brain/CSF ~3–3.2 Log ₁₀ ppm; salivary gland/epithelium ~2.1 Log ₁₀ ppm; saliva unknown. Ratio saliva/blood: 0.8	30-90 min. 25 min in patients without ongoing brain injury	Sensitive but non-specific: increased after extracranial trauma, burns, pediatric age. Also expressed in non-neural tissues (adipocytes, melanocytes, lymphocytes, tumors). Oral health may confound salivary detection.
NSE (Neuron Specific Enolase; 46 kDa; pHi 4.5)	Glycolytic enzyme, abundant in neurons/neuroendocrine cells; high levels promote neuroinflammation, ECM degradation	mRNA: ~7-fold higher in brain vs salivary gland. Protein: brain 3.2–3.4 Log ₁₀ ppm; salivary gland ~1.3 Log ₁₀ ppm; oral epithelium ~2 Log ₁₀ ppm. Ratio saliva/blood: unknown	Half-life ~24 h	Difficult to detect due to minimal plasma levels, rapid metabolism, high protein binding. Interference from erythrocytes and cancer cells.
Cortisol (Steroid hormone; 0.36 kDa; 362.46 g/mole)	Stress hormone produced by adrenal gland; regulates metabolism, immunity, HPA-axis circadian rhythm	Passes blood–saliva barrier as free cortisol (biologically active fraction)	Half-life 70–120 min	Non-specific marker; elevated after stress/trauma. Imbalances linked to Cushing's and Addison's disease.

Table 3: Recent evidence published on pTBI using serum biomarkers [6,9-11,24,26-30,71,110-111].

Study / Year	Design / Sample	Biomarkers	Main findings
Marzano 2022 [6]	Systematic review (56 studies, n=798 <19 yrs)	S100B, NSE, GFAP, UCH-L1	S100B most studied; higher levels linked to worse outcome; GFAP and UCH-L1 differentiate TBI severity. Panels of biomarkers more informative than single measures.
Oris 2023 [9]	Review (12 studies)	S100B	Age-specific reference ranges established; levels decline with age; important for interpretation in infants.
Malhorta 2024 [11]	Meta-analysis (32 studies, n=4743)	S100B, GFAP, UCH-L1, NSE, tau, IL-6	Variable diagnostic performance across studies; AUC ranges: S100B 0.67–1.0, GFAP 0.41–0.85, UCH-L1 0.59–0.83.
Morello 2024 [10]	Systematic review (10 studies, n=1616)	S100B	Pooled sensitivity 98%, specificity 45%; excellent NPV (99%), limited PPV.
Chiollaz 2024 [26]	Prospective cohort (n=302 mTBI, 74 controls)	S100B, GFAP, heart fatty-acid binding protein (HFABP)	At 100% sensitivity, specificity, to rule out the need of CT scans, low (~35–40%). GFAP slightly better than S100B.

Tabor 2024 [28]	Cohort (n=154 concussions, 695 controls)	GFAP, UCH-L1, NfL, total tau	Compared to uninjured patient levels GFAP increased by 17% (males and females), UCH-L1 by 43% (females); NfL and tau elevated subacutely. Reliability concerns due to assay variability.
Puravet 2024 [30]	Multicentre trial substudy(n=1249)	GFAP, UCH-L1	Combined GFAP+UCH-L1: sensitivity 100%, specificity 67%; useful to reduce CT scans.
Pereira 2024 [24]	Prospective study (n=15 TBI, 19 controls)	GFAP, NfL, UCH-L1, S-100B, tau, p-tau181	All biomarkers increase in severe TBI vs ctrls; some differentiate severity even in mild/moderate cases.
Mayer 2025 [29]	Case-control (n=59 pmTBI, 41 controls)	GFAP, NFL, Tau, pTau 181 and UCH-L1	GFAP and UCH-L1 not different vs ctrls at 7d; NfL elevated up to 4 months. Timing critical.
Chiollaz 2024 [27]	Prospective multicenter cohort n=285 paediatricTBI (≤24 h), n=74 controls	IL6, IL8, IL10	IL-6 and IL-10 significantly increased in mTBI vs controls. Within mTBI, IL-6 was higher in CT+ than CT- or observation groups. With sensitivity set at 100% (no CT+ missed), IL-6 specificity 48% for identifying CT-/observation; IL-8 not significant.
Kilinc 2025 [110]	Case-control (n=40 mTBI, 26 controls)	CGRP, PACAP-38, VIP, and SP	All increased in TBI, esp. CT+; potential emerging biomarkers.
Chiollaz 2025 [111]	Prospective multicenter cohort; (n=419 mTBI, n=99 controls (≤24 h)	IL6, NfL, NTproBNP, GFAP, IL10, S100b, and HFABP.	IL-6 was the strongest single marker: at 100% sensitivity, specificity 47.6%. Duplex panels: IL-6+NfL 61%, IL-6+NT-proBNP 60%, IL-6+GFAP 57% (all at 100% sensitivity). Age correlation: GFAP, IL-10 and S100B decreased with age; IL-6 and NT-proBNP were not age-dependent.
Wei 2025 [71]	Retrospective (n=532)	Broad lab panel, NT-proBNP, IL-6, CRP/Alb	Prognostic model (AUC ≈0.8) combining lab markers + ML approaches shows promise.

structural damage and secondary neuroinflammatory mechanisms, each associated with distinct temporal and molecular biomarker profiles [15,35,43,61,119-123]. This complexity is further compounded by biological variability [10,26,28,29,92,95], biomarkers extracranial origin (e.g. fracture, neurodegenerative disorders) (Table 2), biological matrix (e.g. plasma, serum, CSF, saliva) [3,12,56-65,92,114], sampling time (Tables 2-4), assay differences [95,96], and interference from autoantibodies or pharmacological agents [96,118,119] (Supplementary Material S5). Anticoagulants use usually result in a CT scan for TBI at medium and high risk; however, only the French recommendation limits CT scan in cases of medium and low levels of S100B, GFAP and UCH-L1 (Table 4).

Finally, although blood remains the preferred matrix, alternative fluids, particularly saliva, have emerged as promising in paediatrics due to their non-invasiveness and the possibility of repeated sampling. However, salivary biomarker analysis requires rigorous control of pre-analytical variables and a deep understanding of the biological mechanisms underlying secretion. Factors such as salivary flow rate, age-related variability, and swallowing dysfunction, common in children or adults with acquired brain injuries, may influence biomarker concentrations [124-126]. These aspects are further discussed in dedicated sections below.

Neuroanatomical and Functional Vulnerabilities in pTBI

The paediatric brain presents unique anatomical and physiological features that increase its vulnerability to TBI.

Factors such as a larger head-to-body ratio, underdeveloped neck musculature, higher water content, and lower myelination contribute to a different biomechanical response to trauma compared to adults [127]. As a result, injury patterns differ, with children exhibiting a higher proportion of diffuse brain injuries and different lesion topographies [127]. This increased prevalence of diffuse brain injuries in children is partly explained by their open basal cisterns, which allow for a different redistribution of traumatic forces [46].

Neuroimaging findings also differ significantly: while most acute lesions involve haemorrhages, injuries in children often affect extracerebral structures, including the skull and facial bones [127]. Despite clinical suspicion, CT scans are frequently negative, normal in up to 78% of paediatric cases, even when other injuries are present [55] (Supplementary material S2a). Skull fractures are reported in approximately 5% of mild and up to 50% of severe pTBI cases, and are associated with poorer clinical outcomes [128]. Beyond its traditional mechanical role in trauma, emerging evidence suggests that skull bone marrow may contribute to the neuroinflammatory response, potentially influencing secondary injury mechanisms [128] (Supplementary Materials S4). Moreover, mild TBI can disrupt age-related brain development, leading to long-term reductions in both grey and white matter volumes and associated neurocognitive impairments [129].

The brainstem, which coordinates vital functions such as respiration, blood pressure, sleep, and swallowing, is particularly susceptible to injury in children due to its

Table 4: Main recommendations using biomarker-based risk stratification of TBI to minimize unnecessary CT scans [36,37,96].

Author	CT Scan required	Biomarker use and its levels	Clinical criteria	Risk and Recommended action
French Guidelines [96]	NO	Not recommended	-GCS 15 -Asymptomatic patient without medium or high risk criteria	- Low risk. - Discharge patient with oral and written instruction for home monitoring
	NO	-S100B within 3 hrs -GFAP+UCH-L1 within 12 hrs -S100B < 0.10µg/L or GFAP and UCH-L1 < cut-off	-Trauma with high kinetic -Retrograde amnesia 30 min before injury -GCS<15 within 2 h post injury with intoxication -Age ≥65 yrs and antiplatelet therapy	- Medium risk. - Discharge patient with oral and written instruction for home monitoring.
	YES within 8 h	-S100B after 3 hrs GFAP+UCH-L1 after 12 hrs -S100B > 0.10µg/L or GFAP and UCH-L1 >cut-off	See above	-Medium risk. -If the first CT scan is normal, discharge patient with oral and written instruction for home monitoring
	YES within 1 h	Not recommended	-GCS<15 within 2 h post injury -Focal neurological deficits -Post traumatic convulsion -Clinical signs of skull fracture -Repeated vomiting -Anticoagulant intake or Antiplatelet therapy -Congenital haemorrhagic disease	-High risk. - Admission for observation ≥24 h -Consultation with neurosurgeon -Repeat CT scan if neurological and/or GCS deterioration
Scandinavian Guidelines [96]	NO	Not recommended	GCS 15	-Minimal risk -Discharge patient with oral and written instruction for home monitoring
	NO	-time injury-S100-B sampling < 6h -100B < 0.10 µg/L	-GCS 14 -GCS 15 + suspected/confirmed loss consciousness -GCS 15 + repeated vomiting (≥ 2 episodes)	-Low risk -Discharge patient with oral and written instruction for home monitoring
	YES	-Time injury-S100-B sampling > 6h -S100B > 0.10 µg/L	See above	-Mediun risk -If CT scan is normal, discharge patient with oral and written instruction for home monitoring
	YES	-Time injury-S100-B sampling > 6h -S100B > 0.10 µg/L	-GCS 14-15 and -Age ≥65 yrs and antiplatelet therapy	-Medium risk -If CT scan is normal, discharge patient with oral and written instruction for home monitoring
	YES	Not recommended	-GCS 14-15 and -Focal traumatic seizures -Clinical signs of depressed or basal skull fracture -Shunt-treated hydrocephalus -Therapeutic anticoagulants or coagulation disorders	-High risk -Admission for observation ≥24 h -Consultation with neurosurgeon -Repeat CT scan if neurological and/or GCS deterioration
Mavoudis et al. 2025 [36]	NO	-S100B<0.1 µg/L -GFAP within normal limits	-No neurological symptoms	-Low risk -Safe discharge with symptoms monitoring instructions and follow-up care if needed
	YES , if symptoms worsen or a history of multiple concussions	-Moderately elevated levels -GFAP> 626pg/mL -UCH-L1> 225 pg/mL	-Mild neurological symptoms	-Moderate risk -Closer observation and a clinical reassessment within a few hours.

	YES, immediate	-S100B significantly elevated -GFAP and UCH-L1 highly elevated	-Persistent or worsening neurological symptoms	-High risk -Possible hospitalization
	Not indicated	-Persistently elevated NfL and tau protein levels, those have been associated with chronic post-concussive symptoms and neurodegenerative risk	-Observation of persistent symptoms beyond four weeks	-Unknown risk -Neurology referral, cognitive rehabilitation, and long-term monitoring for long-term prognosis
Manley et al. 2025 [37]	Very low risk of CT - detectable intracranial injury with sampling up to 24hr after injury	GFAP -Upper reference range (97.5 th) in healthy adults: 51-71 pg/mL -Cut off : 22-65 pg/mL	Not indicated	Not indicated
		UCH-L1 -Upper reference range (97.5 th) in healthy adults: 157-459 pg/mL -Cut off : 327-400 pg/mL	Not indicated	Not indicated
		S100B -Upper reference range (95 th) in healthy adults: 0.105 µg/L -Cutoff : 0.105 µg/L	Not indicated	Not indicated

anatomical orientation [61]. This is clinically relevant, as brainstem damage may influence salivary secretion and the release of brain-derived biomarkers into saliva [81]. Cranial nerves (V, VII, IX, X, XII), which regulate salivation and swallowing, are affected in pTBI (cranial nerve injury: 6.5% (CI 5.0-8.3) and 4.7% in 1-15 yrs of age, respectively) and may contribute to secondary dysfunctions [130,131].

In children, blood–brain barrier (BBB) disruption typically affects the microvascular compartment [132], influencing the passage of molecules into the bloodstream and possibly saliva. Mechanisms allowing the passage of brain-derived molecules into saliva, such as cranial nerve transport, exosomal pathways, and the glymphatic system [5,15,22,25,81,82,87,133-137], are discussed in dedicated sections below and in more detail in Supplementary Material S2. Finally, age-dependent variability in cerebral blood flow may further modulate biomarker kinetics, although its precise impact on biomarker levels remains unclear [138].

Molecular Mechanisms of Injury and Inflammation in pTBI

Paediatric TBI triggers a multifactorial cascade of molecular events that contribute to progressive cellular damage beyond the initial mechanical insult [61,120]. These include excitotoxicity, calcium overload, oxidative stress, mitochondrial dysfunction, and neuroinflammation. Gene expression studies in animal models have revealed altered regulation of pathways involved in energy metabolism, signal transduction, cell adhesion, and transcription [4,7,120]. The characteristics of each fluid brain biomarker (Table 2)

influence its ability to reflect primary versus secondary injury mechanisms. Interpretation is further complicated by pre-analytical factors (e.g., sampling time), biological variability in paediatric patients (Table 3), analytical variability, and potential extracranial confounders (Table 2) [139]. A wide range of fluid biomarkers have been proposed for mTBI, including indicators of astrocytic injury (e.g., S100B, GFAP), neuronal and axonal damage (e.g., NSE, UCH-L1, α II-spectin, tau proteins, neurofilaments), blood–brain barrier disruption (e.g., occludin) [140], and neuroinflammation (e.g., IgA, ILs, MMPs, S100A12) [141]. Neuropeptides including CGRP, PACAP-38, VIP, and substance P were significantly elevated in young children with mTBI, particularly in those with CT-positive findings, suggesting diagnostic utility [110]. Emerging markers such as exosomes and miRNAs are under investigation, but currently lack specificity regarding injury severity or anatomical localization. Genetic factors, including single nucleotide polymorphisms in APOE, BDNF, COMT, and ion channel genes (e.g., CACNA1A, ATP1A2), may contribute to individual susceptibility and outcomes in pTBI [7,142]. The APOE genotype, particularly the ϵ 4 allele, has been associated with pTBI outcomes, although its prognostic impact appears time-dependent and may differ from that observed in adults [143,144]. While the molecular consequences of concussion and subconcussive impacts remain poorly defined, elevated levels of brain-enriched proteins such as NfL, GFAP, and autoantibodies, persisting months after injury, have been associated with long-term complications and may support extended monitoring [13]. Salivary IgA-related immune responses are also being explored as potential biomarkers of asymptomatic brain

injury [141]. A machine learning-based prognostic model for pTBI has been proposed, combining clinical parameters (e.g., GCS, pupillary response, location of the head haematoma) and a risk score based on laboratory indicators (e.g., LDH, NT-proBNP, pH, Hb, Alb, CRP/albumin ratio) [71]. Although coagulopathy is a recognized complication in TBI, paediatric data on the predictive value of biomarkers, such as copeptin, S100A12, the neutrophil to lymphocytes ratio, IL-33 and galectin-3, for coagulopathy and progressive haemorrhagic injury remain limited [139,145]. Beyond structural and metabolic pathways, inflammatory mediators have received increasing attention. Cytokines such as IL-1 β , IL-6, TNF- α , IL-10, and IFN- γ orchestrate both local and systemic immune responses involving resident CNS cells and circulating immune cells. In pTBI, the pro-inflammatory response appears more intense and prolonged than in adults. IL-6, in particular, has pleiotropic effects in the CNS, including modulation of acute-phase proteins, immune cell activation, blood–brain barrier permeability, and cerebral oedema through aquaporin-4 upregulation [4,146]. Recent studies have reported elevated serum levels of IL-6, IL-8, and TNF- α in pmTBI patients compared to healthy controls [27,147,148]. Extracellular vesicle-associated IL-6 was significantly increased within hours after injury in adolescent athletes [149]. Preliminary evidence also suggests that combining IL-6 with other biomarkers—such as NfL, GFAP, or NT-proBNP—may help rule out intracranial injuries, potentially reducing unnecessary CT scans and observation

stays [111]. Conversely, general inflammatory indices (e.g., systemic immune-inflammation index or neutrophil-to-lymphocyte ratio) currently lack validation in this context [106,150].

Saliva as an Emerging Matrix for TBI Biomarkers

Recent studies have explored the use of saliva as a matrix for detecting biomarkers of mTBI, both in adults and children (Table 5; Supplementary Material S6) [5,12,15,17-23,25,151-155]. In pTBI, the focus has been on brain-specific proteins such as S100B, GFAP, and UCH-L1, as well as non-specific biomarkers including Beclin1, IL-6, IL-8, D-dimer, miRNA, mitochondrial DNA, and soluble NCAM [15,92]. In adults, saliva has been investigated as a potential source for detecting cortisol, EVs, GFAP, NF-L, S100B, t-tau, UCH-L1, and CRP (Supplementary Material S6). Although these findings support the feasibility of salivary testing, the detectability of brain biomarkers in saliva varies markedly. This is partly due to differences in molecular weight, which affects their diffusion or transport from blood to saliva. For instance, GFAP and UCH-L1 show low saliva/blood ratios, whereas S100B reaches a ratio of approximately 0.8, indicating more efficient passage or different release mechanisms (Table 2). Notably, these differences are not solely attributable to molecular size, as local release from inflamed oral tissues, altered salivary flux, salivary gland AQP, and altered oral functions may also play a role as reported in details below.

Table 5: Recent evidence published on mTBI and pTBI using salivary biomarkers [5,12,15,17, 19-23,25,152,153].

Authors, publication yrs (ref)	Study	Patients and controls	Biomarkers	Saliva sampling, storage and methods	Main evidence
Ewing-Cobbs et al, 2017 [17]	prospective cohort study to evaluate post-traumatic stress after 6 months	55 children with TBI (8–15 yrs), 29 extracranial injury, 33 healthy controls; GCS:3-8:36%; 9-12: 11%; 13-15:53%	Cortisol, salivary α -Amylase (sAA)	Saliva sampling (by polyolefin swabs) before and after the TSST-C. Frozen samples (–20° C). Analytics using commercially available assay. Intra- and inter-assay coefficients of variation were, on average, less than 10% and 15% respectively.	Injured children showed higher cortisol; TBI children had elevated cortisol, adolescents elevated α -Amylase. Altered stress reactivity associated with PTSS.
Fedorchak et al, (2021) [19]	multicenter study	112 mTBI (8–24 yrs). Sample collection: $\leq$ 14 days post-injury $\geq$ 21 days post-injury	nc RNAs	Non-fasting saliva samples (n=505) collected using OraCollectSwabs; RNA sequencing	Machine learning model with 16 ncRNAs predicted persistent post-concussion symptoms (AUC 0.86). Combined ncRNAs, balance, cognition best predicted recovery.
Ebraimi et al, 2022 [20]	cross-sectional descriptive study	150 mTBI patients (mean 33 yrs; mostly adults)	Salivary α -amylase (sAA)	Unstimulated saliva sample (1-2 mL) collected immediately after patient arrival at ED. Use of amylase assay kit for serum	Higher salivary amylase in patients with CT abnormalities; no correlation with age, sex, or consciousness level.

Tabor et al, 2023 [153]	cohort study in paediatric ice hockey players.	233 ice hockey players; 165 baseline, 68 post-SRC; adolescents	cortisol	Saliva	Post- sport-related concussion athletes had significantly lower cortisol vs. baseline. Cortisol not correlated with symptoms, but females reported more and more severe symptoms.
Hicks et al., 2022 [22]	prospective multi-center study	251 concussion patients (mean age 18±7 yrs; 57% male)	22 salivary miRNAs	Saliva swabs collected in a non-fasting state. See details of molecular methods.	Identified 10 clusters; pathways involved adrenergic, estrogen, fatty acid metabolism, GABAergic, synaptic vesicle, TGF-β signaling.
Hiskens et al., 2022 [5]	systematic review	9 studies, 2018–2021, heterogeneous population (athletes, hospital patients, children, adults). 5 articles (pediatric population).	188 salivary miRNAs, 13 consistent across ≥2 studies	- by saliva expectoration into a container plus sample preservation (5 studies) - by designed sponge (3 studies) - both methods (1 study)	Heterogeneity precluded meta-analysis; 13 candidate miRNAs showed consistent directionality (e.g., let-7i-5p, miR-107, miR-181a-5p up; miR-182-5p, miR-26b-5p down).
Ewing-Cobbs et al, 2023 [21]	study with prospective cohort design	74 TBI, 35 EI, 51 controls (8–15 yrs), 7 months post-injury	Cortisol, salivary α-Amylase (sAA)	As in ref (17)	Altered sAA but not cortisol reactivity; sAA linked with emotion dysregulation and sex differences (greater in girls).
Kvist et al, 2023 [23]	small prospective study	28 pediatric mTBI (mean age 8 yrs), 30 controls	lectin-binding glycan	Saliva (1-2 mL), . collected at least 1 h after eating. saliva collection: - in children aged ≤4 years by using a syringe without a needle from the sublingual space in the mouth; - in patients >4 years by rinsing their mouths twice with pure water and then to spit saliva into a clean plastic cup. Lectin-bound glycan levels were measured by a biochemical glycan-binding analysis and by fluorescence.	Significant changes in 9 salivary glycans in TBI vs. controls; high inter-individual variability.
Mavroudis et al, 2023 [15]	review	12 studies, 83% published in 2020-2022.. only 5 articles concern the pediatric population.	S100B, NfL, miRNA, EVs	saliva	Research promising but insufficient; need validation. Pediatric data: S100B higher in TBI (AUC 0.675); salivary miRNAs (e.g., miR-27a-5p/miR-30a-3p ratio AUC 0.81); mixed findings for S100B in sports.
Feinberg C et al., 2024 [12]	review	29 studies, 1268 mTBI subjects	miRNA, cortisol, melatonin, others	saliva and urine	Identified 8 salivary and 2 urinary biomarkers with diagnostic/ monitoring potential.
Ciancaglini et al., 2024 [25]	a study with case-control design	14 severe pediatric TBI, 9 controls (mean ~6–10 yrs)	miRNA	Preliminary tap water rinse or oral hygiene regimen. Collection of sub-lingual saliva (by P-157 nucleic acid stabilization swabs) within 24 h, 24–48 h and >48 h after injury. Samples stored at -20 °C and processed by a Genomic Sciences Facility.	Clear separation of TBI vs. controls by miRNA profiles; specific miRNAs up- or down-regulated; temporal changes post-injury.

Miller et al, 2024 [152]	prospective cohort study	60 children (11–17 yrs) with Persistent Post- concussive Symptoms (PPCS) and controls	827 salivary miRNAs	Saliva (2 mL) to obtain 300 ng of extracted RNA. See paper for details.	13 miRNAs differed over time between PPCS vs. recovered children, suggesting prognostic role.
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Analytical, Biological and Clinical Challenges in Salivary Biomarker Use

For what has been shown so far, saliva appears to have genuine potential as a diagnostic fluid in the context of traumatic brain injury, offering a non-invasive and physiologically meaningful alternative to blood-based testing [81,82]. However, caution should be exercised when using saliva for TBI biomarker detection, as it is associated with a range of biological, pre-analytical and analytical confounders that may limit its clinical applicability (Supplementary Material S7) [79,99].

The Influence of the Oral Health Status

Biological variability includes oral health status, age, and sex. Conditions such as gingivitis and periodontitis alter salivary concentrations of proteins such as S100 proteins, GFAP, and NSE. For example, increased levels of salivary and GFC S100A8 and S100A9 have been found in individuals with active disease, whereas these proteins were downregulated in patients with gingivitis [156]. Accordingly, the expression of GFAP and amyloid beta peptides expression (which has antimicrobial effects on oral pathogens) is increased in GCF from patients with gingivitis and periodontitis [157,158]. Moreover, increased levels of albumin and haemoglobin subunits in GCF and saliva of patients with gingivitis and periodontitis are consistent with damage to the gingival epithelial barrier, as well as the well-known presence of blood residues after tooth brushing [81,82,159-161]. NSE, being present in neurons but also in erythrocytes and platelets, lacks specificity for TBI when measured in saliva from inflamed oral environments.

Salivary cytokine concentrations, including IL-1 β , IL-6, IL-8, and IL-10, have been shown to remain stable across age (4–18 years) and between sexes [162]. However, they are significantly influenced by periodontal health and salivary flow. Gingivitis, which is common during orthodontic treatment in children, leads to elevated levels of several cytokines [82]. Moreover, higher salivary flow rates are associated with lower cytokine concentrations, complicating their interpretation. A careful assessment of oral inflammatory status is therefore essential when interpreting salivary biomarkers in pTBI. Further studies are needed to better understand the possible links between oral microbial changes and cytokine levels in this context [85,86].

Regarding miRNAs, although several are promising as TBI biomarkers and appear unaffected by oral disease, their expression depends on collection timing, glandular

origin, epithelial cell desquamation, and oral microbiota [163-165]. Some miRNAs associated with post-concussive symptoms overlap with those implicated in oral cancers (let 7a-3p, miRNA 133 a-5p, miR 769-5p, miR21-5), orthodontic remodeling (Let 7a-3p), or cleft lip/palate, limiting their specificity [166,167].

Salivary Flux in Paediatric and Adult Population

Salivary flow rate varies with age, sex, body weight, type of stimulation, and neurological condition [81]. High flow rates are physiological in infants and typically decline with age [81]. Excessive drooling (>3.5 mL/min) becomes pathological after the age of 4 and may occur in children with cerebral palsy, TBI, or other neurodevelopmental disorders. In healthy children, stimulated saliva shows higher flow rates than unstimulated, with older children exhibiting greater differences [162]. The mean salivary flow rate was 0.8 ± 0.5 mL/min in children (7.8 ± 2.4 years) and 1.5 ± 0.8 mL/min in adolescents (15.1 ± 1.7 years) [162]. The median of unstimulated salivary flow rate was 0.87 (0.54, 1.11) mL/min for boys, 0.65 (0.37, 0.98) mL/min for girls and 0.76 (0.49, 1.05) mL/min overall [168]. Obesity is associated with a modest reduction in stimulated flow, particularly during adolescence [169].

Neurological damage affects salivary gland innervation: parasympathetic input drives secretion, while sympathetic tone regulates duct contraction [170]. In this context, chronic sialorrhoea, though relatively rare, may indicate underlying neurological conditions, including TBI [171]. The hypothalamic-pituitary-adrenal (HPA) axis may be altered post-TBI [4]. While data in paediatrics are limited, adult mTBI is associated with a transient increase in salivary cortisol, despite preserved circadian rhythm [103]. Long-term survivors of pTBI generally show normal or recovered HPA function. Chronic fatigue is frequently reported after TBI, but does not appear to be clearly associated with HPA axis dysfunction. In fact, morning salivary cortisone levels were higher in TBI survivors, who have a high prevalence of fatigue, compared to healthy controls.

Although rare, chronic sialorrhoea [172] presents with recognizable clinical signs, including facial skin maceration, oral infections, and respiratory or fluid balance disturbances.

Effects of Drugs

Pharmacological agents may influence biomarker detection through several mechanisms, including upregulation or suppression of protein synthesis, modulation of salivary gland function and flow, drugs [173]. Drugs like aspirin,

clomipramine, curcumin, and methamphetamine affect GFAP expression; others like olopatadine, cocaine, and dopamine have been reported to effect S100B or UCH-L1 expression and then could interfere with the detection of S100B or UCH-L1 in saliva (97) (Supplementary material S5).

Pre-Analytical Factors

The timing of sample collection is one of the main confounding factors in TBI biomarker analysis. Available data indicate substantial heterogeneity: samples are collected on admission in 30.4% of cases, within 6 hours in 10.1%, within 12 hours in 4.1%, within 24 hours in 28.7%, after 24 hours in 16.6%, and are unspecified in 10.1% of cases [92]. This wide variability, especially in delayed sampling (ranging from <30 minutes to 14 days), hinders the interpretation of biomarker kinetics, particularly for miRNAs, and makes it difficult to assess the effects of sex, diet, exercise, and circadian rhythm [5]. French guidelines recommend collecting serum samples within 3 hours for S100B and GFAP, and within 3 to 12 hours for UCH-L1 (Table 4; Supplementary Material S3) [96]. Other studies, such as Manley's, have reported meaningful levels even at 24 hours post-injury [37]. According to the Oris group, S100B, GFAP, and UCH-L1 are robust biomarkers with good pre-analytical stability in serum or plasma EDTA. S100B is stable at room temperature for up to 8 hours or refrigerated for 48 hours; GFAP and UCH-L1 remain stable for at least 3 days at 4–5°C, and for several months when frozen at -20°C or -80°C [9,62,95,96]. S100B tolerates up to five freeze–thaw cycles [174]. From an endogenous interference standpoint, S100B and UCH-L1 are unaffected by haemolysis due to their absence in erythrocytes, while GFAP may be susceptible [96]. IFUs (Instructions for Use) for tests such as Liaison® (CLIA) and Abbott's GFAP/UCH-L1 recommend avoiding haemolysis, lipemia, buffy coat contamination, and mechanical stress (e.g., vortexing) [175,176].

For saliva, stability data are scarce [9,62,95,96]. Salivary proteins may undergo modification (e.g., extensive proteolytic cleavage, partial deglycosylation, and protein-protein complex formation) and degradation by the oral microbiome. Importantly, common sample preservatives (e.g. sodium azide) may interfere with assays [82]. In addition to stability, sample collection protocols themselves remain poorly standardised. Collection methods, such as drool, swab, or unstimulated saliva, are heterogeneous and are seldom adapted to paediatric populations [82]. Important factors like circadian rhythm, flow rate, periodontal status, and, especially, centrifugation are often neglected. These aspects are less relevant in emergency settings for moderate/severe TBI, but are critical in research and clinical use of salivary biomarkers in pmTBI.

Analytics

A wide range of analytical methods has been employed

for the quantification of brain-specific biomarkers, particularly S100B, GFAP, and UCH-L1, primarily in research contexts [2-5,6,10,12,16,18-22, 31,57,82,92,152]. Available techniques for blood-based detection include ELISA (most commonly used), ECLIA, LIA, IRMA, IFMA, each with distinct analytical characteristics [9]. The Oris group provided a comparative overview of commercial platforms in current use, reporting information such as manufacturer, analyser type, sample volume, reaction time, detection technique, and decision thresholds [96]. The analytical variability of GFAP and UCH-L1 assays in serum or plasma has also been documented, including the impact of autoantibodies (e.g., anti-GFAP, anti-S100B), which may lead to overestimation due to analytical interference [62,96,9,25,26]. The analytical performances of commercially available assays for S100B, GFAP, and UCH-L1 have been extensively evaluated and are generally considered acceptable for clinical use. Detailed comparative analytical data are reported in Supplementary Material S8. Although assays from different manufacturers showed strong correlation, they did not exhibit complete agreement, with systematic differences in both measured concentrations and decision thresholds. To date, all manufacturer-approved methods for these brain biomarkers are validated for use in serum or plasma, not saliva. Nonetheless, several exploratory techniques have been applied to saliva in pTBI, including immune enzymatic assays, proteomics, DNA fingerprinting, and RNA sequencing. However, the stability of biomarkers in saliva, especially when entrapped in EV, remains uncertain—as does the influence of salivary pH compared to serum (Supplementary Material S7). Currently, cortisol is the only salivary test that is CE-IVD marked and validated for clinical use. Other salivary assays—such as those for α -amylase, CRP, IL-1, IL-6, cytokine panels, secretory IgA, and markers of blood contamination like transferrin—remain confined to the research setting, with no regulatory approval for diagnostic application [82].

Additional Challenges in the Use of Saliva for Biomarker Assessment of pTBI in Clinical Settings

Here, we aim to discuss other factors that could influence specificity. Currently, the specificity is insufficient at 45% for S100B, 11–41% for GFAP and UCH-L1, even when serum samples are used [96,177]. Beyond analytical and pre-analytical challenges (Table 2; Supplementary Material S5), the interpretation of salivary biomarkers for pTBI is further complicated by a series of additional biological and physiological factors (Table 3-5; Supplementary Material S3). These include the potential extracranial origin of brain proteins [15,31,66], embryological overlap between neural and salivary tissues, protein release and clearance mechanisms, as well as individual variables such as periodontal status, renal function, or local inflammation (Table 6) [82,86,95,96,99,122,137,174,177-195] (Supplementary

Material S9). Taken together, these elements introduce substantial variability and may confound the diagnostic interpretation of salivary measurements in both research and clinical settings.

Experimental biomarkers: exosomes and non-coding RNAs

Non-coding RNAs (ncRNAs), particularly miRNAs, are highly expressed in the nervous system and play key roles in neuronal physiology, including protein regulation, synapse maturation, and neural circuit formation [5,57,66,68,100,149,196-199]. Their detection within EVs — including exosomes — is being explored in TBI as a means to monitor injury mechanisms and recovery [68,100,200]. EV may include brain-specific proteins (e.g., GFAP, NF-L, UCH-L1), nucleic acids, and metabolites, potentially reflecting the cell of origin and ongoing pathological processes. However, clinical use remains experimental due to challenges in EV isolation, assay standardisation, and uncertainties about BBB passage [163,201]. In pTBI, elevated levels of exosomal GFAP and neurofilament light chains have been reported, along with marked changes in salivary

EV RNA profiles [57,121]. In particular, upregulation of complement system mRNAs (e.g., C1QB, C4A, C1QA, C1S) has been observed in patients with acute post-traumatic headache, suggesting that salivary EV analysis may help monitor mTBI complications [54,202].

Conclusion

While the evidence surrounding blood and salivary biomarkers, particularly exosome miRNAs, in the diagnosis and management of pTBI is promising, current data remain insufficient to fully elucidate their roles. Saliva, especially in pediatric populations, offers distinct organizational advantages, including non-invasiveness and the potential for repeated sampling. However, further research is needed to establish standardized protocols and clarify whether salivary biomarkers simply reflect molecules already present in the blood, detect brain-derived molecules earlier than blood tests, or identify molecules that do not appear in blood at all. The biological significance of salivary biomarkers in pTBI remains unclear, and current findings do not provide a complete understanding of the extent to which salivary markers can enhance diagnostic precision. Furthermore, there

Table 6: Additional biological and physiological factors influencing salivary biomarker interpretation [82,86,95,96,99,122,137,139,174,177-195].

Factors	Key points and Rationale
Extracranial origin of brain proteins	S100B and other proteins can derive from extracerebral tissues (e.g., adipose tissue, melanocytes), reducing specificity [177]. Elevations may also occur after fasting, exercise, trauma without brain injury, or surgery (95). Despite this, increases after TBI mainly reflect BBB disruption [178].
Embryological overlap between neural and salivary tissues	Shared neural crest origin explains basal presence of GFAP, UCH-L1, S100B, RAGE, and AQPs in salivary glands [96]. Expression varies across glands and developmental stages (Supplementary Materials S9) [179].
Protein release and clearance mechanisms	GFAP, UCH-L1, NSE are released with cell damage; S100B also has extracellular functions. Entry into blood occurs via glymphatic flow, BBB disruption, or RAGE-mediated transport. Cytokines may further modulate permeability [99]. Similar tight-junction mechanisms regulate salivary glands [180]. Genes involved in brain protein transport or clearance (RAGE, AQP-4, AQP-4-AS1) are expressed in all salivary glands [179]. AQP-4 is localised in myoepithelial cells around salivary lobules and ducts, with reduced expression in primary Sjögren's syndrome [181,182].
Aquaporin expression in brain and salivary glands	AQP4 clears proteins in brain; AQP5 regulates saliva secretion. Expression ratios change with development and inflammation [179]. AQP genes (3, 4, 5, 8, AQP4-AS1) are linked to cerebral edema, salivary dysfunction, and neuropsychiatric conditions [183-186]. Their role in TBI remains under investigation.
Half-life of TBI biomarkers	In blood, half-lives range from 7–36 h; in saliva they are unknown. Simulations suggest rapid S100B kinetics (peaks within 0.2–0.4 h) [178]. Stability may differ for free vs. EV-bound proteins, and is influenced by proteolysis and oxidative stress. Only in blood, three biomarker trajectories have been described: persistently high, persistently low, and reversal of decline (the latter predicting deterioration) [122].
Individual factors	
Renal function	Impaired renal clearance, especially in pediatric abusive TBI, elevates serum S100B. GFAP and UCH-L1 show poor correlation between serum and urine, suggesting limited renal elimination [99,137,139,187-189].
Periodontal disease	Gingivitis and periodontitis allow serum proteins to leak into saliva (82,159) and the first one affects up to 70% of those over seven years (190). Periodontal pathogens (e.g., <i>Porphyromonas</i> , a key driver of periodontal disease) can disrupt BBB and promote neuroinflammation [191-194]. Oral microbiome changes (<i>Lactobacillus</i> , <i>Saccharomyces</i> , <i>Micrococcus</i>) have been linked to symptom burden in pediatric mTBI [86].
Local inflammation	Oral infections may downregulate AQP5, impairing salivary secretion and contributing to neuroinflammation and neurodegeneration [195].

is a pressing need to investigate the impact of biological, pre-analytical, and analytical variability on salivary biomarker levels, including the influence of salivary flow rate, oral health, and circadian rhythms, which may all play a role in the variability observed in both clinical and research settings. To move forward, well-designed studies are required to establish robust pediatric reference intervals and cut-off values for salivary biomarkers, which remain a key area of uncertainty. Additionally, personalized monitoring of concussion in athletes or individuals with chronic conditions should be further explored to optimize the clinical utility of these biomarkers. The development of reliable serum and salivary tests for routine clinical use is still in its early stages, and cross-platform comparisons remain challenging. Emerging technologies such as wireless biosensors and AI-generated feature clusters for diagnostic, prognostic, and therapeutic applications hold great promise but require further validation. As research progresses, it is essential that both pre-analytical and analytical variabilities are well understood and controlled to ensure that future recommendations in clinical practice are evidence-based and applicable across diverse healthcare settings.

Statements

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Author's contribution

Conceptualization, LB, MV; data curation, LB, AB, MV; form analysis, LB, AB, MV; funding acquisition, LB; investigation, LB, M.V; methodology, LB, AB, M.V; project administration, LB; resources LB; supervision, MV; validation, LB, AB, MV; writing-original draft, LB; writing-review and editing, LB, M.V.

Declaration on the use of AI

The authors declare that they have not used AI-tools for writing and editing the manuscript.

Consent for publication

All authors have read and agreed to the published version of the manuscript.

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Supplementary file:

<https://cdn.fortunejournals.com/supply/DROH13502-2nd-version-supplementary-materials-S1-S9.pdf>