National Institute for Health and Care Excellence

Final

Head Injury: assessment and early management

[G] Evidence reviews for brain injury biomarkers for predicting acute post-brain injury complications

NICE guideline NG232

Evidence reviews underpinning a research recommendation in the NICE guideline

May 2023

Final

Developed by National Institute for Health and Care Excellence

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1.1 Review questions

What is the diagnostic accuracy of brain injury biomarkers for predicting acute post-brain injury complications?

What is the clinical and cost effectiveness of biomarkers when followed by the appropriate treatment for acute post-brain injury complications to improve patient outcomes?

1.1.1 Introduction

The potential use of fluid biomarkers in the identification of patients with acute complications, defined here as the presence of intracranial injury on cross sectional imaging, was reviewed in this update. This is an area of significant interest for researchers with a wide variety of different protein and more recently also non-protein biomarkers the subject of investigation in blood and other fluids.

Biomarkers of traumatic brain injury could offer the opportunity to streamline the care of patients by assisting in separating those who would benefit from imaging and admission from the vast majority who will have normal imaging and could be appropriate for discharge. Likewise, developments in analytical methods mean there may be potential for some patients to avoid hospital attendance at all if biomarkers can be sampled and analysed in the pre-hospital setting. Overall, this could result in a significant improvement in the patient journey and emergency department workflow pressures.

1.1.2 Summary of the protocol

For full details see the review protocol in Appendix A.

Table 1: PICO characteristics of review question – Diagnostic accuracy

Population	Inclusion: Infants, children and adult with suspected traumatic brain injury (TBI) Strata: • Adults (aged ≥16 years) • Children (aged ≥1 to <16 years) • Infants (aged <1 year) Mixed population studies will be included but downgraded for indirectness. Cut-off of 60% will be used for all age groups Exclusion: Adults, and children (including infants under 1 year) with superficial injuries to the eye or face without suspected or confirmed head or brain injury.
Target condition	Acute post-brain injury complications
Index tests	• Biomarkers ○ Blood biomarkers - S100 calcium binding protein B (S100B)

	-Ubiquitin C-terminal Hydrolase-L1 (UCHL1) -Neuron Specific Enolase (NSE) -Brain-derived neurotrophic factor (BDNF) -Neurofilament light (NFL) - Neurofilament Heavy (NF-H) - αII-Spectrin breakdown products (SBDP) - Myelin basic protein (MBP) - glial fibrillary acidic protein (GFAP) ○ Salivary biomarkers -salivary microRNAs (miRNAs) -Extracellular vesicles (EVs) -S100B ○ Urine biomarkers -Extracellular vesicles (EVs) Each test must be followed by an appropriate treatment for complication after brain injury Treatment: Admission to hospital for observation + possible neurosurgical management of TBI Timings: Ideally biomarkers to be done before CT. Include studies if biomarkers done after CT but downgrade it for indirectness.
Reference standard	Intra cranial injury and/or complex skull fracture on CT/MRI
Outcomes	• diagnostic accuracy of biomarkers for predicting acute post-brain injury complications Diagnostic accuracy to be reported by test sensitivity/specificity
Study design	Cross-sectional studies Cohort studies (prospective and retrospective) Systematic reviews and meta-analyses of the above

Table 2: PICO characteristics of review question – Test and treat

Population	Inclusion: Infants, children and adult with suspected or confirmed head injury Strata: • Adults (aged ≥16 years) • Children (aged ≥1 to <16 years) • Infants (aged <1 year) Mixed population studies will be included but downgraded for indirectness. Cut-off of 60% will be used for all age groups Exclusion: Adults, and children (including infants under 1 year) with superficial injuries to the eye or face without suspected or confirmed head or brain injury.
Intervention	Biomarkers ○ Blood biomarkers - S100 calcium binding protein B (S100B) -Ubiquitin C-terminal Hydrolase-L1 (UCHL1)

	-Neuron Specific Enolase (NSE) -Brain-derived neurotrophic factor (BDNF) -Neurofilament light (NFL) - Neurofilament Heavy (NF-H) - αII-Spectrin breakdown products (SBDP) - Myelin basic protein (MBP) - glial fibrillary acidic protein (GFAP) ○ Salivary biomarkers -salivary microRNAs (miRNAs) -Extracellular vesicles (EVs) -S100B ○ Urine biomarkers -Extracellular vesicles (EVs) Each test must be followed by an appropriate treatment for complication after brain injury. Treatment: Admission to hospital for observation + possible neurosurgical management of TBI Timings: Biomarkers are used prior to decision for imaging or within 24 hours of injury. Biomarkers will guide the decision to image or not.
Comparison	Comparators: To usual care (no testing with biomarkers) To each other
Outcomes	All outcomes are considered equally important for decision making and therefore have all been rated as critical: • Quality of life - 3 months or more • Objectively applied score of disability e.g. Glasgow Outcome Score (GOS) or extended GOS - at 3 months or more • Time to return to education/work/usual activities Duration of post-injury complications
Study design	Randomised controlled trials (RCTs), systematic reviews of RCTs. If no RCT evidence is available, non-randomised studies will be considered if they adjust for key confounders, starting with prospective cohort studies. Key Confounders: Age Gender GCS (Glasgow Coma Scale) or pupillary response at presentation

1.1.3 Methods and process

This evidence review was developed using the methods and process described in Developing NICE guidelines: the manual. Methods specific to this review question are described in the review protocol in appendix A and the methods document.

Declarations of interest were recorded according to NICE's conflicts of interest policy.

1.1.4 Diagnostic evidence

1.1.4.1 Included studies

A search was conducted for cross-sectional studies and cohort studies (prospective and retrospective) assessing the diagnostic test accuracy of brain injury biomarkers for predicting acute post-brain injury complications in people with suspected traumatic brain injury (TBI).

Sixty-four studies [cross-sectional and prospective/retrospective cohort studies] were included in the review;[1-3, 5-9, 11, 13-44, 46-49, 51-55, 57, 59-67] these are summarised in Table 2 below. Evidence from these studies is summarised in the clinical evidence summary below in Table 5.

Population

Majority of the included studies were in people with mild TBI (GCS score 13-15). Studies with mixed severity populations (mild/moderate/severe TBI) are included but have been downgraded for indirectness as acute post-brain injury complications are most relevant to those with mild TBI. Most studies reported inclusion of people with mild TBI with extracranial injuries.

There were 47 studies in adults and 13 in infants/children. There were some studies with mixed population (adults and children/children and youth); proportion of adults, youth and children were not reported in these papers. These studies were included in adult or children strata based on the reported mean (SD) age. Studies with mixed population have been downgraded for indirectness. Two studies did not report age of the participants.

Index tests

In adults most of the studies assessed diagnostic accuracy of serum S100 calcium binding protein B (S100 B), other biomarkers assessed were urinary S100B, glial fibrillary acidic protein (GFAP), glial fibrillary acidic protein and break down products (GFAP-BDP), Ubiquitin C-terminal Hydrolase-L1 (UCHL1), neuron-specific enolase (NSE), Neurofilament light (NFL), small neuronal protein neurogranin (NRGN), phosphorylated NFL-H (pNFL-H) and Interleukin 10 (IL-10).

No relevant studies investigating the effects of the following biomarkers were identified in adults: brain-derived neurotrophic factor (BDNF), Neurofilament Heavy (NF-H), αII-Spectrin breakdown products (SBDP), Myelin basic protein (MBP) and salivary biomarkers.

In children studies assessed diagnostic accuracy of serum S100 B, urinary S100B, GFAP, NSE and UCHL-1.

No relevant studies investigating the effects of the following biomarkers were identified in children: Neurofilament light (NFL) brain-derived neurotrophic factor (BDNF), Neurofilament Heavy (NF-H), αII-Spectrin breakdown products (SBDP), Myelin basic protein (MBP), urinary biomarkers and salivary biomarkers.

Timing of blood sampling in studies ranged from 0-32 hours after injury. Most studies reported testing within 3 hours or 3-6 hours after injury.

It was agreed that studies will be pooled based on timing of tests post- injury: 0-3 hours after injury, >3 to 6 hours after injury, >6 -12 hours after injury, >12-24 hours after injury.

Only 2 diagnostic meta-analyses (serum S100 B at 0-3 hours after injury and serum S 100B at >3 to 6 hours after injury) were possible because at least 3 studies are required for a valid pooling of results, and for all other index tests only one or two studies were available. Some

studies reported serum S100 B tests within 0-6 hours after injury, these have been pooled in the strata 0-3 hours or >3-6 hours after injury based on the median time of testing.

All studies reported biomarker testing before CT/MRI.

There was variation in thresholds used for different biomarker tests in the studies. Studies assessing diagnostic accuracy of serum S100B with thresholds 0.10 and 0.105 µg/L were pooled as the thresholds were considered to be close enough to be combined. Studies with all other different biomarkers and thresholds have been analysed/reported separately.

Reference standard

All studies reported CT as reference standard except for 4 studies which also used MRI along with CT as the reference standard (Romner 2020- CT and MRI, Gill 2018- CT and MRI, Linsenmaier, 2016 -CT and MRI, Oh 2007- CT or MRI).

Timing of reference standard: some studies reported that reference standard was done soon after/within the same time frame as biomarker testing, whereas in some studies there was a time interval between biomarker testing and reference standard for example biomarker test within 6 hours and CT within 24h after injury. Many studies did not report exact timing of the reference standard.

Outcomes

There was variation in definition of outcomes reported in papers, with some reporting intra cranial injury/lesions only and others reporting intracranial lesions with complex skull fracture on CT/MRI. All outcomes have been extracted and analysed.

Follow-up/treatment for complication after brain injury

Only a few studies reported on follow-up/observation/ treatment for complication after brain injury, this information where available has been included in the evidence tables.

Outcome measures/statistical measures

Sensitivity and specificity were identified by the committee as the primary measures in guiding decision-making. Clinical decision thresholds for both sensitivity and specificity were set by the committee, at 90% (above which a test would be recommended) and 60% (below which a test is of no clinical use).

Sensitivity and specificity were both considered to be equally important. Biomarker testing is the first stage of a two-step process, followed by CT/MRI if indicated by a positive test. The need for the index test to have a very few false negatives was considered to be important so as to avoid anyone with intracranial injury/lesion exiting at first stage prematurely. Specificity was considered to be important as false positives would mean people who do not have intracranial injury/lesion would receive unnecessary radiation (particularly for children).

Diagnostic test and treat

There was no evidence identified for test and treat component of this review.

1.1.4.2 Excluded studies

See the excluded studies list in Appendix I.

See also the study selection flow chart in Appendix C and study evidence tables in Appendix D.

1.1.5 Summary of studies included in the diagnostic evidence

Table 3: Summary of studies included in the evidence review- Adults

Study	Population	Index test	Reference standard	Outcome	Comments
Asadollahi, 2016 [1] Prospective cohort Iran.	n = 158 Age, mean (SD): 35.5 (15.8) years Gender (male): 69.6% GCS: breakdown of GCS not reported but mild traumatic brain injury (TBI) defined as GCS score 13-15 for inclusion in the study 9 (5.7%) on anticoagulation Setting: adult ED of a teaching hospital Inclusion criteria: isolated mild traumatic brain	Serum S100B measured at 3 and 6 hours post injury	CT scan within 6 hours post injury.	Positive CT scan (at least one trauma-relevant lesion (epidural, subdural, subarachnoid, intracerebral haemorrhage, cerebral contusion, brain oedema, depressed skull fracture))	Follow up: no mention of follow up but states that 2 participants underwent neurologic deterioration and needed surgical treatment

Study	Population	Index test	Reference standard	Outcome	Comments
	injury (TBI) (GCS score 13-15 with loss of consciousness (LOC) <30 minutes and post traumatic amnesia (PTA) <1 hour;) >18 years of age; presented to the ED within 2 hours after the injury				
Bazarian, 2006 [4] Nested cohort	n = 96 Age, mean (SD): 35.9 (19.5) years, range 8-79 years, median 39.5 years Gender (male): 62.5% GCS score 15: 91.7% GCS score 14: 5.2% GCS score 13: 3.1% Ethnicity: 100% white Setting: emergency department of the	Serum S100B measured within 4 hours of injury Serum S100B measured within 4 hours of injury, correct for creatine kinase	Initial head CT Follow up: assessment of post-concussive symptoms using the Rivermead Post Concussion Questionnaire via telephone at 3 months after ED visit	Traumatic abnormality on initial CT scan – Serum S100B within 4 hours Specificity (when sensitivity set at 70%)	

Study	Population	Index test	Reference standard	Outcome	Comments
	University of Rochester School of Medicine Country: USA Inclusion criteria for this study: consent for blood draw for analysis in the ED; head CT scan performed in ED as part of clinical care; completed 3-month follow up Inclusion criteria for the larger NIH study met case definition for mild traumatic brain injury (TBI)(blow to the head or acceleration/deceleration movement of the head resulting in one or more of the following: loss of consciousness <30 minutes; amnesia <24 hours or any alteration in mental state at the time of				

Study	Population	Index test	Reference standard	Outcome	Comments
	injury); GCS score >13 measured ≥30 minutes after injury				
ALERT-TBI Bazarian, 2018 ; Bazarian, 2021 [5 7] Prospective cohort	n = 1959 (results reported separately for n=1920 with GCS score 14-15) Age, mean (SD): 48.9 (20.9) years, range 18-98 years Gender (male): 57% GCS score 15: 93% GCS score 14: 5% GCS score 13: 1% GCS score 12: 1% GCS score 11: <1% GCS score 10: 0% GCS score 11: <1% Ethnicity: white 70%; black or African American 27%; Hispanic 5%; other/unknown 4% Setting: ED at 22 sites	Index test: Combined serum UCH-L1 and GFAP measured within 12 hours post injury (cut-off concentration values of 327 pg/mL for UCH-L1 and 22 pg/mL for GFAP)	Reference standard: CT scan within 12 hours post injury.	Positive CT scan (presence of one or more of the following injuries: acute epidural haematoma, acute subdural haematoma, indeterminate extra-axial haemorrhage, intraventricular haemorrhage, parenchymal haematoma, petechial haemorrhagic or bland sheer injury, subarachnoid haemorrhage, brain oedema, brain herniation, non-haemorrhagic contusion, ventricular compression, ventricular trapping, cranial fractures, depressed skull fractures, facial fractures, scalp injury, or skull base fractures).	

Study	Population	Index test	Reference standard	Outcome	Comments
	Country: USA (15) and Europe (7) Inclusion criteria: ≥18 years of age; presenting to ED with suspected non-penetrating TBI resulting from external force; GCS score 9-15; underwent a non-contrast head CT scan within 12 hours of injury; blood sampling within 12 hours of injury; informed consent				
Bazarian, 2013 [6]; Jones 2020 [30]	n = 787 with mild traumatic brain injury (TBI) Age, mean (SD): 38.2 (19.5) Gender (male): 63.5%	Index test: Serum S100B within 6 hours post injury (cut-off >0.29 µg/L) Serum S100B within 6 hours post injury (cut-off >0.06 µg/L) Serum S100B within 6 hours post injury (cut-off >2.391 µg/L)	Reference standard: CT scan.	Presence of intracranial abnormalities. Traumatic CT abnormalities defined as subdural hematomas (SDH), epidural hematomas, subarachnoid haemorrhage (SAH), oedema, skull fracture, and cerebral contusions.	

Study	Population	Index test	Reference standard	Outcome	Comments
	GCS score 15: 89.2% GCS score 14: 6.5% GCS score 13: 1.3% GCS score 13-15": 2.5% Ethnicity: Hispanic/Latino 4.4%; not Hispanic/Latino 92.2%; refused/missing 3.3% Setting: 6 EDs Country: USA Inclusion criteria: ≥1 year of age; met study definition of mild TBI (blow to the head or rapid acceleration/deceleration resulting in at least one of the following: a loss of consciousness (LOC) ≤30 minutes, posttraumatic	Serum S100B within 6 hours post injury (cut-off >0.097 µg/L) Serum S100B within 6 hours post injury (cut-off >0.521 µg/L) Serum S100B within 6 hours post injury (cut-off >0.1 µg/L)			

Study	Population	Index test	Reference standard	Outcome	Comments
	amnesia ≤24 h, neuropsychological abnormality (any transient period of confusion, disorientation, or impaired consciousness; in children ≤2 years old: irritability, lethargy, or vomiting post-injury), or neurological abnormality (seizure acutely after injury, hemiplegia, or diplopia)); GCS score ≥13 within 30 minutes of injury; informed consent				
Biberthaler, 2006 [8] Prospective multi centre cohort study	n = 1309 with minor head injury Age, median (interquartile range): 47 (32-65) years Gender (male): 65% GCS: no breakdown but GCS score 13-	Index test: Serum S100B The median interval between trauma and blood sampling was 60 min (range, 40-80 or 25%-75%)	Reference standard: Cranial CT (CCT) Median interval between blood sampling and CCT scan was 30 min (range, 16-52 or 25%-75%).	CCT-positive (patients with at least one of the pathophysiological trauma-relevant findings (haemorrhage, epidural, subdural, sub arachnoidal, intracerebral, ventricular, cerebellar, brainstem, cortex contusion, haemorrhagic, non-haemorrhagic))	Treatment: 11 individuals required immediate neurosurgical intervention such as implantation of an intraventricular catheter for drainage of cerebrospinal fluid or decompressive craniotomy.

Study	Population	Index test	Reference standard	Outcome	Comments
	15 for inclusion in the study Ethnicity: not reported Setting: 3 trauma centers Country: Germany Inclusion criteria: history of isolated head trauma and admission within 3 hours; GCS score of 13 to 15 upon admission; one or more of 10 clinical risk factors (brief loss of consciousness, post-traumatic amnesia, nausea, vomiting, severe headache, dizziness, vertigo, intoxication, anticoagulation, age above 60 years)				

Study	Population	Index test	Reference standard	Outcome	Comments
Biberthaler, 2001 [9] Prospective cohort	N=52 Age, mean (SD): not reported Gender (male): 73% GCS: no breakdown but GCS score 13-15 for inclusion in the study Ethnicity: not reported Setting: single ED Country: Germany Inclusion criteria: presented to ED with a history of isolated MHT; GCS score 13-15 at admission; at least one of the following symptoms: amnesia, loss of consciousness (LOC), nausea,	Index test: Serum S100B at admission (cut-off 0.1 µg/ml) The interval between trauma and admission was 73.46 (47) minutes; and the interval between trauma and blood sampling was 116 (18.8) minutes.	Reference standard: Spiral cranial CT scan within 6 hours post injury.	Pathologic findings (intracerebral haemorrhage, skull fracture, or diffuse brain swelling) on CT scan	

Study	Population	Index test	Reference standard	Outcome	Comments
	vomiting, vertigo, or severe headache				
Biberthaler, 2002 [10] Prospective cohort	n = 104 Age, mean (SD): not reported Gender (male): not reported GCS: no breakdown but GCS score 13-15 for inclusion in the study Ethnicity: not reported Setting: single ED Country: Germany Inclusion criteria: presented to ED with a history of isolated MHT; GCS score 13-15 at admission; at least one of the following symptoms: transient loss of	Index test: Serum S100B Plasma S100B Blood sampling within 2 hours of injury	Reference standard: Cranial CT scan	Positive CCT scan (diffuse injury I-IV, evacuated mass lesion and non-evacuated mass lesion)	Results reported for two different test systems

Study	Population	Index test	Reference standard	Outcome	Comments
	consciousness (LOC) <5 minutes, amnesia for the traumatic event, nausea, vomiting, vertigo and severe headache; interval below 2 hours between traumatic event and blood sampling				
Cervellin, 2012 [14] Prospective cohort	Age, mean (range): 58 (14-80) years Gender (male): 68% GCS: no breakdown reported but GCS score 14-15 for inclusion in the study Ethnicity: not reported Setting: EDs of a single hospital Country: Italy	Index test: Serum S100B measured within 3 hours post injury	Reference standard: CT scan performed 30 minutes from blood collection.	Positive CT scan (any intracranial pathology associated with an injury (acute subdural, epidural or parenchymal hematoma, traumatic subarachnoid haemorrhage, cerebral contusion and brain swelling))	

Study	Population	Index test	Reference standard	Outcome	Comments
	Inclusion criteria: 14-80 years of age; presenting at the ED with a history of MHI requiring CT scanning according to local guideline (criteria included GCS score 14-15)				
Cevik, 2019 [15] Cross-sectional study	N=48 Mixed adults and children. Mean age (Adults). Not reported proportion of adults and children. Age, mean (SD): 24 ± 22 (range, 5–65) years Gender: 48 patients [38 (79%) males and 10 (21%) females] with “pure” mild TBI GCS:	Index test: Serum biomarkers: - S100 beta (S100B) - glial fibrillary acidic protein (GFAP) - small neuronal protein neurogranin (NRGN) Venous blood samples were collected within the first 4 h following the trauma	Reference standard: CT	Abnormal cerebral CT findings The primary end point of the study was to investigate the relationship between the levels of biomarkers such as S100B, GFAP and NRGN in patients with mild head injury in the first 4 h after the trauma with abnormal traumatic CT findings.	Mixed population (adults and children). No information on follow-up/treatment reported

Study	Population	Index test	Reference standard	Outcome	Comments
	The Glasgow coma score (GCS score) for all patients was 14–15 GCS score -15: 39 (81.25%) GCS score -14: 9(18.75%) Setting: ED Country: Turkey Inclusion criteria: People with mild TBI				
Chen, 2022 [16] Prospective cohort study	n = 644 (mostly mild TBI) Out of 644 patients, 52 had a Glasgow Coma Scale (GCS) score <13 n=462 with blood samples (Analysed) No-OMEI (other major extra- cranial	Index test: Index test: Distribution of time from injury to ED admission/ blood draw spanned 0–6 h with a median at 1 h -glial fibrillary acidic protein (GFAP) -ubiquitin C-terminal hydrolase-L1 (UCH-L1)	Reference standard: Head CT	Acute brain injuries	Adults No information on treatment

Study	Population	Index test	Reference standard	Outcome	Comments
	injuries) sub-cohort (n = 245) Age, mean (SD) years: With blood sample:50.7±22.7 Gender (male): 286 (61.9%) GCS score: 14 (14-15) Country: USA Setting: ED Inclusion criteria for this study: Adult patients (> 18 years old) transported by ambulance or helicopter, for whom a trauma alert was triggered and who underwent a non-contrast head CT seeking care for suspected TBI, were enrolled	-S100B The pre-specified cut-off values of GFAP, UCH-L1, and S100B were 22 pg/mL3, 327 pg/mL3, and 105 pg/mL13, respectively. A negative test result referred to markers falling at or below their pre-specified cut-off value, whereas a positive test result indicated that markers exceeded their pre-specified cut-off value.			
Czeiter, 2020 [17] CENTER-TBI	n = 2867	Index test:	Reference standard: Head CT scan	Positive CT scan (presence of any	

Study	Population	Index test	Reference standard	Outcome	Comments
Prospective cohort study	Age, median (interquartile range): 49 (30-66) years Gender (male): 67.9% GCS score 15: 52.1% GCS score 13-14: 15.9% GCS score 9-12: 7.7% GCS score 3-8: 21% Setting: 65 clinical sites, patients stratified by care path (emergency department, hospital admission and intensive care unit) Country: 17 European countries and Israel Inclusion criteria: all severities of TBI;	Serum S100B measured within 24 hours post injury Serum neuron-specific enolase (NSE) measured within 24 hours post injury Serum GFAP measured within 24 hours post injury Serum UCH-L1 measured within 24 hours post injury Serum neurofilament protein-light (NFL) measured within 24 hours post injury		traumatic intracranial abnormality; skull fractures in isolation were not considered as intracranial abnormality)	

Study	Population	Index test	Reference standard	Outcome	Comments
	presenting within 24 hours of injury and scheduled for CT scanning				
David, 2017 [18] Prospective cohort	n = 308 Age, mean (SD): 79.1 (10.5) years Gender (male): 49% GCS score 15: 97.4% GCS score 13-14: 2.6% All participants were on antithrombotic medication Setting: ED of a single teaching hospital Country: France Inclusion criteria: ≥18 years of age;	Index test: Serum S100B measured within 6 hours post injury (cut-off 0.105 µg/L)	Reference standard: Cranial CT scan performed within 24 hours post injury	Positive CT scan (any trauma related intracranial haemorrhage, including epidural, subdural or subarachnoid haemorrhage, or intracerebral bleeding (petechial haemorrhage, contusion or hematoma))	All study participants were on antithrombotic medication – limited applicability to the wider review population.

Study	Population	Index test	Reference standard	Outcome	Comments
	pre-injury antiplatelet and/or anticoagulant use; mild blunt head trauma (any blunt head injury regardless of loss of consciousness or amnesia)				
Diaz-Arrastia, 2014 [19]Multicentre prospective cohort study (Transforming Research and Clinical Knowledge in Traumatic Brain Injury [TRACK-TBI]).	n = 206 Age, mean (SD): 42 (18) years, Gender (male): 73% GCS: majority of subjects (83%) were classified as having had mild TBI (admission GCS score 13–15), 4% as having had a moderate TBI (GCS score 9–12), and 13% as having had a severe TBI (GCS score 3–8).	Index test: - Serum Ubiquitin C-terminal hydrolase L1 (UCH-L1) - Serum glial fibrillary acidic protein (GFAP) Upper limits of normal were defined as mean + 3 standard deviations. For UCH-L1 mean (SD) was 0.073 (0.057) ng/mL, and for GFAP mean (SD) was 0.038 (0.059 ng/mL). Therefore, the upper limits of normal for UCH-L1 and GFAP were 0.244 and 0.215 ng/mL, respectively.	Reference standard: CT All patients underwent CT imaging of the brain at the time of initial presentation to the ED. Each patient's head CT was characterized using the recommendations of the TBI-CDE Neuroimaging Working Group. Each CT was de-identified, electronically uploaded to a central imaging database, and reviewed by a blinded central reader who was a board certified neuroradiologist	Intracranial pathology on CT	Included mixed severity

Study	Population	Index test	Reference standard	Outcome	Comments
	Setting: trauma centre Country: International Inclusion criteria: patients had to present within 24 h of injury with a history of trauma to the head sufficient to triage to non-contrast head CT using the American College of Emergency Physicians/Centres for Follow up: 6 months after injury Disease Control (ACEP/CDC) evidence-based joint practice guideline.22 All levels of GCS scores were eligible.	Blood samples were collected from subjects who consented to genetic and proteomic analysis within 24 hours of injury.			
Dickens, 2018 [20] Prospective cohort	n = 210 (discovery cohort- Turku, Finland = 144) and	Index test:	Reference standard: CT	Traumatic intracranial findings	Mixed severity TBI based on GCS (mild, moderate and severe)

Study	Population	Index test	Reference standard	Outcome	Comments
	validation cohort- Cambridge, UK (n = 66) Age, mean (SD): Discovery cohort Mild: 48.37 (20.18) Moderate:59.57 (17.32) Severe: 55.05 (15.25) validation cohort- Mild: 36.75 (18.20) Moderate: 41.57 (20.49) Severe: 44.87 (17.71) Gender (male/female): Discovery cohort Mild: 74/34 Moderate: 8/6 Severe: 19/3 validation cohort	• Serum GFAP • Serum UCH-L1 The blood samples were collected within 12 hours of admission to hospital. There were some patients who were found unconscious and transferred to hospital and patients who sustained mild TBI and sought for medical attention with latency. In these patients, the exact time of injury is unknown.	The CT scans were analysed by neuroradiologists and double-read by a senior neurosurgeon (JPP) and a neurologist (OT). Marshall classification was chosen, because it can be appropriately used for the patient group division and to address the clinical questions.		Study reports that all patients received treatment based on local standards and current international guidelines and recommendations.

Study	Population	Index test	Reference standard	Outcome	Comments
	Mild: 27/9 Moderate: 7/0 Severe: 17/6 GCS: Included all severities Discovery cohort: (N=108) Mean GCS score (mild):14.19 (N=14) Mean GCS score (moderate):9.77 (N=22) Mean GCS score (severe):4.44 Validation cohort: (N=36) Mean GCS score (mild): 14.54 (N=7) Mean GCS score (moderate): 10.44 (N=23) Mean GCS score (severe): 5.68 Setting: trauma centres				

Study	Population	Index test	Reference standard	Outcome	Comments
	Country: UK, Finland Inclusion criteria: Patients were included if they were older than 18 years (16 in the UK) and had a clinical diagnosis of TBI and indications on a head CT according to the National Institute for Health and Care Excellence (NICE) criteria.				
Egea-Guerrero, 2012 [21] Prospective cohort	n = 143 Age, mean (SD): 49 (20.6) years Gender: Eighty-nine patients were male (62.20%) and 54 females (37.80%). GCS score: 15: 143 (100%)	Index test: Serum S100B A blood sample was drawn at 6-hours post-TBI .	Reference standard: CT In this study, IL included cerebral contusion, traumatic subarachnoid haemorrhage, epidural haematoma, and subdural haematoma. A venous blood sample was taken during the first 6 hours post-trauma for posterior analysis of S100B serum	Intracranial lesion (IL) on CT	Includes people over 14 years. Not specified proportion of adults/children. Mean age suggests adults.

Study	Population	Index test	Reference standard	Outcome	Comments
	Twelve patients were under hypocoagulation therapy at the time of injury. All patients had normal levels of consciousness (GCS score = 15) at hospital admission and at least one neurological symptom after TBI Setting: hospital Country: Spain Inclusion criteria: aged 14 or over, GCS score = 15 at hospital admission and one or more of the following symptoms: (1) transitory loss of consciousness; (2) amnesia; (3)		level. A CT scan to identify IL was performed within 24 hours of the accident (never prior to 1-hour post-trauma) [9-12]. Neuroradiological findings were reviewed and classified by a neuroradiologist blind to study goals and data		

Study	Population	Index test	Reference standard	Outcome	Comments
	persistent headache; (4) nausea or vomiting; and (5) vertigo				
Egea-Guerrero, 2018 [22] Prospective cohort study	n = 260 Age, mean (SD): Age > 65 years, n (%)- 50 (19.2%) Gender, male, n (%): 166 (63.8) GCS: GCS score =15 (mild TBI) Isolated TBI, n (%): 171 (65.8) S100B (pg/L), median (IQR): 0.18 (0.09-037) Setting: hospital trauma centre Country: Spain	Index test: Serum S100B Blood sampling ~ 3 h and 25 min post-TBI A 5-mL sample of blood was drawn from each patient. Once collected, samples were centrifuged at 1800g for 10 min. The sera were separated and frozen in aliquots at —80°C until batch evaluation. Venous blood samples for S100B were collected approximately 3 hours and 25 min post-TBI (IQR: 2.0-4.3).	Reference standard: CT CT scan within 24 hours post-TBI (never prior to 1 hour post-accident)	Presence of intracranial lesion (IL) on CT	Mixed population adults and children- includes above 14 years of age. Not reported proportion of adults and children.

Study	Population	Index test	Reference standard	Outcome	Comments
	Inclusion criteria: age ≥ 14; GCS score = 15 at hospital admission and at least one of the following symptoms/findings: transitory loss of consciousness, amnesia, persistent headache, nausea or vomiting ; extraction of serum sample within 6 hours post-trauma and CT scan within 24 hours post-TBI (never prior to 1 hour post-accident)				
Ernstbrunner, 2016 [23]	n = 382 (no ICH 378; Secondary ICH n=4) Age, mean (SD): No ICH: 82 (±9) Secondary ICH: 76 (±11) Gender (female%): No ICH: 60	Index test: Serum S100B Peripheral venous blood was obtained directly after the primary CT within an average of 3 hours after initial trauma	Reference standard: CT scan Primary head CT and RRHCT (repeated head computed tomography (RRHCT) scans within 3 and 48 hours to trauma were performed. The CT scans were reviewed without delay by	Secondary intracranial haemorrhagic events (SIHE) on CT	No information on management

Study	Population	Index test	Reference standard	Outcome	Comments
	Secondary ICH: 50 GCS: Mild TBI (GCS score 14-15) Ethnicity: not reported Setting: Level Trauma centre Country: Austria Inclusion criteria: (1) ≥60 years of age, (2) intake of daily LDA prophylaxis (50-100 mg day-1), (3) isolated mHI with a GCS score of 14-15, (4) negative pHCT within 3 hours and (5) no hypertensive irregularities during the in-hospital observation period (systolic blood pressure <150 mm Hg).		an in-house attending senior radiologist. After the CT scan, all patients with mild HI were kept under observation for a minimum of 24 hours.		

Study	Population	Index test	Reference standard	Outcome	Comments
Forouzan, 2021 [24] Prospective cohort	n = 176 Age, mean (SD): 36.4 (16) years (range 16-90 years) Gender (male): 80.1% GCS score 14-15: 100% Setting: 2 hospitals Country: Iran Inclusion criteria: ≥16 years of age; clinical diagnosis of traumatic brain injury (TBI); those who have indications for brain CT scan in terms of the National Institute for Clinical Excellence Criteria (NICE); <6 hours elapsed between the event and examination; GCS	Index test: Serum GFAP Within 6 hours of injury (not stated mean timing of sampling)	Reference standard: CT scan	Positive CT scan (acute epidural or subdural hematoma, cortical contusion, ventricular compression, ventricular trapping, cerebral herniation, intraventricular haemorrhage, hydrocephalus, subarachnoid haemorrhage, cerebral oedema, post-traumatic ischemia, intracranial hematoma, and cerebral venous sinus thrombosis)	

Study	Population	Index test	Reference standard	Outcome	Comments
	score 15- 13 (mild TBI)				
Gardner, 2018 [26] TRACK-TBI Pilot study Prospective cohort study	n = 586 (n=169 analysed - people with mild TBI) Age < 40 years: n=79. Mean (SD): 25.8 (7.3) years Age 40-59 years: n=60. Mean (SD): 50.0 (5.9) years Age ≥ 60 years: n=30. Mean (SD): 68.0 (8.4) years Gender (female): Age < 40 years: 21 (26.6) Age 40-59 years: 20 (33.3) Age ≥ 60 years: 11 (36.7) GCS: mild TBI (GCS score 13-15) GCS score 13 Age < 40 years: 2 (2.5)	Index test: Serum GFAP All blood samples were obtained within 24 hours of injury Sample collection hours post-injury (hours): Age < 40 years: 8.6 – 5.6 (1.0–23.9) Age 40-59 years: 10.8 – 6.9 (0.5–23.5) Age ≥ 60 years: 13.6 – 6.8 (2.1–23.5)	Reference standard: Head CT	Acute intra cranial trauma For this study, evidence of acute intracranial trauma (i.e., CT+) was defined as presence of at least one of the following: epidural haemorrhage (EDH), subdural haemorrhage (SDH), subarachnoid haemorrhage (SAH), brain contusion, intracerebral haemorrhage (ICH), intraventricular haemorrhage (IVH), traumatic or diffuse axonal injury (TAI/DAI), midline shift >5 mm, partial or complete effacement of basal cisterns, or cerebral oedema. CT- was defined as having none of these aforementioned findings. Additionally, intra-parenchymal injury was	

Study	Population	Index test	Reference standard	Outcome	Comments
	Age 40-59 years: 0 (0.0) Age ≥ 60 years: 0 (0.0) GCS score 14 Age < 40 years: 16 (20.3) Age 40-59 years: 10 (16.7) Age ≥ 60 years: 2 (6.7) GCS score 15 Age < 40 years: 61 (77.2) Age 40-59 years: 50 (83.3) Age ≥ 60 years: 28 (93.3) Setting: trauma centre Country: USA Inclusion criteria: age 16 years and older with mild TBI			defined as contusion, ICH, TAI/DAI, or oedema; extra-parenchymal injury, as EDH, SDH, SAH, or IVH	

Study	Population	Index test	Reference standard	Outcome	Comments
	(GCS score 13-15) and ability to provide informed consent either independently or via a proxy.				
Gatson, 2014 [27] (Mild and Moderate TBI Biomarker [MAMBA] study) Prospective cohort	n = 34 Age, mean (SD): CT negative: 33.4 ± 9.8 CT positive: 35.1 ± 1.6 Gender (male): CT negative: 7 (43.8) CT positive: 14 (77.7) GCS: mild TBI CT (negative): GCS score 13: 2 (12.5) GCS score 14: 6 (37.5) GCS score 15: 8 (50) CT positive:	Index test: Serum neurofilament-H (NFL-H) Study measured the serum levels of pNFL-H in patients with mild TBI at Day 1 (18-24 hours) or Day 3 (66-72 hours) after injury. The range of detection is 0.0293 ng/ml to 15 ng/ ml.	Reference standard: CT scan	Intracranial findings on CT (skull fractures, subdural/ epidural/subarachnoid haemorrhaging, oedema, and/or contusions)	

Study	Population	Index test	Reference standard	Outcome	Comments
	GCS score 13: 10 (55.5) GCS score 14: 1 (5.6) GCS score 15: 7 (38.9) Setting: hospital Country: USA Inclusion criteria: TBI patients with a GCS score between 13 and 15 who were admitted to Parkland Hospital (Dallas, Texas) were identified and screened using the patient database. Both men and women between the ages of 18 and 50 years with an mTBI were screened.				
Gill, 2018 [28] Prospective cohort	n = 277	Index test:	Reference standard: CT scan within 48 hours post injury	Neuroimaging findings (CT and MRI)	No follow-up reported

Study	Population	Index test	Reference standard	Outcome	Comments
	Age, mean (SD): MRI+, CT+ 52.03 (19.83); MRI+, CT- 46.04 (16.08); MRI-, CT- 41.48 (15.25) years Gender (male): MRI+, CT+ 65%; MRI+, CT- 60%; MRI-, CT- 57% GCS score mean (SD): MRI+, CT+ 14.07 (1.53); MRI+, CT- 14.42 (1.11); MRI-, CT- 14.51 (7.22) Setting: emergency department Country: USA Inclusion criteria: seeking care for a suspected brain injury; 18–85 years of age; GCS score 13–15	Plasma NFL within 48 hours post injury Plasma GFAP within 48 hours post injury Plasma UCH-L1 within 48 hours post injury	MRI scan within 48 hours post injury		

Study	Population	Index test	Reference standard	Outcome	Comments
Ingebrigtsen, 2000 [29] Prospective cohort	n = 182 Age, mean (range): 33 (15-78) years Gender (male): 61% GCS score 13: 5.5% GCS score 14: 18.7% GCS score 15: 75.8% Setting: Departments of Neurosurgery/Neurology at 3 centres Country: Finland Inclusion criteria: head injury with brief (≤10 minutes) loss of consciousness; GCS score 13-15 at admission; no focal neurological deficits; age 15-80 years;	Index test: Serum S100B measured immediately after admission (cut-off ≥0.2 µg/L) (mean 3 hours (range 0.5-12 hours) after injury)	Reference standard: CT scan within 24 hours post injury	Intracranial pathology on CT scan	Follow up: Rivermead Post Concussion Symptoms questionnaire measured 3 months post injury Population indirectness as children and adults were included, although the mean suggests majority were adults.

Study	Population	Index test	Reference standard	Outcome	Comments
	admitted within 12 hours post injury; CT performed within 24 hours after injury				
Kahouadji, 2020 [31] Prospective cohort	n = 130 Age, mean (SD): 44.8 (20.4) years Gender (male): 62% GCS score 13/14: 17% GCS score 15: 83% Setting: single centre ED Country: Switzerland Inclusion criteria: Adult (≥18 years) mild TBI patients with a clinical indication for a CT scan, as described in the Canadian CT	Index test: Serum S100B measured 3 hours post injury	Reference standard: Cranial CT scan	Positive CT scan (at least one pathophysiological trauma-relevant intracranial lesion - any signs of cranial (skull fracture) or intracranial pathology (hematoma, air, or contusion), subgaleal hematomas were also considered positive to prevent disregarding abnormalities that may influence S100B levels	Study reports Serum S100B did not influence patients' clinical management

Study	Population	Index test	Reference standard	Outcome	Comments
	Head Rule; mild TBI defined as head trauma with GCS score of 13–15				
Kaneko, 2019 [32] Prospective cohort	n =57 Age, years: 70 (57-81) Gender (male): 22 (39%) Severity: mild to moderate TBI GCS score mean (range): 15 (14-15) Setting: ED Country: Japan Inclusion criteria: admission to the emergency department of Kumamoto Medical Center, single blunt head trauma, mild-to-moderate TBI with Glasgow coma	Index test: Serum biomarkers - glial fibrillary acidic protein (GFAP) (ng/mL) - phosphorylated neurofilament heavy subunit (pNF-H) (pg/mL) - heart-type fatty acid binding protein (H-FABP) (ng/mL) - neuron-specific enolase (NSE) (ng/mL) - S 100B protein(S100B) (pg/mL)	Reference standard: CT	Positive head CT findings Positive head CT findings were defined as intra-cranial haemorrhagic findings	No information on treatment

Study	Population	Index test	Reference standard	Outcome	Comments
	scale score of 9-15, and head computed tomography (CT) scheduled before collecting informed consent				
Kotlyar, 2011 [34] Prospective cohort study	n = 346 Age, mean (SD): 48 Gender (male): 62% GCS: GCS score 15: 89% (303) GCS score <15: 10% (35) Ethnicity: White: 63% (219) Hispanic: 20% (68) Black: 13% (45) Other: 4% (14) Setting: ED Country: USA Inclusion criteria: Patients presenting within 6 hours of	Index test: Serum S100B Blood was collected upon admission (within 6 hours of injury) and immediately sent to the laboratory for processing	Reference standard: Head CT ED HCT was performed within 3 h of ED presentation	Positive CT results (subarachnoid haemorrhage, epidural haemorrhage, subdural haemorrhage, intraparenchymal haemorrhage, diffuse brain oedema , diffuse axonal injury, skull fracture)	No information on treatment

Study	Population	Index test	Reference standard	Outcome	Comments
	injury and undergoing HCT for evaluation of minor head trauma (GCS score of 13-15) were eligible for enrollment. Patients with concomitant trauma were eligible for enrollment. Alcohol- and drug-intoxicated patients were also eligible for enrollment if time of injury was known. Non-focal neurologic examination				
Lagerstedt, 2017 [35] Prospective cohort	n = 172 [CT +: 140 (81%); CT -: 32 (19%)] Mixed population (age > 14 years-adults and children) Age, years: CT+: 46 (20) years CT -: 61 (25) years 261 mild TBI patients with a GCS score of 15. Of	Index test: - Serum S100B - Serum Heart fatty-acid binding protein (H-FABP) Blood samples collected ≤6 hours after trauma. Reference standard:	Head CT CT scan performed within 24 hours of the trauma	CT positive (not defined)	Population indirectness as children and adults were included, although the mean suggests majority were adults. No information on treatment

Study	Population	Index test	Reference standard	Outcome	Comments
	these, 172 patients came to the hospital ≤ 6 h after trauma, with a mean time (± SD) of 198 min ± 88 Gender (male): CT+: 101 (72%) CT-: 23 (72%) Setting: hospital Country: Switzerland and Spain Inclusion criteria: diagnosis of mild TBI with a GCS score of 15; presence of at least one clinical symptom (loss of consciousness, amnesia, vomiting or nausea, headache or equilibrium disorder); CT scan performed within 24				

Study	Population	Index test	Reference standard	Outcome	Comments
	h of the trauma (where the presence of epidural haemorrhage, subdural haemorrhage, subarachnoid haemorrhage, intracerebral haemorrhage, contusion with haemorrhage, cerebral oedema or skull fracture was classified as CT-positive); blood sample collected at admission; and age above 14 years old.				
Lagerstedt, 2018 [36] Prospective cohort study	n = 132 (CT negative scan, n (%) 111 (84) 2; CT positive n=21 (16)) Age, mean (SD) year: mixed population (mean age -adults) CT negative: 46 (21) CT positive: 63 (24)	Index test: Serum —H-FABP, MMP-1, MMP-3, MMP-9, VCAM, ICAM, SAA, CRP, GSTP, NKDA, PRDX1, DJ-1 and IL-10 ≤6 hours following a TBI event	Reference standard: CT scan	CT positive (Epidural haemorrhage, Subdural haemorrhage, Subarachnoid haemorrhage, Intracerebral haemorrhage Contusion with haemorrhage Skull fracture)	mixed population includes adults and children. mean age -adults

Study	Population	Index test	Reference standard	Outcome	Comments
	Gender (male): CT negative: 82 (74) CT positive: 14 (67) GCS: GCS score of 15 and at least one clinical symptom Setting: ED Country: Spain Inclusion criteria: patients were diagnosed with mild TBI and had a GCS score of 15 and at least one additional clinical symptom (vomiting or nausea, loss of consciousness, amnesia, an equilibrium disorder or a headache) and age above 14 years old. Each patient had a blood sample taken at hospital	Mean (SD): CT negative: 195 (86); CT positive: 177 (100)			

Study	Population	Index test	Reference standard	Outcome	Comments
	admission 6 hours post trauma and a CT scan was performed within 24 hours post trauma .				
Lagerstedt, 2018 [37] Prospective cohort study	N=133 CT negative: 111 (83) CT positive: 22 (17) Age, mean (SD) year: (mixed population) CT negative: 46 (21) years CT positive: 61 (26) years Gender (male): CT negative: 82 (74) CT positive: 15 (68) GCS score: 15 (mild TBI) Setting: ED	Index test: o S100B o IL-10 ≤6 hours following TBI A serum (Seville) or plasma (Geneva) sample was collected from patients at hospital admission.	Reference standard: CT scan Participating patients gave a blood sample at hospital admission and underwent a CT scan within 24 hours of their trauma event	CT positive (Epidural haemorrhage, Subdural haemorrhage, Subarachnoid haemorrhage, Intracerebral haemorrhage, Contusion with haemorrhage, Skull fracture).	mixed population includes adults and children. mean age -adults No information on treatment

Study	Population	Index test	Reference standard	Outcome	Comments
	Country: Spain Inclusion criteria: diagnosis of mild TBI, a GCS score of 15 at hospital admission and at least one of the following symptoms: headache, nausea or vomiting, loss of consciousness (< 30 min) and amnesia (< 24 hours).				
Laribi, 2014 [38] Prospective cohort study	N=431 Patients included were aged 18 years or older presenting to the ED within 3 hours after injury. A 3-hour cut-off was chosen as S100B is rapidly cleared from the serum, with a half-life between 0.5 and 2 hours Age: The median age (IQR) of the	Index test: S 100B Venous blood samples were collected immediately at patients' arrival to the ED within 3 hours after the clinical event ((HO) and 3 hours (H3) after the first sampling.	Reference standard: Cranial CT Patients underwent a CT scan within 6 hours after clinical examination.	CT findings of intracranial lesions.	Fifty-one patients were hospitalised either in the ED observation unit or in another hospitalisation unit for ≥ 24 hours, mostly for the treatment of extracranial lesions. Six patients in the CT+ group were hospitalised in a neurological/neurosurgical unit.

Study	Population	Index test	Reference standard	Outcome	Comments
	participants was 36 (24-54) years. Gender (male): 269 (65) GCS: mild TBI GCS score 13: 7 (2) GCS score 14: 48 (11) GCS score 15: 376 (87) Reason for MHI was a fall in 263 patients. Setting: ED Country: International Inclusion criteria: history of MHI defined by a Glasgow Coma Scale score (GCS) from 13 to 15 with one or more of the				

Study	Population	Index test	Reference standard	Outcome	Comments
	following risk factors: amnesia, loss of consciousness, nausea, vomiting, vertigo, anticoagulation before injury or severe headache on admission				
Li, 2022 [39] Retrospective cohort study	n = 463 Age, mean (SD): 50.8 ± 22.7 years Gender (female): 177 (38.2) GCS score, median {Q1,Q3] : 15 [14, 15] Setting: ED Country: USA Setting: ED Country: USA	Index test: Distribution of time from injury to ED admission/blood draw spanned 0 through 6 hours with a median at 1 hour. Plasma GFAP (22°pg/mL) Serum GFAP (22°pg/mL) Plasma UCH-L1 (327°pg/mL) Serum UCH-L1 (327 pg/mL) Serum S1003 (105°pg/mL) Composite plasma biomarker	Reference standard: Non-contrast head CTs	Abnormalities on CT	Adults No information on treatment

Study	Population	Index test	Reference standard	Outcome	Comments
	Inclusion criteria for this study: (1) at least 18 years old at admission, (2)presented to the ED with suspected TBI, and (3) have a blood draw as part of the standard of care.	Plasma GFAP (22°pg/mL) and UCH-L1 (327) Serum GFAP (22°pg/mL) and UCH-L1 (327)			
Linsenmaier, 2016 [40] Prospective cohort study	N=41 Age mean (SD) years: 54.6 6 23.3 Gender (male): 21 (51.2) GCS: mild TBI GCS score 15: 36 (87.8) GCS score 14: 4 (9.8) GCS score 13: 1 (2.4) Setting: ED	Index test: S100B A cut-off value of 0.1mgl-1 not time specified	Reference standard: Cranial CT MRI	CCT positive (abnormal findings)	Admitted for observation: 11 (26.8) Discharged: 30 (73.2)

Study	Population	Index test	Reference standard	Outcome	Comments
	Country: Germany Inclusion criteria: a history of minor head trauma (Glasgow Coma Scale on admission: 13–15)				
Mahan, 2019 [41] Prospective cohort	n = 104 Age, years: with mean (SD) age of 52.7 years (19.6) ranging from 18.5 to 92.8 Gender: 31 female, 73 male GCS: mixed severity TBI. Majority were with mild TBI GCS score 3-8: 28 (19.2) GCS score 9-12: 5 (4.8) GCS score 13-15: 79 (76.0)	Index test: • glial fibrillary acidic protein (GFAP) • ubiquitin C-terminal hydrolase L1 (UCH-L1) • S100 calcium-binding protein B (S100B) The initial blood sample was taken within 8 hours of the reported head injury. Specimen collection was repeated 12-32 hours after the reported time of injury.	Reference standard: Head CT CT scans of the head receiving a Marshall Classification of Diffuse Injury I were labelled CT negative whereas all others were labelled CT positive.	CT positive	Mixed severity -mild moderate and severe

Study	Population	Index test	Reference standard	Outcome	Comments
	Setting: ED Country: USA Inclusion criteria: those with suspected head trauma resulting in a clinically ordered CT scan of the head at the time of admission, and those with a blood specimen collected within 32 hours of time of injury with valid GFAP, S100B, and UCH-L1 biomarker concentrations.				
McMahon, 2015 [43] TRACK-TBI Prospective cohort study	N=215 Age mean (SD) years: 42 (18) years Gender (male): 73% (156)	Index test: Glial fibrillary acidic protein and its breakdown products (GFAP-BDP) Serum GFAP-BDP levels were drawn	Reference standard: CT imaging All patients underwent CT imaging of the brain at the time of initial presentation to the ED.	Intracranial injury on CT	mixed severity GCS (mild moderate and severe)

Study	Population	Index test	Reference standard	Outcome	Comments
	GCS: mixed GCS. Majority with mild TBI Mild (GCS score 13-15): 83 (179) Moderate GCS score 9-12: 4(9) Severe GCS score 3-8: 13 (27) Setting: trauma centre Country: International Inclusion criteria: patients must have presented to an ED within 24 h of their injury and had a positive clinical screen for acute TBI necessitating a non-contrast head CT according to American College of Emergency Physicians/Centres for Disease Control and Prevention (ACEP/CDC)	within 24 hours and analysed. Plasma samples obtained within 24 hours of injury (mean 10.9 hours, SD 6.4 hours, min 0.5 hours, max 23.4 hours)			

Study	Population	Index test	Reference standard	Outcome	Comments
	evidence-based joint practice guideline.				
Morochovic, 2009 [44]	n = 102 Age, years: mean age 42.0 (SD 19.7, range 12—84 years) Gender: 71 males and 31 females, GCS: mild TBI (GCS score 13-15) GCS score 13: 3 GCS score 14: 23 GCS score 15: 76 Setting: ED Country: Slovak Republic Inclusion criteria: adults with mild TBI. Patients with chronic intracerebral lesions were	Index test: Serum S100B Peripheral venous blood samples were taken within 6 hours of the injury and were sent to biochemical laboratory within 30 min.	Reference standard: Cranial CT (CCT) CCT scan was performed in all patients involved in the study within 30 min of blood drawing	Any intracranial pathology associated with an injury (acute subdural, epidural or parenchymal hematoma, traumatic subarachnoid haemorrhage, cerebral contusion and brain swelling) detectable on CCT scan was considered positive (CCT +).	Three patients from CCT + group had negative plasma level of S100B, two of whom required surgical treatment.

Study	Population	Index test	Reference standard	Outcome	Comments
	included to the study except suspected/visible brain tumour.				
Mozafari, 2020 [45] Cross-sectional study	n = 40 Age, mean (SD) years: 35 ± 2.1 years Gender (male): 92.5% GCS: mild TBI Glasgow coma score of 14 and 15. Eleven of the study participants had a GCS score of 9 or 12 at the time of referral Ethnicity: not reported Setting: ED Country: Iran Inclusion criteria: presence for an	Index test: serum neuron-specific enolase A venous blood sample was first taken by the ward nurse during the first three hours of the accident just before performing the cranial CT scan, and then the second sample was taken just before performing the CT scan again (as secondary CT scan, 7 hours after performing the primary CT scan). Within 6 hours (not specifically mentioned about timings)	Reference standard: Cranial CT scan	CT positive (not defined)	

Study	Population	Index test	Reference standard	Outcome	Comments
	indication of a brain CT scan, aged 6 months to 18 years and a Glasgow coma score of 14 or 15. Injuries included those from traffic and home or sport events, and referrals less than 6 hours of the incident. Inclusion criteria were no previous history of alcohol or drug abuse, the absence of a history of previous neurological disease such as seizure or epilepsy, the absence of severe traffic injury and multiple trauma from motor vehicles, and absence of melanoma				
Muller, 2011 [48] Prospective cohort	n = 233 Age, years: 48.4 years (range 11–97;	Index test: S100B	Reference standard: Cranial CT (CCT)	Positive CT findings (not defined)	No information on treatment

Study	Population	Index test	Reference standard	Outcome	Comments
	25–75% quartile 24–72). Gender: 143 were men and 90 were women GCS: mild TBI (GCS score 13 o 15) Setting: ED Country: Switzerland Inclusion criteria: All adult patients (≥16 years) with mild head trauma (GCS score of 13–15) were included in the study.	Median time between admission and blood sampling was 77 min (25–75% quartile 60–120).	After blood sampling, all patients underwent a CCT scan		
Muller, K., (2007) Muller, 2007 [49] Prospective cohort	n = 226 Age, years: Mean 39 (range, 18-92) years	Index test: Serum S100B Blood samples for S100B analysis and head CT were obtained within 12 hours after the injury	Reference standard: Head CT	Intracranial pathologic findings revealed by CT scan (not defined)	No information on treatment

Study	Population	Index test	Reference standard	Outcome	Comments
	Gender: 168 (74.3%) men, and 58 (25.7%) GCS: mild TBI (GCS score 13 o 15) GCS score 13: 16 (7) GCS score 14: 30 (13) GCS score 15: 180 (78) Setting: ED Country: 4 centres in Europe Inclusion criteria: Patients with head injury were assessed for possible inclusion during a 4-year period (2001-2005). The inclusion criteria were the following: History of head injury, Loss of consciousness				

Study	Population	Index test	Reference standard	Outcome	Comments
	(LOC) or retrograde amnesia, GCS score of 13 to 15 at admission, Blood sampling within 12 hours of trauma, First CT scan within 12 hours of trauma, Signed written informed consent (optional, according to local ethical committee's requirements)				
Oh, 2007 [51] Prospective cohort	n = 101 (n= 45 patients with traumatic ABI and= 56 patients with nontraumatic ABI) Age, years, mean (SD): 45 years [31–59] Gender (male): 57.9% male GCS: mixed severity (mild, moderate and severe). Majority with mild TBI (80%)	Index test: Serum S100 levels (by Elecsys S100 immunoassay) Measurement within 6 hours after symptom onset	Reference standard: Cranial CT (CCT) or MRI All patients underwent initial CCT or MRI testing. The patients who showed negative findings in cranial CT (CCT) were confirmed by MRI.	Acute traumatic brain injury (CCT negative or MRI positive)	Mixed severity population (mild, moderate and severe) No information on treatment

Study	Population	Index test	Reference standard	Outcome	Comments
	The patients were stratified into three subgroups on the GCS score: mild (13–15), moderate (8–12), and severe (<8) GCS score 13–15: 82 GSC score 9–12: 9 GCS score <8:10 Setting: emergency department (ED) Country: Korea Inclusion criteria: admitted to emergency department within 6 hr after the onset of ABI symptoms (no further details)				
Okonkwo, 2020 [52] TRACK-TBI Prospective cohort	n = 1497 (810 CT negative, CT positive n = 549) Age, mean (SD) years: CT (-): 37.7 (15.9) CT (+): 43.7 (17.9)	Index test: Serum: • GFAP • S100B	Reference standard: Head CT	Intracranial injury on admission CT scan	Mixed severity (mild, moderate and severe) Point of care testing

Study	Population	Index test	Reference standard	Outcome	Comments
	Gender (male): CT (-):516 (63.7%) CT (+):408 (74.3%) GCS: mixed severity (mild, moderate and severe). Majority with mild TBI CT (-): GCS score 13-15: 779 (98%) GCS score 9-12: 8 (1%) GCS score 3-8: 8 (1%) CT (+): GCS score 13-15: 358 (74%) GCS score 9-12: 40 (8%) - GCS score 3-8: 85 (18%) Setting: trauma centre Country: USA	Blood samples were collected from subjects who consented to genetic and proteomic analysis within a 24 hour window from time of injury Time to blood draw (hours): 13.1 (6.8)			

Study	Population	Index test	Reference standard	Outcome	Comments
	Inclusion criteria: presentation within 24 h of injury with head trauma warranting clinical evaluation with a non-contrast head CT evaluation based on the 2008 American College of Emergency Physicians/Centers for Disease Control (ACEP/CDC) guidelines for neuroimaging and decision making in TBI.				
Okonkwo, 2013 [53] Prospective cohort study	Total n = 215 Mild n= 179 Moderate n=9 Severe n = 27 Age, mean (SD): Mild 42.5 (18) Moderate 44.1 (19.5) Severe 39.2 (18.9)	Index test GFAP and breakdown products (GFAP-BDP) Blood samples collected within 24 hours of injury, mean (SD) 10.9 h (6.4 h) [min 0.5 h, max 23.4 h]	Reference standard All patients underwent CT imaging of the brain at the time of initial presentation to the ED. CT scans performed at time of initial presentation the ED Time between measurement of index test	Intracranial pathology on CT	Included patients with GCS score 9-12

Study	Population	Index test	Reference standard	Outcome	Comments
	Gender (male to female ratio): Mild 69.8% M: 30.2% F Moderate 100% M: 0% F Severe 81.5% M: 18.5% F GCS score, mean (SD): mixed severity. majority with mild TBI- 83% had GCS score 13-15 Mild 14.8 (0.44) Moderate 11.22 (0.67) Severe 3.59 (1.31) Positive CT findings on admission Mild 42.5% Moderate 77.8% Severe 96.3% Ethnicity: Not reported		and reference standard: unclear		

Study	Population	Index test	Reference standard	Outcome	Comments
	Setting: Multicentre – 3 Level I trauma centres participating in the TRACK-TBI study Country: USA Inclusion criteria: patients presenting within 24 h of injury with a history of trauma to the head sufficient to be triaged to non-contrast head CT using the American College of Emergency Physicians/Centres for Disease Control (ACEP/CDC) evidence-based joint practice guideline.				
Papa, 2012 [54] Prospective cohort	n = 108 Age, years mean (SD): 39 (±15) Gender (male): 70(65%)	Index test: Serum GFAP-BDP Blood samples were obtained after arrival to the ED and within 4 hours of the reported	Reference standard: Head CT Patients underwent standard CT scan of the head according to the	Intracranial lesions on CT	Mixed severity mild and moderate Neurosurgical intervention was performed on 14 patients (13%), 6 (43%) presented with a GCS

Study	Population	Index test	Reference standard	Outcome	Comments
	GCS: mixed severity. Majority with mild TBI GCS score 13–15: 97 GCS score 9–12: 11 Setting: Emergency Departments (ED) Country: USA Inclusion criteria: Eligibility for suspected mild TBI was determined by the treating physician based on the history of blunt head trauma followed by either loss of consciousness, amnesia, or disorientation and presenting to the emergency department within 4	time of injury. There was only one serum GFAP-BDP biomarker level analysed per patient in the 4-hour post-injury period. The average time to serum collection for TBI patients was 2.6 hours (95%CI 2.4–2.9)	judgment of the treating physician		score 13–15 and 8 (57%) with GCS score 9–12

Study	Population	Index test	Reference standard	Outcome	Comments
	hours of injury with a GCS of 9 to 15.				
Papa, 2012 [55] Prospective cohort	n = 96 Age, years, mean (SD):39 (±15) Gender (male/female): 64/36 GCS: Mild and moderate TBI n= 86 with GCS score 13–15 n= 10 with GCS score 9–12 Setting: ED Country: USA Inclusion criteria: adult patients with blunt head trauma followed by either loss of consciousness,	Index test: Ubiquitin C-terminal hydrolase (UCH-L1) Blood samples were obtained shortly after arrival to the ED and within 4 hours of the reported time of injury. The average time to serum collection for TBI patients was 2.7 hours (95%CI 2.4–2.9)	Reference standard: Head CT	Intracranial lesions on CT	Mixed severity (mild and moderate) Neurosurgical intervention was performed on 14 (14%) patients: 6 (43%) presented with GCS score 13–15 and 8 (57%) with GCS 9–12. Neurosurgical intervention was defined as either death within 7 days secondary to head injury or the need for any of the following procedures within 7 days: craniotomy, elevation of skull fracture, intracranial pressure monitoring, or intubation for head injury

Study	Population	Index test	Reference standard	Outcome	Comments
	amnesia, or disorientation and presenting to the emergency department within 4 hours of injury with a GCS score of 9 to 15				
Poli-de-Figueiredo, 2006 [59] Prospective cohort study (pilot study)	n = 50 Age, median (IQR): Not reported Gender (male to female ratio): 28 M: 22 F Setting: ED Country: Brazil GSC score: n=37 GCS score 15, n= 11 GCS score 14, n=2 GCS score 13 Inclusion criteria: patients who had sustained isolated	Index test(s) S100B levels A cut-off point at a concentration of 0.1 µg/L of S100B was used. Venous blood samples were drawn on admission and processed to serum (median 82 minutes, (25%-75% quartiles: 60-110 min). Reference standard Time between measurement of index test and reference standard: Unclear	Cranial computed tomography (CCT) was performed within 6 hours of emergency room admission, and radiological findings were defined as pathological (CCT+) if intracranial haemorrhage, skull fracture, and/or diffuse brain swelling (oedema) were detected.	signs of intracranial injury at the initial CCT scan	Age not reported No information on treatment

Study	Population	Index test	Reference standard	Outcome	Comments
	minor head injury (GCS 13 -15) and presented at least one of the following symptoms: amnesia, loss of consciousness, nausea, vomiting, vertigo, or severe headache on admission.				
Posti, 2019 [60] Prospective cohort	n = 160 Age, years mean (SD): 47.2 (19.6) years Gender: 117 males (73.1%) and 43 females (26.9%), with a mean age of GCS: mixed severity Isolated all severities: n = 94 Mild TBI: n = 93 Isolated mild TBI: n = 55 Setting: ED	Index test: • Glial fibrillary acidic protein (GFAP) • Heart fatty-acid binding protein (H-FABP) • Anti-inflammatory mediator interleukin 10 (IL-10) • neurofilament light chain (NF-L) • S100B Samples were obtained within 24 h of admission	Reference standard: CT	CT positive CT scans were classified according to the Marshall grading system. Diffuse injury/grade I (no visual pathology) was considered CT-, whereas the other grades (II-VI) were regarded as CT+.	Mixed severity No information on treatment

Study	Population	Index test	Reference standard	Outcome	Comments
	Country: Finland Inclusion criteria: age >18 years, clinical diagnosis of TBI, and indications for acute head CT according to the National Institute for Health and Care Excellence (NICE) criteria				
Romner, 2000 [61] Prospective cohort	Age: 32 (range, 1–84) years Gender: 175 (63%) men and 103 (37%) GCS: mixed severity, majority with mild TBI The head injuries were classified according to the HISS as either severe (GCS score 3–8), moderate	Index test: S100B A serum sample for S-100 analysis was drawn immediately (mean 3.8 hours after injury; range, 0.5–24.0 hours) after admission to the emergency room in the head-injured patients.	Reference standard: CT scans of the brain and cranium In a subgroup of 45 patients with mild head injury (GCS score 14–15, LOC for 20 min, absence of focal neurological deficits, and no signs of acute intracranial abnormality revealed by a CT scan), MRI was also performed.	Intra cranial findings on CT	Patients with severe head injury (GCS score ≤ 9) were intubated and ventilated in the emergency room before the initial CT scan. All patients were admitted for at least overnight observation Mixed severity (mild, moderate and severe)

Study	Population	Index test	Reference standard	Outcome	Comments
	(GCS score 9–13), or mild (GCS score 14–15) Mild: 254 Moderate: 16 Severe 8 Setting: ED-three centers in Scandinavia Country: Norway Inclusion criteria: head injury with loss of conscious- ness (LOC), (2) blood sample for S-100 analysis collected within 24 h after injury, and (3) CT scan performed within24 h after the injury. LOC was considered to have occurred when the patient had amnesia for the trauma event and if accompanying persons reported LOC				

Study	Population	Index test	Reference standard	Outcome	Comments
Thaler, 2015 [62] Prospective cohort study	n = 782 Age median (IQR): 83 (74–88) years Patients with minor head injury (MHI) who were receiving antiplatelet medication or who were older than 65 years were either admitted or observed for at least 6 hours. All patients underwent CCT. The decision whether a patient had to be admitted and the point in time at which CCT was performed depended on the clinical findings. W GCS: mild TBI (13-15) GCS score 13: 48 (6.1%)	Index test: Serum S100B a venous blood sample was drawn within 3 hours after injury and the S100B level was determined. The result of S100B analysis was not known to the attending physician. All clinical assessments were completed before the CCT scans were performed The median interval between event and blood drawing was 2:05 hours (IQR 1:30–2:30).	Reference standard: Cranial CT (CCT) The median interval between trauma and CCT was 15:40 hours (IQR 3:54– 21:30).	CCT positive (patients with MHI who had at least 1 trauma-related intracranial haemorrhage: i.e., epidural, subdural, subarachnoidal, or intracerebral bleeding).	All patients were treated as they normally would have been, following the standard operating procedures of the institutions Patients with MHI who were receiving antiplatelet medication

Study	Population	Index test	Reference standard	Outcome	Comments
	GCS score 14: 147 (18.8%) GCS score 15: 587 (75.1%) Gender (male): 245 (31.3%) Ethnicity: not reported Setting: trauma centres Country: Austria Inclusion criteria: minor head injury (MHI) (GCS Score 13–15) in patients on medication with h platelet aggregation inhibitors (PAI) who were older than 18 years, and MHI in patients age 65 years and older independent of PAI intake				

Study	Population	Index test	Reference standard	Outcome	Comments
Vedin, 2021 [63] Prospective cohort study	n = 243 (n=13 with intracranial haemorrhage (ICH)) Age, years: 60.8 years (±44.96 years) GCS: mild TBI (GCS score 13–15). All patients were awake when they arrived in the emergency room. Gender: not reported Setting: hospital ED Country: Sweden Inclusion criteria: Population 1: Population 1 was selected for the study on S100B serum and urine levels of patients with isolated head trauma. patients	Index test: Urine and Serum S 100B Sampled < 6 hours or less from trauma.	Reference standard: Head CT	Intracranial haemorrhage on CT	Of the 243 patients, 37 (15.2%) took warfarin or an oral anticoagulant, 24 (9.9%) took 75 mg of aspirin, 2 (0.8%) were administered clopidogrel, 3 (1.2%) were given a combination of aspirin (75 mg once daily) and ticagrelor (90 mg twice daily), and 1 (0.4%) had a serious bleeding disorder

Study	Population	Index test	Reference standard	Outcome	Comments
	who were 18 years or above and seeking emergency medical care due to isolated head trauma population 2: Population 2 was selected for the study on the serum and the urine S100B temporal profiles of patients with intracranial haemorrhage. patients who were 18 years or above and had CT-verified intracranial haemorrhage due to head trauma.				
Welch, 2016 [64] Prospective cohort study	n = 251 Age, years: 45.6 (18.4) years GCS: mild and moderate TBI Of the 251 study patients, 225 (89.6%; 95% CI 85.2%–93.1%) had	Index test: Serum: - GFAP - UCH-L1 - S100B Within 6 hours of injury	Reference standard: Head CT	Intracranial lesion on CT	Mixed severity includes mild and moderate TBI Assay results were not available to the treating clinician and were not used to guide treatment

Study	Population	Index test	Reference standard	Outcome	Comments
	an initial GCS score of 15 of whom 24 (10.7%) had a positive CT scan. Among patients with a GCS score <15 (n=26) 12 (46.2%) had a positive CT scan. Gender: 60.2% (95% CI; 53.8%–66.3%) were male Setting: ED Country: US Inclusion criteria: patients were those with an initial Glasgow Coma Scale (GCS) score of 9–15 who underwent emergency head CT scan for evaluation of the head injury as deemed necessary by the attending ED physician. The subject was ‡18	Blood samples were collected at time of study enrolment and every 6 hours up to the time of discharge (either ED or hospital) or up to 24 hours (maximum of five samples during index visit). Patients who were seen at follow-up (Day 35 – 5 days) had another sample obtained when feasible.			

Study	Population	Index test	Reference standard	Outcome	Comments
	years of age and no more than 80 years of age. Acceleration or deceleration closed injury to the head that was either self-reported or witnessed. Presented to an emergency department (ED) within 4 hours of injury. An initial Glasgow Coma Scale score of 9–15 in the ED performed by the Principal Investigator (PI) or trained study personnel. Included patients presented within 4 hours of injury, completed the required CT scan as part of routine care, and had blood drawn for analysis within 6 hours of injury				

Study	Population	Index test	Reference standard	Outcome	Comments
Wolf, 2013 [65] Prospective cohort study	n = 107 Age, mean (SD): 59 ± 23 years Gender: 60 male and 47 females GCS: GCS score 13-15 (mild GCS) Ethnicity: not reported Setting: academic, Level I trauma centre Country: Austria Inclusion criteria: injury within 3 hours prior to admission to the emergency room, blunt head trauma, and a GCS score of 13-15.	Index test: Serum: -S100B protein -neuron-specific enolase (NSE) Peripheral venous blood was obtained from each patient within 3 hours after the accident and prior to cranial CT.	Reference standard: Cranial CT An emergency cranial CT study was performed in all patients. The timing was usually within 30 minutes after the first examination by a physician. Prior to the CT a venous blood sample was drawn.	CT positive (patients with epidural, subdural, subarachnoid, or intracerebral haemorrhage, including contusions)	Seven patients from the CT-positive group were on anticoagulants at the time of injury. Eight patients required a neurosurgical operation to evacuate the ICH; only 1 of these 8 patients was on anti- coagulation therapy prior to the accident
Zongo, 2012 [67] Prospective cohort	n = 1560	Index test: S100B	Reference standard: CT head scan	CT scan abnormality (CT positive)	At 0.10 and 0.12 µg/L, only 1 patient with plasma S100-B below the cut-off

Study	Population	Index test	Reference standard	Outcome	Comments
	Age, years mean (range): 57 (32-82) years Gender (male): 870 (55.8%) GCS: mild TBI GCS score 13: 39 (2.5) GCS score 14: 335 (21.5) GCS score 15: 1186 (76.0) Ethnicity: not reported Setting: ED Country: France Inclusion criteria: Patients included were aged 15 years or older, presenting to the ED within 6 hours of isolated head trauma, with a GCS score of 13 to 15 as determined by	Testing within 6 hours of head trauma	CT scan was performed within 6 hours after the head trauma		value had a positive CT scan result: a 28-year-old man with a cerebral contusion that proved to be a petechia and with a blood alcohol concentration of 3.0 g/L at admission. The patient required no further neurosurgery or intensive care. He stayed in the hospital for 30 hours for surveillance. The delay between trauma and blood drawing was 120 minutes. Between 0.12 and 0.14 µg/L, 2 patients had a positive CT scan result: a cerebral petechia and a chronic subdural haemorrhage with recent bleeding. No neurosurgical care was required, and there was no further neurologic deterioration. The delay between trauma and blood drawing was 152 minutes and 255 minutes for these 2 patients

Study	Population	Index test	Reference standard	Outcome	Comments
	the attending physician, and with one or more of the following risk factors: loss of consciousness, posttraumatic amnesia, repeated vomiting, severe headache, dizziness, vertigo, alcohol intoxication, anticoagulation, and age older than 65 years				

Table 4: Summary of studies included in the evidence review- children

Study	Population	Index test	Reference standard	outcome	Comments
Babcock, 2012 [2] Secondary analysis of prospective cohort study	n = 679 (children in TBI registry) n = 360 (underwent cranial CT) n = 155 (serum S100B measurement)	Index Test S100B S 100B level > 0.006µg L-1 S 100B level of 0.1 µg L-1 Within 6 hours of injury	Reference Standard CT scan	Abnormal cranial CT was defined by the presence of any intracranial injury, including subdural haematomas, epidural haematomas and cerebral contusions, as well as the	

Study	Population	Index test	Reference standard	outcome	Comments
	n = 109 (eligible patients with cranial CT and serum S100B measurement) Age, mean (SD): Normal CT 14.7 (3.9); Abnormal CT 14.2 (4.2) Gender, male (%) Normal CT 52 (57.8); Abnormal CT 10 (52.6) Among the children with both CT and serum S100B, a majority (86.2%) of children had mild TBI; 83 had a GCS score of 15, eight had a GCS score of 14 and three had a GCS score of 13 Setting: Paediatric emergency department at a university medical centre. Country: USA Inclusion criteria: Patients aged 0-18 years were eligible for inclusion in the primary study if they met a modified case definition of TBI			presence of skull fractures.	

Study	Population	Index test	Reference standard	outcome	Comments
	developed by the American Congress of Rehabilitation Medicine (blow to the head or acceleration/deceleration movement of the head resulting in one or more of the following: LOC <30 minutes, amnesia <24 hours or any alteration in mental state at the time of the injury. Exclusion criteria: Patients presenting to the ED >6 hours after injury or with pre-existing medical or psychiatric conditions known to be associated with elevated S100B level in the absence of TBI (specifically, Alzheimer's disease, Down's syndrome and schizophrenia). Additionally, those who had run >10 miles in the past 12 hours were excluded.				
Bandyopadhyay, 2005 [3]Retrospective analysis of a prospectively enrolled cohort study	n = 86 Age, mean (SD): 8.2(6 5.5) years (range 11 months to 18 years). Gender: Approximately two thirds were male and white.	Index test: Serum Neuron-specific Enolase (NSE) Blood for serum NSE assay was drawn at the time of ED evaluation.	Reference standard: CT Timing of CT not reported	Abnormal CT scan was defined as a CT scan with cerebral contusions, cerebral oedema, or parenchymal, subarachnoidal, subdural, or epidural bleeding. Presence of skull fracture alone was not sufficient to	Mixed severity TBI included however majority of patients had mild TBI

Study	Population	Index test	Reference standard	outcome	Comments
	GCS: Among 86 enrolled subjects, ten had Glasgow Coma Scale (GCS) scores <13 (moderate and severe cTBI). Ethnicity: not reported Setting: ED Country: USA Inclusion criteria: Subjects between 0 and 18 years of age, evaluated within 24 hours of sustaining closed traumatic brain injury (TBI), and requiring a cranial computed tomography (CT) scan in accordance with the written ED protocol were enrolled.	The mean time interval from the time of reported injury and the time blood was drawn for NSE measurement was 3.8 hours (range 0.4 to 14.8).		classify a CT as abnormal.	
Bouvier, 2012 [11] Prospective cohort	n = 446 Age, median (IQR): 5.2 (2.1-9.0) Gender, male: female ratio: 1.68	Index Test S100B Recently established reference intervals were used: the upper serum S100B reference limits (95th percentile) were derived for 3 age groups:	Reference standard CT scan	Intracerebral lesion on CT	Includes a population with mixed TBI severity

Study	Population	Index test	Reference standard	outcome	Comments
	Severity: 3 severity groups according to the Masters classification Masters 1: 183 (41%) Masters 2: 241 (54%) Masters 3: 22 (5%) Ethnicity: Not reported Setting: Paediatric emergency department Country: France Inclusion criteria: All children (age 0 –16 years, admission within 3 h) with closed head trauma were eligible for enrolment and were ranked in 3 severity groups according to the Masters classification. Masters group 3 (severe TBI), which formed a positive control group, was composed of children with a Glasgow Coma Scale (GCS) <13 or loss of consciousness or progressive decrease in consciousness. Masters group 2 (mild TBI) comprised children with a GCS score of 13–15 on admission	0.35 µg/L for age 0 –9 months, 0.23 µg/L for age 10 –24 months, and 0.18 µg/L for age >24 months. Patients exhibiting serum concentrations below the specific age-range cut-off were counted as S100B negative (S100B), and those with concentrations above as S100B positive (S100B). The median interval between trauma and blood sampling was 2 hours 05 min (range 1 hour 30 min to 2 hours 45 min or 25%–75%).			

Study	Population	Index test	Reference standard	outcome	Comments
	and 1 or more of 12 clinical risk factors: brief loss of consciousness, posttraumatic amnesia, nausea, vomiting, severe or progressive headache, dizziness, vertigo, intoxication, anticoagulation, skull fracture, seizure, age <2 years. Masters group 1 (minimal TBI) was made up of children with a GCS score of 15 without symptoms or with only headache or bruising.				
Castellani, 2009 [13] Prospective cohort	n = 928 n = 109 (included in study) Age, mean (SD): 9.5 (4.7) Gender, male, n (%): 73 (67) On admission, a GCS score of 15 was recorded in 86 (78.9%), of 14 in 13 (11.9%) and of 13 in 10 (9.2%) patients. Ethnicity: Not reported Setting: Hospital emergency department	Index Test S100B According to an analysis in healthy children recently conducted by the authors, the upper reference of serum S-100B was set to 0.16 µg/L. All patients with MTBI and clinical symptoms who had their serum S-100B measured within 6 hours after trauma and subsequently went on to require a CT during their in-patient episode were	Reference Standard CT scan	.Pathological CT	After clinical examination and S100B sampling, all patients were admitted for inpatient observation

Study	Population	Index test	Reference standard	outcome	Comments
	Country: Austria Inclusion criteria: Patients <18 years with a GCS score 13-15 (in combination with vomiting, loss of consciousness, persisting headache, retrograde amnesia, and vertigo) with serum S100B measured within 6 hours of blunt head trauma who went on to require a CT scan during their inpatient episode.	selected from the database for this study			
Fridriksson, 2000 [25] prospective pilot study	n = 50 Age, mean (SD): aged 2 months to 16 years Presence of intracranial lesion group (PICL) (n=22): 9.16 (5.7) years No intra cranial lesion (NICL) group (n=27): 7.66 (5.3) years Gender: Presence of intracranial lesion (n=22): males 12; females 10 No intra cranial lesion (n=27): males 15; females 12 Mixed severity population GCS score mean (SD):	Index test: serum neuron-specific enolase (NSE) The mean time from injury to obtaining blood samples for NSE was 256 (310) minutes in the PICL group and 242 (147) in the NICL group (p = 0.82).	Reference standard: Head CT	Head CT was reported as positive for ICL when cerebral oedema, parenchymal bleeding, cerebral contusion, or sub arachnoidal, subdural, or epidural bleeding was identified. Enrolled patients were assigned to one of two groups based on the presence or absence of ICL on head CT. The PICL (presence of ICL) group consisted of patients with evidence of ICL. The NICL (no ICL) group consisted of patients with no evidence	One patient in the NICL group required surgery for elevation of a minor depressed skull fracture. In the PICL group, five patients (23%) underwent craniotomy: two for evacuation of an intra cranial hematoma, two for elevation of a de-pressed skull fracture, and one for insertion of a ventriculostomy catheter. Twenty-one of the 22 patients in the PICL group and 14 of the 27

Study	Population	Index test	Reference standard	outcome	Comments
	Presence of intracranial lesion: 11.96 (4.2) No intracranial lesion: 13.96 (2.6) GCS score >12 Presence of intracranial lesion: 14/22 No intracranial lesion: 25/27 Setting: ED of an academic tertiary care children's hospital Country: USA Inclusion criteria: All patients presenting with blunt head trauma within 24 hours of injury and requiring head CT evaluation in accordance with the written ED practice guidelines were eligible for enrolment			of ICL or isolated skull fracture only.	patients in the NICL group were admitted to the hospital. The mean hospital stay was 7.4 days (range 1–48) for the PICL group and 2.1 days (range 1–6) for the NICL group (p =0.89).
Kelmendi, 2018 [33] single-centre prospective cohort study	n = 80 Age, mean (SD): 9.1 (3.8) years	Index test: S 100 B At 3 hours of injury	Reference standard: Head CT The CT was usually performed within 30 minutes	Trauma related cerebral lesions on CT	The study reports that S100B levels had no effect on clinical decisions or patient management in the study.

Study	Population	Index test	Reference standard	outcome	Comments
	Gender: Forty-six patients were male (57.5%), and 34 patients were female (42.5%). GCS: Patients were diagnosed with mild TBI if they presented with a GCS score of 13–15, loss of consciousness (LOC) lasting < 30 mins and posttraumatic amnesia (PTA) lasting < 1 hour GCS score 15: 25 (31.3%) GCS score 14: 26 (32.5%) GCS score 13: 27 (33.8%) Ethnicity: not reported Setting: emergency department and the neurosurgery clinic Country: Kosovo Inclusion criteria: Children with head trauma alone who were between 2 and 16 years of age were included in the study	Blood samples were obtained from each patient via a cubital vein at 3 hours after head injury.	after the patient was first examined by an emergency physician. A venous blood sample was drawn prior to every CT. The CT examination involved the acquisition of parenchymal and bone window images. All head CTs were reviewed for signs of TBI by a radiologist blinded to the patients' clinical signs and S100B levels		

Study	Population	Index test	Reference standard	outcome	Comments
Manzano, 2016 [42] Prospective multicentre cohort study	N=73 Without intracranial injury (ICI) (n=53); With ICI (n=20) Age mean (SD) months: Without ICI: 94.0 (56.5) With ICI: 78.1 (44.4) Gender (male): Without ICI: 35 (66.0) With ICI: 16 (80.0) GCS score <15: mild TBI Without ICI: 19 (35.8) With ICI: 8 (40.0) Ethnicity: not reported Setting: ED Country: Switzerland Inclusion criteria: children aged <16 years with a mild TBI (GCS≥13) for whom a head CT was requested by the attending physician.	Index test: S100B Venous blood was obtained within 6 hours of the trauma in all children for S100B measurement before a head CT was performed. As the S100B value was not available during the acute care period, the patient's management was not altered.	Reference standard: Cranial CT	Primary outcome was evaluation of the diagnostic value of S100B in detecting intracranial injuries in children aged <16 years with mild head trauma.	No surgical intervention was required.

Study	Population	Index test	Reference standard	outcome	Comments
Mozafari, 2019 [46] cross-sectional study	n = 40 Age: Median age in years (range) Group A (positive CT) (n=20): 9 (2-18) years Group B (negative CT) (n=20): 6.6 (0.5 - 18) years Gender: Group A (positive CT) (n=20): females -4 (20%) Group B (negative CT) (n=20): females – 8 (40%) GCS score %: GCS score 14 Group A (positive CT) (n=20): 4 (20%) Group B (negative CT) (n=20): 13 (65%) GCS score 15 Group A (positive CT) (n=20): 16 (80%) Group B (negative CT) (n=20): 7 (35%) Ethnicity: not reported	Index test: Urine and serum S 100B Within 6 hours of injury	Reference standard: Brain CT	Positive pathologic findings associated with isolated head trauma on CT (not defined positive pathological findings)	No information on treatment reported.

Study	Population	Index test	Reference standard	outcome	Comments
	Setting: Country: Iran Inclusion criteria: presence for an indication of a brain CT scan, aged 6 months to 18 years and a Glasgow coma score of 14 or 15. Injuries included those from traffic and home or sport events, and referrals less than 6 hours of the incident. Inclusion criteria were no previous history of alcohol or drug abuse, the absence of a history of previous neurological disease such as seizure or epilepsy, the absence of severe traffic injury and multiple trauma from motor vehicles, and absence of melanoma				
Mozafari, 2020 [47] Prospective cohort study	n = 62 Age, mean (SD): CT positive: 8.57 (5.16) years CT negative: 8.32 (4.72) years Gender: CT positive: 22 (71%)	Index test: neuron-specific enolase (NSE) A venous blood sample was immediately taken by the ward nurse from all the	Reference standard: Cranial CT	Positivefortrauma pathological findings on CT scan	

Study	Population	Index test	Reference standard	outcome	Comments
	CT negative: 24 (77.4%) GCS score: Positive CT scan group: the frequency of a GCS score of 14 was 17 (54.8%) and that of a GCS score of 15 was 14 (45.2%), Negative CT scan group: frequency of a GCS score of 14 was 6 (19.4%) and that of a GCS score of 15 was 25 (80.6%) Ethnicity: not reported Setting: ED Country: Iran Inclusion criteria: After the initial examinations and stabilisation of the patients with TBIs by a senior emergency medicine resident, CT scans of the brain were performed according to the latest guidelines in case the indications appeared, including an age of 6 months to 18 years, a GCS score of 14 and	eligible patients within 6 hrs of the incident after obtaining their information, performing initial examinations and their initial stabilisation. The patients were then referred to an imaging unit for cranial CT scan.			

Study	Population	Index test	Reference standard	outcome	Comments
	15, the mechanism of damage being of the type of traffic accidents and domestic or sport injuries, the incident occurring within the previous 6 hrs, the parents giving consent for the participation of their children in the study, lack of pregnancy, no history of alcohol or drug abuse, no history of neurological diseases such as seizure and epilepsy and the absence of severe road traffic injuries such as overturned vehicle or being thrown out of the car				
Papa, 2017 [57] Prospective cohort study	n = 196 head trauma patients Patients with head trauma with and without TBI symptoms (n=196): Age in years, mean (SD): 11.51 (7) Gender (male to female ratio): 130 M: 18 F GCS score in ED, n (%): mixed severity TBI GCS score 9-12 = 3 (1.5%) GCS score 13 = 1 (0.5%)	Index test(s) Ubiquitin C-terminal hydrolase (UCH-L1) Blood samples were obtained in all patients within 6 hours of injury	Reference standard CT scan Time between measurement of index test and reference standard: Unclear	Presence of intracranial lesions on initial CT scan	Mixed population (birth-21 years) After assessment and treatment in the ED, patients were either discharged home or admitted to the hospital based on severity of their injuries and patient management was not altered by the study

Study	Population	Index test	Reference standard	outcome	Comments
	GCS score 14 = 13 (6.5%) GCS score 15 = 179 (91.5%) Setting: EDs of three level 1 trauma centres (2 paediatric and 1 adult) Country: USA Inclusion criteria: children and young people (birth–21 years of age) with blunt head trauma presenting to the ED within 6 h of injury with a GCS score of 9–15. The control cohort included trauma patients without blunt head trauma and with a GCS score of 15 presenting to the ED within 6 h of injury.				
Papa, 2015 [58] Prospective cohort study	Age (years), mean (SD): 11.51 (7) Gender (male to female ratio): 131 M: 66 F	Index test(s) Glial fibrillary acidic protein (GFAP) Cut-off level of 0.15 ng/mL	Reference standard: CT scan Time between measurement of index test and reference standard: unclear	presence of intracranial lesions on initial CT scan	Mixed population (birth-21 years) After assessment and treatment in the ED, patients were either discharged home or admitted to hospital

Study	Population	Index test	Reference standard	outcome	Comments
	GCS score in ED, n (%). Mixed severity TBI (majority with mild TBI) GCS score 9-12 = 3 (1.5%) GCS score 13 = 1 (0.5%) GCS score 14 = 13 (6.5%) GCS score 15 = 180 (91.5%) Setting: EDs of three level 1 trauma centres (2 paediatric and 1 adult) Country: USA Inclusion criteria: children and young people (birth–21 years of age) with blunt head trauma presenting to the ED within 6 h of injury with a GCS score of 9–15. The control cohort included trauma patients without blunt head trauma and with a GCS score of 15 presenting to the ED within 6 h of injury.	Blood samples were obtained in all patients within six hours of injury and measured by ELISA for GFAP (ng/ml)			based on severity of their injuries and patient management was not altered by the study
Papa, 2016 [56] Prospective cohort study	n = 155 Age, mean (SD): 13 (7) years	Index test: Glial fibrillary acidic protein (GFAP)	Reference standard: Head CT	Presence of intracranial lesions on initial CT scan.	Mixed population of adults and children (six months to 21 years).

Study	Population	Index test	Reference standard	outcome	Comments
	range from six months to 21 years. Gender (male): 100 (65%) GCS: mixed severity but 99% with GCS score 13-15 GCS score 9-12: 2 (1%) GCS score 13: 2 (1%) GCS score 14: 6 (4%) GCS score 15: 146 (94%) Ethnicity: not reported Setting: ED Country: USA Inclusion criteria: history of blunt head trauma presenting to the ED within 6 h of injury with an initial GCS score of 9 to 15. Head trauma patients were further categorised into children with TBI symptoms (loss of consciousness, amnesia, disorientation, or change in behaviour) and children without TBI symptoms.	S100B Blood samples were obtained within 6 hours of the reported time of injury.	Trauma patients underwent standard CT scan of the head according to the judgment of the treating physician. CT examinations were interpreted by board-certified radiologists who recorded location, extent, and type of brain injury.		After assessment and treatment in the ED, patients were either discharged home or admitted to hospital based on severity of their injuries, and patient management was not altered by the study. No further details on treatment.

Study	Population	Index test	Reference standard	outcome	Comments
Yeung, 2020 [66] Prospective cohort study	n = 24 children < 18 years Age, median IQR: age of 5 years (3.5, 1—8.8 years) Gender: 67% males GCS: Mixed severity (mild, moderate and severe). Majority with mild TBI mild TBI with GCS score of 13 to 15: 15 (62.5%) moderate TBI with GCS score of 9 to 12: 4 (16.7%) severe TBI with GCS score of 8 or less: 5 (20.8%) Ethnicity: not reported Setting: tertiary care hospital Country: USA Inclusion criteria: Children 0 to 18 years with an isolated, acute (<24 hours) TBI who presented to a paediatric trauma referral centre were	Index test: Salivary biomarkers • GFAP • S100B • NSE Study reports variable timing of sample collection. Timing of index test not reported	Reference standard: Head CT	Significant brain injury on CT scan	All patients with SBI were admitted to the paediatric intensive care unit; 1 patient (7.1%) subsequently expired

Study	Population	Index test	Reference standard	outcome	Comments
	eligible for participation if they required inpatient hospitalisation following ED management.				

1.1.6 Summary of the diagnostic evidence

Table 5: Clinical evidence summary: diagnostic test accuracy of biomarkers in adults

Index Test/study	Number of studies	N	Ref. standard	Time-point	Outcome definition	Sensitivity (95% CI)	Specificity (95% CI)	Risk of bias	Indirectness	Inconsistency	Imprecision	GRADE
S100B <3 hours after injury												
Adults- serum S 100 B (cut-offs 0.10 and 0.105 µg/L) (within 3 hours after injury) – meta-analysis performed												
serum S 100 B (cut-offs 0.10 and 0.105 µg/L)	6 Biberthaler,2006 Ernstbrunner, 2016 Laribi, 2014 Li, 2022 Muller 2011 Thaler, 2015 Wolf, 2013	3676	CT	Within 3 hours	Varies across studies: see footnotes[d]	0.99 [0.94, 1.00] 0.75 [0.19, 0.99] 1.00 [0.87, 1.00] 0.93 [0.81, 0.99] 0.86 [0.65, 0.97] 0.98 [0.89, 1.00] 0.73 [0.54, 0.88] **Pooled:** (0.94 (0.83, 0.99) [e]	0.33 [0.30, 0.36] 0.19 [0.15, 0.23] 0.38 [0.33, 0.43] 0.17 [0.11, 0.24] 0.32 [0.26, 0.38] 0.35 [0.32, 0.39] 0.36 [0.26, 0.48] **Pooled:** 0.29 (0.20, 0.40) [e]	Sensitivity Very Serious[a]	None	None	None	LOW
								Specificity Very Serious[a]	None	None	None	LOW
Adults - serum S100B (cut-off 0.15 µg/L) - within 3 hours after injury												
	1	524	CT			0.96 [0.81, 1.00]	0.44 [0.39, 0.49]	Sensitivity				

Index Test/study	Number of studies	N	Ref. standard	Time-point	Outcome definition	Sensitivity (95% CI)	Specificity (95% CI)	Risk of bias	Indirectness	Inconsistency	Imprecision	GRADE
serum S100B cut-off 0.15, µg/L)	Laribi 2014 Biberthaler, 2002			within 3 hours	CT findings of intracranial lesions. positive CCT scan (diffuse injury I-IV, evacuated mass lesion and non-evacuated mass lesion)	1.00 [0.86, 1.00]	0.46 [0.35, 0.58]	Very serious[a]	None	None	Serious[c]	VERY LOW
								Specificity				
								Very serious[a]	None	None	None	LOW
Adults - serum S100B (optimal cut off 0.115 µg/L)- at 3 hours after injury												
serum S100B (optimal cut off 0.115 µg/L)	1 Asadollahi, 2016	158	CT	at 3 hours	Positive CT scan (at least one trauma-relevant lesion (epidural, subdural, subarachnoid, intracerebral haemorrhage, cerebral contusion, brain oedema, depressed skull fracture))	0.95 [0.88, 0.99]	0.35 [0.25, 0.47]	Sensitivity				
								Very serious[a]	None	Serious[b]	Serious[c]	VERY LOW
								Specificity				
								Very serious[a]	None	Serious[b]	Very serious[c]	VERY LOW
Adults-serum S100B measured immediately after admission (cut-off ≥0.2 µg/L) mean 3 hours after injury												

Index Test/study	Number of studies	N	Ref. standard	Time-point	Outcome definition	Sensitivity (95% CI)	Specificity (95% CI)	Risk of bias	Indirectness	Inconsistency	Imprecision	GRADE
serum S100B measured immediately after admission (cut-off ≥0.2 µg/L)	1 Ingebrigtsen, 2000	182	CT	mean 3 hours	intracranial pathology on CT scan	0.90 [0.55, 1.00]	0.65 [0.57, 0.72]	Sensitivity				
								Very Serious[a]	Serious[b]	None	Very serious[c]	VERY LOW
								Specificity				
								Very Serious[a]	Serious[b]	None	Serious[c]	VERY LOW
Adults- serum S100B (cut-off 0.12 µg/L)- within 2 hours after injury												
serum S100B (cut-off 0.12 µg/L)	1 Biberthaler, 2002	104	CT scan	within 2 hours	positive CCT scan (diffuse injury I-IV, evacuated mass lesion and non-evacuated mass lesion)	1.00 [0.86, 1.00]	0.46 [0.35, 0.58]	Sensitivity				
								Very Serious[a]	None	None	Serious[c]	VERY LOW
								Specificity				
								Very Serious[a]	None	None	None	LOW
Adults- serum S100B (a cut-off of 0.48 µg/L)- within 3 hours after injury												
serum S 100B (a cutoff of 0.48 µg/L)	1 Wolf, 2013	107	CT	within 3 hours	CT positive (patients with epidural, subdural, subarachnoid,	0.33 [0.17, 0.53]	0.91 [0.82, 0.96]	Sensitivity				
								Very serious[a]	None	None	none	LOW
								Specificity				

Index Test/study	Number of studies	N	Ref. standard	Time-point	Outcome definition	Sensitivity (95% CI)	Specificity (95% CI)	Risk of bias	Indirectness	Inconsistency	Imprecision	GRADE
					or intracerebral haemorrhage, including contusions)			Very serious[a]	None	None	Serious[c]	VERY LOW
Adults- serum S100B post injury (cut-off 0.38 µg/L)- within 3 hours after injury												
serum S100B post injury (cut-off 0.38 µg/L)	1 Cervellin, 2012	60	CT	within 3 hours	positive CT scan (any intracranial pathology associated with an injury (acute subdural, epidural or parenchymal hematoma, traumatic subarachnoid haemorrhage, cerebral contusion and brain swelling))	1.00 [0.83, 1.00]	0.57 [0.41, 0.73]	Sensitivity				
								None	Serious[b]	None	Serious[c]	LOW
								Specificity				
								None	Serious[b]	None	Serious[c]	LOW
Adults- serum S100B (cut-off 2.31 µg/L)-within 3 hours after injury												
serum S100B (cut-off 2.31 µg/L)	1 Cervellin, 2012	60	CT	within 3 hours	positive CT scan (any intracranial pathology associated with	0.15 [0.03, 0.38]	1.00 [0.91, 1.00]	Sensitivity				
								None	Serious[b]	None	None	MODERATE

Index Test/study	Number of studies	N	Ref. standard	Time-point	Outcome definition	Sensitivity (95% CI)	Specificity (95% CI)	Risk of bias	Indirectness	Inconsistency	Imprecision	GRADE
					an injury (acute subdural, epidural or parenchymal hematoma, traumatic subarachnoid haemorrhage, cerebral contusion and brain swelling))			Specificity				
								None	Serious[b]	None	Serious[c]	LOW
S100B - > 3 to 6 hours after injury												
Adults- serum S 100 B (cut-offs 0.10 and 0.105 µg/L) (> 3 - 6 hours after injury) – meta-analysis performed												
serum S 100 B (cut-offs 0.10	10 Bazarian 2013	3994	CT	>3 hours to 6 hours	Varies across studies: see footnotes[d]	0.87 [0.73, 0.95] 0.85 [0.68, 0.95] 1.00 [0.78, 1.00] 0.95 [0.77, 1.00]	0.36 [0.32, 0.39] 0.30 [0.25, 0.36] 0.27 [0.19, 0.35] 0.31 [0.25, 0.37]	Sensitivity				
								Very serious[a]	Very serious[b]	None	Serious[c]	VERY LOW
								Specificity				

Index Test/study	Number of studies	N	Ref. standard	Time-point	Outcome definition	Sensitivity (95% CI)	Specificity (95% CI)	Risk of bias	Indirectness	Inconsistency	Imprecision	GRADE
and 0.105 µg/L)	David, 2017 Egea-Guerrero, 2012 Egea-Guerrero, 2018 Kahouadji, 2020 Lagerstedt, 2017 Laribi, 2014 Morochovic, 2009 Vedin, 2021 Zongo 2012					0.97 [0.84, 1.00] 0.81 [0.64, 0.93] 0.68 [0.46, 0.85] 0.83 [0.59, 0.96] 1.00 [0.75, 1.00] 0.99 [0.95, 1.00] **Pooled:** 0.93 [0.84,0.98][e]	0.10 [0.05, 0.18] 0.42 [0.34, 0.51] 0.52 [0.46, 0.57] 0.30 [0.20, 0.41] 0.14 [0.10, 0.20] 0.14 [0.13, 0.16] **Pooled:** 0.27 [0.17, 0.38][e]	Very serious[a]	Very serious[b]	None	none	VERY LOW
Adults- serum S100B (cut-off >0.29 µg/L)- within 6hours after injury												
serum S100B (cut-off >0.29 µg/L)	1 Bazarian 2013	787	CT	within 6 hours	Traumatic CT abnormalities defined as subdural	0.52 [0.37, 0.66]	0.76 [0.73, 0.79]	Sensitivity Serious[a]	Serious[b]	none	Serious[c]	VERY LOW
								Specificity				

Index Test/study	Number of studies	N	Ref. standard	Time-point	Outcome definition	Sensitivity (95% CI)	Specificity (95% CI)	Risk of bias	Indirectness	Inconsistency	Imprecision	GRADE
					hematomas (SDH), epidural hematomas, subarachnoid haemorrhage (SAH), oedema, skull fracture, and cerebral contusions.			Serious[a]	Serious[b]	none	None	LOW
Adults- serum S100B (cut-off >0.06 µg/L)- within 6hours after injury												
serum S100B (cut-off >0.06 µg/L)	1 Bazarian 2013	787	Cranial CT	within 6 hours	Traumatic CT abnormalities defined as subdural hematomas (SDH), epidural hematomas, subarachnoid haemorrhage (SAH), oedema, skull fracture, and cerebral contusions.	1.00 [0.93, 1.00]	0.12 [0.10, 0.15]	Sensitivity				
								Serious[a]	Serious[b]	none	none	LOW
								Specificity				
								Serious[a]	Serious[b]	none	none	LOW
Adults- serum S100B (cut-off >2.391 µg/L)- within 6 hours after injury												
serum S100B (cut-		787	CT	within 6 hours	Traumatic CT abnormalities	0.04 [0.00, 0.14]	0.99 [0.98, 1.00]	Sensitivity				
								Serious[a]	Serious[b]	none	none	LOW

Index Test/study	Number of studies	N	Ref. standard	Time-point	Outcome definition	Sensitivity (95% CI)	Specificity (95% CI)	Risk of bias	Indirectness	Inconsistency	Imprecision	GRADE
off >2.391 µg/L)	1 Bazarian 2013				defined as subdural hematomas (SDH), epidural hematomas, subarachnoid haemorrhage (SAH), oedema, skull fracture, and cerebral contusions.			Specificity				
								Serious[a]	Serious[b]	none	none	LOW
Adults- serum S100B (cut-off >0.097 µg/L)-within 6 hours after injury												
serum S100B (cut-off >0.097 µg/L)	1 Bazarian 2013	787	CT	within 6 hours	Traumatic CT abnormalities defined as subdural hematomas (SDH), epidural hematomas, subarachnoid haemorrhage (SAH), oedema, skull fracture, and cerebral contusions.	0.90 [0.78, 0.97]	0.32 [0.29, 0.36]	Sensitivity				
								Serious[a]	Serious[b]	none	Serious[c]	LOW
								Specificity				
								Serious[a]	Serious[b]	none	none	LOW
Adults- serum S100B (cut-off >0.521 µg/L)-within 6hours after injury												

Index Test/study	Number of studies	N	Ref. standard	Time-point	Outcome definition	Sensitivity (95% CI)	Specificity (95% CI)	Risk of bias	Indirectness	Inconsistency	Imprecision	GRADE
serum S100B (cut-off >0.521 µg/L)	1 Bazarian 2013	787	CT	within 6 hours	Traumatic CT abnormalities defined as subdural hematomas (SDH), epidural hematomas, subarachnoid haemorrhage (SAH), oedema, skull fracture, and cerebral contusions.	0.24 [0.13, 0.38]	0.90 [0.88, 0.92]	Sensitivity				
								Serious[a]	Serious[b]	none	none	LOW
								Specificity				
								Serious[a]	Serious[b]	none	Serious[c]	VERY LOW
Adults- serum S100B (cut-off 0.130 µg/L) at 6 hours after injury												
serum S100 B (cut-off 0.130 µg/L)	1 Egea-Guerrero, 2012	143	CT	at 6 hours	intracranial lesion (IL) on CT	1.00 [0.78, 1.00]	0.33 [0.25, 0.42]	Sensitivity				
								none	Serious[b]	none	Serious[c]	LOW
								Specificity				
								none	Serious[b]	none	none	MODERATE
Adults- serum S100B (cut-off 0.2 µ g/L) -mean 3.8 hours after injury												
serum S100 B	1 Romner, 2000	278	CT and MRI	mean 3.8 hours	Intra cranial findings on CT	0.92 [0.74, 0.99]	0.66 [0.60, 0.72]	Sensitivity				
								Very serious[a]	Serious[b]	none	Serious[c]	VERY LOW

Index Test/study	Number of studies	N	Ref. standard	Time-point	Outcome definition	Sensitivity (95% CI)	Specificity (95% CI)	Risk of bias	Indirectness	Inconsistency	Imprecision	GRADE
(cut-off 0.2 µ g/L)				after injury				Specificity				
								Very serious[a]	Serious[b]	none	Serious[c]	VERY LOW
Adults-serum S100B (optimal cut off 0.21 µg/L)- at 6 hours after injury												
serum S100B (optimal cut off 0.21 µg/L)	1 Asadollahi, 2016	236	CT	at 6 hours	Positive CT scan (at least one trauma-relevant lesion (epidural, subdural, subarachnoid, intracerebral haemorrhage, cerebral contusion, brain oedema, depressed skull fracture))	0.99 [0.93, 1.00]	0.20 [0.14, 0.27]	Sensitivity				
								Serious[a]	none	none	none	MODERATE
								Specificity				
								Serious[a]	none	none	none	MODERATE
Adults- serum S100 B (cut-off 0.230 µg/L) at 6 hours after injury												
serum S100 B (cut-off 0.230 µg/l)	1 Egea-Guerrero, 2012	143	CT	at 6 hours	intracranial lesion (IL) on CT	0.93 [0.68, 1.00]	0.52 [0.43, 0.60]	Sensitivity				
								None	Serious[b]	None	serious[c]	LOW
								Specificity				

Index Test/study	Number of studies	N	Ref. standard	Time-point	Outcome definition	Sensitivity (95% CI)	Specificity (95% CI)	Risk of bias	Indirectness	Inconsistency	Imprecision	GRADE
								None	Serious[b]	None	None	MODERATE
Adults- serum S100 B (cut-off 0.254 µg/l) at 6hours after injury												
serum S100 B (cut-off 0.254 µg/l)	1 Egea-Guerrero, 2012	143	CT	at 6 hours	intracranial lesion (IL) on CT	0.87 [0.60, 0.98]	0.57 [0.48, 0.66]	Sensitivity				
								None	Serious[b]	None	Serious[c]	MODERATE
								Specificity				
								None	Serious[b]	None	Serious[c]	LOW
Adults- serum S100B (cut-off 0.15, µg/L)- at 6 hours after injury (second sampling 3 hrs after 1st sampling)												
serum S100B (cut-off 0.15, µg/L)	1 Laribi, 2014	412	CT	at 6 hours	CT findings of intracranial lesions.	0.85 [0.65, 0.96]	0.63 [0.58, 0.68]	Sensitivity				
								Serious[a]	None	None	Serious[c]	LOW
								Specificity				
								Serious[a]	None	None	Serious[c]	LOW
Adults (mean age 24 yrs) [mixed children and adults] - serum S100B- optimal cut-off value of 0.47 µg/L -within 4hours after injury												
serum S100B-optimal cut-	1 Cevik 2019	48	CT	within 4 hours	abnormal cerebral CT findings	0.96 [0.79, 1.00]	0.63 [0.41, 0.81]	Sensitivity				
								Very serious[a]	Serious[b]	None	Serious[c]	LOW
								Specificity				

Index Test/study	Number of studies	N	Ref. standard	Time-point	Outcome definition	Sensitivity (95% CI)	Specificity (95% CI)	Risk of bias	Indirectness	Inconsistency	Imprecision	GRADE
off value of 0.47 µg/L								Very serious[a]	Serious[b]	None	Serious[c]	VERY LOW
Adults- serum S100B (cut-off 0.42 µg/L)-within 6 hours after injury												
serum S100B (cut-off 0.42 µg/L	1 Kotlyar, 2011	346	CT	within 6 hours	Positive CT results (subarachnoid haemorrhage, epidural haemorrhage, subdural haemorrhage, intraparenchymal haemorrhage, diffuse brain oedema , diffuse axonal injury, skull fracture)	0.86 [0.65, 0.97]	0.37 [0.32, 0.43]	Sensitivity				
								Very serious[a]	None	None	Serious[c]	VERY LOW
								Specificity				
								Very serious[a]	None	None	None	LOW
Adults- serum S100B (cut-off 0.32 µg/L)-within 6 hours after injury												
serum S100B (cut-off 0.32 µg/L)	1 Kotlyar, 2011	346	CT	within 6 hours	Positive CT results (subarachnoid haemorrhage,	0.91 [0.71, 0.99]	0.24 [0.20, 0.29]	Sensitivity				
								Very serious[a]	None	None	Serious[c]	VERY LOW
								Specificity				

Index Test/study	Number of studies	N	Ref. standard	Time-point	Outcome definition	Sensitivity (95% CI)	Specificity (95% CI)	Risk of bias	Indirectness	Inconsistency	Imprecision	GRADE
					epidural haemorrhage, subdural haemorrhage, intraparenchymal haemorrhage, diffuse brain oedema, diffuse axonal injury, skull fracture)			Very serious[a]	None	None	None	LOW
Adults- serum S100B (cut-off 0.24 µg/L) - within 6 hours after injury												
serum S100B (cut-off 0. 24 µg/L)	1 Kotlyar, 2011	346	CT	within 6 hours	Positive CT results (subarachnoid haemorrhage, epidural haemorrhage, subdural haemorrhage, intraparenchymal haemorrhage, diffuse brain oedema, diffuse axonal injury, skull fracture)	0.97 [0.82, 1.00]	0.13 [0.10, 0.17]	Sensitivity				
								Very serious[a]	None	None	Serious[c]	VERY LOW
								Specificity				
								Very serious[a]	None	None	None	LOW
Adults- serum S100B (cut-off value, 0.12 µg/L) - within 6 hours after injury												

Index Test/study	Number of studies	N	Ref. standard	Time-point	Outcome definition	Sensitivity (95% CI)	Specificity (95% CI)	Risk of bias	Indirectness	Inconsistency	Imprecision	GRADE
serum S100B (cut-off Value, 0.12 µg/L)	1 Zongo 2012	1560	CT	within 6 hours	CT scan abnormality (CT positive)	0.99 [0.95, 1.00]	0.20 [0.18, 0.22]	Sensitivity				
								None	None	None	None	HIGH
								Specificity				
								None	None	None	None	HIGH
Adults- serum S100B (cut-off 0.14 µg/L)-within 6 hours after injury												
serum S100B (cut-off Value, 0.14 µg/L)	1 Zongo 2012	1560	CT	within 6 hours	CT scan abnormality (CT positive)	0.97 [0.92, 0.99]	0.27 [0.25, 0.29]	Sensitivity				
								None	None	None	None	HIGH
								Specificity				
								None	None	None	None	HIGH
Adults- serum S100B (Cut-off 0.105 µg/L)- within 6 hours after injury (CCT negative or MRI positive)												
serum S100B (Cut-off 0.105 mg/ L)	1 Oh, 2007	101	CT or MRI	within 6 hours	acute traumatic brain injury (CCT negative or MRI positive)	1.00 [0.92, 1.00]	0.54 [0.40, 0.67]	Sensitivity				
								Very serious[a]	Serious[b]	None	None	LOW
								Specificity				
								Very serious[a]	Serious[b]	None	Serious[c]	VERY LOW
Adults (mixed adults and children -mean age adults) – serum S100B (cut-off 0.072 ug/L)- within 6 hours after injury												
serum S100B (cut-off	1 Lagerstedt, 2018	133	CT	within 6 hours	CT positive (Epidural haemorrhage, Subdural	sensitivity set at 100%	specificity: 18.4% (95% CI 12.9– 24.6)[f]	sensitivity				
								very serious[a]	Serious[b]	none	none	VERY LOW
								specificity				

Index Test/study	Number of studies	N	Ref. standard	Time-point	Outcome definition	Sensitivity (95% CI)	Specificity (95% CI)	Risk of bias	Indirectness	Inconsistency	Imprecision	GRADE
0.072 0. 24 µg/L)					haemorrhage, Subarachnoid haemorrhage, Intracerebral haemorrhage, Contusion with haemorrhage, Skull fracture).			very serious[a]	Serious[b]	none	none	VERY LOW
Adults- serum S100B (0. 03 µg/L threshold) [6 hours -time from reported injury to blood sample obtained]												
serum S100B (0.03 µg/L threshold)	1 Welch, 2016	231	CT	at 6 hours -	CT scan was positive—defined as the presence of an acute trauma-related intracranial lesion	sensitivity set at 100%	0.02 (95% CI 0.00, 0.04)[f]	sensitivity				
								Serious[a]	Serious[b]	none	none	LOW
								specificity				
								Serious[a]	Serious[b]	none	none	LOW
Adults- serum S100B (0.10 µg/l] threshold) [6 hours -time from reported injury to blood sample obtained]												
serum S100B (0.10 µg/l] threshold)	1 Welch, 2016	231	CT	at 6 hours	CT scan was positive—defined as the presence of an acute trauma-related intracranial lesion	0.91 (0.75, 0.98)	0.44 (0.37, 0.51)[f]	sensitivity				
								Serious[a]	Serious[b]	none	Serious[c]	VERY LOW
								specificity				
								Serious[a]	Serious[b]	none	none	LOW

Index Test/study	Number of studies	N	Ref. standard	Time-point	Outcome definition	Sensitivity (95% CI)	Specificity (95% CI)	Risk of bias	Indirectness	Inconsistency	Imprecision	GRADE
Adults-serum S100B (cut-off NR) within 4 hours after injury												
serum S100B (cut-off NR)	1 Bazarian, 2006	96	CT	within 4 hours	Traumatic abnormality on initial CT scan	when sensitivity set at 70%	Specificity%: 26% (CI not reported)[f]	sensitivity				
								Very serious[a]	none	none	Cannot assess	LOW
								specificity				
								Very serious[a]	none	none	Cannot assess	LOW
S100B >6 to 12 hours after injury												
Adults- serum S100 B- 0 to 8-hours after injury (cut-off value NR)												
serum S100 B- (cut-off value NR)	1 Mahan, 2019	104	CT	0- to 8-hours	CT positive (not defined)	0.64 [0.46, 0.79]	0.54 [0.42, 0.67]	Sensitivity				
								None	Serious[b]	None	Serious[c]	LOW
								Specificity				
								None	Serious[b]	None	Serious[c]	LOW
Adults-serum S100B (Cut-off ≥0.10 µg/L) -within 12 hours after injury												
serum S100B (Cut-off ≥0.10 µg/L)	1 Muller 2007	226	CT	within 12 hours	Intracranial pathologic findings revealed by CT	0.95 [0.76, 1.00]	0.31 [0.25, 0.38]	Sensitivity				
								Very serious[a]	None	None	Serious[c]	VERY LOW
								Specificity				

Index Test/study	Number of studies	N	Ref. standard	Time-point	Outcome definition	Sensitivity (95% CI)	Specificity (95% CI)	Risk of bias	Indirectness	Inconsistency	Imprecision	GRADE
					scan (not defined)			Very serious[a]	None	None	None	LOW
S100B> 12 hours after injury												
Adults- serum S100 B - 12 to 32-hours after injury (cut-off value NR)												
serum S100 B - cut-off value NR)	1 Mahan, 2019	104	CT	12- to 32-hours	CT positive (not defined)	0.72 [0.55, 0.86]	0.57 [0.45, 0.69]	Sensitivity				
								None	Serious[b]	None	Serious[c]	VERY LOW
								Specificity				
								None	Serious[b]	None	Serious[c]	VERY LOW
Adults- serum S100B (cut-off [0.179 µg/L])- within 24 hours of admission												
serum S100B (cut-off 0.179 µg/L) Patients with Isolated Mild TBI	1 Posti 2019	55	CT	Within 24 hours	CT scans were classified according to the Marshall grading system. Diffuse injury/grade I (no visual pathology) was considered CT-, whereas the other grades (II-VI) were regarded as CT+.	sensitivity set at 100%	Specificity 11.1 (CI not reported)[f]	sensitivity				
								Serious[a]	Serious[b]	none	Cannot assess	LOW
								Specificity				
								Serious[a]	Serious[b]	none	Cannot assess	LOW

Index Test/study	Number of studies	N	Ref. standard	Time-point	Outcome definition	Sensitivity (95% CI)	Specificity (95% CI)	Risk of bias	Indirectness	Inconsistency	Imprecision	GRADE
S100B- timing of test NR												
Adults-serum S100B (cut-off value of 0.1µg/L)- not time specified [reference standard CT and MRI]												
serum S100B (cut-off value of 0.1mg/L)	1 Linsenmaier, 2016	41	CT and MRI	not time specified	CCT positive. If intracranial haemorrhage could not be excluded safely, the patient was also considered as "CCT positive" because of an equivocal CT finding deserving further evaluation.	1.00 [0.48, 1.00]	0.25 [0.12, 0.42]	Sensitivity				
								Very serious[a]	None	None	Very serious[c]	VERY LOW
								Specificity				
								Very serious[a]	None	None	None	LOW
Urine S100B												
Adults- urine S100B (cut-off ≥0.09 µg/L)- within 6 hours after injury												
Adults-urine - S100B (cut-	1 Vedin, 2021	243	CT	within 6 hours	Intracranial haemorrhage on CT	0.92 [0.64, 1.00]	0.11 [0.08, 0.16]	Sensitivity				
								Very serious[a]	None	None	Serious[c]	VERY LOW
								Specificity				

Index Test/study	Number of studies	N	Ref. standard	Time-point	Outcome definition	Sensitivity (95% CI)	Specificity (95% CI)	Risk of bias	Indirectness	Inconsistency	Imprecision	GRADE
off ≥0.09 µg/L)								Very serious[a]	None	None	None	LOW
GFAP												
within 6hours after injury												
Adults [mixed adults and children (mean 24 years)]- serum GFAP - cut-off 0.23 µg/L -within 4hours after injury												
serum GFAP - cut-off 0.23 0.23 µg/L	1 Cevik 2019	48	CT	within 4 hours	abnormal cerebral CT findings	0.75 [0.53, 0.90]	0.63 [0.41, 0.81]	Sensitivity				
								Very serious[a]	Serious[b]	none	Serious[c]	VERY LOW
								Specificity				
								Very serious[a]	Serious[b]	none	Serious[c]	VERY LOW
Adults- serum GFAP (cut-off 1.35 µg/L)- within 6 hours after injury												
serum GFAP (cut-off 1.35 µg/L)	1 Forouzan, 2021	176	CT	within 6 hours	positive CT scan (acute epidural or subdural hematoma,	0.50 [0.16, 0.84]	0.44 [0.36, 0.52]	Sensitivity				
								Serious[a]	none	none	Serious[c]	LOW
								Specificity				

Index Test/study	Number of studies	N	Ref. standard	Time-point	Outcome definition	Sensitivity (95% CI)	Specificity (95% CI)	Risk of bias	Indirectness	Inconsistency	Imprecision	GRADE
					cortical contusion, ventricular compression, ventricular trapping, cerebral herniation, intraventricular haemorrhage, hydrocephalus, subarachnoid haemorrhage, cerebral oedema, post-traumatic ischemia, intracranial hematoma, and cerebral venous sinus thrombosis)			Serious[a]	none	none	none	MODERATE
Adults- plasma GFAP (cut-off 0.022 µg/ml) -within 6 hours after injury												
plasma GFAP (cut-off 0.022 µg/ml)	1 Li, 2022	463	CT	0-6 hours	Abnormalities on CT-presence/absence of closed	0.97 [0.92, 0.99]	0.50 [0.45, 0.56]	sensitivity Serious[a]	none	none	none	MODERATE

Index Test/study	Number of studies	N	Ref. standard	Time-point	Outcome definition	Sensitivity (95% CI)	Specificity (95% CI)	Risk of bias	Indirectness	Inconsistency	Imprecision	GRADE
					head injuries including skull fracture, pneumocephalus, haemorrhage, mass effect, and brain parenchymal injuries.			Specificity				
								Serious[a]	none	none	none	MODERATE
Adults- serum GFAP (cut-off 0.022 µg/ml) -within 6 hours after injury												
serum GFAP (cut-off 0.022 µg/ml)	1 Li, 2022	463	CT	0-6 hours	Abnormalities on CT-presence/absence of closed head injuries including skull fracture, pneumocephalus, haemorrhage, mass effect, and brain parenchymal injuries.	0.93 [0.82, 0.98]	0.51 [0.43, 0.59]	Sensitivity				
								Serious[a]	none	none	none	MODERATE
								Specificity				
								Serious[a]	none	none	none	MODERATE
Adults-serum GFAP (cut-off of 0 µg/L) [6 hours -time from reported injury to blood sample obtained]												
		231	CT	a6 hours				sensitivity				

Index Test/study	Number of studies	N	Ref. standard	Time-point	Outcome definition	Sensitivity (95% CI)	Specificity (95% CI)	Risk of bias	Indirectness	Inconsistency	Imprecision	GRADE
serum GFAP (cut-off of 0 µg/L)	1 Welch 2016				CT scan was positive—defined as the presence of an acute trauma-related intracranial lesion	Sensitivity set at 100%	0.00 (0.00, 0.02)[f]	Serious[a]	Serious[b]	none	Cannot assess	LOW
								specificity				
								Serious[a]	Serious[b]	none	Cannot assess	LOW
within 8h after injury												
Adults- serum GFAP - 0 to 8-hours after injury (cut-off value NR)												
serum GFAP - (cut-off value NR)	1 Mahan, 2019	104	CT	within 8-hours	CT positive (not defined)	0.89 [0.74, 0.97]	0.62 [0.49, 0.73]	Sensitivity				
								None	Serious[b]	none	Serious[c]	LOW
								Specificity				
								None	Serious[b]	none	Serious[c]	LOW
within 24 h after injury												
Adults- serum GFAP cut-off 0.013 µg/L -within 24h after injury												
serum GFAP cut-off 0.013 µg/L	1 Okonkwo, 2020	1359	CT	within 24 hours	intracranial injury on admission CT scan	0.99 [0.98, 1.00]	0.16 [0.13, 0.18]	Sensitivity				
								Very serious[a]	Serious[b]	none	none	VERY LOW
								Specificity				

Index Test/study	Number of studies	N	Ref. standard	Time-point	Outcome definition	Sensitivity (95% CI)	Specificity (95% CI)	Risk of bias	Indirectness	Inconsistency	Imprecision	GRADE
								Very serious[a]	Serious[b]	none	none	VERY LOW
Adults- serum GFAP cut-off 0.038 µg/L) -within 24 hours after injury												
serum GFAP cut-off 0.038 µg/L)	1 Okonkwo, 2020	1359	CT	within 24 hours	intracranial injury on admission CT scan	0.96 [0.94, 0.98]	0.30 [0.27, 0.34]	Sensitivity				
								Very serious[a]	Serious[b]	none	none	VERY LOW
								Specificity				
								Very serious[a]	Serious[b]	none	none	VERY LOW
Adults-serum GFAP cut-off 0.113 µg/L)- within 24hours after injury												
serum GFAP cut-off 0.113 µg/L)	1 Okonkwo, 2020	1359	CT	within 24 hours	intracranial injury on admission CT scan	0.90 [0.87, 0.93]	0.50 [0.46, 0.53]	Sensitivity				
								Very serious[a]	Serious[b]	none	Serious[c]	VERY LOW
								Specificity				
								Very serious[a]	Serious[b]	none	none	VERY LOW
Adults- serum GFAP cut-off 0.190 µg/L)- within 24hours after injury												
serum GFAP cut-off 0.190 µg/L)	1 Okonkwo, 2020	1359	CT	within 24 hours of injury	intracranial injury on admission CT scan	0.85 [0.81, 0.87]	0.59 [0.56, 0.63]	Sensitivity				
								Very serious[a]	Serious[b]	none	none	VERY LOW
								Specificity				

Index Test/study	Number of studies	N	Ref. standard	Time-point	Outcome definition	Sensitivity (95% CI)	Specificity (95% CI)	Risk of bias	Indirectness	Inconsistency	Imprecision	GRADE
								Very serious[a]	Serious[b]	none	Serious[c]	VERY LOW
Adults- serum GFAP [cut-off 0.43 µg/L)]- within 24 hours after injury - middle age (40-59)												
serum GFAP 0.43 µg/L)	1 Gardner, 2018	60	CT	within 24 hours	acute intra cranial trauma	0.90 [0.68, 0.99]	0.78 [0.62, 0.89]	Sensitivity				
								Serious[a]	none	none	Serious[c]	LOW
								Specificity				
								Serious[a]	none	none	none	MODERATE
Adults- serum GFAP [cut-off point 0.43 µg/L) -within 24 hours after injury- young (<40 yr)												
serum GFAP [cut-off point 0.43 µg/L)	1 Gardner, 2018	79	CT	within 24 hours	acute intra cranial trauma	0.83 [0.59, 0.96]	0.84 [0.72, 0.92]	Sensitivity				
								Serious[a]	none	none	Very serious[c]	VERY LOW
								Specificity				
								Serious[a]	none	none	Serious[c]	LOW
Adults- serum GFAP [cut-off 0.43 µg/L)] -within 24 hours after injury- older age (>60)												
serum GFAP [cut-off 0.43 µg/L)]	1 Gardner, 2018	30	CT	within 24 hours	acute intra cranial trauma	0.67 [0.41, 0.87]	0.67 [0.35, 0.90]	Sensitivity				
								Serious[a]	none	none	Serious[c]	LOW
								Specificity				

Index Test/study	Number of studies	N	Ref. standard	Time-point	Outcome definition	Sensitivity (95% CI)	Specificity (95% CI)	Risk of bias	Indirectness	Inconsistency	Imprecision	GRADE
								Serious[a]	none	none	Serious[c]	LOW
Adults- serum GFAP cut-off 0.0666 µg/L - within 24 hours of admission												
serum GFAP cut-off 0.0666 µg/L Patients with Isolated Mild TBI	1 Posti 2019	55	CT	Within 24 hours	CT scans were classified according to the Marshall grading system. Diffuse injury/grade I (no visual pathology) was considered CT-, whereas the other grades (II-VI) were regarded as CT+.	sensitivity set at 100%	Specificity 19.4 (no CI reported)[f]	Sensitivity				
								Serious[a]	Serious[b]	none	Not applicable	LOW
								Specificity				
								Serious[a]	Serious[b]	None	Cannot assess	LOW
Adults- serum GFAP (cut-off 0.06662 µg/L)- within 24 hours after injury												
serum GFAP (cut-off 0.0662 µg/L	1 Posti 2019	93	CT	within 24 hours	CT scans were classified according to the Marshall grading system. Diffuse	Sensitivity set at 100%	Specificity 16.1 (CI not reported)[f]	Sensitivity				
								Serious[a]	Serious[b]	none	Cannot assess	LOW
								Specificity				

Index Test/study	Number of studies	N	Ref. standard	Time-point	Outcome definition	Sensitivity (95% CI)	Specificity (95% CI)	Risk of bias	Indirectness	Inconsistency	Imprecision	GRADE
Patients with Mild TBI					injury/grade I (no visual pathology) was considered CT-, whereas the other grades (II-VI) were regarded as CT+.			Serious[a]	Serious[b]	none	Cannot assess	LOW
within 32 hours after injury												
Adults- serum GFAP -12 to 32-hours after injury- (cut-off value NR)												
serum GFAP (12- to 32-hour) (cut-off value NR)	1 Mahan, 2019	104	CT	12- to 32-hours	CT positive (not defined)	0.94 [0.81, 0.99]	0.68 [0.55, 0.78]	Sensitivity				
								None	Serious[b]	none	Serious[c]	LOW
								Specificity				
								None	Serious[b]	none	Serious[c]	LOW
GFAP-BDP												
Adults-Serum GFAP-BDP (cut-off level of 0.035 µg/L) - within 4 hours of injury												
Serum GFAP-BDP (cut-off	1 Papa 2012	117	CT	within 4 hours	intra cranial lesions on CT	0.97 [0.84, 1.00]	0.18 [0.10, 0.27]	Sensitivity				
								None	Serious[b]	none	Serious[c]	LOW
								Specificity				

Index Test/study	Number of studies	N	Ref. standard	Time-point	Outcome definition	Sensitivity (95% CI)	Specificity (95% CI)	Risk of bias	Indirectness	Inconsistency	Imprecision	GRADE
level of 0.035 µg/L)								None	Serious[b]	none	none	MODERATE
Adults- serum GFAP-BDP at a 0.68 µg/L - within 24 hours after injury												
serum GFAP-BDP at a 0.68 µg/L	1 Okonkwo 2013	215	CT	within 24 hours	intracranial pathology on CT	0.73 [0.64, 0.81]	0.89 [0.81, 0.94]	Sensitivity				
								None	Serious[b]	none	none	MODERATE
								Specificity				
								None	Serious[b]	none	Serious[c]	LOW
Adults- serum GFAP-BDP (a cut-off of 0.6 µg/L)- within 24 hours after injury												
serum GFAP-BDP (a cut-off of 0.6 µg/L)	1 McMahon, 2015	215	CT	within 24 hours	intracranial injury on CT	0.67 [0.58, 0.76]	0.89 [0.81, 0.94]	Sensitivity				
								None	Serious[b]	none	Serious[c]	LOW
								Specificity				
								None	Serious[b]	none	Serious[c]	LOW
Adults- serum GFAP-BDP level (a cut-off of 1.66 µg/L) - within 24 hours after injury												
serum GFAP-BDP level (a cut-	1 McMahon, 2015	215	CT	within 24 hours	intracranial injury on CT	0.45 [0.36, 0.55]	0.99 [0.95, 1.00]	Sensitivity				
								None	Serious[b]	none	none	MODERATE

Index Test/study	Number of studies	N	Ref. standard	Time-point	Outcome definition	Sensitivity (95% CI)	Specificity (95% CI)	Risk of bias	Indirectness	Inconsistency	Imprecision	GRADE
off of 1.66 µg/L)								Specificity				
								None	Serious[b]	none	none	MODERATE
NSE												
Adults- serum NSE (a cut-off limit of 14.7 µg/L)- within 3 hours after injury												
serum NSE (a cut-off limit of 14.7 µg/L)	1 Wolf, 2013	107	CT	within 3 hours	CT positive (patients with epidural, subdural, subarachnoid, or intracerebral hemorrhage, including contusions)	0.57 [0.37, 0.75]	0.77 [0.66, 0.86]	Sensitivity				
								Very serious[a]	none	none	Serious[c]	VERY LOW
								Specificity				
								Very serious[a]	none	none	none	LOW
Adults- serum NSE (cut-off limit of 16.4 µg/L) - within 3 hours after injury												
serum NSE (cut-off limit of 16.4 µg/L)	1 Wolf, 2013	107	CT	within 3 hours	CT positive (patients with epidural, subdural, subarachnoid, or intracerebral haemorrhage, including contusions)	0.53 [0.34, 0.72]	0.16 [0.08, 0.26]	Sensitivity				
								Very serious[a]	none	none	Serious[c]	VERY LOW
								Specificity				
								Very serious[a]	none	none	none	LOW

Index Test/study	Number of studies	N	Ref. standard	Time-point	Outcome definition	Sensitivity (95% CI)	Specificity (95% CI)	Risk of bias	Indirectness	Inconsistency	Imprecision	GRADE
UCH-L1												
Adults- serum UCH-L1 (cut-off level of 0.09 µg/L) – within 4hours of injury												
serum UCH-L1 (cut-off level of 0.09 µg/L)	1 Papa 2012	105	CT	within 4 hours	Intracranial lesions on CT	1.00 [0.88, 1.00]	0.21 [0.12, 0.32]	Sensitivity				
								None	Serious[b]	none	Serious[c]	LOW
								Specificity				
								None	Serious[b]	none	none	MODERATE
Adults plasma UCH-L1 (cut-off 0.327 µg/ml)- within 6 hours after injury												
plasma UCH-L1 (cut-off 0.327 µg/ml)	1 Li, 2022	463	CT	0-6 hours	Abnormalities on CT-presence/absence of closed head injuries including skull fracture, pneumocephalus, haemorrhage, mass effect, and brain parenchymal injuries.	0.95 [0.89, 0.98]	0.18 [0.14, 0.22]	sensitivity				
								Serious[a]	none	none	none	MODERATE
								specificity				
								Serious[a]	none	none	none	MODERATE
Adults serum UCH-L1 (cut-off 0.327 µg/ml)- within 6 hours after injury												

Index Test/study	Number of studies	N	Ref. standard	Time-point	Outcome definition	Sensitivity (95% CI)	Specificity (95% CI)	Risk of bias	Indirectness	Inconsistency	Imprecision	GRADE
serum UCH-L1 (cut-off 0.327 µg/ml)	1 Li, 2022	463	CT	0-6 hours	Abnormalities on CT-presence/absence of closed head injuries including skull fracture, pneumocephalus, haemorrhage, mass effect, and brain parenchymal injuries.	0.89 [0.78, 0.96]	0.27 [0.21, 0.35]	sensitivity				
								Serious[a]	none	none	none	MODERATE
								specificity				
								Serious[a]	none	none	none	MODERATE
Adults- serum UCH-L1 (cut-off of 0.041 µg/L) [6 hours -time from reported injury to blood sample obtained]												
serum UCH-L1 (cut-off of 0.041 µg/L)	1 Welch 2016	231	CT	at 6 hours -	CT scan was positive—defined as the presence of an acute trauma-related intracranial lesion	Sensitivity set at 100%	0.40 (0.33, 0.47) [f]	Sensitivity				
								Serious[a]	Serious[b]	none	none	LOW
								Specificity				
								Serious[a]	Serious[b]	none	none	LOW
Adults- serum UCH-L1 - 0 to 8-hours after injury (cut-off value NR)												
		104	CT			0.53 [0.35, 0.70]	0.50 [0.38, 0.62]	Sensitivity				

Index Test/study	Number of studies	N	Ref. standard	Time-point	Outcome definition	Sensitivity (95% CI)	Specificity (95% CI)	Risk of bias	Indirectness	Inconsistency	Imprecision	GRADE
serum UCH-L1 (cut-off value NR)	1Mahan, 2019			Within 8-hours	CT positive (not defined)			None	Serious[b]	none	Serious[c]	LOW
								Specificity				
								None	Serious[b]	none	Serious[c]	LOW
Adults- serum UCH-L1 – 12 to 32 hours after injury - (cut-off value NR)												
serum UCH-L1 (cut-off value NR)	1 Mahan, 2019	104	CT	12 to 32hours	CT positive (not defined)	0.61 [0.43, 0.77]	0.51 [0.39, 0.64]	Sensitivity				
								None	Serious[b]	none	Serious[c]	LOW
								Specificity				
								None	Serious[b]	none	Serious[c]	LOW
Combined serum UCH-L1 and GFAP												
Adults -combined plasma GFAP (cut-off 0.022 µg/ml) and UCH-L1 (cut-off 0.327 µg/ml) - within 6 hours after injury												
combined plasma GFAP (cut-off 0.022 µg/ml) and	1 Li, 2022	463	CT	0-6 hours	Abnormalities on CT-presence/absence of closed head injuries	1.00 [0.97, 1.00]	0.11 [0.08, 0.15]	Sensitivity				
								Serious[a]	none	none	none	MODERATE
								Specificity				

Index Test/study	Number of studies	N	Ref. standard	Time-point	Outcome definition	Sensitivity (95% CI)	Specificity (95% CI)	Risk of bias	Indirectness	Inconsistency	Imprecision	GRADE
UCH-L1 (cut-off 0.327 µg/ml)					including skull fracture, pneumocephalus, haemorrhage, mass effect, and brain parenchymal injuries.			Serious[a]	none	none	none	MODERATE
Adults -combined serum GFAP (cut-off 0.022 µg/ml) and UCH-L1 (cut-off 0.327 µg/ml) - within 6 hours after injury												
combined serum GFAP (cut-off 0.022 µg/ml) and UCH-L1 (cut-off 0.327 µg/ml)	1 Li, 2022	463	CT	0-6 hours	Abnormalities on CT-presence/absence of closed head injuries including skull fracture, pneumocephalus, haemorrhage, mass effect, and brain parenchymal injuries.	1.00 [0.94, 1.00]	0.17 [0.12, 0.24]	Sensitivity				
								Serious[a]	none	none	none	MODERATE
								specificity				
								Serious[a]	none	none	none	MODERATE
Adults- combined serum UCH-L1 and GFAP measured within 12 hours after injury (0.327 µg/Lfor UCH-L1 and 0.022 for GFAP µg/L)												
Combined serum UCH-L1	1 Bazarian 2018	1920	CT	within 12 hours	Positive CT scan (presence of one or more	0.97 [0.92, 0.99]	0.37 [0.34, 0.39]	Sensitivity				
								None	none	none	none	HIGH
								Specificity				

Index Test/study	Number of studies	N	Ref. standard	Time-point	Outcome definition	Sensitivity (95% CI)	Specificity (95% CI)	Risk of bias	Indirectness	Inconsistency	Imprecision	GRADE
and GFAP measured (0.327 µg/L for UCH-L1 and 0.022 for GFAP µg/L)					of the following injuries: acute epidural haematoma, acute subdural haematoma, indeterminate extra-axial haemorrhage, intraventricular haemorrhage, parenchymal haematoma, petechial haemorrhagic or bland sheer injury, subarachnoid haemorrhage, brain oedema, brain herniation, non-haemorrhagic contusion,			None	none	none	none	HIGH

Index Test/study	Number of studies	N	Ref. standard	Time-point	Outcome definition	Sensitivity (95% CI)	Specificity (95% CI)	Risk of bias	Indirectness	Inconsistency	Imprecision	GRADE
					ventricular compression, ventricular trapping, cranial fractures, depressed skull fractures, facial fractures, scalp injury, or skull base fractures).							
Adults- serum GFAP & UCH-L1N (Threshold for positive test 0.1 µg/L for GFAP and 0.040 µg/L UCH-L1) [4 hours- time from reported injury to blood sample obtained]												
serum GFAP & UCH-L1N (Threshold for positive test 0.1 µg/Lfor GFAP and 0.040 µg/L UCH-L1)	1 welch 2016	231	CT	at 4 hours	CT scan was positive—defined as the presence of an acute trauma-related intracranial lesion	Sensitivity set at 100%	0.37 (0.30, 0.44) [f]	Sensitivity				
								Serious[a]	Serious[b]	none	none	LOW
								specificity				
								Serious[a]	Serious[b]	none	none	LOW
Adults- serum GFAP & UCH-L1N (Threshold for positive test- 0.1 µg/Lfor GFAP and 0.040 µg/L UCH-L1)) [6 hours - time from reported injury to blood sample obtained]												

Index Test/study	Number of studies	N	Ref. standard	Time-point	Outcome definition	Sensitivity (95% CI)	Specificity (95% CI)	Risk of bias	Indirectness	Inconsistency	Imprecision	GRADE
serum GFAP & UCH-L1N (Threshold for positive test 0.1 µg/Lfor GFAP and 0.040 µg/L UCH-L1)	1 welch 2016	231	CT	at 6 hours	CT scan was positive—defined as the presence of an acute trauma-related intracranial lesion	Sensitivity set at 100%	0.39 (0.33, 0.46) [f]	Sensitivity				
								Serious[a]	Serious[b]	none	none	LOW
								specificity				
								Serious[a]	Serious[b]	none	none	LOW
serum small neuronal protein neurogranin (NRGN)												
Adults (mixed adults and children (mean 24 years))- serum small neuronal protein neurogranin (NRGN)- cut-off 1.87 µg/L - within 4hours after injury												
serum small neuronal protein neurograni n (NRGN)-cut-off 1.87 µg/L	1 Cevik 2019	48	CT	within 4hours	abnormal cerebral CT findings	0.83 [0.63, 0.95]	0.58 [0.37, 0.78]	Sensitivity				
								Very serious[a]	Serious[b]	None	Seriou s[c]	VERY LOW
								Specificity				
								Very serious[a]	serious[2]	None	Seriou s[c]	VERY LOW
serum pNFL-H												
Adults- serum pNFL-H (1.071 µg/L) – 18-24 hours after injury												
serum pNFL-H	1Gatson, 2014	34	CT	18-24 hours	Intracranial findings on CT (skull fractures,	0.89 [0.65, 0.99]	0.69 [0.41, 0.89]	Sensitivity				
								Serious[a]	none	none	Seriou s[c]	LOW

Index Test/study	Number of studies	N	Ref. standard	Time-point	Outcome definition	Sensitivity (95% CI)	Specificity (95% CI)	Risk of bias	Indirectness	Inconsistency	Imprecision	GRADE
(1.071 µg/L)					subdural/ epidural/subarachnoid haemorrhaging, oedema, and/or con¬tusions)			Specificity				
								Serious[a]	none	none	Serious[c]	LOW
Serum IL-10												
Adults (mixed adults and children- mean age adults) IL-10 (cut-off 0.000 159 µg/L))- within 6 hours after injury												
serum IL-10 (cut-off 0.000 159 µg/L))-	1 Lagerstedt, 2018	133	CT	within 6 hours	CT positive (Epidural haemorrhage, Subdural haemorrhage, Subarachnoid haemorrhage, Intracerebral haemorrhage, Contusion with haemorrhage, Skull fracture).	Sensitivity set at 100%	specificity 25.8% (95% CI 19.7–32.0)[f]	Sensitivity				
								Very serious[a]	Serious[b]	none	Not applicable	VERY LOW
								Specificity				
								Very serious[a]	Serious[b]	none	none	VERY LOW
Adults- serum IL-10 (cut-off 0.00014 µg/L)- within 24 hours of admission												
		55	CT					Sensitivity				

Index Test/study	Number of studies	N	Ref. standard	Time-point	Outcome definition	Sensitivity (95% CI)	Specificity (95% CI)	Risk of bias	Indirectness	Inconsistency	Imprecision	GRADE
serum IL-10 (cut-off 0.00014 µg/L)- Patients with isolated TBI	1 Posti 2019			Within 24 hours of admission	Ability of the Individual Biomarkers in Discriminating CT-Negative and CT-Positive Patients with Isolated Mild TBI	Sensitivity set at 100%	Specificity 2.8% (CI not reported) [f]	Serious[a]	Serious[b]	none	Cannot assess	LOW
								Specificity				
								Serious[a]	Serious[b]	none	Cannot assess	LOW
Adults- serum IL-10 (cut-off 0.00014 µg/) – within 24 hours of admission												
serum IL-10 (cut-off 0.00014 µg/) Patients with Mild TB	1 Posti 2019	55	CT	within 24 hours of admission	CT scans were classified according to the Marshall grading system. Diffuse injury/grade I (no visual pathology) was considered CT-, whereas the other grades (II-VI) were regarded as CT+.	sensitivity set at 100%	Specificity 5.4% (no CI) [f]	Sensitivity				
								Serious[a]	Serious[b]	none	Cannot assess	LOW
								Specificity				
								Serious[a]	Serious[b]	none	Cannot assess	LOW
Neurofilament light chain (NF-L)												

Index Test/study	Number of studies	N	Ref. standard	Time-point	Outcome definition	Sensitivity (95% CI)	Specificity (95% CI)	Risk of bias	Indirectness	Inconsistency	Imprecision	GRADE
Adults- serum NF-L (cut-off 0.00418 µg/L)- within 24 hours of admission												
serum NF-L (cut-off 0.00418 µg/L)- Patients with isolated Mild TBI	1 Posti 2019	55	CT	Within 24 hours of admission	CT scans were classified according to the Marshall grading system. Diffuse injury/grade I (no visual pathology) was considered CT-, whereas the other grades (II-VI) were regarded as CT+.	sensitivity set at 100%	Specificity 5.6% (CI not reported)[f]	Sensitivity				
								Serious[a]	Serious[b]	none	Cannot assess	LOW
								Specificity				
								Serious[a]	Serious[b]	none	Cannot assess	LOW
Adults- serum NF-L (cut-off 0.0043 µg/L) - within 24 hours of admission												
serum NF-L (cut-off 0.0043 µg/L) Patients	1 Posti 2019	93	CT	Within 24 hours of admission	CT scans were classified according to the Marshall grading system. Diffuse	Sensitivity set at 100%	7.1 % (CI not reported)[f]	Sensitivity				
								Serious[a]	Serious[b]	none	Cannot assess	LOW
								Specificity				

Index Test/study	Number of studies	N	Ref. standard	Time-point	Outcome definition	Sensitivity (95% CI)	Specificity (95% CI)	Risk of bias	Indirectness	Inconsistency	Imprecision	GRADE
with mild TBI					injury/grade I (no visual pathology) was considered CT-, whereas the other grades (II-VI) were regarded as CT+.			Serious[a]	Serious[b]	none	Cannot assess	LOW

[a] *Risk of bias was assessed using the QUADAS-2 checklist. The evidence was downgraded by 1 increment if the majority of studies were rated at high risk of bias, and downgraded by 2 increments if the majority of studies were rated at very high risk of bias.*

[b] *Indirectness was assessed using the QUADAS-2 checklist items referring to applicability. The evidence was downgraded by 1 increment if the majority of studies were seriously indirect and downgraded by 2 increments if the majority of studies are very seriously indirect. Studies were downgraded for indirectness if it included mixed population (adults and children or youth and children) or mixed severity population (mild, moderate and severe TBI).*

[c] *The evidence was downgraded by 1 increment when the confidence interval around the point estimate crossed one of the clinical thresholds (0.90 or 0.60), and downgraded by 2 increments when the confidence interval around the point estimate crossed both of the clinical thresholds. The upper clinical threshold marked the point above which recommendations would be possible, and the lower clinical threshold marked the point below which the tool would be regarded as of little clinical use.*

[d] *Outcome definitions: Biberthaler,2006- intracerebral lesions on CT; Ernstbrunner, 2016- secondary intracranial haemorrhagic events (SIHE) on CT; Laribi, 2014- CT findings of intracranial lesions;Muller 2011- positive CT findings (not defined); Thaler, 2015- CCT positive (patients with MHI who had at least 1 trauma-related intracranial haemorrhage: i.e., epidural, subdural, subarachnoidal, or intracerebral bleeding);Wolf, 2013- CT positive (patients with epidural, subdural, subarachnoid, or intracerebral hemorrhage, including contusions);Bazarian 2013- presence of intracranial abnormalities. Traumatic CT abnormalities defined as subdural hematomas (SDH), epidural hematomas, subarachnoid*

haemorrhage (SAH), oedema, skull fracture, and cerebral contusions.;David, 2017- positive CT scan (any trauma related intracranial haemorrhage, including epidural, subdural or subarachnoid haemorrhage, or intracerebral bleeding (petechial haemorrhage, contusion or hematoma));Egea-Guerrero, 2012- intracranial lesion (IL) on CT; Egea-Guerrero, 2018- presence of intracranial lesion (IL) on CT; Kahouadji, 2020-positive CT scan (at least one pathophysiological trauma-relevant intracranial lesion - any signs of cranial (skull fracture) or intracranial pathology (hematoma, air, or contusion), subgaleal hematomas were also considered positive to prevent disregarding abnormalities that may influence S100B levels; Lagerstedt, 2017 -CT positive (not defined); Lin 2022-abnormalities on CT- presence/absence of closed head injuries including skull fracture, pneumocephalus, haemorrhage, mass effect, and brain parenchymal injuries. ; Vedin, 2021- Intracranial haemorrhage on CT; Zongo 2012- CT scan—positive (minor head injury patients with at least 1 trauma-relevant lesion).

[e]*Pooled sensitivity/specificity from diagnostic meta-analysis*
[f] *raw data were not provided by the paper and were not able to be calculated by NGC. Data as reported in the paper.*

Clinical evidence summary: diagnostic test accuracy of biomarkers in children

Index Test/study	Number of studies	n	Ref. standard	Time-point	Outcome definition	Sensitivity (95% CI)	Specificity (95% CI)	Risk of bias	Indirectness	Inconsistency	Imprecision	GRADE
Serum S100B												
children- serum S100B cut-off (0.35 µg/L for age 0 to 9 months; 0.23 µg/L for age 10 to 24 months; 0.18 µ g/L for age >24 months)- median 2 hours after injury												
serum S100B cut-off (0.35	1 Bouvier, 2012	65	CT	median 2 hours	Intracerebral lesion on CT	1.00 [0.85, 1.00]	0.33 [0.20, 0.50]	Sensitivity				
								Serious[a]	Serious[b]	None	Serious[c]	VERY LOW
								Specificity				

Index Test/study	Number of studies	n	Ref. standard	Time-point	Outcome definition	Sensitivity (95% CI)	Specificity (95% CI)	Risk of bias	Indirectness	Inconsistency	Imprecision	GRADE
µg/L for age 0 –9 months; 0.23 µg/L for age 10 – 24 months; 0.18 µg/L for age >24 months)-								Serious[a]	Serious[b]	None	None	LOW
children- serum S100B (Cut-off value 0.14 µg/L) - All children <16 years – within 6hours after injury												
serum S100B (Cut-off value 0.14 µg/L) - All children <16 years	1 Manzano, 2016	73	CT	within 6 hours	intracranial injuries	0.95 [0.75, 1.00]	0.34 [0.22, 0.48]	Sensitivity				
								None	None	None	Serious[c]	MODERATE
								Specificity				
								None	None	None	None	HIGH
children- serum S100B (cut-off 0.16µg/L)- within 6 hours after injury												
		109	CT		pathological CT		0.42 [0.31, 0.55]	Sensitivity				

Index Test/study	Number of studies	n	Ref. standard	Time-point	Outcome definition	Sensitivity (95% CI)	Specificity (95% CI)	Risk of bias	Indirectness	Inconsistency	Imprecision	GRADE
serum S100B (cut-off 0.16µg/L)	1 Castellani, 2009			within 6 hours	CT was classified as pathological in the presence of a skull fracture or intra cranial hemorrhage (ICH).	1.00 [0.90, 1.00]		Very serious [a]	None	None	Serious [c]	VERY LOW
								Specificity				
								Very Serious [a]	None	None	none	LOW
children- serum S 100B level of 0.1 µg/L- within 6 hours after injury												
serum S 100B level of 0.1 µg/L	1 Babock 2012	109	CT	within 6 hours	An abnormal cranial CT was defined by the presence of any intracranial injury, including subdural haematomas, epidural haemato-mas and cerebral contusions, as well as the presence of skull fractures.	0.47 [0.24, 0.71]	0.89 [0.81, 0.95]	Sensitivity				
								Very serious [a]	None	None	Serious [c]	VERY LOW
								Specificity				
								Very serious [a]	None	None	Serious [c]	VERY LOW
children and youth (mean 13 years) - serum S100b cut-off level of 0.020 µg/L - within 6hours after injury												
children and youth	1 Papa 2016	92	CT	within 6 hours	presence of intracranial lesions on initial CT scan.	1.00 [0.63, 1.00]	0.26 [0.17, 0.37]	Sensitivity				
								None	Serious [b]	None	Serious [c]	LOW

Index Test/study	Number of studies	n	Ref. standard	Time-point	Outcome definition	Sensitivity (95% CI)	Specificity (95% CI)	Risk of bias	Indirectness	Inconsistency	Imprecision	GRADE
(mean 13 yrs)- serum S100b cut-off level of 0.020 µg/L								Specificity				
								None	Serious[b]	None	none	MODERATE
children- serum S 100 B (cut-off of 172.15µg/L)- within 6 hours after injury												
serum S 100 B (cut-off of 172.15µg/L	1 Mozafari, 2019	40	CT	within 6 hours	positive pathologic findings associated with isolated head trauma on CT (not defined positive pathological findings)	0.95 [0.75, 1.00]	1.00 [0.83, 1.00]	Sensitivity				
								Serious[a]	None	None	Serious[c]	LOW
								Specificity				
								Serious[a]	None	None	Serious[c]	LOW
Children- serum S 100B level cut-off > 0.006µg/L- within 6 hours after injury												
serum S 100B level cut-off > 0.006µg/L	1 Babock 2012	109	CT	within 6 hours	Abnormal cranial CT	0.89 [0.67, 0.99]	0.31 [0.22, 0.42]	Sensitivity				
								Very serious[a]	None	None	Serious[c]	VERY LOW
								Specificity				

Index Test/study	Number of studies	n	Ref. standard	Time-point	Outcome definition	Sensitivity (95% CI)	Specificity (95% CI)	Risk of bias	Indirectness	Inconsistency	Imprecision	GRADE
								Very serious [a]	None	None	None	LOW
Urinary S100B												
children- urinary S100B (cut-off levels of 56.4 ng/L)- within 6 hours after injury												
urinary S100B (cut-off levels of 56.4 µg/L	1 Mozafari, 2019	40	CT	within 6 hours	positive pathologic findings associated with isolated head trauma on CT (not defined positive pathological findings)	0.95 [0.75, 1.00]	0.90 [0.68, 0.99]	Sensitivity				
								Serious [a]	None	None	Serious [c]	LOW
								Specificity				
								Serious [a]	None	None	Serious [c]	LOW
Children- urinary S100B (cut-off levels of 67.75 ng/L)- within 6 hours after injury												
urinary S100B (cut-off levels of 67.75 ng/L)	1 Mozafari, 2019	40	CT	within 6 hours	positive pathologic findings associated with isolated head trauma on CT (not defined positive pathological findings)	0.90 [0.68, 0.99]	0.95 [0.75, 1.00]	Sensitivity				
								Serious [a]	None	None	Serious [c]	LOW
								Specificity				
								Serious [a]	None	None	Serious [c]	LOW
GFAP												
children and youth (median age 12)- serum GFAP (cut-off 0.15 µg/L) within 6 hours after injury: Isolated skull fracture+ICL												

Index Test/study	Number of studies	n	Ref. standard	Time-point	Outcome definition	Sensitivity (95% CI)	Specificity (95% CI)	Risk of bias	Indirectness	Inconsistency	Imprecision	GRADE
Serum GFAP (cut-off 0.15 µg/L)	1 Papa 2015	152	CT	within 6 hours	presence of intracranial lesions on initial CT scan	0.95 [0.76, 1.00]	0.48 [0.39, 0.57]	Sensitivity				
								Very serious [a]	Serious [b]	None	Serious [c]	VERY LOW
								Specificity				
								Very serious [a]	Serious [b]	None	none	VERY LOW
children and youth (mean 13 years)- serum GFAP cut-off level of 0.15 µg/L - within 6hours after injury (ICL only no skull fracture)												
serum GFAP cut-off level of	1Papa 2016	92	CT	within 6 hours	presence of intracranial lesions on initial CT scan.	1.00 [0.63, 1.00]	0.36 [0.26, 0.47]	Sensitivity				
								None	Serious [b]	None	Serious [c]	LOW
								Specificity				

Index Test/study	Number of studies	n	Ref. standard	Time-point	Outcome definition	Sensitivity (95% CI)	Specificity (95% CI)	Risk of bias	Indirectness	Inconsistency	Imprecision	GRADE
0.15 µg/L					Intracranial lesions on CT included any acute traumatic intracranial lesions visualised on CT scan such haemorrhages (epidural, subdural, subarachnoid, ventricular, and parenchymal), contusions, oedema, and pneumocephalus but excluded facial fractures and isolated skull fractures without intracranial lesions.			None	Serious[b]	None	none	MODERATE
children and youth (median age 12)- serum GFAP (cut-off 0.15 µg/L) within 6hours after injury: ICL only (no skull fracture)												
serum GFAP (cut-off 0.15 µg/L)	1 Papa 2015	152	CT	within 6 hours	presence of intracranial lesions on initial CT scan	0.94 [0.73, 1.00]	0.47 [0.38, 0.56]	Sensitivity				
								Very serious[a]	Serious[b]	none	Serious[c]	VERY LOW
								Specificity				

Index Test/study	Number of studies	n	Ref. standard	Time-point	Outcome definition	Sensitivity (95% CI)	Specificity (95% CI)	Risk of bias	Indirectness	Inconsistency	Imprecision	GRADE
								Very serious [a]	Serious [b]	none	none	VERY LOW
UCH-L1												
children and youth (mean age 12) -serum UCH-L1 (cu-off ≥ 0.18 µg/L])- within 6hours after injury												
serum UCH-L1 (Cut -off ≥ 0.18 µg/L])	1 Papa, 2017	152	CT	within 6 hours	presence of intracranial lesions on initial CT scan	1.00 [0.80, 1.00]	0.47 [0.39, 0.56]	Sensitivity				
								Very serious [a]	Serious [b]	none	Serious [c]	VERY LOW
								Specificity				
								Very serious [a]	Serious [b]	none	none	VERY LOW
NSE												
children- serum NSE (at a level of NSE ≥ 15.3 µg/L) - mean 4hours after injury												
serum NSE (at a level of NSE ≥ 15.3 µg/L)-	1 Fridriksson, 2000	49	CT	mean 4 hours	Presence of intracranial lesion (ICL)	0.77 [0.55, 0.92]	0.52 [0.32, 0.71]	Sensitivity				
								None	Serious [b]	none	Very serious [c]	VERY LOW
								Specificity				
								None	Serious [b]	none	Serious [c]	LOW
children- serum NSE (cut-off points 5.74 µg/L)-within 6hours after injury												

Index Test/study	Number of studies	n	Ref. standard	Time-point	Outcome definition	Sensitivity (95% CI)	Specificity (95% CI)	Risk of bias	Indirectness	Inconsistency	Imprecision	GRADE
serum NSE (cut-off points 5.74 µg/L)-	1 Mozafari 2020	62	CT	within 6 hours	CT positive (not defined)	1.00 [0.89, 1.00]	0.87 [0.70, 0.96]	Sensitivity				
								Serious[a]	none	none	Serious[c]	LOW
								Specificity				
								Serious[a]	none	none	Serious[c]	LOW
children- serum NSE (cut-off points of 6.97 µg/L) - within 6hours after injury												
serum NSE (cut-off points of 6.97 µg/L)	1 Mozafari 2020	62	CT	within 6 hours	CT positive (not defined)	0.94 [0.79, 0.99]	1.00 [0.89, 1.00]	Sensitivity				
								Serious[a]	none	none	Serious[c]	LOW
								Specificity				
								Serious[a]	none	none	Serious[c]	LOW

[a]Risk of bias was assessed using the QUADAS-2 checklist. The evidence was downgraded by 1 increment if the majority of studies were rated at high risk of bias, and downgraded by 2 increments if the majority of studies were rated at very high risk of bias.

[b]Indirectness was assessed using the QUADAS-2 checklist items referring to applicability. The evidence was downgraded by 1 increment if the majority of studies were seriously indirect and downgraded by 2 increments if the majority of studies are very seriously indirect. Studies were downgraded for indirectness if it included mixed population (adults and children or youth and children) or mixed severity population (mild, moderate and severe TBI).

[c]The evidence was downgraded by 1 increment when the confidence interval around the point estimate crossed one of the clinical thresholds (0.90 or 0.60), and downgraded by 2 increments when the confidence interval around the point estimate crossed both of the clinical thresholds. The upper clinical threshold marked the point above which recommendations would be possible, and the lower clinical threshold marked the point below which the tool would be regarded as of little clinical use.

Clinical evidence summary: diagnostic test accuracy of biomarkers in studies where age was not reported in studies

Index Test/study	Number of studies	n	Ref. standard	Time-point	Outcome definition	Sensitivity (95% CI)	Specificity (95% CI)	Risk of bias	Indirectness	Inconsistency	Imprecision	GRADE
S100B												
age NR- serum100B (cut-off 0.1 µg/L) -median 82 minutes after injury												
age NR-serum S100B (cut-off 0.1 µg/L)	1 Poli-de-Figueiredo 2006	50	CT	median 82 minutes	signs of intracranial injury at the initial CCT scan	1.00 [0.54, 1.00]	0.20 [0.10, 0.35]	Sensitivity				
								Serious[a]	Serious[b]	None	Very serious[c]	VERY LOW
								Specificity				
								Serious[a]	Serious[b]	None	None	LOW
age NR-serum S100B at admission (cut-off 0.1 µg/L- within 3 hours after injury												
serum S100B (cut-off 0.1 µg/L)	1 Bibertha ler, 2001	52	CT	within 3 hours	pathologic findings (intracerebral haemorrhage, skull fracture, or diffuse brain swelling) on CT scan	1.00 [0.78, 1.00]	0.41 [0.25, 0.58]	Sensitivity				
								serious[a]	Serious[b]	None	Serious[c]	VERY LOW
								Specificity				
								Serious[a]	Serious[b]	None	None	LOW

[a] Risk of bias was assessed using the QUADAS-2 checklist. The evidence was downgraded by 1 increment if the majority of studies were rated at high risk of bias, and downgraded by 2 increments if the majority of studies were rated at very high risk of bias.

[b]Indirectness was assessed using the QUADAS-2 checklist items referring to applicability. The evidence was downgraded by 1 increment if the majority of studies were seriously indirect and downgraded by 2 increments if the majority of studies are very seriously indirect. Studies were downgraded for indirectness if it included mixed population (adults and children or youth and children) or mixed severity population (mild, moderate and severe TBI).
[c]The evidence was downgraded by 1 increment when the confidence interval around the point estimate crossed one of the clinical thresholds (0.90 or 0.60), and downgraded by 2 increments when the confidence interval around the point estimate crossed both of the clinical thresholds. The upper clinical threshold marked the point above which recommendations would be possible, and the lower clinical threshold marked the point below which the tool would be regarded as of little clinical use.

1.1.7 Economic evidence

1.1.7.1 Included studies

No health economic studies were included.

1.1.7.2 Excluded studies

One economic study relating to this review question was identified but was excluded due to methodological limitations[12]. See Appendix I, for reasons for exclusion given.

See also the health economic study selection flow chart in Appendix F.

1.1.8 Summary of included economic evidence

None.

1.1.9 Economic model

Modelling was not conducted for this review.

1.1.10 Unit costs

Relevant unit costs are provided below to aid consideration of cost effectiveness.

Code	Description	Unit cost
RD01A	Magnetic Resonance Imaging Scan of One Area, without Contrast, 19 years and over	£146.75
RD01B	Magnetic Resonance Imaging Scan of One Area, without Contrast, between 6 and 18 years	£215.63
RD01C	Magnetic Resonance Imaging Scan of One Area, without Contrast, 5 years and under	£140.83
RD20A	Computerised Tomography Scan of One Area, without Contrast, 19 years and over	£88.06
RD20B	Computerised Tomography Scan of One Area, without Contrast, between 6 and 18 years	£159.25
RD20C	Computerised Tomography Scan of One Area, without Contrast, 5 years and under	£104.27
PF	Plain Film (including x-ray)	£28.62

Direct access costs from NHS Reference costs: 2019-2020 version 2

1.1.11 Evidence statements

Economic

- No relevant economic evaluations were identified.

1.1.12 The committee's discussion and interpretation of the evidence

1.1.12.1. The outcomes that matter most

Diagnostic accuracy

Intracranial injury and/or complex skull fracture on CT or MRI was the outcome relevant for the diagnostic accuracy component of this review. Sensitivity and specificity were identified by the committee as the primary measures in guiding decision-making. Clinical decision thresholds for sensitivity and specificity were set at 0.90 (above which a test would be recommended) and 0.60 (below which a test is of no clinical use).

Sensitivity and specificity were both considered to be equally important. Biomarker testing is the first stage of a two-step process, followed by CT/MRI if indicated by a positive test. The need for the index test to have a very few false negatives was considered to be important so as to avoid anyone with intracranial injury/lesion exiting at first stage prematurely. Specificity was considered to be important as false positives would mean people who do not have intracranial injury/lesion would receive unnecessary radiation (particularly for children).

Diagnostic test and treat

All outcomes are considered equally important for decision making and therefore have all been rated as critical: quality of life at 3 months or more, objectively applied score of disability e.g. Glasgow Outcome Score (GOS) or extended GOS - at 3 months or more and time to return to education/work/usual activities.

There was no evidence identified for this component of the review.

1.1.12.2 The quality of the evidence

Diagnostic accuracy

Sixty-four cross-sectional and prospective/retrospective cohort studies were included in the diagnostic accuracy component of the review.

Population

The majority of the included studies were in people with mild TBI (GCS score 13-15). Studies with mixed severity populations (mild/moderate/severe TBI) were included but were downgraded for indirectness as acute post-brain injury complications are most relevant to those with mild TBI. Most studies reported inclusion of people with mild TBI with extracranial injuries: a couple of studies reported results separately for isolated TBI (without extracranial injuries) and mild TBI (with extracranial injuries). Most of the studies were in adults (49 studies in adults and 13 in infants/children). There were some studies with mixed population (adults and children/children and young people); proportion of adults, young people and children were not reported in these papers. These studies were included in either adult or children strata based on the reported mean (SD) age. Studies with mixed populations have been downgraded for indirectness. Two studies did not report age of the participants.

Index tests

In adults most of the studies assessed diagnostic accuracy of serum S100 calcium binding protein B (S100 B); other biomarkers assessed were urinary S100B, glial fibrillary acidic protein (GFAP), glial fibrillary acidic protein and break down products (GFAP-BDP), Ubiquitin C-terminal Hydrolase-L1 (UCHL1), neuron-specific enolase (NSE), Neurofilament light (NFL), small neuronal protein neurogranin (NRGN), pNFL-H and IL-10. No relevant studies investigating the effects of the following biomarkers were identified in adults: brain-derived neurotrophic factor (BDNF), Neurofilament Heavy (NF-H), αII-Spectrin breakdown products (SBDP), Myelin basic protein (MBP) and salivary biomarkers.

In children, studies assessed diagnostic accuracy of serum S100 B, urinary S100B, GFAP, NSE and UCHL-1. No relevant studies investigating the effects of the following biomarker were identified in children: Neurofilament light (NFL) brain-derived neurotrophic facto (BDNF), Neurofilament Heavy (NF-H), αII-Spectrin breakdown products (SBDP), Myeli basic protein (MBP), urinary biomarkers and salivary biomarkers. Only one of the biomarkers was point of care testing and all were lab-based testing. Timing of blood sampling in studies ranged from 0-32 hours after injury. Most studies reported testing within 3 hours or 3-6 hours after injury, with only 8 studies reporting testing 6 hours after injury. In clinical practice most people with traumatic head injury present to the hospital within 3 hours of injury and manufacturers recommend this time frame for optimal test results.

There was variation in thresholds used for different biomarker tests in the studies, as these depend on the assays (platforms) used for testing. All studies reported biomarker testing before CT/MRI.

Reference standard

All studies reported CT as reference standard except for 4 studies which also used MRI along with CT as the reference standard. Three of the 4 studies used MRI when CT was negative, only one study used CT and MRI in all participants. Many studies did not report exact timing of the reference standard. Some studies reported that reference standard was done soon after/within the same time frame as biomarker testing, whereas in some studies there was a time interval between biomarker testing and reference standard, for example biomarker test within 6 hours and CT within 24h after injury. In clinical practice ideally head CT scan should be performed within one hour of the risk factor being identified.

Outcomes

There was variation in definition of outcomes reported in papers, with some reporting intra cranial injury/lesions only and others reporting intracranial lesions with complex skull fracture on CT/MRI. All outcomes were extracted and analysed.

Meta-analysis

Analysis was stratified by population in the studies: adults and children. For each of the above strata studies were pooled/classified based on the biomarkers and timings (0-3 hours, >3-6 hours, >6-12 hours and >12 to 24 hours) of the test post-injury. Only 2 diagnostic meta-analyses (S100 B at 0-3 hours after injury and S 100B at >3 to 6 hours after injury) were possible because at least 3 studies are required for a valid pooling of results, and for all other index tests only one or two studies were available as there was variation in the thresholds (cut-offs) and timing of index tests. Studies assessing diagnostic accuracy of serum S100B with thresholds 0.10 and 0.105 µg/L were pooled as the thresholds were considered to be close enough to be combined. Studies with all other different biomarkers and thresholds have been analysed/reported separately.

Risk of bias

The quality of evidence for diagnostic accuracy studies varied from very low to high quality using the QUADAS checklist; the majority of the evidence was downgraded due to risk of bias, imprecision and indirectness. The most common reasons that studies were downgraded for risk of bias were due to selection bias or because of a lack of blinding in some studies which may have caused detection bias. The committee also acknowledged that some uncertainty existed across the effect sizes seen within the evidence, with some confidence intervals crossing the MID thresholds (0.90 as the upper threshold and 0.60 as lower threshold for both sensitivity and specificity was used for assessing imprecision), or line of no effect. Studies were downgraded for indirectness if the study included a mixed population (adults and children/youth and children) or mixed severity (mild, moderate and severe TBI). The committee took quality of the evidence into account while interpreting the evidence for decision making.

1.1.12.3 Benefits and harms

The diagnostic accuracy data for the different biomarker tests in adults and children were discussed. The evidence for biomarkers was insufficient and not consistent in both adults and children.

There were high sensitivity values for some biomarkers at certain thresholds; however, the specificity values were not high enough across the evidence; the committee agreed that this was equally important given the consequences of unnecessary radiation particularly in children.

Many biomarkers were tested in small samples leading to imprecise estimates. Alternatively, such estimates were from large but single studies. The committee noted that accuracy differed quite widely between different studies looking at the same biomarker test, measured with different assays on different platforms.

The evidence included in the review was heterogenous with different biomarkers with variable thresholds and time-points. Most people with head injury present to the hospital within 3 hours and the manufacturers recommend this time frame for optimal test results. Many studies assessed biomarkers beyond this time point.

The population in the included studies was mixed; medium risk and high-risk people (decision algorithms for whether or not to perform a CT scan in the acute phase, which stratifies patients into high/medium/low risk based on history and clinical findings) are already indicated for CT imaging according to current NICE guidelines (rec 1.4.7 and 1.4.8). Studies also included very low risk patients who are currently ineligible for CT in NHS practice.

Many studies included people with mild TBI with extracranial injuries. The committee noted that some biomarkers, particularly S100B, can also rise in the presence of extracranial injury due to injury to peripheral nerves and this could have contributed to increased sensitivity of these biomarker tests.

The committee noted that most studies assessed lab-based biomarker testing and only one of them was point of care testing. In lab-based testing results would be available only after a few hours which could potentially lead to delays in CT scanning. Point of care test with immediate results would help the clinician to determine a quicker course of action or treatment. Currently the technology for point of care testing for biomarkers for traumatic head injury is still in development hence the committee agreed that further research is needed in this area.

Considering the limitations of the evidence the committee were unable to make recommendations for the use of biomarkers in the prediction of acute post-injury complications in those with mild traumatic brain injury. However, the committee agreed that biomarker tests had promise, which might be manifested in further high-quality research, and so a research recommendation was proposed.

Biomarkers are not currently used within the NHS in the prediction of acute post brain injury complications. In current practice the decision on whether to conduct a CT to detect intracranial injury is made on the basis of two clinical decision rules (CDRs). The adult rule is a modification of the Canadian CT head rule to allow applicability to all adults with head injury, not just people with mild TBI (GCS score 13-15). The paediatric rule is a modification of the CHALICE rule to permit observation rather than radiation in medium risk children.

1.1.12.4 Cost effectiveness and resource use

Biomarker tests can prevent potentially harmful exposure to radiation, if they can identify those patients that do not require a CT scan. There are several different biomarker tests, although none are routinely used in the NHS at present. Their costs will vary but are likely to be a fraction of the cost of a CT scan.

No economic evaluations were included and so unit costs were presented to the committee. For a biomarker test to be cost effective it would need to be accurate (both sensitive and specific). If it is not specific enough then it would not prevent enough CT scans to justify the cost of the biomarker. Unless it is highly sensitive, a significant proportion of people with clinically important injuries will be discharged without a CT scan, some of whom would deteriorate before getting access to neurosurgical treatment.

In the clinical review there was no evidence of clinical effectiveness from test and treat studies, which would have been ideal. There tended to be high sensitivity values for some biomarkers at certain thresholds, although in most studies, especially the larger ones, these were below 100%. The specificity values were not considered by the committee to be high enough. There were other limitations to the clinical evidence. In particular, the population included moderate and high-risk patients, where CT scanning is already strongly indicated.

All the research was laboratory-based. Since CT scanning can usually be conducted quite quickly in the emergency department, the use of biomarker testing could potentially increase the time it takes to get scanned, which might lead to deterioration and worse patient outcomes. The committee therefore decided that more research was needed before biomarkers could be recommended either as a replacement or as a supplement to current

prediction rules. The research would need to focus on point-of-care testing in appropriate populations. This research would need to be incorporated into an economic model that evaluates the trade-off between the cost of the test, the cost savings in terms of reduced imaging and the net impact on patient outcomes.

1.1.12.5 Other factors the committee took into account

None.

References

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Appendices

Appendix A – Review protocols

Review protocol for diagnostic accuracy of brain injury biomarkers for predicting acute post-brain injury complications

ID	Field	Content
1.	Review title	2.3 a What is the diagnostic accuracy of brain injury biomarkers for predicting acute post-brain injury complications? Post-brain injury complications may be defined in two main ways: • Intracranial injury on CT/MRI (does not usually include fracture within this definition) • Abnormal CT/MRI which includes intracranial injury and skull fracture (depends on definition of the study) [Some papers will include fractures but not all] The committee wanted to limit only to the ability of biomarkers to identify CT/MRI findings as it was agreed that even if biomarkers could identify or predict signs or symptoms such as seizures, this would only be relevant if they also had a confirmed abnormality on CT/MRI This will group will include people with mild traumatic brain injury Time frame: • Up to 1 week for children • Up to 1 month for adults

2.	Review question	What is the diagnostic accuracy of brain injury biomarkers for predicting acute post-brain injury complications?
3.	Objective	To determine the diagnostic accuracy of brain injury biomarkers for predicting acute post-brain injury complications.
4.	Searches	The following databases (from inception) will be searched: • Cochrane Database of Systematic Reviews (CDSR) • Embase • MEDLINE • Epistemonikos Searches will be restricted by: • English language studies • Human studies Other searches: • Inclusion lists of systematic reviews The searches may be re-run 6 weeks before the final committee meeting and further studies retrieved for inclusion if relevant. The full search strategies will be published in the final review. Medline search strategy to be quality assured using the PRESS evidence-based checklist (see methods chapter for full details).

5.	Condition or domain being studied	Acute post-brain injury complications in patients who have experienced a head injury.
6.	Population	Inclusion: Infants, children and adult with suspected traumatic brain injury (TBI) Strata: • Adults (aged ≥16 years) • Children (aged ≥1 to <16 years) • Infants (aged <1 year) Mixed population studies will be included but downgraded for indirectness. Cut-off of 60% will be used for all age groups Exclusion: Adults, and children (including infants under 1 year) with superficial injuries to the eye or face without suspected or confirmed head or brain injury.
7.	Test	• Biomarkers o Blood biomarkers - S100 calcium binding protein B (S100B) -Ubiquitin C-terminal Hydrolase-L1 (UCHL1) -Neuron Specific Enolase (NSE) -Brain-derived neurotrophic factor (BDNF) -Neurofilament light (NFL) - Neurofilament Heavy (NF-H) - αII-Spectrin breakdown products (SBDP) - Myelin basic protein (MBP)

		- glial fibrillary acidic protein (GFAP) o Salivary biomarkers -salivary microRNAs (miRNAs) -Extracellular vesicles (EVs) -S100B o -Urine biomarkers -Extracellular vesicles (EVs) Each test must be followed by an appropriate treatment for complication after brain injury Treatment: Admission to hospital for observation + possible neurosurgical management of TBI Timings: Ideally biomarkers to be done before CT. Include studies if biomarkers done after CT but downgrade it for indirectness. Biomarkers are used prior to decision for imaging or within 24 hours of injury. Biomarkers will guide the decision to image or not. Studies will be pooled based on timing of tests: 0-3 hours after injury, >3 to 6 hours after injury, >6-12hours after injury, >12-24hours after injury. Biomarkers of TBI are often measured in body fluids. Measurements are obtained from CSF, saliva, blood (serum or plasma) and urine. CSF not in common use hence it is not included. There will be access to CSF only in people with significant (severe) head injury. Most of the patients with post-brain injury complications have mild head injury (GCS score 13-15) and lumbar puncture to analyse CSF biomarkers is not indicated for such patients.

8.	Reference standard	• Intra cranial injury and/or complex skull fracture on CT/MRI
9.	Types of study to be included	Cross-sectional studies Cohort studies (prospective and retrospective) Systematic reviews and meta-analyses of the above
10.	Other exclusion criteria	Non-English language studies. Conference abstracts will be excluded as it is expected there will be sufficient full text published studies available.
11.	Context	To determine diagnostic accuracy of brain injury biomarkers for predicting acute post-brain injury complications.
12.	Primary outcomes (critical outcomes)	All outcomes are considered equally important for decision making and therefore have all been rated as critical: diagnostic accuracy outcomes • diagnostic accuracy of biomarkers for predicting acute post-brain injury complications
13.	Data extraction (selection and coding)	All references identified by the searches and from other sources will be uploaded into EPPI reviewer and de-duplicated. 10% of the abstracts will be reviewed by two reviewers, with any disagreements resolved by discussion or, if necessary, a third independent reviewer. This review will make use of the priority screening functionality within the EPPI-reviewer software. The full text of potentially eligible studies will be retrieved and will be assessed in line with the criteria outlined above. A standardised form will be used to extract data from studies (see Developing NICE guidelines: the manual section 6.4).

		10% of all evidence reviews are quality assured by a senior research fellow. This includes checking: • papers were included /excluded appropriately • a sample of the data extractions • correct methods are used to synthesise data • a sample of the risk of bias assessments Disagreements between the review authors over the risk of bias in particular studies will be resolved by discussion, with involvement of a third review author where necessary.
14.	Risk of bias (quality) assessment	For diagnostic reviews • Diagnostic test accuracy studies: QUADAS-2 Assessment will be independently quality assured by a second reviewer. Disagreements between the reviewers will be resolved by discussion, with involvement of a third party where necessary.
15.	Strategy for data synthesis	For diagnostic accuracy evidence: • Aggregate data on diagnostic accuracy of investigations will be collected and synthesized in a quantitative data analysis. • Endnote will be used for bibliography, citations, sifting and reference management. • WinBUGS will be used for meta-analysis of diagnostic accuracy studies if included studies are sufficiently homogeneous. • If meta-analysis is not possible, data will be presented as individual values in adapted GRADE profile tables and plots of un-pooled sensitivity and specificity from RevMan software. • Where available, outcome data from new studies will be meta-analysed with corresponding data included in CG 176.

16.	Analysis of sub-groups	NA		
17.	Type and method of review	☐	Intervention	
		☒	Diagnostic	
		☐	Prognostic	
		☐	Qualitative	
		☐	Epidemiologic	
		☐	Service Delivery	
		☐	Other (please specify)	
18.	Language	English		
19.	Country	England		
20.	Anticipated or actual start date			
21.	Anticipated completion date			
22.	Stage of review at time of this submission	Review stage	Started	Completed
		Preliminary searches	☐	☑
		Piloting of the study selection process	☐	☑
		Formal screening of search results	☐	☑

		against eligibility criteria		
		Data extraction	☐	☑
		Risk of bias (quality) assessment	☐	☑
		Data analysis	☐	☑
23.	Named contact	5a. Named contact National Guideline Centre 5b Named contact e-mail headinjury@nice.org.uk 5e Organisational affiliation of the review National Institute for Health and Care Excellence (NICE) and National Guideline Centre		
24.	Review team members	From the National Guideline Centre: Guideline lead: Sharon Swain Senior systematic reviewer: Sharangini Rajesh Senior systematic reviewer: Julie Neilson Health economist: David Wonderling Information specialist: Joseph Runicles		

25.	Funding sources/sponsor	This systematic review is being completed by the National Guideline Centre which receives funding from NICE.
26.	Conflicts of interest	All guideline committee members and anyone who has direct input into NICE guidelines (including the evidence review team and expert witnesses) must declare any potential conflicts of interest in line with NICE's code of practice for declaring and dealing with conflicts of interest. Any relevant interests, or changes to interests, will also be declared publicly at the start of each guideline committee meeting. Before each meeting, any potential conflicts of interest will be considered by the guideline committee Chair and a senior member of the development team. Any decisions to exclude a person from all or part of a meeting will be documented. Any changes to a member's declaration of interests will be recorded in the minutes of the meeting. Declarations of interests will be published with the final guideline.
27.	Collaborators	Development of this systematic review will be overseen by an advisory committee who will use the review to inform the development of evidence-based recommendations in line with section 3 of Developing NICE guidelines: the manual. Members of the guideline committee are available on the NICE website: 1 (nice.org.uk) .
28.	Other registration details	
29.	Reference/URL for published protocol	
30.	Dissemination plans	NICE may use a range of different methods to raise awareness of the guideline. These include standard approaches such as: • notifying registered stakeholders of publication • publicising the guideline through NICE's newsletter and alerts • issuing a press release or briefing as appropriate, posting news articles on the NICE website, using social media channels, and publicising the guideline within NICE.
31.	Keywords	Brain injury biomarkers

32.	Details of existing review of same topic by same authors	[Give details of earlier versions of the systematic review if an update of an existing review is being registered, including full bibliographic reference if possible. NOTE: most NICE reviews will not constitute an update in PROSPERO language. To be an update it needs to be the same review question/search/methodology. If anything has changed it is a new review]	
33.	Current review status	☐	Ongoing
		☒	Completed but not published
		☐	Completed and published
		☐	Completed, published and being updated
		☐	Discontinued
34.	Additional information	NA	
35.	Details of final publication	www.nice.org.uk	

Review protocol for clinical and cost effectiveness of brain injury biomarkers for acute post-brain injury complications (test and treat)

ID	Field	Content
1.	Review title	2.3b What is the clinical and cost effectiveness of biomarkers when followed by the appropriate treatment for acute post-brain injury complications to improve patient outcomes? Post-brain injury complications may be defined in two main ways: • Intracranial injury on CT/MRI (does not usually include fracture within this definition)

		• Abnormal CT/MRI which includes intracranial injury and skull fracture (depends on definition of the study) [Some papers will include fractures but not all] The committee wanted to limit only to the ability of biomarkers to identify CT/MRI findings as it was agreed that even if biomarkers could identify or predict signs or symptoms such as seizures, this would only be relevant if they also had a confirmed abnormality on CT/MRI This will group will include people with mild traumatic brain injury Time frame: • Up to 1 week for children • Up to 1 month for adults
2.	Review question	What is the clinical and cost effectiveness of biomarkers when followed by the appropriate treatment for acute post-brain injury complication to improve patient outcomes?
3.	Objective	To understand the clinical and cost efficacy of brain injury biomarkers for acute post-brain injury complications.
4.	Searches	The following databases (from inception) will be searched: • Cochrane Central Register of Controlled Trials (CENTRAL) • Cochrane Database of Systematic Reviews (CDSR) • Embase • MEDLINE • Epistemonikos

		Searches will be restricted by: • English language studies • Human studies Other searches: • Inclusion lists of systematic reviews The searches may be re-run 6 weeks before the final committee meeting and further studies retrieved for inclusion if relevant. The full search strategies will be published in the final review. Medline search strategy to be quality assured using the PRESS evidence-based checklist (see methods chapter for full details).
5.	Condition or domain being studied	Acute post-brain injury complication in patients who have experienced a head injury.
6.	Population	Inclusion: Infants, children and adult with suspected or confirmed head injury Strata: • Adults (aged ≥16 years) • Children (aged ≥1 to <16 years)

		• Infants (aged <1 year) Mixed population studies will be included but downgraded for indirectness. Cut-off of 60% will be used for all age groups Exclusion: Adults, and children (including infants under 1 year) with superficial injuries to the eye or face without suspected or confirmed head or brain injury.
7.	Intervention	Biomarkers o Blood biomarkers - S100 calcium binding protein B (S100B) -Ubiquitin C-terminal Hydrolase-L1 (UCHL1) -Neuron Specific Enolase (NSE) -Brain-derived neurotrophic factor (BDNF) -Neurofilament light (NFL) - Neurofilament Heavy (NF-H) - αII-Spectrin breakdown products (SBDP) - Myelin basic protein (MBP) - glial fibrillary acidic protein (GFAP) o Salivary biomarkers -salivary microRNAs (miRNAs) -Extracellular vesicles (EVs) -S100B o Urine biomarkers -Extracellular vesicles (EVs)

		Each test must be followed by an appropriate treatment for complication after brain injury. Treatment: Admission to hospital for observation + possible neurosurgical management of TBI Timings: Biomarkers are used prior to decision for imaging or within 24 hours of injury. Biomarkers will guide the decision to image or not. Biomarkers of TBI are often measured in body fluids. Measurements are obtained from CSF, saliva, blood (serum or plasma) and urine. CSF not in common use hence it is not included. There will be access to CSF only in people with significant (severe) head injury. Most of the patients with post-brain injury complications have mild head injury (GCS score 13-15) and lumbar puncture to analyse CSF biomarkers is not indicated for such patients.
9.	Comparator	Comparators: • To usual care (no testing with biomarkers) • To each other
9.	Types of study to be included	• Randomised controlled trials (RCTs), systematic reviews of RCTs. • If no RCT evidence is available, non-randomised studies will be considered if they adjust for key confounders, starting with prospective cohort studies. Key Confounders: • Age • Gender • GCS or pupillary response at presentation

10.	Other exclusion criteria	Non-English language studies. Conference abstracts will be excluded as it is expected there will be sufficient full text published studies available.
11.	Context	To determine the clinical effectiveness of brain injury biomarkers for predicting acute post-brain injury complications.
12.	Primary outcomes (critical outcomes)	All outcomes are considered equally important for decision making and therefore have all been rated as critical: • Quality of life - 3 months or more • Objectively applied score of disability e.g. Glasgow Outcome Score (GOS) or extended GOS - at 3 months or more • Time to return to education/work/usual activities • Duration of post-injury complications
13.	Data extraction (selection and coding)	All references identified by the searches and from other sources will be uploaded into EPPI reviewer and de-duplicated. 10% of the abstracts will be reviewed by two reviewers, with any disagreements resolved by discussion or, if necessary, a third independent reviewer. This review will make use of the priority screening functionality within the EPPI-reviewer software. The full text of potentially eligible studies will be retrieved and will be assessed in line with the criteria outlined above. A standardised form will be used to extract data from studies (see Developing NICE guidelines: the manual section 6.4). 10% of all evidence reviews are quality assured by a senior research fellow. This includes checking: • papers were included /excluded appropriately • a sample of the data extractions • correct methods are used to synthesise data

		• a sample of the risk of bias assessments Disagreements between the review authors over the risk of bias in particular studies will be resolved by discussion, with involvement of a third review author where necessary.
14.	Risk of bias (quality) assessment	Risk of bias will be assessed using the appropriate checklist as described in Developing NICE guidelines: the manual. For Intervention reviews • Systematic reviews: Risk of Bias in Systematic Reviews (ROBIS) • Randomised Controlled Trial: Cochrane RoB (2.0) • Non randomised study, including cohort studies: Cochrane ROBINS-I
15.	Strategy for data synthesis	For clinical effectiveness evidence: • Pairwise meta-analyses will be performed using Cochrane Review Manager (RevMan5). Fixed-effects (Mantel-Haenszel) techniques will be used to calculate risk ratios for the binary outcomes where possible. Continuous outcomes will be analysed using an inverse variance method for pooling weighted mean differences. • Heterogeneity between the studies in effect measures will be assessed using the I^2 statistic and visually inspected. An I^2 value greater than 50% will be considered indicative of substantial heterogeneity. Sensitivity analyses will be conducted based on pre-specified subgroups using stratified meta-analysis to explore the heterogeneity in effect estimates. If this does not explain the heterogeneity, the results will be presented pooled using random-effects. • GRADEpro will be used to assess the quality of evidence for each outcome, taking into account individual study quality and the meta-analysis results. The 4 main quality elements (risk of bias, indirectness, inconsistency and imprecision) will be appraised for each outcome. Publication bias is tested for when there are more than 5 studies for an outcome. • The risk of bias across all available evidence was evaluated for each outcome using an adaptation of the 'Grading of Recommendations Assessment, Development and Evaluation (GRADE) toolbox' developed by the international GRADE working group http://www.gradeworkinggroup.org/

		Where meta-analysis is not possible, data will be presented and quality assessed individually per outcome.		
16.	Analysis of sub-groups	Subgroups that will be investigated if heterogeneity is present: Older adults • older/frail adults who have suffered a fall		
17.	Type and method of review	☒	Intervention	
		☐	Diagnostic	
		☐	Prognostic	
		☐	Qualitative	
		☐	Epidemiologic	
		☐	Service Delivery	
		☐	Other (please specify)	
18.	Language	English		
19.	Country	England		
20.	Anticipated or actual start date			
21.	Anticipated completion date			
22.	Stage of review at time of this submission	Review stage	Started	Completed
		Preliminary searches	☐	☑

		Piloting of the study selection process	☐	☑
		Formal screening of search results against eligibility criteria	☐	☑
		Data extraction	☐	☑
		Risk of bias (quality) assessment	☐	☑
		Data analysis	☐	☑
23.	Named contact	5a. Named contact National Guideline Centre 5b Named contact e-mail headinjury@nice.org.uk 5e Organisational affiliation of the review National Institute for Health and Care Excellence (NICE) and National Guideline Centre		
24.	Review team members	 From the National Guideline Centre: Guideline lead: Sharon Swain Senior systematic reviewer: Sharangini Rajesh		

		Senior systematic reviewer: Julie Neilson Health economist: David Wonderling Information specialist: Joseph Runicles Project manager: Giulia Zuodar
25.	Funding sources/sponsor	This systematic review is being completed by the National Guideline Centre which receives funding from NICE.
26.	Conflicts of interest	All guideline committee members and anyone who has direct input into NICE guidelines (including the evidence review team and expert witnesses) must declare any potential conflicts of interest in line with NICE's code of practice for declaring and dealing with conflicts of interest. Any relevant interests, or changes to interests, will also be declared publicly at the start of each guideline committee meeting. Before each meeting, any potential conflicts of interest will be considered by the guideline committee Chair and a senior member of the development team. Any decisions to exclude a person from all or part of a meeting will be documented. Any changes to a member's declaration of interests will be recorded in the minutes of the meeting. Declarations of interests will be published with the final guideline.
27.	Collaborators	Development of this systematic review will be overseen by an advisory committee who will use the review to inform the development of evidence-based recommendations in line with section 3 of Developing NICE guidelines: the manual. Members of the guideline committee are available on the NICE website: 1 (nice.org.uk) .
28.	Other registration details	
29.	Reference/URL for published protocol	
30.	Dissemination plans	NICE may use a range of different methods to raise awareness of the guideline. These include standard approaches such as: • notifying registered stakeholders of publication • publicising the guideline through NICE's newsletter and alerts • issuing a press release or briefing as appropriate, posting news articles on the NICE website, using social media channels, and publicising the guideline within NICE.

31.	KeywordsS	Brain injury biomarkers	
32.	Details of existing review of same topic by same authors	[Give details of earlier versions of the systematic review if an update of an existing review is being registered, including full bibliographic reference if possible. NOTE: most NICE reviews will not constitute an update in PROSPERO language. To be an update it needs to be the same review question/search/methodology. If anything has changed it is a new review]	
33.	Current review status	☐	Ongoing
		☒	Completed but not published
		☐	Completed and published
		☐	Completed, published and being updated
		☐	Discontinued
34.	Additional information	NA	
35.	Details of final publication	www.nice.org.uk	

Health economic review protocol

Review question	All questions – health economic evidence
Objectives	To identify health economic studies relevant to any of the review questions.
Search criteria	• Populations, interventions and comparators must be as specified in the clinical review protocol above. • Studies must be of a relevant health economic study design (cost–utility analysis, cost-effectiveness analysis, cost–benefit analysis, cost–consequences analysis, comparative cost analysis). • Studies must not be a letter, editorial or commentary, or a review of health economic evaluations. (Recent reviews will be ordered although not reviewed. The bibliographies will be checked for relevant studies, which will then be ordered.)

	• Unpublished reports will not be considered unless submitted as part of a call for evidence. • Studies must be in English.
Search strategy	A health economic study search will be undertaken using population-specific terms and a health economic study filter – see appendix B below. The search covered all years
Review strategy	Studies not meeting any of the search criteria above will be excluded. Studies published before 2006, abstract-only studies and studies from non-OECD countries or the USA will also be excluded. Studies published in 2006 or later that were included in the previous guidelines will be reassessed for inclusion and may be included or selectively excluded based on their relevance to the questions covered in this update and whether more applicable evidence is also identified. Each remaining study will be assessed for applicability and methodological limitations using the NICE economic evaluation checklist which can be found in appendix H of Developing NICE guidelines: the manual (2014).[50] **Inclusion and exclusion criteria** • If a study is rated as both 'Directly applicable' and with 'Minor limitations' then it will be included in the guideline. A health economic evidence table will be completed and it will be included in the health economic evidence profile. • If a study is rated as either 'Not applicable' or with 'Very serious limitations' then it will usually be excluded from the guideline. If it is excluded then a health economic evidence table will not be completed and it will not be included in the health economic evidence profile. • If a study is rated as 'Partially applicable', with 'Potentially serious limitations' or both then there is discretion over whether it should be included. **Where there is discretion** The health economist will make a decision based on the relative applicability and quality of the available evidence for that question, in discussion with the guideline committee if required. The ultimate aim is to include health economic studies that are helpful for decision-making in the context of the guideline and the current NHS setting. If several studies are considered of sufficiently high applicability and methodological quality that they could all be included, then the health economist, in discussion with the committee if required, may decide to include only the most applicable studies and to selectively exclude the remaining studies. All studies excluded on the basis of applicability or methodological limitations will be listed with explanation in the excluded health economic studies appendix below.

	The health economist will be guided by the following hierarchies. *Setting:* • UK NHS (most applicable). • OECD countries with predominantly public health insurance systems (for example, France, Germany, Sweden). • OECD countries with predominantly private health insurance systems (for example, Switzerland). • Studies set in non-OECD countries or in the USA will be excluded before being assessed for applicability and methodological limitations. *Health economic study type:* • Cost–utility analysis (most applicable). • Other type of full economic evaluation (cost–benefit analysis, cost-effectiveness analysis, cost–consequences analysis). • Comparative cost analysis. • Non-comparative cost analyses including cost-of-illness studies will be excluded before being assessed for applicability and methodological limitations. *Year of analysis:* • The more recent the study, the more applicable it will be. • Studies published in 2006 or later (including any such studies included in the previous guidelines) but that depend on unit costs and resource data entirely or predominantly from before 2006 will be rated as 'Not applicable'. • Studies published before 2006 (including any such studies included in the previous guidelines) will be excluded before being assessed for applicability and methodological limitations. *Quality and relevance of effectiveness data used in the health economic analysis:* • The more closely the clinical effectiveness data used in the health economic analysis match with the outcomes of the studies included in the clinical review the more useful the analysis will be for decision-making in the guideline.

Appendix B – Literature search strategies

This literature search strategy was used for the following questions:

- What is the diagnostic accuracy of brain injury biomarkers for predicting acute post-brain injury complications?
- What is the clinical and cost effectiveness of biomarkers when followed by the appropriate treatment for acute post-brain injury complications to improve patient outcomes?

The literature searches for this review are detailed below and complied with the methodology outlined in Developing NICE guidelines: the manual.[50]

For more information, please see the Methodology review published as part of the accompanying documents for this guideline.

B.1 Clinical search literature search strategy

Searches were constructed using a PICO framework where population (P) terms were combined with Intervention (I) and in some cases Comparison (C) terms. Outcomes (O) are rarely used in search strategies as these concepts may not be indexed or described in the title or abstract and are therefore difficult to retrieve. Search filters were applied to the search where appropriate.

Table 6: Database parameters, filters and limits applied

Database	Dates searched	Search filter used
Medline (OVID)	1946 – 22 June 2022	Randomised controlled trials Systematic review studies Observational studies Diagnostic tests studies Exclusions (animal studies, letters, comments, editorials, case studies/reports) English language
Embase (OVID)	1974 – 22 June 2022	Randomised controlled trials Systematic review studies Observational studies Diagnostic tests studies Exclusions (animal studies, letters, comments, editorials, case studies/reports, conference abstracts) English language
The Cochrane Library (Wiley)	Cochrane Reviews to 2022 Issue 6 of 12 CENTRAL to 2022 Issue 6 of 12	
Epistemonikos (The Epistemonikos Foundation)	Inception to 22 June 2022	Exclusions (Cochrane reviews)

Medline (Ovid) search terms

1.	craniocerebral trauma/ or exp brain injuries/ or coma, post-head injury/ or exp head injuries, closed/ or head injuries, penetrating/ or exp intracranial hemorrhage, traumatic/ or exp skull fractures/
2.	((skull or cranial) adj3 fracture*).ti,ab.
3.	((head or brain or craniocerebral or cranial or cerebral or skull) adj4 (injur* or trauma*)).ti,ab.
4.	(trauma* and ((subdural or intracranial) adj2 (h?ematoma* or h?emorrhage* or bleed*))).ti,ab.
5.	or/1-4
6.	letter/
7.	editorial/
8.	news/
9.	exp historical article/
10.	Anecdotes as Topic/
11.	comment/
12.	case report/
13.	(letter or comment*).ti.
14.	or/6-13
15.	randomized controlled trial/ or random*.ti,ab.
16.	14 not 15
17.	animals/ not humans/
18.	exp Animals, Laboratory/
19.	exp Animal Experimentation/
20.	exp Models, Animal/
21.	exp Rodentia/
22.	(rat or rats or mouse or mice or rodent*).ti.
23.	or/16-22
24.	5 not 23
25.	limit 24 to English language
26.	exp Biomarkers/
27.	exp S100 Proteins/
28.	Glial Fibrillary Acidic Protein/
29.	Phosphopyruvate Hydratase/
30.	Ubiquitin Thiolesterase/
31.	exp MicroRNAs/
32.	Brain-Derived Neurotrophic Factor/
33.	Neurofilament Proteins/
34.	Spectrin/
35.	Myelin Basic Protein/
36.	exp Extracellular Vesicles/
37.	tau Proteins/
38.	(Ubiquitin Thiolesterase* or "Ubiquitin C-terminal hydrolase*" or "Ubiquitin C-Terminal Esterase*" or "Ubiquitin Carboxy-Terminal Hydrolase*" or "Ubiquitin Carboxy-Terminal Esterase*" or uch-l1 or UCHL1).ti,ab.
39.	(S100* or GFAP or "glial fibrillary acid* protein*" or "brain-derived neurotrophic factor*" or "brain-derived nerve growth factor*" or BDNF or spectrin* or tau or proteomic* or microRNA* or miRNA* or micro-rna*).ti,ab.

40.	((muscle or nervous or neuron* or alpha or beta or gamma) adj3 enolase*).ti,ab.
41.	(Phosphopyruvate Hydratase* or 2-phosphoglycerate* or 2-phospho-D-glycerate* or NSE).ti,ab.
42.	((neurofilament* adj3 (protein* or chain* or polypeptide*)) or NF-L or NF-H).ti,ab.
43.	((myelin basic or nerve tissue* or golli* or hog* or mbp*) adj2 protein*).ti,ab.
44.	(((extracellular or secretory) adj vesicle*) or exovesicle* or apoptotic bod* or exosome* or endosome* or ectosome* or microvesicle* or ((membrane or cell-derived) adj microparticle*)).ti,ab.
45.	biomarker*.ti,ab,kf.
46.	marker*.ti,ab.
47.	or/26-46
48.	25 and 47
49.	randomized controlled trial.pt.
50.	controlled clinical trial.pt.
51.	randomi#ed.ti,ab.
52.	placebo.ab.
53.	randomly.ti,ab.
54.	Clinical Trials as topic.sh.
55.	trial.ti.
56.	or/49-55
57.	Meta-Analysis/
58.	exp Meta-Analysis as Topic/
59.	(meta analy* or metanaly* or metaanaly* or meta regression).ti,ab.
60.	((systematic* or evidence*) adj3 (review* or overview*)).ti,ab.
61.	(reference list* or bibliograph* or hand search* or manual search* or relevant journals).ab.
62.	(search strategy or search criteria or systematic search or study selection or data extraction).ab.
63.	(search* adj4 literature).ab.
64.	(medline or pubmed or cochrane or embase or psychlit or psyclit or psychinfo or psycinfo or cinahl or science citation index or bids or cancerlit).ab.
65.	cochrane.jw.
66.	((multiple treatment* or indirect or mixed) adj2 comparison*).ti,ab.
67.	or/57-66
68.	Epidemiologic studies/
69.	Observational study/
70.	exp Cohort studies/
71.	(cohort adj (study or studies or analys* or data)).ti,ab.
72.	((follow up or observational or uncontrolled or non randomi#ed or epidemiologic*) adj (study or studies or data)).ti,ab.
73.	((longitudinal or retrospective or prospective or cross sectional) and (study or studies or review or analys* or cohort* or data)).ti,ab.
74.	Controlled Before-After Studies/
75.	Historically Controlled Study/
76.	Interrupted Time Series Analysis/
77.	(before adj2 after adj2 (study or studies or data)).ti,ab.
78.	exp case control study/

79.	case control*.ti,ab.
80.	Cross-sectional studies/
81.	(cross sectional and (study or studies or review or analys* or cohort* or data)).ti,ab.
82.	or/68-81
83.	exp "Sensitivity and Specificity"/
84.	(sensitivity or specificity).ti,ab.
85.	((pre test or pretest or post test) adj probability).ti,ab.
86.	(predictive value* or PPV or NPV).ti,ab.
87.	likelihood ratio*.ti,ab.
88.	Likelihood Functions/
89.	((area under adj4 curve) or AUC).ti,ab.
90.	(receive* operat* characteristic* or receive* operat* curve* or ROC curve*).ti,ab.
91.	gold standard.ab.
92.	exp Diagnostic Errors/
93.	(false positiv* or false negativ*).ti,ab.
94.	Diagnosis, Differential/
95.	(diagnos* adj3 (performance* or accurac* or utilit* or value* or efficien* or effectiveness or precision or validat* or validity or differential or error*)).ti,ab.
96.	or/83-95
97.	48 and (56 or 67 or 82 or 96)

Embase (Ovid) search terms

1.	head injury/
2.	exp brain injury/
3.	skull injury/ or exp skull fracture/
4.	((head or brain or craniocerebral or cranial or cerebral or skull) adj4 (injur* or trauma*)).ti,ab.
5.	((skull or cranial) adj3 fracture*).ti,ab.
6.	(trauma* and ((subdural or intracranial) adj2 (h?ematoma* or h?emorrhage* or bleed*))).ti,ab.
7.	or/1-6
8.	letter.pt. or letter/
9.	note.pt.
10.	editorial.pt.
11.	(conference abstract or conference paper).pt.
12.	case report/ or case study/
13.	(letter or comment*).ti.
14.	or/8-13
15.	randomized controlled trial/ or random*.ti,ab.
16.	14 not 15
17.	animal/ not human/
18.	nonhuman/
19.	exp Animal Experiment/
20.	exp Experimental Animal/
21.	animal model/
22.	exp Rodent/

23.	(rat or rats or mouse or mice or rodent*).ti.
24.	or/16-23
25.	7 not 24
26.	limit 25 to English language
27.	*biological marker/
28.	*protein S 100/
29.	*glial fibrillary acidic protein/
30.	*enolase/
31.	*ubiquitin thiolesterase/
32.	exp *microRNA/
33.	*brain derived neurotrophic factor/
34.	*neurofilament protein/
35.	*spectrin/
36.	*myelin basic protein/
37.	*exosome/
38.	*tau protein/
39.	(Ubiquitin Thiolesterase* or "Ubiquitin C-terminal hydrolase*" or "Ubiquitin C-Terminal Esterase*" or "Ubiquitin Carboxy-Terminal Hydrolase*" or "Ubiquitin Carboxy-Terminal Esterase*" or uch-l1 or UCHL1).ti,ab.
40.	(S100* or GFAP or "glial fibrillary acid* protein*" or "brain-derived neurotrophic factor*" or "brain-derived nerve growth factor*" or BDNF or spectrin* or tau or proteomic* or microRNA* or miRNA* or micro-rna*).ti,ab.
41.	((muscle or nervous or neuron* or alpha or beta or gamma) adj3 enolase*).ti,ab.
42.	(Phosphopyruvate Hydratase* or 2-phosphoglycerate* or 2-phospho-D-glycerate* or NSE).ti,ab.
43.	((neurofilament* adj3 (protein* or chain* or polypeptide*)) or NF-L or NF-H).ti,ab.
44.	((myelin basic or nerve tissue* or golli* or hog* or mbp*) adj2 protein*).ti,ab.
45.	(((extracellular or secretory) adj vesicle*) or exovesicle* or apoptotic bod* or exosome* or endosome* or ectosome* or microvesicle* or ((membrane or cell-derived) adj microparticle*)).ti,ab.
46.	biomarker*.ti,ab,kw.
47.	marker*.ti,ab.
48.	or/27-47
49.	26 and 48
50.	random*.ti,ab.
51.	factorial*.ti,ab.
52.	(crossover* or cross over*).ti,ab.
53.	((doubl* or singl*) adj blind*).ti,ab.
54.	(assign* or allocat* or volunteer* or placebo*).ti,ab.
55.	crossover procedure/
56.	single blind procedure/
57.	randomized controlled trial/
58.	double blind procedure/
59.	or/50-58
60.	systematic review/
61.	Meta-Analysis/
62.	(meta analy* or metanaly* or metaanaly* or meta regression).ti,ab.

63.	((systematic* or evidence*) adj3 (review* or overview*)).ti,ab.
64.	(reference list* or bibliograph* or hand search* or manual search* or relevant journals).ab.
65.	(search strategy or search criteria or systematic search or study selection or data extraction).ab.
66.	(search* adj4 literature).ab.
67.	(medline or pubmed or cochrane or embase or psychlit or psyclit or psychinfo or psycinfo or cinahl or science citation index or bids or cancerlit).ab.
68.	cochrane.jw.
69.	((multiple treatment* or indirect or mixed) adj2 comparison*).ti,ab.
70.	or/60-69
71.	Clinical study/
72.	Observational study/
73.	Family study/
74.	Longitudinal study/
75.	Retrospective study/
76.	Prospective study/
77.	Cohort analysis/
78.	Follow-up/
79.	cohort*.ti,ab.
80.	78 and 79
81.	(cohort adj (study or studies or analys* or data)).ti,ab.
82.	((follow up or observational or uncontrolled or non randomi#ed or epidemiologic*) adj (study or studies or data)).ti,ab.
83.	((longitudinal or retrospective or prospective or cross sectional) and (study or studies or review or analys* or cohort* or data)).ti,ab.
84.	(before adj2 after adj2 (study or studies or data)).ti,ab.
85.	exp case control study/
86.	case control*.ti,ab.
87.	cross-sectional study/
88.	(cross sectional and (study or studies or review or analys* or cohort* or data)).ti,ab.
89.	or/71-77,80-89
90.	exp "sensitivity and specificity"/
91.	(sensitivity or specificity).ti,ab.
92.	((pre test or pretest or post test) adj probability).ti,ab.
93.	(predictive value* or PPV or NPV).ti,ab.
94.	likelihood ratio*.ti,ab.
95.	((area under adj4 curve) or AUC).ti,ab.
96.	(receive* operat* characteristic* or receive* operat* curve* or ROC curve*).ti,ab.
97.	diagnostic accuracy/
98.	diagnostic test accuracy study/
99.	gold standard.ab.
100.	exp diagnostic error/
101.	(false positiv* or false negativ*).ti,ab.
102.	differential diagnosis/
103.	(diagnos* adj3 (performance* or accurac* or utilit* or value* or efficien* or effectiveness or precision or validat* or validity or differential or error*)).ti,ab.

104.	or/90-103
105.	49 and (59 or 70 or 89 or 104)

Cochrane Library (Wiley) search terms

#1.	MeSH descriptor: [Craniocerebral Trauma] this term only
#2.	MeSH descriptor: [Brain Injuries] explode all trees
#3.	MeSH descriptor: [Coma, Post-Head Injury] this term only
#4.	MeSH descriptor: [Head Injuries, Closed] explode all trees
#5.	MeSH descriptor: [Head Injuries, Penetrating] this term only
#6.	MeSH descriptor: [Intracranial Hemorrhage, Traumatic] explode all trees
#7.	MeSH descriptor: [Skull Fractures] explode all trees
#8.	((skull or cranial) near/3 fracture*):ti,ab
#9.	((head or brain or craniocerebral or cranial or skull) near/3 (injur* or trauma*)):ti,ab
#10.	(trauma* and ((subdural or intracranial) near/2 (h?ematoma* or h?emorrhage* or bleed*))):ti,ab
#11.	#1 or #2 or #3 or #4 or #5 or #6 or #7 or #8 or #9 or #10
#12.	MeSH descriptor: [Biomarkers] explode all trees
#13.	MeSH descriptor: [S100 Proteins] explode all trees
#14.	MeSH descriptor: [Glial Fibrillary Acidic Protein] this term only
#15.	MeSH descriptor: [Phosphopyruvate Hydratase] this term only
#16.	MeSH descriptor: [Ubiquitin Thiolesterase] this term only
#17.	MeSH descriptor: [MicroRNAs] explode all trees
#18.	MeSH descriptor: [Brain-Derived Neurotrophic Factor] this term only
#19.	MeSH descriptor: [Neurofilament Proteins] this term only
#20.	MeSH descriptor: [Spectrin] this term only
#21.	MeSH descriptor: [Myelin Basic Protein] this term only
#22.	MeSH descriptor: [Extracellular Vesicles] explode all trees
#23.	MeSH descriptor: [tau Proteins] this term only
#24.	(Ubiquitin Thiolesterase* or "Ubiquitin C-terminal hydrolase*" or "Ubiquitin C-Terminal Esterase*" or "Ubiquitin Carboxy-Terminal Hydrolase*" or "Ubiquitin Carboxy-Terminal Esterase*" or uch-l1 or UCHL1):ti,ab
#25.	(S100* or GFAP or "glial fibrillary acid* protein*" or "brain-derived neurotrophic factor*" or "brain-derived nerve growth factor*" or BDNF or spectrin* or tau or proteomic* or microRNA* or miRNA* or micro-rna*):ti,ab
#26.	((muscle or nervous or neuron* or alpha or beta or gamma) near/3 enolase*):ti,ab
#27.	(Phosphopyruvate Hydratase* or 2phosphoglycerate* or 2phospho-D-glycerate* or NSE):ti,ab
#28.	((neurofilament* near/3 (protein* or chain* or polypeptide*)) or NF-L or NF-H):ti,ab
#29.	((myelin basic or nerve tissue* or golli* or hog* or mbp*) near/2 protein*):ti,ab
#30.	(((extracellular or secretory) near vesicle*) or exovesicle* or apoptotic bod* or exosome* or endosome* or ectosome* or microvesicle* or ((membrane or cell-derived) near microparticle*)):ti,ab
#31.	biomarker*:ti,ab,kw
#32.	marker*:ti,ab
#33.	#12 or #13 or #14 or #15 or #16 or #17 or #18 or #19 or #20 or #21 or #22 or #23 or #24 or #25 or #26 or #27 or #28 or #29 or #30 or #31 or #32
#34.	#11 and #33

Epistemonikos search terms

1.	(advanced_title_en:(((trauma OR traumatic) AND (injury OR injuries))) OR advanced_abstract_en:(((trauma OR traumatic) AND (injury OR injuries)))) OR (advanced_title_en:(((skull OR cranial) AND fracture*)) OR advanced_abstract_en:(((skull OR cranial) AND fracture*))) OR (advanced_title_en:(((head OR brain OR craniocerebral OR cranial OR cerebral OR skull) AND (injur* OR trauma*))) OR advanced_abstract_en:(((head OR brain OR craniocerebral OR cranial OR cerebral OR skull) AND (injur* OR trauma*)))) AND (advanced_title_en:((biomarker* OR marker*)) OR advanced_abstract_en:((biomarker* OR marker*))) OR (advanced_title_en:((S100* OR GFAP OR "glial fibrillary acid* protein*" OR "brain-derived neurotrophic factor*" OR "brain-derived nerve growth factor*" OR BDNF OR spectrin* OR tau OR proteomic* OR microRNA* OR miRNA* OR micro-rna*)) OR advanced_abstract_en:((S100* OR GFAP OR "glial fibrillary acid* protein*" OR "brain-derived neurotrophic factor*" OR "brain-derived nerve growth factor*" OR BDNF OR spectrin* OR tau OR proteomic* OR microRNA* OR miRNA* OR micro-rna*))) OR (advanced_title_en:((Ubiquitin Thiolesterase* OR "Ubiquitin C-terminal hydrolase*" OR "Ubiquitin C-Terminal Esterase*" OR "Ubiquitin Carboxy-Terminal Hydrolase*" OR "Ubiquitin Carboxy-Terminal Esterase*" OR uch-l1 OR UCHL1)) OR advanced_abstract_en:((Ubiquitin Thiolesterase* OR "Ubiquitin C-terminal hydrolase*" OR "Ubiquitin C-Terminal Esterase*" OR "Ubiquitin Carboxy-Terminal Hydrolase*" OR "Ubiquitin Carboxy-Terminal Esterase*" OR uch-l1 OR UCHL1))) OR (advanced_title_en:((enolase* OR Phosphopyruvate Hydratase* OR 2-phosphoglycerate* OR 2-phospho-D-glycerate* OR NSE)) OR advanced_abstract_en:((enolase* OR Phosphopyruvate Hydratase* OR 2-phosphoglycerate* OR 2-phospho-D-glycerate* OR NSE))) OR (advanced_title_en:((neurofilament protein* OR myelin basic protein* OR extracellular vesicle* OR exovesicle* OR apoptotic bod* OR exosome* OR endosome* OR ectosome* OR microvesicle* OR cell-derived microparticle*)) OR advanced_abstract_en:((neurofilament protein* OR myelin basic protein* OR extracellular vesicle* OR exovesicle* OR apoptotic bod* OR exosome* OR endosome* OR ectosome* OR microvesicle* OR cell-derived microparticle*)))

B.2 Health Economics literature search strategy

Health economic evidence was identified by conducting searches using terms for a broad Head Injury population. The following databases were searched: NHS Economic Evaluation Database (NHS EED - this ceased to be updated after 31st March 2015), Health Technology Assessment database (HTA - this ceased to be updated from 31st March 2018) and The International Network of Agencies for Health Technology Assessment (INAHTA). Searches for recent evidence were run on Medline and Embase from 2014 onwards for health economics, and all years for quality-of-life studies.

Table 7: Database parameters, filters and limits applied

Database	Dates searched	Search filters and limits applied
Medline (OVID)	Health Economics 1 January 2014 – 22 June 2022	Health economics studies Quality of life studies Exclusions (animal studies, letters, comments, editorials, case studies/reports) English language
	Quality of Life 1946 – 22 June 2022	
Embase (OVID)	Health Economics 1 January 2014 – 22 June 2022	Health economics studies Quality of life studies

Database	Dates searched	Search filters and limits applied
	Quality of Life 1974 – 22 June 2022	Exclusions (animal studies, letters, comments, editorials, case studies/reports, conference abstracts) English language
NHS Economic Evaluation Database (NHS EED) (Centre for Research and Dissemination - CRD)	Inception –31st March 2015	
Health Technology Assessment Database (HTA) (Centre for Research and Dissemination – CRD)	Inception – 31st March 2018	
The International Network of Agencies for Health Technology Assessment (INAHTA)	Inception – 22 June 2022	English language

Medline (Ovid) search terms

1.	craniocerebral trauma/ or exp brain injuries/ or coma, post-head injury/ or exp head injuries, closed/ or head injuries, penetrating/ or exp intracranial hemorrhage, traumatic/ or exp skull fractures/
2.	((skull or cranial) adj3 fracture*).ti,ab.
3.	((head or brain or craniocerebral or intracranial or cranial or skull) adj3 (injur* or trauma*)).ti,ab.
4.	(trauma* and ((subdural or intracranial or brain) adj2 (h?ematoma* or h?emorrhage* or bleed*))).ti,ab.
5.	or/1-4
6.	letter/
7.	editorial/
8.	news/
9.	exp historical article/
10.	Anecdotes as Topic/
11.	comment/
12.	case report/
13.	(letter or comment*).ti.
14.	or/6-13
15.	randomized controlled trial/ or random*.ti,ab.
16.	14 not 15
17.	animals/ not humans/
18.	exp Animals, Laboratory/
19.	exp Animal Experimentation/
20.	exp Models, Animal/
21.	exp Rodentia/
22.	(rat or rats or mouse or mice or rodent*).ti.

23.	or/16-22
24.	5 not 23
25.	limit 24 to English language
26.	economics/
27.	value of life/
28.	exp "costs and cost analysis"/
29.	exp Economics, Hospital/
30.	exp Economics, medical/
31.	Economics, nursing/
32.	economics, pharmaceutical/
33.	exp "Fees and Charges"/
34.	exp budgets/
35.	budget*.ti,ab.
36.	cost*.ti.
37.	(economic* or pharmaco?economic*).ti.
38.	(price* or pricing*).ti,ab.
39.	(cost* adj2 (effectiv* or utilit* or benefit* or minimi* or unit* or estimat* or variable*)).ab.
40.	(financ* or fee or fees).ti,ab.
41.	(value adj2 (money or monetary)).ti,ab.
42.	or/26-41
43.	quality-adjusted life years/
44.	sickness impact profile/
45.	(quality adj2 (wellbeing or well being)).ti,ab.
46.	sickness impact profile.ti,ab.
47.	disability adjusted life.ti,ab.
48.	(qal* or qtime* or qwb* or daly*).ti,ab.
49.	(euroqol* or eq5d* or eq 5*).ti,ab.
50.	(qol* or hql* or hqol* or h qol* or hrqol* or hr qol*).ti,ab.
51.	(health utility* or utility score* or disutilit* or utility value*).ti,ab.
52.	(hui or hui1 or hui2 or hui3).ti,ab.
53.	(health* year* equivalent* or hye or hyes).ti,ab.
54.	discrete choice*.ti,ab.
55.	rosser.ti,ab.
56.	(willingness to pay or time tradeoff or time trade off or tto or standard gamble*).ti,ab.
57.	(sf36* or sf 36* or short form 36* or shortform 36* or shortform36*).ti,ab.
58.	(sf20 or sf 20 or short form 20 or shortform 20 or shortform20).ti,ab.
59.	(sf12* or sf 12* or short form 12* or shortform 12* or shortform12*).ti,ab.
60.	(sf8* or sf 8* or short form 8* or shortform 8* or shortform8*).ti,ab.
61.	(sf6* or sf 6* or short form 6* or shortform 6* or shortform6*).ti,ab.
62.	or/43-61

63.	25 and (42 or 62)

Embase (Ovid) search terms

1.	head injury/
2.	exp brain injury/
3.	skull injury/ or exp skull fracture/
4.	((head or brain or craniocerebral or intracranial or cranial or skull) adj3 (injur* or trauma*)).ti,ab.
5.	((skull or cranial) adj3 fracture*).ti,ab.
6.	(trauma* and ((subdural or intracranial or brain) adj2 (h?ematoma* or h?emorrhage* or bleed*))).ti,ab.
7.	or/1-6
8.	letter.pt. or letter/
9.	note.pt.
10.	editorial.pt.
11.	(conference abstract or conference paper).pt.
12.	case report/ or case study/
13.	(letter or comment*).ti.
14.	or/8-13
15.	randomized controlled trial/ or random*.ti,ab.
16.	14 not 15
17.	animal/ not human/
18.	nonhuman/
19.	exp Animal Experiment/
20.	exp Experimental Animal/
21.	animal model/
22.	exp Rodent/
23.	(rat or rats or mouse or mice or rodent*).ti.
24.	or/16-23
25.	7 not 24
26.	limit 25 to English language
27.	health economics/
28.	exp economic evaluation/
29.	exp health care cost/
30.	exp fee/
31.	budget/
32.	funding/
33.	budget*.ti,ab.
34.	cost*.ti.
35.	(economic* or pharmaco?economic*).ti.
36.	(price* or pricing*).ti,ab.
37.	(cost* adj2 (effectiv* or utilit* or benefit* or minimi* or unit* or estimat* or variable*)).ab.
38.	(financ* or fee or fees).ti,ab.
39.	(value adj2 (money or monetary)).ti,ab.

40.	or/27-39
41.	quality-adjusted life years/
42.	"quality of life index"/
43.	short form 12/ or short form 20/ or short form 36/ or short form 8/
44.	sickness impact profile/
45.	(quality adj2 (wellbeing or well being)).ti,ab.
46.	sickness impact profile.ti,ab.
47.	disability adjusted life.ti,ab.
48.	(qal* or qtime* or qwb* or daly*).ti,ab.
49.	(euroqol* or eq5d* or eq 5*).ti,ab.
50.	(qol* or hql* or hqol* or h qol* or hrqol* or hr qol*).ti,ab.
51.	(health utility* or utility score* or disutilit* or utility value*).ti,ab.
52.	(hui or hui1 or hui2 or hui3).ti,ab.
53.	(health* year* equivalent* or hye or hyes).ti,ab.
54.	discrete choice*.ti,ab.
55.	rosser.ti,ab.
56.	(willingness to pay or time tradeoff or time trade off or tto or standard gamble*).ti,ab.
57.	(sf36* or sf 36* or short form 36* or shortform 36* or shortform36*).ti,ab.
58.	(sf20 or sf 20 or short form 20 or shortform 20 or shortform20).ti,ab.
59.	(sf12* or sf 12* or short form 12* or shortform 12* or shortform12*).ti,ab.
60.	(sf8* or sf 8* or short form 8* or shortform 8* or shortform8*).ti,ab.
61.	(sf6* or sf 6* or short form 6* or shortform 6* or shortform6*).ti,ab.
62.	or/41-61
63.	26 and (40 or 62)

NHS EED and HTA (CRD) search terms

#1.	MeSH DESCRIPTOR Brain Injuries EXPLODE ALL TREES
#2.	MeSH DESCRIPTOR Craniocerebral Trauma
#3.	MeSH DESCRIPTOR Coma, Post-Head Injury
#4.	MeSH DESCRIPTOR Head Injuries, Closed EXPLODE ALL TREES
#5.	MeSH DESCRIPTOR Head Injuries, Penetrating
#6.	MeSH DESCRIPTOR Intracranial Hemorrhage, Traumatic EXPLODE ALL TREES
#7.	MeSH DESCRIPTOR Skull Fractures EXPLODE ALL TREES
#8.	(((skull or cranial) adj3 fracture*))
#9.	(((head or brain or craniocerebral or intracranial or cranial or skull) adj3 (injur* or trauma*)))
#10.	((trauma* and ((subdural or intracranial or brain) adj2 (h?ematoma* or h?emorrhage* or bleed*))))
#11.	#1 OR #2 OR #3 OR #4 OR #5 OR #6 OR #7 OR #8 OR #9 OR #10

INAHTA search terms

1.	((((trauma* and ((subdural or intracranial or brain) and (haematoma* or hematoma* or haemorrhage* or hemorrhage* or bleed*))))[Title]) AND (((trauma* and ((subdural or intracranial or brain) and (haematoma* or hematoma* or haemorrhage* or hemorrhage* or bleed*))))[Title])) OR ((((skull or cranial) and fracture*))[Title] OR (((skull or cranial) and fracture*))[abs]) OR ((((head or brain or craniocerebral or intracranial or cranial or skull) and (injur* or trauma*)))[Title] OR (((head or brain or

	craniocerebral or intracranial or cranial or skull) and (injur* or trauma*)))[abs]) OR ("Skull Fractures"[mhe]) OR ("Intracranial Hemorrhage, Traumatic"[mhe]) OR ("Head Injuries, Penetrating"[mh]) OR ("Head Injuries, Closed"[mhe]) OR ("Coma, Post-Head Injury"[mh]) OR ("Brain Injuries"[mhe]) OR ("Craniocerebral Trauma"[mh])

Appendix C – Diagnostic evidence study selection

Figure 1: Flow chart of clinical study selection for the review of biomarkers for acute post-injury complications (diagnostic accuracy)

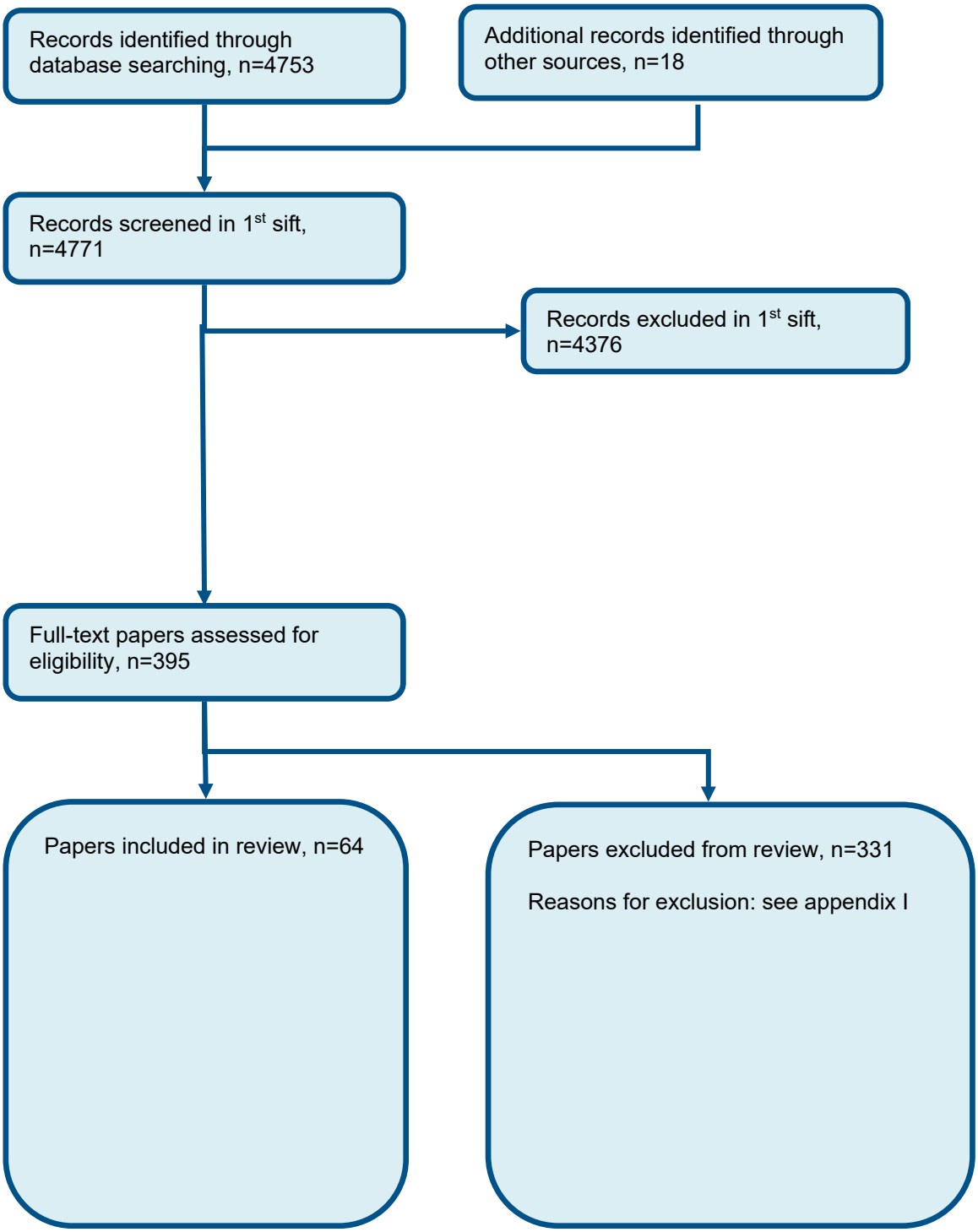
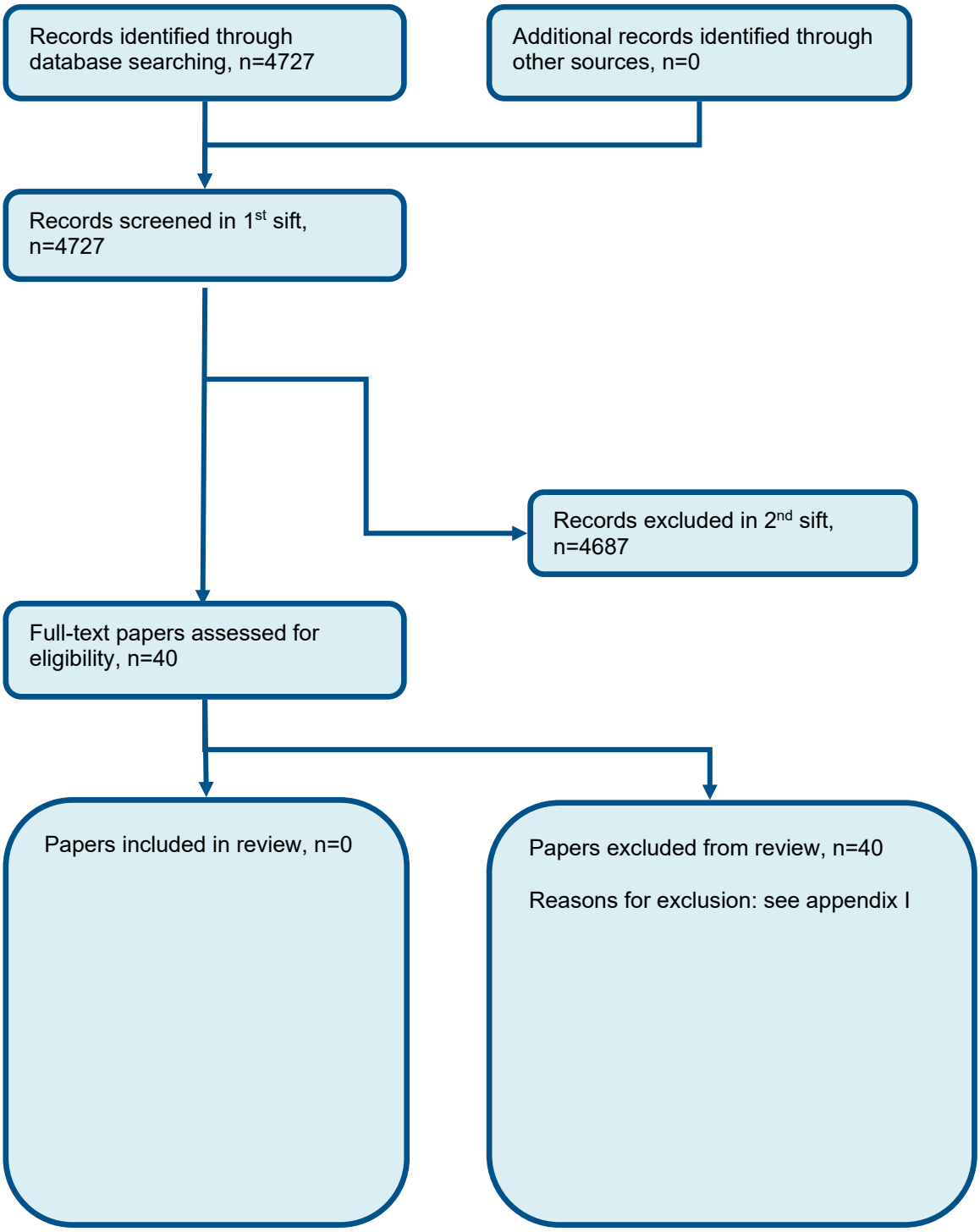


Figure 2: Flow chart of clinical study selection for the review of biomarkers for acute post-injury complications (test and treat)



Appendix D – Diagnostic evidence

Biomarkers in adults

Reference	**Asadollahi, 2016** [1]
Study type	Prospective cohort
Study methodology	Data source: prospective study carried out from December 2013 to June 2014 at an adult emergency department (ED) of a teaching hospital in Tehran, Iran. Consecutive patients with isolated mild TBI were recruited.
Number of patients	n = 158
Patient characteristics	Age, mean (SD): 35.5 (15.8) years Gender (male): 69.6% GCS: breakdown of GCS not reported but mild TBI defined as GCS score 13-15 for inclusion in the study 9 (5.7%) on anticoagulation Ethnicity: not reported Setting: adult ED of a teaching hospital Country: Iran Inclusion criteria: isolated mild TBI (GCS score 13-15 with loss of consciousness (LOC) <30 minutes and post traumatic amnesia (PTA) <1 hour;) >18 years of age; presented to the ED within 2 hours after the injury Exclusion criteria: GCS score of 15 without LOC or PTA; pregnancy; history of severe neurologic or psychiatric disorder; history of neurosurgical procedures; focal neurologic deficit; multiple injuries (trauma to the face, chest, abdomen, extremities or pelvic girdle requiring immediate therapeutic intervention); renal or liver disease; severe injury (abbreviated injury score ≥2); acute non-traumatic intracranial lesions
Target condition(s)	Acute post-brain injury complications

Reference	**Asadollahi, 2016** [1]
Index test(s) and reference standard	Index test: Serum S100B measured at 3 and 6 hours post injury Reference standard: CT scan within 6 hours post injury. Follow up: no mention of follow up but states that 2 participants underwent neurologic deterioration and needed surgical treatment
Results	Outcome: Positive CT scan (at least one trauma-relevant lesion (epidural, subdural, subarachnoid, intracerebral haemorrhage, cerebral contusion, brain oedema, depressed skull fracture). Positive CT scan – Serum S100B at 3 hours (optimal cut off 0.115 µg L^{-1}) TP: 75 FP: 51 TN: 28 FN: 4 Sensitivity%: 94.9 Specificity%: 35.4 PPV%: 59.5 NPV%: 87.5 AUC (95% CI): 0.7 (0.618-0.782)

Reference	Asadollahi, 2016 [1]
	SN/SP calculated by NGC Sensitivity: 0.95 [0.88, 0.99] Specificity: 0.35 [0.25, 0.47] Positive CT scan – Serum S100B at 6 hours (optimal cut off 0.21 µg L^{-1}) TP: 78 FP: 126 TN: 31 FN: 1 Sensitivity%: 98.7 Specificity%: 39.2 PPV%: 61.9 NPV%: 96.8 AUC (95% CI): 0.74 (0.662-0.817) SN/SP Calculated by NGC Sensitivity: 0.99 [0.93, 1.00] Specificity: 0.20 [0.14, 0.27]
Source of funding	Financially supported by a grant from Sina Trauma and Surgery Research Center, Tehran University of Medical Sciences
Limitations	Risk of bias *(QUADAS 2 – risk of bias)*: serious. Unclear whether the results of the index test were interpreted without knowledge of the results of the reference standard and vice versa Indirectness *(QUADAS 2 – applicability)*: none

Reference	**Asadollahi, 2016** [1]
Comments	-

Reference	**Bazarian, 2006** [4]
Study type	Nested cohort
Study methodology	Data source: subjects identified by research assistants from a larger cohort of consecutive mild TBI patients participating in a NIH registry study between February and September 2003.
Number of patients	n = 96
Patient characteristics	Age, mean (SD): 35.9 (19.5) years, range 8-79 years, median 39.5 years Gender (male): 62.5% GCS score 15: 91.7% GCS score 14: 5.2% GCS score 13: 3.1% Ethnicity: 100% white Setting: emergency department of the University of Rochester School of Medicine Country: USA Inclusion criteria for this study: consent for blood draw for analysis in the ED; head CT scan performed in ED as part of clinical care; completed 3-month follow up Inclusion criteria for the larger NIH study met case definition for mild TBI (blow to the head or acceleration/deceleration movement of the head resulting in one or more of the following: loss of consciousness <30 minutes; amnesia <24 hours or any alteration in mental state at the time of injury); GCS score >13 measured ≥30 minutes after injury

Reference	**Bazarian, 2006** [4]
	Exclusion criteria: presenting >4 hours after injury; pre-existing medical or psychiatric conditions known to be associated with elevated S100B (Alzheimer's, Down's syndrome, Schizophrenia); those who had run >10 miles in the last 12 hours
Target condition(s)	Acute post-brain injury complications
Index test(s) and reference standard	Index test: Serum S100B measured within 4 hours of injury Serum S100B measured within 4 hours of injury, correct for creatine kinase Reference standard: Initial head CT Follow up: assessment of post-concussive symptoms using the Rivermead Post Concussion Questionnaire via telephone at 3 months after ED visit
Results	Outcome: traumatic abnormality on initial CT scan Traumatic abnormality on initial CT scan – Serum S100B within 4 hours Specificity%: 26 (when sensitivity set at 70%) PPV%: not reported NPV% (95% CI): 75 (2.6-67) AUC (95% CI): 0.49 (CI not reported) Traumatic abnormality on initial CT scan – Serum S100B within 4 hours, corrected for creatine kinase Specificity%: 42 (when sensitivity set at 70%) PPV%: not reported

Reference	**Bazarian, 2006** [4]
	NPV% (95% CI): 96 (83.5-99.8) AUC (95% CI): 0.54 (CI not reported)
Source of funding	National Institutes of Health grant
Limitations	Risk of bias *(QUADAS 2 – risk of bias)*: very serious. Unclear whether the results of the index test were interpreted without knowledge of the results of the reference standard and vice versa; unclear sequencing and time interval between index test and reference standard Indirectness *(QUADAS 2 – applicability)*: none
Comments	-

Reference	**ALERT-TBI Bazarian, 2018 ; Bazarian, 2021** [5 7]
Study type	Prospective cohort
Study methodology	Data source: prospective study carried out at 22 sites from December 2012 to March 2014. Patients presenting to participating emergency departments (ED) with suspected non-penetrating TBI resulting from an external force were recruited. Secondary analysis using only the mild TBI subjects (n=1901). Index test was a rapid version of the UCH-L1 and GFAP combined test.

Reference	**ALERT-TBI Bazarian, 2018 ; Bazarian, 2021** [5 7]
Number of patients	n = 1959 (results reported separately for n=1920 with GCS score 14-15)
Patient characteristics	Age, mean (SD): 48.9 (20.9) years, range 18-98 years Gender (male): 57% GCS score 15: 93% GCS score 14: 5% GCS score 13: 1% GCS score 12: 1% GCS score 11: <1% GCS score 10: 0% GCS score 11: <1% Ethnicity: white 70%; black or African American 27%; Hispanic 5%; other/unknown 4% Setting: ED at 22 sites Country: USA (15) and Europe (7) Inclusion criteria: ≥18 years of age; presenting to ED with suspected non-penetrating TBI resulting from external force; GCS 9-15; underwent a non-contrast head CT scan within 12 hours of injury; blood sampling within 12 hours of injury; informed consent Exclusion criteria: time of injury could not be determined; head CT scan not performed; venepuncture not feasible; informed consent not obtainable
Target condition(s)	Acute post-brain injury complications
Index test(s) and reference standard	<u>Index test:</u> Combined serum UCH-L1 and GFAP measured within 12 hours post injury (cut-off concentration values of 327 pg/mL for UCH-L1 and 22 pg/mL for GFAP)

Reference	**ALERT-TBI Bazarian, 2018 ; Bazarian, 2021** [5 7]
	Reference standard: CT scan within 12 hours post injury. Follow up: no mention of follow up
Results	Outcome: Positive CT scan (presence of one or more of the following injuries: acute epidural haematoma, acute subdural haematoma, indeterminate extra-axial haemorrhage, intraventricular haemorrhage, parenchymal haematoma, petechial haemorrhagic or bland sheer injury, subarachnoid haemorrhage, brain oedema, brain herniation, non-haemorrhagic contusion, ventricular compression, ventricular trapping, cranial fractures, depressed skull fractures, facial fractures, scalp injury, or skull base fractures). 113 participants with GCS score 14–15 TBI had a traumatic intracranial injury on head CT. without intra cranial injury in GCS score 14–15 n=1,807 Positive CT scan – Combined serum UCH-L1 and GFAP measured within 12 hours post injury (cut-off concentration values of 327 pg/mL for UCH-L1 and 22 pg/mL for GFAP) n=1920 with GC score S 14-15: Sensitivity%: 97.3 (95% CI 92.4-99.4) Specificity%: 36.7 (95% CI 34.5-39) PPV%: 8.8 (95% CI 7.3-10) NPV%: 99.5 (95% CI 98.7-99.9) Back calculation of 2x2 table done by NGC: TP: 110

Reference	**ALERT-TBI Bazarian, 2018 ; Bazarian, 2021** [5 7]
	FP: 1144 FN: 3 TN: 663
Source of funding	Sponsored by Banyan Biomarkers (involved in the design of the study)
Limitations	Risk of bias *(QUADAS 2 – risk of bias)*: none Indirectness *(QUADAS 2 – applicability)*: none
Comments	-

Reference	**Bazarian, 2013** [6]**; Jones 2020** [30]
Study type	Prospective cohort
Study methodology	Data source: prospective study carried out at 6 emergency departments (ED) from 2008 to 2010. Patients presenting to participating EDs with mild TBI were recruited. Patients presenting to a single centre for routine blood work were enrolled as control subjects.
Number of patients	n = 787 with mild TBI, n = 467 controls (only mild TBI case data extracted for this review)
Patient characteristics	Age, mean (SD): 38.2 (19.5) Gender (male): 63.5% GCS score 15: 89.2% GCS 14 score: 6.5% GCS score 13: 1.3% GCS score “13-15”: 2.5%

Reference	**Bazarian, 2013 [6]; Jones 2020 [30]**
	Ethnicity: Hispanic/Latino 4.4%; not Hispanic/Latino 92.2%; refused/missing 3.3% Setting: 6 EDs Country: USA Inclusion criteria: ≥1 year of age; met study definition of mild TBI (blow to the head or rapid acceleration/deceleration resulting in at least one of the following: a loss of consciousness (LOC) ≤30 minutes, posttraumatic amnesia ≤24 h, neuropsychological abnormality (any transient period of confusion, disorientation, or impaired consciousness; in children ≤2 years old: irritability, lethargy, or vomiting post-injury), or neurological abnormality (seizure acutely after injury, hemiplegia, or diplopia)); GCS score ≥13 within 30 minutes of injury; informed consent Exclusion criteria: history of brain tumour, melanoma, Alzheimer's disease, bone fracture, stroke/surgery within the previous month
Target condition(s)	Acute post-brain injury complications
Index test(s) and reference standard	Index test: Serum S100B within 6 hours post injury (cut-off >0.29 µg/L) Serum S100B within 6 hours post injury (cut-off >0.06 µg/L) Serum S100B within 6 hours post injury (cut-off >2.391 µg/L) Serum S100B within 6 hours post injury (cut-off >0.097 µg/L) Serum S100B within 6 hours post injury (cut-off >0.521 µg/L) Serum S100B within 6 hours post injury (cut-off >0.1 µg/L) Reference standard: CT scan. Follow up: no mention of follow up

Reference	**Bazarian, 2013 [6]; Jones 2020 [30]**
Results	Outcome: presence of intracranial abnormalities. Traumatic CT abnormalities defined as subdural hematomas (SDH), epidural hematomas, subarachnoid haemorrhage (SAH), oedema, skull fracture, and cerebral contusions. N=737 with normal CT, n=45 abnormal CT, n=5 missing Presence of intracranial abnormalities – Serum S100B within 6 hours post injury AUC: 0.694 (0.62-0.77) Presence of intracranial abnormalities – Serum S100B within 6 hours post injury (cut-off >0.29 µg/L) Sensitivity%: 51.1 (95% CI 35-66) Specificity%: 76.3 (95% CI 73-79) Back calculation of 2x2 table done by NGC (for Serum S100B within 6 hours post injury (cut-off >0.29 µg/L) TP: 26 FP: 177 FN: 24 TN: 560 Presence of intracranial abnormalities – Serum S100B within 6 hours post injury (cut-off >0.06 µg/L) Sensitivity%: 100 (95% CI 92-100) Specificity%: 12.3 (95% CI 10-15)

Reference	Bazarian, 2013 [6]; Jones 2020 [30]
	Back calculation of 2x2 table done by NGC (for Serum S100B within 6 hours post injury (cut-off >0.06 µg/L) TP: 50 FP: 649 FN: 0 TN: 88 Presence of intracranial abnormalities – Serum S100B within 6 hours post injury (cut-off >2.391 µg/L) Sensitivity%: 4.4 (95% CI 0.5-15) Specificity%: 99.1 (95% CI 98-99.6) Back calculation of 2x2 table done by NGC (for Serum S100B within 6 hours post injury (cut-off >2.391 µg/L) TP: 2 FP: 7 FN: 48 TN: 730 Presence of intracranial abnormalities – Serum S100B within 6 hours post injury (cut-off >0.097 µg/L) Sensitivity%: 88.9 (95% CI 76-96) Specificity%: 31.7 (95% CI 28-35)

Reference	Bazarian, 2013 [6]; Jones 2020 [30]
	Back calculation of 2x2 table done by NGC (for Serum S100B within 6 hours post injury (cut-off >0.097 µg/L) TP: 45 FP: 501 FN: 5 TN: 236 Presence of intracranial abnormalities – Serum S100B within 6 hours post injury (cut-off >0.521 µg/L) Sensitivity%: 24.4 (95% CI 13-40) Specificity%: 90.2 (95% CI 88-92) Back calculation of 2x2 table done by NGC (for Serum S100B within 6 hours post injury (cut-off >0.521 µg/L) TP: 12 FP: 74 FN: 38 TN: 663 Presence of intracranial abnormalities – Serum S100B within 6 hours post injury (cut-off >0.1 µg/L) Sensitivity%: 86.7 (95% CI 73-95) Specificity%: 35.8 (95% CI 32-39)

Reference	**Bazarian, 2013 [6]; Jones 2020 [30]**
	Back calculation of 2x2 table done by NGC (for Serum S100B within 6 hours post injury (cut-off >0.1 µg/L): TP: 39 FP: 476 FN: 6 TN: 266
Source of funding	Supported by funds from the New York State Department of Health, the Academic Health Center Consortium, and the Emergency Research Network of the Empire State (ERNIES)
Limitations	Risk of bias *(QUADAS 2 – risk of bias)*: serious. Unclear whether index test results were interpreted without knowledge of the reference standard Indirectness *(QUADAS 2 – applicability)*: serious. Included adults and children. Reported mean (SD) age suggests majority were adults
Comments	No information on treatment

Reference	**Biberthaler, 2002 [10]**
Study type	Prospective cohort
Study methodology	Data source: prospective study carried out at a single level 1 University trauma center over 18 months. Patients presenting to the emergency room of the Department of Surgery and Traumatology with a history of isolated minor head trauma (MHT) were recruited.

Reference	**Biberthaler, 2002** [10]
Number of patients	n = 104
Patient characteristics	Age, mean (SD): not reported Gender (male): not reported GCS: no breakdown but GCS score 13-15 for inclusion in the study Ethnicity: not reported Setting: single ED Country: Germany Inclusion criteria: presented to ED with a history of isolated MHT; GCS score 13-15 at admission; at least one of the following symptoms: transient loss of consciousness (LOC) <5 minutes, amnesia for the traumatic event, nausea, vomiting, vertigo and severe headache; interval below 2 hours between traumatic event and blood sampling Exclusion criteria: not reported
Target condition(s)	Acute post-brain injury complications
Index test(s) and reference standard	Index test: Serum S100B (LIA-mat® and LIAISON®) Plasma S100B (LIA-mat® and LIAISON®) Reference standard: Cranial CT scan. Follow up: no mention of follow up

Reference	Biberthaler, 2002 [10]
Results	Outcome: positive CCT scan (diffuse injury I-IV, evacuated mass lesion and non-evacuated mass lesion) N=80 CCT negative N=24 CCT positive <u>Positive CCT scan – Serum S100B within 2 hours post injury (cut-off 0.12ng/ml) (LIA-mat®)</u> Sensitivity%: 100 (95% CI not reported) Specificity%: 46 (95% CI not reported) AUC (95% CI): 0.77 (95% CI 0.68-0.87) <u>Back calculation of 2x2 table by NGC:</u> TP: 24 FP: 43 FN: 0 TN: 37 <u>Positive CCT scan – Serum S100B within 2 hours post injury (cut-off 0.12ng/ml) (LIAISON®)</u> Sensitivity%: 100 (95% CI not reported) Specificity%: 46 (95% CI not reported)

Reference	Biberthaler, 2002 [10]
	AUC (95% CI): 0.79 (95% CI 0.7-0.89) Back calculation of 2x2 table by NGC: TP: 24 FP: 43 FN: 0 TN: 37 Positive CCT scan – Plasma S100B within 2 hours post injury (cut-off 0.15ng/ml) (LIA-mat®) Sensitivity%: 100 (95% CI not reported) Specificity%: 46 (95% CI not reported) AUC (95% CI): 0.77 (95% CI 0.68-0.87) Back calculation of 2x2 table by NGC: TP: 24 FP: 43 FN: 0 TN: 37

Reference	**Biberthaler, 2002** [10]
	<u>Positive CCT scan – Plasma S100B within 2 hours post injury (cut-off 0.12ng/ml) (LIAISON®)</u> Sensitivity%: 100 (95% CI not reported) Specificity%: 46 (95% CI not reported) AUC (95% CI): 0.76 (95% CI 0.67-0.85) <u>Back calculation of 2x2 table by NGC:</u> TP: 24 FP: 43 FN: 0 TN: 37
Source of funding	Supported in part by the Deutsche Forschungs-Gemeinschaft, Sonderforschungsbereich 469 of the Ludwig-Maximilians-University Munich
Limitations	Risk of bias *(QUADAS 2 – risk of bias)*: very serious. Unclear whether selection of patients could have introduced bias; unclear whether index test results were interpreted without knowledge of the reference standard and vice versa Indirectness *(QUADAS 2 – applicability)*: serious. Age not reported
Comments	Results reported for two different test systems

Reference	**Biberthaler, 2006** [8]
Study type	Prospective multi centre cohort study
Study methodology	Data source: prospective study carried out at 3 level 1 trauma centers from June 2002 to October 2003. Consecutive patients presenting with minor head injury (MHI) were recruited, as well as a negative control group (healthy individuals) and a positive control group (moderate to severe head injury).
Number of patients	n = 1309 with minor head injury [study also included n = 504 healthy individuals and n = 55 with moderate and severe TBI- data has not been extracted for these groups as they are not relevant to our review population]
Patient characteristics	Age, median (interquartile range): 47 (32-65) years Gender (male): 65% GCS: no breakdown but GCS score 13-15 for inclusion in the study Ethnicity: not reported Setting: 3 trauma centers Country: Germany Inclusion criteria: history of isolated head trauma and admission within 3 hours; GCS score of 13 to 15 upon admission; one or more of 10 clinical risk factors (brief loss of consciousness, post-traumatic amnesia, nausea, vomiting, severe headache, dizziness, vertigo, intoxication, anticoagulation, age above 60 years) Exclusion criteria: <18 years of age; pregnant women; prisoners; multiple injured patients
Target condition(s)	Acute post-brain injury complications
Index test(s) and reference standard	Index test: Serum S100B The median interval between trauma and blood sampling was 60 min (range, 40-80 or 25%-75%) Reference standard:

Reference	Biberthaler, 2006 [8]
	Cranial CT (CCT) Median interval between blood sampling and CCT scan was 30 min (range, 16-52 or 25%-75%). Patients classified as : CCT-negative (CCT^-; MHI patients without any signs of trauma-relevant intracerebral lesions) and CCT-positive (patients with at least one of the pathophysiological trauma-relevant findings (hemorrhage, epidural, subdural, sub arachnoidal, intracerebral, ventricular, cerebellar, brainstem, cortex contusion, haemorrhagic, non-hemorrhagic))
Results	Outcome: intracerebral lesions on CT Of these n=1309 MHI patients, 1216 (93%) were proved to be CCT^- and 93 (7%) were proved to be CCT^+ on the initial CCT scan. Of the latter group, 11 individuals required immediate neurosurgical intervention such as implantation of an intraventricular catheter for drainage of cerebrospinal fluid or decompressive craniotomy. <u>S100B (cut-off value of 0.10 µg/L) – median time was 60 min:</u> Sensitivity: 99% (95% CI, 96%-100%) Specificity: 30% (95% CI, 29%-31%) AUC: 0.80 (95% CI, 0.75-0.84) <u>Back calculation of 2x2 table done by NGC</u> TP: 92 FP: 815 FN: 1

Reference	**Biberthaler, 2006** [8]
	TN: 401
Source of funding	Not reported
Limitations	Risk of bias *(QUADAS 2 – risk of bias)*: very serious. Unclear whether selection of patients could have introduced bias; unclear whether index test results were interpreted without knowledge of the reference standard and vice versa Indirectness *(QUADAS 2 – applicability)*: none
Comments	Treatment: 11 individuals required immediate neurosurgical intervention such as implantation of an intraventricular catheter for drainage of cerebrospinal fluid or decompressive craniotomy.

Reference	**Cervellin, 2012** [14]
Study type	Prospective cohort
Study methodology	Data source: prospective study carried out at a single hospital between January and May 2011. Consecutive patients presenting to the emergency department (ED) with a history of minor head injury (MHI) were recruited.
Number of patients	n = 60
Patient characteristics	Age, mean (range): 58 (14-80) years Gender (male): 68% GCS: no breakdown reported but GCS score 14-15 for inclusion in the study Ethnicity: not reported Setting: EDs of a single hospital

Reference	Cervellin, 2012 [14]
	Country: Italy Inclusion criteria: 14-80 years of age; presenting at the ED with a history of MHI requiring CT scanning according to local guideline (criteria included GCS score 14-15) Exclusion criteria: suspected/visible brain tumour
Target condition(s)	Acute post-brain injury complications
Index test(s) and reference standard	Index test: Serum S100B measured within 3 hours post injury Reference standard: CT scan performed 30 minutes from blood collection. Follow up: no mention of follow up
Results	Outcome: positive CT scan (any intracranial pathology associated with an injury (acute subdural, epidural or parenchymal hematoma, traumatic subarachnoid haemorrhage, cerebral contusion and brain swelling)) N=20 CT+ N=40 CT - Positive CT scan – Serum S100B within 3 hours post injury (cut-off 0.38 µg/L) Sensitivity%: 100 (95% CI not reported) Specificity%: 58 (95% CI not reported) PPV%: 54 (95% CI not reported) NPV%: 100 (95% CI not reported)

Reference	Cervellin, 2012 [14]
	AUC: 0.8 (p<0.001) <u>Back calculation of 2x2 table by NGC:</u> TP: 20 FP: 17 FN: 0 TN: 23 <u>Positive CT scan – Serum S100B within 3 hours post injury (cut-off 2.31 µg/L)</u> Sensitivity%: 15 (95% CI not reported) Specificity%: 100 (95% CI not reported) PPV%: 100 (95% CI not reported) NPV%: 70 (95% CI not reported) <u>Back calculation of 2x2 table by NGC:</u> TP: 3 FP: 0

Reference	**Cervellin, 2012** [14]
	FN: 17 TN: 40
Source of funding	Not reported
Limitations	Risk of bias *(QUADAS 2 – risk of bias)*: none Indirectness *(QUADAS 2 – applicability)*: serious. Study included children and adults; mean age suggests majority were adults
Comments	-

Reference	**Cevik, 2019** [15]
Study type	Cross-sectional study
Study methodology	Data source: patients who were treated in the Emergency Department of hospital for mild TBI between February 2016 and September 2016
Number of patients	n = 48
Patient characteristics	Mixed adults and children. Mean age (Adults). Not reported proportion of adults and children. Age, mean (SD): 24 ± 22 (range, 5–65) years Gender: 48 patients [38 (79%) males and 10 (21%) females] with “pure” mild TBI GCS:

Reference	**Cevik, 2019** [15]
	The Glasgow coma score (GCS) score for all patients was 14–15 and all presented with one or more symptoms of post-traumatic amnesia which is accepted as the presence of any elapsed time between the return of continuous memory and the accident, nausea/vomiting, post-traumatic seizure, persistent headache, and transient loss of consciousness at the time of referral to emergency service, but no injury to other tissues or organs (solid organ injury, bone fracture). GCS score -15: 39 (81.25%) GCS score -14: 9(18.75%) Ethnicity: not reported Setting: ED Country: Turkey Inclusion criteria: People with mild TBI 24 patients with intracranial traumatic pathology (CT+) were included as the pathological group and 24 age-matched patients without intracranial traumatic pathology (CT-). Exclusion criteria: GCS score at hospital admission of < 14, pregnancy or possibility of pregnancy, renal failure, multiple trauma, admission to hospital > 4 h after trauma, and previous concurrent nervous system disorders.
Target condition(s)	Acute post-brain injury complications
Index test(s) and reference standard	Index test: Serum biomarkers: - S100 beta (S100B) - glial fibrillary acidic protein (GFAP) - small neuronal protein neurogranin (NRGN) Venous blood samples were collected within the first 4 h following the trauma Reference standard: CT

Reference	Cevik, 2019 [15]
	Follow up: Venous blood samples were collected within the first 4 h following the trauma and 16-slice CT scans of the head without contrast enhancement were obtained (Somatom Emotion; Siemens AG, Erlangen, Germany).
Results	Outcome: abnormal cerebral CT findings The primary end point of the study was to investigate the relationship between the levels of biomarkers such as S100B, GFAP and NRGN in patients with mild head injury in the first 4 h after the trauma with abnormal traumatic CT findings. Of the 48 patients with mild TBI, 24 were CT + and 24 were CT-. Regarding haemorrhage, 11 patients had epidural hematoma two of which was having accompanying pneumocephalus, one patient had intracerebral hematoma, two patients had subarachnoid hematoma, and three patients had subdural hematoma one of which was having accompanying subarachnoid haematoma. In addition, seven patients had cerebral contusions and 18 had cranial fractures with accompanying intracranial pathology. NRGN levels were significantly higher in patients who were CT+ (n = 24) than in those who were CT- (n = 24) (5.79 ± 4.14 vs. 2.95 ± 2.38 ng/mL, respectively, p = 0.001). Mean S100B levels were significantly higher in the 24 patients who were CT+ than the 24 patients who were CT- (1.72 ± 1.05 µg/L vs. 0.73 ± 0.64 µg/L, respectively, p < 0.001). The mean GFAP level was significantly higher in the 24 patients who were CT+ than in the 24 patients who were CT- (0.60 ± 0.38 vs.0.36 ± 0.25ng/mL, respectively, p = 0.026. S100 beta (S100B) - optimal cut-off value of 0.47 µg/L -within 4h Sensitivity: 95.8% (95% CI 78.9%–99.9%) specificity: 62.5% (95% CI 40.6%–81.2%) Positive predictive value (PPV): 71.9 % Negative predictive value (NPV): 93.7 % AUC: 0. 84 (95% CI 0.72- 0.95)

Reference	Cevik, 2019 [15]
	Back calculation of 2x2 table done by NGC TP: 23 FP: 9 FN: 1 TN: 15 glial fibrillary acidic protein (GFAP) - cut-off value of GFAP 0.23 ng/mL - within 4h Sensitivity: 75% [95% CI 53.3%–90.2%] Specificity: 62.5% (95% CI 40.6%–81.2%). Positive predictive value (PPV): 66.7%. Negative predictive value (NPV): 71.4 % AUC: 0.69 (95% CI 0.54- 0.84) Back calculation of 2x2 table done by NGC TP: 18 FP: 9 FN: 6 TN: 15 small neuronal protein neurogranin (NRGN)- optimal cut-off value for 1.87 ng/mL- within 4h Sensitivity: 83.3% (95%CI 62.6%–95.3%) Specificity: 58.3% (95% CI 36.6%–77.9%) PPV: 66.7% NPV: 77.8 % AUC: 0.77 (95% CI 0.63 -0.90) Back calculation of 2x2 table done by NGC TP: 20 FP: 10

Reference	**Cevik, 2019** [15]
	FN: 4 TN: 14 At NRGN concentration of > 1.63 ng/mL[N=48]- within 4h Sensitivity: 100% (no CI reported) Specificity: 17% (no CI reported) PPV: 55% (no CI reported) NPV: 100% (no CI reported) At NRGN concentration of > 1.95 ng/mL in the paediatric group (age, ≤ 16 years) [N=20]- within 4h Sensitivity: 100% (no CI reported) Specificity: 70% (no CI reported) PPV: 77% (no CI reported) NPV: 100% (no CI reported) ROC analysis of NRGN values showed that NRGN serum levels accurately discriminated between patients with pathologic versus normal findings on CT
Source of funding	No funding
Limitations	Risk of bias *(QUADAS 2 – risk of bias)*: very serious. Unclear whether the selection of patients could have introduced bias; unclear whether the index test was interpreted without knowledge of the reference standard and vice versa Indirectness *(QUADAS 2 – applicability)*: serious. Mixed population (adults and children).
Comments	No information on treatment

Reference	**Chen, 2022** [16]
Study type	Prospective cohort study USA
Study methodology	Data source: This prospective study involved patients admitted to Stanford Hospital's Emergency Department for suspicion of TBI between November 2015 and April 2017.

Reference	**Chen, 2022** [16]
Number of patients	n = 644 (mostly mild TBI) Out of 644 patients, 52 had a Glasgow Coma Scale (GCS) score <13 n=462 with blood samples (Analysed) No-OMEI (other major extra- cranial injuries) sub-cohort (n = 245)
Patient characteristics	Age, mean (SD) years: With blood sample:50.7±22.7 Gender (male): 286 (61.9%) GCS score: 14 (14-15) Ethnicity: Setting: ED Country: USA Inclusion criteria for this study: Adult patients (> 18 years old) transported by ambulance or helicopter, for whom a trauma alert was triggered and who underwent a non-contrast head CT seeking care for suspected TBI, were enrolled Exclusion criteria: NR
Target condition(s)	Acute post-brain injury complications
Index test(s) and reference standard	<u>Index test:</u> Distribution of time from injury to ED admission/ blood draw spanned 0–6 h with a median at 1 h -glial fibrillary acidic protein (GFAP) -ubiquitin C-terminal hydrolase-L1 (UCH-L1) -S100B

Reference	**Chen, 2022** [16]
	The pre-specified cut-off values of GFAP, UCH-L1, and S100B were 22 pg/mL3, 327 pg/mL3, and 105 pg/mL13, respectively. A negative test result referred to markers falling at or below their pre-specified cut-off value, whereas a positive test result indicated that markers exceeded their pre-specified cut-off value. Reference standard: Head CT
Results	Outcome: acute brain injuries In the full cohort, 440 patients had plasma samples of GFAP, and 442 patients had plasma samples of UCH-L1; 189 patients had serum samples of S100B Full cohort (n=440) Plasma GFAP: AUC 0.868 (N=119) Plasma UCH-L1 :AUC 0.624 (n=119) Serum 100B: AUC 0.607 (n=44) No OMEI (n=245) Plasma GFAP: AUC 0.873 (N=78) Plasma UCH-L1 :AUC 0.660 (n=78) Serum 100B: AUC 0.662 (n=30)
Source of funding	No funding
Limitations	Risk of bias *(QUADAS 2 – risk of bias)*: very serious. Unclear whether the results of the index test were interpreted without knowledge of the results of the reference standard and vice versa; unclear sequencing and time interval between index test and reference standard Indirectness *(QUADAS 2 – applicability)*: none
Comments	No information on treatment

Reference	**Czeiter, 2020 [17] CENTER-TBI**
Study type	Prospective cohort
Study methodology	Data source: prospective study conducted at 65 clinical sites between December 2014 and December 2017. Patients with all severities of TBI presenting to a study centre within 24 hours of injury and scheduled for CT scanning were recruited.
Number of patients	n = 2867
Patient characteristics	Age, median (interquartile range): 49 (30-66) years Gender (male): 67.9% GCS score 15: 52.1% GCS score 13-14: 15.9% GCS score 9-12: 7.7% GCS score 3-8: 21% Ethnicity: not reported Setting: 65 clinical sites, patients stratified by care path (emergency department, hospital admission and intensive care unit) Country: 17 European countries and Israel Inclusion criteria: all severities of TBI; presenting within 24 hours of injury and scheduled for CT scanning Exclusion criteria: severe pre-existing neurological disorder
Target condition(s)	Acute post-brain injury complications
Index test(s) and reference standard	<u>Index test:</u> Serum S100B measured within 24 hours post injury Serum neuron-specific enolase (NSE) measured within 24 hours post injury

Reference	Czeiter, 2020 [17] CENTER-TBI
	Serum GFAP measured within 24 hours post injury Serum UCH-L1 measured within 24 hours post injury Serum neurofilament protein-light (NFL) measured within 24 hours post injury Reference standard: Head CT scan Follow up: no mention of follow up
Results	Outcome: positive CT scan (presence of any traumatic intracranial abnormality; skull fractures in isolation were not considered as intracranial abnormality) Positive CT scan – Serum S100B within 24 hours post injury (mild TBI subgroup GCS score 13-15) AUC (95% CI): 0.68 (0.65-0.71) Positive CT scan – Serum NSE within 24 hours post injury (mild TBI subgroup GCS score 13-15) AUC (95% CI): 0.62 (0.6-0.65) Positive CT scan – Serum GFAP within 24 hours post injury (mild TBI subgroup GCS score 13-15) AUC (95% CI): 0.85 (0.83-0.87) Positive CT scan – Serum UCH-L1 within 24 hours post injury (mild TBI subgroup GCS score 13-15) AUC (95% CI): 0.76 (0.74-0.79)

Reference	**Czeiter, 2020 [17] CENTER-TBI**
	Positive CT scan – Serum NFL within 24 hours post injury (mild TBI subgroup GCS score 13-15) AUC (95% CI): 0.75 (0.72-0.77) Positive CT scan – all biomarkers combined within 24 hours post injury (mild TBI subgroup GCS score 13-15) AUC (95% CI): 0.85 (0.84-0.87) Positive CT scan – Serum GFAP + UCH-L1 within 24 hours post injury (mild TBI subgroup GCS score 13-15) AUC (95% CI): 0.85 (0.84-0.87) Positive CT scan – Serum GFAP + S100B within 24 hours post injury (mild TBI subgroup GCS score 13-15) AUC (95% CI): 0.85 (0.84-0.87) Positive CT scan – Serum GFAP + UCH-L1 + S100B within 24 hours post injury (mild TBI subgroup GCS score 13-15) AUC (95% CI): 0.85 (0.84-0.87)
Source of funding	Supported by the European Union 7th Framework program (EC grant 602150). Additional funding was obtained from the Hannelore Kohl Stiftung (Germany), from OneMind (USA), from Integra LifeSciences Corporation (USA) and from Neurotrauma Sciences (USA). The funders had no role in the study design, collection, analysis and interpretation of data, nor in the writing of the report or in publication decisions.
Limitations	Risk of bias *(QUADAS 2 – risk of bias)*: none Indirectness *(QUADAS 2 – applicability)*: none
Comments	Results extracted from supplementary tables 6 and 7

Reference	**David, 2017** [18]
Study type	Prospective cohort
Study methodology	Data source: prospective study conducted at a single teaching hospital between January 2014 and October 2015. Consecutive patients presenting to the adult emergency department (ED) with pre-injury antiplatelet/anticoagulant use and mild blunt head trauma were recruited.
Number of patients	n = 308
Patient characteristics	Age, mean (SD): 79.1 (10.5) years Gender (male): 49% GCS score 15: 97.4% GCS score 13-14: 2.6% Ethnicity: not reported All participants were on antithrombotic medication Setting: ED of a single teaching hospital Country: France Inclusion criteria: ≥18 years of age; pre-injury antiplatelet and/or anticoagulant use; mild blunt head trauma (any blunt head injury regardless of loss of consciousness or amnesia) Exclusion criteria: unable to give informed consent; known injuries transferred from outside facilities; history of coagulation disorder; cranial CT scan performed >24 hours after index trauma; serum sampling >6 hours post-injury; unknown time of trauma; missing informed consent
Target condition(s)	Acute post-brain injury complications

Reference	**David, 2017** [18]
Index test(s) and reference standard	Index test: Serum S100B measured within 6 hours post injury (cut-off 0.105 µg/L) Reference standard: Cranial CT scan performed within 24 hours post injury Follow up: no mention of follow up
Results	Outcome: positive CT scan (any trauma related intracranial haemorrhage, including epidural, subdural or subarachnoid haemorrhage, or intracerebral bleeding (petechial haemorrhage, contusion or hematoma)) Positive CT scan – Serum S100B within 6 hours post injury (cut-off 0.105 µg/L) TP: 28 FP: 192 TN: 83 FN: 5 Sensitivity% (95% CI): 84.8 (68.1-94.9) Specificity% (95% CI): 30.2 (24.8-36) PPV% (95% CI): 12.7 (8.6-17.9) NPV% (95% CI): 94.3 (87.2-98.1) SN/SP calculated by NGC: Sensitivity: 0.85 [0.68, 0.95]

Reference	**David, 2017** [18]
	Specificity: 0.30 [0.25, 0.36]
Source of funding	Not reported
Limitations	Risk of bias *(QUADAS 2 – risk of bias)*: serious. Unclear whether the results of the index test were interpreted without knowledge of the results of the reference standard and vice versa Indirectness *(QUADAS 2 – applicability)*: serious. All study participants were on antithrombotic medication – limited applicability to the wider review population.
Comments	Methods section states sensitivity, specificity, PPV and NPV of S100B blood level for the detection of ICH requiring a medical and/or surgical treatment were estimated in the study population, but this is not clearly reported in the results. Main results extracted for positive CT scan

Reference	**Dickens, 2018** [20]
Study type	Prospective cohort
Study methodology	Data source: The patients were recruited as part of the EU funded TBI care (Evidence-based Diagnostic and Treatment Planning Solution for Traumatic Brain Injuries) project.
Number of patients	n = 210 (discovery cohort- Turku, Finland = 144) and validation cohort- Cambridge, UK (n = 66)
Patient characteristics	Age, mean (SD): Discovery cohort Mild: 48.37 (20.18) Moderate:59.57 (17.32) Severe: 55.05 (15.25)

Reference	Dickens, 2018 [20]
	validation cohort- Mild: 36.75 (18.20) Moderate: 41.57 (20.49) Severe: 44.87 (17.71) Gender (male/female): Discovery cohort Mild: 74/34 Moderate: 8/6 Severe: 19/3 validation cohort Mild: 27/9 Moderate: 7/0 Severe: 17/6 GCS: Included all severities Discovery cohort: (N=108) Mean GCS score (mild):14.19 (N=14) Mean GCS score (moderate):9.77 (N=22) Mean GCS score (severe):4.44 Validation cohort: (N=36) Mean GCS score (mild): 14.54 (N=7) Mean GCS score (moderate): 10.44 (N=23) Mean GCS score (severe): 5.68

Reference	**Dickens, 2018** [20]
	Ethnicity: not reported Setting: trauma centres Country: UK, Finland Inclusion criteria: Patients were included if they were older than 18 years (16 in the UK) and had a clinical diagnosis of TBI and indications on a head CT according to the National Institute for Health and Care Excellence (NICE) criteria. Exclusion criteria: Patients were excluded if the injuries were blast-induced or penetrating injury, chronic subdural hematoma, pre-existing brain injuries or conditions, which caused non-independent living, TBI or suspected TBI two weeks prior to recruitment, non-native speaker, and no if no consent was obtained
Target condition(s)	Acute post-brain injury complications
Index test(s) and reference standard	<u>Index test:</u> • Serum GFAP • Serum UCH-L1 The blood samples were collected within 12 h of admission to hospital. There were some patients who were found unconscious and transferred to hospital and patients who sustained mTBI and sought for medical attention with latency. In these patients, the exact time of injury is unknown. <u>Reference standard:</u> CT The CT scans were analysed by neuroradiologists and double-read by a senior neurosurgeon (JPP) and a neurologist (OT). Marshall classification was chosen, because it can be appropriately used for the patient group division and to address the clinical questions. Classification: Marshall Grade 1 (CT negative) vs. Marshall Grade 2-6 (CT positive)

Reference	**Dickens, 2018** [20]
Results	Outcome: traumatic intracranial findings (CT positive) Predict abnormalities on a CT scan (Marshall Grade > 1) Turku data (discovery cohort, Finland) GFAP AUC = 0.73; 95% CI: 0.64-0.83 UCH-L1 AUC = 0.71; 95% CI: 0.62-0.85) a combination of both AUC = 0.73; 95% CI: 0.62-0.86),). Cambridge data (validation cohort, UK). GFAP AUC = 0.64; 95% CI: 0.64-0.64) UCH-L1

Reference	**Dickens, 2018** [20]
	AUC = 0.58; 95% CI: 0.58-0.58 <u>combination model</u> AUC = 0.64; 95% CI: 0.63-0.64) all giving poorer predictive accuracy in validation cohort
Source of funding	The work was supported by the EU FP7 project TBI care (Project ref. 270259 to MO DM and OT), by the GE-NFL Head Health Challenge I Award (grant no. 7620 to MO and TH), by Goverment's Special Financial Transfer tied to academic research in Health Sciences (Finland) (to JPP and RSKT.), and by personal grant from Emil Aaltonen Foundation and Finnish Brain Foundation (to JPP). Support for P.J.H. – National Institute for Health Research (NIHR) Research Professorship; NIHR Biomedical Research Centre (NIHR BRC) Cambridge. Support for K.L.H.C. – NIHR BRC Cambridge. This work was supported by the NIHR Biomedical Research Centre in Cambridge. VFJN is supported by an Academy of Medical Sciences / The Health Foundation Fellowship.
Limitations	Risk of bias *(QUADAS 2 – risk of bias)*: none Indirectness *(QUADAS 2 – applicability)*: serious. Mixed severity TBI based on GCS (mild, moderate and severe)
Comments	All patients received treatment based on local standards and current international guidelines and recommendations

Reference	**Diaz-Arrastia, 2014** [19]
Study type	Multicentre prospective cohort study (Transforming Research and Clinical Knowledge in Traumatic Brain Injury [TRACK-TBI]).
Study methodology	Data source: Subjects were identified and recruited upon arrival at one of three level 1 trauma centres as part of the multicentre prospective TRACK-TBI study

Reference	**Diaz-Arrastia, 2014** [19]
Number of patients	n = 206
Patient characteristics	Age, mean (SD): 42 (18) years, Gender (male): 73% GCS: majority of subjects (83%) were classified as having had mild TBI (admission GCS score 13–15), 4% as having had a moderate TBI (GCS score 9–12), and 13% as having had a severe TBI (GCS score 3–8). Ethnicity: not reported Setting: trauma centre Country: International Inclusion criteria: patients had to present within 24 h of injury with a history of trauma to the head sufficient to triage to non-contrast head CT using the American College of Emergency Physicians/Centres for Disease Control (ACEP/CDC) evidence-based joint practice guideline.22 All levels of GCS scores were eligible. Exclusion criteria: not reported
Target condition(s)	Acute post-brain injury complications
Index test(s) and reference standard	<u>Index test:</u> - Serum Ubiquitin C-terminal hydrolase L1 (UCH-L1) - Serum glial fibrillary acidic protein (GFAP) Upper limits of normal were defined as mean + 3 standard deviations. For UCH-L1 mean (SD) was 0.073 (0.057) ng/mL, and for GFAP mean (SD) was 0.038 (0.059 ng/mL). Therefore, the upper limits of normal for UCH-L1 and GFAP were 0.244 and 0.215 ng/mL, respectively. Blood samples were collected from subjects who consented to genetic and proteomic analysis within 24 h of injury. All samples were date and time stamped to compare with time of injury.

Reference	**Diaz-Arrastia, 2014** [19]
	Reference standard: CT All patients underwent CT imaging of the brain at the time of initial presentation to the ED. Each patient's head CT was characterized using the recommendations of the TBI-CDE Neuroimaging Working Group. Each CT was de-identified, electronically uploaded to a central imaging database, and reviewed by a blinded central reader who was a board certified neuroradiologist. Follow up: 6 months after injury
Results	Outcome: Intracranial pathology on CT CT scans demonstrated intracranial pathology in 43% of those with mild TBI, in 78% of those with moderate TBI, and in 96% of those with severe TBI. Ubiquitin C-terminal hydrolase L1 (UCH-L1) AUC: 0.71 (95% CI 0.64–0.78) glial fibrillary acidic protein (GFAP) AUC: 0.88 (95% CI 0.84–0.93) Combined UCH-L1 and GFAP AUC: 0.88 (95% CI 0.83–0.93)
Source of funding	This study was funded by National Institutes of Health (NIH) Grant 1RC2 NS069409
Limitations	Risk of bias *(QUADAS 2 – risk of bias)*: none Indirectness *(QUADAS 2 – applicability)*: serious. Included mixed severity

Reference	**Diaz-Arrastia, 2014** [19]
Comments	-

Reference	**Egea-Guerrero, 2012** [21]
Study type	Prospective cohort
Study methodology	Data source: One hundred and forty-three post-TBI patients without a decrease in consciousness (GCS score =15) and with at least one neurological symptom (e.g. transitory loss of consciousness, amnesia, headache, dizziness or vomiting) were prospectively included.
Number of patients	Includes people over 14 years. Not specified proportion of adults/children. Mean age suggests adults. n = 143
Patient characteristics	Age, mean (SD): 49 (20.6) years Gender: Eighty-nine patients were male (62.20%) and 54 females (37.80%). GCS score: 15: 143 (100%) Twelve patients were under hypocoagulation therapy at the time of injury. All patients had normal levels of consciousness (GCS score = 15) at hospital admission and at least one neurological symptom after TBI Ethnicity: not reported Setting: hospital

Reference	**Egea-Guerrero, 2012** [21]
	Country: Spain Inclusion criteria: aged 14 or over, GCS score = 15 at hospital admission and one or more of the following symptoms: (1) transitory loss of consciousness; (2) amnesia; (3) persistent headache; (4) nausea or vomiting; and (5) vertigo Exclusion criteria: under 14 years of age, pregnancy or possibility of pregnancy, previous history of drug/alcohol abuse, renal failure, GCS score below 15 at hospital admission, drug interference in GCS evaluation, hospital admission after 6 hours post-trauma, history of syncope or seizure before head trauma, other previous concurrent nervous system disorders, absence of post-trauma head CT scan, hospital discharge before the first 24 hours post-TBI and ICU admission/transfer due to associated severe extracranial lesions.
Target condition(s)	Acute post-brain injury complications
Index test(s) and reference standard	Index test: Serum S100B A blood sample was drawn at 6-hours post-TBI Reference standard: CT In this study, IL included cerebral contusion, traumatic subarachnoid haemorrhage, epidural haematoma, and subdural haematoma. A venous blood sample was taken during the first 6 hours post-trauma for posterior analysis of S100B serum level. A CT scan to identify IL was performed within 24 hours of the accident (never prior to 1-hour post-trauma) [9-12]. Neuroradiological findings were reviewed and classified by a neuroradiologist blind to study goals and data.
Results	Outcome: intracranial lesion (IL) on CT The mean S100B value in this series was 0.392 µg L-1, with 95% Confidence Interval (CI) at 0.327-0.456 µg L-1. A total of 15 patients (10.5%) showed IL.

Reference	Egea-Guerrero, 2012 [21]

Patients with IL detected by CT scan had significantly higher S100B protein levels than those without IL (p= 0.007). The mean S100B value in serum from patients without IL was 0.369 µg with 95% CI at 0.302-0.436141.-1. Patients with pathological CT findings had an S100B mean value of 0.585 µgL-1, with 95% CI at 0.363-0.806 µg L-1.

No patient suffered neurological deterioration, and none required emergency neurosurgery

Different cut-off values for S 100B for detecting IL 6 hours post-TBI

Value (µg L-1)	Sensitivity	Specificity	PPV	NPV
0.105	100	26.56	13.76	100
0.130	100	32.81	14.85	100
0.230	93.33	51.56	18.42	98.51
0.254	86.66	57.03	19.11	97.33

Raw data:

S100 B (cut-off 0.105 µg L-1) 6h post-TBI
TP: 15
FP: 94
FN: 0
TN: 34

SN/SP ccalculated by NGC:
Sensitivity: 1.00 [0.78, 1.00]
Specificity: 0.27 [0.19, 0.35]

S100 B (cut-off 0.130 µg L-1) 6h post-TBI
TP: 15
FP: 86

Reference	Egea-Guerrero, 2012 [21]

FN: 0
TN: 42

SN/SP calculated by NGC:
Sensitivity: 1.00 [0.78, 1.00]
Specificity: 0.33 [0.25, 0.42]

S100 B (cut-off 0.230 µg L-1) at 6h post-TBI
TP: 14
FP: 62
FN:1
TN: 66

SN/SP calculated by NGC:
Sensitivity: 0.93 [0.68, 1.00]
Specificity: 0.52 [0.43, 0.60]

S100 B (cut-off 0.254 µg L-1) at 6h post-TBI
TP: 13
FP: 55
FN: 2
TN: 73

SN/SP calculated by NGC:
Sensitivity: 0.87 [0.60, 0.98]
Specificity: 0.57 [0.48, 0.66]

Different cut-off values for S 100B detecting IL 3-hours post- TBI

Value (µg L-1)	Sensitivity	Specificity	PPV	NPV

Reference	**Egea-Guerrero, 2012** [21]
	0.105 | 100 | 25 | 15.29 | 100 0.130 | 100 | 32.29 | 16.67 | 100 0.230 | 92.31 | 51.04 | 20.34 | 98 0.254 | 84.62 | 57.29 | 21.15 | 96.49 No raw data available No significant differences were found between AUCs for the 6-hour and 3-hour screening intervals.
Source of funding	Donation of Protein S100B Electrochemiluminescence Assay Kits from Roche Diagnostics, Mannheim, Germany
Limitations	Risk of bias *(QUADAS 2 – risk of bias)*: none Indirectness *(QUADAS 2 – applicability)*: serious. Mixed population of adults and children.
Comments	No information on treatment for patients.

Reference	**Egea-Guerrero, 2018** [22]
Study type	Prospective cohort study
Study methodology	Data source: Two centres contributed to this study, the Virgen del Rocio University Hospital in Seville and the Virgen de las Nieves University Hospital in Granada. Subjects with a Glasgow Coma Scale (GCS) score of 15 and at least one neurological symptom post-trauma was selected.
Number of patients	n = 260
Patient characteristics	Mixed population includes above 14 years. Not reported proportion of adults and children. Age, mean (SD): Age > 65 years, n (%)- 50 (19.2%) Gender, male, n (%): 166 (63.8)

Reference	**Egea-Guerrero, 2018** [22]
	GCS: GCS score =15 (mild TBI) Symptom findings, n (%) Amnesia: 143 (55.0) Loss of consciousness: 190 (73.1) Nausea: 69 (26.5) Vomits: 40 (15.4) Headache: 145 (55.8) Isolated TBI, n (%): 171 (65.8) S100B (pg/L), median (IQR): 0.18 (0.09-037) Ethnicity: not reported Setting: hospital trauma centre Country: Spain Inclusion criteria: age ≥ 14; GCS score = 15 at hospital admission and at least one of the following symptoms/findings: transitory loss of consciousness, amnesia, persistent headache, nausea or vomiting (17); extraction of serum sample within 6 h post-trauma (2) and CT scan within 24 h post-TBI (never prior to 1 h post-accident) Exclusion criteria: anticoagulated or anti-aggregated treatment, pregnancy or possibility of pregnancy, previous history of drug/alcohol abuse, renal failure, drug interference in the evaluation of GCS, history of syncope or seizure before head trauma, previous cerebrovascular accident or TBI, epilepsy, schizophrenia, depression or degenerative disease and multiple trauma with an Abbreviated Injury Score ≥ 3 in organs excluding the brain
Target condition(s)	Acute post-brain injury complications
Index test(s) and reference standard	Index test: Serum S100B A 5-mL sample of blood was drawn from each patient. Once collected, samples were centrifuged at 1800g for 10 min. The sera were separated and frozen in aliquots at —80°C until batch evaluation.

Reference	Egea-Guerrero, 2018 [22]
	Venous blood samples for S100B were collected approximately 3 h and 25 min post-TBI (IQR: 2.0-4.3). Reference standard: CT CT scan within 24 h post-TBI (never prior to 1 h post-accident)
Results	Outcome: presence of intracranial lesion (IL) on CT For the analysis of the clinical variables of mild TBI, patients were divided according to findings in the CT scan indicating presence or no presence of IL. N=22. Twenty-two patients (8.5%) presented ILs in CT scans, including epidural hemorrhage (18.2% of ILs), subdural haemorrhage (54.5%), subarachnoid haemorrhage (27.3%) and contusion (50.0%). None of these patients underwent emergency neurosurgery. N=238 normal CT S100 B - cut-off for S 100B in patients with mild TBI (S100B = 0.10 µg/L) ~ 3 h and 25 min post-TBI ROC analysis showed that levels of S100B within 6 h post-mild TBI could differentiate between patients with and without IL (AUC [area under the curve] = 0.671; 95% CI = 0.5740.769; *p* = 0.008). Seventy patients (26.92%) showed values of S100B <0.10 µg/L. Sensitivity- 95.5% (no CI reported) specificity- 30.7% (no CI reported)

Reference	**Egea-Guerrero, 2018** [22]
	positive predictive value (PPV)- 11.1% (no CI reported) negative predictive value (NPV)- 98.6% (no CI reported) <u>Back calculation of 2x2 table by NGC:</u> TP: 21 FP: 164 FN: 1 TN: 74
Source of funding	Not reported
Limitations	Risk of bias *(QUADAS 2 – risk of bias)*: none Indirectness *(QUADAS 2 – applicability)*: serious. Mixed population of adults and children.
Comments	None of these patients underwent emergency neurosurgery.

Reference	**Ernstbrunner, 2016** [23]
Study type	Retrospective cohort
Study methodology	Data source: Data were collected retrospectively of all patients admitted from November 2008 to May 2012.
Number of patients	n = 382 (no ICH 378; Secondary ICH n=4)
Patient characteristics	Age, mean (SD): No ICH: 82 (±9) Secondary ICH: 76 (±11)

Reference	**Ernstbrunner, 2016** [23]
	No ICH :207 lacerations, 25 fractures Secondary ICH : 3 lacerations Gender (female%): No ICH: 60 Secondary ICH: 50 GCS: Mild (GCS score 14-15) Ethnicity: not reported Setting: Level Trauma centre Country: Austria Inclusion criteria: (1) ≥60 years of age, (2) intake of daily LDA prophylaxis (50-100 mg day^{-1}) (low dose acetyl salicylic acid prophylaxis), (3) isolated mHI with a GCS score of 14-15, (4) negative pHCT within 3 hours and (5) no hypertensive irregularities during the in-hospital observation period (systolic blood pressure <150 mm Hg). Exclusion criteria: patients taking anticoagulants such as heparin, warfarin, coumarin, clopidogrel or non-steroidal anti-inflammatory drugs; haematological or onco-logical diseases; and moderate or severe head injuries. On admission, the LDA therapy was paused and replaced by low-dose heparin for 14 days after the event
Target condition(s)	Acute post-brain injury complications
Index test(s) and reference standard	Index test: Serum S100B Peripheral venous blood was obtained directly after the primary CT within an average of 3 hours after initial trauma Reference standard: CT scan

Reference	**Ernstbrunner, 2016** [23]
	Primary head CT and RRHCT (repeated head computed tomography (RRHCT) scans within 3 and 48 hours to trauma were performed. The CT scans were reviewed without delay by an in-house attending senior radiologist. After the CT scan, all patients with mHI were kept under observation for a minimum of 24 hours.
Results	Outcome: secondary intracranial haemorrhagic events (SIHE) on CT Four patients (corresponds to 1.0%) developed SIHEs S100B cut-off value of 0.10 µg 1^{-1} -within an average of 3 hours Sensitivity: 75.0% Specificity: 19.0% negative predictive value (NPV): 98.6% positive predictive value (PPV) 1.0% area under the curve (AUC) for detection of SIHEs: 0.399 (95% CI = 0.079-0.720; *p*> 0.05) Back calculation of 2x2 table by NGC: TP: 3 FP: 306 FN: 1 TN: 72

Reference	**Ernstbrunner, 2016** [23]
Source of funding	No funding
Limitations	Risk of bias *(QUADAS 2 – risk of bias)*: very serious. Unclear whether selection of patients could have introduced bias; unclear whether index test results were interpreted without knowledge of the reference standard and vice versa Indirectness *(QUADAS 2 – applicability)*: none
Comments	No information on management

Reference	**Forouzan, 2021** [24]
Study type	Prospective cohort
Study methodology	Data source: patients with TBI who were referred to 2 trauma hospitals in 2019
Number of patients	n = 176
Patient characteristics	Age, mean (SD): 36.4 (16) years (range 16-90 years) Gender (male): 80.1% GCS score 14-15: 100% Ethnicity: not reported Setting: 2 hospitals Country: Iran

Reference	**Forouzan, 2021** [24]
	Inclusion criteria: ≥16 years of age; clinical diagnosis of TBI; those who have indications for brain CT scan in terms of the National Institute for Clinical Excellence Criteria (NICE); <6 hours elapsed between the event and examination; GCS score 15- 13 (mild TBI) Exclusion criteria: <16 years old; explosive or penetrating damage; chronic subdural hematoma; previous brain disorders; TBI requiring no CT; living in another province that made it difficult to follow-up
Target condition(s)	Acute post-brain injury complications
Index test(s) and reference standard	Index test: Serum GFAP Within 6 hours of injury (not stated mean timing of sampling) Reference standard: CT scan Follow up: post-concussion symptoms measured 3 months after injury
Results	Outcome: positive CT scan (acute epidural or subdural hematoma, cortical contusion, ventricular compression, ventricular trapping, cerebral herniation, intraventricular hemorrhage, hydrocephalus, subarachnoid hemorrhage, cerebral edema, post-traumatic ischemia, intracranial hematoma, and cerebral venous sinus thrombosis) The results of the CT scan in the patients under study were found to be normal for 168 individuals and positive for 8 individuals with intracranial trauma-related lesions. A Positive CT scan – Serum GFAP (optimal cut-off derived from AUC 1.35 ng/ml)- within 6 hours Sensitivity%: 50 (95% CI not reported) Specificity%: 44 (95% CI not reported)

Reference	**Forouzan, 2021** [24]
	AUC% (95% CI): 42.5 (95% CI 73.5-11.5) <u>Back calculation of 2x2 table by NGC:</u> TP: 4 FP: 94 FN: 4 TN: 74
Source of funding	Supported by the vice-chancellor of research affairs of the Ahvaz Jundishapur University of Medical Sciences
Limitations	Risk of bias *(QUADAS 2 – risk of bias)*: serious. Unclear whether the reference standard was interpreted without knowledge of the index test Indirectness *(QUADAS 2 – applicability)*: none
Comments	Methods section states sensitivity, specificity, PPV and NPV of S100B blood level for the detection of ICH requiring a medical and/or surgical treatment were estimated in the study population, but this is not clearly reported in the results. Main results extracted for positive CT scan.

Reference	**Gardner, 2018** [26] **TRACK-TBI Pilot study**
Study type	Prospective cohort study
Study methodology	Data source: Study enrolled 586 patients with TBI across the spectrum of age and TBI severity who presented to the emergency department (ED) of one of the three participating Level 1 trauma centres within 24 h of head trauma.
Number of patients	n = 586 (n=169 analysed - people with mild TBI)
Patient characteristics	Age < 40 years: n=79. Mean (SD): 25.8 (7.3) years

Reference	Gardner, 2018 [26] TRACK-TBI Pilot study
	Age 40-59 years: n=60. Mean (SD): 50.0 (5.9) years Age ≥ 60 years: n=30. Mean (SD): 68.0 (8.4) years Gender (female): Age < 40 years: 21 (26.6) Age 40-59 years: 20 (33.3) Age ≥ 60 years: 11 (36.7) GCS: mild TBI (GCS score 13-15) GCS score 13 Age < 40 years: 2 (2.5) Age 40-59 years: 0 (0.0) Age ≥ 60 years: 0 (0.0) GCS score 14 Age < 40 years: 16 (20.3) Age 40-59 years: 10 (16.7) Age ≥ 60 years: 2 (6.7) GCS score 15 Age < 40 years: 61 (77.2) Age 40-59 years: 50 (83.3) Age ≥ 60 years: 28 (93.3) Ethnicity: White Age < 40 years: 62 (78.5) Age 40-59 years: 47 (78.3) Age ≥ 60 years: 29 (96.7) Black Age < 40 years: 7 (8.9)

Reference	**Gardner, 2018 [26] TRACK-TBI Pilot study**
	Age 40-59 years: 8 (13.3) Age ≥ 60 years: 0 (0.0) <u>Asian</u> Age < 40 years: 5 (6.3) Age 40-59 years: 2 (3.3) Age ≥ 60 years: 0 (0.0) <u>Other/unknown</u> Age < 40 years: 5 (6.3) Age 40-59 years: 3 (5.0) Age ≥ 60 years: 1 (3.3) Setting: trauma centre Country: USA Inclusion criteria: age 16 years and older with mild TBI (GCS score 13-15) and ability to provide informed consent either independently or via a proxy. Exclusion criteria: Patients were excluded if they were non-English speaking, pregnant, in custody, undergoing psychiatric evaluation, had contraindications to magnetic resonance imaging (MRI), or had pre-existing medical or neurological conditions that would interfere with evaluation of TBI (such as pre-existing dementia or severe psychiatric illness)
Target condition(s)	Acute post-brain injury complications
Index test(s) and reference standard	<u>Index test:</u> Serum GFAP All blood samples were obtained within 24 h of injury Sample collection hours post-injury (hours): Age < 40 years: 8.6 – 5.6 (1.0–23.9) Age 40-59 years: 10.8 – 6.9 (0.5–23.5) Age ≥ 60 years: 13.6 – 6.8 (2.1–23.5)

Reference	Gardner, 2018 [26] TRACK-TBI Pilot study
	Reference standard: Head CT A single board-certified neuroradiologist (ELY), blinded to demographic, socioeconomic, and clinical data (except age and sex), reviewed each head CT and scored evidence of acute intracranial trauma according to expert consensus recommendations of the TBI CDE Neuroimaging Working Group.
Results	Outcome: acute intra cranial trauma For this study, evidence of acute intracranial trauma (i.e., CT+) was defined as presence of at least one of the following: epidural haemorrhage (EDH), subdural haemorrhage (SDH), subarachnoid haemorrhage (SAH), brain contusion, intracerebral haemorrhage (ICH), intraventricular haemorrhage (IVH), traumatic or diffuse axonal injury (TAI/DAI), midline shift >5 mm, partial or complete effacement of basal cisterns, or cerebral oedema. CT- was defined as having none of these aforementioned findings. Additionally, intra-parenchymal injury was defined as contusion, ICH, TAI/DAI, or oedema; extra-parenchymal injury, as EDH, SDH, SAH, or IVH. Patients were categorised as young (age< 40 years), middle aged (40-59 years) and older age (age ≥60 years). While GCS score did not significantly differ across age categories, older adults had the highest prevalence of CT findings (especially SDH and SAH) and the highest prevalence of intensive care unit and stepdown admission GFAP levels were found to be significantly higher with increasing age group. Young: CT negative n=61; CT positive n=18

Reference	Gardner, 2018 [26] TRACK-TBI Pilot study
	Middle aged: CT negative: n=40, CT positive n= 20 Older age: CT negative n=12, CT positive n= 18 Results: For diagnosis of intracranial trauma on CT GFAP ng/ml [cut-off point 0.43 (0.25-0.60)] (all patients) AUC (95% confidence interval [CI]): 0.88 (0.82- 0.93) GFAP ng/mL [cut-off point 0.43 (0.25-0.60)]- within 24 hrs Young age (< 40 years), Sensitivity: 83.3 (no CI reported) specificity: 83.6 (no CI reported) Back calculation of 2x2 table by NGC: TP: 15 FP: 10 FN: 3 TN: 51

Reference	Gardner, 2018 [26] TRACK-TBI Pilot study
	GFAP ng/mL [cut-off point 0.43 (0.25-0.60)]- within 24 hrs Middle age (40-59 years) Sensitivity: 90.0 (no CI reported) specificity: 77.5 (no CI reported) Back calculation of 2x2 table by NGC: TP: 18 FP: 9 FN: 2 TN: 31 GFAP ng/mL [cut-off point 0.43 (0.25-0.60)]- within 24 hrs Old age (age ≥60 years). Sensitivity: 66.7 (no CI reported) specificity: 66.7 (no CI reported) Back calculation of 2x2 table by NGC: TP: 12 FP: 4 FN: 6 TN: 8

Reference	**Gardner, 2018 [26] TRACK-TBI Pilot study**
	<u>AUC</u> <u>Young age</u> 0.93, 0.88– 0.99 <u>Middle-aged</u> 0.92, 0.86–0.99 <u>old age</u> 0.73, 0.54-0.91
Source of funding	This study was supported by the National Institutes of Neurological Disorders and Stroke (Beeson K23NS095755 to RCG, RC2 NS069409 to GTM, U01 NS086090 to GTM, and R21NS085455 to KWW), the American Federation for Aging Research (to RCG), the Department of Defence (DoD; W81XWH-13-1-0441 to GTM and W81XWH-14-2-0176 to GTM), the University of Florida McKnight Brain Institute BSCIRTF fund (to KWW), and the SUNY Downstate Medical Centre (to RR). This study was also supported in part by the Office of the Assistant Secretary of Defence for Health Affairs through the DoD Broad Agency Announcement under award numbers W81XWH-11-2-0069 (RR) and W81XWH-14-2-0166 (RR).
Limitations	Risk of bias *(QUADAS 2 – risk of bias)*: serious. Unclear whether the selection of patients could have introduced bias Indirectness *(QUADAS 2 – applicability)*: none
Comments	No information on treatment

Reference	**Gatson, 2014 [27] (Mild and Moderate TBI Biomarker [MAMBA] study)**
Study type	Prospective cohort
Study methodology	Data source: patients with an mild TBI (mTBI) consisting of periods of loss of consciousness, loss of memory before or after the event, altered mental status, and/or neurological deficits that are acute or chronic were enrolled into this TBI bio-marker study. The Glasgow Coma Scale (GCS) scoring system was also used to identify the mTBI patients.
Number of patients	n = 34
Patient characteristics	Age, mean (SD): CT negative: 33.4 ± 9.8 CT positive: 35.1 ± 1.6 Gender (male): CT negative: 7 (43.8) CT positive: 14 (77.7) GCS: mild TBI CT (negative): GCS score 13: 2 (12.5) GCS score 14: 6 (37.5) GCS score 15: 8 (50) CT positive: GCS score 13: 10 (55.5) GCS score 14: 1 (5.6) GCS score 15: 7 (38.9) Ethnicity: not reported Setting: hospital Country: USA

Reference	**Gatson, 2014 [27] (Mild and Moderate TBI Biomarker [MAMBA] study)**
	Inclusion criteria: TBI patients with a GCS score between 13 and 15 who were admitted to Parkland Hospital (Dallas, Texas) were identified and screened using the patient database .Both men and women between the ages of 18 and 50 years with an mTBI were screened. Exclusion criteria: Patients with penetrating injuries or those included in an interventional clinical trial were excluded
Target condition(s)	Acute post-brain injury complications
Index test(s) and reference standard	Index test: Serum neurofilament-H (NFL-H) Study measured the serum levels of pNFL-H in patients with mTBI at Day 1 (18-24 hours) or Day 3 (66-72 hours) after injury. The range of detection is 0.0293 ng/ml to 15 ng/ ml. Reference standard: CT scan A scan was deemed to be positive if there was evidence of skull fractures, subdural/ epidural/subarachnoid hemorrhaging, edema, and/or contusions.
Results	Outcome: Intracranial findings on CT (skull fractures, subdural/ epidural/subarachnoid haemorrhaging, oedema, and/or contusions) Of the mTBI patients who were admitted to the hospital, 47% of subjects (n = 16) had normal findings on CT scans (CT— group), and intracranial findings were documented on the CT scans of 53% (n = 18; CT+ group). pNFL-H (1071 pg/ml) – (18-24 hours) Day 1

Reference	**Gatson, 2014 [27] (Mild and Moderate TBI Biomarker [MAMBA] study)**
	AUC: 82.5% Sensitivity: 87.5% Specificity :70%. <u>Back calculation of 2x2 table by NGC:</u> TP: 16 FP: 5 FN: 2 TN: 11 <u>pNFL-H (1071 pg/ml) - Day 3</u> AUC: 71.7%
Source of funding	Funding was provided by the Division of Bum/Trauma/Critical Care; Department of Surgery; UT Southwestern Medical Center, Dallas, Texas.
Limitations	Risk of bias *(QUADAS 2 – risk of bias)*: serious. Unclear whether the selection of patients could have introduced bias Indirectness *(QUADAS 2 – applicability)*: none
Comments	No information on management

Reference	**Gill, 2018** [28]
Study type	Prospective cohort
Study methodology	Data source: patients presenting to the emergency department (ED) seeking care for a suspected brain injury and healthy controls without a history of TBI or neurologic disease were recruited from the National Institute of Health protocols: NCT01762475 and 09-NR-0131. Part of the Traumatic Head Injury Neuroimaging Classification study
Number of patients	n = 277 (n = 49 controls)
Patient characteristics	Age, mean (SD): MRI+, CT+ 52.03 (19.83); MRI+, CT- 46.04 (16.08); MRI-, CT- 41.48 (15.25) years Gender (male): MRI+, CT+ 65%; MRI+, CT- 60%; MRI-, CT- 57% GCS mean (SD): MRI+, CT+ 14.07 (1.53); MRI+, CT- 14.42 (1.11); MRI-, CT- 14.51 (7.22) Ethnicity: not reported Setting: emergency department Country: USA Inclusion criteria: seeking care for a suspected brain injury; 18–85 years of age; GCS score 13–15 Exclusion criteria: not reported
Target condition(s)	Acute post-brain injury complications
Index test(s) and reference standard	<u>Index test:</u> Plasma NFL within 48 hours post injury Plasma GFAP within 48 hours post injury Plasma UCH-L1 within 48 hours post injury <u>Reference standard:</u> CT scan within 48 hours post injury

Reference	**Gill, 2018** [28]
	MRI scan within 48 hours post injury Follow up: no mention of follow up
Results	Outcome: neuroimaging findings (CT and MRI) <u>Findings on CT scan – Plasma GFAP within 48 hours post injury</u> AUC (95% CI): 0.77 (0.7-0.84) <u>Findings on CT scan – Plasma NFL within 48 hours post injury</u> AUC: 0.65 (95% CI not reported) <u>Findings on MRI scan – Plasma GFAP within 48 hours post injury</u> AUC: 0.8 (95% CI not reported) <u>Findings on MRI scan – Plasma NFL within 48 hours post injury</u> AUC: 0.66 (95% CI not reported) <u>Findings on MRI scan with negative CT scan – Plasma GFAP within 48 hours post injury</u> AUC: 0.74 (95% CI not reported) <u>Findings on MRI scan with negative CT scan – Plasma NFL within 48 hours post injury</u>

Reference	**Gill, 2018** [28]
	AUC: 0.64 (95% CI not reported)
Source of funding	National Institute of Nursing Research (NINR) Intramural Research Program, National Institute of Neurological Disease and Stroke (NINDS) Team, Center for Neuroscience and Regenerative Medicine, Acute Studies and Biomarker Core, National Football League and General Electric, Head to Head Grant
Limitations	Risk of bias *(QUADAS 2 – risk of bias)*: very serious. Unclear whether selection of patients could have introduced bias; no description of what was considered to be a positive finding on CT/MRI scans; unclear whether reference standard was interpreted without knowledge of the index test Indirectness *(QUADAS 2 – applicability)*: none
Comments	UCH-L1 also measured but findings not reported as approximately one third did not meet quality control specifications. Diagnostic accuracy of a combination of biomarkers was also reported, but not extracted as it included tau which is not included in the review protocol.

Reference	**Ingebrigtsen, 2000** [29]
Study type	Prospective cohort
Study methodology	Data source: consecutive adult patients with head injury evaluated in the emergency department (ED) of a single university hospital from November 2015 to November 2016.
Number of patients	n = 182
Patient characteristics	Age, mean (range): 33 (15-78) years Gender (male): 61% GCS score 13: 5.5% GCS score 14: 18.7% GCS score 15: 75.8%

Reference	**Ingebrigtsen, 2000** [29]
	Ethnicity: not reported Setting: Departments of Neurosurgery/Neurology at 3 centres Country: Finland Inclusion criteria: head injury with brief (≤10 minutes) loss of consciousness; GCS score 13-15 at admission; no focal neurological deficits; age 15-80 years; admitted within 12 hours post injury; CT performed within 24 hours after injury Exclusion criteria: history of neurological disease
Target condition(s)	Acute post-brain injury complications
Index test(s) and reference standard	<u>Index test:</u> Serum S100B measured immediately after admission (cut-off ≥0.2 µg/L) (mean 3 hours (range 0.5-12 hours) after injury) <u>Reference standard:</u> CT scan within 24 hours post injury Follow up: Rivermead Post Concussion Symptoms questionnaire measured 3 months post injury
Results	Outcome: intracranial pathology on CT scan <u>Intracranial pathology on CT scan – Serum S100B measured immediately after admission (cut-off ≥0.2 µg/L) mean 3 hours</u> TP: 9 FP: 60 TN: 112 FN: 1

Reference	**Ingebrigtsen, 2000** [29]
	Sensitivity%: 90 Specificity%: 65 PPV: 0.13 NPV: 0.99 <u>SN/SP calculated by NGC</u> Sensitivity: 0.90 [0.55, 1.00] Specificity: 0.65 [0.57, 0.72]
Source of funding	The Lærdal Foundation for Acute Medicine and the Skane County Council's Research and Development Foundation
Limitations	Risk of bias *(QUADAS 2 – risk of bias)*: very serious. Unclear whether the index test was interpreted without knowledge of the reference standard and vice versa; unclear no description of what was considered intracranial pathology on CT scan Indirectness *(QUADAS 2 – applicability)*: serious. Population indirectness as children and adults were included, although the mean suggests majority were adults.
Comments	-

Reference	**Kahouadji, 2020** [31]
Study type	Prospective cohort
Study methodology	Data source: adult patients presenting to the emergency department (ED) at a single centre with mild TBI and clinical indication for a CT scan from February 2018 to April 2019
Number of patients	n = 130
Patient characteristics	Age, mean (SD): 44.8 (20.4) years Gender (male): 62%

Reference	**Kahouadji, 2020** [31]
	GCS score 13/14: 17% GCS score 15: 83% Ethnicity: not reported Setting: single centre ED Country: Switzerland Inclusion criteria: Adult (≥18 years) mild TBI patients with a clinical indication for a CT scan, as described in the Canadian CT Head Rule; mild TBI defined as head trauma with GCS score of 13–15 Exclusion criteria: not reported
Target condition(s)	Acute post-brain injury complications
Index test(s) and reference standard	<u>Index test:</u> Serum S100B measured 3 hours post injury <u>Reference standard:</u> Cranial CT scan Follow up: no mention of follow up
Results	Outcome: positive CT scan (at least one pathophysiological trauma-relevant intracranial lesion - any signs of cranial (skull fracture) or intracranial pathology (hematoma, air, or contusion), subgaleal hematomas were also considered positive to prevent disregarding abnormalities that may influence S100B levels <u>Positive CT scan – Serum S100B measured 3 hours post injury (cut-off 0.1 µg/L)</u> TP: 32

Reference	Kahouadji, 2020 [31]
	FP: 87 TN: 10 FN: 1 Sensitivity% (95% CI): 97 (84.2-99.9) Specificity% (95% CI): 11 (5.8-19.4) PPV% (95% CI): 27 (19.3-36.1) NPV% (95% CI): 92 (61.5-99.8) AUC (95% CI): 0.71 (0.6-0.81) SN/SP calculated by NGC Sensitivity: 0.97 [0.84, 1.00] Specificity: 0.10 [0.05, 0.18] Positive CT scan – Serum S100B measured 3 hours post injury (cut-off 0.08 µg/L) Sensitivity% (95% CI): 100 (89.4-100) Specificity% (95% CI): 7 (2.3-13) Positive CT scan – Serum S100B measured 3 hours post injury (cut-off 0.14 µg/L) Sensitivity% (95% CI): 91 (76-98)

Reference	**Kahouadji, 2020** [31]
	Specificity% (95% CI): 31 (21.9-41.1)
Source of funding	Supported by the program in the Auvergne Rhône-Alpes region for international academic and scientific cooperation between French and Swiss teams
Limitations	Risk of bias *(QUADAS 2 – risk of bias)*: very serious. Unclear whether the selection of patients could have introduced bias; unclear whether the index test was interpreted without knowledge of the reference standard and vice versa Indirectness *(QUADAS 2 – applicability)*: none
Comments	Serum S100B did not influence patients' clinical management

Reference	**Kaneko, 2019** [32]
Study type	Prospective cohort
Study methodology	Data source: patients with mild-to-moderate TBI admitted to emergency department, using blood samples obtained upon admission. The study was performed between May 2014 and June 2016 in the emergency department at Kumamoto Medical Center
Number of patients	n =57
Patient characteristics	Age, years: 70 (57-81) Gender (male): 22 (39%) Severity: mild to moderate TBI GCS score mean (range): 15 (14-15) Ethnicity: not reported

Reference	Kaneko, 2019 [32]
	Setting: ED Country: Japan Inclusion criteria: admission to the emergency department of Kumamoto Medical Center, single blunt head trauma, mild-to-moderate TBI with Glasgow coma scale score of 9-15, and head computed tomography (CT) scheduled before collecting informed consent Exclusion criteria: Pregnant women were excluded.
Target condition(s)	Acute post-brain injury complications
Index test(s) and reference standard	Index test: Serum biomarkers - glial fibrillary acidic protein (GFAP) (ng/mL) - phosphorylated neurofilament heavy subunit (pNF-H) (pg/mL) - heart-type fatty acid binding protein (H-FABP) (ng/mL) - neuron-specific enolase (NSE) (ng/mL) - S 100B protein (S100B) (pg/mL) After obtaining informed consent, the blood sample taken upon emergency department admission was stored at -80 °C Reference standard: CT Follow up: not reported
Results	Outcome: Positive head CT findings Positive head CT findings were defined as intra-cranial haemorrhagic findings CT positive: (n=12) CT negative: (n=45)

Reference	Kaneko, 2019 [32]
	Positive head CT findings: 12 (21%) - Traumatic subarachnoid haemorrhage (SAH): 4 - subdural haematoma (SDH): 7 - Brain contusion: 1 The Glasgow coma scale score was significantly different between the two groups, with median values of 14 and 15 in the head CT-positive and -negative groups. Serum biomarkers value: - GFAP (ng/mL) 0.11 (0.00-0.22) - pNF-H (pg/mL) 53.9 (0.0-265.4) - H-FABP (ng/mL) 5.2 (3.1-9.6) - NSE (ng/mL) 12.6 (7.9-15.6) - S 100B (pg/mL) 7.2 (0.0-48.7) Biomarker: AUC curve (95% CI) GFAP: 0.845 (0.698-0.991) pNF-H: 0.569 (0.398-0.739) H-FABP: 0.518 (0.315-0.721) NSE: 0.744 (0.565-0.923)

Reference	**Kaneko, 2019** [32]
	S100B: 0.753 (0.582-0.924)
Source of funding	study was supported by JSPS KAKENHI (Grant Number JP16K11409)
Limitations	Risk of bias *(QUADAS 2 – risk of bias)*: very serious. Unclear whether the selection of patients could have introduced bias; unclear whether the index test was interpreted without knowledge of the reference standard and vice versa Indirectness *(QUADAS 2 – applicability)*: none
Comments	No information on treatment

Reference	**Kotlyar, 2011** [34]
Study type	Prospective cohort study
Study methodology	Data source: This was a prospective observational study of patients aged 18 years or older with minor head trauma presenting to an urban Level I ED (> 90,000 visits per year) from March 2006-April 2007. Minor head trauma was defined as blunt head injury with a Glasgow Coma Scale score (GCS) of 13-15, with or without loss of consciousness, and a non-focal neurologic examination.
Number of patients	n = 346
Patient characteristics	Age, mean (SD): 48 Gender (male): 62% GCS: GCS score 15: 89% (303) GCS score <15: 10% (35) Ethnicity: White: 63% (219) Hispanic: 20% (68) Black: 13% (45)

Reference	**Kotlyar, 2011** [34]
	Other: 4% (14) Setting: ED Country: USA Inclusion criteria: Patients presenting within 6 h of injury and undergoing HCT for evaluation of minor head trauma (GCS score of 13-15) were eligible for enrollment. Patients with concomitant trauma were eligible for enrollment. Alcohol- and drug-intoxicated patients were also eligible for enrollment if time of injury was known. Non-focal neurologic examination Exclusion criteria: Major trauma, non-English-speaking patients, head trauma occurring > 6 h before ED presentation, altered mental status of unclear etiology (e.g., seizure, stroke, sepsis)
Target condition(s)	Acute post-brain injury complications
Index test(s) and reference standard	Index test: Serum S100B Blood was collected upon admission (within 6 hours of injury) and immediately sent to the laboratory for processing Reference standard: Head CT ED HCT was performed within 3 h of ED presentation
Results	Outcome: Positive CT results (subarachnoid haemorrhage, epidural haemorrhage, subdural haemorrhage, intraparenchymal haemorrhage , diffuse brain oedema , diffuse axonal injury, skull fracture)

Reference	Kotlyar, 2011 [34]
	Head CT negative: 324 Head CT positive: 22 S100B (cut-off 42 ng/dL)- within 6 hours Sensitivity (95% CI): 86% (95% CI 67-96%) Specificity (95% CI): 37% (95% CI 29-45%) PPV (95% CI): 18% (95% CI 12-27%) NPV (95% CI): 94% (95% CI 87-98%) Back calculation of 2x2 table done by NGC: TP: 19 FP: 204 FN: 3 TN: 120 S100B (cut-off 32 ng/dL)- within 6 hours Sensitivity (95% CI): 91% (95% CI 72-98%) Specificity (95% CI): 24% (95% CI 17-31%) PPV (95% CI): 16% (95% CI 11-24%)

Reference	Kotlyar, 2011 [34]
	NPV (95% CI): 94% (95% CI 81-98%) <u>Back calculation of 2x2 table done by NGC:</u> TP: 20 FP: 246 FN: 2 TN: 78 <u>S100B (cut-off 24 ng/dL)- within 6 hours</u> Sensitivity (95% CI): 96% (95% CI 78-100%) Specificity (95% CI): 13% (95% CI 9-20%) PPV (95% CI): 15% (95% CI 10-22%) NPV (95% CI): 95% (95% CI 76-100%) <u>Back calculation of 2x2 done by NGC:</u> TP: 21 FP: 282 FN: 1 TN: 42
Source of funding	Not reported

Reference	**Kotlyar, 2011** [34]
Limitations	Risk of bias *(QUADAS 2 – risk of bias)*: very serious. Unclear whether the selection of patients could have introduced bias; unclear whether the index test was interpreted without knowledge of the reference standard and vice versa Indirectness *(QUADAS 2 – applicability)*: none
Comments	No information on treatment

Reference	**Lagerstedt, 2017** [35]
Study type	Prospective cohort
Study methodology	Data source: This study recruited a total of 261 patients at three different European sites: Geneva (Switzerland), Barcelona (Spain) and Seville (Spain)
Number of patients	n = 172 [CT +: 140 (81%); CT -: 32 (19%)]
Patient characteristics	Mixed population (age > 14 years- adults and children) Age, years: CT+: 46 (20) years CT -: 61 (25) years 261 mild TBI patients with a GCS score of 15. Of these, 172 patients came to the hospital ≤ 6 h after trauma, with a mean time (± SD) of 198 min ± 88 Gender (male): CT+: 101 (72%) CT-: 23 (72%) Ethnicity: not reported Setting: hospital Country: Switzerland and Spain

Reference	**Lagerstedt, 2017** [35]
	Inclusion criteria: diagnosis of mTBI with a GCS score of 15; presence of at least one clinical symptom (loss of consciousness, amnesia, vomiting or nausea, headache or equilibrium disorder); CT scan performed within 24 h of the trauma (where the presence of epidural haemorrhage, subdural haemorrhage, subarachnoid haemorrhage, intracerebral haemorrhage, contusion with haemorrhage, cerebral oedema or skull fracture was classified as CT-positive); blood sample collected at admission; and age above 14 years old. Exclusion criteria: pregnancy; GCS score below 15 at admission to hospital; absence of clinical symptoms; no head CT scan; and no signed informed consent form
Target condition(s)	Acute post-brain injury complications
Index test(s) and reference standard	Index test: - Serum S100B - Serum Heart fatty-acid binding protein (H-FABP) Blood samples collected ≤6 h after trauma. Reference standard: Head CT CT scan performed within 24 h of the trauma
Results	Outcome: CT positive (not defined) S100B and H-FABP levels were measured for all patients (at ≤ 6 h) and showed significantly higher concentrations in CT-positive than in CT-negative patients (p = 0.003 and p = 0.004, respectively). A total of 32 patients (19%) were classified as CT-positive and n=140 CT negative. Results:

Reference	Lagerstedt, 2017 [35]
	S100 B (cut-off 0.1 µg/L)- within 6h Sensitivity: 81.3 (95% CI 65.6–93.8) Specificity: 42.1 (95% CI 34.3–50.0) Back calculation of 2x2 tables by NGC TP: 26 FP: 81 FN: 6 TN: 59 S100 B (cut-off 0.042 µg/L) [sensitivity set at 100%)- within 6h Sensitivity: 100 (95% CI 100–100) Specificity: 6.4 (95% CI 2.8–10.7) S100B: AUC: 66.9% (95% CI 56 -77.8) H-FABP (cut-off 2.62 µg/L) [sensitivity set at 100%)- within 6h

Reference	**Lagerstedt, 2017** [35]
	Sensitivity: 100 (95% CI 100–100) Specificity: 29.3 (95% CI 21.4–37.1) H-FABP AUC: 66.4% (95% CI 57.2-75.5%)
Source of funding	Not stated
Limitations	Risk of bias *(QUADAS 2 – risk of bias)*: very serious. Unclear whether the selection of patients could have introduced bias; unclear whether the index test was interpreted without knowledge of the reference standard and vice versa Indirectness *(QUADAS 2 – applicability)*: serious. Population indirectness as children and adults were included, although the mean suggests majority were adults.
Comments	No information on treatment

Reference	**Lagerstedt, 2018** [36]
Study type	Prospective cohort study
Study methodology	Data source: Patients were recruited from three different European sites: Geneva, Seville and Barcelona.
Number of patients	n = 132 (CT negative scan, n (%) 111 (84) 2; CT positive n=21 (16))
Patient characteristics	Age, mean (SD) year: mixed population (mean age -adults) CT negative: 46 (21)

Reference	Lagerstedt, 2018 [36]
	CT positive: 63 (24) Gender (male): CT negative: 82 (74) CT positive: 14 (67) GCS: GCS score of 15 and at least one clinical symptom Ethnicity: not reported Setting: ED Country: Spain Inclusion criteria: patients were diagnosed with mTBI and had a GCS score of 15 and at least one additional clinical symptom (vomiting or nausea, loss of consciousness, amnesia, an equilibrium disorder or a headache) and age above 14 years old. Each patient had a blood sample taken at hospital admission 6 h post trauma and a CT scan was performed within 24h post trauma Exclusion criteria: no CT scan, no clinical symptoms, GCS score below 15, pregnancy and no signed informed consent form.
Target condition(s)	Acute post-brain injury complications
Index test(s) and reference standard	<u>Index test:</u> Serum biomarkers - Thirteen proteins —H-FABP, MMP-1, MMP-3, MMP-9, VCAM, ICAM, SAA, CRP, GSTP, NKDA, PRDX1, DJ-1 and IL-10 ≤6 h following a TBI event Time, trauma to blood, (min):

Reference	**Lagerstedt, 2018** [36]
	Mean (SD): CT negative: 195 (86); CT positive: 177 (100) <u>Reference standard:</u> CT scan
Results	Outcome: CT positive (Epidural haemorrhage, Subdural haemorrhage, Subarachnoid haemorrhage, Intracerebral haemorrhage Contusion with haemorrhage Skull fracture) N= 132 patients, of whom 21 were CT-positive (16%). Analyses were performed on the first 62 patients recruited in the 132 mTBI patients of Cohort 1, of whom were 48 CT-negative and 14 CT-positive. Results: (All protein concentrations in ng/mL except for IL-10, which is in pg/mL) **Sensitivity set at 100%** <u>IL-10 – (cut-off 0.06)</u> Sensitivity: 100 (95% CI 100–100) Specificity: 27.1 (95% CI 14.6–39.6) <u>H-FABP- (cut-off 2.0)</u> Sensitivity: 100 (95% CI 100–100) Specificity: 33.3 (95% CI 20.8–47.9)

Reference	Lagerstedt, 2018 [36]
	VCAM-(cut-off 359.2) Sensitivity: 100 (95% CI 100–100) Specificity: 12.5 (95% CI 4.2–22.9) GSTP -(cut-off 42.1) Sensitivity: 100 (95% CI 100–100) Specificity: 10.4 (95% CI 2.1–18.8) CRP -(cut-off 132.4) Sensitivity: 100 (95% CI 100–100) Specificity: 4.2 (95% CI 0.0–10.4) SAA – (cut-off 279.2) Sensitivity: 100 (95% CI 100–100) Specificity: 8.3 (2.1–16.7) DJ-1 (cut-off 50.8) Sensitivity: 100 (95% CI 100–100) Specificity: 16.7 (95% CI 6.3–27.1) PRDX1-(cut-off 23.5)

Reference	Lagerstedt, 2018 [36]
	Sensitivity: 100 (95% CI 100–100) Specificity: 6.3 (95% CI 0.0–14.6) NDKA- cut-off NR Sensitivity: 100 (100–100) - Specificity: NR ICAM cut-off NR Sensitivity: 100 (95% CI 100–100) Specificity: NR MMP-3-cut-off NR Sensitivity: 100 (95% CI 100–100) Specificity: NR MMP-1 -cut-off 5.5 Sensitivity: 100 (95% CI 100–100) Specificity: 10.4 (95% CI 2.1–18.8) MMP-9- (cut-off 505.0)

Reference	**Lagerstedt, 2018** [36]
	Sensitivity: 100 (95% CI 100–100) Specificity: 8.3 (95% CI 2.1–16.7) Among the 13 biomarkers, only the H-FABP and IL-10 proteins were found at significantly higher levels in CT positive patients than in CT-negative patients
Source of funding	Not reported
Limitations	Risk of bias *(QUADAS 2 – risk of bias)*: very serious. Unclear whether the selection of patients could have introduced bias; unclear whether the index test was interpreted without knowledge of the reference standard and vice versa Indirectness *(QUADAS 2 – applicability)*: mixed population includes adults and children. mean age -adults
Comments	To increase specificity four proteins were further analysed when combined in panels. This data has not been extracted.

Reference	**Lagerstedt, 2018** [37]
Study type	Prospective cohort study
Study methodology	Data source: Patients were recruited from Geneva (Switzerland) and Seville (Spain).
Number of patients	-For verification, plasma samples were collected from n=52 patients in Geneva and for validation; n= 133 patients, with either plasma or serum samples, were collected in Geneva and Seville.
Patient characteristics	CT negative: 111 (83) CT positive: 22 (17) Age, mean (SD) year: (mixed population) CT negative: 46 (21) years CT positive: 61 (26) years

Reference	**Lagerstedt, 2018** [37]
	Gender (male): CT negative: 82 (74) CT positive: 15 (68) GCS score: 15 (mild TBI) Ethnicity: not reported Setting: ED Country: Spain Inclusion criteria: diagnosis of mTBI, a GCS score of 15 at hospital admission and at least one of the following symptoms: headache, nausea or vomiting, loss of consciousness (< 30 min) and amnesia (< 24 h). Exclusion criteria: NR
Target condition(s)	Acute post-brain injury complications
Index test(s) and reference standard	Index test: o S100B o IL-10 ≤6 hours following TBI A serum (Seville) or plasma (Geneva) sample was collected from patients at hospital admission. Reference standard: CT scan

Reference	**Lagerstedt, 2018** [37]
	Participating patients gave a blood sample at hospital admission and underwent a CT scan within 24 h of their trauma event
Results	Outcome: CT positive (Epidural haemorrhage, Subdural haemorrhage, Subarachnoid haemorrhage, Intracerebral haemorrhage, Contusion with haemorrhage, Skull fracture). Not all patients suffering from mTBI seek immediate clinical help, thereby increasing the time between trauma and blood sampling. The markers' performances were evaluated on patients admitted to hospital within 24 h of their trauma event. This raised the cohort population to 207 mTBI patients, of whom 29 (14%) were CT-positive and 178 (86%) were CT-negative Both IL-10 and S100B were significantly higher in CT-positive than in CT-negative patients ($p < 0.001$). Results **sensitivity set at 100%** S100B (cut-off 0.072 ug/uL) specificity: 18.4% (95% CI 12.9– 24.6) IL-10 (cut-off 0.159 pg/mL): specificity 25.8% (95% CI 19.7–32.0)
Source of funding	Not reported
Limitations	Risk of bias *(QUADAS 2 – risk of bias)*: very serious. Unclear whether the selection of patients could have introduced bias; unclear whether the index test was interpreted without knowledge of the reference standard and vice versa Indirectness *(QUADAS 2 – applicability)*: mixed population includes adults and children. mean age -adults

Reference	**Lagerstedt, 2018** [37]
Comments	No information on treatment

Reference	**Laribi, 2014** [38]
Study type	Prospective cohort study
Study methodology	Data source: prospective observational study was carried out from June 2008 to June 2010 in adult emergency departments (ED) of seven hospitals in France, including five teaching hospitals (Cochin, Henri Mondor, Lariboisiere, Poitiers, Reims) and two general hospitals (Orleans, Pontoise).
Number of patients	N=431
Patient characteristics	Patients included were aged 18 years or older presenting to the ED within 3 h after injury. A 3-h cut-off was chosen as S100B is rapidly cleared from the serum, with a half-life between 0.5 and 2 h Age,: The median age (IQR) of the participants was 36 (24-54) years. Extra cranial injuries: 201 (47%) Gender (male): 269 (65) GCS: mild TBI GCS score 13: 7 (2) GCS score 14: 48 (11) GCS score 15: 376 (87) Reason for MHI was a fall in 263 patients. Ethnicity: not reported Setting: ED

Reference	**Laribi, 2014** [38]
	Country: International Inclusion criteria: history of MHI defined by a Glasgow Coma Scale score (GCS) from 13 to 15 with one or more of the following risk factors: amnesia, loss of consciousness, nausea, vomiting, vertigo, anticoagulation before injury or severe headache on admission Exclusion criteria: no need for a CT scan as decided by the treating physician, renal failure with a serum creatinine level above 150 µmol/L, malignant melanoma, focal neurological deficit, pregnant women, age older than 80 years, and loss of consciousness more than 10 min. Patients with concomitant injuries of the extremities were also excluded.
Target condition(s)	Acute post-brain injury complications
Index test(s) and reference standard	Index test: S 100B Venous blood samples were collected immediately at patients' arrival to the ED within 3 h after the clinical event ((HO) and 3 h (H3) after the first sampling. Reference standard: Cranial CT Patients underwent a CT scan within 6 h after clinical examination. Need for hospitalisation or neurosurgery was also recorded. According to the CT radiological findings, patients were divided into two groups: CT— corresponding to MHI patients without relevant cerebral lesion and CT+ corresponding to MHI patients with at least one trauma-relevant cerebral lesion. Trauma relevant lesions were confirmed by a board-certified radiologist blinded to the serum S100B level. The mean time interval between MHI and CT scanning was 3 h; time intervals between MHI and the HO/H3 blood samplings were near 2 h and 5 h, respectively.

Reference	**Laribi, 2014** [38]
	Four patients initially classified as CT+, were reclassified as CT— after a second CT examination by a senior radiologist/ED physician that excluded cerebral lesions. Fifty-one patients were hospitalised either in the ED observation unit or in another hospitalisation unit for ≥ 24 h, mostly for the treatment of extracranial lesions. Six patients in the CT+ group were hospitalized in a neurological/neurosurgical unit.
Results	Outcome: CT positive- CT findings of intracranial lesions. Of the total cohort, 404 patients had a negative CT and 27 had a positive one. **At H0** (at **ED within 3 hours)** S100B (Diasorin)(cut-off 0.15, µg/L) Sensitivity: 96.3% (95% CI 81.0-99.9) Specificity: 44.3% (95% CI 39.4-49.1) TP: 26 FP: 219 FN: 174 TN: 1 SN/SP calculated by NGC:

Reference	Laribi, 2014 [38]
	Sensitivity: 0.96 [0.81, 1.00] Specificity: 0.44 [0.39, 0.49] S100B (Roche Diagnostics assay) cut-off 0.10, µg/L) Sensitivity: 100% (95% CI 86.8-100) Specificity: 38.2% (95% CI 33.3-43.1) TP: 26 FP: 231 FN: 0 TN: 143 SN/SP calculated by NGC: Sensitivity: 1.00 [0.87, 1.00] Specificity: 0.38 [0.33, 0.43] **At H3 (3 h after the first sampling)** S100B (Diasorin)(cut-off 0.15, µg/L) Sensitivity: 84.6% (95% CI 65.1-95.6)

Reference	Laribi, 2014 [38]
	Specificity: 63.0% (95% CI 58.1-67.7) TP: 22 FP: 143 FN: 4 TN: 243 SN/SP calculated by NGC: Sensitivity: 0.85 [0.65, 0.96] Specificity: 0.63 [0.58, 0.68] S100B (Roche Diagnostics assay) (cut-off 0.10, µg/L) Sensitivity=68.0% (95% CI 49.7-86.3) Specificity=51.2% (95% CI 46.1-56.4) TP: 17 FP: 177 FN: 8 TN: 189

Reference	**Laribi, 2014** [38]
	SN/SP calculated by NGC: Sensitivity: 0.68 [0.46, 0.85] Specificity: 0.52 [0.46, 0.57]
Source of funding	No funding
Limitations	Risk of bias *(QUADAS 2 – risk of bias)*: serious. Unclear whether the selection of patients could have introduced bias Indirectness *(QUADAS 2 – applicability)*: none
Comments	Information on treatment

Reference	**Li, 2022** [39]
Study type	Retrospective cohort study USA
Study methodology	Data source: retrospective enrolment of patients transported to Stanford Health Care's emergency department (ED) by ambulance or helicopter, for whom a trauma alert was triggered per established criteria and who underwent a non-contrast head CT scan due to suspicion of TBI, between December 2015 and April 2017
Number of patients	n = 463
Patient characteristics	Age, mean (SD): 50.8 ± 22.7 years Gender (female): 177 (38.2) GCS score , median {Q1,Q3] : 15 [14, 15] Ethnicity: NR

Reference	**Li, 2022** [39]
	Setting: ED Country: USA Inclusion criteria for this study: (1) at least 18 years old at admission, (2) presented to the ED with suspected TBI, and (3) have a blood draw as part of the standard of care. Exclusion criteria: non-English–speaking patients were excluded, as well as patients without the capacity to consent (including those with altered mental status and hearing impairments) if no legal authorized representative was available
Target condition(s)	Acute post-brain injury complications
Index test(s) and reference standard	<u>Index test:</u> Distribution of time from injury to ED admission/blood draw spanned 0 through 6 h with a median at 1 h. Plasma GFAP (22°pg/mL) Serum GFAP (22°pg/Ml) Plasma UCH-L1 (327°pg/mL) Serum UCH-L1 (327 pg/mL) Serum S1003 (105°pg/mL) Composite plasma biomarker Plasma GFAP (22°pg/mL) and UCH-L1 (327) Serum GFAP (22°pg/mL) and UCH-L1 (327) Thresholds for GFAP (22 pg/mL) and UCH-L1(327 pg/mL) were taken from Bazarian et al.'s multicenter study for the prediction of intracranial injuries on head CT, and the threshold for S100β (105 pg/mL) was taken from Welch et al.'s study on differentiating normal and abnormal head CT findings in patients with suspected mild or moderate TBI. <u>Reference standard:</u>

Reference	**Li, 2022** [39]
	Non-contrast head CTs All patients underwent a noncontrast head CT as part of their initial workup in the emergency room
Results	Outcome: Abnormalities on CT N=122 (26.3%) had one or more abnormalities presenting on head CT Normal head CT: N=341 Plasma and serum samples were available—and levels detectable—in 442 (95%) and 222 (48%) of the patients, respectively. Plasma GFAP (22°pg/mL) TP: 115 FP: 159 TN: 162 FN: 4 Serum GFAP (22°pg/Ml) TP: 51 FP: 81 TN: 85 FN: 4 Plasma UCH-L1 (327°pg/mL) TP: 113 FP: 266

Reference	Li, 2022 [39]
	TN: 57 FN: 6 Serum UCH-L1 (327 pg/mL) TP:50 FP: 121 TN: 45 FN: 6 Serum S100 (105°pg/mL) TP: 41 FP: 120 TN: 25 FN: 3 Composite plasma biomarker Plasma GFAP (22°pg/mL) [0.022 µg/ml] and UCH-L1 (327 pg/mL) [0.327 µg/ml] TP: 119 FP: 287 TN: 35 FN: 0 Serum GFAP (22°pg/mL) [0.022 µg/ml] or UCH-L1 (327) [0.327 µg/ml] TP: 56 FP: 137

Reference	**Li, 2022** [39]
	TN: 29 FN: 0 Any head CT abnormalities, n (%) 122 (26.3) Skull fracture, n (%) 31 (6.7) Pneumocephalus, n (%) 10 (2.2) Intracranial hemorrhage, n (%) 114 (24.6) Mass effect, n (%) 24 (5.2) Brain parenchymal injuries, n (%) 36 (7.8)
Source of funding	no financial support for the research, authorship, and/or publication of this article.
Limitations	Risk of bias *(QUADAS 2 – risk of bias)*: very serious. Unclear whether the results of the index test were interpreted without knowledge of the results of the reference standard and vice versa; unclear sequencing and time interval between index test and reference standard Indirectness *(QUADAS 2 – applicability)*: none
Comments	No information on treatment

Reference	**Linsenmaier, 2016** [40]
Study type	Prospective cohort study
Study methodology	Data source: 41 patients with a history of minor head trauma (Glasgow Coma Scale on admission: 13–15) were examined using CCT and underwent MRI of the head within 48 h of admission.
Number of patients	N=41
Patient characteristics	Age mean (SD) years: 54.6 6 23.3 Gender (male): 21 (51.2)

Reference	Linsenmaier, 2016 [40]
	GCS: mild TBI GCS score 15: 36 (87.8) GCS score 14: 4 (9.8) GCS score 13: 1 (2.4) Ethnicity: not reported Setting: ED Country: Germany Inclusion criteria: a history of minor head trauma (Glasgow Coma Scale on admission: 13–15) Exclusion criteria: under the age of 18 years, pregnant females and patients with multiple injuries were exclude
Target condition(s)	Acute post-brain injury complications
Index test(s) and reference standard	Index test: S100B A cut-off value of 0.1mgl^{-1} blood samples were obtained on admission for the measurement of S-100B concentration (time not specified) Patients with raised serum concentrations were grouped as S-100B positive (S-100B1) and concentrations below the cut-off were considered as S-100B negative (S-100B2). Reference standard: Cranial CT MRI

Reference	**Linsenmaier, 2016** [40]
	The patients were grouped into CCT negative (CCT- no abnormal findings) and CCT positive (CCT+, abnormal findings). If intracranial haemorrhage could not be excluded safely, the patient was also considered as “CCT positive” because of an equivocal CT finding deserving further evaluation. patients were grouped into MRI negative (MRI- no abnormality) and MRI positive (MRI+ at least one trauma-related finding).
Results	Outcome: CCT positive (abnormal findings) Of 41 (100%) patients, 12 (29.3%, CCT1) patients were categorized as CCT positive with a total of 14 trauma-related lesions: contusions (n =8); subarachnoid (n =1), subdural (n = 2) and epidural (n =1) haemorrhages; and fractures (n = 2). 5 (12.2%, MRI) patients had abnormal MRI with a total of 15 trauma-related lesions: contusions (n = 7); subarachnoid (n =5), subdural (n =2) and epidural (n =1) haemorrhages. Five patients had trauma-related findings diagnosed by both CCT and MRI; another seven patients had positive or questionable CCT scans and lesions were not confirmed by MRI. In the latter group, one haemorrhage suspected on CCT was then correctly diagnosed as cavernoma by MRI. The remaining six positive CCT were assessed as artefacts, in retrospect, because of the negative MRI scan. The rate of CCT scans that were supposed to be false-positive CCT findings was 17%. Compared with CCT, MRI detected 10 additional lesions: 6 contusions and 4 subarachnoid haemorrhages. Compared with CCT, MRI detected 10 additional lesions: 6 contusions and 4 subarachnoid haemorrhages. However, none of both skull fractures was detected. Outcome Admitted for observation: 11 (26.8) Discharged: 30 (73.2) Results: S100B and MRI (cut-off value of 0.1mgl^{-1})- not time specified Sensitivity:100% Specificity: 25% TP: 5 FP: 27 FN: 0

Reference	**Linsenmaier, 2016** [40]
	TN: 9 PPV: 16% NPV: 100% <u>SN/SP calculated by NGC:</u> Sensitivity: 1.00 [0.48, 1.00] Specificity: 0.25 [0.12, 0.42]
Source of funding	No funding
Limitations	Risk of bias *(QUADAS 2 – risk of bias)*: very serious. Unclear whether the selection of patients could have introduced bias; unclear whether the index test was interpreted without knowledge of the reference standard and vice versa Indirectness *(QUADAS 2 – applicability)*: none
Comments	No information on treatment

Reference	**Mahan, 2019** [41]
Study type	Prospective cohort
Study methodology	Data source: The study enrolled patients presenting to the emergency department of Hennepin County Medical Center (Minneapolis, Minnesota, USA), an adult level I trauma center, from May i6, 2016, to May t, 2017.
Number of patients	n = 104
Patient characteristics	Age, years: with mean (SD) age of 52.7 years (19.6) ranging from 18.5 to 92.8

Reference	**Mahan, 2019** [41]
	Gender: 31 female, 73 male GCS: mixed severity TBI. Majority mild TBI GCS score 3-8: 28 (19.2) GCS score 9-12: 5 (4.8) GCS score 13-15: 79 (76.0) Ethnicity: Hispanic or Latino: 4 (3.8) Non-Hispanic or Latino: 96 (92.3) Unknown: 4 (3.8) Setting: ED Country: USA Inclusion criteria: those with suspected head trauma resulting in a clinically ordered CT scan of the head at the time of admission, and those with a blood specimen collected within 32 hours of time of injury with valid GFAP, StooB, and UCH-Li biomarker concentrations. Exclusion criteria: excluded if their time of injury could not be accurately identified, if they had a history of head trauma 6 months before admission, or if they were participating in another clinical study. Those with active psychiatric, neurologic, and/or developmental disorders also were excluded
Target condition(s)	Acute post-brain injury complications
Index test(s) and reference standard	Index test: • glial fibrillary acidic protein (GFAP) • ubiquitin C-terminal hydrolase L1 (UCH-L1) • S100 calcium-binding protein B (S100B)

Reference	**Mahan, 2019** [41]
	The initial blood sample was taken within 8 hours of the reported head injury. Specimen collection was repeated 12-32 hours after the reported time of injury. Reference standard: Head CT CT scans of the head receiving a Marshall Classification of Diffuse Injury I were labeled CT negative whereas all others were labeled CT positive.
Results	Outcome: CT positive CT scan of the head (68 CT negative and 36 CT positive) At the 0- to 8-hour timepoint GFAP -0- to 8-hour (cut-off value NR) Sensitivity: 0.89 (95% CI not reported) Specificity: 0.62 (95% CI not reported) Back calculation of 2x2 table by NGC: TP: 32 FP: 26 FN: 4 TN: 42

Reference	Mahan, 2019 [41]
	UCH-L1 -0- to 8-hour (cut-off value NR) Sensitivity: 0.52 (95% CI not reported) specificity**:** 0.50 (95% CI not reported) Back calculation of 2x2 table by NGC: TP: 19 FP: 34 FN: 17 TN: 34 S100 B alone -0- to 8-hour (cut-off value NR) Sensitivity: 0.63 (95% CI not reported) Specificity: 0.54 (95% CI not reported) Back calculation of 2x2 table by NGC: TP: 23 FP: 31 FN: 13

Reference	Mahan, 2019 [41]
	TN: 37 <u>The combination of GFAP and UCH-L1 -0- to 8-hour-(cut-off value NR)</u> Sensitivity: 0.87 (95% CI not reported) Specificity: 0.61 (95% CI not reported) <u>Back calculation of 2x2 table by NGC:</u> TP: 31 FP: 27 FN: 5 TN: 41 <u>Combination of all biomarkers 0- to 8-hour-(cut-off value NR)</u> sensitivity:0.86 (95% CI not reported) specificity: 0.61 (95% CI not reported) <u>Back calculation of 2x2 table by NGC:</u> TP: 31 FP: 27 FN: 5

Reference	Mahan, 2019 [41]
	TN: 41 <u>GFAP and S1ooB combination -0- to 8-hour-(cut-off value NR)</u> Sensitivity: 0.84 (95% CI not reported) Specificity: 0.60 (95% CI not reported) <u>Back calculation of 2x2 table by NGC:</u> TP: 30 FP: 27 FN: 6 TN: 41 <u>combination of UCH-L1 and S1ooB -0- to 8-hour-(cut-off value NR)</u> sensitivity: 0.56 (95% CI not reported) specificity: 0.51 (95% CI not reported) <u>Back calculation of 2x2 table by NGC:</u> TP: 20 FP: 33

Reference	Mahan, 2019 [41]
	FN: 16 TN: 35 At the 12- to 32-hour timepoint <u>GFAP -12- to 32-hour-(cut-off value NR)</u> Sensitivity: 0.94 (95% CI not reported) Specificity: 0.67 (95% CI not reported) <u>Back calculation of 2x2 table by NGC:</u> TP: 34 FP: 22 FN: 2 TN: 46 <u>S100 B alone -12- to 32-hour-(cut-off value NR)</u> Sensitivity: 0.72 (95% CI not reported) Specificity: 0.57 (95% CI not reported) <u>Back calculation of 2x2 table by NGC:</u>

Reference	Mahan, 2019 [41]
	TP: 26 FP: 29 FN: 10 TN: 39 UCH-L1 alone-12- to 32-hour-(cut-off value NR) Sensitivity: 0.61 (95% CI not reported) Specificity: 0.52 (95% CI not reported) Back calculation of 2x2 table by NGC: TP: 22 FP: 33 FN: 14 TN: 35 combination of GFAP and UCH-L1 -12- to 32-hour-(cut-off value NR) sensitivity: 0.93 (95% CI not reported) specificity: 0.67 (95% CI not reported)

Reference	Mahan, 2019 [41]
	Back calculation of 2x2 table by NGC: TP: 33 FP: 22 FN: 3 TN: 46 combination of all biomarkers -12- to 32-hour-(cut-off value NR) sensitivity: 0.93 (95% CI not reported) specificity: 0.67 (95% CI not reported) Back calculation of 2x2 table by NGC: TP: 33 FP: 22 FN: 3 TN: 46 combination of GFAP and S1ooB -12- to 32-hour-(cut-off value NR) sensitivity: 0.91 (95% CI not reported) specificity: 0.66 (95% CI not reported)

Reference	**Mahan, 2019** [41]
	Back calculation of 2x2 table by NGC: TP: 33 FP: 23 FN: 3 TN: 45 combination of UCH-L1 and S100 B -12- to 32-hour-(cut-off value NR) sensitivity: 0.74 (95% CI not reported) specificity: 0.59 (95% CI not reported) Back calculation of 2x2 table by NGC: TP: 27 FP: 28 FN: 9 TN: 40
Source of funding	Not stated
Limitations	Risk of bias *(QUADAS 2 – risk of bias)*: none Indirectness *(QUADAS 2 – applicability)*: serious. Mixed severity -mild moderate and severe
Comments	No information on treatment

Reference	**McMahon, 2015 [43] TRACK-TBI**
Study type	Prospective cohort study
Study methodology	Data source: This multi-centre, prospective, cohort study included patients 16–93 years of age presenting to three level 1 trauma centres with suspected TBI (loss of consciousness, post-trauma amnesia, and so on).
Number of patients	N=215
Patient characteristics	Age mean (SD) years: 42 (18) years Gender (male): 73% (156) GCS: mixed GCS. Majority with mild TBI Mild (GCS score 13-15): 83 (179) Moderate GCS score 9-12: 4(9) Severe GCS score 3-8: 13 (27) Ethnicity: not reported Setting: trauma centre Country: International Inclusion criteria: patients must have presented to an ED within 24 h of their injury and had a positive clinical screen for acute TBI necessitating a non-contrast head CT according to American College of Emergency Physicians/Centers for Disease Control and Prevention (ACEP/CDC) evidence-based joint practice guideline. Exclusion criteria: NR
Target condition(s)	Acute post-brain injury complications
Index test(s) and reference standard	<u>Index test:</u> Glial fibrillary acidic protein and its breakdown products (GFAP-BDP)

Reference	**McMahon, 2015 [43] TRACK-TBI**
	Serum GFAP-BDP levels were drawn within 24 h and analysed. Plasma samples obtained within 24 h of injury (mean 10.9 h, SD 6.4 h, min 0.5 h, max 23.4 h) Reference standard: CT imaging All patients underwent CT imaging of the brain at the time of initial presentation to the ED.
Results	Outcome: intracranial injury on CT Fifty-one percent (n = 110) of patients presenting with positive clinical screen for TBI had intracranial pathology demonstrated on admission CT. n=105 CT negative Results: GFAP-BDP (a cut-off value to maximize accuracy in the mild and moderate injury range specifically yielded a GFAP-BDP level of 0.6 ng/mL)- within 24 hrs Sensitivity: 67%, Specificity: 89% Back calculation of 2x2 table by NGC: TP: 74 FP:12 FN: 36

Reference	**McMahon, 2015 [43] TRACK-TBI**
	TN:93 GFAP-BDP level (A cut-off value to maximise specificity was calculated at a GFAP-BDP concentration of 1.66 ng/mL) - within 24 hrs Sensitivity: 45% Specificity: 99% Back calculation of 2x2 table by NGC: TP: 50 FP: 1 FN: 60 TN: 104 To assess the diagnostic performance of GFAP-BDP, the AUC for GFAP-BDP that was calculated to discriminate patients with traumatic lesions on head CT was 0.88 (95% confidence interval [CI], 0.84–0.93, p < 0.000001)
Source of funding	This work was funded by the National Institutes of Health (grant no.: 1RC2 NS069409).
Limitations	Risk of bias *(QUADAS 2 – risk of bias)*: none Indirectness *(QUADAS 2 – applicability)*: mixed severity GCS (mild moderate and severe)
Comments	No information on treatment

Reference	**Muller, 2007** [49]
Study type	Prospective cohort
Study methodology	Data source: This prospective study recruited patients from four centers in Europe (Department of Neurosurgery, University Hospital of North Norway, Troms0, Norway; Emergency Department, Hope University Hospital, Salford, UK, Department of Orthopaedic Surgery, Spital Oberen-gadin, St. Moritz, Switzerland; and Department of Neurosurgery, University Hospital of Lund, Sweden
Number of patients	n = 226
Patient characteristics	Age, years: Mean 39 (range, 18-92) years Gender: 168 (74.3%) men, and 58 (25.7%) GCS: mild TBI (GCS 13 o 15) GCS score 13: 16 (7) GCS score 14: 30 (13) GCS score 15: 180 (78) Ethnicity: not reported Setting: ED Country: 4 centres in Europe Inclusion criteria: Patients with head injury were assessed for possible inclusion during a 4-year period (2001-2005). The inclusion criteria were the following: History of head injury, Loss of consciousness (LOC) or retrograde amnesia, GCS score of 13 to 15 at admission, Blood sampling within 12 hours of trauma, First CT scan within 12 hours of trauma, Signed written informed consent (optional, according to local ethical committee's requirements) Exclusion criteria: History of neurologic or psychiatric disorder, Focal neurologic deficit· Multiple injuries, defined as trauma to the face, chest, abdomen, extremities, or pelvic girdle requiring immediate therapeutic intervention, Renal or liver disease, Age <18 years

Reference	**Muller, 2007** [49]
Target condition(s)	Acute post-brain injury complications
Index test(s) and reference standard	Index test: Serum S100B Blood samples for S100B analysis and head CT were obtained within 12 hours after the injury Reference standard: Head CT
Results	Outcome: Intracranial pathologic findings revealed by CT scan (not defined) CT showed intracranial abnormality in 21 (9%) patients. Seventeen patients (82%) had contusion, two (9%) had subdural hematoma, and two patients (9%) had epidural hematoma. None underwent neurologic deterioration, and none needed surgical treatment. S100B (Cut-off ≥0.10 µg/L) (within 12 hours of injury) area under the curve, 0.73; 95% CI, 0.62-0.84; p = 0.001 ROC analysis showed S100B to be a significant discriminator of CT abnormality Sensitivity: 0.95 (95% CI, 0.76-1.0) Specificity: 0.31 (95% CI, 0.25-0.38) positive predictive value: 0.12 (95% CI, 0.080.19) negative predictive value: 0.98 (95% CI, 0.92-1.0) TP: 20 FP:141 FN: 1 TN: 64 SN/SP calculated by NGC: sensitivity: 0.95 [0.76, 1.00] specificity: 0.31 [0.25, 0.38]
Source of funding	Not stated

Reference	**Muller, 2007** [49]
Limitations	Risk of bias *(QUADAS 2 – risk of bias)*: very serious. Unclear whether the selection of patients could have introduced bias; unclear whether the index test was interpreted without knowledge of the reference standard and vice versa Indirectness *(QUADAS 2 – applicability)*: none
Comments	No information on treatment

Reference	**Morochovic, 2009** [44]
Study type	Prospective cohort
Study methodology	Data source: The prospective study comprised consecutive patients of all ages, who presented to the trauma emergency department with history of MTBI between December 2006 and December 2007
Number of patients	n = 102
Patient characteristics	Age, years: mean age 42.0 (SD 19.7, range 12—84 years) Gender: 71 males and 31 females, GCS: mild TBI (GCS 13-15) GCS score 13: 3 GCS score 14: 23 GCS score 15: 76 Ethnicity: not reported Setting: ED Country: Slovak Republic

Reference	**Morochovic, 2009 [44]**
	Inclusion criteria: adults with mild TBI. Patients with chronic intracerebral lesions were included to the study except suspected/visible brain tumour. Exclusion criteria: Any patients with unknown time of injury or acute non-traumatic intracerebral lesions were excluded from the study.
Target condition(s)	Acute post-brain injury complications
Index test(s) and reference standard	Index test: Serum S100B Peripheral venous blood samples were taken within 6 h of the injury and were sent to biochemical laboratory within 30 min. Reference standard: Cranial CT (CCT) CCT scan was performed in all patients involved in the study within 30 min of blood drawing.
Results	Outcome: Any intracranial pathology on CCT Any intracranial pathology associated with an injury (acute subdural, epidural or parenchymal hematoma, traumatic subarachnoid haemorrhage, cerebral contusion and brain swelling) detectable on CCT scan was considered positive (CCT +). Intracranial injuries detectable on CCT scans were present in eighteen (17.6%) patients and negative CCT scans in 84 (82.4%) patients. There were 74 (72.5%) patients with serum S 1 00B level above 0.1 ng/ml and 28 (27.5%) below cut off point. S100B- cut-off (≥ 0.1 ng/ml) Sensitivity: 83.3% (95% CI 0.58-0.96)

Reference	**Morochovic, 2009** [44]
	Specificity: 29.8% (95% CI 0.21-0.41) positive predictive value: 20.3% (95% CI 0.12-0.32) negative predictive value: 89.3% (95% CI 0.71-0.97) TP: 15 FP: 59 FN: 3 TN: 25 SN/SP calculated by NGC Sensitivity: 0.83 [0.59, 0.96] Specificity: 0.30 [0.20, 0.41]
Source of funding	This work was supported by the scientific grant agency of The Ministry of Education of the Slovak Republic (ME SR) and of The Slovak Academy of Sciences (SAS) No. 1/4260/07
Limitations	Risk of bias *(QUADAS 2 – risk of bias)*: none Indirectness *(QUADAS 2 – applicability)*: none
Comments	Three patients from CCT + group had negative plasma level of S100B, two of whom required surgical treatment.

Reference	**Muller, 2011** [48]
Study type	Prospective cohort

Reference	**Muller, 2011** [48]
Study methodology	Data source: Between January 2008 and August 2009, all patients with mild head trauma admitted to the ER of our regional trauma centre were consecutively enrolled. According to our in-house policy, all patients with head injuries undergo CCT
Number of patients	n = 233
Patient characteristics	Age, years: 48.4 years (range 11–97; 25–75% quartile 24–72). Gender: 143 were men and 90 were women GCS: mild TBI (GCS score 13 o 15) Ethnicity: not reported Setting: ED Country: Switzerland Inclusion criteria: All adult patients (≥16 years) with mild head trauma (GCS score of 13–15) were included in the study. Exclusion criteria: Patients suffering from cancer, stroke or other neurological diseases, or presenting with intracranial bleeds with a diameter greater than 5 mm or >1 bleed, a history of inherited coagulopathy or anticoagulant therapy, platelet aggregation inhibitor therapy or intoxication were excluded. Patients with late admissions to the ER and/or multiple associated injuries were also excluded from the study group
Target condition(s)	Acute post-brain injury complications
Index test(s) and reference standard	Index test: S100B Median time between admission and blood sampling was 77 min (25–75% quartile 60–120). Reference standard: Cranial CT (CCT)

Reference	**Muller, 2011** [48]
	After blood sampling, all patients underwent a CCT scan
Results	Outcome: positive CT findings (not defined) A positive S-100B level in the blood was found in 169/233 (72.5%) mild head injury patients (>0.105 µg/l). Findings in the remaining patients were negative. There were 22/233 (9.4%) positive CCT scans and the remainder were negative. <u>S100B (cut-off 0.105 µg/l)- median time 77 min</u> Sensitivity: 86.4% (no CI reported) Specificity: 12.2% (no CI reported) PPV :12.8% (no CI reported) NPV: 85.7% (no CI reported) <u>Raw data:</u> TP: 19 FP: 144 FN: 3

Reference	**Muller, 2011** [48]
	TN: 67 SN/SP calculated by NGC Sensitivity: 0.86 [0.65, 0.97] Specificity: 0.32 [0.26, 0.38]
Source of funding	Not stated
Limitations	Risk of bias *(QUADAS 2 – risk of bias)*: very serious. Unclear whether the selection of patients could have introduced bias; unclear whether the index test was interpreted without knowledge of the reference standard and vice versa Indirectness *(QUADAS 2 – applicability)*: none
Comments	No information on treatment

Reference	**Oh, 2007** [51]
Study type	Prospective cohort
Study methodology	Data source: This study consisted of 101 patients who were admitted to the emergency department within 6 hr after the onset of acute brain injury (ABI) symptoms
Number of patients	n = 101 (n= 45 patients with traumatic ABI and= 56 patients with nontraumatic ABI)
Patient characteristics	Age, years, mean (SD): 45 years [31–59] Gender (male): 57.9% male GCS: mixed severity (mild, moderate and severe). Majority with mild TBI (80%) The patients were stratified into three subgroups on the GCS: mild (13–15), moderate (8–12), and severe (<8) GCS score 13–15: 82 GSC score 9–12: 9 GCS score <8:10

Reference	**Oh, 2007** [51]
	Ethnicity: not reported Setting: ED Country: Korea Inclusion criteria: admitted to emergency department within 6 hr after the onset of ABI symptoms (no further details) Exclusion criteria: The patients with metabolic disorders or seizure did not take imaging test and were excluded in this study
Target condition(s)	Acute post-brain injury complications
Index test(s) and reference standard	Index test: Serum S100 levels (by Elecsys S100 immunoassay) Measurement within 6 hr after symptom onset Reference standard: Cranial CT (CCT) or MRI All patients underwent initial CCT or MRI testing. The patients who showed negative findings in CCT were confirmed by MRI.
Results	Outcome: acute traumatic brain injury (CCT negative or MRI positive) Traumatic acute brain injury: n= 45. There were 45 patients with traumatic ABI and 56 patients with nontraumatic ABI. S100B (Cut-off 0.105 mg/ L) (n=45 people with acute TBI)- within 6 hr (CCT negative or MRI positive) Sensitivity%: 96.9 (no CI reported)

Reference	**Oh, 2007** [51]
	Specificity%: 53.8 (no CI reported) PPV%: 83.8 (no CI reported) NPV%: 87.5 (no CI reported) <u>Back calculation of 2x2 table by NGC:</u> TP: 45 FP: 26 FN: 0 TN: 30
Source of funding	Not stated
Limitations	Risk of bias *(QUADAS 2 – risk of bias)*: very serious. Unclear whether the selection of patients could have introduced bias; unclear whether the index test was interpreted without knowledge of the reference standard and vice versa Indirectness *(QUADAS 2 – applicability)*: serious. Mixed severity population (mild, moderate and severe)
Comments	No information on treatment

Reference	**Okonkwo, 2020** [52] **TRACK-TBI**
Study type	Prospective cohort
Study methodology	Data source: Subjects with TBI (GCS 3–15) were identified and enrolled prospectively in the TRACK-TBI study (TRACK-TBI. Subjects presenting to one of 18 participating level I United States trauma centers were enrolled from February 26, 2014 to July 27, 2018. T
Number of patients	n = 1497 (810 CT negative, CT positive n = 549)

Reference	**Okonkwo, 2020 [52] TRACK-TBI**
Patient characteristics	Age, mean (SD) years: CT (-): 37.7 (15.9) CT (+): 43.7 (17.9) Gender (male): CT (-):516 (63.7%) CT (+):408 (74.3%) GCS: mixed severity (mild, moderate and severe). Majority with mild TBI CT (-): GCS score 13-15: 779 (98%) GCS score 9-12: 8 (1%) GC score S 3-8: 8 (1%) CT (+): GCS score 13-15: 358 (74%) GCS score 9-12: 40 (8%) - GCS score 3-8: 85 (18%) Ethnicity: not reported Setting: trauma centre Country: USA Inclusion criteria: presentation within 24 h of injury with head trauma warranting clinical evaluation with a non-contrast head CT evaluation based on the 2008 American College of Emergency Physicians/Centers for Disease Control (ACEP/CDC) guidelines for neuroimaging and decision making in TBI. Exclusion criteria: NR
Target condition(s)	Acute post-brain injury complications

Reference	**Okonkwo, 2020 [52] TRACK-TBI**
Index test(s) and reference standard	Index test: Serum: • GFAP • S100B Blood samples were collected from subjects who consented to genetic and proteomic analysis within a 24 h window from time of injury Time to blood draw (hours): 13.1 (6.8) Reference standard: Head CT
Results	Outcome: intracranial injury on admission CT scan CT- (n = 810) CT+ (n = 549) GFAP cut-off 13.1 pg/ml (within 24h of injury) Sensitivity: 0.994 (95% CI 0.986, 1) Specificity: 0.157 (95% CI 0.131, 0.182) PPV: 0.351 (95% CI 0.344, 0.359) NPV: 0.985 (95% CI 0.961, 1) Back calculation of 2x2 table done by NGC: TP: 546 FP: 683

Reference	Okonkwo, 2020 [52] TRACK-TBI
	FN: 3 TN: 127 <u>GFAP cut-off 37.8 pg/ml (within 24h of injury)</u> Sensitivity: 0.964 (95% CI 0.944, 0.980) Specificity: 0.303 (95% CI 0.271, 0.340) PPV: 0.389 (95% CI 0.377, 0.402) NPV: 0.949 (95% CI 0.921, 0.973) <u>Back calculation of 2x2 table done by NGC:</u> TP: 529 FP: 565 FN: 20 TN: 245 <u>GFAP cut-off 113.3 pg/ml (within 24h of injury)</u> Sensitivity: 0.902 (95% CI 0.869, 0.933) Specificity: 0.498 (95% CI 0.466, 0.530) PPV: 0.452 (95% CI 0.435, 0.470) NPV: 0.917 (95% CI 0.891, 0.941)

Reference	Okonkwo, 2020 [52] TRACK-TBI
	Back calculation of 2x2 table done by NGC: TP: 495 FP: 407 FN: 54 TN: 403 GFAP cut-off 190.1 pg/ml (within 24h of injury) Sensitivity: 0.846 (95% CI 0.810, 0.883) Specificity: 0.594 (95% CI 0.561, 0.630) PPV: 0.490 (95% CI 0.466, 0.515) NPV: 0.894 (95% CI 0.872, 0.917) Back calculation of 2x2 table done by NGC: TP: 464 FP: 329 FN: 85 TN: 481 AUCs for GFAP and S100B of CT+ versus CT-

Reference	Okonkwo, 2020 [52] TRACK-TBI
	0-6 h post-injury GFAP AUC: 0.93 (95% CI 0.880-0.977) S100B AUC: 0.77 (95% CI 0.681-0.859) 7-12 h post-injury GFAP AUC: 0.81 (95% CI 0.761-0.865) S100B AUC:0.67 (95% CI 0.601-0.743) 13-18 h post-injury GFAP AUC: 0.84 (95% CI 0.800-0.881) S100B AUC: 0.71 (95% CI 0.652-0.760)

Reference	**Okonkwo, 2020 [52] TRACK-TBI**
	19-24 h post-injury GFPA AUC: 0.85 (95% CI 0.815-0.882) S100B AUC: 0.68 (95% CI 0.634-0.730)
Source of funding	This work was supported by the following grants: National Institute of Neurological Disorders (NINDS) 1RC2NS069409-01, 3RC2NS069409-02S1, 5RC2NS069409-02, 1U01NS086090-01, 3U01NS086090-02S1, 3U01NS086090-02S2, 3U01NS086090- 03S1, 5U01NS086090-02, and 5U01NS086090-03; US Department of Defense (DOD) W81XWH-13-1-0441, and US DOD W81XWH-14-2-0176.
Limitations	Risk of bias *(QUADAS 2 – risk of bias)*: very serious. Unclear whether the selection of patients could have introduced bias; unclear whether the index test was interpreted without knowledge of the reference standard and vice versa Indirectness *(QUADAS 2 – applicability)*: serious. Mixed severity (mild, moderate and severe)
Comments	No information on treatment

Reference	**Okonkwo, 2013 [53]**
Study type	Prospective cohort study
Study methodology	Mixed (mild, moderate and severe) traumatic brain injury patients Data source: Transforming Research and Clinical Knowledge in Traumatic Brain Injury (TRACK-TBI) study which is a National Institute of Neurological Disorders and Stroke (NINDS)-funded multicentre, prospective, collaboration among four United States centres to develop, test and refine TBI common data elements (TBI-CDEs) for research in four domains: demographics, neuroimaging, biomarkers, and outcome measures.

Reference	Okonkwo, 2013 [53]
	Recruitment: Subjects were identified and recruited upon arrival at one of three level I trauma centres involved in the TRACK-TBI study
Number of patients	Total n = 215 Mild n= 179 Moderate n=9 Severe n = 27
Patient characteristics	Age, mean (SD): Mild 42.5 (18) Moderate 44.1 (19.5) Severe 39.2 (18.9) Gender (male to female ratio): Mild 69.8% M: 30.2% F Moderate 100% M: 0% F Severe 81.5% M: 18.5% F GCS score, mean (SD): mixed severity. majority with mild TBI- 83% had GCS score 13-15 Mild 14.8 (0.44)

Reference	Okonkwo, 2013 [53]
	Moderate 11.22 (0.67) Severe 3.59 (1.31) Positive CT findings on admission Mild 42.5% Moderate 77.8% Severe 96.3% Ethnicity: Not reported Setting: Multicentre – 3 Level I trauma centres participating in the TRACK-TBI study Country: USA Inclusion criteria: patients presenting within 24 h of injury with a history of trauma to the head sufficient to be triaged to non-contrast head CT using the American College of Emergency Physicians/Centres for Disease Control (ACEP/CDC) evidence-based joint practice guideline. Exclusion criteria: Not stated in paper These were reported on clinical trials.gov: presentation to Emergency Department > 24 hours post-injury, custody or Incarceration, 5150 Psychiatric Hold.

Reference	**Okonkwo, 2013** [53]
Target condition(s)	Acute post brain injury complications
Index test(s) and reference standard	Index test GFAP and breakdown products (GFAP-BDP) Reference standard All patients underwent CT imaging of the brain at the time of initial presentation to the ED. Each patient's head CT was characterized using the recommendations of the TBI-CDE Neuroimaging Working Group, a set of consensus-based recommendations for data collection regarding specific radiological features, data definitions needed to characterise injuries, and best practices needed to optimise and harmonise imaging data acquisition for TBI research. Time between measurement of index test and reference standard: unclear CT scans performed at time of initial presentation the ED Blood samples collected within 24 hours of injury, mean (SD) 10.9 h (6.4 h) [min 0.5 h, max 23.4 h]
Results	Outcomes: Intracranial pathology on CT CT+ = 109 CT -= 106 Diagnostic accuracy of GFAP-BDP: GFAP-BDP at a 0.68ng/mL optimal value - within 24 hrs Sensitivity (%) = 73 (95%CI =64–81) Specificity (%) = 89 (95%CI = 81–94)

Reference	**Okonkwo, 2013** [53]
	PPV (%) = 87 (95%CI =78–92) NPV (%) = 76 (95%CI =68–83) <u>Back calculation of 2x2 table done by NGC:</u> TP: 80 FP: 12 FN: 29 TN: 94 The ability of GFAP-BDP level to for diagnosing TBI subjects with pathological CT features AUC 0.88 (95% confidence interval [CI], 0.84–0.93).
Source of funding	National Institute of Neurological Disorders and Stroke (NINDS)
Limitations	Risk of bias (QUADAS 2 – risk of bias): none Indirectness (QUADAS 2 – applicability): serious. Included patients with GCS score 9-12
Comments	Demographic analysis of patients lost to follow-up at 6 months revealed no significant difference in age or gender, but a significant difference in admission GCS score (p = 0.019). Of patients lacking 6 month data, 94% sustained mild TBI, whereas 78% of patients with 6 month follow-up data sustained mild TBI.

Reference	**Papa, 2012** [54]
Study type	Prospective cohort
Study methodology	Data source: study enrolled a convenience sample of adult patients with suspected TBI following blunt head trauma presenting to the emergency department within 4 hours of injury with a GCS score of 9 to 15.
Number of patients	n = 108
Patient characteristics	Age, years mean (SD): 39 (±15) Gender (male): 70(65%) GCS: mixed severity. Majority with mild TBI GCS score 13–15: 97 GCS score 9–12: 11 Ethnicity: not reported Setting: Emergency Departments (ED) of three Level I Trauma Centers; Shands at University of Florida in Gainesville, Florida; Orlando Regional Medical Center in Orlando, Florida; and Washington University in St. Louis, Missouri. Country: USA Inclusion criteria: Eligibility for suspected mild TBI was determined by the treating physician based on the history of blunt head trauma followed by either loss of consciousness, amnesia, or disorientation and presenting to the emergency department within 4 hours of injury with a GCS score of 9 to 15. Exclusion criteria: Patients were excluded if: 1) they were less than 18 years old; 2) there was no history of trauma as their primary event (e.g. syncope or seizure); 3) they had known dementia, chronic psychosis or active CNS pathology; or 4) were pregnant.
Target condition(s)	Acute post-brain injury complications
Index test(s) and reference standard	Index test: Serum GFAP-BDP

Reference	**Papa, 2012** [54]
	Blood samples were obtained after arrival to the ED and within 4 hours of the reported time of injury. There was only one serum GFAP-BDP biomarker level analysed per patient in the 4-hour post-injury period. The average time to serum collection for TBI patients was 2.6 hours (95%CI 2.4–2.9) <u>Reference standard:</u> Head CT Patients underwent standard CT scan of the head according to the judgment of the treating physician. Radiologists were blinded to the study protocol but had the usual clinical information. Lab personnel running the samples were blinded to the clinical data.
Results	Outcome: intra cranial lesions on CT CT scan of the head was performed in all TBI patients and traumatic intracranial lesions on CT scan were evident in 32 (30%): 24 (75%) of patients presented with a GCS score 13–15 and 8 (25%) with GCS score 9–12. GFAP-BDP demonstrated a rapid appearance in serum post-injury with levels detectible within an hour of injury. Some of the higher levels were seen starting at approximately 2 hours post-injury. <u>Serum GFAP-BDP (cut-off level of 0.035 ng/ml) -within 4h of injury</u> Sensitivity: 97% (95%CI 82–100) Specificity: 18% (95%CI 11–28) negative predictive value: 94% (95%CI 68–100) positive predictive value: 31% (95%CI 22–41)

Reference	**Papa, 2012** [54]
	AUC: 0.79 (95%CI 0.69–0.89) The area under the curve for discriminating between CT scan positive and CT scan negative intracranial lesions was 0.79 (95%CI 0.69–0.89) <u>2x2 table</u> TP: 31 FP: 70 FN: 1 TN: 15 <u>SN/SP calculated by NGC</u> Sensitivity: 0.97 [0.84, 1.00] Specificity: 0.18 [0.10, 0.27]
Source of funding	This study was supported in part by Department of Defense Award number DoD W81XWH-06-1-0517. The project was supported in part by Award Number R01NS057676 from the National Institute of Neurological Disorders and Stroke
Limitations	Risk of bias *(QUADAS 2 – risk of bias)*: none Indirectness *(QUADAS 2 – applicability)*: serious. Mixed severity mild and moderate
Comments	Neurosurgical intervention was performed on 14 patients (13%), 6 (43%) presented with a GCS score 13–15 and 8 (57%) with GCS score 9–12

Reference	**Papa, 2012** [55]
Study type	Prospective cohort
Study methodology	Data source: This prospective controlled cohort study enrolled a convenience sample of adult patients with blunt head trauma followed by either loss of consciousness, amnesia, or disorientation and presenting to the emergency department within 4 hours of injury with a GCS of 9 to 15
Number of patients	n = 96
Patient characteristics	Age, years, mean (SD):39 (±15) Gender (male/female): 64/36 GCS: Mild and moderate TBI n= 86 with GCS score 13–15 n= 10 with GCS score 9–12 Ethnicity: not reported Setting: Study sites included the Emergency Departments (ED) of three Level I Trauma Centers; Shands at University of Florida in Gainesville, Florida; Orlando Regional Medical Center in Orlando, Florida; and Washington University in St. Louis, Missouri. Country: USA Inclusion criteria: adult patients with blunt head trauma followed by either loss of consciousness, amnesia, or disorientation and presenting to the emergency department within 4 hours of injury with a GCS score of 9 to 15 Exclusion criteria: Patients were excluded if: 1) they were less than 18 years old; 2) there was no history of trauma as their primary event (e.g. syncope or seizure); 3) they had known dementia, chronic psychosis or active CNS pathology; 4) were pregnant, or 5) were incarcerated.
Target condition(s)	Acute post-brain injury complications

Reference	**Papa, 2012** [55]
Index test(s) and reference standard	Index test: Ubiquitin C-terminal hydrolase (UCH-L1) Blood samples were obtained shortly after arrival to the ED and within 4 hours of the reported time of injury. The average time to serum collection for TBI patients was 2.7 hours (95%CI 2.4–2.9) Reference standard: Head CT
Results	Outcome: Intracranial lesions on CT Intracranial lesions on CT included any acute traumatic intracranial lesions visualized on CT scan. CT scan of the head scan was performed in all TBI patients and traumatic intracranial lesions on CT scan were evident in 28 (29%): 23% of patients with GCS score 13–15 and 80% of those with GCS score 9–12. UCH-L1 (cut-off level of 0.09 ng/ml) – within 4h of injury TP: 28 FP: 61 FN: 0 TN: 16 Sensitivity:100% (95%CI 88–100) Specificity: 21% (95%CI 13–32) Negative predictive value: 100% (95%CI 76–100)

Reference	**Papa, 2012** [55]
	Positive predictive value: 31% (95%CI 22–42) AUC: 0.73 (95%CI 0.62–0.83) The area under the curve for discriminating between CT scan positive and CT scan negative intracranial lesions was 0.73 (95%CI 0.62–0.83) <u>SN/SP calculated by NGC</u> Sensitivity: 1.00 [0.88, 1.00] Specificity: 0.21 [0.12, 0.32]
Source of funding	This study was supported in part by Department of Defense Award number DoD W81XWH-06-1-0517. The project was supported in part by Award Number R01NS057676 from the National Institute of Neurological Disorders and Stroke.
Limitations	Risk of bias *(QUADAS 2 – risk of bias)*: none Indirectness *(QUADAS 2 – applicability)*: serious. Mixed severity (mild and moderate)
Comments	Neurosurgical intervention was performed on 14 (14%) patients: 6 (43%) presented with GCS score 13–15 and 8 (57%) with GCS score 9–12. Neurosurgical intervention was defined as either death within 7 days secondary to head injury or the need for any of the following procedures within 7 days: craniotomy, elevation of skull fracture, intracranial pressure monitoring, or intubation for head injury

Reference	**Posti, 2019** [60]
Study type	Prospective cohort
Study methodology	Data source: Recruitment of patients with TBIs of all severities at Turku University Hospital, Finland, during November 2011 to October 2013
Number of patients	n = 160
Patient characteristics	Age, years mean (SD): 47.2 (19.6) years Gender: 117 males (73.1%) and 43 females (26.9%), with a mean age of GCS: mixed severity Isolated all severities: n = 94 Mild TBI: n = 93 Isolated mild TBI: n = 55 Ethnicity: not reported Setting: ED Country: Finland Inclusion criteria: age >18 years, clinical diagnosis of TBI, and indications for acute head CT according to the National Institute for Health and Care Excellence (NICE) criteria Exclusion criteria: blast-induced or penetrating injury, chronic subdural hematoma, inability to live independently as a result of pre-existing brain disease, TBI or suspected TBI not needing head CT, >2 weeks from the injury, not speaking the local language, and no consent obtained.
Target condition(s)	Acute post-brain injury complications

Reference	**Posti, 2019** [60]
Index test(s) and reference standard	Index test: • Glial fibrillary acidic protein (GFAP) • Heart fatty-acid binding protein (H-FABP) • Anti-inflammatory mediator interleukin 10 (IL-10) • neurofilament light chain (NF-L) • S100B Samples were obtained within 24 h of admission Reference standard: CT
Results	Outcome: CT positive CT scans were classified according to the Marshall grading system. Diffuse injury/grade I (no visual pathology) was considered CT-, whereas the other grades (II-VI) were regarded as CT+. Among all patients, a negative CT was found in 65 patients (40.6%) and a positive CT in 95 (59.4%). Ability of the Individual Biomarkers in Discriminating CT-Negative and CT-Positive Patients with Isolated Mild TBI (All, n = 55; CT-Negative, n = 36; CT-Positive, n = 19) GFAP AUC: 0.749 (95% CI 0.614–0.883) Specificity (at 100% sensitivity): 19.4 (cut-off 66.6)

Reference	Posti, 2019 [60]
	H-FABP AUC: 0.699 (95% CI 0.559–0.839) Specificity (at 100% sensitivity): 19.4 (cut-off 2520) S100B AUC: 0.689 (95% CI 0.833–0.544) Specificity (at 100% sensitivity): 11.1 (cut-off 179) NF-L AUC: 0.662 (95% CI 0.512–0.812) Specificity (at 100% sensitivity): 5.6 (cut-off 4.18) IL-10 AUC: 0.515 (95% CI 0.347–0.683) Specificity (at 100% sensitivity): 2.8 (cut-off 0.14) Ability of the Individual Biomarkers in Discriminating CT-Negative and CT-Positive Patients with Mild TBI (All, n = 93; CT-Negative n = 56; CT-Positive n = 37) GFAP AUC: 0.720 (95% CI 0.616–0.820)

Reference	Posti, 2019 [60]
	Specificity (at 100% sensitivity): 16.1 (cut-off 66.62) <u>NF-L</u> AUC: 0.676 (95% CI 0.563–0.780) Specificity (at 100% sensitivity):7.1 (cut-off 4.43) <u>H-FABP</u> AUC: 0.642 (95% CI 0.525–0.750) Specificity (at 100% sensitivity): 1.8 (cut-off 1709.61) <u>S100B</u> AUC: 0.569 (95% CI 0.445–0.693) Specificity (at 100% sensitivity): 0 (-) <u>IL-10</u> AUC: 0.583 (95% CI 0.463–0.703) Specificity (at 100% sensitivity): 5.4 (cut-off 0.14)
Source of funding	Partially funded by the European Commission under the 7th Framework Programme (FP7-270259- TBIcare), Government's Special Financial Transfer tied to academic research in Health Sciences (Finland) (JPP), Emil Aaltonen Foundation (JPP), Finnish Brain Foundation (JPP), Integra EANS Research Grant (IH), University of Turku Graduate School funding (MM), NIHR Research Professorship and the NIHR Cambridge BRC (PJH), NIHR Research UK (through a Senior Investigator

Reference	**Posti, 2019** [60]
	Award and the Cambridge Biomedical Research Centre) (DKM), Academy of Medical Sciences/The Health Foundation Clinician Scientist Fellowship (VFN); Wallenberg Academy Fellowship and grants from the Swedish and European Research Councils (HZ), Torsten So¨derberg Professorship in Medicine, award by the Royal Swedish Academy of Sciences, grants from the Swedish Research Council (KB).
Limitations	Risk of bias *(QUADAS 2 – risk of bias)*: serious. Unclear whether the selection of patients could have introduced bias Indirectness *(QUADAS 2 – applicability)*: serious. Mixed severity
Comments	No information on treatment

Reference	**Romner, 2000** [61]
Study type	Prospective cohort
Study methodology	Data source: hospital admitted patients with mild, moderate, and severe head injury during a 5-year period (January 1993 to December 1997).
Number of patients	n = 278
Patient characteristics	Age: 32 (range, 1–84) years Gender: 175 (63%) men and 103 (37%) GCS: mixed severity, majority with mild TBI The head injuries were classified according to the HISS as either severe (GCS 3–8), moderate (GCS 9–13), or mild (GCS 14–15) Mild: 254 Moderate: 16 Severe 8 Ethnicity: not reported

Reference	**Romner, 2000** [61]
	Setting: ED-three centers in Scandinavia Country: Norway Inclusion criteria: head injury with loss of conscious- ness (LOC), (2) blood sample for S-100 analysis collected within 24 h after injury, and (3) CT scan performed within24 h after the injury. LOC was considered to have oc- curred when the patient had amnesia for the trauma event and if accompanying persons reported LOC Exclusion criteria: Patients with a history of neurological disease (e.g., multiple sclerosis, cerebral tumor, epilepsy, stroke, serious head injury) were excluded.
Target condition(s)	Acute post-brain injury complications
Index test(s) and reference standard	Index test: S100B A serum sample for S-100 analysis was drawn immediately (mean 3.8 h after injury; range, 0.5–24.0 h) after admission to the emergency room in the head-injured patients. Reference standard: CT scans of the brain and cranium In a subgroup of 45 patients with mild head injury (GCS score 14–15, LOC for , 20 min, absence of focal neurological deficits, and no signs of acute intracranial abnormality revealed by a CT scan), MRI was also performed.
Results	Outcome: Intra cranial findings on CT head-injured patients were dichotomized into those with nondetectable serum levels (S-100 negative group) and those with a serum level of at least 0.2 m g/L (S-100 positive group). Forty (14%) of the 278 patients demonstrated a pathologic CT scan. Fifteen (5%) had isolated skull fracture without intracranial pathology, and 25 (9%) showed intracranial lesions (main pathology: brain contusion n=13, subdural hematoma n 5 6, epidural hematoma n =2, traumatic subarachnoid hemorrhage n=2, and brain edema n= 2. Among the 45 mildly injured patients who underwent MRI, brain contusion was detected in five (11%). S100 B (cut-off 0.2 µ g/L) -mean 3.8 h after injury

Reference	**Romner, 2000** [61]
	TP: 23 FP: 85 FN: 2 TN: 168 Sensitivity: 92% Specificity: 66% positive predictive value: 0.23 negative predictive value: 0.99 SN/SP calculated by NGC Sensitivity: 0.92 [0.74, 0.99] Specificity: 0.66 [0.60, 0.72]
Source of funding	The study was supported by The Lærdal Foundation for Acute Medicine (Grant number 1629) and The Skane County Council's Research and Development Foundation.
Limitations	Risk of bias *(QUADAS 2 – risk of bias)*: very serious. Unclear whether the selection of patients could have introduced bias; unclear whether the index test was interpreted without knowledge of the reference standard and vice versa Indirectness *(QUADAS 2 – applicability)*: serious. Mixed severity (mild, moderate and severe)
Comments	Patients with severe head injury (GCS score ≤ 9) were intubated and ventilated in the emergency room before the initial CT scan. All patients were admitted for at least overnight observation

Reference	**Thaler, 2015** [62]
Study type	Prospective cohort study
Study methodology	Data source: This prospective, observational study was conducted from May 2011 to October 2013 at 2 trauma centers in Vienna, Austria
Number of patients	n = 782

Reference	**Thaler, 2015** [62]
Patient characteristics	Age median (IQR): 83 (74–88) years Patients with MHI who were receiving antiplatelet medication or who were older than 65 years were either admitted or observed for at least 6 hours. All patients underwent CCT. The decision whether a patient had to be admitted and the point in time at which CCT was performed depended on the clinical findings. W GCS: mild TBI (13-15) GCS score 13: 48 (6.1%) GCS score 14: 147 (18.8%) GCS score 15: 587 (75.1%) Gender (male): 245 (31.3%) Ethnicity: not reported Setting: trauma centres Country: Austria Inclusion criteria: minor head injury (MHI) (GCS Score 13–15) in patients on medication with h platelet aggregation inhibitors (PAI) who were older than 18 years, and MHI in patients age 65 years and older independent of PAI intake Exclusion criteria: Patients with MHI and severe trauma (open fractures, large open wounds, clinical signs of basal skull fracture, polytrauma), focal neurological deficits, posttraumatic seizures, anticoagulant therapy (vitamin K antagonists, direct oral anticoagulants), alcohol intoxication, and with a history of a coagulation disorder were excluded. Additional exclusion criteria were blood sampling more than 3 hours after index event, unknown point in time of the trauma, and missing informed consent.
Target condition(s)	Acute post-brain injury complications
Index test(s) and reference standard	<u>Index test:</u> Serum S100B

Reference	Thaler, 2015 [62]
	a venous blood sample was drawn within 3 hours after injury and the S100B level was determined. The result of S100B analysis was not known to the attending physician. All clinical assessments were completed before the CCT scans were performed The median interval between event and blood drawing was 2:05 hours (IQR 1:30–2:30). Reference standard: Cranial CT (CCT) The median interval between trauma and CCT was 15:40 hours (IQR 3:54– 21:30). Patients were classified into 2 groups: CCT negative (patients with MHI who had no signs of trauma-related intracranial bleeding) and CCT positive (patients with MHI who had at least 1 trauma-related intracranial haemorrhage: i.e., epidural, subdural, subarachnoidal, or intracerebral bleeding). Each CCT scan was interpreted by a consultant radiologist who was blinded to the S100B level. The CCT results were spot-checked (25%) by an independent, experienced radiologist who was blinded to patient data.
Results	Outcome: CCT positive (patients with MHI who had at least 1 trauma-related intracranial haemorrhage: i.e., epidural, subdural, subarachnoidal, or intracerebral bleeding). Of the 782 patients included, 732 (93.6%) proved to be CCT negative and 50 (6.4%) showed intracranial bleeding on CCT scans. Six hundred thirty-one patients (80.7%) were being treated with PAIs. Of the 631 patients on PAIs, 526 (83.3%) were taking low-dose aspirin, 68 (10.8%) were on clopidogrel, and 37 (5.9%) were taking both drugs. The respective number of intracranial haemorrhages was 32 (6.1%) in the aspirin group, 3 (4.4%) in the clopidogrel group, and 2 (5.4%) in the group on both drugs. Patients treated with PAIs had a lower rate of intracranial bleeding (5.9%) than those without PAIs (8.6%). This difference, however, is not statistically significant (p = 0.215) S100B (Cut-off 0.105 µg/L)- within 3 h

Reference	Thaler, 2015 [62]
	Sensitivity: 98.0% (CI 89.5%–99.7%) Specificity: 35.3% (CI 31.9%–38.8%) NPV 99.6% (CI 97.9%–99.9%) PPV 9.4% (CI 7.2%–12.2%) AUC 0.73 (CI 0.67–0.79, p < 0.001) Raw data: TP: 49 FP: 474 FN: 1 TN: 258 SN/SP calculated by NGC: Sensitivity: 0.98 [0.89, 1.00] Specificity: 0.35 [0.32, 0.39]
Source of funding	Not reported
Limitations	Risk of bias *(QUADAS 2 – risk of bias)*: none Indirectness *(QUADAS 2 – applicability)*: none

Reference	**Thaler, 2015** [62]
Comments	All patients were treated as they normally would have been, following the standard operating procedures of the institutions

Reference	**Vedin, 2021** [63]
Study type	Prospective cohort study
Study methodology	Data source: The study was conducted in Helsingborg General Hospital, Helsingborg, Sweden. The catchment area included 350,000 people.
Number of patients	n = 243 (n=13 with intracranial haemorrhage)
Patient characteristics	Age, years: 60.8 years (±44.96 years) GCS: mild TBI (GCS score 13–15). All patients were awake when they arrived in the emergency room. Gender: not reported Ethnicity: not reported Setting: hospital ED Country: Sweden Inclusion criteria: Population 1: Population 1 was selected for the study on S100B serum and urine levels of patients with isolated head trauma. patients who were 18 years or above and seeking emergency medical care due to isolated head trauma population 2: Population 2 was selected for the study on the serum and the urine S100B temporal profiles of patients with intracranial haemorrhage. patients who were 18 years or above and had CT-verified intracranial haemorrhage due to head trauma.

Reference	**Vedin, 2021** [63]
	Exclusion criteria: The patients who underwent neurosurgical intervention were excluded, because they were transferred to another hospital. patients under 18 years were excluded, as well as those with multi-trauma, as it might lead to false positive S100B levels due to extracerebral S100B (mainly from adipocytes and chondrocytes)
Target condition(s)	Acute post-brain injury complications
Index test(s) and reference standard	Index test: Urine and Serum S 100B Sampled < 6 hours or less from trauma. Reference standard: Head CT Timing of CT not reported.
Results	Outcome: Intracranial haemorrhage on CT The CT frequency was 151/243 (62.1%). Unconsciousness was confirmed in 58/243 (23.9%) cases, but in 38/243 (15.6%) cases, it could not be ascertained. Amnesia was present in 71/243 (29.2%) cases. All patients were awake when they arrived in the emergency room (13–15 on the Glasgow Coma Scale). Of the 243 patients, 37 (15.2%) took warfarin or an oral anticoagulant, 24 (9.9%) took 75 mg of aspirin, 2 (0.8%) were administered clopidogrel, 3 (1.2%) were given a combination of aspirin (75 mg once daily) and ticagrelor (90 mg twice daily), and 1 (0.4%) had a serious bleeding disorder The mean S100B concentrations were 0.151 (±0.225) µg/l in serum and 0.067 (±0.200) µg/l in urine. The median S-S100B of the 230 patients without intracranial haemorrhage was 0.12 (0.07–0.22 IQR) µg/l, and their median U-S100B was 0.07 (0.05–0.09 IQR) µg/l (41.7% lower than the serum level). The median S-S100B of the 13 patients with

Reference	Vedin, 2021 [63]
	intracranial haemorrhage was 0.18 (0.12–0.35 IQR) µg/l, and their median U-S100B was 0.08 (0.045–0.10 IQR) µg/l (66% lower than the serum level). 13 patients with CT-verified intracranial haemorrhage were included in population 2. Serum -S100B (cut-off ≥0.10 µg/L) - within 6h (population 2-with CT verified intracranial haemorrhage) Sensitivity: 97.0% (95% CI 89.5–99.2%) Specificity: 14.5% (95% CI 12.6–16.6%) Back calculation of 2x2 done by NGC: TP: 13 FP: 197 FN: 0 TN: 33 Urine -S100B (cut-off ≥0.09 µg/L)- within 6h (population 2-with CT verified intracranial haemorrhage) Sensitivity: 89.1% (95% CI 85.5–91.9%) specificity: 11.1% (95% CI 6.2–19.2%)

Reference	**Vedin, 2021** [63]
	Back calculation of 2x2 done by NGC: TP: 12 FP: 204 FN: 1 TN: 26 Serum S100B In population 1, the serum S100B in the samples drawn 6 h or less from trauma (12/201 patients with intracranial haemorrhage) had the best cut-off at 0.1 µg/l (AUC=0.589, 95% CI 0.436–0.741, p=0.304). In the combined populations 1 and 2, for the serum S100B samples drawn 6 h or less from trauma (23 samples from patients with intracranial haemorrhage), the AUC was 0.628 (95% CI 0.523–0.734, p=0.044). Urine S100B In population 1, the best cut-off for urine S100B, sampled within 6 h from trauma (10/180 patients with intracranial haemorrhage), was 0.09 µg/l (AUC=0.635, 95% CI 0.454–0.816, p=0.151). For the urine S100B samples drawn 6 h or less from trauma (21 samples from patients with intracranial haemorrhage), the AUC was 0.502 (95% CI 0.371–0.633, p=0.977).
Source of funding	The study was funded by the Gorthon Foundation, Helsingborg and Thelma Zoega Foundation for Medical Research, Helsingborg.
Limitations	Risk of bias *(QUADAS 2 – risk of bias)*: very serious. Unclear whether the selection of patients could have introduced bias; unclear whether the index test was interpreted without knowledge of the reference standard and vice versa

Reference	**Vedin, 2021** [63]
	Indirectness *(QUADAS 2 – applicability)*: none
Comments	Information on treatment

Reference	**Welch, 2016** [64]
Study type	Prospective cohort study
Study methodology	Data source: Study included patients 18–80 years of age who were evaluated and treated at one of seven study site hospital emergency departments (EDs) for a blunt closed head injury and potential mild to moderate TBI. The hospitals were composed of Level 1 and 2 trauma centres, a non-trauma centre, and included both U.S. and European sites
Number of patients	n = 251
Patient characteristics	Age, years: 45.6 (18.4) years GCS: mild and moderate TBI Of the 251 study patients, 225 (89.6%; 95% CI 85.2%–93.1%) had an initial GCS score of 15 of whom 24 (10.7%) had a positive CT scan. Among patients with a GCS score <15 (n=26) 12 (46.2%) had a positive CT scan. Gender: 60.2% (95% CI; 53.8%–66.3%) were male Ethnicity: not reported Setting: ED Country: US Inclusion criteria: patients were those with an initial Glasgow Coma Scale (GCS) score of 9–15 who underwent emergency head CT scan for evaluation of the head injury as deemed necessary by the attending ED physician. The subject was ‡18 years of age and no more than 80 years of age. Acceleration or deceleration closed injury to the head that was either self-

Reference	**Welch, 2016** [64]
	reported or witnessed. Presented to an emergency department (ED) within 4 h of injury. An initial Glasgow Coma Scale score of 9–15 in the ED performed by the Principal Investigator (PI) or trained study personnel. ED workup included a head computed tomography (CT) scan (based on standard practice and/or decision rules). Informed consent was obtained from the subject or his or her legal representative; oral consent for the initial blood draw and/or deferred consent to 24 h was allowed for patients who were unable to consent at initial evaluation or exception from the informed consent requirement by use of ‘‘community consent’’ if approved by an Institutional Review Board. The PI deemed the subject to be an appropriate study candidate. Included patients presented within 4 h of injury, completed the required CT scan as part of routine care, and had blood drawn for analysis within 6 h of injury Exclusion criteria: Participation in another clinical study that may affect the results of either study. Time of injury was not able to be accurately determined. Head CT not done as part of clinical emergency care. Primary diagnosis of ischemic or haemorrhagic infarct. Not available for 35-day follow-up visit. Venipuncture not feasible. Blood donation within 1 week of screening. The subject was otherwise determined medically unsuitable for study participation.
Target condition(s)	Acute post-brain injury complications
Index test(s) and reference standard	<u>Index test:</u> Serum: - GFAP - UCH-L1 - S100B Within 6 hours of injury Blood samples were collected at time of study enrolment and every 6 hours up to the time of discharge (either ED or hospital) or up to 24 h (maximum of five samples during index visit). Patients who were seen at follow-up (Day 35 – 5 days) had another sample obtained when feasible. <u>Reference standard:</u> Head CT

Reference	**Welch, 2016** [64]
	The neuroradiologists determined whether a CT scan was positive—defined as the presence of an acute trauma-related intracranial lesion Two of the neuroradiologists who had no access to any other clinical or laboratory data, except subject age and sex, reviewed all of the study subjects' CT scans.
Results	Outcome: Intracranial lesion on CT results of the head CT scan (positive/negative) among patients with the first blood sample drawn within 6 h of injury. CT scan was positive—defined as the presence of an acute trauma-related intracranial lesion After final diagnosis, adjudicated by a third independent radiologist, 36 patients (14.3%; 95% CI 10.3%–19.3%) had a CT scan that was positive for an acute intracranial lesion. <u>UCH-L1 (cut-off of 41 pg/mL) [6 hours -time from reported injury to blood sample obtained]</u> Sensitivity: 1.00 (0.90, 1.00) specificity: 0.40 (0.33, 0.47) <u>GFAP (cut-off of 0 pg/mL) [6 hours -time from reported injury to blood sample obtained]</u> Sensitivity: 1.00 (0.90, 1.00) Specificity: 0.00 (0.00, 0.02)

Reference	Welch, 2016 [64]
	Indicating that using the GFAP value associated with 100% sensitivity within 6 h of injury, the test could not reliably determine which patients had negative head CTs. S100B (30 pg/mL threshold) [6 hours -time from reported injury to blood sample obtained] Sensitivity:1.00 (0.89, 1.00) specificity: 0.02 (0.00, 0.04) S100B (100 pg/mL threshold) [6 hours -time from reported injury to blood sample obtained] Sensitivity: 0.91 (0.75, 0.98) Specificity: 0.44 (0.37, 0.51) GFAP & UCH-L1N (Threshold for positive test- 100 GFAP 40 UCH-L1) [6 hours -time from reported injury to blood sample obtained] Sensitivity: 1.00 (0.90, 1.00) Specificity: 0.39 (0.33, 0.46) GFAP & UCH-L1N (Threshold for positive test 100 GFAP 40 UCH-L1) [4 hours- time from reported injury to blood sample obtained.] Sensitivity: 1.00 (0.89, 1.00) Specificity: 0.37 (0.30, 0.44)

Reference	**Welch, 2016** [64]
	The combined biomarker strategy (both GFAP and UCH-L1) resulted in the same sensitivity and specificity estimates as that of UCH-L1 alone. <u>AUC</u> GFAP: 0.79 (95% CI 0.70–0.88) UCH-L1: 0.80 (95% CI 0.71–0.89) S100B: 0.75 (95% CI 0.65–0.85)
Source of funding	No funding
Limitations	Risk of bias *(QUADAS 2 – risk of bias)*: serious. Unclear whether the selection of patients could have introduced bias Indirectness *(QUADAS 2 – applicability)*: serious. Mixed severity includes mild and moderate TBI
Comments	Assay results were not available to the treating clinician and were not used to guide treatment

Reference	**Wolf, 2013** [65]
Study type	Prospective cohort study
Study methodology	Data source: prospective study of patients with suspected TBI who had been admitted to the Level I trauma center of the Vienna General Hospital, Medical University of Vienna.
Number of patients	n = 107
Patient characteristics	Age, mean (SD): 59 ± 23 years Gender: 60 male and 47 females GCS: GCS score 13-15 (mild GCS)

Reference	**Wolf, 2013** [65]
	Ethnicity: not reported Setting: academic, Level I trauma center Country: Austria Inclusion criteria: injury within 3 hours prior to admission to the emergency room, blunt head trauma, and a GCS score of 13-15. Exclusion criteria: penetrating head injury; severe TBI; unstable vital signs; acute focal neurological deficit; pregnancy; significant extracerebral injury including, for example, fractures of the long bones, soft tissue injuries, and hematomas; malignant melanoma; polytrauma; inherited coagulopathy; cancer; and multiple sclerosis. Patients for whom blood samples were obtained more than 3 hours after injury were also excluded from the study
Target condition(s)	Acute post-brain injury complications
Index test(s) and reference standard	Index test: Serum: -S100B protein -neuron-specific enolase (NSE) Peripheral venous blood was obtained from each patient within 3 hours after the accident and prior to cranial CT. Reference standard: Cranial CT An emergency cranial CT study was performed in all patients. The timing was usually within 30 minutes after the first examination by a physician. Prior to the CT a venous blood sample was drawn.

Reference	Wolf, 2013 [65]
	Radiological data were recorded, and the patients were assigned to 1 of the 2 following groups: CT negative, that is, patients without any trauma-related radiological sign; and CT positive, that is, patients with epidural, subdural, subarachnoid, or intracerebral hemorrhage, including contusions. Protocol did not include plain radiographs of the skull, because a negative result does not exclude intracranial bleeding. Therefore, skull fractures, which are risk factors for the development of ICH, were detected on CT scanning in both groups. No MRI was performed within the first 24 hours after injury.
Results	Outcome: CT positive (patients with epidural, subdural, subarachnoid, or intracerebral hemorrhage, including contusions) 25 (23.4%) had intracranial bleeding. Among these 25 patients, the bleeding was subarachnoid in 7, subdural in 6, and intracerebral in 7. In 5 patients, a cerebral contusion was detected by CT. Patients with intra-cranial bleeding had significantly higher S100B and NSE values and had nausea and vomiting more often. In patients with a subdural haemorrhage, the mean serum level of S100B and NSE was 0.28 and 8.46 µg/L, respectively. The mean serum level of S100B in patients with ICH was 0.34 µg/L; the mean serum level of NSE was 22.51 µg/L. In patients with a subarachnoid haemorrhage, the mean serum level of S100B was 0.98 µg/L and the mean serum level of NSE was 18.14 µg/L. In 12 CT-positive patients, the mean alcohol serum level was 1.24 per thousand. Seven patients from the CT-positive group were on anticoagulants at the time of injury. Eight patients required a neurosurgical operation to evacuate the ICH; only 1 of these 8 patients was on anti- coagulation therapy prior to the accident <u>S 100B (a cutoff of 0.48 µg/L)- within 3 hrs</u> Sensitivity: 33% (no CI reported) Specificity: 91% (no CI reported)

Reference	Wolf, 2013 [65]
	Back calculation of 2x2 table by NGC: TP: 10 FP:7 FN: 20 TN: 70 S 100B (cut-off 0.105 µg/L)- within 3 hrs Sensitivity: 72% (no CI reported) Specificity: 37% (no CI reported) Back calculation of 2x2 table by NGC: TP: 22 FP: 49 FN: 8 TN: 28 NSE (a cutoff limit of 14.7 µg/L)- within 3 hrs Sensitivity: 56% (no CI reported) Specificity: 77% (no CI reported) Back calculation of 2x2 table by NGC:

Reference	**Wolf, 2013** [65]
	TP: 17 FP: 18 FN: 13 TN: 59 <u>NSE (cutoff limit of 16.4 µg/L) - within 3 hrs</u> Sensitivity: 53% (no CI reported) Specificity: 15% (no CI reported) <u>Back calculation of 2x2 table by NGC:</u> TP: 16 FP: 65 FN: 14 TN: 12
Source of funding	No funding
Limitations	Risk of bias *(QUADAS 2 – risk of bias)*: very serious. Unclear whether the selection of patients could have introduced bias; unclear whether the index test was interpreted without knowledge of the reference standard and vice versa Indirectness *(QUADAS 2 – applicability)*: none
Comments	Information on treatment reported

Reference	**Zongo, 2012** [67]
Study type	Prospective cohort
Study methodology	Data source: Patients with minor head injury were consecutively included from December 2007 to February 2009 in a prospective study
Number of patients	n = 1560
Patient characteristics	Age, years mean (range): 57 (32-82) years Gender (male): 870 (55.8%) GCS: mild TBI GCS score 13: 39 (2.5) GCS score 14: 335 (21.5) GCS score 15: 1186 (76.0) Ethnicity: not reported Setting: ED Country: France Inclusion criteria: Patients included were aged 15 years or older, presenting to the ED within 6 hours of isolated head trauma, with a GCS score of 13 to 15 as determined by the attending physician, and with one or more of the following risk factors: loss of consciousness, posttraumatic amnesia, repeated vomiting, severe headache, dizziness, vertigo, alcohol intoxication, anticoagulation, and age older than 65 years Exclusion criteria: Patients were excluded on admission if a severe injury was suspected (Abbreviated Injury Score obviously >2). Severe injury included open fracture, large open wounds, and intrathoracic or abdominal contusion.
Target condition(s)	Acute post-brain injury complications
Index test(s) and reference standard	<u>Index test:</u> S100B

Reference	**Zongo, 2012 [67]**
	Testing within 6 hours of head trauma <u>Reference standard:</u> CT head scan CT scan was performed within 6 hours after the head trauma
Results	Outcome: CT scan abnormality (CT positive) CT scan—negative (minor head injury patients without any sign of trauma-relevant lesions) and CT scan—positive (minor head injury patients with at least 1 trauma-relevant lesion). CT scan result was positive for 111 (7%) participants, 12 of whom afterwards had a clinically important traumatic brain injury : 3 required a neurosurgical intervention and 3 died from their head trauma. Patients with positive CT scan results had higher median S100-B levels than those with negative CT scan results: median value 0.46 µg/L (0.27 to 0.72) versus 0.22 µg/L (0.14 to 0.36) <u>S100B (cut-off Value, 0.10 µg/L)- within 6 h</u> Sensitivity: 99.1(95% CI 95.0-100) Specificity: 12.2 (95% CI 10.6-14.0) Negative predictive value: 99.4 (95% CI 96.9-100) Positive predictive value: 8 (95% CI 6.6-9.5) <u>Back calculation of 2x2 table by NGC:</u>

Reference	Zongo, 2012 [67]
	TP: 110 FP: 1239 FN: 1 TN: 210 <u>S100B (cut-off Value, 0.12 µg/L) -within 6 h</u> Sensitivity :99.1(95% CI 95.0-100) Specificity: 19.7 (95% CI 17.7-21.9) Negative predictive value: 99.7 (95% CI 98.1-100) Positive predictive value: 8.6 (95% CI 7.1-10.3) <u>Back calculation of 2x2 table by NGC:</u> TP: 110 FP: 1164 FN: 1 TN: 285 <u>S100B (cut-off Value, 0.14 µg/L)-within 6 h</u> Sensitivity :97.3 (95% CI 92.3-99.4) Specificity: 26.8 (95% CI 24.5-29.1)

Reference	**Zongo, 2012** [67]
	Negative predictive value: 99.2 (95% CI 97.8-99.8) Positive predictive value: 9.2 (95% CI 7.6-11.0) <u>Back calculation of 2x2 table by NGC:</u> TP: 108 FP: 1061 FN: 3 TN: 388 AUC: 0.76; 95% CI 0.72 to 0.80 S100-B test to be a significant discriminator of CT scan abnormality (area under the curve value 0.76; 95% CI 0.72 to 0.80).
Source of funding	This study was funded by INSERM, the Reunica Group, and the teaching hospital of Bordeaux (PHRC 2007).
Limitations	Risk of bias *(QUADAS 2 – risk of bias)*: none Indirectness *(QUADAS 2 – applicability)*: none
Comments	At 0.10 and 0.12 µg/L, only 1 patient with plasma S100-B below the cut-off value had a positive CT scan result: a 28-year-old man with a cerebral contusion that proved to be a petechia and with a blood alcohol concentration of 3.0 g/L at admission. The patient required no further neurosurgery or intensive care. He stayed in the hospital for 30 hours for surveillance. The delay between trauma and blood drawing was 120 minutes. Between 0.12 and 0.14 µg/L, 2 patients had a positive CT scan result: a cerebral petechia and a chronic subdural haemorrhage with recent bleeding. No neurosurgical care was required, and there was no further neurologic deterioration. The delay between trauma and blood drawing was 152 minutes and 255 minutes for these 2 patients

Biomarkers in children

Reference	**Babcock, 2012** [2]
Study type	Secondary analysis of prospective cohort study
Study methodology	Data source: consecutive patients of all ages who consented to participate in a NIH funded registry study of patients with mild TBI between January 2003 and September 2004.
Number of patients	n = 679 (children in TBI registry) n = 360 (underwent cranial CT) n = 155 (serum S100B measurement) n = 109 (eligible patients with cranial CT and serum S100B measurement)
Patient characteristics	Age, mean (SD): Normal CT 14.7 (3.9); Abnormal CT 14.2 (4.2) Gender, male (%) Normal CT 52 (57.8); Abnormal CT 10 (52.6) Among the children with both CT and serum S100B, a majority (86.2%) of children had mild TBI; 83 had a GCS score of 15, eight had a GCS score of 14 and three had a GCS score of 13 Ethnicity: Not reported Setting: Paediatric emergency department at a university medical centre. Country: USA Inclusion criteria: Patients aged 0-18 years were eligible for inclusion in the primary study if they met a modified case definition of TBI developed by the American Congress of Rehabilitation Medicine (blow to the head or acceleration/deceleration movement of the head resulting in one or more of the following: LOC <30 minutes, amnesia <24 hours or any alteration in mental state at the time of the injury.

Reference	**Babcock, 2012** [2]
	Exclusion criteria: Patients presenting to the ED >6 hours after injury or with pre-existing medical or psychiatric conditions known to be associated with elevated S100B level in the absence of TBI (specifically, Alzheimer's disease, Down's syndrome and schizophrenia). Additionally, those who had run >10 miles in the past 12 hours were excluded.
Target condition(s)	Acute post-injury complications
Index test(s) and reference standard	Index Test S100B S 100B level > 0.006µg L^{-1} S 1OOB level of 0.1 µg L^{-1} Within 6 hours of injury Reference Standard CT scan
Results	Outcome: Abnormal cranial CT An abnormal cranial CT was defined by the presence of any intracranial injury, including subdural haematomas, epidural haemato-mas and cerebral contusions, as well as the presence of skull fractures. N=19 with abnormal CT N=90 with normal cranial CT For all patients:

Reference	Babcock, 2012 [2]
	<u>S 100B level cut-off > 0.006µg L^{-1} within 6 hours</u> Sensitivity: 90% (no CI reported) Specificity: 31% (no CI reported) Positive predictive value: 22% (no CI reported) Negative predictive value: 93% (no CI reported) <u>Back calculation of 2x2 table by NGC:</u> TP: 17 FP: 62 FN: 2 TN: 28 <u>S 1OOB level of 0.1 µg L^{-1}, - within 6 hours</u> Sensitivity: 47% (no CI reported) Specificity: 89% (no CI reported) <u>Back calculation of 2x2 table by NGC</u> TP: 9 FP: 10

Reference	Babcock, 2012 [2]
	FN: 10 TN: 80 Using the cut-off of 0.006 µg L^{-1} derived from the data, a positive S 1 00B value (>0.006 jig L^{-1}) failed to predict an abnormal CT in two children. Both of these children had a serum Sl00B level of 0. One of these children was a 9-year-old male with a GCS score of 15 who had LOC, nausea/vomiting and a headache; and the second child was a 17-year-old female who was intubated in the field and was pronounced dead shortly after presentation. If a Sl00B level of >0.006 µg L^{-1} was used as the sole criterion to order a cranial CT, 28% (30/109) of children in this cohort would not have undergone CT imaging. For GCS score 15 (n=83): S 100B levels >0.006 µg L^{-1} Sensitivity: 85.7% (no CI reported) Specificity: 34.2% (no CI reported) Area under the curve: 0.53 (95% CI = 0.36, 0.71).
Source of funding	Part funded by Career Development K23 Award from the NOH/National Institute of Neurological Disorders and Stroke (NIH/NINDS K23 NS41952-02) and a research grant from the Ronald McDonald Charities of Rochester. S100B analysis was funded through a grant by the New York State Department of Health (NYS DOH C806001). Analysis was part funded by KL2 Mentored Career Development Program in clinical and Translational Research from the University of Cincinnati Center for Clinical and Translational Science and Training, as well as the Division of Emergency Medicine at Cincinnati Children's Hospital Medical Center.
Limitations	Risk of bias *(QUADAS 2 – risk of bias)*: very serious. Unclear whether the selection of patients could have introduced bias; unclear whether the index test was interpreted without knowledge of the reference standard and vice versa

Reference	**Babcock, 2012** [2]
	Indirectness *(QUADAS 2 – applicability)*: none
Comments	-

Reference	**Bandyopadhyay, 2005** [3]
Study type	Retrospective analysis of a prospectively enrolled cohort study
Study methodology	Data source: The study cohort was obtained from an academic children's hospital emergency department (ED) head trauma study database with enrolment between December 1997 and November 2000.
Number of patients	n = 86
Patient characteristics	Age, mean (SD): 8.2(6 5.5) years (range 11 months to 18 years). Gender: Approximately two thirds were male and white. GCS: Among 86 enrolled subjects, ten had Glasgow Coma Scale (GCS) scores <13 (moderate and severe cTBI). Ethnicity: not reported Setting: ED Country: USA Inclusion criteria: Subjects between 0 and 18 years of age, evaluated within 24 hours of sustaining closed traumatic brain injury (TBI), and requiring a cranial computed tomography (CT) scan in accordance with the written ED protocol were enrolled. Exclusion criteria: Patients excluded if they had penetrating TBIs, intentional head trauma, multisystem injuries, pelvic or lower extremity fractures, spinal cord injuries, or bleeding disorders. Patients who sustained injury more than 24 hours prior to presentation or with a history of cerebral palsy, mental retardation, developmental delay, or ventricular shunts

Reference	**Bandyopadhyay, 2005** [3]
Target condition(s)	Acute post-brain injury complications
Index test(s) and reference standard	Index test: Serum Neuron-specific Enolase (NSE) Blood for serum NSE assay was drawn at the time of ED evaluation. The mean time interval from the time of reported injury and the time blood was drawn for NSE measurement was 3.8 hours (range 0.4 to 14.8). Reference standard: CT Timing of CT not reported
Results	Outcome: abnormal CT scan An abnormal CT scan was defined as a CT scan with cerebral contusions, cerebral oedema, or parenchymal, subarachnoidal, subdural, or epidural bleeding. Presence of skull fracture alone was not sufficient to classify a CT as abnormal. Results: NSE level cut-off value of 21.2 ng/mL was a relatively poor predictor of abnormal CT scan, with a c statistic of 0.66. (no other diagnostic accuracy measures reported for this outcome)
Source of funding	Not reported
Limitations	Risk of bias *(QUADAS 2 – risk of bias)*: serious. Unclear whether the selection of patients could have introduced bias

Reference	**Bandyopadhyay, 2005** [3]
	Indirectness *(QUADAS 2 – applicability)*: serious. Mixed severity TBI included however majority of patients had mild TBI
Comments	No information on treatment reported.

Reference	**Bouvier, 2012** [11]
Study type	Prospective cohort
Study methodology	Data source: Children younger than 16 years presenting at a paediatric emergency department within 3 h after TBI were enrolled prospectively for blood sampling to determine serum S100B concentrations. This prospective study was carried out from April 2010 to April 2011 in the Department of Paediatric Emergency of Clermont–Ferrand Hospital
Number of patients	n = 446
Patient characteristics	Age, median (IQR): 5.2 (2.1-9.0) Gender, male: female ratio: 1.68 Severity: 3 severity groups according to the Masters classification Masters 1: 183 (41%) Masters 2: 241 (54%) Masters 3: 22 (5%) Ethnicity: Not reported Setting: Paediatric emergency department Country: France Inclusion criteria: All children (age 0 –16 years, admission within 3 h) with closed head trauma were eligible for enrolment and were ranked in 3 severity groups according to the Masters classification. Masters group 3 (severe TBI), which formed a positive control group, was composed of children with a Glasgow Coma Scale (GCS) <13 or loss of consciousness or

Reference	**Bouvier, 2012** [11]
	progressive decrease in consciousness. Masters group 2 (mild TBI) comprised children with a GCS score of 13–15 on admission and 1 or more of 12 clinical risk factors: brief loss of consciousness, posttraumatic amnesia, nausea, vomiting, severe or progressive headache, dizziness, vertigo, intoxication, anticoagulation, skull fracture, seizure, age <2 years. Masters group 1 (minimal TBI) was made up of children with a GCS score of 15 without symptoms or with only headache or bruising. Exclusion criteria: Pregnant women, children whose TBI occurred >3 h before presentation, and multiply injured patients were excluded.
Target condition(s)	Acute post-injury complications
Index test(s) and reference standard	Index Test S100B Recently established reference intervals were used: the upper serum S100B reference limits (95th percentile) were derived for 3 age groups: 0.35 µg/L for age 0 –9 months, 0.23 µg/L for age 10 –24 months, and 0.18 µg/L for age >24 months. Patients exhibiting serum concentrations below the specific age-range cut-off were counted as S100B negative (S100B), and those with concentrations above as S100B positive (S100B). The median interval between trauma and blood sampling was 2 h 05 min (range 1 h 30 min to 2 h 45 min or 25%–75%). Reference standard CT scan To determine whether a patient had a trauma-relevant intracerebral lesion, the radiological parameters were recorded, and the patients divided into 2 groups: CCT-negative (CCT) mild TBI patients with no signs of trauma relevant intracerebral lesions and CCT-positive (CCT) mild TBI patients with at least 1 pathophysiological trauma–relevant intracerebral lesion
Results	Outcome: Intracerebral lesion on CT

Reference	Bouvier, 2012 [11]
	CCT-: mTBI patients with no signs of trauma-relevant intracerebral lesions. CCT+: mTBI patients with at least 1 pathophysiological trauma–relevant intracerebral lesion The median concentrations of S100B were 0.21 (interquartile range 0.15–0.29), 0.31 (range 0.18 –0.47), and 0.44 µg/L (range 0.30 –0.66) in Masters groups 1, 2, and 3, respectively. The difference across these 3 groups was statistically significant (P <0.05) S100B [serum concentrations below the cut-off 0.35 µg/L for age 0 –9 months; 0.23 µg/L for age 10 –24 months; 0.18 µ g/L for age >24 months were counted as S100B-, and those above as S100B+)- median 2 hours. TP: 23 FP: 28 FN:0 TN: 14 Sensitivity: 100% (CI 85.2-100) Specificity: 33% (CI 20-50) AUC: 0.72 (CI 85.2-100) Positive predictive value: 45% (31%–60%) Negative predictive value: 100% (77%–100%)

Reference	**Bouvier, 2012** [11]
	SN/SP calculated by NGC Sensitivity: 1.00 [0.85, 1.00] Specificity: 0.33 [0.20, 0.50]
Source of funding	None
Limitations	Risk of bias *(QUADAS 2 – risk of bias)*: serious. unclear whether the index test was interpreted without knowledge of the reference standard and vice versa Indirectness *(QUADAS 2 – applicability)*: serious. includes a population with mixed TBI severity
Comments	-

Reference	**Castellani, 2009** [13]
Study type	Prospective cohort
Study methodology	All patients <18 years presenting to hospital with mild TBI between December 2004 and April 2008 had venous blood samples taken within 6h of trauma for the determination of S100B. MTBI was defined as follows: GCS score of 13-15 at admission in combination with associated clinical symptoms (vomiting, loss of consciousness - and in patients >4 years persisting headache, retrograde amnesia and vertigo).
Number of patients	n = 928 n = 109 (included in study)
Patient characteristics	Age, mean (SD): 9.5 (4.7) Gender, male, n (%): 73 (67) On admission, a GCS score of 15 was recorded in 86 (78.9%), of 14 in 13 (11.9%) and of 13 in 10 (9.2%) patients. Ethnicity: Not reported

Reference	**Castellani, 2009** [13]
	Setting: Hospital emergency department Country: Austria Inclusion criteria: Patients <18 years with a GCS score 13-15 (in combination with vomiting, loss of consciousness, persisting headache, retrograde amnesia, and vertigo) with serum S100B measured within 6 hours of blunt head trauma who went on to require a CT scan during their inpatient episode. Exclusion criteria: None specified
Target condition(s)	Acute post-injury complications
Index test(s) and reference standard	Index Test S100B According to an analysis in healthy children recently conducted by the authors, the upper reference of serum S-100B was set to 0.16 µg/L. All patients with MTBI and clinical symptoms who had their serum S-100B measured within 6 h after trauma and subsequently went on to require a CT during their in-patient episode were selected from the database for this study Reference Standard CT scan
Results	Outcome: pathological CT CT was classified as pathological in the presence of a skull fracture or intra cranial hemorrhage (ICH). Thirty-six patients (30.3%) showed abnormalities on their CCT: 22 (20.2%) had skull fractures without ICH, 12 (11.9%) had skull fractures in combination with ICH and two (1.8%) had isolated ICH without fracture. ICH was limited to single locations in 11 patients: four patients epidural, four subarachnoid and two subdural hemorrhages and one patient with shear

Reference	**Castellani, 2009** [13]
	bleedings. Three patients showed hemorrhages in multiple locations: one epi- and subdural, one subdural and shear bleeding and one sub-arachnoidal and shear bleeding. <u>S100B (cut-off 0.16µg/L)-within 6 h</u> TP = 36 FP = 42 FN = 0 TN = 31 Sensitivity 100% (CI 92-100) Specificity 42% (CI 38-43) AUC 0.68 (CI 0.58-0.78) <u>SN/SP calculated by NGC</u> Sensitivity: 1.00 [0.90, 1.00] Specificity: 0.42 [0.31, 0.55]
Source of funding	None
Limitations	Risk of bias *(QUADAS 2 – risk of bias)*: very serious. Unclear whether the selection of patients could have introduced bias; unclear whether the index test was interpreted without knowledge of the reference standard and vice versa Indirectness *(QUADAS 2 – applicability)*: None
Comments	After clinical examination and S100B sampling, all patients were admitted for inpatient observation.

Reference	**Fridriksson, 2000** [25]
Study type	prospective pilot study
Study methodology	Data source: A prospective pilot study was conducted of patients 0 to 18 years of age presenting to a children's hospital emergency department (ED) between December1997 and October 1998 were consecutively enrolled. Children presenting within 24 hours of injury who required head computed tomography (CT) were eligible.
Number of patients	n = 50
Patient characteristics	Age, mean (SD): aged 2 months to 16 years Presence of intracranial lesion (n=22): 9.16 (5.7) years No intra cranial lesion (n=27): 7.66 (5.3) years Gender: Presence of intracranial lesion (n=22): males 12; females 10 No intra cranial lesion (n=27): males 15; females 12 Mixed severity population GCS score mean (SD): Presence of intracranial lesion: 11.96 (4.2) No intracranial lesion: 13.96 (2.6) GCS score >12 Presence of intracranial lesion: 14/22 No intracranial lesion: 25/27 Ethnicity: Presence of intracranial lesion: White- 14; African American-5; Hispanic-1; Other- 2 No intracranial lesion: White- 15; African American: 10; Hispanic – 1; Other- 1 Setting: ED of an academic tertiary care children's hospital Country: USA

Reference	**Fridriksson, 2000** [25]
	Inclusion criteria: All patients presenting with blunt head trauma within 24 hours of injury and requiring head CT evaluation in accordance with the written ED practice guidelines were eligible for enrolment Exclusion criteria: Patients with penetrating head trauma, injury sustained more than 24 hours prior to presentation, or bleeding disorders were not eligible for enrolment in the study
Target condition(s)	Acute post-brain injury complications
Index test(s) and reference standard	Index test: serum neuron-specific enolase (NSE) Serum NSE levels were determined using standard radio-immunoassay technique (SpecialtyLaboratories, Santa Monica, CA). The NSE reference range provided by Specialty Laboratories was undetectable (<10 ng/mL), indeterminate (10–15 ng/mL), and abnormal(>15 ng/mL). For the purpose of the study, NSE levels of >15 ng/mL were considered abnormal. The mean time from injury to obtaining blood samples for NSE was 256 (310) minutes in the PICL group and 242 (147) in the NICL group (p = 0.82). Reference standard: Head CT
Results	Outcome: Presence of intracranial lesion (ICL) Head CT was reported as positive for ICL when cerebral oedema, parenchymal bleeding, cerebral contusion, or sub arachnoidal, subdural, or epidural bleeding was identified. Enrolled patients were assigned to one of two groups based on the presence or absence of ICL on head CT. The PICL (presence of ICL) group consisted of patients with evidence of ICL. The NICL (no ICL) group consisted of patients with no evidence of ICL or isolated skull fracture only. Results:

Reference	Fridriksson, 2000 [25]
	Intra-cranial abnormalities were identified on head CT in 22 patients (45%) One patient in the NICL group required surgery for elevation of a minor depressed skull fracture. In the PICL group, five patients (23%) underwent craniotomy: two for evacuation of an intra cranial hematoma, two for elevation of a de-pressed skull fracture, and one for insertion of a ventriculostomy catheter. Overall NSE levels ranged from4.3 to >100 ng/mL for all patients, and 63% of the patients had levels ≥15 ng/Ml. Of the 39 patients with GCS score of >12, 22 (56%) had NSE levels > 15 ng/mL. Of ten patients with GCS score < 12, eight (80%) had levels > 15 ng/mL. The mean level in patients with GCS score of >12 was 18.4ng/mL, compared with 36.6 ng/mL inpatients with GCS score < 12 (p = 0.003;95% CI =25.8 to226.7). Mean NSE level was 26.7621.4 ng/mL in the PICL group and 17.867.8 ng/mL in the NICL group (p = 0.48; CI = 0.10to 17.9). 17 patients (77%) in the PICL group had NSE levels > 15.3 ng/mL compared with 14 (52%) in the NICL group (p = 0.034). Twenty-one of the 22 patients in the PICL group and 14 of the 27 patients in the NICL group were admitted to the hospital. The mean hospital stay was 7.4 days (range 1–48) for the PICL group and 2.1 days (range 1–6) for the NICL group (p =0.89). None of the patients in the study group died. <u>NSE (at a level of NSE ≥15.3 ng/mL)- mean 4h</u>

Reference	**Fridriksson, 2000** [25]
	TP: 17 FP: 13 FN: 5 TN: 14 Sensitivity: 77% (no CI reported) Specificity: 52% (no CI reported) negative predictive value: 74% (no CI reported) <u>SN/SP calculated by NGC:</u> Sensitivity: 0.77 [0.55, 0.92] Specificity: 0.52 [0.32, 0.71]
Source of funding	Not reported
Limitations	Risk of bias *(QUADAS 2 – risk of bias)*: serious. None Indirectness *(QUADAS 2 – applicability)*: serious. Mixed severity TBI
Comments	Information on treatment reported.

Reference	**Kelmendi, 2018** [33]
Study type	single-centre prospective cohort study
Study methodology	Data source: study that was carried out from December 2016 to December 2017. The study was conducted in the emergency department and the neurosurgery clinic. The study site is a tertiary neurosurgical centre
Number of patients	n = 80
Patient characteristics	Age, mean (SD): 9.1 (3.8) years Gender: Forty-six patients were male (57.5%), and 34 patients were female (42.5%). GCS: Patients were diagnosed with mild TBI if they presented with a GCS score of 13–15, loss of consciousness (LOC) lasting < 30 mins and posttraumatic amnesia (PTA) lasting < 1 hour GCS score 15: 25 (31.3%) GCS score 14: 26 (32.5%) GCS score 13: 27 (33.8%) Ethnicity: not reported Setting: emergency department and the neurosurgery clinic Country: Kosovo Inclusion criteria: Children with head trauma alone who were between 2 and 16 years of age were included in the study Exclusion criteria: Children who were admitted to the hospital more than three hours after trauma, children with a history of syncope or seizure before the head trauma, children with Down syndrome (S100B is overexpressed in such patients), children who had previously undergone a neurosurgical procedure, children with multiple injuries (involving the chest, abdomen, extremities, or pelvis), children with renal or liver disease, children with a history of a severe neurologic or psychiatric disorder, children suffering from cancer, and children who either had a history of an inherited coagulopathy or had received anticoagulant therapy were excluded from the study.

Reference	**Kelmendi, 2018** [33]
	Serum S100B protein has a short half-life; thus, patients whose blood samples were drawn more than 3 hours after head trauma were excluded from the analysis
Target condition(s)	Acute post-brain injury complications
Index test(s) and reference standard	Index test: S 100 B At 3 hours of injury Blood samples were obtained from each patient via a cubital vein at 3 hours after head injury. The blood samples were processed to separate the serum from the plasma, and then the serum was deep-frozen at −20◦ C (−4◦ F) until analysed with an electrochemiluminescence immunoassay kit (Elecsys S100; Roche Diagnostics, Mannheim, Germany). Reference standard: Head CT The CT was usually performed within 30 minutes after the patient was first examined by an emergency physician. A venous blood sample was drawn prior to every CT. The CT examination involved the acquisition of parenchymal and bone window images. All head CTs were reviewed for signs of TBI by a radiologist blinded to the patients' clinical signs and S100B levels
Results	Outcome: Trauma related cerebral lesions on CT Patients found to have any signs of trauma-related cerebral lesions (skull-cap fracture, skull-base fracture, or both; epidural haematoma; subdural haematoma; traumatic subarachnoid bleeding; cerebral haematoma; brain contusion; or pneumocephalus) were considered to have a positive head CT. The patients were divided into the following two groups: (a) a negative CT group (CT−), which included patients without any signs of cranial injury on CT; and (b) a positive CT group (CT+), which included patients with at least one trauma-related lesion on CT

Reference	Kelmendi, 2018 [33]
	The patients were classified according to the number of injuries rather than the size of the injury. Many of the patients had multiple injuries, but these injuries affected only a small volume of brain tissue (always considering that one injury can damage a large area of brain tissue). plain radiographs of the skull and MRI were not performed. Results: The mean S100B level was 0.398 μg L−1 (SD ± 0.298 μg L−1), and the 95% CI ranged from 0.332 to 0.465 μg L−1. A total of 53 patients (66.3%) had cranial lesions. Patients with cranial injury, as demonstrated by CT, had higher S100B protein levels than those without cranial injury ($p < 0.0001$). The mean serum S100B protein level in patients without cranial injury (head CT−) was 0.145 g L−1 (95% CI 0.138–0.152 μg L−1), while the mean serum S100B protein level in patients with cranial injury (head CT+) was 0.527 μg L−1 (95% CI 0.447–0.607 μg L−1). <u>S 100 B:</u> AUC: = 0.893, 95% CI 0.786–0.987 S100B levels differed significantly between the patients with and without cranial injury at 3 hours after TBI (AUC = 0.893, 95% CI 0.786–0.987, $p = 0.0001$).

Reference	**Kelmendi, 2018** [33]
Source of funding	Not reported
Limitations	Risk of bias *(QUADAS 2 – risk of bias)*: none Indirectness *(QUADAS 2 – applicability)*: none
Comments	The study reports that S100B levels had no effect on clinical decisions or patient management in the study.

Reference	**Manzano, 2016** [42]
Study type	Prospective multicentre cohort study
Study methodology	Data source: The study was conducted in the paediatric emergency departments of three tertiary hospitals in Switzerland on a consecutive sample of patients between January 2009 and December 2011
Number of patients	N=73
Patient characteristics	Without intracranial injury (ICI) (n=53); With ICI (n=20) Age mean (SD) months: Without ICI: 94.0 (56.5) With ICI: 78.1 (44.4) Gender (male): Without ICI: 35 (66.0) With ICI: 16 (80.0) GCS score <15: mild TBI Without ICI: 19 (35.8) With ICI: 8 (40.0) Ethnicity: not reported Setting: ED

Reference	**Manzano, 2016** [42]
	Country: Switzerland Inclusion criteria: children aged <16 years with a mild TBI (GCS score ≥13) for whom a head CT was requested by the attending physician. Exclusion criteria: e children who arrived at the hospital more than 6 h after the trauma, children with Down syndrome (since in these patients S100B is overexpressed) or patients with a history of convulsion in the past 7 days.
Target condition(s)	Acute post-brain injury complications
Index test(s) and reference standard	Index test: S100B Venous blood was obtained within 6 h of the trauma in all children for S100B measurement before a head CT was performed. As the S100B value was not available during the acute care period, the patient's management was not altered. Reference standard: Cranial CT
Results	Outcome: Primary outcome was evaluation of the diagnostic value of S100B in detecting intracranial injuries in children aged <16 years with mild head trauma. Of the 73 included children, 20 (27.4%) had an ICI detected on CT. The lesion was an epidural haematoma in nine children, a subarachnoid haemorrhage in four, an epidural haematoma and subarachnoid haemorrhage in three. The remaining four children had respectively a subdural haematoma, an epidural and subdural haematoma, a subdural haematoma and subarachnoid haemorrhage and haemorrhagic parenchymal contusion.

Reference	**Manzano, 2016** [42]
	No surgical intervention was required. <u>S100B (Cut-off value 0.14 µg/L) - All children <16 years – within 6h</u> Sensitivity: 95.0% (95% CI 77% to 100%) Specificity: 34.0% (95% CI 27% to 36%) <u>Back calculation of 2x2 table done by NGC:</u> TP: 19 FP: 35 FN: 1 TN: 18 AUC: 0.73 (95% CI 0.60–0.86)
Source of funding	Roche Switzerland supplied the S100 reagents without charge
Limitations	Risk of bias *(QUADAS 2 – risk of bias)*: none Indirectness *(QUADAS 2 – applicability)*: none
Comments	Information on treatment reported

Reference	**Mozafari, 2019** [46]
Study type	cross-sectional study

Reference	**Mozafari, 2019** [46]
Study methodology	Data source: parents/ guardians were approached for informed consent for children and adolescent with head injuries referred to the Emergency Department of the Ahvaz Golestan Hospital, Iran during April to September 2017.
Number of patients	n = 40
Patient characteristics	Age: Median age in years (range) Group A (positive CT) (n=20): 9 (2-18) years Group B (negative CT) (n=20): 6.6 (0.5 - 18) years Gender: Group A (positive CT) (n=20): females -4 (20%) Group B (negative CT) (n=20): females – 8 (40%) GCS score %: GCS score 14 Group A (positive CT) (n=20): 4 (20%) Group B (negative CT) (n=20): 13 (65%) GCS score 15 Group A (positive CT) (n=20): 16 (80%) Group B (negative CT) (n=20): 7 (35%) Ethnicity: not reported Setting: Country: Iran Inclusion criteria: presence for an indication of a brain CT scan, aged 6 months to 18 years and a Glasgow coma score of 14 or 15. Injuries included those from traffic and home or sport events, and referrals less than 6 hours of the incident. Inclusion

Reference	**Mozafari, 2019** [46]
	criteria were no previous history of alcohol or drug abuse, the absence of a history of previous neurological disease such as seizure or epilepsy, the absence of severe traffic injury and multiple trauma from motor vehicles, and absence of melanoma Exclusion criteria: if patients had any of the following conditions: injuries except the brain mild trauma damage such as organ damage, previous illnesses such as diabetes, heart disease, asthma, pregnancy or recent febrile illness.
Target condition(s)	Acute post-brain injury complications
Index test(s) and reference standard	Index test: Urine and serum S 100B The blood and urine samples were immediately transferred to the central laboratory of Golestan Hospital. Blood samples were centrifuged at room temperature for 10 minutes at 2200 g and the separated serum was stored at -70 ° C until analysis. Urine samples were centrifuged at room temperature for 10 minutes at 900 g and the supernatant was stored at -70° C until analysis. The S100B in both serum and urine samples were determined using S100B ELISA kits (Shanghai Crystal Day Biotech Co., Ltd). The concentration of S100B in serum and urine of each sample was recorded independently without knowledge of the brain CT scan results. Reference standard: Brain CT The primary brain CT scans of all patients was interpreted using a 64 slice CTs can device and independently interpreted by a consultant neurologist who was not aware of the results of the corresponding S100B results. Patients were assigned to either Group A or Group B according to their CT scan results. Not reported timing of CT.
Results	Outcome: positive pathologic findings associated with isolated head trauma on CT (not defined positive pathological findings)

Reference	Mozafari, 2019 [46]
	20 who had positive pathologic findings associated with isolated head trauma (Group A) and those who lacked these findings in brain CT scans (Group B). In Group A the mean (±1SD) serum level of S100B was 561±283 ng/L, whereas in Group B it was 79.8±22.8 ng/L (p <0.001). In group A, the mean urinary level of S100B was 134±63.5 ng/ L, whereas in group B it was 25±19 ng/L (p <0.001) Results: Serum and urine S 100B Serum S 100 B (cut-off of 172.15 ng/L)- within 6 hours Sensitivity: 95% (no CI reported) Specificity: 100% (no CI reported) Back calculation of 2x2 table by NGC: TP: 19 FP: 0 FN: 1 TN: 20 Urinary S100B (cut-off levels of 67.75 ng/L)- within 6 hours Sensitivity: 90% (no CI reported) Specificity: 95% (no CI reported) Back calculation of 2x2 table by NGC: TP: 18 FP: 1

Reference	**Mozafari, 2019** [46]
	FN: 2 TN: 19 <u>Urinary S100B cut-off levels of 56.4 ng/L)- within 6 hours</u> Sensitivity: 95% (no CI reported) Specificity: 90% (no CI reported) <u>Back calculation of 2x2 table by NGC:</u> TP: 19 FP: 2 FN: 1 TN: 18 The area under the ROC curve of 0.998 (P <0.0001) indicated a high predictive value of serum S100B in the differentiation between positive and negative patients. The area under the ROC curve with a value of 0. 985 (P <0.0001) indicated a high accuracy of the urine S100B level in differentiating between positive and negative patients.
Source of funding	support of the deputy of research affairs of the Ahvaz Jundishapur University of Medical Sciences as part of Kourosh Mohammadi's thesis under the research code: U-94138.
Limitations	Risk of bias *(QUADAS 2 – risk of bias)*: serious. Unclear whether the selection of patients could have introduced bias Indirectness *(QUADAS 2 – applicability)*: none
Comments	No information on treatment reported.

Reference	**Mozafari, 2020** [47]
Study type	Prospective cohort study
Study methodology	Data source: conducted on all children with head traumas presenting to the ED of Golestan Hospital in Ahvaz in 2016.
Number of patients	n = 62
Patient characteristics	Age, mean (SD): CT positive: 8.57 (5.16) years CT negative: 8.32 (4.72) years Gender: CT positive: 22 (71%) CT negative: 24 (77.4%) GCS: Positive CT scan group: the frequency of a GCS score of 14 was 17 (54.8%) and that of a GCS score of 15 was 14 (45.2%), Negative CT scan group: frequency of a GCS score of 14 was 6 (19.4%) and that of a GCS score of 15 was 25 (80.6%) Ethnicity: not reported Setting: ED Country: Iran Inclusion criteria: After the initial examinations and stabilisation of the patients with TBIs by a senior emergency medicine resident, CT scans of the brain were performed according to the latest guidelines in case the indications appeared, including an age of 6 months to 18 years, a GCS score of 14 and 15, the mechanism of damage being of the type of traffic accidents and domestic or sport injuries, the incident occurring within the previous 6 hrs, the parents giving consent for the participation of their children in the study, lack of pregnancy, no history of alcohol or drug abuse, no history of neurological diseases such as seizure and epilepsy and the absence of severe road traffic injuries such as overturned vehicle or being thrown out of the car

Reference	**Mozafari, 2020** [47]
	Exclusion criteria: a history or clinical evidence for stroke, cerebral haemorrhage, head trauma and infection of central nervous system within the previous 3 months, a history of brain tumours, having injuries other than mild brain trauma such as limb fractures, having a history of major diseases such as diabetes, heart problems and asthma, a BMI below the fifth percentile or above the 95th percentile, severe traffic injuries such as overturned vehicle or being thrown out of the car
Target condition(s)	Acute post-brain injury complications
Index test(s) and reference standard	Index test: neuron-specific enolase (NSE) a venous blood sample was immediately taken by the ward nurse from all the eligible patients within 6 hrs of the incident after obtaining their information, performing initial examinations and their initial stabilisation. The patients were then referred to an imaging unit for cranial CT scan. Reference standard: Cranial CT Done after biomarker test The initial CT scans of the brain of all the patients were performed by a CT scan machine, the results interpreted by an emergency medicine specialist, and the films subsequently interpreted independently by one neuroradiologist, who was unaware of the results of enolase levels
Results	Outcome: Positive-for-trauma pathological findings on CT scan Serum levels of enolase were found to be 9.74±2.7 µg/ L in the positive CT scan group and 4.23±1.33 µg/L in the negative group, suggesting a statistically significant difference (P<0.0001).

Reference	Mozafari, 2020 [47]
	Results: The area under the ROC curve for serum levels of enolase was found to be 0.992 (P<0.007) in diagnosing brain lesions caused by mild head traumas. N=31 CT positive NSE (cut-off points 5.74 µg/L)-within 6h Sensitivity: 100% (no CI reported) Specificity: 87.1% (no CI reported) Back calculation of 2x2 table by NGC: TP: 31 FP:4 FN:0 TN: 27 NSE (cut-off points of 6.97 µg/L) - within 6h Sensitivity: 93.55% (no CI reported) Specificity: 100% (no CI reported) Back calculation of 2x2 table by NGC: TP: 29 FP:0 FN: 2

Reference	**Mozafari, 2020** [47]
	TN: 31
Source of funding	support of the deputy of research affairs of the Ahvaz Jundishapur University of Medical Sciences as part of Bita Fatehifar's thesis under the research code GP95230.
Limitations	Risk of bias *(QUADAS 2 – risk of bias)*: serious. Unclear whether the selection of patients could have introduced bias Indirectness *(QUADAS 2 – applicability)*: none
Comments	No information on treatment reported.

Reference	**Papa, 2015** [58]
Study type	Prospective cohort study
Study methodology	Data source: convenience sample of children and young people presenting to Level 1 trauma centres with blunt head trauma. An additional control cohort of trauma patients without blunt head trauma was also recruited from these centres to examine biomarker levels in patients who were exposed to traumatic forces without direct blunt head trauma.
Number of patients	n = 197 head trauma patients
Patient characteristics	Age (years), mean (SD): 11.51 (7) Gender (male to female ratio): 131 M: 66 F GCS score in ED, n (%). Mixed severity TBI (majority with mild TBI) GCS score 9-12 = 3 (1.5%)

Reference	Papa, 2015 [58]
	GCS score 13 = 1 (0.5%) GCS score 14 = 13 (6.5%) GCS score 15 = 180 (91.5%) Ethnicity: Ethnicity (n): Asian= 3 Black = 53 Hispanic = 42 White = 96 Other = 3 Setting: EDs of three level 1 trauma centres (2 paediatric and 1 adult) Country: USA Inclusion criteria: children and young people (birth–21 years of age) with blunt head trauma presenting to the ED within 6 h of injury with a GCS score of 9–15. The control cohort included trauma patients without blunt head trauma and with a GCS score of 15 presenting to the ED within 6 h of injury.

Reference	**Papa, 2015** [58]
	Exclusion criteria: syncope or seizure preceding head trauma, known chronic psychosis, neurological disorder, or active CNS pathology; pregnancy; incarceration; spinal cord injury; or hemodynamic instability.
Target condition(s)	Acute post brain injury complications
Index test(s) and reference standard	Index test(s) Glial fibrillary acidic protein (GFAP) Cut-off level of 0.15 ng/mL derived from the ROC curves for detecting intracranial lesions on CT scan to maximize the sensitivity and to correctly classify all traumatic intracranial lesions GFAP > 0.15 ng/mL = positive GFAP ≤ 0.15 ng/mL= negative Blood samples were obtained in all patients within six hours of injury and measured by ELISA for GFAP (ng/ml) Reference standard: CT scan Time between measurement of index test and reference standard: unclear CT within and serum were within 6 hours of injury

Reference	Papa, 2015 [58]
	Mean time from injury to serum sample collection at 3.5 hours (95% CI = 3.3 to 3.7 hours). The mean time to serum collection for head trauma patients was 3.3 hours (95% CI = 3.1 to 3.5 hours) and for non–head-injured trauma controls it was 4.1 hours (95% CI = 3.7 to 4.5 hours).
Results	Outcome: presence of intracranial lesions on initial CT scan Only children who had actual CTs performed (at the discretion of the treating physician) were included in this analysis; no surrogate measures were used. CT scans of the head were performed in 152 patients, and traumatic intracranial lesions on CT scan were evident in 18 (11%), all of whom had GCS scores of 13 to 15. <u>GFAP (cut-off 0.15 ng/mL) within 6h of injury: Isolated skull fractures excluded from intracranial lesions</u> TP = 17 FP = 71 TN = 63 FN = 1 CT + = 18 Sensitivity (%) = 94 (95%CI 71-100) Specificity (%) = 47 (95%CI 38-56) PPV (%) = 19 (95%CI 12-29) NPV (%) = 98 (95%CI 90-100)

Reference	**Papa, 2015** [58]
	<u>GFAP (cut-off 0.15 ng/mL) within 6h of injury: Isolated skull fractures included with intracranial lesions</u> TP = 20 FP =68 TN = 63 FN = 1 Sensitivity (%) = 95 (95%CI 74-100) Specificity (%) = 48 (95%CI 39-56) PPV (%) = 22 (95%CI 16-33) NPV (%) = 98 (95%CI 90-100) Performance of serum GFAP in detecting intracranial lesions on CT, AUC by age group Birth–5 years 0.83 (95%CI 0.56–1.00) 5.1–10 years 0.87 (95%CI 0.70–1.00) 10.1–15 years 0.78 (95%CI 0.60–0.95) 15.1–21 years 0.91 (95%CI 0.83–0.99)
Source of funding	This study was supported in part by Award Number R01NS057676 from the National Institute of Neurological Disorders and Stroke. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institute of Neurological Disorders And Stroke or the National Institutes of Health.
Limitations	Risk of bias (QUADAS 2 – risk of bias): very serious. Unclear whether the selection of patients could have introduced bias. Not all participants had the reference standard.

Reference	**Papa, 2015** [58]
	Indirectness (QUADAS 2 – applicability): serious. Mixed population (birth-21 years)
Comments	After assessment and treatment in the ED, patients were either discharged home or admitted to hospital based on severity of their injuries and patient management was not altered by the study

Reference	**Papa, 2017** [57]
Study type	Prospective cohort study
Study methodology	The aim of the study was to assess whether Ubiquitin C-terminal hydrolase (UCH-L1) was significantly elevated in serum of children and young people with mild and moderate traumatic brain injury (TBI) compared to other trauma patients without mild and moderate TBI. It also investigated the relationship between UCH-L1 levels and traumatic intracranial lesions on CT scan. Data source: convenience sample of children and young people presenting to Level 1 trauma centres with blunt head trauma.
Number of patients	n = 196 head trauma patients
Patient characteristics	Patients with head trauma with and without TBI symptoms (n=196): Age in years, mean (SD): 11.51 (7) Gender (male to female ratio): 130 M: 18 F GCS score in ED, n (%): mixed severity TBI GCS score 9-12 = 3 (1.5%) GCS score 13 = 1 (0.5%) GCS score 14 = 13 (6.5%) GCS score 15 = 179 (91.5%)

Reference	Papa, 2017 [57]
	Ethnicity (n): Asian= 3 Black = 53 Hispanic = 42 White = 95 Other = 3 Setting: EDs of three level 1 trauma centres (2 paediatric and 1 adult) Country: USA Inclusion criteria: children and young people (birth–21 years of age) with blunt head trauma presenting to the ED within 6 h of injury with a GCS score of 9–15. The control cohort included trauma patients without blunt head trauma and with a GCS score of 15 presenting to the ED within 6 h of injury. Exclusion criteria: syncope or seizure preceding head trauma, known chronic psychosis, neurological disorder, or active CNS pathology; pregnancy; incarceration; spinal cord injury; or hemodynamic instability.
Target condition(s)	Acute post brain injury complications
Index test(s) and reference standard	Index test(s) Ubiquitin C-terminal hydrolase (UCH-L1) Blood samples were obtained in all patients within 6 h of injury

Reference	Papa, 2017 [57]
	Reference standard CT scan Time between measurement of index test and reference standard: Unclear Both the head trauma and trauma controls had serum samples drawn within 6 h of injury with the average time from injury to serum sample collection at 3.5 h (95% CI, 3.3–3.7). The average time to serum collection for head trauma patients was 3.3 hours (95% CI, 3.1–3.5) and for non-head-injured trauma controls was 4.1 hours (95% CI, 3.7–4.5).
Results	Outcome: presence of intracranial lesions on initial CT scan Ubiquitin C-terminal hydrolase (UCH-L1) Cut -off ≥0.18 ng/mL = positive. <0.18 ng/mL= negative (was derived from the ROC curves for detecting intracranial lesions on CT scan to maximize the sensitivity and to correctly classify all traumatic intracranial lesions). Only children who had actual CTs performed (at the discretion of the treating physician) were included in this analysis; no surrogate measures were used. Intracranial CT scan of the head was performed in 151 patients and traumatic intracranial lesions on CT scan were evident in 17 (11%), all of whom had a GCS score 13–15. Classification performance of serum UCH-L1 in detecting Intracranial lesions on CT (Cut -off ≥0.18 ng/mL)- within 6h of injury TP = 17 FP = 71 TN = 64

Reference	Papa, 2017 [57]
	FN = 0 CT + = 17 Sensitivity = 100 (95%CI 77-100) Specificity= 47 (95%CI 39-56) PPV (%) = 19 (95%CI 12-29) NPV (%) = 100 (95%CI 93-100) <u>Calculated by NGC</u> Sensitivity: 1.00 [0.80, 1.00] Specificity: 0.47 [0.39, 0.56] <u>Performance of serum UCH-L1 in detecting intracranial lesions on CT AUC by age group</u> Birth–5 years 0.79 (0.59–1.00) 5.1–10 years 1.00 (1.00–1.00) 10.1–15 years 0.59 (0.34–0.85) 15.1–21 years 0.95 (0.85–1.00)
Source of funding	Supported in part by an award from the National Institute of Neurological Disorders and Stroke (NINDS).
Limitations	Risk of bias (QUADAS 2 – risk of bias): very serious. Unclear whether the selection of patients could have introduced bias; not all participants had the reference standard.

Reference	**Papa, 2017** [57]
	Indirectness (QUADAS 2 – applicability): serious. Mixed population (birth-21 years)
Comments	After assessment and treatment in the ED, patients were either discharged home or admitted to the hospital based on severity of their injuries and patient management was not altered by the study

Reference	**Papa, 2016** [56]
Study type	Prospective cohort study
Study methodology	Data source: study enrolled a convenience sample of children and youth with head trauma (Glasgow Coma Scale [GSC] score of 9 to 15) presenting to the emergency department (ED) within 6 h of trauma.
Number of patients	n = 155
Patient characteristics	Age, mean (SD): 13 (7) years range from six months to 21 years. Gender (male): 100 (65%) GCS: mixed severity but 99% with GCS score 13-15 GCS score 9-12: 2 (1%) GCS score 13: 2 (1%) GCS score 14: 6 (4%) GCS score 15: 146 (94%) Ethnicity: not reported Setting: ED Country: USA

Reference	**Papa, 2016** [56]
	Inclusion criteria: history of blunt head trauma presenting to the ED within 6 h of injury with an initial GCS score of 9 to 15. Head trauma patients were further categorised into children with TBI symptoms (loss of consciousness, amnesia, disorientation, or change in behaviour) and children without TBI symptoms. Exclusion criteria: if patients had: 1) had syncope or seizure prior to their head trauma; 2) had known chronic psychosis, neurological disorder, or active central nervous system pathology; 3) were pregnant; 4) were incarcerated; 5) had spinal cord injury; or 6) had hemodynamic instability
Target condition(s)	Acute post-brain injury complications
Index test(s) and reference standard	Index test: Glial fibrillary acidic protein (GFAP) S100B Blood samples were obtained within 6 h of the reported time of injury. A single vial of approximately 5 mL of blood was collected and placed in a serum separator tube and allowed to clot at room temperature before being centrifuged. The serum was placed in barcoded aliquot containers and stored in a freezer at - 70C until it was transported to a central laboratory where samples were analysed in batches using sandwich enzyme-linked immunosorbent assays (ELISA) for GFAP and S100b. Reference standard: Head CT Trauma patients underwent standard CT scan of the head according to the judgment of the treating physician. CT examinations were interpreted by board-certified radiologists who recorded location, extent, and type of brain injury. Radiologists were blinded to the study protocol but had the usual clinical information. Lab personnel running the samples were blinded to the clinical data.
Results	Outcome: presence of intracranial lesions on initial CT scan.

Reference	Papa, 2016 [56]
	Intracranial lesions on CT included any acute traumatic intracranial lesions visualised on CT scan such haemorrhages (epidural, subdural, subarachnoid, ventricular, and parenchymal), contusions, oedema, and pneumocephalus but excluded facial fractures and isolated skull fractures without intracranial lesions. Of the 114 subjects with head trauma, 112 (98%) had a GCS score of 13-15 and two had a GCS score of 9-12. CT scan of the head was performed in 92 patients and traumatic intracranial lesions on CT scan were evident in eight (9%), all of whom had a GCS score of 13-15: one had a GCS score of 13, two had a GCS score of 14, and five had a GCS score of 15. Cut-off points for GFAP and S100b were derived from the ROC curves for detecting intracranial lesions on CT scan to maximise the sensitivity and correctly classify all CT positive lesion <u>Results:</u> <u>GFAP</u> AUC: 0.85 (95% CI 0.72-0.98 <u>GFAP cut-off level of 0.15 ng/mL- within 6h</u> TP: 8 FP: 54 FN:0

Reference	Papa, 2016 [56]
	TN: 30 Sensitivity:100% (95% CI 60-100) Specificity: 36% (95% CI 26-47) SN/SP calculated by NGC: Sensitivity: 1.00 [0.63, 1.00] Specificity: 0.36 [0.26, 0.47] S100B: AUC: 0.67 (95% CI 0.50-0.85) S100b cut-off level of 0.020 ng/mL- within 6h TP: 8 FP: 62 FN:0 TN: 22

Reference	**Papa, 2016** [56]
	Sensitivity: 100% (95% CI 60-100) Specificity: 26% (95% CI 5-22) <u>SN/SP calculated by NGC:</u> Sensitivity: 1.00 [0.63, 1.00] Specificity: 0.26 [0.17, 0.37]
Source of funding	No funding
Limitations	Risk of bias *(QUADAS 2 – risk of bias)*: none Indirectness *(QUADAS 2 – applicability)*: serious. Mixed population of adults and children (six months to 21 years).
Comments	After assessment and treatment in the ED, patients were either discharged home or admitted to hospital based on severity of their injuries, and patient management was not altered by the study. No further details on treatment.

Reference	**Yeung, 2020** [66]
Study type	Prospective cohort study
Study methodology	Data source: A convenience sample of pediatric patients with TBI was recruited prospectively
Number of patients	n = 24
Patient characteristics	children < 18 years

Reference	**Yeung, 2020** [66]
	Age, median IQR: age of 5 years (3.5, 1—8.8 years) Gender: 67% males GCS: Mixed severity (mild, moderate and severe). Majority with mild TBI mild TBI with GCS score of 13 to 15: 15 (62.5%) moderate TBI with GCS score of 9 to 12: 4 (16.7%) severe TBI with GCS score of 8 or less: 5 (20.8%) Ethnicity: not reported Setting: tertiary care hospital Country: USA Inclusion criteria: Children 0 to 18 years with an isolated, acute (<24 hours) TBI who presented to a paediatric trauma referral centre were eligible for participation if they required inpatient hospitalisation following ED management. Exclusion criteria: multisystem trauma, presence of preexisting neurological conditions such as cerebral palsy, chronic seizure disorder, ventriculo-peritoneal shunts, history suggestive of head trauma due to chronic abuse, incarcerated patients, and refusal to participate.
Target condition(s)	Acute post-brain injury complications
Index test(s) and reference standard	Index test: Salivary biomarkers • GFAP • S100B • NSE Study reports variable timing of sample collection.

Reference	**Yeung, 2020** [66]
	Timing of index test not reported Reference standard: Head CT
Results	Outcome: Significant brain injury on CT scan Significant brain injury on CT scan was defined as evidence of intracranial haemorrhage or contusion, cerebral oedema, traumatic infarction, diffuse axonal injury, shearing injury, sigmoid sinus thrombosis, midline shift of intracranial contents or signs of brain herniation, diastasis of the skull pneumocephalus, and skull fracture depressed by at least the width of the table of the skull. Of the 24 TBI patients, 100% underwent a head CT scan; 14 (58.3%) were diagnosed to have SBI on CT scan. All patients with SBI were admitted to the paediatric intensive care unit; 1 patient (7.1%) subsequently expired. Among the SBI patients, 7 (50%) had mild TBI with GCS score of 13 to 15, 3 (21.4%) had moderate TBI with GCS score of 9 to 12, and 4 (28.6%) had severe TBI with GCS score of 8 or less. S100B in predicting SBI detected by CT scan AUC: 0.675 (no CI reported) No sensitivity/specificity/AUC data reported for GFAP and NSE.

Reference	**Yeung, 2020** [66]
Source of funding	Not reported
Limitations	Risk of bias *(QUADAS 2 – risk of bias)*: serious. Unclear whether the index test was interpreted without knowledge of the reference standard and vice versa Indirectness *(QUADAS 2 – applicability)*: serious. Mixed severity TBI included however majority of patients had mild TBI
Comments	Six TBI patients (25%) required neurosurgical intervention, with 2 (8.3%) requiring external ventricular drain placement, 1 (4.2%) requiring craniectomy, and 3 (12.5%) requiring both interventions. All 6 patients requiring neurosurgical intervention had SBI on CT scan.

Biomarkers in studies where age was not reported

Reference	**Biberthaler, 2001** [9]
Study type	Prospective cohort
Study methodology	Data source: prospective study carried out at a single level 1 urban surgical emergency department (ED) between October and December 1998. Patients presenting to the ED with a history of minor head trauma (MHT) were recruited. A positive control group of patients with severe head trauma (GCS score <8) and a negative control group of healthy volunteers (n = 20) were also recruited.
Number of patients	n = 52
Patient characteristics	Age, mean (SD): not reported Gender (male): 73% GCS: no breakdown but GCS score 13-15 for inclusion in the study Ethnicity: not reported Setting: single ED

Reference	**Biberthaler, 2001** [9]
	Country: Germany Inclusion criteria: presented to ED with a history of isolated MHT; GCS score 13-15 at admission; at least one of the following symptoms: amnesia, loss of consciousness (LOC), nausea, vomiting, vertigo, or severe headache Exclusion criteria: focal neurologic deficits
Target condition(s)	Acute post-brain injury complications
Index test(s) and reference standard	Index test: Serum S100B at admission (cut-off 0.1 µg/ml) The interval between trauma and admission was 73.46 (47) minutes; and the interval between trauma and blood sampling was 116 (18.8) minutes. Reference standard: Spiral cranial CT scan within 6 hours post injury. Follow up: no mention of follow up
Results	Outcome: pathologic findings (intracerebral haemorrhage, skull fracture, or diffuse brain swelling) on CT scan Pathologic findings on CT scan – Serum S100B at admission (cut-off 0.1 µg /ml) TP: 15 FP: 22 TN: 15 FN: 0

Reference	**Biberthaler, 2001** [9]
	Sensitivity%: 100 Specificity%: 40.5 PPV%: 40.5 NPV%: 100 <u>SN/SP calculated by NGC:</u> Sensitivity: 1.00 [0.78, 1.00] Specificity: 0.41 [0.25, 0.58]
Source of funding	Supported by the Deutsche Forschungs-Gemeinschaft, Sonderforschungsbereich 469 of the Ludwig-Maximilians University Munich
Limitations	Risk of bias *(QUADAS 2 – risk of bias)*: serious. Unclear whether index test results were interpreted without knowledge of the reference standard and vice versa Indirectness *(QUADAS 2 – applicability)*: serious. Age not reported
Comments	-

Reference	**Poli-de-Figueiredo, 2006** [59]
Study type	Prospective cohort study (pilot study)
Study methodology	This was a pilot study investigating the hypothesis that S100B may be a rapid and useful screening tool for the management of minor head injury (MHI) patients and thus reducing any unnecessary and costly CCT scans.

Reference	Poli-de-Figueiredo, 2006 [59]
	Data source: consecutive patients presenting with MHI to a large emergency centre in João XXIII Hospital between September and October 2003. A negative control group of healthy volunteers was also studied to compare the levels of S100B in these patients to those in patients with head injury.
Number of patients	n = 50
Patient characteristics	Age, median (IQR): Not reported Gender (male to female ratio): 28 M: 22 F Ethnicity: not reported Setting: ED Country: Brazil GSC: n=37 GCS score 15, n= 11 GCS score 14, n=2 GCS score 13 Inclusion criteria: patients who had sustained isolated minor head injury (GCS score 13 -15) and presented at least one of the following symptoms: amnesia, loss of consciousness, nausea, vomiting, vertigo, or severe headache on admission.

Reference	**Poli-de-Figueiredo, 2006** [59]
	Exclusion criteria: patients with focal neurological deficits
Target condition(s)	Acute post brain injury complications
Index test(s) and reference standard	Index test(s) S100B levels A cut-off point at a concentration of 0.1 µg/L of S100B was used. This was based on the highest level measured in healthy volunteers without any sign of intracranial injury. Therefore, patients presenting a S100B level below 0.1 µg/L were defined as "negative," and those with concentration above 0.1 µg/L were defined as "positive." Venous blood samples were drawn on admission and processed to serum (median 82 minutes, (25%-75% quartiles: 60-110 min). Reference standard Cranial computed tomography (CCT) was performed within 6 hours of emergency room admission, and radiological findings were defined as pathological (CCT+) if intracranial haemorrhage, skull fracture, and/or diffuse brain swelling (oedema) were detected. Time between measurement of index test and reference standard: Unclear

Reference	Poli-de-Figueiredo, 2006 [59]
Results	Outcome: signs of intracranial injury at the initial CCT scan The median time interval from trauma to blood sampling for the S100B assay was 82 minutes (25%-75% quartiles: 60-110 min) 6 patients had trauma-relevant intracranial lesions according to the radiological criteria and were thereby counted as CCT+. S100B cut-off 0.1 µg/L TP = 6 FP = 35 TN = 9 FN = 0 CT + = 6 Sensitivity= 100% (95%CI not reported) Specificity= 20% (95%CI not reported) PPV= 100% (95%CI not reported) NPV= 100% (95%CI not reported) AUC (95%CI)= 0.82% (0.69-0.96)

Reference	**Poli-de-Figueiredo, 2006** [59]
	Calculated by NGC Sensitivity: 1.00 [0.54, 1.00] Specificity: 0.20 [0.10, 0.35]
Source of funding	The study was supported by a grant from the program “CAPES-BAVARIA” which is a project of the Bavarian ministry of science, research and art (Staatsministerium für Wissenschaft, Forschung und Kunst) and the “Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES)” administration in Brazil, to increase scientific exchange between both countries. The test systems were provided by ROCHE Diagnostics, Mannheim, Germany.
Limitations	Risk of bias (QUADAS 2 – risk of bias): serious. Unclear whether the selection of patients could have introduced bias Indirectness (QUADAS 2 – applicability): serious. Age not reported
Comments	No information on treatment

Appendix E – Forest plots

E.1 Coupled sensitivity and specificity forest plots

Biomarkers in adults

S100 B within 3 hours after injury

Figure 3: serum S 100 B (cut-offs 0.10 and 0.105 µg/L) (within 3 hours)- Adults

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Biberthaler, 2006	92	815	1	401	0.99 [0.94, 1.00]	0.33 [0.30, 0.36]
Ernstbrunner, 2016	3	306	1	72	0.75 [0.19, 0.99]	0.19 [0.15, 0.23]
Laribi, 2014	26	231	0	143	1.00 [0.87, 1.00]	0.38 [0.33, 0.43]
Li, 2022	41	120	3	25	0.93 [0.81, 0.99]	0.17 [0.11, 0.24]
Muller 2011	19	144	3	67	0.86 [0.65, 0.97]	0.32 [0.26, 0.38]
Thaler, 2015	49	474	1	258	0.98 [0.89, 1.00]	0.35 [0.32, 0.39]
Wolf, 2013	22	49	8	28	0.73 [0.54, 0.88]	0.36 [0.26, 0.48]

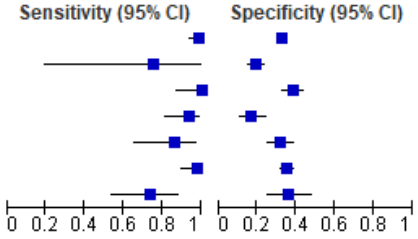


Figure 4: serum S100B (cut-off 0.15, µg/L)-within 3 hours after injury- Adults

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Biberthaler, 2002	24	43	0	37	1.00 [0.86, 1.00]	0.46 [0.35, 0.58]
Laribi, 2014	26	219	1	174	0.96 [0.81, 1.00]	0.44 [0.39, 0.49]

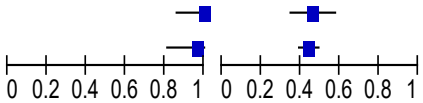


Figure 5: Serum S100B (optimal cut off 0.115 µg/L)- at 3 hours after injury- Adults

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Asadollahi, 2016	75	51	4	28	0.95 [0.88, 0.99]	0.35 [0.25, 0.47]

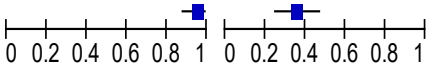


Figure 6: Serum S100B measured immediately after admission (cut-off ≥0.2 µg/L) mean 3 hours after injury- Adults

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Ingebrigtsen, 2000	9	60	1	112	0.90 [0.55, 1.00]	0.65 [0.57, 0.72]

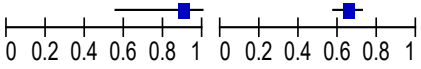


Figure 7: Serum S100B (cut-off 0.12 µg/L)-within 2 hours post injury after injury- Adults

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Biberthaler, 2002	24	43	0	37	1.00 [0.86, 1.00]	0.46 [0.35, 0.58]

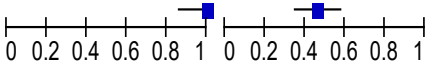


Figure 8: serum S 100B (a cut-off of 0.48 µg/L)- within 3 hrs after injury- Adults

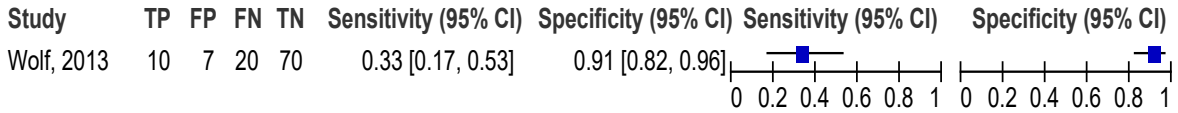


Figure 9: Serum S100B post injury (cut-off 0.38 µg/L)- within 3 hours after injury- Adults

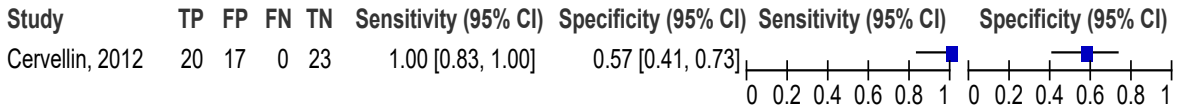
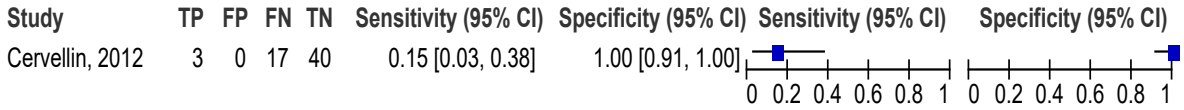


Figure 10: Serum S100B (cut-off 2.31 µg/L)-within 3 hours after injury -Adults



S100 B >3 -6 hours post injury

Figure 11: serum S 100 B (cut-offs 0.10 and 0.105 µg/L) (> 3 hours -6 hours after injury) -Adults

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Bazarian 2013	39	476	6	266	0.87 [0.73, 0.95]	0.36 [0.32, 0.39]
David, 2017	28	192	5	83	0.85 [0.68, 0.95]	0.30 [0.25, 0.36]
Egea-Guerrero, 2012	15	94	0	34	1.00 [0.78, 1.00]	0.27 [0.19, 0.35]
Egea-Guerrero, 2018	21	164	1	74	0.95 [0.77, 1.00]	0.31 [0.25, 0.37]
Kahouadji, 2020	32	87	1	10	0.97 [0.84, 1.00]	0.10 [0.05, 0.18]
Lagerstedt, 2017	26	81	6	59	0.81 [0.64, 0.93]	0.42 [0.34, 0.51]
Laribi, 2014	17	177	8	189	0.68 [0.46, 0.85]	0.52 [0.46, 0.57]
Morochovic, 2009	15	59	3	25	0.83 [0.59, 0.96]	0.30 [0.20, 0.41]
Vedin, 2021	13	197	0	33	1.00 [0.75, 1.00]	0.14 [0.10, 0.20]
Zongo 2012	110	1239	1	210	0.99 [0.95, 1.00]	0.14 [0.13, 0.16]

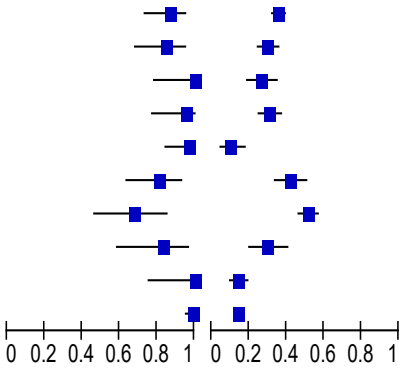


Figure 12: Serum S100B (cut-off >0.29 µg/L)- within 6 hours after injury- Adults

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Bazarian 2013	26	177	24	560	0.52 [0.37, 0.66]	0.76 [0.73, 0.79]

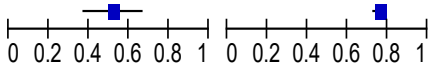


Figure 13: Serum S100B (cut-off >0.06 µg/L)- within 6 hours after injury -Adults

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Bazarian 2013	50	649	0	88	1.00 [0.93, 1.00]	0.12 [0.10, 0.15]

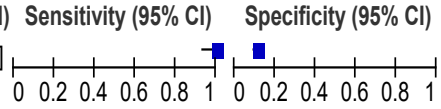


Figure 14: Serum S100B (cut-off >2.391 µg/L)- within 6 hours after injury- Adults

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Bazarian 2013	2	7	48	730	0.04 [0.00, 0.14]	0.99 [0.98, 1.00]

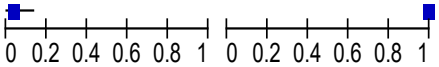


Figure 15: Serum S100B (cut-off >0.097 µg/L)-within 6 hours after injury -Adults

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Bazarian 2013	45	501	5	236	0.90 [0.78, 0.97]	0.32 [0.29, 0.36]

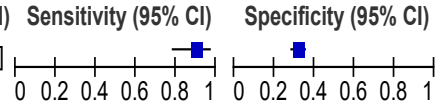


Figure 16: Serum S100B (cut-off >0.521 µg/L)-within 6 hours after injury- Adults

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Bazarian 2013	12	74	38	663	0.24 [0.13, 0.38]	0.90 [0.88, 0.92]

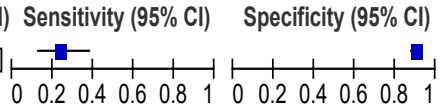


Figure 17: serum S100 B (cut-off 0.130 µg/L) at 6 hours after injury- Adults

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Egea-Guerrero, 2012	15	86	0	42	1.00 [0.78, 1.00]	0.33 [0.25, 0.42]

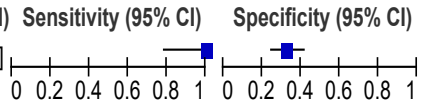


Figure 18: serum S100 B (cut-off 0.2 µ g/L) -mean 3.8 hours after injury (reference standard CT and MRI) -Adults

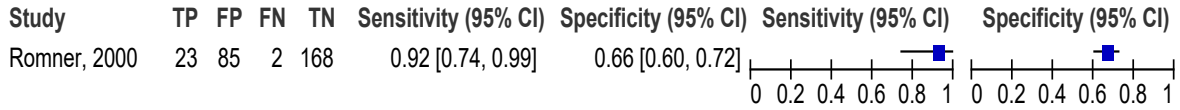


Figure 19: Serum S100B (optimal cut off 0.21 µg/L)- at 6 hours after injury- Adults

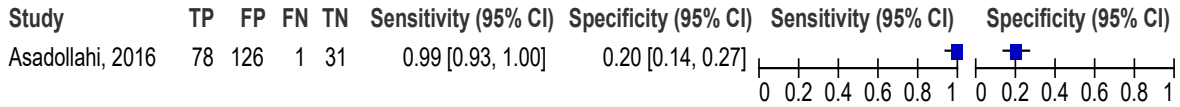


Figure 20: serum S100 B (cut-off 0.230 µg/L) at 6 hours after injury -Adults

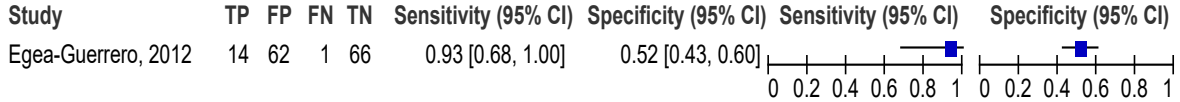


Figure 21: serum S100 B (cut-off 0.254 µg/L) at 6 hours after injury- Adults

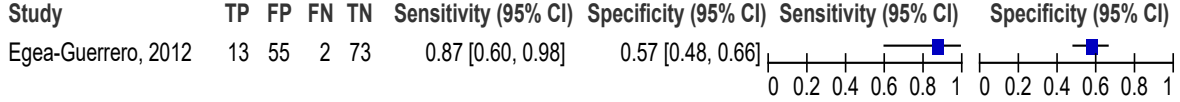


Figure 22: serum S100B (cut-off 0.15, µg/L)- 6 hours after injury (second sampling 3 hrs after 1st sampling) -Adults

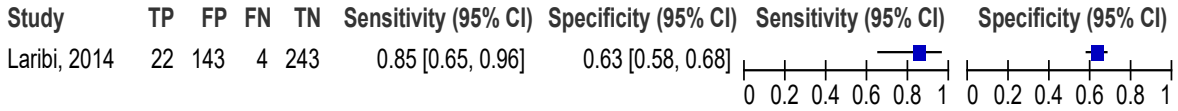


Figure 23: serum S100B- optimal cut-off value of 0.47 µg/L-within 4 hours after injury -Adults [mixed children and adults(mean age 24 years)]

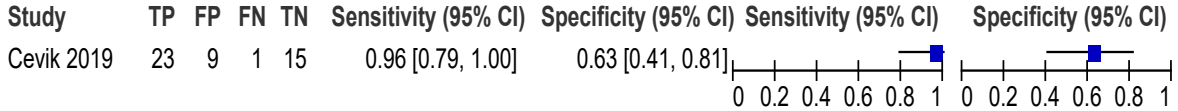


Figure 24: serum S100B (cut-off 0.42 µg/)-within 6 hours after injury- Adults

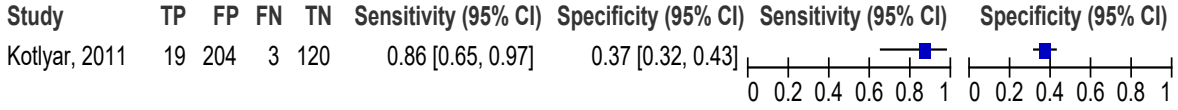


Figure 25: serum S100B (cut-off 0.32 µg/L)-within 6 hours after injury- Adults

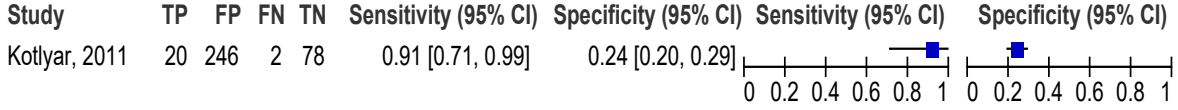


Figure 26: serum S100B (cut-off 0. 24 µg/L)- within 6 hours after injury -Adults

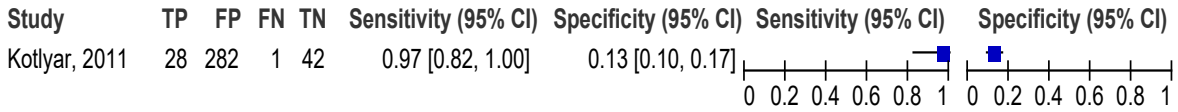


Figure 27: serum S100B (cut-off Value, 0.12 µg/L) -within 6 hours after injury - Adults

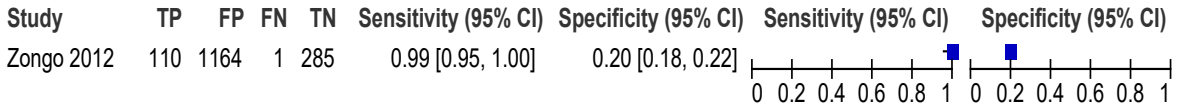


Figure 28: serum S100B (cut-off Value, 0.14 µg/L)-within 6 hours after injury - Adults

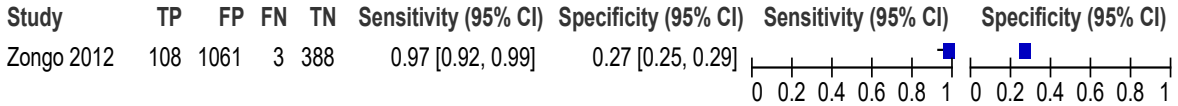


Figure 29: serum S100B (Cut-off 0.105 µg/L)- within 6 hours after injury (reference standard CCT or MRI) -Adults

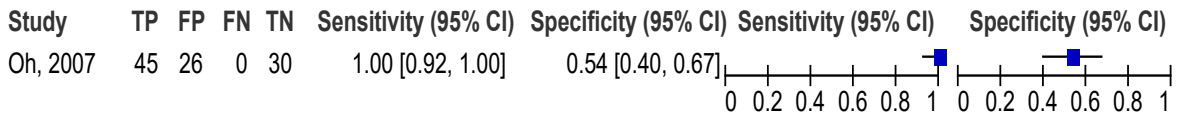


Figure 30: serum S100 B- 0 to 8-hours after injury (cut-off value NR) -Adults

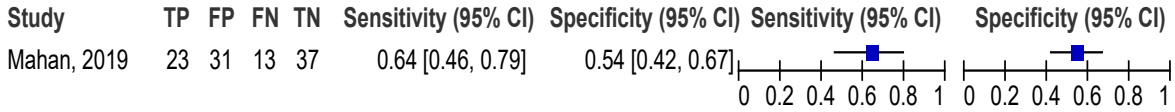
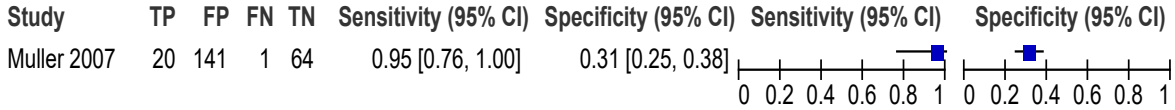


Figure 31: serum S100B (Cut-off ≥0.10 µg/L) (within 12 hours after injury) -Adults



>12 hours after injury

Figure 32: S100 B -12- to 32-hour- after injury (cut-off value NR) -Adults

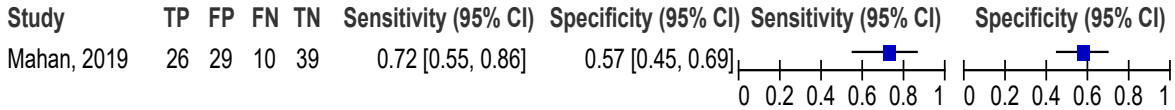
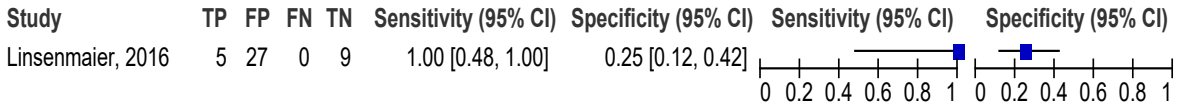
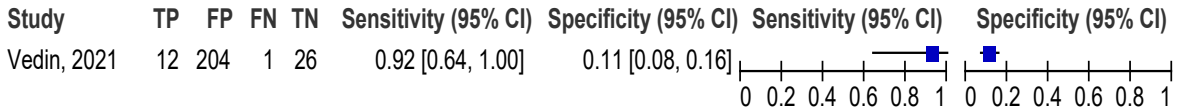


Figure 33: serum S100B (cut-off value of 0.1 µg/L)- not time specified [reference standard CT and MRI] -Adults



Urine -S100B

Figure 34: Urine -S100B (cut-off ≥0.09 µg/L)- within 6 hours after injury -Adults



GFAP

Figure 35: (GFAP) - cut-off GFAP 0.23 µg/L -within 4 hours after injury -mixed adults and children (mean 24 yrs)

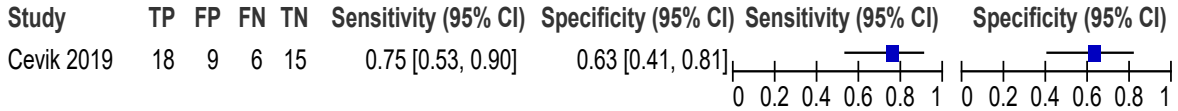


Figure 36: Serum GFAP (cut-off 1.35 µg/L)- within 6 hours after injury- Adults

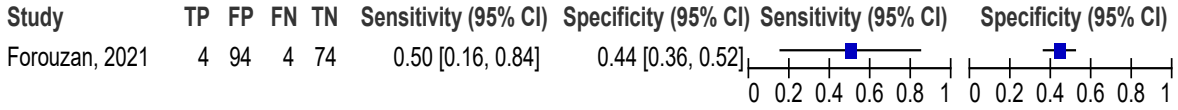


Figure 37: Plasma GFAP (cut-off 0.022 µg/ml) -within 6 hours after injury- Adults

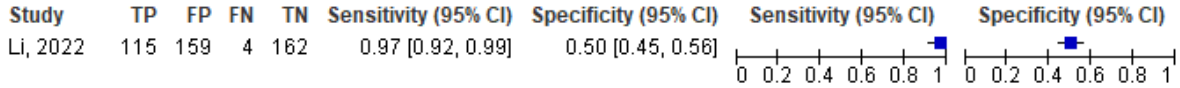


Figure 38: serum GFAP (cut-off 0.022 µg/ml) -within 6 hours after injury- Adults

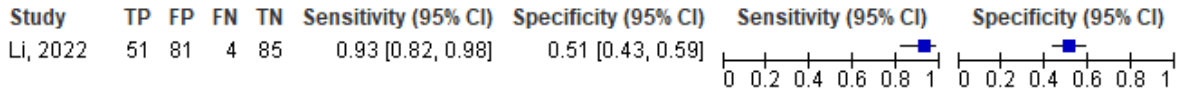


Figure 39: serum GFAP -0- to 8 hours after injury (cut-off value NR)- Adults

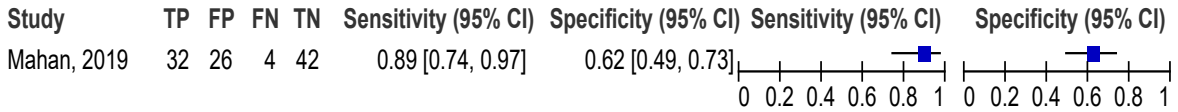


Figure 40: serum GFAP cut-off 0.013 µg/L (within 24 hours after injury)- Adults-

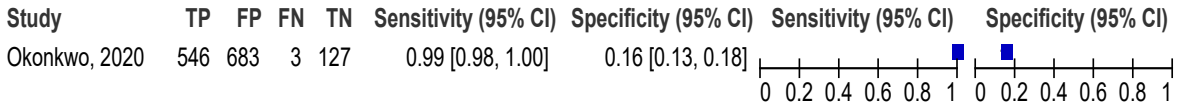


Figure 41: serum GFAP cut-off 0.038 µg/ (within 24 hours after injury)- Adults

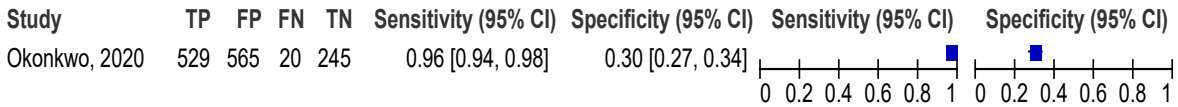


Figure 42: serum GFAP cut-off 0.113 µg/L (within 24 hours after injury)- Adults

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
Okonkwo, 2020	495	407	54	403	0.90 [0.87, 0.93]	0.50 [0.46, 0.53]		

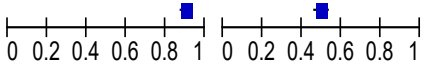


Figure 43: serum GFAP cut-off 0.190 µg/L (within 24 hours after injury)- Adults

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
Okonkwo, 2020	464	329	85	481	0.85 [0.81, 0.87]	0.59 [0.56, 0.63]		

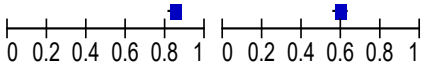


Figure 44: serum GFAP [cut-off 0.43 µg/L] (within 24 hours after injury) - middle age (40-59)- Adults

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
Gardner, 2018	18	9	2	31	0.90 [0.68, 0.99]	0.78 [0.62, 0.89]		

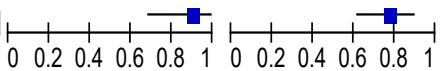


Figure 45: serum GFAP [cut-off point 0.43 µg/L) (within 24 hours after injury) young (<40 yr)- Adults

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
Gardner, 2018	15	10	3	51	0.83 [0.59, 0.96]	0.84 [0.72, 0.92]		

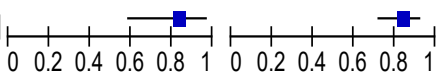


Figure 46: serum GFAP [cut-off 0.43 µg/L] (within 24 hours after injury) older age (>60)- Adults

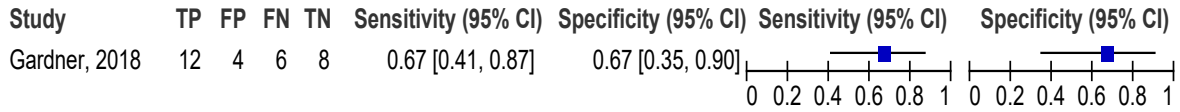
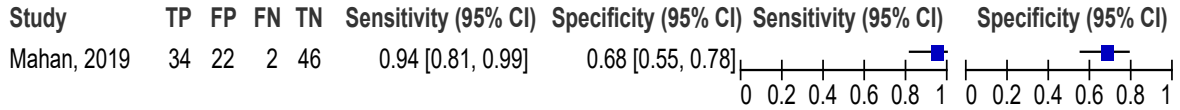


Figure 47: serum GFAP (12- to 32-hours after injury) (cut-off value NR)- Adults



GFAP-BDP

Figure 48: Serum GFAP-BDP (cut-off level of 0.035 µg/L) -within 4 hours after injury- Adults

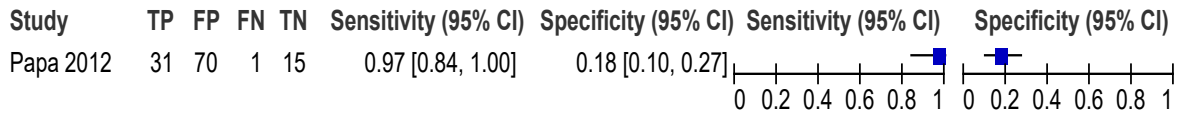


Figure 49: serum GFAP-BDP at a 0.68 µg/L - within 24 hours after injury- Adults

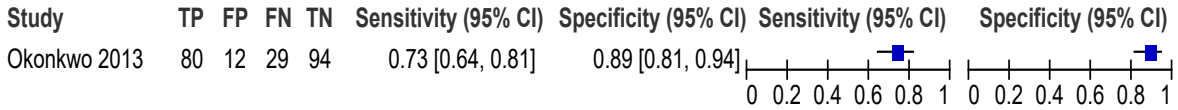


Figure 50: serum GFAP-BDP (a cut-off of 0.6 µg/L)- within 24 hours after injury- Adults

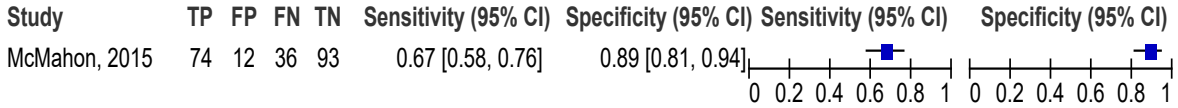
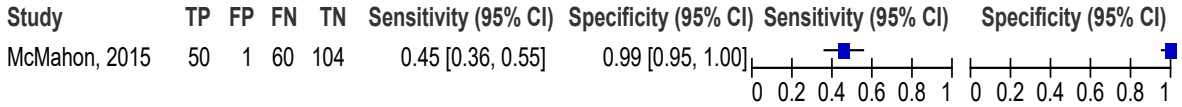


Figure 51: serum GFAP-BDP level (a cut-off of 1.66 µg/L) - within 24 hours after injury- Adults



NSE

Figure 52: serum NSE (a cut-off limit of 14.7 µg/L)- within 3 hours after injury- Adults

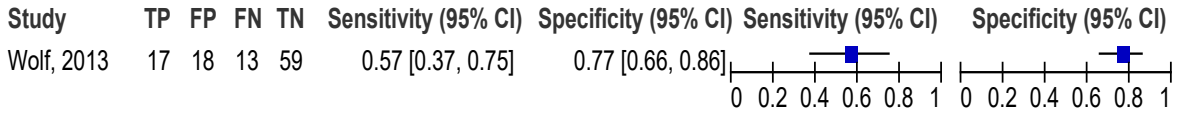
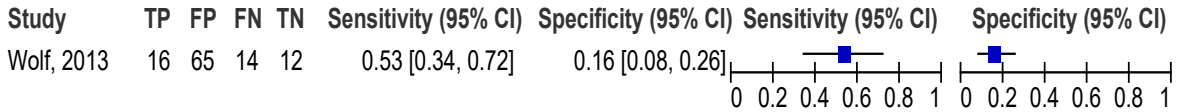


Figure 53: serum NSE (cut-off limit of 16.4 µg/L) - within 3 hours after injury -Adults



UCH-L1

Figure 54: serum UCH-L1 (cut-off level of 0.09 µg/L) – within 4 hours of injury after injury- Adults

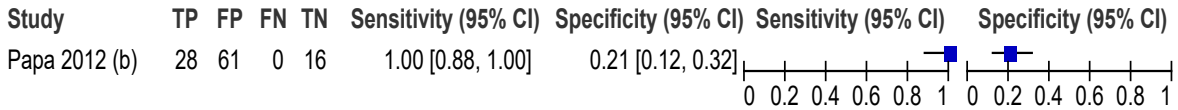
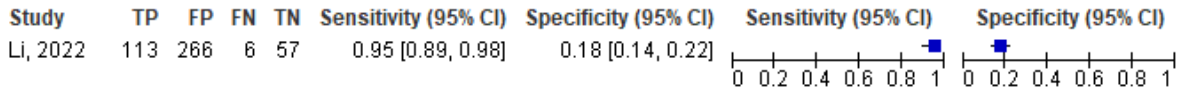


Figure 55: Plasma UCH-L1 (cut-off 0.327 µg/ml)- within 6 hours after injury- Adults



<Insert Note here>

Figure 56: serum UCH-L1 (cut-off 0.327 µg/ml)- within 6 hours after injury- Adults

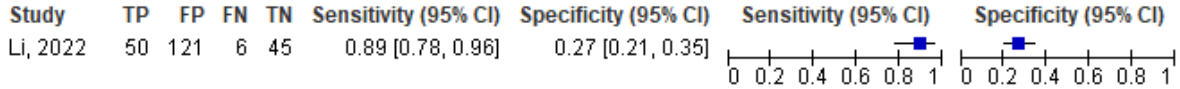


Figure 57: serum UCH-L1 -0- to 8 hours after injury (cut-off value NR)- Adults

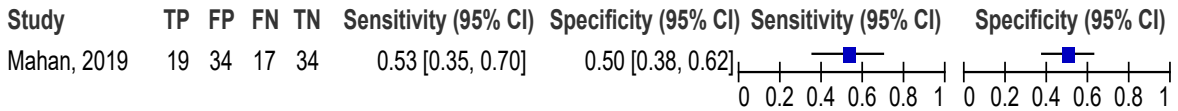
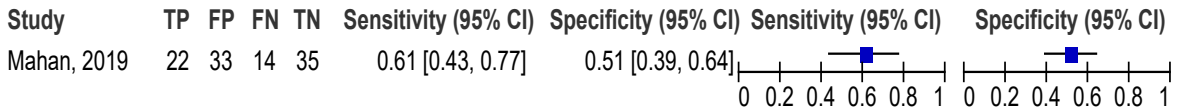


Figure 58: serum UCH-L1 -12- to 32hours- after injury (cut-off value NR) -Adults



Combined serum UCH-L1 and GFAP

Figure 59: combined Plasma GFAP (cut-off 0.022 µg/ml) and UCH-L1 (cut-off 0.327 µg/ml) -within 6 hours after injury -Adults

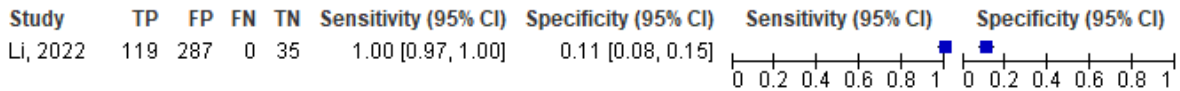
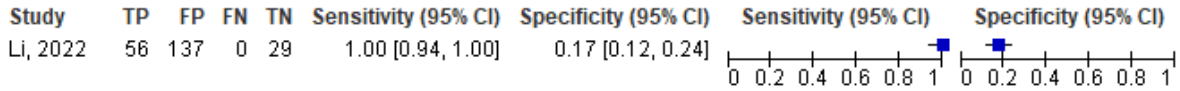
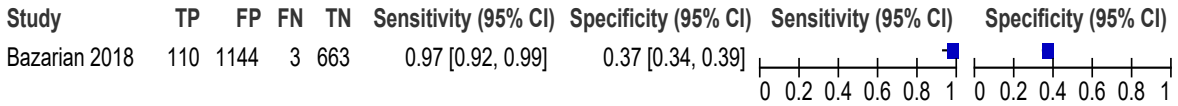


Figure 60: combined Plasma GFAP (cut-off 0.022 µg/ml) and UCH-L1 (cut-off 0.327 µg/ml) -within 6 hours after injury -Adults



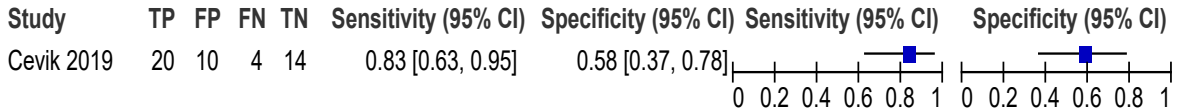
<Insert Note here>

Figure 61: Combined serum UCH-L1 and GFAP measured within 12 h after injury (cut-off 0.327 µg/L for UCH-L1 and 0.022 for GFAP µg/L)) -Adults



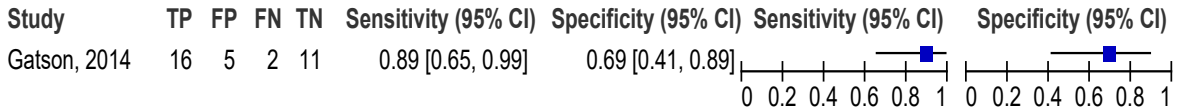
<u>serum small neuronal protein neurogranin (NRGN)</u>

Figure 62: serum small neuronal protein neurogranin (NRGN)- cut-off 1.87 µg/L - within 4h after injury -mixed adults and children (mean 24 yrs)-



<u>serum pNFL-H</u>

Figure 63: serum pNFL-H (1.071 µg/L) – (18-24 hours after injury)- Adults



Biomarkers in children

serum S100B

Figure 64: serum S100B cut-off (0.35 µg/L for age 0 –9 months; 0.23 µg/L for age 10 –24 months; 0.18 µ g/L for age >24 months)- median 2 hours after injury- children

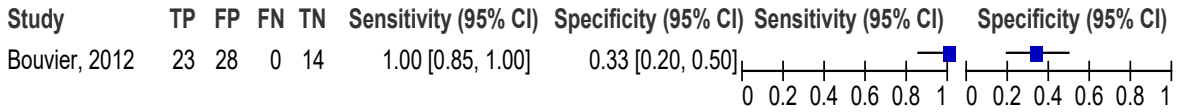


Figure 65: S100B (Cut-off value 0.14 µg/L) - All children <16 years – within 6 hours after injury -children

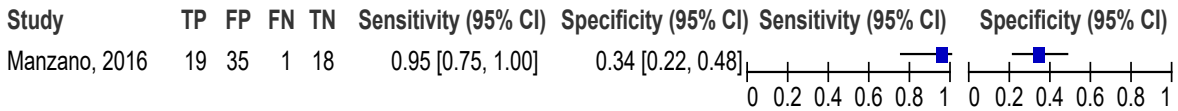


Figure 66: S100B (cut-off 0.16µg/L)-within 6 hours after injury -children

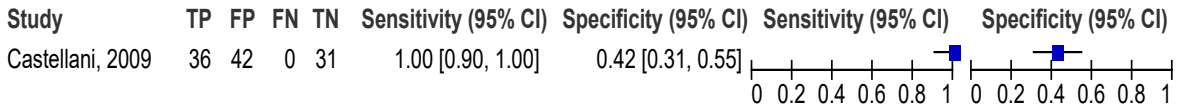


Figure 67: serum S 100B level of 0.1 µg/L- within 6 hours after injury- children

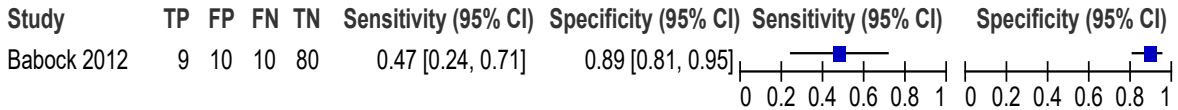


Figure 68: S100b cut-off level of 0.020 µg/L - within 6 hours after injury -children [mixed children and youth (mean 13 years)]

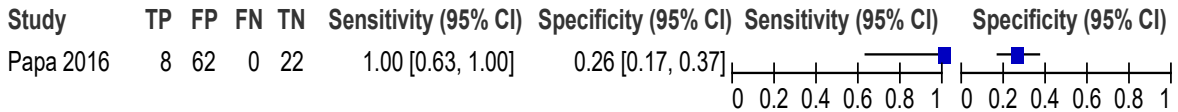


Figure 69: Serum S 100 B (cut-off of 172.15 µg/L)- within 6 hours after injury - children

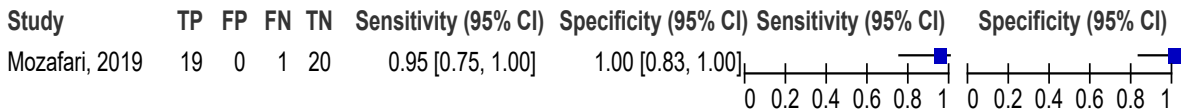
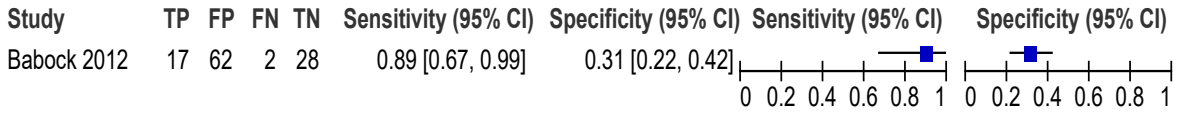


Figure 70: serum S 100B level cut-off > 0.006µg/L- within 6 hours after injury- children



Urinary S100B

Figure 71: Urinary S100B (cut-off levels of 56.4 ng/L)- within 6 hours after injury- children

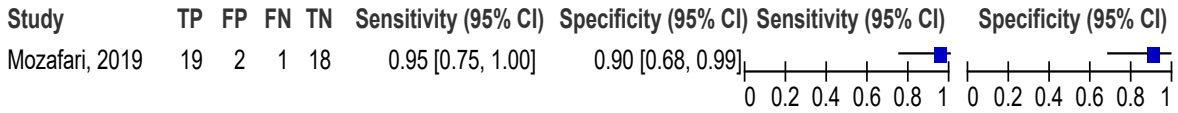
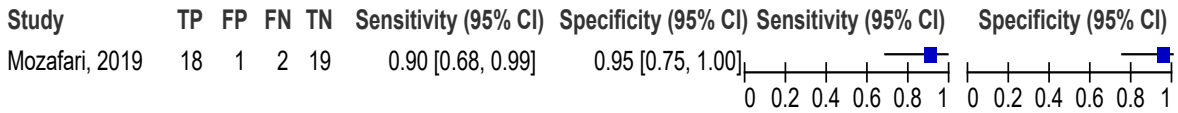


Figure 72: Urinary S100B (cut-off levels of 67.75 ng/L)- within 6 hours after injury- children



GFAP

Figure 73: GFAP (cut-off 0.15 µg/L) within 6 hours after injury: Isolated skull fracture+ICL- children [mixed children and youth (median age 12)]

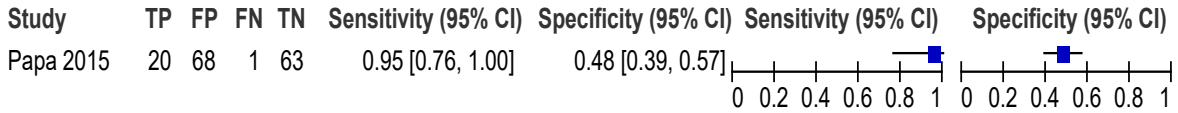


Figure 74: GFAP cut-off level of 0.15 µg/L - within 6 hours after injury - children [mixed children and youth (mean 13 years)

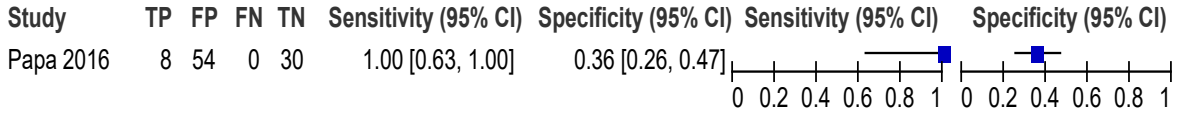
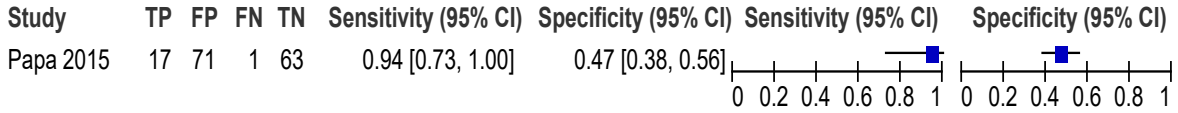
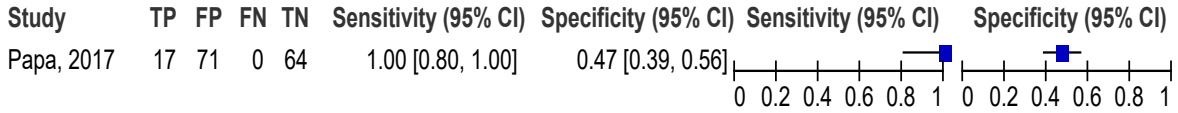


Figure 75: GFAP (cut-off 0.15 µg/L) within 6 hours after injury: ICL only (no skull fracture) -children [mixed children and youth (median age 12)



UCH-L1

Figure 76: -UCH-L1 (Cut -off ≥0.18 µg/L)- within 6 hours after injury- children and youth (mean age 12)



NSE

Figure 77: NSE (at a level of NSE ≥15.3 µg/L)- mean 4 hours after injury- children

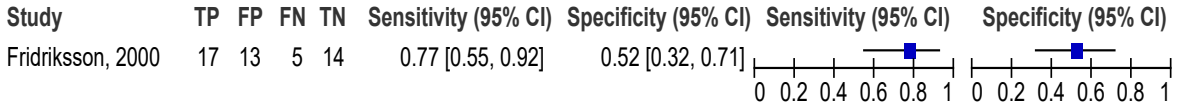


Figure 78: NSE (cut-off points 5.74 µg/L)-within 6 hours after injury -children

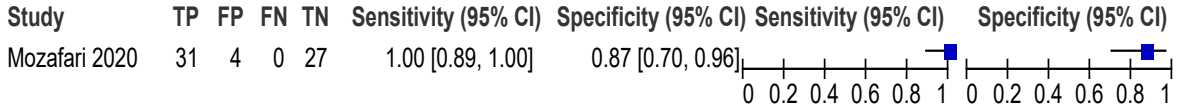
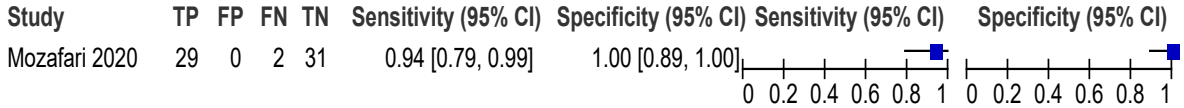


Figure 79: NSE (cut-off points of 6.97 µg/L) - within 6 hours after injury -children



Biomarkers in studies where age was not reported

Figure 80: Serum100B (cut-off 0.1 µg/L) (median 82 minutes after injury) -age NR

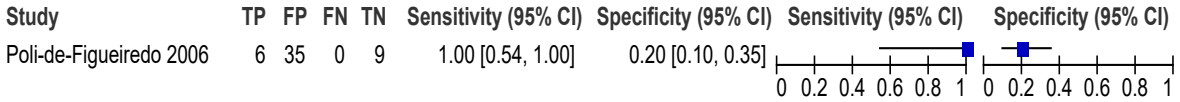
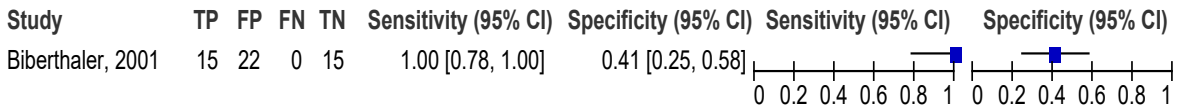


Figure 81: Serum S100B at admission (cut-off 0.1 µg/L) [within 3 hours after injury]- Age NR



E.2 ROC curves

Figure 82: Meta-analysis of S100 B 0.10 µg/L and 0.105 µg/L (<3 hours after injury)

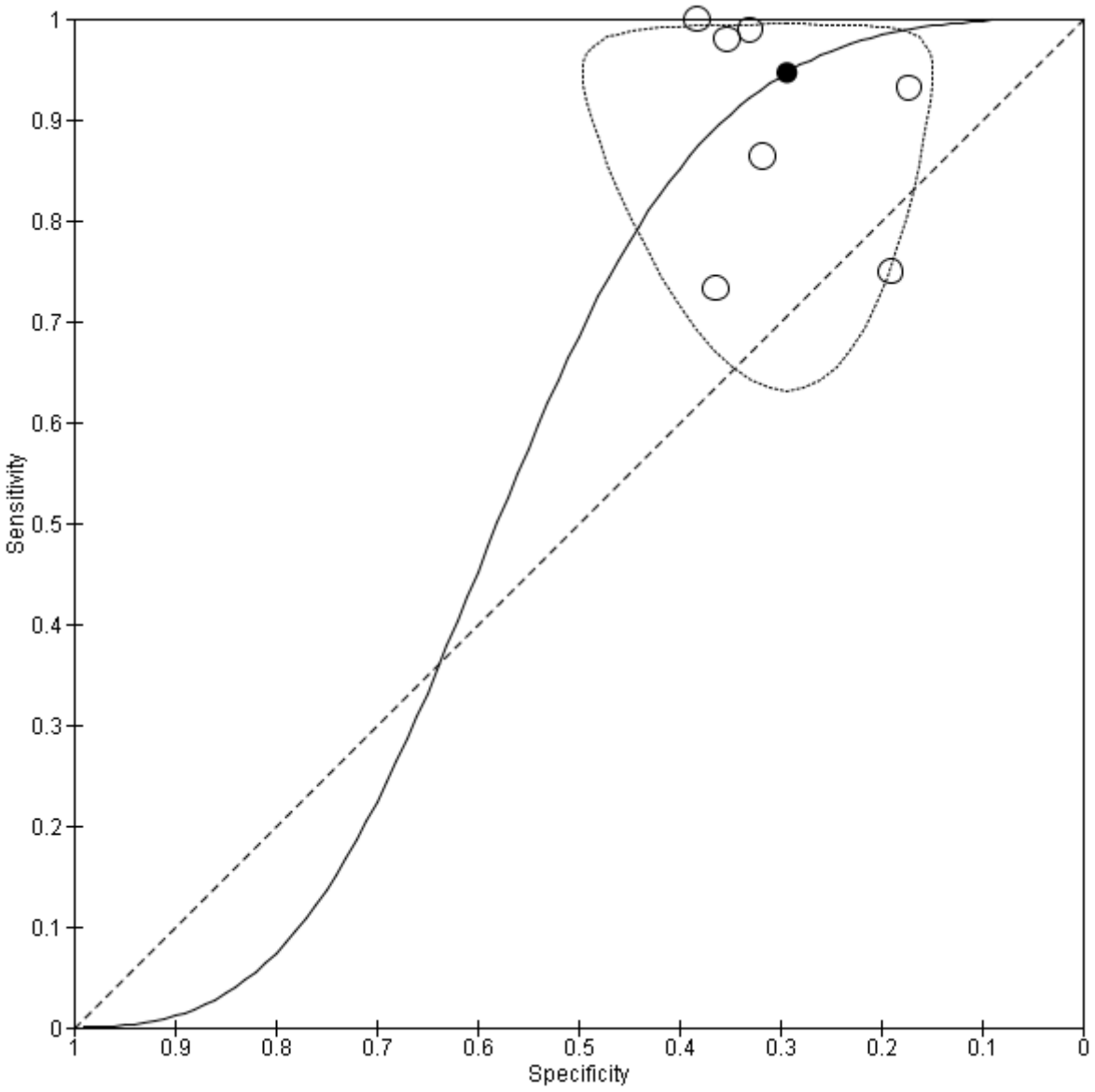
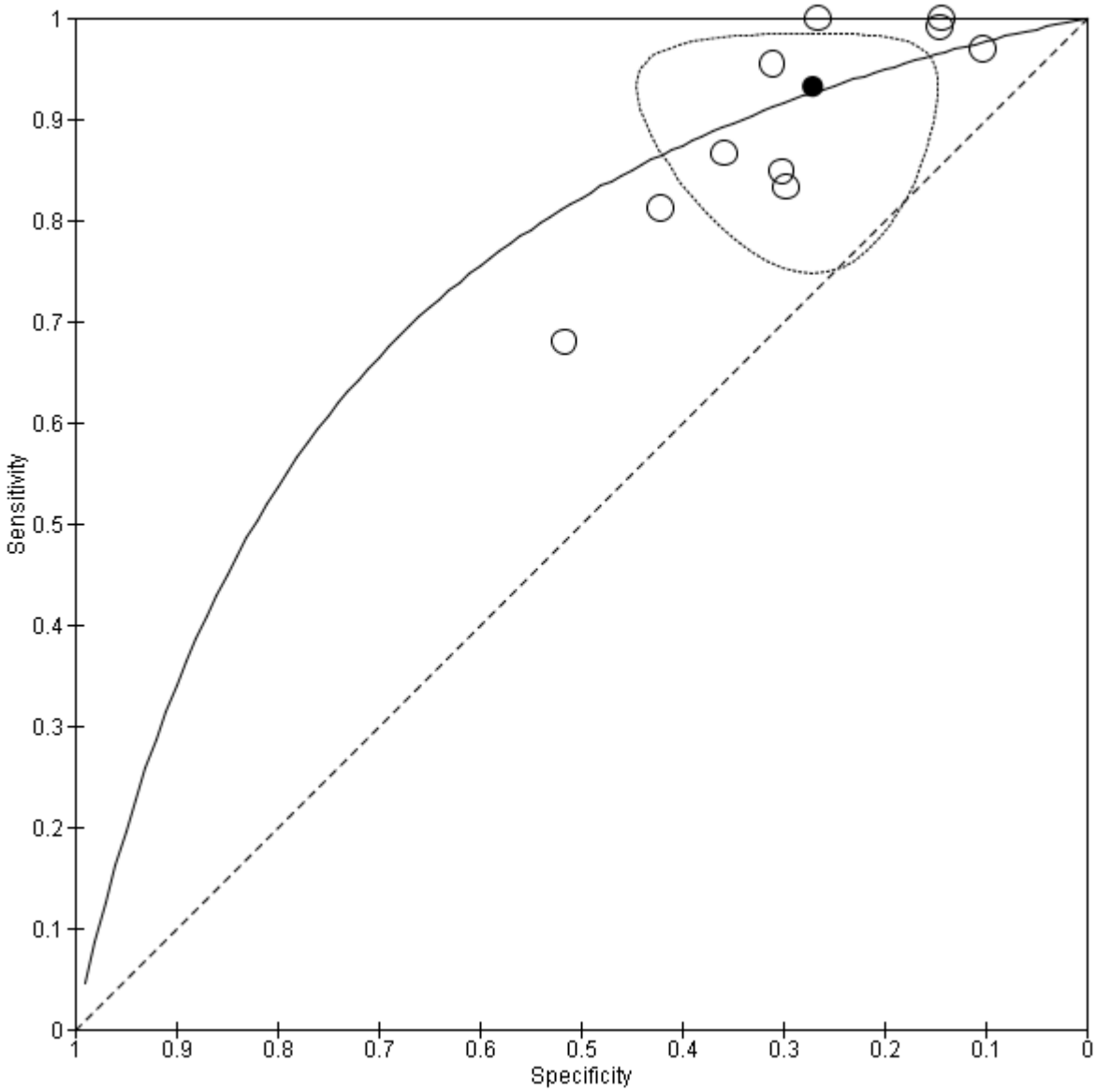


Figure 83: Meta-analysis of S100 B 0.10 µg/L and 0.105 µg/L (>3 to 6 hours after injury)



Key:

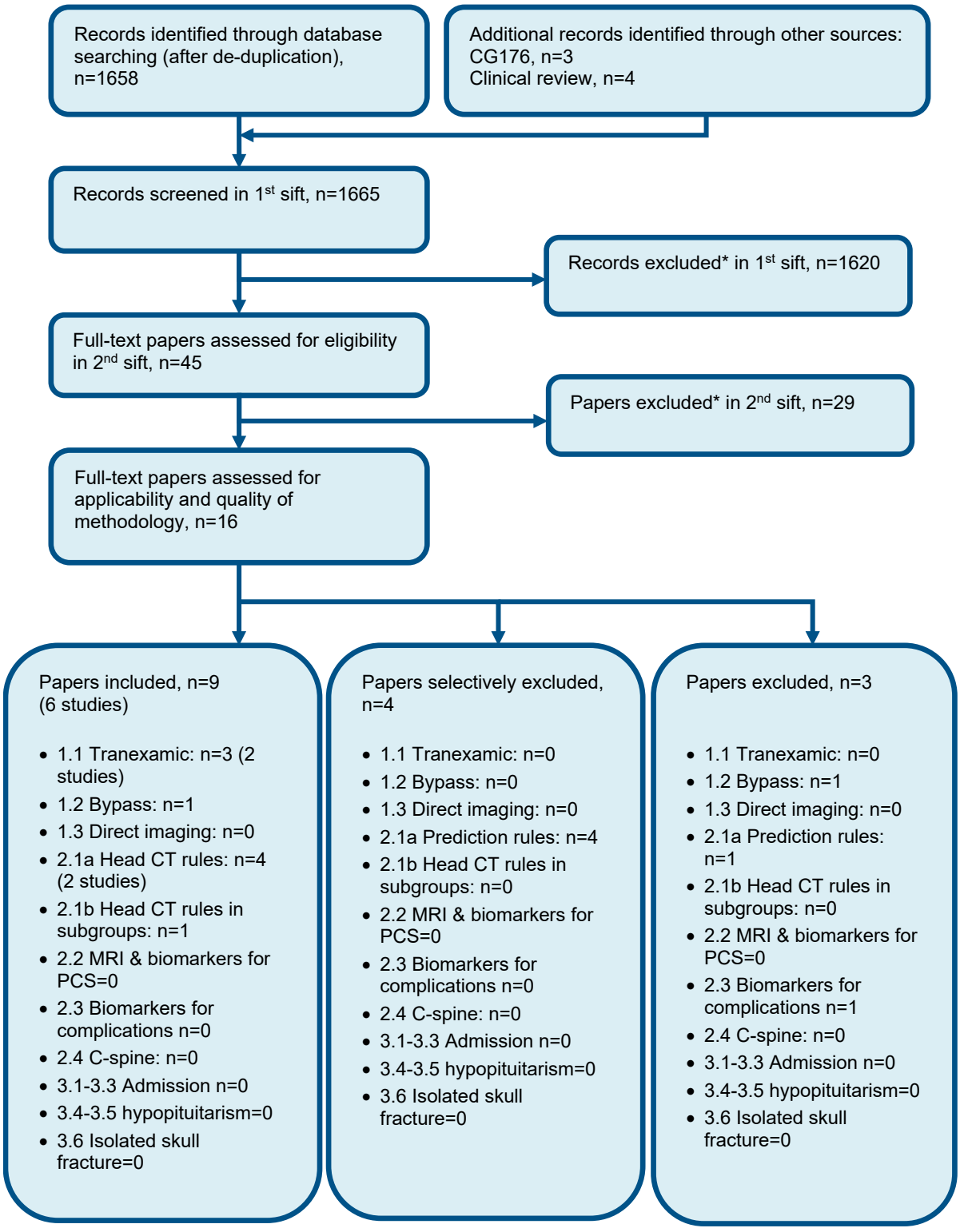
Solid line represents the ROC summary curve

Dotted line represents the 95% confidence region of the ROC

Solid circle represents pooled ROC

Clear circles represent ROC of individual studies

Appendix F – Economic evidence study selection



* Non-relevant population, intervention, comparison, design or setting; non-English language

Appendix G – Economic evidence tables

None.

Appendix H – Health economic model

None.

Appendix I – Excluded studies

Clinical studies

Table 7: Studies excluded from the clinical review (diagnostic accuracy)

Study	Code [Reason]
Abbasi, M., Sajjadi, M., Fathi, M. et al. (2014) Serum S100B Protein as an Outcome Prediction Tool in Emergency Department Patients with Traumatic Brain Injury. Turkish Journal of Emergency Medicine 14(4): 147-52	- No diagnostic accuracy measures reported
Agoston, D. V.; Shutes-David, A.; Peskind, E. R. (2017) Biofluid biomarkers of traumatic brain injury. Brain Injury 31(9): 1195-1203	- Review article but not a systematic review
Akhtar, J. I., Spear, R. M., Senac, M. O. et al. (2003) Detection of traumatic brain injury with magnetic resonance imaging and S-100B protein in children, despite normal computed tomography of the brain. Pediatric Critical Care Medicine 4(3): 322-6	- No diagnostic accuracy measures reported
Al-Adli, N., Akbik, O. S., Rail, B. et al. (2021) The Clinical Use of Serum Biomarkers in Traumatic Brain Injury: A Systematic Review Stratified by Injury Severity. World Neurosurgery 23: 23	- Systematic review - screened for relevant references
Alatas, O. D., Gurger, M., Atescelik, M. et al. (2015) Neuron-Specific Enolase, S100 Calcium-Binding Protein B, and Heat Shock Protein 70 Levels in Patients With Intracranial Hemorrhage. Medicine 94(45): e2007	- Population not relevant to this review protocol
Alexiou, G. A., Lianos, G. D., Sotiropoulos, A. et al. (2019) Novel biomarkers may aid the decision for CT scan in emergency settings in mild head trauma. Biomarkers in Medicine 13(13): 1055-1057	- Editorial
Allouchery, G., Moustafa, F., Roubin, J. et al. (2018) Clinical validation of S100B in the management of a mild traumatic brain injury: issues from an interventional cohort of 1449 adult patients. Clinical Chemistry & Laboratory Medicine 56(11): 1897-1904	- Unclear reference standard *not all participants received CT scan (those with S100B- results)*
Amoo, M., Henry, J., O'Halloran, P. J. et al. (2022) S100B, GFAP, UCH-L1 and NSE as	- Systematic review - screened for relevant references

Study	Code [Reason]
predictors of abnormalities on CT imaging following mild traumatic brain injury: a systematic review and meta-analysis of diagnostic test accuracy. Neurosurgical Review 45(2): 1171-1193	
Anderson, R. E., Hansson, L. O., Nilsson, O. et al. (2001) High serum S100B levels for trauma patients without head injuries. Neurosurgery 48(6): 1255-8; discussion 1258	- Population not relevant to this review protocol
Anderson, T. N., Hwang, J., Munar, M. et al. (2020) Blood-based biomarkers for prediction of intracranial hemorrhage and outcome in patients with moderate or severe traumatic brain injury. The Journal of Trauma and Acute Care Surgery 89(1): 80-86	- Severe or moderate TBI. Not relevant to post-injury complications
Asken, B. M., Bauer, R. M., DeKosky, S. T. et al. (2018) Concussion BASICS III: Serum biomarker changes following sport-related concussion. Neurology 91(23): e2133-e2143	- Not relevant to post-injury complications
Avci, A., Yilmaz, H. L., Satar, S. et al. (2013) The correlation between S-100B protein levels and prognosis in children with head trauma. Turkiye Klinikleri Journal of Medical Sciences 33(1): 149-158	- Study not reported in English
Bak, Hyeun Uk, Sung, Won Young, Lee, Jang Young et al. (2008) The Usefulness of Serum S-100 beta Levels as a Screening Test for Pediatric Minor Head Trauma. Journal of The Korean Society of Emergency Medicine 19(2): 185-191	- Study not reported in English
Ballesteros, M. A., Rubio-Lopez, M. I., San Martin, M. et al. (2018) Serum levels of S100B from jugular bulb as a biomarker of poor prognosis in patients with severe acute brain injury. Journal of the Neurological Sciences 385: 109-114	- Population not relevant to this review protocol
Bazarian, J. J., Zemlan, F. P., Mookerjee, S. et al. (2006) Serum S-100B and cleaved-tau are poor predictors of long-term outcome after mild traumatic brain injury. Brain Injury 20(7): 759-65	- No appropriate reference standard
Bechtel, K., Frasure, S., Marshall, C. et al. (2009) Relationship of serum S100B levels and intracranial injury in children with closed head trauma. Pediatrics 124(4): e697-704	- Data not reported in an extractable format or a format that can be analysed

Study	Code [Reason]
Berger, R. P., Adelson, P. D., Pierce, M. C. et al. (2005) Serum neuron-specific enolase, S100B, and myelin basic protein concentrations after inflicted and noninflicted traumatic brain injury in children. Journal of Neurosurgery 103(1suppl): 61-8	- Study design not relevant to this review protocol *case control study*
Berger, R. P., Beers, S. R., Richichi, R. et al. (2007) Serum biomarker concentrations and outcome after pediatric traumatic brain injury. Journal of Neurotrauma 24(12): 1793-801	- Comparator in study does not match that specified in this review protocol
Berger, R. P. and Kochanek, P. M. (2006) Urinary S100B concentrations are increased after brain injury in children: A preliminary study. Pediatric Critical Care Medicine 7(6): 557-61	- Comparator in study does not match that specified in this review protocol
Berger, R. P., Pierce, M. C., Wisniewski, S. R. et al. (2002) Serum S100B concentrations are increased after closed head injury in children: a preliminary study. Journal of Neurotrauma 19(11): 1405-9	- No diagnostic accuracy measures reported
Berger, R. P., Ta'asan, S., Rand, A. et al. (2009) Multiplex assessment of serum biomarker concentrations in well-appearing children with inflicted traumatic brain injury. Pediatric Research 65(1): 97-102	- Study design not relevant to this review protocol
Bernard, F., Al-Tamimi, Y. Z., Chatfield, D. et al. (2008) Serum albumin level as a predictor of outcome in traumatic brain injury: potential for treatment. Journal of Trauma-Injury Infection & Critical Care 64(4): 872-5	- No relevant diagnostic factor
Bhomia, M., Balakathiresan, N. S., Wang, K. K. et al. (2016) A Panel of Serum MiRNA Biomarkers for the Diagnosis of Severe to Mild Traumatic Brain Injury in Humans. Scientific Reports 6: 28148	- No relevant diagnostic factor
Blais Lecuyer, J., Mercier, E., Tardif, P. A. et al. (2021) S100B protein level for the detection of clinically significant intracranial haemorrhage in patients with mild traumatic brain injury: a subanalysis of a prospective cohort study. Emergency Medicine Journal 38(4): 285-289	- Reference standard not measured in all participants
Bogoslovsky T, Wilson D, Chen Y et al. Increases of Plasma Levels of Glial Fibrillary Acidic Protein, Tau, and Amyloid β up to 90	- Study design not relevant to this review protocol

Study	Code [Reason]
Days after Traumatic Brain Injury. Journal of neurotrauma 34(1): 66-73	
Bogoslovsky, T., Wilson, D., Chen, Y. et al. (2017) Increases of Plasma Levels of Glial Fibrillary Acidic Protein, Tau, and Amyloid beta up to 90 Days after Traumatic Brain Injury. Journal of Neurotrauma 34(1): 66-73	- Study design not relevant to this review protocol
Bohmer, A. E., Oses, J. P., Schmidt, A. P. et al. (2011) Neuron-specific enolase, S100B, and glial fibrillary acidic protein levels as outcome predictors in patients with severe traumatic brain injury. Neurosurgery 68(6): 1624-30; discussion 1630	- Severe or moderate TBI. Not relevant to post-injury complications
Bouvier D, Oddoze C, Ben Haim D et al. (2009) [Interest of S100B protein blood level determination for the management of patients with minor head trauma]. Annales de biologie clinique 67(4): 425-431	- Study not reported in English *Article in French*
Bouvier, D., Giguere, Y., Pereira, B. et al. (2020) Cord blood S100B: reference ranges and interest for early identification of newborns with brain injury. Clinical Chemistry & Laboratory Medicine 58(2): 285-293	- Population not relevant to this review protocol
Bouvier, D., Oris, C., Brailova, M. et al. (2020) Interest of blood biomarkers to predict lesions in medical imaging in the context of mild traumatic brain injury. Clinical Biochemistry 85: 5-11	- Review article but not a systematic review
Bulut, M., Koksal, O., Dogan, S. et al. (2006) Tau protein as a serum marker of brain damage in mild traumatic brain injury: preliminary results. Advances in Therapy 23(1): 12-22	- No relevant diagnostic factor
Buonora JE, Yarnell AM, Lazarus RC et al. (2015) Multivariate analysis of traumatic brain injury: development of an assessment score. Frontiers in neurology 6: 68	- Not relevant to post-injury complications
Calcagnile O, Holmén A, Chew M et al. (2013) S100B levels are affected by older age but not by alcohol intoxication following mild traumatic brain injury. Scandinavian journal of trauma, resuscitation and emergency medicine 21: 52	- Reference standard not measured in all participants
Calcagnile, O.; Anell, A.; Unden, J. (2016) The addition of S100B to guidelines for management	- Reference standard not measured in all participants

Study	Code [Reason]
of mild head injury is potentially cost saving. BMC Neurology 16(1)	
Calcagnile, O.; Unden, L.; Unden, J. (2012) Clinical validation of S100B use in management of mild head injury. BMC Emergency Medicine 12: 13	- Reference standard not measured in all participants
Carabias, C. S., Castano-Leon, A. M., Blanca Navarro, B. et al. (2020) Serum Amyloid A1 as a Potential Intracranial and Extracranial Clinical Severity Biomarker in Traumatic Brain Injury. Journal of Intensive Care Medicine 35(11): 1180-1195	- Comparator in study does not match that specified in this review protocol
Carabias, C. S., Gomez, P. A., Panero, I. et al. (2020) Chitinase-3-Like Protein 1, Serum Amyloid A1, C-Reactive Protein, and Procalcitonin Are Promising Biomarkers for Intracranial Severity Assessment of Traumatic Brain Injury: Relationship with Glasgow Coma Scale and Computed Tomography Volumetry. World Neurosurgery 134: e120-e143	- Data not reported in an extractable format or a format that can be analysed
Cervellin, G., Benatti, M., Carbucicchio, A., Aloe, R., and Lippi G (2014) Protein S100B and neuron-specific enolase (NSE) for the initial evaluation of mild head trauma in adults: Ready for prime time? . Biochim. Clin. 38: 227-233	- Full text paper not available
Chen, D., Bao, L., Lu, S. Q. et al. (2014) Serum albumin and prealbumin predict the poor outcome of traumatic brain injury. PLoS ONE [Electronic Resource] 9(3): e93167	- No relevant diagnostic factor
Chen, S., Chen, X. C., Lou, X. H. et al. (2019) Determination of serum neutrophil gelatinase-associated lipocalin as a prognostic biomarker of acute spontaneous intracerebral hemorrhage. Clinica Chimica Acta 492: 72-77	- No relevant diagnostic factor
Cheng, F., Yuan, Q., Yang, J. et al. (2014) The prognostic value of serum neuron-specific enolase in traumatic brain injury: systematic review and meta-analysis. PLoS ONE [Electronic Resource] 9(9): e106680	- Systematic review - screened for relevant references
Clarke, G. J. B., Skandsen, T., Zetterberg, H. et al. (2021) One-Year Prospective Study of Plasma Biomarkers From CNS in Patients With Mild Traumatic Brain Injury. Frontiers in neurology [electronic resource]. 12: 643743	- Not relevant to post-injury complications

Study	Code [Reason]
Cnossen, M. C., Winkler, E. A., Yue, J. K. et al. (2017) Development of a Prediction Model for Post-Concussive Symptoms following Mild Traumatic Brain Injury: A TRACK-TBI Pilot Study. Journal of Neurotrauma 34(16): 2396-2409	- Not relevant to post-injury complications
Cui, X., Zhou, B., Wu, J. et al. (2021) Changes in amplitude-integrated electroencephalography, neuron-specific enolase, and S100B in neonates with brain injury induced by neonatal hyperbilirubinemia and their significance. Brain Injury 35(8): 943-948	- Population not relevant to this review protocol
Czeiter, E., Mondello, S., Kovacs, N. et al. (2012) Brain injury biomarkers may improve the predictive power of the IMPACT outcome calculator. Journal of Neurotrauma 29(9): 1770-8	- Severe or moderate TBI. Not relevant to post-injury complications
da Rocha, A. B., Schneider, R. F., de Freitas, G. R. et al. (2006) Role of serum S100B as a predictive marker of fatal outcome following isolated severe head injury or multitrauma in males. Clinical Chemistry & Laboratory Medicine 44(10): 1234-42	- No appropriate reference standard
da Rocha, A. B., Zanoni, C., de Freitas, G. R. et al. (2005) Serum Hsp70 as an early predictor of fatal outcome after severe traumatic brain injury in males. Journal of Neurotrauma 22(9): 966-77	- Severe or moderate TBI. Not relevant to post-injury complications
Daoud, H., Alharfi, I., Alhelali, I. et al. (2014) Brain injury biomarkers as outcome predictors in pediatric severe traumatic brain injury. Neurocritical Care 20(3): 427-35	- No appropriate reference standard
Davis, T. S., Nathan, J. E., Tinoco Martinez, A. S. et al. (2020) -----Comparison of T1-Post and FLAIR-Post MRI for identification of traumatic meningeal enhancement in traumatic brain injury patients. PLoS ONE [Electronic Resource] 15(7): e0234881	- No relevant diagnostic factor
De Kruijk, J. R., Leffers, P., Menheere, P. P. et al. (2002) Prediction of post-traumatic complaints after mild traumatic brain injury: early symptoms and biochemical markers. Journal of Neurology, Neurosurgery & Psychiatry 73(6): 727-32	- No relevant diagnostic factor

Study	Code [Reason]
de Kruijk, J. R., Leffers, P., Menheere, P. P. et al. (2001) S-100B and neuron-specific enolase in serum of mild traumatic brain injury patients. A comparison with health controls. Acta Neurologica Scandinavica 103(3): 175-9	- Not relevant to post-injury complications
De Kruijk, J. R.; Twijnstra, A.; Leffers, P. (2001) Diagnostic criteria and differential diagnosis of mild traumatic brain injury. Brain Injury 15(2): 99-106	- Review article but not a systematic review
DeFazio, M. V., Rammo, R. A., Robles, J. R. et al. (2014) The potential utility of blood-derived biochemical markers as indicators of early clinical trends following severe traumatic brain injury. World Neurosurgery 81(1): 151-8	- Severe or moderate TBI. Not relevant to post-injury complications
Dey, S., Gangadharan, J., Deepika, A. et al. (2017) Correlation of ubiquitin C terminal hydrolase and S100beta with cognitive deficits in young adults with mild traumatic brain injury. Neurology India 65(4): 761-766	- Comparator in study does not match that specified in this review protocol
Di Battista, A. P., Buonora, J. E., Rhind, S. G. et al. (2015) Blood Biomarkers in Moderate-To-Severe Traumatic Brain Injury: Potential Utility of a Multi-Marker Approach in Characterizing Outcome. Frontiers in neurology [electronic resource]. 6: 110	- Severe or moderate TBI. Not relevant to post-injury complications
Di Pietro V, Porto E, Ragusa M et al. (2018) Salivary MicroRNAs: Diagnostic Markers of Mild Traumatic Brain Injury in Contact-Sport. Frontiers in molecular neuroscience 11: 290	- Not relevant to post-injury complications
Di Pietro, V., Porto, E., Ragusa, M. et al. (2018) Salivary MicroRNAs: Diagnostic Markers of Mild Traumatic Brain Injury in Contact-Sport. Frontiers in Molecular Neuroscience 11: 290	- Not relevant to post-injury complications
Di Pietro, V., Ragusa, M., Davies, D. et al. (2017) MicroRNAs as Novel Biomarkers for the Diagnosis and Prognosis of Mild and Severe Traumatic Brain Injury. Journal of Neurotrauma 34(11): 1948-1956	- Comparator in study does not match that specified in this review protocol
Duda, I.; Wiorek, A.; Krzych, L. J. (2020) Biomarkers Facilitate the Assessment of Prognosis in Critically Ill Patients with Primary Brain Injury: A Cohort Study. International	- Population not relevant to this review protocol

Study	Code [Reason]
Journal of Environmental Research & Public Health [Electronic Resource] 17(12): 21	
Eagle, S. R., Womble, M. N., Elbin, R. J. et al. (2020) Concussion Symptom Cutoffs for Identification and Prognosis of Sports-Related Concussion: Role of Time Since Injury. American Journal of Sports Medicine 48(10): 2544-2551	- No relevant diagnostic factor
Efstathiou, N., Slavakis, A., Drossou, V. et al. (2021) Can we delineate brain injury in full-term neonates using serum biomarkers?. Brain Injury 35(7): 821-830	- Population not relevant to this review protocol
Efstathiou, N., Soubasi, V., Koliakos, G. et al. (2015) Mobilization of circulating progenitor cells following brain injury in premature neonates could be indicative of an endogenous repair process. A pilot study. Hippokratia 19(2): 141-7	- Population not relevant to this review protocol
Egea-Guerrero, J. J., Murillo-Cabezas, F., Gordillo-Escobar, E. et al. (2013) S100B protein may detect brain death development after severe traumatic brain injury. Journal of Neurotrauma 30(20): 1762-9	- Severe or moderate TBI. Not relevant to post-injury complications
Eisele, A., Hill-Strathy, M., Michels, L. et al. (2020) Magnetic Resonance Spectroscopy following Mild Traumatic Brain Injury: A Systematic Review and Meta-Analysis on the Potential to Detect Posttraumatic Neurodegeneration. Neurodegenerative Diseases 20(1): 2-11	- No relevant diagnostic factor
El-Maraghi, S., Yehia, H., Hossam, H. et al. (2013) The prognostic value of neuron specific enolase in head injury. Egyptian Journal of Critical Care Medicine 1(1): 25-32	- Severe or moderate TBI. Not relevant to post-injury complications
Erickson, J. A. and Grenache, D. G. (2011) Comparison of three assays for quantifying S-100B in serum. Clinica Chimica Acta 412(2324): 2122-7	- Study design not relevant to this review protocol
Falk, H., Bechtold, K. T., Peters, M. E. et al. (2021) A Prognostic Model for Predicting One-Month Outcomes among Emergency Department Patients with Mild Traumatic Brain Injury and a Presenting Glasgow Coma Scale of Fifteen. Journal of Neurotrauma 38(19): 2714-2722	- No relevant diagnostic factor

Study	Code [Reason]
Fedorchak, G., Rangnekar, A., Onks, C. et al. (2021) Saliva RNA biomarkers predict concussion duration and detect symptom recovery: a comparison with balance and cognitive testing. Journal of Neurology 268(11): 4349-4361	- Comparator in study does not match that specified in this review protocol
Feng, M. J., Ning, W. B., Wang, W. et al. (2018) Serum S100A12 as a prognostic biomarker of severe traumatic brain injury. Clinica Chimica Acta 480: 84-91	- No relevant diagnostic factor
Filippidis, A. S., Papadopoulos, D. C., Kapsalaki, E. Z. et al. (2010) Role of the S100B serum biomarker in the treatment of children suffering from mild traumatic brain injury. Neurosurgical Focus 29(5): e2	- Systematic review - screened for relevant references
Forouzan, A., Motamed, H., Delirrooyfard, A. et al. (2020) Serum Cleaved Tau Protein and Clinical Outcome in Patients with Minor Head Trauma. Open Access Emergency Medicine 12: 7-12	- No relevant diagnostic factor
Foulady, P., Shakeri, M., Yarand, K. K. et al. (2014) Prognostic importance of serum S100 protein (B dimer) in patients with severe head trauma. Journal of Medical Sciences (Faisalabad) 14(1): 41-45	- Severe or moderate TBI. Not relevant to post-injury complications
Frankel, M., Fan, L., Yeatts, S. D. et al. (2019) Association of Very Early Serum Levels of S100B, Glial Fibrillary Acidic Protein, Ubiquitin C-Terminal Hydrolase-L1, and Spectrin Breakdown Product with Outcome in ProTECT III. Journal of Neurotrauma 36(20): 2863-2871	- Severe or moderate TBI. Not relevant to post-injury complications
Galovski, T. E., Werner, K. B., Iverson, K. M. et al. (2021) A Multi-Method Approach to a Comprehensive Examination of the Psychiatric and Neurological Consequences of Intimate Partner Violence in Women: A Methodology Protocol. Frontiers in Psychiatry 12 (no pagination)	- Not relevant to post-injury complications
Gan, Z. S., Stein, S. C., Swanson, R. et al. (2019) Blood Biomarkers for Traumatic Brain Injury: A Quantitative Assessment of Diagnostic and Prognostic Accuracy. Frontiers in neurology [electronic resource]. 10: 446	- Review article but not a systematic review

Study	Code [Reason]
Gandhi, S. S., Mann, M., Jain, S. et al. (2018) A Prospective Analysis of Derangement of Coagulation Profile in Adult and Pediatric Age Group in Moderate-to-Severe Traumatic Brain Injury. Indian Journal of Neurotrauma 15(2-3): 87-93	- No relevant diagnostic factor
Gando, S.; Nanzaki, S.; Kemmotsu, O. (1999) Coagulofibrinolytic changes after isolated head injury are not different from those in trauma patients without head injury. Journal of Trauma-Injury Infection & Critical Care 46(6): 1070-6; discussion 1076	- No relevant diagnostic factor
Gao, W., Zhang, Z., Lv, X. et al. (2020) Neurofilament light chain level in traumatic brain injury: A system review and meta-analysis. Medicine 99(38): e22363	- Not relevant to post-injury complications
Gao, Y., Duan, J., Ji, H. et al. (2021) Levels of S100 calcium binding protein B (S100B), neuron-specific enolase (NSE), and cyclophilin A (CypA) in the serum of patients with severe craniocerebral injury and multiple injuries combined with delirium transferred from the ICU and their prognostic value. Annals of Palliative Medicine 10(3): 3371-3378	- Severe or moderate TBI. Not relevant to post-injury complications
Gasparroni, G., Graziosi, A., Bersani, I. et al. (2021) S100B protein, cerebral ultrasound and magnetic resonance imaging patterns in brain injured preterm infants. Clinical Chemistry & Laboratory Medicine 59(9): 1527-1534	- No relevant diagnostic factor
Gazzolo, D., Marinoni, E., Di Iorio, R. et al. (2003) Measurement of Urinary S100B Protein Concentrations for the Early Identification of Brain Damage in Asphyxiated Full-term Infants. Archives of Pediatrics and Adolescent Medicine 157(12): 1163-1168	- Population not relevant to this review protocol
Gazzolo, D., Pluchinotta, F., Bashir, M. et al. (2015) Neurological abnormalities in full-term asphyxiated newborns and salivary S100B testing: the "Cooperative Multitask against Brain Injury of Neonates" (CoMBINe) international study. PLoS ONE [Electronic Resource] 10(1): e0115194	- Population not relevant to this review protocol
Genet, G. F., Johansson, P. I., Meyer, M. A. S. et al. (2013) Trauma-induced coagulopathy: Standard coagulation tests, biomarkers of	- Severe or moderate TBI. Not relevant to post-injury complications

Study	Code [Reason]
coagulopathy, and endothelial damage in patients with traumatic brain injury. Journal of Neurotrauma 30(4): 301-306	
Geyer, C., Ulrich, A., Grafe, G. et al. (2009) Diagnostic value of S100B and neuron-specific enolase in mild pediatric traumatic brain injury. Journal of Neurosurgery. Pediatrics. 4(4): 339-44	- No appropriate reference standard
Ghai, V., Fallen, S., Baxter, D. et al. (2020) Alterations in Plasma microRNA and Protein Levels in War Veterans with Chronic Mild Traumatic Brain Injury. Journal of Neurotrauma 37(12): 1418-1430	- No relevant diagnostic factor
Ghonemi, M. O., Rabah, A. A., Saber, H. M. et al. (2013) Role of Phosphorylated Neurofilament H as a diagnostic and prognostic marker in traumatic brain injury. Egyptian Journal of Critical Care Medicine 1(3): 139-144	- Severe or moderate TBI. Not relevant to post-injury complications
Giuseppe, D., Sergio, C., Pasqua, B. et al. (2009) Perinatal asphyxia in preterm neonates leads to serum changes in protein S-100 and neuron specific enolase. Current Neurovascular Research 6(2): 110-6	- Population not relevant to this review protocol
Giza, C. C., McCrea, M., Huber, D. et al. (2021) Assessment of Blood Biomarker Profile After Acute Concussion During Combative Training Among US Military Cadets: A Prospective Study From the NCAA and US Department of Defense CARE Consortium. JAMA Network Open 4(2): e2037731	- Not relevant to post-injury complications
Goergen, S. K., Ang, H., Wong, F. et al. (2014) Early MRI in term infants with perinatal hypoxic-ischaemic brain injury: interobserver agreement and MRI predictors of outcome at 2 years. Clinical Radiology 69(1): 72-81	- Population not relevant to this review protocol
Goetzl, E. J., Elahi, F. M., Mustapic, M. et al. (2019) Altered levels of plasma neuron-derived exosomes and their cargo proteins characterize acute and chronic mild traumatic brain injury. FASEB Journal 33(4): 5082-5088	- No diagnostic accuracy measures reported - Not relevant to post-injury complications
Goetzl, E. J., Peltz, C. B., Mustapic, M. et al. (2020) Neuron-Derived Plasma Exosome Proteins after Remote Traumatic Brain Injury. Journal of Neurotrauma 37(2): 382-388	- Not relevant to post-injury complications

Study	Code [Reason]
Golden, N., Mahadewa, T. G. B., Aryanti, C. et al. (2018) S100B Serum Level as a Mortality Predictor for Traumatic Brain Injury: A Meta-Analysis. Open Access Macedonian Journal of Medical Sciences 6(11): 2239-2244	- Not relevant to post-injury complications
Gonzclez-Mao, M. C., Reparaz-Andrade, A., Del Campo-Perez, V. et al. (2011) Model predicting survival/exitus after traumatic brain injury: biomarker S100B 24h. Clinical Laboratory 57(78): 587-97	- No appropriate reference standard
Goyal, K., Tomar, G. S., Sengar, K. et al. (2021) Prognostic Value of Serially Estimated Serum Procalcitonin Levels in Traumatic Brain Injury Patients With or Without Extra Cranial Injury on Early In-hospital Mortality: A Longitudinal Observational Study. Neurocritical Care 34(1): 182-192	- Severe or moderate TBI. Not relevant to post-injury complications
Gozt, A., Licari, M., Halstrom, A. et al. (2020) Towards the development of an integrative, evidence-based suite of indicators for the prediction of outcome following mild traumatic brain injury: Results from a pilot study. Brain Sciences 10(1)	- Comparator in study does not match that specified in this review protocol
Gradisek, P., Osredkar, J., Korsic, M. et al. (2012) Multiple indicators model of long-term mortality in traumatic brain injury. Brain Injury 26(12): 1472-81	- Severe or moderate TBI. Not relevant to post-injury complications
Gradisek, P., Osredkar, J., Kremzar, B. et al. (2011) Biochemical markers of traumatic brain injury. Zdravniski Vestnik 80(4): 293-301	- Study not reported in English
Guedes, V. A., Kenney, K., Shahim, P. et al. (2020) Exosomal neurofilament light: A prognostic biomarker for remote symptoms after mild traumatic brain injury?. Neurology 94(23): e2412-e2423	- Comparator in study does not match that specified in this review protocol
Gul, H. F., Simsek, A. T., Dolanbay, T. et al. (2021) Evaluation of blood glucose and inflammation markers in pediatric head injuries. Eastern Journal of Medicine 26(1): 67-74	- No relevant diagnostic factor
Guzel, A., Er, U., Tatli, M. et al. (2008) Serum neuron-specific enolase as a predictor of short-term outcome and its correlation with Glasgow Coma Scale in traumatic brain injury.	- Data not reported in an extractable format or a format that can be analysed

Study	Code [Reason]
Neurosurgical Review 31(4): 439-44; discussion 444	
Guzel, A., Karasalihoglu, S., Aylanc, H. et al. (2010) Validity of serum tau protein levels in pediatric patients with minor head trauma. American Journal of Emergency Medicine 28(4): 399-403	- No relevant diagnostic factor
Hack, D., Huff, J. S., Curley, K. et al. (2017) Increased prognostic accuracy of TBI when a brain electrical activity biomarker is added to loss of consciousness (LOC). American Journal of Emergency Medicine 35(7): 949-952	- No relevant diagnostic factor
Hahn, G. H., Maroun, L. L., Larsen, N. et al. (2012) Cerebral autoregulation in the first day after preterm birth: no evidence of association with systemic inflammation. Pediatric Research 71(3): 253-60	- Population not relevant to this review protocol
Hallen, M., Karlsson, M., Carlhed, R. et al. (2010) S-100B in serum and urine after traumatic head injury in children. Journal of Trauma-Injury Infection & Critical Care 69(2): 284-9	- Reference standard not measured in all participants
Hansen-Schwartz, J. and Bouchelouche, P. N. (2014) Use of biomarker S100B for traumatic brain damage in the emergency department may change observation strategy. Danish Medical Journal 61(9): a4894	- No diagnostic accuracy measures reported
Hardy, J. J., Mooney, S. R., Pearson, A. N. et al. (2017) Assessing the accuracy of blood RNA profiles to identify patients with post-concussion syndrome: A pilot study in a military patient population. PLoS ONE [Electronic Resource] 12(9): e0183113	- No relevant diagnostic factor
Haselmann, V., Schamberger, C., Trifonova, F. et al. (2021) Plasma-based S100B testing for management of traumatic brain injury in emergency setting. Practical Laboratory Medicine 26: e00236	- Data not reported in an extractable format or a format that can be analysed
Hatefi, M., Behzadi, S., Dastjerdi, M. M. et al. (2017) Correlation of Homocysteine with Cerebral Hemodynamic Abnormality, Endothelial Dysfunction Markers, and Cognition Impairment in Patients with Traumatic Brain Injury. World Neurosurgery 97: 70-79	- No relevant diagnostic factor

Study	Code [Reason]
Heidari, K., Asadollahi, S., Jamshidian, M. et al. (2015) Prediction of neuropsychological outcome after mild traumatic brain injury using clinical parameters, serum S100B protein and findings on computed tomography. Brain Injury 29(1): 33-40	- Data not reported in an extractable format or a format that can be analysed
Heidari, K., Vafaee, A., Rastekenari, A. M. et al. (2015) S100B protein as a screening tool for computed tomography findings after mild traumatic brain injury: Systematic review and meta-analysis. Brain Injury 29(10): 1146-1157	- Systematic review - screened for relevant references
Hellewell, S. C., Conquest, A., Little, L. et al. (2020) EPO treatment does not alter acute serum profiles of GFAP and S100B after TBI: A brief report on the Australian EPO-TBI clinical trial. Journal of Clinical Neuroscience 76: 5-8	- No appropriate reference standard
Hergenroeder, G., Redell, J. B., Moore, A. N. et al. (2008) Identification of serum biomarkers in brain-injured adults: potential for predicting elevated intracranial pressure. Journal of Neurotrauma 25(2): 79-93	- Severe or moderate TBI. Not relevant to post-injury complications
Herrmann M (2001) High serum S100B levels for trauma patients without head injuries. Neurosurgery 49(5): 1272-1273	- Editorial
Herrmann, M., Curio, N., Jost, S. et al. (1999) Protein S-100B and neuron specific enolase as early neurobiochemical markers of the severity of traumatic brain injury. Restorative Neurology & Neuroscience 14(23): 109-114	- Data not reported in an extractable format or a format that can be analysed
Herrold, A. A., Kletzel, S. L., Foecking, E. M. et al. (2021) miRNAs as Potential Biomarkers for Traumatic Brain Injury: Pathway From Diagnosis to Neurorehabilitation. Journal of Head Trauma Rehabilitation 36(3): E155-E169	- Review article but not a systematic review
Hicks, S. D., Johnson, J., Carney, M. C. et al. (2018) Overlapping MicroRNA Expression in Saliva and Cerebrospinal Fluid Accurately Identifies Pediatric Traumatic Brain Injury. Journal of Neurotrauma 35(1): 64-72	- Study design not relevant to this review protocol
Hicks, S. D., Olympia, R. P., Onks, C. et al. (2020) Saliva microRNA Biomarkers of Cumulative Concussion. International Journal of Molecular Sciences 21(20): 20	- Study design not relevant to this review protocol

Study	Code [Reason]
Hicks, S. D., Onks, C., Kim, R. Y. et al. (2021) Refinement of saliva microRNA biomarkers for sports-related concussion. Journal of sport and health science. 27	- Study design not relevant to this review protocol
Hicks, S. D., Onks, C., Kim, R. Y. et al. (2020) Diagnosing mild traumatic brain injury using saliva RNA compared to cognitive and balance testing. Clinical and Translational Medicine 10(6): e197	- Study design not relevant to this review protocol
Hill, L. J., Di Pietro, V., Hazeldine, J. et al. (2017) Cystatin D (CST5): An ultra-early inflammatory biomarker of traumatic brain injury. Scientific Reports 7(1): 5002	- Study design not relevant to this review protocol
Honda, M., Tsuruta, R., Kaneko, T. et al. (2010) Serum glial fibrillary acidic protein is a highly specific biomarker for traumatic brain injury in humans compared with S-100B and neuron-specific enolase. Journal of Trauma-Injury Infection & Critical Care 69(1): 104-9	- Not relevant to post-injury complications
Hossain, I., Mohammadian, M., Takala, R. S. K. et al. (2020) Admission Levels of Total Tau and beta-Amyloid Isoforms 1-40 and 1-42 in Predicting the Outcome of Mild Traumatic Brain Injury. Frontiers in neurology [electronic resource]. 11: 325	- No relevant diagnostic factor
Hossain, I., Mohammadian, M., Takala, R. S. K. et al. (2019) Early Levels of Glial Fibrillary Acidic Protein and Neurofilament Light Protein in Predicting the Outcome of Mild Traumatic Brain Injury. Journal of Neurotrauma 36(10): 1551-1560	- Comparator in study does not match that specified in this review protocol
Huang, H. B., Yang, S. B., Shen, L. J. et al. (2019) A prospective study on serum secreted protein acidic and rich in cysteine-like 1 as a prognostic marker for severe traumatic brain injury. Clinica Chimica Acta 491: 19-23	- No relevant diagnostic factor
Huang, J. J., Qiu, S. Z., Zheng, G. R. et al. (2019) Determination of serum tissue kallikrein levels after traumatic brain injury. Clinica Chimica Acta 499: 93-97	- No relevant diagnostic factor
Huang, M., Dong, X. Q., Hu, Y. Y. et al. (2010) High S100B levels in cerebrospinal fluid and peripheral blood of patients with acute basal	- Population not relevant to this review protocol

Study	Code [Reason]
ganglial hemorrhage are associated with poor outcome. World journal of emergency medicine 1(1): 22-31	
Huang, X., Dai, Y., Ma, X. et al. (2020) Different changes in granulocyte-colony stimulating factor and its correlation with inflammatory biomarkers in patients after traumatic brain injury. Neuroreport 31(4): 293-299	- No relevant diagnostic factor
Huebschmann, N. A., Luoto, T. M., Karr, J. E. et al. (2020) Comparing Glial Fibrillary Acidic Protein (GFAP) in Serum and Plasma Following Mild Traumatic Brain Injury in Older Adults. Frontiers in neurology [electronic resource]. 11: 1054	- Reference standard not measured in all participants
Huie, J. R., Diaz-Arrastia, R., Yue, J. K. et al. (2019) Testing a Multivariate Proteomic Panel for Traumatic Brain Injury Biomarker Discovery: A TRACK-TBI Pilot Study. Journal of Neurotrauma 36(1): 100-110	- No relevant diagnostic factor
Huseynova, S. A., Panakhova, N. F., Hajiyeva, A. S. et al. (2017) Endothelial dysfunction and developmental outcomes of very low birth weight newborns with hypoxic encephalopathy. JPMA - Journal of the Pakistan Medical Association 67(12): 1857-1863	- Population not relevant to this review protocol
Ingebrigtsen, T. and Romner, B. (2003) Biochemical serum markers for brain damage: a short review with emphasis on clinical utility in mild head injury. Restorative Neurology & Neuroscience 21(34): 171-6	- Review article but not a systematic review
Ingebrigtsen, T., Waterloo, K., Jacobsen, E. A. et al. (1999) Traumatic brain damage in minor head injury: relation of serum S-100 protein measurements to magnetic resonance imaging and neurobehavioral outcome. Neurosurgery 45(3): 468-75; discussion 475	- Data not reported in an extractable format or a format that can be analysed
Iverson, G. L., Posti, J. P., Ohman, J. et al. (2020) Reliability of serum S100B measurement following mild traumatic brain injury: a comparison of assay measurements from two laboratories. Brain Injury 34(9): 1237-1244	- Reference standard not measured in all participants
Iverson, G. L., Reddi, P. J., Posti, J. P. et al. (2019) Serum Neurofilament Light Is Elevated Differentially in Older Adults with Uncomplicated	- Study design not relevant to this review protocol

Study	Code [Reason]
Mild Traumatic Brain Injuries. Journal of Neurotrauma 36(16): 2400-2406	*case control study*
Jacquin, A., Kanakia, S., Oberly, D. et al. (2018) A multimodal biomarker for concussion identification, prognosis and management. Computers in Biology & Medicine 102: 95-103	- No relevant diagnostic factor
Jagoda, A. S., Bazarian, J. J., Bruns, J. J., Jr. et al. (2009) Clinical policy: neuroimaging and decisionmaking in adult mild traumatic brain injury in the acute setting. Journal of Emergency Nursing 35(2): e5-40	- Systematic review - screened for relevant references
Jeter, C. B., Hergenroeder, G. W., Hylin, M. J. et al. (2013) Biomarkers for the diagnosis and prognosis of mild traumatic brain injury/concussion. Journal of Neurotrauma 30(8): 657-670	- Review article but not a systematic review
Johnson, J. J., Loeffert, A. C., Stokes, J. et al. (2018) Association of Salivary MicroRNA Changes With Prolonged Concussion Symptoms. JAMA Pediatrics 172(1): 65-73	- Comparator in study does not match that specified in this review protocol
Juengst, S. B., Kumar, R. G., Failla, M. D. et al. (2015) Acute inflammatory biomarker profiles predict depression risk following moderate to severe traumatic brain injury. Journal of Head Trauma Rehabilitation 30(3): 207-18	- No relevant diagnostic factor
Kavalci, C., Pekdemir, M., Durukan, P. et al. (2007) The value of serum tau protein for the diagnosis of intracranial injury in minor head trauma. American Journal of Emergency Medicine 25(4): 391-5	- No relevant diagnostic factor
Kawata, K.; Mitsuhashi, M.; Aldret, R. (2018) A preliminary report on brain-derived extracellular vesicle as novel blood biomarkers for sport-related concussions. Frontiers in Neurology 9(APR)	- Population not relevant to this review protocol
Kazakova, M., Pavlov, G., Dichev, V. et al. (2021) Relationship between YKL-40, neuron-specific enolase, tumor necrosis factor-A, interleukin-6, and clinical assessment scores in traumatic brain injury. Archives of Trauma Research 10(1): 23-29	- Severe or moderate TBI. Not relevant to post-injury complications

Study	Code [Reason]
Kellermann, I., Kleindienst, A., Hore, N. et al. (2016) Early CSF and Serum S100B Concentrations for Outcome Prediction in Traumatic Brain Injury and Subarachnoid Hemorrhage. Clinical Neurology & Neurosurgery 145: 79-83	- Severe or moderate TBI. Not relevant to post-injury complications
Khong, E., Odenwald, N., Hashim, E. et al. (2016) Diffusion Tensor Imaging Findings in Post-Concussion Syndrome Patients after Mild Traumatic Brain Injury: A Systematic Review. Frontiers in neurology [electronic resource]. 7: 156	- No relevant diagnostic factor
Kochanek, P. M., Berger, R. P., Bayir, H. et al. (2008) Biomarkers of primary and evolving damage in traumatic and ischemic brain injury: diagnosis, prognosis, probing mechanisms, and therapeutic decision making. Current Opinion in Critical Care 14(2): 135-41	- Review article but not a systematic review
Korley, F. K., Datwyler, S. A., Jain, S. et al. (2021) Comparison of GFAP and UCH-L1 Measurements from Two Prototype Assays: The Abbott i-STAT and ARCHITECT Assays. Neurotrauma Reports 2(1): 193-199	- Not relevant to post-injury complications
Korley, F. K., Yue, J. K., Wilson, D. H. et al. (2019) Performance Evaluation of a Multiplex Assay for Simultaneous Detection of Four Clinically Relevant Traumatic Brain Injury Biomarkers. Journal of Neurotrauma 36(1): 182-187	- Secondary publication of an included study (TRACK TBI) that does not provide any additional relevant information
Kou, Z., Gattu, R., Kobeissy, F. et al. (2013) Combining biochemical and imaging markers to improve diagnosis and characterization of mild traumatic brain injury in the acute setting: Results from a pilot study. PLoS ONE 8(11)	- No diagnostic accuracy measures reported
Kovesdi, E., Luckl, J., Bukovics, P. et al. (2010) Update on protein biomarkers in traumatic brain injury with emphasis on clinical use in adults and pediatrics. Acta Neurochirurgica 152(1): 1-17	- Systematic review - screened for relevant references
Kristman, V. L., Brison, R. J., Bedard, M. et al. (2016) Prognostic Markers for Poor Recovery After Mild Traumatic Brain Injury in Older Adults: A Pilot Cohort Study. Journal of Head Trauma Rehabilitation 31(6): E33-E43	- No relevant diagnostic factor

Study	Code [Reason]
Lange RT; Iverson GL; Brubacher JR (2012) Clinical utility of the protein S100B to evaluate traumatic brain injury in the presence of acute alcohol intoxication. The Journal of head trauma rehabilitation 27(2): 123-134	- Population not relevant to this review protocol *S100B as a biomarker for traumatic brain injury (TBI) in the presence of acute alcohol intoxication.*
Lange, R. T., Brubacher, J. R., Iverson, G. L. et al. (2010) Differential effects of alcohol intoxication on S100B levels following traumatic brain injury. Journal of Trauma-Injury Infection & Critical Care 68(5): 1065-71	- Not relevant to post-injury complications
Langness, S., Ward, E., Halbach, J. et al. (2018) Plasma D-dimer safely reduces unnecessary CT scans obtained in the evaluation of pediatric head trauma. Journal of Pediatric Surgery 53(4): 752-757	- No relevant diagnostic factor
Le Sage, N., Tardif, P. A., Frenette, J. et al. (2019) Detection of S-100beta Protein in Plasma and Urine After a Mild Traumatic Brain Injury. Canadian Journal of Neurological Sciences 46(5): 599-602	- Not relevant to post-injury complications
Lee JY, Lee CY, Kim HR et al. (2015) A Role of Serum-Based Neuronal and Glial Markers as Potential Predictors for Distinguishing Severity and Related Outcomes in Traumatic Brain Injury. Journal of Korean Neurosurgical Society 58(2): 93-100	- Comparator in study does not match that specified in this review protocol
Lee, T., Chikkabyrappa, S. M., Reformina, D. et al. (2018) Ubiquitin C-Terminal Hydrolase 1 and Phosphorylated Axonal Neurofilament Heavy Chain in Infants Undergoing Cardiac Surgery: Preliminary Assessment as Potential Biomarkers of Brain Injury. World Journal for Pediatric & Congenital Heart Surgery 9(4): 412-418	- Population not relevant to this review protocol
Lei, J., Gao, G., Feng, J. et al. (2015) Glial fibrillary acidic protein as a biomarker in severe traumatic brain injury patients: a prospective cohort study. Critical Care (London, England) 19: 362	- Severe or moderate TBI. Not relevant to post-injury complications
Leon-Lozano, M. Z., Arnaez, J., Valls, A. et al. (2020) Cerebrospinal fluid levels of neuron-specific enolase predict the severity of brain damage in newborns with neonatal hypoxic-ischemic encephalopathy treated with	- Population not relevant to this review protocol

Study	Code [Reason]
hypothermia. PLoS ONE [Electronic Resource] 15(6): e0234082	
Lesko, M. M., O'Brien, S. J., Childs, C. et al. (2014) Comparison of several prognostic tools in traumatic brain injury including S100B. Brain Injury 28(7): 987-94	- No appropriate reference standard
Levitt, M. A., Cook, L. A., Simon, B. C. et al. (1995) Biochemical markers of cerebral injury in patients with minor head trauma and ethanol intoxication. Academic Emergency Medicine 2(8): 675-80	- No relevant diagnostic factor
Lewis, L. M., Schloemann, D. T., Papa, L. et al. (2017) Utility of Serum Biomarkers in the Diagnosis and Stratification of Mild Traumatic Brain Injury. Academic Emergency Medicine 24(6): 710-720	- Secondary publication of an included study that does not provide any additional relevant information *Primary study (Welch 2016) included in our reveiw. Comparison is mild TBI vs no mild TBI population. This study analysed the subset of subjects with a GCS* score *of 13 to 15 inclusive, with the aim of determining sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of these biomarkers to differentiate subjects with concussion from those without.*
Li, N., Shen, J. K., Zhao, W. G. et al. (2004) S-100B and neuron specific enolase in outcome prediction of severe head injury. Chinese Journal of Traumatology 7(3): 156-8	- Severe or moderate TBI. Not relevant to post-injury complications
Lima, D. P., Simao Filho, C., Abib Sde, C. et al. (2008) Quality of life and neuropsychological changes in mild head trauma. Late analysis and correlation with S100B protein and cranial CT scan performed at hospital admission. Injury 39(5): 604-11	- Not relevant to post-injury complications
Lin, C., Huang, S. J., Wang, N. et al. (2012) Relationship between plasma leptin levels and clinical outcomes of pediatric traumatic brain injury. Peptides 35(2): 166-71	- No relevant diagnostic factor
Lippa, S. M., Werner, J. K., Miller, M. C. et al. (2020) Recent Advances in Blood-Based Biomarkers of Remote Combat-Related Traumatic Brain Injury. Current Neurology & Neuroscience Reports 20(12): 54	- Review article but not a systematic review

Study	Code [Reason]
Liu, H. and Zhang, X. (2020) Correlation between platelet parameters, platelet/lymphocyte ratio, the severity and prognosis of patients with traumatic brain injury. International Journal of Clinical and Experimental Medicine 13(7): 5187-5192	- Not relevant to post-injury complications
Liu, L., Wei, H., Chen, F. et al. (2011) Endothelial progenitor cells correlate with clinical outcome of traumatic brain injury. Critical Care Medicine 39(7): 1760-5	- No relevant diagnostic factor
Lo, T. Y.; Jones, P. A.; Minns, R. A. (2010) Combining coma score and serum biomarker levels to predict unfavorable outcome following childhood brain trauma. Journal of Neurotrauma 27(12): 2139-45	- Severe or moderate TBI. Not relevant to post-injury complications
Lomas, J. P. and Dunning, J. (2005) S-100b protein levels as a predictor for long-term disability after head injury. Emergency Medicine Journal 22(12): 889-891	- Review article but not a systematic review
Lorton, F., Simon-Pimmel, J., Masson, D. et al. (2021) Impact of routine S100B protein assay on CT scan use in children with mild traumatic brain injury. Clinical Chemistry & Laboratory Medicine 59(5): 875-882	- Study design not relevant to this review protocol *before after study*
Lu, W., Jiang, C., Wang, Z. et al. (2020) Lactic acid, neuron-specific enolase, and blood-brain barrier index after a severe traumatic brain injury: a prospective study. British Journal of Neurosurgery: 1-5	- No diagnostic accuracy measures reported
Lumpkins, K. M., Bochicchio, G. V., Keledjian, K. et al. (2008) Glial fibrillary acidic protein is highly correlated with brain injury. Journal of Trauma-Injury Infection & Critical Care 65(4): 778-82; discussion 782	- Population not relevant to this review protocol *Not mild TBI. Includes critically injured trauma patients. GCS on admission (mean (SD) : 6 (3). Mean ISS: 30 (14)*
Luo, H. C., Fu, Y. Q., You, C. Y. et al. (2019) Comparison of admission serum albumin and hemoglobin as predictors of outcome in children with moderate to severe traumatic brain injury: A retrospective study. Medicine 98(44): e17806	- Severe or moderate TBI. Not relevant to post-injury complications
Luoto, T. M., Raj, R., Posti, J. P. et al. (2017) A Systematic Review of the Usefulness of Glial Fibrillary Acidic Protein for Predicting Acute Intracranial Lesions following Head Trauma.	- Systematic review - screened for relevant references

Study	Code [Reason]
Frontiers in neurology [electronic resource]. 8: 652	
Mannix, R., Levy, R., Zemek, R. et al. (2020) Fluid Biomarkers of Pediatric Mild Traumatic Brain Injury: A Systematic Review. Journal of Neurotrauma 37(19): 2029-2044	- Systematic review - screened for relevant references
Marklund, N., Vedung, F., Lubberink, M. et al. (2021) Tau aggregation and increased neuroinflammation in athletes after sports-related concussions and in traumatic brain injury patients - A PET/MR study. NeuroImage: Clinical 30 (no pagination)	- Population not relevant to this review protocol
Martinez, B. and Peplow, P. V. (2017) MicroRNAs as diagnostic markers and therapeutic targets for traumatic brain injury. Neural Regeneration Research 12(11): 1749-1761	- Review article but not a systematic review
Marzano, L. A. S., Batista, J. P. T., de Abreu Arruda, M. et al. (2021) Traumatic brain injury biomarkers in pediatric patients: a systematic review. Neurosurgical Review 25: 25	- Systematic review screened for relevant references
Massaeli, M., Nava, A. O., Hejripour Rafsanjani, S. Z. et al. (2021) Diagnostic value of neuron-specific enolase in patients with traumatic brain injury referring to emergency departments in 2015-2016. Journal of Kerman University of Medical Sciences 28(3): 319-329	- Severe or moderate TBI. Not relevant to post-injury complications
Massaro, A. N., Wu, Y. W., Bammler, T. K. et al. (2018) Plasma Biomarkers of Brain Injury in Neonatal Hypoxic-Ischemic Encephalopathy. Journal of Pediatrics 194: 67-75.e1	- Population not relevant to this review protocol
Mayer, A. R. and Quinn, D. K. (2021) Neuroimaging Biomarkers of New-Onset Psychiatric Disorders Following Traumatic Brain Injury. Biological Psychiatry 12: 12	- Review article but not a systematic review
Mehta, S. S. (2010) Biochemical serum markers in head injury: an emphasis on clinical utility. Clinical Neurosurgery 57: 134-40	- No diagnostic accuracy measures reported
Mendoza, D. A., Lopez, K. D., Echeverri, R. A. et al. (2020) Utility of biomarkers in traumatic brain injury: A narrative review. Colombian Journal of Anesthesiology 48(3): 155-161	- Review article but not a systematic review

Study	Code [Reason]
Mercier, E., Boutin, A., Lauzier, F. et al. (2013) Predictive value of S-100beta protein for prognosis in patients with moderate and severe traumatic brain injury: systematic review and meta-analysis. BMJ 346: f1757	- Severe or moderate TBI. Not relevant to post-injury complications
Mercier, E., Boutin, A., Shemilt, M. et al. (2016) Predictive value of neuron-specific enolase for prognosis in patients with moderate or severe traumatic brain injury: a systematic review and meta-analysis. CMAJ open 4(3): E371-E382	- Severe or moderate TBI. Not relevant to post-injury complications
Mercier, E., Tardif, P. A., Cameron, P. A. et al. (2018) Prognostic Value of S-100beta Protein for Prediction of Post-Concussion Symptoms after a Mild Traumatic Brain Injury: Systematic Review and Meta-Analysis. Journal of Neurotrauma 35(4): 609-622	- Not relevant to post-injury complications
Meshcheryakov, S. V., Semenova, Z. B., Lukianov, V. I. et al. (2018) Prognosis of Severe Traumatic Brain Injury Outcomes in Children. Acta Neurochirurgica - Supplement 126: 11-16	- Full text paper not available
Meshkini, A., Haghjo, A. G., Segherlou, Z. H. et al. (2021) S100 calcium-binding protein b and glial fibrillary acidic protein in patients with mild traumatic brain injury. Bulletin of Emergency and Trauma 9(4): 183-187	- No diagnostic accuracy measures reported
Metting, Z., Wilczak, N., Rodiger, L. A. et al. (2012) GFAP and S100B in the acute phase of mild traumatic brain injury. Neurology 78(18): 1428-33 - No diagnostic accuracy measures reported [not all patients received MRI. No diagnostic accuracy measures for CT as references standard.]	- No diagnostic accuracy measures reported [CT or MRI reference standards. Not all patients received MRI. No diagnostic accuracy measures for CT as reference standard.]
Metzger, R. R., Sheng, X., Niedzwecki, C. M. et al. (2018) Temporal response profiles of serum ubiquitin C-terminal hydrolase-L1 and the 145-kDa alpha II-spectrin breakdown product after severe traumatic brain injury in children. Journal of Neurosurgery. Pediatrics. 22(4): 369-374	- Severe or moderate TBI. Not relevant to post-injury complications
Middleton, J. (2022) UCH-L1 and GFAP Testing (i-STAT TBI Plasma) for the Detection of Intracranial Injury Following Mild Traumatic Brain Injury. American Family Physician 105(3): 313-314	No diagnostic accuracy measures reported [cannot calculate specificity. only sensitivity reported.]

Study	Code [Reason]
Minkkinen, M., Iverson, G. L., Kotilainen, A. K. et al. (2019) Prospective Validation of the Scandinavian Guidelines for Initial Management of Minimal, Mild, and Moderate Head Injuries in Adults. Journal of Neurotrauma 36(20): 2904-2912	- Reference standard not measured in all participants
Mondello, S., Guedes, V. A., Lai, C. et al. (2020) Circulating Brain Injury Exosomal Proteins following Moderate-To-Severe Traumatic Brain Injury: Temporal Profile, Outcome Prediction and Therapy Implications. Cells 9(4): 15	- Severe or moderate TBI. Not relevant to post-injury complications
Mondello, S., Kobeissy, F., Vestri, A. et al. (2016) Serum Concentrations of Ubiquitin C-Terminal Hydrolase-L1 and Glial Fibrillary Acidic Protein after Pediatric Traumatic Brain Injury. Scientific Reports 6: 28203	- Severe or moderate TBI. Not relevant to post-injury complications
Mondello, S., Schmid, K., Berger, R. P. et al. (2014) The challenge of mild traumatic brain injury: role of biochemical markers in diagnosis of brain damage. Medicinal Research Reviews 34(3): 503-31	- Review article but not a systematic review
Mondello, S., Sorinola, A., Czeiter, E. et al. (2021) Blood-Based Protein Biomarkers for the Management of Traumatic Brain Injuries in Adults Presenting to Emergency Departments with Mild Brain Injury: A Living Systematic Review and Meta-Analysis. Journal of Neurotrauma 38(8): 1086-1106	- Systematic review - screened for relevant references
Mozafari, J., Barzegari, H., Motamed, H. et al. (2020) The diagnostic value of neuron-specific enolase in patients with mild head injury requiring cranial CT scan. New Zealand Journal of Medical Laboratory Science 74(2): 95-97	- Population not relevant to this review protocol *Includes people with moderate and severe TBI (GCS score 9-12). Population not relevant to post-injury complications.*
Mozaffari, K., Dejam, D., Duong, C. et al. (2021) Systematic Review of Serum Biomarkers in Traumatic Brain Injury. Cureus 13(8): e17056	- Systematic review - screened for relevant references
Murillo-Cabezas, F., Munoz-Sanchez, M. A., Rincon-Ferrari, M. D. et al. (2010) The prognostic value of the temporal course of S100beta protein in post-acute severe brain injury: A prospective and observational study. Brain Injury 24(4): 609-19	- No appropriate reference standard

Study	Code [Reason]
Mussack T, Biberthaler P, Kanz KG et al. (2002) Immediate S-100B and neuron-specific enolase plasma measurements for rapid evaluation of primary brain damage in alcohol-intoxicated, minor head-injured patients. Shock (Augusta, Ga.) 18(5): 395-400	- Population not relevant to this review protocol *People with alcohol intoxicated minor head injury (GCS score 13-15)*
Mussack, T., Biberthaler, P., Wiedemann, E. et al. (2000) S-100b as a screening marker of the severity of minor head trauma (MHT)--a pilot study. Acta Neurochirurgica - Supplement 76: 393-6	- Full text paper not available
Naeimi, Z. S., Weinhofer, A., Sarahrudi, K. et al. (2006) Predictive value of S-100B protein and neuron specific-enolase as markers of traumatic brain damage in clinical use. Brain Injury 20(5): 463-8	- No diagnostic accuracy measures reported
Neher, M. D., Keene, C. N., Rich, M. C. et al. (2014) Serum biomarkers for traumatic brain injury. Southern Medical Journal 107(4): 248-55	- Review article but not a systematic review
Nekludov, M., Bellander, B. M., Gryth, D. et al. (2017) Brain-Derived Microparticles in Patients with Severe Isolated TBI. Brain Injury 31(1314): 1856-1862	- No diagnostic accuracy measures reported
Nekludov, M., Mobarrez, F., Gryth, D. et al. (2014) Formation of microparticles in the injured brain of patients with severe isolated traumatic brain injury. Journal of Neurotrauma 31(23): 1927-1933	- Severe or moderate TBI. Not relevant to post-injury complications
Nygren De Boussard, C., Fredman, P., Lundin, A. et al. (2004) S100 in mild traumatic brain injury. Brain Injury 18(7): 671-83	- No appropriate reference standard
Nylen, K., Ost, M., Csajbok, L. Z. et al. (2006) Increased serum-GFAP in patients with severe traumatic brain injury is related to outcome. Journal of the Neurological Sciences 240(12): 85-91	- No diagnostic accuracy measures reported
Nylen, K., Ost, M., Csajbok, L. Z. et al. (2008) Serum levels of S100B, S100A1B and S100BB are all related to outcome after severe traumatic brain injury. Acta Neurochirurgica 150(3): 221-7; discussion 227	- Severe or moderate TBI. Not relevant to post-injury complications

Study	Code [Reason]
Ohrt-Nissen, S., Friis-Hansen, L., Dahl, B. et al. (2011) How does extracerebral trauma affect the clinical value of S100B measurements?. Emergency Medicine Journal 28(11): 941-4	- Population not relevant to this review protocol
Olivecrona, Z.; Bobinski, L.; Koskinen, L. O. (2015) Association of ICP, CPP, CT findings and S-100B and NSE in severe traumatic head injury. Prognostic value of the biomarkers. Brain Injury 29(4): 446-54	- No diagnostic accuracy measures reported
Oris, C., Bouillon-Minois, J. B., Pinguet, J. et al. (2021) Predictive Performance of Blood S100B in the Management of Patients Over 65 Years Old With Mild Traumatic Brain Injury. Journals of Gerontology Series A-Biological Sciences & Medical Sciences 76(8): 1471-1479	- Unclear reference standard *Not all people received CCT*
Oris, C., Pereira, B., Durif, J. et al. (2018) The Biomarker S100B and Mild Traumatic Brain Injury: A Meta-analysis. Pediatrics 141(6): 06	- Systematic review - screened for relevant references
Osier, N. D., Ziari, M., Puccio, A. M. et al. (2019) Elevated cerebrospinal fluid concentrations of N-acetylaspartate correlate with poor outcome in a pilot study of severe brain trauma. Brain Injury 33(10): 1364-1371	- Severe or moderate TBI. Not relevant to post-injury complications
Ost, M., Nylen, K., Csajbok, L. et al. (2006) Initial CSF total tau correlates with 1-year outcome in patients with traumatic brain injury. Neurology 67(9): 1600-4	- No relevant diagnostic factor
Papa, L., Ramia, M. M., Edwards, D. et al. (2015) Systematic review of clinical studies examining biomarkers of brain injury in athletes after sports-related concussion. Journal of Neurotrauma 32(10): 661-73	- Not relevant to post-injury complications
Papa, L., Robinson, G., Oli, M. et al. (2008) Use of biomarkers for diagnosis and management of traumatic brain injury patients. Expert Opinion on Medical Diagnostics 2(8): 937-945	- Review article but not a systematic review
Papa, L., Slobounov, S. M., Breiter, H. C. et al. (2019) Elevations in MicroRNA Biomarkers in Serum Are Associated with Measures of Concussion, Neurocognitive Function, and Subconcussive Trauma over a Single National Collegiate Athletic Association Division I Season	- Not relevant to post-injury complications

Study	Code [Reason]
in Collegiate Football Players. Journal of Neurotrauma 36(8): 1343-1351	
Papa, L., Zonfrillo, M. R., Welch, R. D. et al. (2019) Evaluating glial and neuronal blood biomarkers GFAP and UCH-L1 as gradients of brain injury in concussive, subconcussive and non-concussive trauma: a prospective cohort study. BMJ Paediatrics Open 3(1): e000473	- Not relevant to post-injury complications
Park, S. H. and Hwang, S. K. (2018) Prognostic Value of Serum Levels of S100 Calcium-Binding Protein B, Neuron-Specific Enolase, and Interleukin-6 in Pediatric Patients with Traumatic Brain Injury. World Neurosurgery 118: e534-e542	- No diagnostic accuracy measures reported
Pelinka, L. E., Kroepfl, A., Leixnering, M. et al. (2004) GFAP versus S100B in serum after traumatic brain injury: relationship to brain damage and outcome. Journal of Neurotrauma 21(11): 1553-61	- Not relevant to post-injury complications
Pelinka, L. E., Kroepfl, A., Schmidhammer, R. et al. (2004) Glial fibrillary acidic protein in serum after traumatic brain injury and multiple trauma. Journal of Trauma-Injury Infection & Critical Care 57(5): 1006-12	- No diagnostic accuracy measures reported
Pelinka, L. E., Petto, H., Kroepfl, A. et al. (2003) Serum Procalcitonin and S100B Are Associated with Mortality after Traumatic Brain Injury. European Journal of Trauma 29(5): 316-323	- No appropriate reference standard
Pelinka, L. E., Toegel, E., Mauritz, W. et al. (2003) Serum S 100 B: a marker of brain damage in traumatic brain injury with and without multiple trauma. Shock 19(3): 195-200	- Severe or moderate TBI. Not relevant to post-injury complications
Peters, A. J., Schnell, E., Saugstad, J. A. et al. (2021) Longitudinal Course of Traumatic Brain Injury Biomarkers for the Prediction of Clinical Outcomes: A Review. Journal of Neurotrauma 38(18): 2490-2501	- Not relevant to post-injury complications
Peters, M. E., Rao, V., Bechtold, K. T. et al. (2017) Head injury serum markers for assessing response to trauma: Design of the HeadSMART study. Brain Injury 31(3): 370-378	- No diagnostic accuracy measures reported

Study	Code [Reason]
Petrone, A. B., Gionis, V., Giersch, R. et al. (2017) Immune biomarkers for the diagnosis of mild traumatic brain injury. Neurorehabilitation 40(4): 501-508	- No relevant diagnostic factor
Petzold, A., Green, A. J., Keir, G. et al. (2002) Role of serum S100B as an early predictor of high intracranial pressure and mortality in brain injury: a pilot study. Critical Care Medicine 30(12): 2705-10	- No appropriate reference standard
Pfortmueller, C. A., Drexel, C., Krahenmann-Muller, S. et al. (2016) S-100 B Concentrations Are a Predictor of Decreased Survival in Patients with Major Trauma, Independently of Head Injury. PLoS ONE [Electronic Resource] 11(3): e0152822	- Not relevant to post-injury complications
Piazza, O., Storti, M. P., Cotena, S. et al. (2007) S100B is not a reliable prognostic index in paediatric TBI. Pediatric Neurosurgery 43(4): 258-64	- No diagnostic accuracy measures reported
Polito, F., Fama, F., Oteri, R. et al. (2020) Circulating miRNAs expression as potential biomarkers of mild traumatic brain injury. Molecular Biology Reports 47(4): 2941-2949	- No relevant diagnostic factor
Posti, J. P., Hossain, I., Takala, R. S. et al. (2017) Glial Fibrillary Acidic Protein and Ubiquitin C-Terminal Hydrolase-L1 Are Not Specific Biomarkers for Mild CT-Negative Traumatic Brain Injury. Journal of Neurotrauma 27: 27	- Study design not relevant to this review protocol
Posti, J. P., Takala, R. S. K., Raj, R. et al. (2020) Admission Levels of Interleukin 10 and Amyloid beta 1-40 Improve the Outcome Prediction Performance of the Helsinki Computed Tomography Score in Traumatic Brain Injury. Frontiers in neurology [electronic resource]. 11: 549527	- Comparator in study does not match that specified in this review protocol
Posti, J. P., Takala, R. S., Runtti, H. et al. (2016) The Levels of Glial Fibrillary Acidic Protein and Ubiquitin C-Terminal Hydrolase-L1 During the First Week After a Traumatic Brain Injury: Correlations With Clinical and Imaging Findings. Neurosurgery 79(3): 456-64	- Severe or moderate TBI. Not relevant to post-injury complications

Study	Code [Reason]
Puffer, R. C., Cumba Garcia, L. M., Himes, B. T. et al. (2021) Plasma extracellular vesicles as a source of biomarkers in traumatic brain injury. Journal of Neurosurgery 134(6): 1921-1928	- Not relevant to post-injury complications
Raabe, A.; Grolms, C.; Seifert, V. (1999) Serum markers of brain damage and outcome prediction in patients after severe head injury. British Journal of Neurosurgery 13(1): 56-9	- Severe or moderate TBI. Not relevant to post-injury complications
Raabe, A. and Seifert, V. (2000) Protein S-100B as a serum marker of brain damage in severe head injury: preliminary results. Neurosurgical Review 23(3): 136-8	- Severe or moderate TBI. Not relevant to post-injury complications
Radwan, T. A. M., Fahmy, R. S., El Emady, M. F. M. et al. (2021) Ischemia-modified Albumin as a Biomarker for Prediction of Poor Outcome in Patients With Traumatic Brain Injury: An Observational Cohort Study. Journal of Neurosurgical Anesthesiology 33(3): 254-257	- No relevant diagnostic factor
Raheja, A., Sinha, S., Samson, N. et al. (2016) Serum biomarkers as predictors of long-term outcome in severe traumatic brain injury: analysis from a randomized placebo-controlled Phase II clinical trial. Journal of Neurosurgery 125(3): 631-41	- No appropriate reference standard
Rahimian, S., Potteiger, S., Loynd, R. et al. (2020) The utility of S100B level in detecting mild traumatic brain injury in intoxicated patients. American Journal of Emergency Medicine 38(4): 799-805	- Systematic review - screened for relevant references
Rainey, T., Lesko, M., Sacho, R. et al. (2009) Predicting outcome after severe traumatic brain injury using the serum S100B biomarker: results using a single (24h) time-point. Resuscitation 80(3): 341-5	- No appropriate reference standard
Ramezani, F., Bahrami-Amiri, A., Babahajian, A. et al. (2018) Ubiquitin C-Terminal Hydrolase-L1 (UCH-L1) in Prediction of Computed Tomography Findings in Traumatic Brain Injury; a Meta-Analysis. Emergency (Tehran, Iran) 6(1): e62	- Systematic review - screened for relevant references
Ressel, V., Berati, D., Raselli, C. et al. (2020) Magnetic resonance imaging markers reflect cognitive outcome after rehabilitation in children	- No relevant diagnostic factor

Study	Code [Reason]
with acquired brain injury. European Journal of Radiology 126: 108963	
Rhine, T., Babcock, L., Zhang, N. et al. (2016) Are UCH-L1 and GFAP promising biomarkers for children with mild traumatic brain injury?. Brain Injury 30(10): 1231-8	- No appropriate reference standard
Rodriguez-Rodriguez, A., Egea-Guerrero, J. J., Leon-Justel, A. et al. (2012) Role of S100B protein in urine and serum as an early predictor of mortality after severe traumatic brain injury in adults. Clinica Chimica Acta 414: 228-33	- No appropriate reference standard
Rogan, A., O'Sullivan, M. B., Holley, A. et al. (2022) Can serum biomarkers be used to rule out significant intracranial pathology in emergency department patients with mild traumatic brain injury? A Systemic Review & Meta-Analysis. Injury 53(2): 259-271	- Systematic review - screened for relevant references
Rothoerl, R. D.; Woertgen, C.; Brawanski, A. (2000) S-100 serum levels and outcome after severe head injury. Acta Neurochirurgica - Supplement 76: 97-100	- Full text paper not available
Rothoerl, R. D., Woertgen, C., Holzschuh, M. et al. (1998) S-100 serum levels after minor and major head injury. Journal of Trauma-Injury Infection & Critical Care 45(4): 765-7	- No diagnostic accuracy measures reported
Rowland, B., Savarraj, J. P. J., Karri, J. et al. (2020) Acute Inflammation in Traumatic Brain Injury and Polytrauma Patients Using Network Analysis. Shock 53(1): 24-34	- No relevant diagnostic factor
Roy, D., Peters, M. E., Everett, A. et al. (2019) Loss of consciousness and altered mental state predicting depressive and post-concussive symptoms after mild traumatic brain injury. Brain Injury 33(8): 1064-1069	- No diagnostic accuracy measures reported
Ryan, E., Kelly, L., Stacey, C. et al. (2021) Traumatic Brain Injury in Children: Glial fibrillary Acidic Protein and Clinical Outcomes. Pediatric emergency care. 30	- No diagnostic accuracy measures reported
Ryb, G. E., Dischinger, P. C., Auman, K. M. et al. (2014) S-100beta does not predict outcome after mild traumatic brain injury. Brain Injury 28(11): 1430-5	- No appropriate reference standard

Study	Code [Reason]
Salmi, L., Gavelli, F., Gardino, C. A. et al. (2020) Plasma microvesicles in patients admitted to the emergency department for mild traumatic brain injury: First clues to understand their role. Minerva Biotecnologica 32(3): 89-94	- No relevant diagnostic factor
Sandmo, S. B., Filipcik, P., Cente, M. et al. (2020) Neurofilament light and tau in serum after head-impact exposure in soccer. Brain Injury 34(5): 602-609	- No appropriate reference standard
Sapin, V., Gaulmin, R., Aubin, R. et al. (2021) Blood biomarkers of mild traumatic brain injury: State of art. Neuro-Chirurgie 67(3): 249-254	- Review article but not a systematic review
Savola, O. and Hillbom, M. (2003) Early predictors of post-concussion symptoms in patients with mild head injury. European Journal of Neurology 10(2): 175-81	- No appropriate reference standard
Schiff, L., Hadker, N., Weiser, S. et al. (2012) A literature review of the feasibility of glial fibrillary acidic protein as a biomarker for stroke and traumatic brain injury. Molecular Diagnosis & Therapy 16(2): 79-92	- No relevant diagnostic factor
Schindler, C. R., Woschek, M., Vollrath, J. T. et al. (2020) miR-142-3p Expression Is Predictive for Severe Traumatic Brain Injury (TBI) in Trauma Patients. International Journal of Molecular Sciences 21(15): 29	- No relevant diagnostic factor
Schultke, E., Sadanand, V., Kelly, M. E. et al. (2009) Can admission S-100beta predict the extent of brain damage in head trauma patients?. Canadian Journal of Neurological Sciences 36(5): 612-6	- No diagnostic accuracy measures reported
Seidenfaden, S. C., Kjerulff, J. L., Juul, N. et al. (2021) Diagnostic accuracy of prehospital serum S100B and GFAP in patients with mild traumatic brain injury: a prospective observational multicenter cohort study - "the PreTBI I study". Scandinavian Journal of Trauma, Resuscitation & Emergency Medicine 29(1): 75	- Reference standard not measured in all participants
Sezer, A. A., Akinci, E., Ozturk, M. et al. (2012) The role of blood S100B and lactate levels in minor head traumas in children and adults and correlation with brain computerized tomography.	- Study not reported in English

Study	Code [Reason]
Ulusal Travma ve Acil Cerrahi Dergisi 18(5): 411-416	
Shahim, P., Darin, N., Andreasson, U. et al. (2013) Cerebrospinal fluid brain injury biomarkers in children: a multicenter study. Pediatric Neurology 49(1): 31-39.e2	- No relevant diagnostic factor
Shahim, P., Politis, A., van der Merwe, A. et al. (2020) Neurofilament light as a biomarker in traumatic brain injury. Neurology 95(6): e610-e622	- Not relevant to post-injury complications
Shahim, P., Politis, A., van der Merwe, A. et al. (2020) Time course and diagnostic utility of NfL, tau, GFAP, and UCH-L1 in subacute and chronic TBI. Neurology 95(6): e623-e636	- Severe or moderate TBI. Not relevant to post-injury complications
Shahim, P., Tegner, Y., Marklund, N. et al. (2018) Neurofilament light and tau as blood biomarkers for sports-related concussion. Neurology 90(20): e1780-e1788	- No appropriate reference standard
Shahim, P., Zetterberg, H., Tegner, Y. et al. (2017) Serum neurofilament light as a biomarker for mild traumatic brain injury in contact sports. Neurology 88(19): 1788-1794	- No appropriate reference standard
Shahjouei, S., Sadeghi-Naini, M., Yang, Z. et al. (2018) The diagnostic values of UCH-L1 in traumatic brain injury: A meta-analysis. Brain Injury 32(1): 1-17	- Systematic review - screened for relevant references
Shakeri, M., Dokht, Y. G. M., Panahi, F. et al. (2014) S100B protein value in predicting brain death after head trauma. Neurosurgery Quarterly 24(4): 291-296	- No diagnostic accuracy measures reported
Shakeri, M.; Mahdkhah, A.; Panahi, F. (2013) S100B Protein as a Post-traumatic Biomarker for Prediction of Brain Death in Association With Patient Outcomes. Archives of Trauma Research 2(2): 76-80	- No diagnostic accuracy measures reported
Sharma, R., Rosenberg, A., Bennett, E. R. et al. (2017) A blood-based biomarker panel to risk-stratify mild traumatic brain injury. PLoS ONE [Electronic Resource] 12(3): e0173798	- No relevant diagnostic factor
Shehab, H. A. and Nassar, Y. H. (2010) Neuromarkers as diagnostic adjuvant to cranial	- No diagnostic accuracy measures reported

Study	Code [Reason]
CT in closed traumatic brain injury patients admitted to ICU: A preliminary comparative study. Egyptian Journal of Anaesthesia 26(4): 267-272	
Shemilt, M., Boutin, A., Lauzier, F. et al. (2019) Prognostic Value of Glial Fibrillary Acidic Protein in Patients With Moderate and Severe Traumatic Brain Injury: A Systematic Review and Meta-Analysis. Critical Care Medicine 47(6): e522-e529	- Systematic review screened for relevant references
Shetty, T., Cogsil, T., Dalal, A. et al. (2019) High-Sensitivity C-Reactive Protein: Retrospective Study of Potential Blood Biomarker of Inflammation in Acute Mild Traumatic Brain Injury. Journal of Head Trauma Rehabilitation 34(3): E28-E36	- No relevant diagnostic factor
Shi, R., Wang, P. Y., Li, X. Y. et al. (2015) Exosomal levels of miRNA-21 from cerebrospinal fluids associated with poor prognosis and tumor recurrence of glioma patients. Oncotarget 6(29): 26971-81	- Population not relevant to this review protocol
Shibahashi, K., Doi, T., Tanaka, S. et al. (2016) The Serum Phosphorylated Neurofilament Heavy Subunit as a Predictive Marker for Outcome in Adult Patients after Traumatic Brain Injury. Journal of Neurotrauma 33(20): 1826-1833	- No appropriate reference standard
Shibata, A., Matano, F., Saito, N. et al. (2021) Serum glucose-to-potassium ratio as a prognostic predictor for severe traumatic brain injury. Journal of Nippon Medical School 88(4): 342-346	- No relevant diagnostic factor
Shore, P. M., Berger, R. P., Varma, S. et al. (2007) Cerebrospinal fluid biomarkers versus glasgow coma scale and glasgow outcome scale in pediatric traumatic brain injury: the role of young age and inflicted injury. Journal of Neurotrauma 24(1): 75-86	- No relevant diagnostic factor
Siman, R., Cui, H., Wewerka, S. S. et al. (2020) Serum SNTF, a Surrogate Marker of Axonal Injury, Is Prognostic for Lasting Brain Dysfunction in Mild TBI Treated in the Emergency Department. Frontiers in neurology [electronic resource]. 11: 249	- No appropriate reference standard

Study	Code [Reason]
Siman, R., Giovannone, N., Hanten, G. et al. (2013) Evidence That the Blood Biomarker SNTF Predicts Brain Imaging Changes and Persistent Cognitive Dysfunction in Mild TBI Patients. Frontiers in neurology [electronic resource]. 4: 190	- No appropriate reference standard
Simon-Pimmel, J., Lorton, F., Guiziou, N. et al. (2015) Serum S100beta Neuroprotein Reduces Use of Cranial Computed Tomography in Children After Minor Head Trauma. Shock 44(5): 410-6	- No relevant diagnostic factor
Singh, A., Singh, K., Sahu, A. et al. (2021) Serum Concentration of Myelin Basic Protein as a Prognostic Marker in Mild-to-moderate Head Injury Patients: A Prospective Study in a Tertiary Care Center. Indian Journal of Neurosurgery: 1-5	- No appropriate reference standard
Sojka, P., Stalnacke, B. M., Bjornstig, U. et al. (2006) One-year follow-up of patients with mild traumatic brain injury: occurrence of post-traumatic stress-related symptoms at follow-up and serum levels of cortisol, S-100B and neuron-specific enolase in acute phase. Brain Injury 20(6): 613-20	- No diagnostic accuracy measures reported
Sood, S., Azad, C., Kaur, J. et al. (2021) Role of cerebrospinal fluid tau protein levels as a biomarker of brain injury in pediatric status epilepticus. International Journal of Neuroscience: 1-9	- Population not relevant to this review protocol
Spinella, P. C., Dominguez, T., Drott, H. R. et al. (2003) S-100beta protein-serum levels in healthy children and its association with outcome in pediatric traumatic brain injury. Critical Care Medicine 31(3): 939-45	- No appropriate reference standard
Stalnacke, B. M., Bjornstig, U., Karlsson, K. et al. (2005) One-year follow-up of mild traumatic brain injury: post-concussion symptoms, disabilities and life satisfaction in relation to serum levels of S-100B and neurone-specific enolase in acute phase. Journal of Rehabilitation Medicine 37(5): 300-5	- Not relevant to post-injury complications
Stapert S, de Kruijk J, Houx P et al. (2005) S-100B concentration is not related to neurocognitive performance in the first month	- Not relevant to post-injury complications

Study	Code [Reason]
after mild traumatic brain injury. European neurology 53(1): 22-26	
Stefanovic, B., Duric, O., Stankovic, S. et al. (2017) Elevated Serum Protein S100B and Neuron Specific Enolase Values as Predictors of Early Neurological Outcome After Traumatic Brain Injury. Journal of Medical Biochemistry 36(4): 314-321	- No appropriate reference standard
Stein, D. M., Lindell, A. L., Murdock, K. R. et al. (2012) Use of serum biomarkers to predict cerebral hypoxia after severe traumatic brain injury. Journal of Neurotrauma 29(6): 1140-9	- No appropriate reference standard
Stranjalis, G., Korfias, S., Papapetrou, C. et al. (2004) Elevated serum S-100B protein as a predictor of failure to short-term return to work or activities after mild head injury. Journal of Neurotrauma 21(8): 1070-5	- No appropriate reference standard
Studer, M., Goeggel Simonetti, B., Heinks, T. et al. (2015) Acute S100B in serum is associated with cognitive symptoms and memory performance 4 months after paediatric mild traumatic brain injury. Brain Injury 29(1314): 1667-73	- Not relevant to post-injury complications
Su, S. H., Xu, W., Li, M. et al. (2014) Elevated C-reactive protein levels may be a predictor of persistent unfavourable symptoms in patients with mild traumatic brain injury: a preliminary study. Brain, Behavior, & Immunity 38: 111-7	- No relevant diagnostic factor
Sun, Y., Wang, S., Gan, S. et al. (2021) Serum Neuron-Specific Enolase Levels Associated with Connectivity Alterations in Anterior Default Mode Network after Mild Traumatic Brain Injury. Journal of Neurotrauma 38(11): 1495-1505	- No diagnostic accuracy measures reported
Takala, R. S., Posti, J. P., Runtti, H. et al. (2016) Glial Fibrillary Acidic Protein and Ubiquitin C-Terminal Hydrolase-L1 as Outcome Predictors in Traumatic Brain Injury. World Neurosurgery 87: 8-20	- No appropriate reference standard
Talypov, A. E., Puras, Y. V., Godkov, M. A. et al. (2010) Levels of S100beta protein in patients with mild traumamic brain injury. Zhurnal Nevrologii i Psihiatrii imeni S.S Korsakova. 110(12): 4-8	- Study not reported in English

Study	Code [Reason]
Tas, D.; Kaplan, O.; Sogut, O. (2020) Validity of Serum miRNA 93 and miRNA 191 to Reduce Unnecessary Computed Tomography in Patients With Mild Head Trauma. Journal of Clinical Medicine Research 12(9): 579-589	- No relevant diagnostic factor
Thelin, E. P., Jeppsson, E., Frostell, A. et al. (2016) Utility of neuron-specific enolase in traumatic brain injury; relations to S100B levels, outcome, and extracranial injury severity. Critical Care (London, England) 20: 285	- No appropriate reference standard
Thelin, E. P., Johannesson, L., Nelson, D. et al. (2013) S100B is an important outcome predictor in traumatic brain injury. Journal of Neurotrauma 30(7): 519-28	- No appropriate reference standard
Thelin, E. P.; Nelson, D. W.; Bellander, B. M. (2014) Secondary peaks of S100B in serum relate to subsequent radiological pathology in traumatic brain injury. Neurocritical Care 20(2): 217-29	- No relevant diagnostic factor
Thelin, E., Al Nimer, F., Frostell, A. et al. (2019) A Serum Protein Biomarker Panel Improves Outcome Prediction in Human Traumatic Brain Injury. Journal of Neurotrauma 36(20): 2850-2862	- No appropriate reference standard
Thorngren-Jerneck, K., Alling, C., Herbst, A. et al. (2004) S100 protein in serum as a prognostic marker for cerebral injury in term newborn infants with hypoxic ischemic encephalopathy. Pediatric Research 55(3): 406-12	- Population not relevant to this review protocol
Tokshilykova, A. B., Sarkulova, Z. N., Kabdrakhmanova, G. B. et al. (2020) Neuron-Specific Markers and their Correlation with Neurological Scales in Patients with Acute Neuropathologies. Journal of Molecular Neuroscience 70(8): 1267-1273	- Population not relevant to this review protocol
Topolovec-Vranic, J., Pollmann-Mudryj, M. A., Ouchterlony, D. et al. (2011) The value of serum biomarkers in prediction models of outcome after mild traumatic brain injury. Journal of Trauma-Injury Infection & Critical Care 71(5suppl1): S478-86	- No appropriate reference standard
Townend, W. J., Guy, M. J., Pani, M. A. et al. (2002) Head injury outcome prediction in the	- Not relevant to post-injury complications

Study	Code [Reason]
emergency department: a role for protein S-100B?. Journal of Neurology, Neurosurgery & Psychiatry 73(5): 542-6	
Townend, W. and Ingebrigtsen, T. (2006) Head injury outcome prediction: a role for protein S-100B?. Injury 37(12): 1098-108	- No appropriate reference standard
Tremblay, S., Iturria-Medina, Y., Mateos-Perez, J. M. et al. (2017) Defining a multimodal signature of remote sports concussions. European Journal of Neuroscience 46(4): 1956-1967	- Population not relevant to this review protocol
Ucar, T., Baykal, A., Akyuz, M. et al. (2004) Comparison of serum and cerebrospinal fluid protein S-100b levels after severe head injury and their prognostic importance. Journal of Trauma-Injury Infection & Critical Care 57(1): 95-8	- No appropriate reference standard
Unden, J., Astrand, R., Waterloo, K. et al. (2007) Clinical significance of serum S100B levels in neurointensive care. Neurocritical Care 6(2): 94-9	- No diagnostic accuracy measures reported
Unden, J. and Romner, B. (2009) A new objective method for CT triage after minor head injury--serum S100B. Scandinavian Journal of Clinical & Laboratory Investigation 69(1): 13-7	- Review article but not a systematic review
Unden, J. and Romner, B. (2010) Can low serum levels of S100B predict normal CT findings after minor head injury in adults?: an evidence-based review and meta-analysis. Journal of Head Trauma Rehabilitation 25(4): 228-40	- Systematic review - screened for relevant references
Unden, L., Calcagnile, O., Unden, J. et al. (2015) Validation of the Scandinavian guidelines for initial management of minimal, mild and moderate traumatic brain injury in adults. BMC Medicine 13: 292	- No relevant diagnostic factor
van Geel, W. J., de Reus, H. P., Nijzing, H. et al. (2002) Measurement of glial fibrillary acidic protein in blood: an analytical method. Clinica Chimica Acta 326(12): 151-4	- Study design not relevant to this review protocol
Visser, K., Koggel, M., Blaauw, J. et al. (2022) Blood-based biomarkers of inflammation in mild	- Systematic review - screened for relevant references

Study	Code [Reason]
traumatic brain injury: A systematic review. Neuroscience & Biobehavioral Reviews 132: 154-168	
Vos, P. E., Jacobs, B., Andriessen, T. M. et al. (2010) GFAP and S100B are biomarkers of traumatic brain injury: an observational cohort study. Neurology 75(20): 1786-93	- No appropriate reference standard
Vos, P. E., Lamers, K. J., Hendriks, J. C. et al. (2004) Glial and neuronal proteins in serum predict outcome after severe traumatic brain injury. Neurology 62(8): 1303-10	- No appropriate reference standard
Wang, J., Li, J., Han, L. et al. (2016) Serum tau protein as a potential biomarker in the assessment of traumatic brain injury. Experimental & Therapeutic Medicine 11(3): 1147-1151	- No relevant diagnostic factor
Wang, X. (2021) Correlation between serum neuron specific enolase level and neuron injury index and neuron apoptosis index in patients with brain injury. Acta Medica Mediterranea 37(2): 791-794	- No diagnostic accuracy measures reported
Wang, X. H. and Zhang, X. D. (2006) Evaluating the prognosis and degree of brain injury by combined S-100 protein and neuron specific enolase determination. Neural Regeneration Research 1(7): 649-652	- Study design not relevant to this review protocol *case-control study*
Ward, M. D., Weber, A., Merrill, V. D. et al. (2020) Predictive Performance of Traumatic Brain Injury Biomarkers in High-Risk Elderly Patients. The Journal of Applied Laboratory Medicine 5(1): 91-100	- No relevant diagnostic factor
Welch, R. D., Ellis, M., Lewis, L. M. et al. (2017) Modeling the Kinetics of Serum Glial Fibrillary Acidic Protein, Ubiquitin Carboxyl-Terminal Hydrolase-L1, and S100B Concentrations in Patients with Traumatic Brain Injury. Journal of Neurotrauma 34(11): 1957-1971	- Data not reported in an extractable format or a format that can be analysed
Wiesmann, M., Steinmeier, E., Magerkurth, O. et al. (2010) Outcome prediction in traumatic brain injury: comparison of neurological status, CT findings, and blood levels of S100B and GFAP. Acta Neurologica Scandinavica 121(3): 178-85	- No diagnostic accuracy measures reported

Study	Code [Reason]
Wijanarko, F., Alifianto, U., Setyono, H. et al. (2021) S100beta protein levels as a parameter to assess the clinical development of adult patients with mild traumatic brain injury in Dr. Moewardi Public Hospital, Surakarta. Surgical neurology international 12: 342	-No diagnostic accuracy measures reported
Woertgen, C., Rothoerl, R. D., Holzschuh, M. et al. (1997) Comparison of serial S-100 and NSE serum measurements after severe head injury. Acta Neurochirurgica 139(12): 1161-4; discussion 1165	- Severe or moderate TBI. Not relevant to post-injury complications
Woertgen, C., Rothoerl, R. D., Metz, C. et al. (1999) Comparison of clinical, radiologic, and serum marker as prognostic factors after severe head injury. Journal of Trauma-Injury Infection & Critical Care 47(6): 1126-30	- Severe or moderate TBI. Not relevant to post-injury complications
Xu, L. B., Yue, J. K., Korley, F. et al. (2021) High-Sensitivity C-Reactive Protein is a Prognostic Biomarker of Six-Month Disability after Traumatic Brain Injury: Results from the TRACK-TBI Study. Journal of Neurotrauma 38(7): 918-927	- No appropriate reference standard
Yakoub, K. M., O'Halloran, P., Davies, D. J. et al. (2018) Study of Concussion in Rugby Union through MicroRNAs (SCRUM): a study protocol of a prospective, observational cohort study. BMJ Open 8(11): e024245	- Not a peer-reviewed publication
Yokobori, S., Hosein, K., Burks, S. et al. (2013) Biomarkers for the clinical differential diagnosis in traumatic brain injury--a systematic review. CNS Neuroscience & Therapeutics 19(8): 556-65	- No protocol outcomes
Yoon, S. M., Choi, Y. J., Kim, H. J. et al. (2008) Prognostic value of serum S100 protein by elecsys S100 immunoassay in patients with spontaneous subarachnoid and intracerebral hemorrhages. Journal of Korean Neurosurgical Society 44(5): 308-313	- Population not relevant to this review protocol
Yu, L., Wu, X., Wang, H. et al. (2014) Diagnostic and prognostic significance of suPAR in traumatic brain injury. Neurology India 62(5): 498-502	- No relevant diagnostic factor

Study	Code [Reason]
Yue, J. K., Upadhyayula, P. S., Avalos, L. N. et al. (2020) The Role of Blood Biomarkers for Magnetic Resonance Imaging Diagnosis of Traumatic Brain Injury. Medicina 56(2): 22	- Review article but not a systematic review
Yue, J. K., Yuh, E. L., Korley, F. K. et al. (2019) Association between plasma GFAP concentrations and MRI abnormalities in patients with CT-negative traumatic brain injury in the TRACK-TBI cohort: a prospective multicentre study. Lancet Neurology 18(10): 953-961	- Data not reported in an extractable format or a format that can be analysed
Yuh, E. L., Mukherjee, P., Lingsma, H. F. et al. (2013) Magnetic resonance imaging improves 3-month outcome prediction in mild traumatic brain injury. Annals of Neurology 73(2): 224-35	- No relevant diagnostic factor
Zhang, J., Wang, H., Li, Y. et al. (2021) The diagnosis and prognostic value of plasma copeptin in traumatic brain injury: a systematic review and meta-analysis. Neurological Sciences 42(2): 539-551	- No relevant diagnostic factor
Zhang, Z. Y., Zhang, L. X., Dong, X. Q. et al. (2014) Comparison of the performances of copeptin and multiple biomarkers in long-term prognosis of severe traumatic brain injury. Peptides 60: 13-7	- No appropriate reference standard
Zhao, J., Chen, H., Zhang, M. et al. (2016) Early expression of serum neutrophil gelatinase-associated lipocalin (NGAL) is associated with neurological severity immediately after traumatic brain injury. Journal of the Neurological Sciences 368: 392-8	- No relevant diagnostic factor
Zhou, Q., Yin, J., Wang, Y. et al. (2021) MicroRNAs as potential biomarkers for the diagnosis of Traumatic Brain Injury: A systematic review and meta-analysis. International Journal of Medical Sciences 18(1): 128-136	- No relevant diagnostic factor
Zurek, J.; Bartlova, L.; Fedora, M. (2011) Hyperphosphorylated neurofilament NF-H as a predictor of mortality after brain injury in children. Brain Injury 25(2): 221-6	- No appropriate reference standard
Zurek, J. and Fedora, M. (2011) Dynamics of glial fibrillary acidic protein during traumatic	- No appropriate reference standard

Study	Code [Reason]
brain injury in children. Journal of Trauma-Injury Infection & Critical Care 71(4): 854-9	
Zurek, J. and Fedora, M. (2012) The usefulness of S100B, NSE, GFAP, NF-H, secretagogin and Hsp70 as a predictive biomarker of outcome in children with traumatic brain injury. Acta Neurochirurgica 154(1): 93-103; discussion 103	- No appropriate reference standard

Table 8: Studies excluded from the clinical review (test and treat)

Study	Code [Reason]
Al-Adli, N., Akbik, O. S., Rail, B. et al. (2021) The Clinical Use of Serum Biomarkers in Traumatic Brain Injury: A Systematic Review Stratified by Injury Severity. World Neurosurgery 23: 23	- Systematic review- screened for relevant references
Anderson, T. N., Hwang, J., Munar, M. et al. (2020) Blood-based biomarkers for prediction of intracranial hemorrhage and outcome in patients with moderate or severe traumatic brain injury. The Journal of Trauma and Acute Care Surgery 89(1): 80-86	- Population not relevant to this review protocol *moderate and severe TBI*
Bouvier, D., Balayssac, D., Durif, J. et al. (2019) Assessment of the advantage of the serum S100B protein biomonitoring in the management of paediatric mild traumatic brain injury-PROS100B: protocol of a multicentre unblinded stepped wedge cluster randomised trial. BMJ Open 9(5): e027365	- study protocol
Bratu, L. M., Rogobete, A. F., Papurica, M. et al. (2016) Literature Research Regarding miRNAs' Expression in the Assessment and Evaluation of the Critically Ill Polytrauma Patient with Traumatic Brain and Spinal Cord Injury. Clinical Laboratory 62(10): 2019-2024	- Review article but not a systematic review
Cheng, F., Yuan, Q., Yang, J. et al. (2014) The prognostic value of serum neuron-specific enolase in traumatic brain injury: systematic review and meta-analysis. PLoS ONE [Electronic Resource] 9(9): e106680	- Systematic review- screened for relevant references
Daoud, H., Alharfi, I., Alhelali, I. et al. (2014) Brain injury biomarkers as outcome predictors in	- Systematic review- screened for relevant references

Study	Code [Reason]
pediatric severe traumatic brain injury. Neurocritical Care 20(3): 427-35	
Edalatfar, M., Piri, S. M., Mehrabinejad, M. M. et al. (2021) Biofluid Biomarkers in Traumatic Brain Injury: A Systematic Scoping Review. Neurocritical Care 05: 05	- Systematic review- screened for relevant references
Frankel, M., Fan, L., Yeatts, S. D. et al. (2019) Association of Very Early Serum Levels of S100B, Glial Fibrillary Acidic Protein, Ubiquitin C-Terminal Hydrolase-L1, and Spectrin Breakdown Product with Outcome in ProTECT III. Journal of Neurotrauma 36(20): 2863-2871	- Population not relevant to this review protocol *moderate and severe TBI*
Gan, Z. S., Stein, S. C., Swanson, R. et al. (2019) Blood Biomarkers for Traumatic Brain Injury: A Quantitative Assessment of Diagnostic and Prognostic Accuracy. Frontiers in neurology [electronic resource]. 10: 446	- Review article but not a systematic review
Ghonemi, M. O., Rabah, A. A., Saber, H. M. et al. (2013) Role of Phosphorylated Neurofilament H as a diagnostic and prognostic marker in traumatic brain injury. Egyptian Journal of Critical Care Medicine 1(3): 139-144	- Population not relevant to this review protocol *- Severe or moderate TBI. Not relevant to post-injury complications*
Gradisek, P., Carrara, G., Antiga, L. et al. (2021) Prognostic Value of a Combination of Circulating Biomarkers in Critically Ill Patients with Traumatic Brain Injury: Results from the European CREACTIVE Study. Journal of Neurotrauma 11: 11	- Population not relevant to this review protocol *Not relevant to post-injury complications*
Hendoui, N., Beigmohammadi, M. T., Mahmoodpoor, A. et al. (2013) Reliability of calcium-binding protein S100B measurement toward optimization of hyperosmolal therapy in traumatic brain injury. European Review for Medical & Pharmacological Sciences 17(4): 477-85	- Population not relevant to this review protocol *Not relevant to post-injury complications. Not relevant intervention- study compares administration ways of hypertonic saline 5% (bolus and infusion) with mannitol upon S100 as a therapeutic tool for monitoring treatment in TBI patients.*
Herrold, A. A., Kletzel, S. L., Foecking, E. M. et al. (2021) miRNAs as Potential Biomarkers for Traumatic Brain Injury: Pathway From Diagnosis to Neurorehabilitation. Journal of Head Trauma Rehabilitation 36(3): E155-E169	- Systematic review- screened for relevant references
Ingebrigtsen, T. and Romner, B. (2003) Biochemical serum markers for brain damage: a short review with emphasis on clinical utility in	- Review article but not a systematic review

Study	Code [Reason]
mild head injury. Restorative Neurology & Neuroscience 21(34): 171-6	
Karakulova, Y. V. and Selyanina, N. V. (2017) Monitoring of neurotrophic factors and cognitive function in patients with traumatic brain injury. Zhurnal nevrologii i psikhiatrii imeni S.S. Korsakova 117(10): 34-37	- Study does not contain an intervention relevant to this review protocol *study aims to determine the neurological and cognitive status in comparison to the quantitative content of blood serum neurotrophic factors of patients with acute and long-term brain injury during treatment with cerebrolysin.*
Kovesdi, E., Luckl, J., Bukovics, P. et al. (2010) Update on protein biomarkers in traumatic brain injury with emphasis on clinical use in adults and pediatrics. Acta Neurochirurgica 152(1): 1-17	- Systematic review- screened for relevant references
Leidel, B. A. (2013) Serological protein S100B for diagnostic management of adults with mild head injury-a meta-analysis. Langenbeck's Archives of Surgery: 656	- Full text paper not available
Lugones, M., Parkin, G., Bjelosevic, S. et al. (2018) Blood biomarkers in paediatric mild traumatic brain injury: a systematic review. Neuroscience & Biobehavioral Reviews 87: 206-217	- Systematic review- screened for relevant references
Lumba-Brown, A., Yeates, K. O., Sarmiento, K. et al. (2018) Diagnosis and Management of Mild Traumatic Brain Injury in Children: A Systematic Review. JAMA Pediatrics 172(11): e182847	- Systematic review- screened for relevant references
Luoto, T. M., Raj, R., Posti, J. P. et al. (2017) A Systematic Review of the Usefulness of Glial Fibrillary Acidic Protein for Predicting Acute Intracranial Lesions following Head Trauma. Frontiers in neurology [electronic resource]. 8: 652	- Systematic review- screened for relevant references
Mannix, R., Levy, R., Zemek, R. et al. (2020) Fluid Biomarkers of Pediatric Mild Traumatic Brain Injury: A Systematic Review. Journal of Neurotrauma 37(19): 2029-2044	- Systematic review- screened for relevant references
Marzano, L. A. S., Batista, J. P. T., de Abreu Arruda, M. et al. (2021) Traumatic brain injury biomarkers in pediatric patients: a systematic review. Neurosurgical Review 25: 25	- Systematic review- screened for relevant references

Study	Code [Reason]
Mercier, E., Boutin, A., Lauzier, F. et al. (2013) Predictive value of S-100beta protein for prognosis in patients with moderate and severe traumatic brain injury: systematic review and meta-analysis. BMJ 346: f1757	- Population not relevant to this review protocol *Severe or moderate TBI. Not relevant to post-injury complications*
Mercier, E., Boutin, A., Shemilt, M. et al. (2016) Predictive value of neuron-specific enolase for prognosis in patients with moderate or severe traumatic brain injury: a systematic review and meta-analysis. CMAJ open 4(3): E371-E382	- Population not relevant to this review protocol *Severe or moderate TBI. Not relevant to post-injury complications*
Meyer, J., Bartolomei, C., Sauer, A. et al. (2020) The relationship between fluid biomarkers and clinical outcomes in sports-related concussions: a systematic review. Brain Injury 34(11): 1435-1445	- Systematic review- screened for relevant references
Mitra, B., Rau, T. F., Surendran, N. et al. (2017) Plasma micro-RNA biomarkers for diagnosis and prognosis after traumatic brain injury: A pilot study. Journal of Clinical Neuroscience 38: 37-42	- Not appropriate study design *Not RCT. Not adjusted for key confounders*
Mondello, S., Sorinola, A., Czeiter, E. et al. (2021) Blood-Based Protein Biomarkers for the Management of Traumatic Brain Injuries in Adults Presenting to Emergency Departments with Mild Brain Injury: A Living Systematic Review and Meta-Analysis. Journal of Neurotrauma 38(8): 1086-1106	- Systematic review- screened for relevant references
Mozaffari, K., Dejam, D., Duong, C. et al. (2021) Systematic Review of Serum Biomarkers in Traumatic Brain Injury. Cureus 13(8): e17056	- Systematic review- screened for relevant references
O'Connell, B., Kelly, A. M., Mockler, D. et al. (2018) Use of Blood Biomarkers in the Assessment of Sports-Related Concussion-A Systematic Review in the Context of Their Biological Significance. Clinical Journal of Sport Medicine 28(6): 561-571	- Systematic review- screened for relevant references
Oris, C., Pereira, B., Durif, J. et al. (2018) The Biomarker S100B and Mild Traumatic Brain Injury: A Meta-analysis. Pediatrics 141(6): 06	- Systematic review- screened for relevant references
Pandor, A., Goodacre, S., Harnan, S. et al. (2011) Diagnostic management strategies for adults and children with minor head injury: a systematic review and an economic evaluation.	- Systematic review- screened for relevant references

Study	Code [Reason]
Health Technology Assessment (Winchester, England) 15(27): 1-202	
Papa, L., Ramia, M. M., Kelly, J. M. et al. (2013) Systematic review of clinical research on biomarkers for pediatric traumatic brain injury. Journal of Neurotrauma 30(5): 324-38	- Systematic review- screened for relevant references
Papurica, M., Rogobete, A. F., Sandesc, D. et al. (2016) Advances in Biomarkers in Critical Ill Polytrauma Patients. Clinical Laboratory 62(6): 977-86	- Population not relevant to this review protocol *critically ill trauma patients*
Reuter-Rice, K., Eads, J. K., Berndt, S. B. et al. (2015) Chapter 6 state of the science of pediatric traumatic brain injury: biomarkers and gene association studies. Annual Review of Nursing Research 33: 185-217	- Systematic review- screened for relevant references
Salehpoor, F., Meshkini, A., Razmgiri, A. et al. (2016) Prognostic serum factors in patients with traumatic brain injury: A systematic review. Neurosurgery Quarterly 26(1): 19-36	- Population not relevant to this review protocol *moderate or severe TBI*
Sapin, V., Gaulmin, R., Aubin, R. et al. (2021) Blood biomarkers of mild traumatic brain injury: State of art. Neuro-Chirurgie 67(3): 249-254	- Review article but not a systematic review
Shahim, P., Gill, J. M., Blennow, K. et al. (2020) Fluid Biomarkers for Chronic Traumatic Encephalopathy. Seminars in Neurology.	- Population not relevant to this review protocol *biomarkers for diagnosing chronic traumatic encephalopathy*
Shehab, H. A. and Nassar, Y. H. (2010) Neuromarkers as diagnostic adjuvant to cranial CT in closed traumatic brain injury patients admitted to ICU: A preliminary comparative study. Egyptian Journal of Anaesthesia 26(4): 267-272	- Population not relevant to this review protocol *severe TBI*
Shemilt, M., Boutin, A., Lauzier, F. et al. (2019) Prognostic Value of Glial Fibrillary Acidic Protein in Patients With Moderate and Severe Traumatic Brain Injury: A Systematic Review and Meta-Analysis. Critical Care Medicine 47(6): e522-e529	- Population not relevant to this review protocol *moderate and severe TBI*
Wang, X. H. and Zhang, X. D. (2006) Evaluating the prognosis and degree of brain injury by combined S-100 protein and neuron specific enolase determination. Neural Regeneration Research 1(7): 649-652	- Not appropriate study design *case-control study*

Health Economic studies

Published health economic studies that met the inclusion criteria (relevant population, comparators, economic study design, published 2006 or later and not from non-OECD country or USA) but that were excluded following appraisal of applicability and methodological quality are listed below. See the health economic protocol for more details.

Table 9: Studies excluded from the health economic review

Reference	Reason for exclusion
Calcagnile 2016[12]	Excluded as rated very serious limitations due to the no biomarkers arm being hypothetical.

Appendix J – Research recommendations – full details

J.1 Research recommendation

What is the diagnostic accuracy of brain injury biomarkers for predicting acute post-brain injury complications after a brain injury?

J.1.1 Why this is important

Acute post-brain injury complications here refer to the presence of an abnormality on CT/MRI head. The use of MRI head is not routine in NHS emergency departments. However, the use of CT head is routine in emergency departments all over the world in the management of head injury patients. In the context of mild TBI, around 85% of CT scans performed will be normal. Despite the use of decision rules to rationalise the use of CT scans after head injury, several studies indicate that they are "over-used" outside of these recommendations. As a result in children and in adults, many thousand CT scans are performed annually, after mild TBI, which do not demonstrate any abnormality. In hospital this has a negative impact on emergency and radiology department workflow, results in longer waits for patients while scans are carried out and reported, longer wait times for other types of CT scanning and prevention of patients exiting emergency departments. Pre hospital the JRCALC head injury management reflects CT decision rules with regards to the destination of patients. In paediatric populations in particular, there are concerns about exposure to radiation with CT scanning as well as the need for sedation in order to perform a CT head in younger children. Because of the large number of scans performed in this context nationwide, biomarkers which can distinguish lower risk patients who can, therefore, not undergo a scan and instead can be discharged under the supervision of a responsible adult has the potentially to dramatically reduce costs and pressure on emergency departments and improve patient experience.

J.1.2 Rationale for research recommendation

Importance to ‘patients’ or the population	If accurate test using biomarkers could be established this would reduce the number of people undergoing scanning. This is important to reduce exposure to radiation.
Relevance to NICE guidance	Evidence would support recommendations on the use of biomarkers in the NHS for detecting post injury complications.
Relevance to the NHS	Acute post-brain injury complications" here refers to the presence of an abnormality on CT/MRI head. -The use of MRI head is not routine in NHS emergency departments -The use of CT head is routine in emergency departments all over the world in the management of head injury patients -In the context of mild TBI, around 85% of CT scans performed will be normal. -Despite the use of decision rules to rationalise the use of CT scans after head injury, several studies indicate that they are "over-used" outside of these recommendations -As a result in children and in adults, many thousand CT scans are performed annually, after mild TBI, which do not demonstrate any abnormality

	-In hospital this has a negative impact on emergency and radiology department workflow, results in longer waits for patients while scans are carried out and reported, longer wait times for other types of CT scanning and prevention of patients exiting emergency departments -Pre hospital the JRCALC head injury management reflects CT decision rules with regards to the destination of patients -In paediatric populations in particular, there are concerns about exposure to radiation with CT scanning as well as the need for sedation in order to perform a CT head in younger children -Because of the large number of scans performed in this context nationwide, biomarkers which can distinguish lower risk patients who can, therefore, not undergo a scan and instead can be discharged under the supervision of a responsible adult has the potentially to dramatically reduce costs and pressure on emergency departments and improve patient experience.
National priorities	None identified
Current evidence base	There were high sensitivity values for some biomarkers at certain thresholds, however the specificity values were not high enough across the evidence and felt this was equally important given the consequences of unnecessary radiation particularly in children. Many biomarkers were tested in small samples leading to imprecise estimates. Alternatively, such estimates were from large but single studies. The committee noted that accuracy differed quite widely between different studies looking at the same biomarker test. The evidence included in the review was heterogenous with different biomarkers with variable thresholds and time-points. Most people with head injury present to the hospital within 3 hours and the manufacturers recommend this time frame for optimal test results. Many studies assessed biomarkers beyond this time point. The population in the included studies was mild TBI (GCS score 13-15) but they also included medium risk (medium risk includes mild and moderate TBI) and high-risk CDR patients along with very low risk patients who are currently ineligible for CT in NHS practice. Most studies included people with mild injury with extracranial injuries.
Equality considerations	This research is of particular relevance to: -Children -Victims of domestic abuse who may receive repeated head injury

	-Older adults who may fall frequently -Adults with cognitive impairment and learning difficulties who may be scanned more readily due to difficulties assessing change from baseline

J.1.3 Modified PICO table

Population	Inclusion: Infants, children and adult with suspected traumatic brain injury (TBI) Strata: • Adults (aged ≥16 years) • Children (aged ≥1 to <16 years) • Infants (aged <1 year) Mixed population studies will be included but downgraded for indirectness. Cut-off of 60% will be used for all age groups Exclusion: Adults, and children (including infants under 1 year) with superficial injuries to the eye or face without suspected or confirmed head or brain injury.
Target condition	Acute post-brain injury complications
Index tests	• Biomarkers o Blood biomarkers - S100 calcium binding protein B (S100B) -Ubiquitin C-terminal Hydrolase-L1 (UCHL1) -Neuron Specific Enolase (NSE) -Brain-derived neurotrophic factor (BDNF) -Neurofilament light (NFL) - Neurofilament Heavy (NF-H) - αII-Spectrin breakdown products (SBDP) - Myelin basic protein (MBP) - glial fibrillary acidic protein (GFAP) o Salivary biomarkers -salivary microRNAs (miRNAs) -Extracellular vesicles (EVs) -S100B
Reference standard	Intra cranial injury and/or complex skull fracture on CT/MRI
Outcome	• diagnostic accuracy of biomarkers for predicting acute post-brain injury complications Diagnostic accuracy to be reported by test sensitivity/specificity
Study design	Cross-sectional studies Cohort studies (prospective and retrospective) Systematic reviews and meta-analyses of the above
Timeframe	Medium term – required for when the guidance is updated

Additional information	None