

BLOOD-BASED BIOMARKERS TO RULE OUT CEREBRAL LESIONS IN MILD TRAUMATIC BRAIN INJURIES



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LIST OF ABBREVIATIONS

ABBREVIATION	DEFINITION
AUC	Area Under Curve
BIA	Budget Impact Analysis
CCHR	Canadian CT Head Rule
CHEERS	Consolidated Health Economic Evaluation Reporting Standards
CHIP	CT in Head Injury Patients
CI	Confidence Interval
CMIA	Chemiluminescent Microparticle Immunoassay
CT	Computed Tomography
CUA	Cost-Utility Analysis
ED	Emergency Department
ELFA	Enzyme-Linked Fluorescent Assay
ELISA	Enzyme-Linked Immunosorbent Assay
FDA	Food and Drug Administration
GCS	Glasgow Coma Scale
GCSS	Glasgow Coma Scale Score
GFAP	Glial Fibrillary Acidic Protein
GOS	Glasgow Outcome Score
ICI	Intracranial Injury
ICU	Intensive Care Unit
INSEE	Institut national de la statistique et des études économiques
LR+	Positive Likelihood Ratio
LR-	Negative Likelihood Ratio
LYGs	Life Years Gained
MA	Meta-Analysis
mTBI	Mild TBI
NA	Not Applicable
NEXUS	National Emergency X-Radiography Utilization Study
NICE	National Institute for Health and Care Excellence
NIHDI	National Institute for Health and Disability Insurance
NLR	Negative Likelihood Ratio
NOC	New Orleans Criteria
NPV	Negative Predictive Value
PCS	Post-Concussive Syndrome



PECARN	Pediatric Emergency Care Applied Research Network
POC	Point-of-Care
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
QALY	Quality Adjusted Life Year
QUADAS	Quality Assessment of Diagnostic Accuracy Studies
ROC	Receiver Operating Characteristic
RPP	Recommendations for Professional Practice
SA	Sensitivity Analysis
SE	Sensitivity
SFAR	French Society of Anaesthesia and Intensive Care
SFBC	French Society of Clinical Biology
SFMU	French Society of Emergency Medicine
SFR	French Society of Radiology
SNC	Scandinavian Neurotrauma Committee
SOFMER	French Society of Physical Medicine and Rehabilitation
S100 β	S100 Calcium-Binding Protein β
SP	Specificity
SR	Systematic Review
SROC	Summary Receiver Operating Characteristic
TBI	Traumatic Brain Injury
UCH-L1	Ubiquitin Carboxy-terminal Hydrolase L1
WTP	Willingness To Pay

■ SCIENTIFIC REPORT

1 INTRODUCTION

1.1 The public health importance of mild traumatic brain injuries

1.1.1 Definition of mTBI

Traumatic Brain Injury (TBI) is an acute brain injury caused by any external mechanical forces, impact, rapid acceleration-deceleration, or a penetrating injury to the head, and presents a significant health challenge worldwide.¹ Falls are the leading cause of TBI in high-income countries, particularly among older people (age ≥ 65 years), followed by motor vehicle accidents, and blows to the head.² TBI can generally be classified as closed or penetrating. The latter is distinguished by a foreign object penetrating into (or through) the intracranial compartment. Of the two types, closed TBI is far more common. The different types of these injuries can be broken down to include concussion, contusion, diffuse axonal injury, and intracranial hematoma (epidural hematoma, subdural hematoma, subarachnoid haemorrhage, and intraparenchymal haemorrhage).¹

TBI severity is classified into three categories: mild, moderate, and severe. Operational criteria for clinical identification of mild TBI (mTBI) include:

1. One or more of the following elements: confusion or disorientation, loss of consciousness lasting less than 30 minutes, post-traumatic amnesia lasting less than 24 hours, and/or other transient neurological abnormalities such as focal signs, seizure, and intracranial lesion not requiring surgery. Moderate or severe TBI is clinically defined as a loss of consciousness lasting ≥ 30 minutes to prolonged coma, post-traumatic amnesia lasting ≥ 24 hours up to permanently.^{1, 3}
2. A Glasgow Coma Scale (GCS) score is also used in the description of those with TBI. It is a 3 to 15-point scale used to assess patients' level of consciousness and neurological functioning following a head injury.¹ Scoring on this scale is based on motor, verbal, and eye-opening responses upon admission to the emergency department (ED). Mild TBI is most often defined as a score in the GCS of 13-15, although some authors limit the definition of mild to GCS 14-15,⁴ moderate when the GCS score is 9-12, and severe when the GCS score is 3-8.² Around 90% of TBI cases are classified as mild.^{1, 2, 5}

Although the clinical severity of TBI is commonly classified according to the GCS score, a new approach is under development for characterising TBI more accurately and informing individualised clinical management.⁶ This new framework incorporates four pillars: a clinical pillar (full GCS and pupillary reactivity); a biomarker pillar (blood-based measures); an imaging pillar (pathoanatomical measures); and a modifier pillar (features influencing clinical presentation and outcome). This CBI-M framework still needs to be validated.⁶

1.1.2 Incidence of mTBI

A 2016 systematic literature review including 82 studies reported a pooled annual incidence of TBI for all ages of 295 per 100 000 (95%CI: 274-317), corresponding to a pooled incidence rate of 349 per 100 000 person-years (95%CI: 96.2-1 266).⁷ The pooled annual incidence and incidence rate of mTBI were 224 per 100 000 (95% CI: 120-418) and 982 per 100 000 person-years (95% CI: 756-1 276), respectively.⁷ Most TBIs were mild and occurred more frequently in males, particularly in adulthood, and incidence varied widely by age and between countries.⁷

A second systematic review published in 2021, with literature searched up to 15 January 2015, reported crude incidence rates ranging from 47.3 per 100 000 to 694 per 100 000 population per year, across 22 country-level studies.⁸



Another global estimation published in the Lancet in 2019 reported an age-standardised incidence rate of TBI of 369 per 100 000 population (95%CI: 331-412) and showed an increase over time of 8.4% between 1990 and 2016.⁹ In the same year, Belgium had an age-standardised TBI incidence of 344 cases per 100 000 population (95%CI: 287-416), which was higher than the Western European average of 292 cases per 100 000 population.⁹ In absolute terms, this corresponded to an estimated 41 126 TBI cases per year in Belgium (95%CI: 33 848-51 024).

Regarding Belgian figures, the other studies identified were limited to hospitalised TBI cases. Peeters et al. estimated an age and gender standardised TBI incidence of 263.9 per 100 000 population in 2003 that decreased to 187.9 per 100 000 population in the year 2012,¹⁰ while more recent research reported an incidence of hospitalised TBI in 2016 of 136 cases per 100 000, accounting for 17 086 cases, 45% of whom were mTBI.¹¹

As explained in section 1.1.1., mTBI (also known as concussion) accounts for approximately 90% of traumatic brain injury cases.^{1, 2, 5}

The above estimations probably underestimate the true incidence of mTBI because they are hospital-based and do not capture people with mTBI who do not seek medical attention soon after injury.⁹

1.1.3 Consequences of mTBI

A 2015 systematic literature review including 14 studies on patients with GCS score 13-15 (n=23 079) reported that 7.1% (95% CI, 6.8%-7.4%) had severe intracranial injuries (ICIs) visible on CT scan requiring prompt intervention. The proportion of injuries leading to death or requiring neurosurgical intervention was 0.9% (95% CI, 0.78%-1.0%).¹²

Although most CT scans obtained after mTBIs are normal, patients may nevertheless present with a broad range of somatic, cognitive, emotional, and behavioural symptoms.¹³ These frequently include features of altered mental status, such as loss of consciousness, post-traumatic amnesia, confusion, impaired concentration, slowed thinking, and feeling dazed.¹³

While most patients fully recover from their post-concussive symptoms within two to four weeks, some experience persisting symptoms.¹ Around 50% of adult patients with mTBI presenting to hospital and meeting criteria to undergo a CT scan, do not recover to their pre-TBI health levels within 6 months of their injury.^{5, 14} Because persisting symptoms vary greatly, it is challenging to provide an exact estimate of their prevalence. The prognosis of TBI primarily depends on the severity of the injury, but other factors can also play a role such as the injury's location or the individual's age and general health condition. The aim of the new CBI-M framework is to reach a multidimensional characterisation of TBI to inform individualised clinical management.⁶

The current management of mTBIs in clinical practice is limited, as are the available treatment options. It has been estimated that in Europe fewer than 10% of patients discharged from an emergency department (ED) after presenting with a TBI currently receive follow-up care.⁵ Structured follow-up care after mTBI should be considered good practice, and research is needed to identify which patients with a mTBI are at risk for incomplete recovery. There remains a pressing need for more effective therapeutics, given the scarcity of published literature on successful treatments in the clinical setting.²

1.2 Current diagnosis of mTBI

Head CT scans are recognised as the gold standard in TBI due to their high sensitivity and specificity in detecting any lesion that may require neurosurgical intervention, such as skull fracture, epidural hematoma, subdural hematoma, pneumocephalus, subarachnoid haemorrhage, haemorrhagic contusion, and otomastoiditis. However, in mTBI such complications are observed only in a low proportion of patients (less than 10%)¹² and a routine CT scan is therefore not recommended. Another reason to limit the use of CT scans is the radiation-associated risk of cancer, particularly in children and young adults.¹⁵⁻¹⁷ Validated clinical decision rules were developed to identify patients with mTBI

who can safely forgo head CT imaging and those in whom CT is warranted to exclude a clinically important ICI. The most common rules for adults and children are described in this section.

1.2.1 Clinical Head Rules in adults

1.2.1.1 Canadian CT Head Rule (CCHR)

Developed by Stiell et al., in 2001,¹⁸ these rules apply to adults aged 16 years and older, who present to the ED within 24 hours of a blunt head trauma with a GCS of 13-15 accompanied by witnessed loss of consciousness, definite amnesia, or witnessed disorientation. This rule identifies patients at high or medium risk of clinically significant ICIs and guides the use of CT imaging as follows:

CT scanning is recommended in the presence of high-risk factors for neurological intervention, described as: a GCS score below 15 two hours post-injury, a suspected open or depressed skull fracture, signs of basal skull fracture, two or more episodes of vomiting, or age 65 years and older. Patients with medium-risk features are those who could still have clinically important injuries but would not require neurosurgery. These patients should also be considered for a head CT scan, but observation is mentioned as a possible alternative. Medium-risk symptoms are described as: amnesia lasting 30 minutes or more, and dangerous mechanisms of injury (e.g. pedestrians struck by vehicles, ejection from motor vehicles, or falls from a height greater than three feet or five stairs).

1.2.1.2 New Orleans Criteria (NOC)

The New Orleans Criteria published by Haydel et al. in 2000¹⁹ also focused on the need for a CT scan in adults with minor head injuries. Minor head injury was defined more restrictively in these rules than in the CCTH rules as a loss of consciousness in patients with normal findings on a brief neurologic examination (normal cranial nerves and normal strength and sensation in the arms and legs) with a GCS of 15. From these, CT imaging is recommended in cases in which one or more of the following seven symptoms are present: headache, vomiting, aged over 60, drug or alcohol intoxication, persistent anterograde amnesia, visible trauma above the clavicle, or post-traumatic seizure.

1.2.1.3 NICE Guidelines (UK)

The NICE guidelines for head injury assessment in adults (aged ≥ 16) were originally published in 2003 and updated in 2023.²⁰ These guidelines provide a structured approach to identify patients who require urgent CT imaging, versus those for which a CT scan can wait, based on clinical presentation and risk factors.

Thus, urgent CT scanning (i.e. within one hour) is recommended for any patients with a GCS score ≤ 12 on initial assessment, a GCS score below 15 two hours post-injury, suspected open or depressed skull fracture, signs of basal skull fracture, post-traumatic seizure, focal neurological deficit, or repeated vomiting (≥ 2 episodes).

In patients who had some loss of consciousness or amnesia since injury, a CT scan should be performed (within 8 hours from injury) in those aged ≥ 65 , anyone having bleeding or clotting disorders, patients having suffered from a dangerous mechanism of injury, or those with retrograde amnesia exceeding 30 minutes.

1.2.1.4 NEXUS II

The NEXUS II decision rule²⁰ was originally derived from a population with blunt head trauma with or without loss of consciousness. It provides a framework for identifying low-risk patients who may not require CT imaging.

CT scanning is advised if any of the following features are present: age 65 years or older, evidence of significant skull fracture, scalp hematoma, neurological deficit, altered level of alertness, abnormal behaviour, coagulopathy, or repeated vomiting (≥ 2 episodes).



1.2.1.5 *Scandinavian Neurotrauma Committee (SNC) Guidelines*

The Scandinavian guidelines, published by Unden et al. in 2013 are designed for adult patients presenting a moderate to minimal head injury (i.e. GCS 9-15).²¹ They offer a tiered approach to imaging based on risk-stratification and clinical presentation.

In mTBI, CT scanning is recommended in “high-risk” patients, defined as patients with a GCS 14-15 and one or more of the following: post-traumatic seizures, focal neurological deficit, clinical signs of skull fracture, shunt treated hydrocephalus or use of anticoagulants or coagulation disorders. It is also recommended in “medium-risk” patients, defined as GCS 14-15 and both aged ≥ 65 and on antiplatelets. It is important to note that these guidelines are the only ones not including “age” in isolation as a risk factor. In “low-risk” patients, defined as GCS 14-15 and suspected/confirmed loss of consciousness or repeated vomiting (≥ 2 episodes), these guidelines include the consideration of the protein S100 β (within 6 hours from injury) followed by a head CT scan only in those cases in which the result of the biomarker test is positive (i.e. over 0.1 $\mu\text{g/l}$).

1.2.1.6 *SFMU French Guidelines (2022)*

The French guidelines, developed by the French Society of Emergency Medicine (SFMU) and published in 2023 apply to adult patients, presenting within 24 hours at the ED with a non-penetrating TBI and an initial GCS score of 13-15.²²

An immediate CT scan is recommended for mTBI patients considered as “high risk”, according to the following criteria: a GCS score less than 15 two hours after presentation, bleeding or coagulation disorders, repeated vomiting (≥ 2 episodes), post-traumatic seizure, focal neurological deficit and signs or suspicion of skull fracture. CT scanning can also be considered in “intermediate risk patients”, defined according to the following criteria: age over 65 years with mono-antiplatelet therapy, a dangerous mechanism of injury, or amnesia lasting more than 30 minutes. These guidelines also include the consideration of biomarkers (i.e. S100 β and GFAP+UCH-L1) in these “intermediate-risk” mTBI. The guidelines include hospital triage recommendations and emphasize multidisciplinary decision-making.

1.2.1.7 *Dutch CT in Head Injury Patients (CHIP) Rule*

The CHIP rule was developed and updated by van den Brand et al. in 2022 based on a large multicentre prospective cohort study in the Netherlands involving 4 557 adult patients with minor head injury defined as GCS 13-15.²³ The rule aims to guide CT imaging decisions in the ED, using a tiered risk stratification approach. CT imaging is recommended in patients with any major risk factor, or with two or more minor risk factors.

Major risk factors include: GCS < 15 two hours post-injury, suspected skull fracture, post-traumatic seizure, focal neurological deficit, repeated vomiting (≥ 2 episodes), use of anticoagulants, aged ≥ 65 .

Minor risk factors include loss of consciousness, amnesia, dangerous trauma mechanism (e.g. fall from height, pedestrian struck), intoxication (alcohol or drugs), headache, signs of trauma above the clavicles.

The NHG (Nederlands Huisartsen Genootschap) Head Trauma Guidelines published in 2025,²⁴ use the CHIP rule as a supporting tool within a broader framework. However, these broader guidelines are tailored for use in primary care, making them less relevant for the purpose of our research.

Table 1 summarises all these clinical rules for adults. Both the French and the Scandinavian clinical rules include the potential use of biomarkers in mTBI patients. These potential uses and recommendations are discussed in more detail in a subsequent chapter of this report.

Table 1 – Summary of existing clinical assessment rules for CT scans in adults with mTBI

Rules	Inclusion Criteria	Exclusion Criteria	CT Indications
CCTH Rule¹⁸	Age ≥16, GCS 13-15, witnessed LOC, amnesia, or witnessed disorientation.	Age <16; seizure after injury; bleeding disorders or anticoagulant use; open skull fracture; unstable vitals; repeat visit for same injury.	High risk: GCS <15 at 2 hours, suspected open or depressed skull fracture, signs of basal skull fracture, ≥2 vomiting episodes, age ≥65. Medium risk: amnesia ≥30 min, dangerous mechanism of injury.
New Orleans Criteria¹⁹	Age ≥18, GCS 15, LOC or amnesia, normal neuro exam.	GCS <15; age <18; abnormal neurological findings.	Headache, vomiting, age >60, intoxication, anterograde amnesia, trauma above clavicle, post-traumatic seizure.
NICE Guidelines²⁰	Any head injury with concerning symptoms.	None specified.	GCS ≤12, GCS <15 at 2 hours, suspected open or depressed skull fracture, basal skull fracture signs, post-traumatic seizure, focal neurological deficit, repeated vomiting (≥2 episodes). If LOC or amnesia and any of the following: age ≥65, bleeding or clotting disorders, dangerous mechanism, retrograde amnesia >30 min.
NEXUS II²⁵	Blunt head trauma.	None specified.	Age ≥65, skull fracture, scalp hematoma, neuro deficit; altered alertness, abnormal behaviour, coagulopathy, repeated vomiting (≥2 episodes).
Scandinavian Guidelines²¹	GCS 9-15. MTBI limited to GCS 14-15.	GCS <9.	Mild high-risk: GCS 14-15 AND post-traumatic seizures, focal neurological deficit, shunt-treated hydrocephalus, skull fracture signs or on anticoagulants/coagulation disorders. Mild medium-risk: GCS 14-15 AND both aged ≥65 and on antiplatelets. In Mild low-risk (only if S100β test is positive): GCS 14 OR GCS 15 AND suspected/confirmed LOC or repeated vomiting.
SFMU French Guidelines²²	GCS 13-15, LOC <30 min, amnesia <24 hrs.	Age <18, LOC >30 min, amnesia >24 hrs, neurosurgical need.	High risk: GCS <15 at 2 hours after presentation, bleeding or coagulation disorders, repeated vomiting (≥2 episodes), post-injury seizure, neuro deficit, skull fracture



			signs, Medium risk (CT OR biomarker testing): age >65 or >60 with antiplatelets, anticoagulants, dangerous mechanism, amnesia >30 min.
Dutch CHIP guidelines²³	Age ≥ 16, GCS 13-15, blunt head trauma occurring within 24 hours.	Penetrating head trauma, pre-existing neurological disorders, multitrauma requiring immediate surgery, previous neurosurgical intervention.	One or more major risk factors: GCS <15 2 hrs post-injury, suspected skull fracture, post-traumatic seizure, focal neurological deficit, repeated vomiting (≥2 episodes), use of anticoagulants, age ≥ 65 years. OR at least 2 minor risk factors: LOC, amnesia, dangerous trauma mechanism, intoxication, headache, or signs of trauma above the clavicles.

CCTH: Canadian CT Head Rules; CHIP: CT in Head Injury Patients; GCS: Glasgow Coma Scale; LOC: Loss of Consciousness; NCWFNS: Neurotrauma Committee of the World Federation of Neurosurgical Societies; NEXUS: National Emergency X-Radiography Utilization Study; NICE: National Institute for Health and Clinical Excellence; SFMU: French Society of Emergency Medicine

1.2.2 Clinical Head Rules in children

1.2.2.1 PECARN Rule (Pediatric Emergency Care Applied Research Network)

The PECARN rule, developed by Kuppermann et al. in 2009 is the most extensively validated clinical decision rule for paediatric head trauma.²⁶ It aims to identify children at very low risk of clinically important TBI (ciTBI), thereby minimising unnecessary exposure to ionizing radiation from CT scans. The rule applies to children under 18 years of age who present in the ED within 24 hours of blunt head trauma and have a GCS score of 14-15. It excludes cases of trivial injury, penetrating trauma, pre-existing neurological disorders, prior neuroimaging, or signs of intoxication. The PECARN rule stratifies patients into two age groups. For children younger than 2 years, CT imaging is recommended in the presence of GCS < 15, altered mental status, or a palpable skull fracture. Observation or CT may be considered if there is an occipital, parietal, or temporal scalp hematoma, loss of consciousness lasting at least five seconds, a severe mechanism of injury (such as a fall greater than three feet or being struck by a motor vehicle), or abnormal behaviour as reported by the parent. For children aged ≥2 years, CT imaging is indicated when there is a GCS less than 15, altered mental status, or signs of basilar skull fracture. Observation or CT may be considered in cases of repeated vomiting, loss of consciousness, severe headache, or a severe mechanism of injury (such as a fall greater than five feet or being struck by a motor vehicle).

1.2.2.2 CATCH rule

The Canadian Assessment of Tomography for Childhood Head injury (CATCH) rule was developed by Osmond et al.²⁷ and published in 2010. It was designed to help emergency physicians identify children with mTBI, at risk of clinically significant brain injury, and therefore, requiring a CT scan. The rule distinguishes between high-risk (neurosurgical intervention) and medium risk (brain injury on CT).

This rule applies to children aged ≤16 presenting in the ED within 24 hours from blunt head trauma and GCS 13-15, and at least one of the following: witnessed LOC, definite amnesia, witnessed disorientation, repeated vomiting (≥2 episodes, ≥15 minutes apart), irritability (in children <2 years).

High-risk cases in which a CT scan is recommended to ascertain the need for neurosurgical intervention are described as: GCS <15 at 2 hours after injury, suspected open or depressed skull fracture, history of worsening headache, irritability on examination.

Medium-risk cases in which a CT is recommended to detect brain injury on CT were described as: signs of basal skull fracture, large, boggy scalp hematoma or dangerous mechanism of injury (i.e. motor vehicle collision, fall from ≥3 feet or ≥5 stairs, fall from bicycle without helmet).

1.2.2.3 CHALICE rule

The CHALICE rule (Children's Head Injury Algorithm for the Prediction of Important Clinical Events) was published in 2006 by Dunning et al.²⁸ It applies to children <16 years old presenting in the ED with a head injury of any severity.

CT scanning is recommended when any of the following criteria, grouped in three different categories, are met.

Regarding the history of the injury: witnessed LOC or (anterograde or retrograde) amnesia for more than 5 minutes, abnormal drowsiness (more than expected by the clinician), ≥3 episodes of vomiting, suspicion of non-accidental injury, post-injury seizure.

Regarding clinical examination: GCS <14, or GCS <15 in patients younger than one year, suspicion of penetrating or depressed skull injury, or tense fontanelle, signs of basal skull fracture, focal neurological signs (motor, sensory, coordination, or reflex abnormality), bruise, swelling, or laceration >5 cm in patients younger than 1 year.

Regarding the mechanism of the injury: high-speed road traffic accident (>40 mph) as pedestrian, cyclist, or vehicle occupant, fall from >3 meters, high-speed projectile injury.

1.2.2.4 NEXUS II Rule

The NEXUS II decision rule was originally derived in a population of adults with blunt head trauma, but its principles have been extended to paediatric populations.²⁹⁻³¹ CT imaging is recommended if any of the following features are present: evidence of skull fracture, scalp hematoma, neurologic deficit, abnormal level of alertness, abnormal behaviour, persistent vomiting (≥2 episodes), or known coagulopathy. Children who do not exhibit any of these findings are considered low risk for clinically important ICI and may not require CT imaging. In these low-risk cases, observation should be considered.

1.2.2.5 NICE Guidelines (UK) – Paediatric Head Injury

The NICE guidelines for head injury assessment in children under 16 years of age were first published in 2003 and most recently updated in 2023.²⁰ These guidelines offer a structured approach for determining when urgent CT imaging is needed in paediatric patients presenting with head trauma. They apply to all children with head injuries, including both closed and penetrating trauma, but exclude superficial facial injuries without head involvement. CT imaging should be performed within one hour of identifying any high-risk features such as suspected non-accidental injury, post-traumatic seizure in a child without epilepsy, a GCS score less than 14 (or less than 15 in children under one year), a GCS less than 15 two hours post-injury, suspected open or depressed skull fracture, signs of basal skull fracture, focal neurological deficit, or a bruise, swelling, or laceration greater than 5 cm in children under one year. Additionally, CT imaging is also recommended in children with more than one of the following risk features: witnessed loss of consciousness exceeding five minutes, bleeding or clotting disorders, abnormal drowsiness, repeated vomiting (≥3 episodes), amnesia (anterograde or retrograde) lasting more than 5 minutes or a dangerous mechanism of injury such as a high-speed road traffic accident or a fall from height greater than one meter. If only one of these risk features is present, the guidelines recommend leaving patients under observation for minimum 4 hours.



1.2.2.6 Scandinavian Neurotrauma Committee Guidelines (SNC-16) for children

The SNC-16 guidelines were developed by the Scandinavian Neurotrauma Committee and published in 2016 to standardize the initial management of minor and moderate head trauma in children across Scandinavian EDs.³² The aim was to reduce unnecessary CT scans while ensuring safe identification of children needing neurosurgical intervention or further observation.

These guidelines apply to children aged under 18, presenting to the ED within 24 hours of blunt head trauma with GCS scores of 9-15.

Cases are grouped according to risk, and a CT scan is recommended in high-risk cases, defined as GCS 14-15 and any of the following: focal neurological deficit, signs of skull fracture or post-traumatic seizures.

For medium-risk cases, either a CT scan or clinical observation may be considered, depending on clinical judgment. Medium-risk is defined as a GCS of 14 or a GCS of 15 accompanied by any of the following: LOC (>1 minute) or anticoagulation or coagulation disorders.

Table 1 summarises these paediatric clinical rules.

Table 2 – Summary of existing clinical assessment rules for CT scans in children with mTBI

Rules	Inclusion Criteria	Exclusion Criteria	CT Indications
CATCH²⁷	Children ≤16 years, GCS 13-15) with LOC, amnesia, disorientation, repeated vomiting, or persistent irritability.	Penetrating/depressed skull fracture, focal neurological deficit, developmental delay, suspected abuse, pregnancy, reassessment of prior injury.	High-risk: GCS <15 at 2h, suspected open/depressed fracture, worsening headache, irritability. Medium-risk: Signs of basal skull fracture, large boggy scalp hematoma, dangerous mechanism of injury.
CHALICE²⁸	Children <16 years, any severity of head injury.	None explicitly stated.	LOC >5 min, amnesia >5 min, abnormal drowsiness, ≥3 vomiting episodes, post-injury seizure, GCS <14 (<15 if <1 year), suspected skull fracture, signs of basal skull fracture, focal neurological deficit, scalp injury >5 cm (<1 year), dangerous mechanism, suspected NAI.
NEXUS II^{29, 30}	Blunt head trauma, GCS ≥14.	Penetrating trauma, delayed presentations (>24 hours after injury), and patients undergoing imaging for reasons unrelated to trauma.	Evidence of skull fracture, scalp hematoma, neurologic deficit, abnormal alertness, abnormal behaviour, repeated vomiting.
NICE Guidelines²⁰	Children <16 years with blunt head trauma.	Superficial facial injuries without head trauma.	Immediate CT if one or more of the following: suspicion of NAI, post-traumatic seizure, GCS <14, or <15 if <1 year; GCS <15 at 2 hours post-injury, suspected open/ depressed skull fracture, signs of basal skull fracture, focal neurological deficit, scalp injury >5 cm (if <1 year). CT also recommended if more than one of the following: f witnessed LOC >5 min, bleeding or clotting disorders, abnormal drowsiness, repeated vomiting (≥3 episodes), amnesia (anterograde or

			retrograde > 5 min, or dangerous mechanism of injury.
PECARN²⁶	Children <18 years, blunt head trauma, GCS 14-15.	Trivial injury, penetrating trauma, neurological disorders, prior imaging.	GCS < 15, altered mental status, or palpable skull fracture. Depending on age, observation or CT scan should be considered if: <u>Aged <2</u> : non-frontal scalp hematoma, LOS ≥ 5 sec, severe mechanism of injury, or abnormal behaviour reported by parents. <u>Aged ≥ 2</u> : repeated vomiting, LOC, severe headache, or severe mechanism of injury.
SNC-16³²	Children <18 years; blunt head trauma within 24h; GCS 9-15.	GCS ≤8, penetrating trauma, known neurological disorder, prior neurosurgery.	High-risk: GCS 14-15, focal deficit; skull fracture signs, and post-injury seizures. Medium-risk: GCS 14 or a GCS 15 accompanied by any of the following: LOC (>1 minute) or anticoagulation or coagulation disorders.

GCS: Glasgow Coma Scale; LOC: Loss of Consciousness; NAI: Non-Accidental Injury; NEXUS: National Emergency X-Radiography Utilization Study; NICE: National Institute for Health and Clinical Excellence; PECARN: Paediatric Emergency Care Applied Research Network

1.3 The potential role of GFAP and UCH-L1 in ruling out cerebral lesions in mTBI

1.3.1 Potential role

The validated clinical rules previously presented have shown high sensitivity and therefore, high negative predictive value, i.e. a good performance in predicting the absence of ICIs.^{5, 12, 18, 33-35} The CCHR has been particularly evaluated. Various validation studies reported that about one-third to 40% of patients with mTBI could avoid a CT scan if the rule is correctly applied, while maintaining a very high sensitivity for patients requiring neurosurgical intervention.^{12, 36-40} These figures are a little lower than in the pivotal study and probably reflect the average benefit expected in various settings and patterns of patient management (in Stiell 2001, the reduction of CT scan was between 46% and 68%).¹⁸ Even if the CCHR or a similar clinical rule is applied properly, resulting in a 30-40% reduction in unnecessary CT scans, 60% to 70% of patients without ICI will still undergo imaging. Therefore, applying a second screening filter to this group could further reduce the number of unnecessary CT scans. Biomarkers could be very useful in achieving this.

Several blood biomarkers have been evaluated in the management of adult mTBI in EDs, particularly to rule out ICIs. A CT scan is no longer indicated if the biomarker test result is negative, and the patient can be discharged with instructions on what to do if symptoms occur.⁴¹ If the biomarker tested is positive, the patient undergoes a CT scan. Available biomarkers include protein S100 β , glial fibrillary acidic protein (GFAP), ubiquitin C-terminal hydrolase-L1 (UCH-L1), neurofilament light chain (NFL), and Tau.⁴¹

To the publication of this report, the most studied biomarker for TBI remains the protein S100 β , which was incorporated in the years 2013 and 2022 to Scandinavian and French clinical guidelines, respectively,^{21, 22} and recommended in a European consensus paper published in 2024.⁴² Protein S100 β is a small dimeric protein predominantly expressed by astrocytes, but also to some extent by other cells in the central nervous system.⁴³ When measured within 3 to 6 hours after the trauma, it has excellent sensitivity and negative predictive value (NPV) for excluding ICIs in both adults and children.^{44, 45} The addition of protein S100 β measurement to the Scandinavian guidelines allowed a one-third reduction in unnecessary CT scans, resulting in a financial saving of approximately €39 to €71 on the cost of care per patient.⁴⁶ In France, an interventional study assessing the inclusion of



serum protein S100 β measurements into the SFMU's guidelines reported a theoretical reduction in the number of CT scans of 32%.⁴⁷

However, protein S100 β also presents limitations: its short half-life limits its utility beyond 6 hours after the trauma; it is influenced by skin pigmentation; it is also expressed in melanocytes, chondrocytes, adipocytes and skeletal muscle, and therefore lacks neurospecificity.⁴³ In summary, possible extracranial sources should be taken into consideration when assessing S100 β levels in mTBI patients, especially in black individuals, patients with bone fractures and athletes. These limitations may partly explain why protein S100 β has not been integrated in screening algorithms in countries other than Scandinavia and France.

A previous KCE HTA report, concluded that there was high quality evidence that protein S100 β could reliably rule out the presence of ICIs after mTBI in adults, although some points of attention were raised.⁴⁸ Consequently, the use of protein S100 β was recommended under certain circumstances only. However, insights from a limited number of expert consultations in Belgium, along with a hospital survey that will be presented later in this report, suggest that its use remains limited.

GFAP and UCH-L1 are two biomarkers which could overcome the limitations of protein S100 β .⁴⁹ GFAP is an intermediate filament primarily found in astrocytes, but also expressed in fibroblasts, lymphocytes, chondrocytes, and myoepithelial cells. GFAP is released by damaged astrocytes into the subarachnoid cerebrospinal fluid, then enters the peripheral circulation, either through the glymphatic pathway or by diffusion across the blood-brain barrier. A definitive accounting for the pathways that biomarkers follow from the brain to the blood after mTBI has not been performed.⁵⁰ Once in the bloodstream, GFAP is excreted by the kidneys with a half-life of 24 to 48 hours.⁴¹ It is released within 1 hour of injury, peaks about 20 hours after the trauma and declines over 1-2 days.

UCH-L1 is an enzyme expressed by neurons involved in the proteasome degradation pathway. It accounts for 5% of total neuronal proteins. UCH-L1 expression was also observed in kidney cells and gonads.⁴¹ Following brain injury, UCH-L1 enters the bloodstream either through the blood-brain barrier or the glymphatic system and has a half-life of 6 to 7 hours.⁴¹ As for GFAP, an in-depth understanding on how UCH-L1 enters the bloodstream is still lacking.⁵⁰ It is detectable in blood within 30 minutes, peaks about 8 hours after trauma and falls quickly.

A combined panel of these two biomarkers, which are not influenced by skin pigmentation, was approved by the US Food and Drug Administration (FDA) for clinical use in mTBI adult patients (≥ 18 years) with GCS of 13-15, to help determine the need for a CT scan within 12 hours of injury (Table 3).^{22, 41}

Table 3 – Main characteristics of GFAP+UCH-L1 analysers

Feature	Abbott iSTAT Alinity (handheld, POC)	Abbott Alinity I (core lab)	BioMérieux VIDAS® 3 (core lab)
Assay names	i-STAT TBI cartridge (GFAP + UCH-L1)	Alinity i TBI panel (GFAP + UCH-L1)	VIDAS® TBI GFAP + VIDAS® TBI UCH-L1
Setting	Point of care or lab bench	Clinical laboratory (high-throughput IA)	Clinical laboratory (benchtop IA)
Sample	EDTA whole blood or plasma*	Serum / plasma	Serum
Time window post-injury	Up to 24 h	Up to 12 h	Up to 12 h
Measurement principle	Sandwich immunoassay with electrochemical (amperometric) detection on silicon biosensor	CMIA (two-step chemiluminescent microparticle IA; acridinium label)	ELFA (enzyme-linked fluorescent assay; 3-step sandwich)

Reported outputs	Quantitative internal signals → combined semi-quantitative interpretation (“Not Elevated” / “Elevated”); both biomarkers required	Quantitative GFAP & UCH-L1 + instrument combined interpretation vs fixed cut-offs; both biomarkers required	Quantitative GFAP & UCH-L1 + overall qualitative interpretation from table; both biomarkers required
Cut-offs (published)	Plasma: GFAP<30 ng/L, UCH-L1<360 ng/L Whole blood: Algorithm-defined; numeric cut-offs not user-facing	GFAP<35 ng/L, UCH-L1<400 ng/L (per FDA summary)	GFAP<22.0 ng/L, UCH-L1<327.0 ng/L (per FDA summary)
Analytical measuring interval	GFAP ^{**} : 47–10 000 ng/L UCH-L1 ^{**} : 87–3 200 ng/L	GFAP: 6.1–42 000 ng/L UCH-L1: 26.3–25 000 ng/L	GFAP: 10–320 ng/L UCH-L1: 80–2560 ng/L
Coefficient of variation within-lab	GFAP ^{**} : 3.3–6.9% UCH-L1 ^{**} : 7.5–8.5%	GFAP: 3.0–4.5% UCH-L1: 2.6–3.6%	GFAP: 6.2–8.7% UCH-L1: 4.1–6.0%
Time-to-result (assay)	~15 min	Lab-dependent (CMIA; typically short per analyte; depends on workflow/batching)	~40 min
Throughput	Single-cartridge, patient-proximal	High throughput, automated, continuous loading	Moderate throughput, random-access on VIDAS® 3
Regulatory	FDA 510(k) K234143 for whole blood in 2024, K201778 for plasma in 2021; CE-IVD	FDA 510(k) 510(k) K223602 in 2023 Cleared/CE as applicable (assay availability by region)	FDA 510(k) K240279 in 2024; CE-IVD
Intended use (high level)	Aid decision on need for head CT in adults with mTBI; within 24 h; use with clinical info	Aid decision on need for head CT in adults with mTBI; within 12 h; use with clinical info	Aid decision on need for head CT in adults with mTBI; within 12 h; use with clinical info

*Two different cartridges can be used; **: values for whole blood; Abbreviations: Abbott = Abbott Laboratories (manufacturer); Alinity = automated immunoassay platform by Abbott; CE-IVD = Conformité Européenne – in vitro diagnostic (European regulatory marking for in-vitro diagnostic devices); CMIA = chemiluminescent microparticle immunoassay; CT = computed tomography; EDTA = ethylenediaminetetraacetic acid (anticoagulant used in blood collection); ECLIA = electrochemiluminescence immunoassay; ELFA = enzyme-linked fluorescent assay; FDA = U.S. Food and Drug Administration; GFAP = glial fibrillary acidic protein; IA = immunoassay; i-STAT = portable point-of-care analyser by Abbott; IVD = in vitro diagnostic; POC = point of care; TBI = TBI; UCH-L1 = ubiquitin carboxy-terminal hydrolase L1; VIDAS® = Vitek Immuno Diagnostic Assay System (bioMérieux platform)

1.3.2 Dosage of GFAP and UCH-L1

Measurement of GFAP and UCH-L1 is available for clinical use on three analysers which combine the measurement of the two biomarkers: a point-of-care i-STAT Alinity handheld device (electrochemiluminescence; Abbott), a core lab Alinity i TBI platform (chemiluminescent Microparticle Immunoassay, CMIA; Abbott), and the VIDAS TBI platform (enzyme-linked fluorescent assay, bioMérieux).⁴¹ The main characteristics of the three devices are presented in Table 3. A test is considered positive if 1 or both of the biomarkers are above their predefined cut-offs, and negative if both biomarkers are below these cut-offs.

These three devices, suitable for routine use, allow results to be obtained in less than an hour, provided that the organisational conditions are met. All other techniques, such as enzyme-linked



immunosorbent assays (ELISA) or single molecule array, are for research purposes only and cannot be used in routine practice.⁵¹

2 SCOPE AND AIM

2.1 Scope

Although this KCE report originally aimed at addressing a question raised in 2022 by Abbott, to study the combination of GFAP and UCH-L1 in mTBI cases, a broader perspective was considered and discussed during the scoping phase of our research. During such phase, the research team identified some studies that raised questions regarding the added value in terms of accuracy of the combination of GFAP and UCH-L1 over that of the individual biomarkers that form such a combination.^{52, 53} Therefore, in order to clarify such point, the scope of our project was enlarged to also include GFAP and UCH-L1 as individual markers for mTBI.

A decision was made to exclude any studies exclusively on S100 β . The base for this decision was two-fold.

First, the guideline by NICE identified as many as 31 studies in adults and 6 in children focusing on this protein. It concluded that its sensitivity was high, (above 90%) although not 100% and its specificity was low. The added value of updating such a review for this specific biomarker was thought to be limited.

Second, despite the fact that countries such as France, or the Scandinavian countries, have included the S100 β protein in their clinical guidelines, a number of drawbacks have been highlighted in the literature, including its low neurospecificity, the effect of skin pigmentation or age in its results, its short half-life and the risk of false negative results.^{43, 48, 54, 55}

Despite the fact that S100 β is not the focus of our research, any studies comparing GFAP, UCH-L1 or their combination with the protein S100 β , were captured in our review. Similarly, questions regarding the protein S100 β were considered during discussions with Belgian and foreign experts in the field, in order to gain a broader overview on comparative accuracy and acceptability of these biomarkers.

2.2 Aim of this research

The aim of this research is to assess the performance of blood-based biomarkers to rule out the presence of ICIs after mTBI and limit the number of CT scans performed in low-risk populations. The focus is on the combination of GFAP and UCH-L1, as well as GFAP and UCH-L1 individually.

The following research questions are tackled in this research:

1. RQ1: Can the combined use of GFAP and UCH-L1, or either biomarker individually, accurately exclude the presence of CT-confirmed acute ICIs in patients presenting to the ED with mTBI?
2. RQ2: What would be the cost-effectiveness and budgetary impact of using the blood-based biomarkers mentioned in RQ1?
3. RQ3: What is the current practice of Belgian hospitals in the assessment of patients presenting to the ED with mTBI, and what are the impediments to the use of biomarkers in this field?

3 PERFORMANCE OF BIOMARKERS TO RULE OUT CEREBRAL LESIONS IN MTBI PATIENTS

KEY POINTS

- In a **meta-analysis of 15 studies** (n=10 440 tests in adults), the combination GFAP+UCH-L1 displayed a **pooled sensitivity of 98%** (95%CI: 96-99) and a **specificity of 29%** (95%CI: 25-34). The power of a negative result to rule out a brain lesion was very high, with a **negative likelihood ratio of 0.08** (95%CI: 0.05-0.12).
- However, the low specificity limited the **proportion of unnecessary head CT scans that could be avoided at around 30%**. The **specificity was particularly low in patients ≥ 65 years**. Age-stratified cut-offs could improve the performance of the test.
- Evidence in children is currently scarce (only 1 study for GFAP+UCH-L1), but new studies are underway.
- The majority of studies analysed plasma or serum samples that had been previously collected and frozen. **More research is needed on the added value of these biomarkers in real-life situation**, particularly interventional trials with outcomes on patients' management (e.g. waiting time), resources use and costs.
- We conclude that:
 - There is moderate to high quality evidence that the combination GFAP+UCH-L1 can rule out a brain injury reliably in patients with mTBI received in the ED within 12 hours.
 - There is low quality evidence regarding the use of these biomarkers in real world practice.
 - There is insufficient evidence that the point of care device i-STAT Alinity (Abbott) presents an acceptable risk-benefit balance in real world practice.
 - There is low quality evidence that these biomarkers may reduce unnecessary CT scans by approximately 30%.
 - There is very low quality evidence that these biomarkers may reduce unnecessary CT scans by approximately 30% in real life practice.
 - There is insufficient evidence that these biomarkers generate other benefits such as a reduction in observation time or gains in QALYs.

3.1 Methods

3.1.1 Studies selection

A systematic review was performed to assess the performance of GFAP and UCH-L1 (individually or combined) to exclude ICIs in adults and children with mTBI, CT scan or magnetic resonance imaging (MRI) being considered the gold standard (reference test). The protocol of our systematic review was registered at [CRD420251051158](https://www.crd420251051158). We followed the reporting recommendations of the PRISMA-DTA framework.⁵⁶

Given the availability of a recent, broad review of high quality (assessed using AMSTAR; see appendix) published in 2023 by the National Institute of Health and Clinical Excellence (NICE),⁵⁷ a pragmatic approach was pursued by which the search strategy used in their chapter on biomarkers was updated, to both capture more recent data, and to include any relevant manuscripts written in languages other than English which were excluded from their original search. The work by NICE was therefore taken as our starting point to avoid duplication of efforts. Although only primary studies



published after the NICE search date (June 2022) were retrieved in our update, all previously identified primary studies described in their review and supplementary materials were still considered.

For the purpose of our update, the following databases were searched in January 2025, and updated in May 2025: MEDLINE via OVID, Embase, and Cochrane Database of Systematic Reviews. The sites of other HTA agencies were visited to identify any recent assessments in this field and the references of any relevant studies were checked. A full search strategy is available in appendix.

Any original study meeting the following criteria was considered eligible:

Population:

- Any patient (child, adult and elderly) presenting to the ED with a mTBI who is eligible for a CT scan or MRI according to the current diagnostic criteria.

Index test:

- GFAP and UCH-L1 both individually and in combination.

Reference standard:

- CT scan or MRI.

Target conditions to be excluded:

- Any form of intracranial brain injury detected by a CT scan or MRI, including conditions requiring urgent neurosurgery (epidural or subdural haematoma, subarachnoid haemorrhage).

Main outcomes:

- Sensitivity (Se) and specificity (Sp), negative predictive value (NPV), positive (LR+) and negative likelihood ratio (LR-) of GFAP and UCH-L1 individually and combined.

Study types:

- Primary diagnosis studies. Literature reviews were only used for reference checking purposes, and only primary studies were included in our review.

Exclusion criteria:

- Studies on the clinical prognosis of mTBI.
- Studies limited to patients with severe head injuries or mixed populations for which results for patients with mTBI could not be isolated.
- Studies on patients with no injuries (e.g. degenerative disease).
- Studies reporting only the area under the curve (AUC) of the Receiver Operator Curve (ROC), without mentioning specific cut-offs for computation of sensitivity and specificity. These studies were excluded because they do not fit the aim of our research, i.e. assessing if measuring biomarkers allows to rule out a brain injury.

Study selection was performed by two researchers, while data extraction was performed by one researcher and cross-checked by a second one. Study selection was done in two steps, first on title and abstract, and second on content. Excel was used in order to capture information on a standardized way on both the characteristics of the studies and their relevant outcomes. The following information was extracted from each included article: study design; number and characteristics of patients (age, GCS score, incidence of brain lesions confirmed by CT scans, exclusion factors); assay techniques; cut-offs for GFAP and UCH-L1; time interval between trauma and assay; number of test which were true positive, false positive, true negative, and false negative relative to CT results.

3.1.2 Data analysis

The quality appraisal of all included studies was performed by one researcher using the QUADAS-2 tool,⁵⁸ and was checked by a second one. The four domains of bias concerned patient selection, index test, reference standard and flow and timing. We also provided an overall appraisal for each study, where the rating corresponded to the lowest score in any of the four domains, as recommended by the authors of the emerging QUADAS-3 tool.⁵⁹ The results of the QUADAS-2/3 were displayed in figures using the risk-of-bias VISualization tool (robvis).⁶⁰

Data were analysed using the *metadta* command in Stata 15.0. This command implements the bivariate random-effects model for diagnostic test accuracy meta-analysis. The model is a generalized linear mixed model (GLMM) for the binomial family with a logit link, jointly modelling logit-transformed sensitivity and specificity and allowing for their correlation. The binomial distribution specifies the within-study variability, giving more influence to studies with more precise estimates (typically those with larger sample sizes) through the likelihood-based estimation. Because the weighting is implicit in the likelihood, Stata's forest plots do not display study weights, but weighting is indeed performed.

Heterogeneity of sensitivity and specificity across studies was quantified with the I^2 statistics (Higgins index), where a result >50% was considered a substantial variation of the outcomes across studies. The summary receiver operating characteristic space (SROC) was designed with confidence and prediction contours. Bubbles identifying each study have a size proportionate to their sample size. The negative likelihood ratio (LR-) was computed with the command *metandi*, which basically runs the same modelling as *metadta*. This ratio quantifies the strength of a negative test result by comparing the likelihood of a false negative in those with the disease to the likelihood of a true negative in those without the disease. It is usually considered that a negative likelihood ratio smaller than 0.1 reveals a test with a very big exclusive power. Finally, a Fagan's nomogram was designed with the *midas* command. Although a statistical investigation of publication and reporting bias is not routinely recommended in DTA systematic reviews,⁵⁶ we ran a Deeks' test and plotted a Deeks' diagram with the *midas* command. Moreover, three external experts in the field of mTBI were consulted for identification of studies we might have missed. We also consulted ClinicalTrials.gov to search for previous studies whose results had not been published.

In a sensitivity analysis, we aimed to assess the performance of the biomarkers by age category (having more than 65 years versus being aged <65 years), analyser type, incidence of cerebral lesions confirmed by CT scan (<10%, 10-20%, more than 20%), time from injury to biomarker dosage, and study quality.

The strength of overall evidence was evaluated following the GRADE methods on criteria of study limitations (risk of bias), indirectness, inconsistency in study results, imprecision and probability of publication bias.^{61, 62}

3.2 Results

3.2.1 Identification and characteristics of included studies

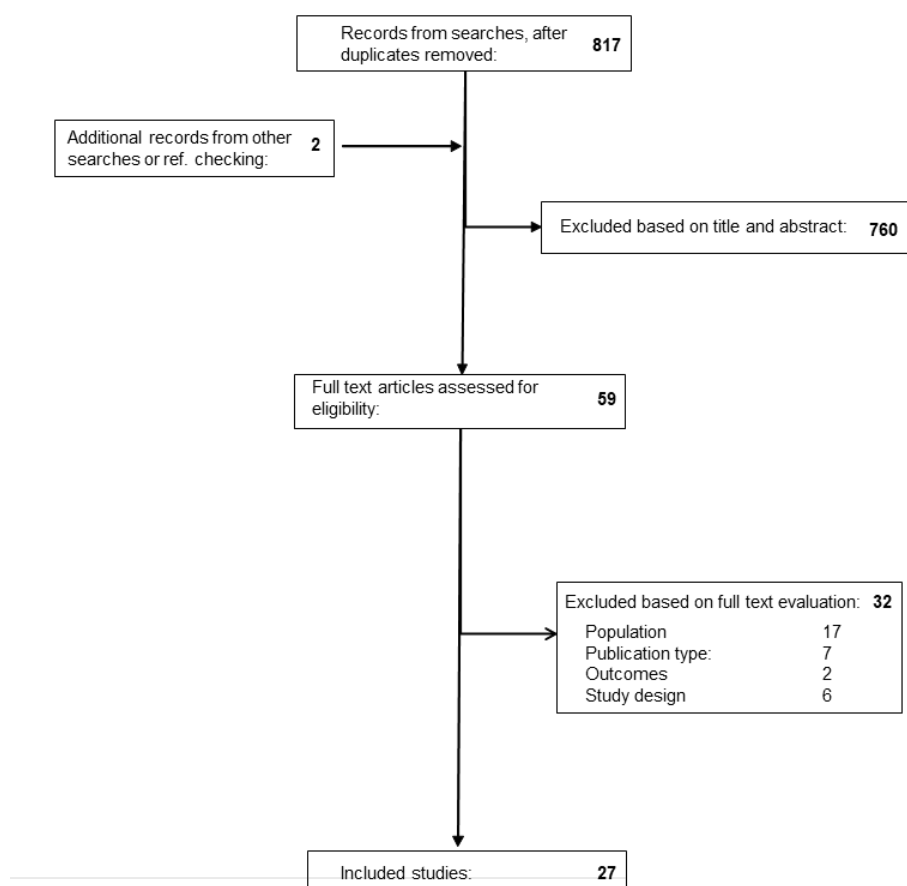
Our search resulted in 817 papers after elimination of duplicates. To these, an additional reference found by hand-searching was added. A total of 760 articles were excluded based on title and abstract, leaving 58 potentially relevant studies, for which full texts were retrieved for consideration.

From the studies left, 5 were systematic reviews (SRs) with or without meta-analysis (MAs) and were used for reference checking purposes only.^{41, 63-66} Although one very recent SR by Puravet et al.,⁴¹ was published in the same field, we proceeded with our own review due to key differences in scope and evidence base. Notably, the existing review was limited to adult populations, whereas our study includes both adult and paediatric data, addressing a broader clinical context. Furthermore, our comprehensive search strategy identified additional relevant studies that were not included in the prior review, thereby enriching the evidence base.



During our reference checking exercise, an additional relevant reference was identified, which left us with 54 studies. Twenty-seven of them were excluded based on full text. The main reasons for such exclusion are provided in a table in our supplement and in the PRISMA flow chart illustrating the screening and selection process (see Figure 1). Finally, 27 studies were included in our review.

Figure 1 – Flow chart for clinical study selection



Only 13 of the 27 studies included in our review were considered in the SR published by Puravet et al. in 2025.⁴¹ Most of our additional studies were recent, which is a reflection of the rapid evolution of this field of knowledge.

An overview of the studies included is displayed in our supplement. Most of the studies focused on adult patients (n=25), with only two centred in paediatric populations.^{67, 68} Two other studies included a small proportion of adolescents aged 15-17 years.^{69, 70}

Only one study focused purely on an elderly population,⁷¹ while three others focused on adult populations but provided sub-analysis in patients aged 65 and over.^{69, 72, 73}

With regards to the biomarkers assessed, 18 studies presented results on the combination of GFAP and UCH-L1, 17 studies looked at GFAP alone, and 12 studied UCH-L1. In addition to this, there were 9 studies that compared at least one of the previously mentioned biomarkers with the protein S100β.

Other relevant study characteristics such as definition of mTBI, type of assays, blood matrix (serum and/or plasma), cut-offs for a positive test, time elapsed from injury to biomarker dosage, incidence of CT-confirmed ICIs, sensitivity, specificity and negative likelihood ratio are presented for each biomarker in Table 4, Table 5, Table 6 and Table 7. Only results for mTBI patients are presented, i.e. we extracted relevant data from studies with mixed populations.



Table 4 – Main results of studies assessing the performance of GFAP+UCH-L1 to rule out a cerebral injury

Study	Population	Cut-offs GFAP and UCH-L1 (pg/ml)	Median time measured from injury in hours	Measured within 'hours'	Analyser (provider)	N	CT+ %	Sensitivity % (95% CI)	Specificity % (95% CI)	LR-
<i>Bazarian 2018</i> ⁷⁴	Adults. Mean age 48.8 GCS: 14-15	22 and 327	3.2	12	Chemiluminescent sandwich ELISA in serum (Banyan)	1920	5.9	97.3 (92.4; 99.4)	36.7 (34.5; 39.0)	0.07 (0.00; 0.16)
<i>Bazarian 2021</i> ⁷⁵	Adults mean age 49.1. GCS: 13-15	30 and 360	3.1	12	i-STAT Alinity in plasma (Banyan)	1901	6.3	95.8 (90.6; 98.2)	40.4 (38.2; 42.7)	0.10 (0.04; 0.23)
<i>Chayoua 2024</i> ⁷⁶	Adults. Median age: 48. GCS: 13-15	30 and 360	Most within 3h	24	i-STAT Alinity in plasma (Abbott)	253	23.3	97 (89; 99)	19 (14; 25)	0.18 (0.04; 0.72)
<i>Curran 2025</i> ⁷⁷	Adults. Mean age: 63. GCS: 13-15	35 and 400	-	12	Alinity i platform in plasma/serum (Abbott)	89	13.5	100 (76; 100)	27 (19; 38)	0.0
<i>Harris 2025</i> ⁷⁸	Patients aged ≥17 GCS: 13-15	30 and 360	-	24	i-STAT Alinity in plasma (Abbott) ARCHITECT platform (Abbott) ^a	599	NA	97 (94; 99)	24 (20; 29)	0.12 (0.05; 0.27)
<i>Iverson 2022</i> ⁷¹	Aged ≥60. Mean age: 79 GCS: 13-15.	323 and 42 AUC	3.1	12	Simoa digital immunoassay in serum (Quanterix)	83	26 .5	100 (85.0; 100)	36.1 (24.2; 49.4)	0
<i>Kopcinovic 2025</i> ⁷²	Adults. Median age: 64 GCS: 13-15	35 and 400	-	12	Alinity i platform in plasma/serum (Abbott)	822	13.6	95.5 (89.9; 98.5)	25.5 (22.3; 28.9)	0.18 (0.07; 0.42)
<i>Kopcinovic 2025 <65 years</i>	Adults aged <65 GCS: 13-15	35 and 400	-	12	Alinity i platform in plasma/serum (Abbott)	418	11.2	93.6 (82.5; 98.7)	43.4 (38.3; 48.6)	0.15 (0.05; 0.44)

^a I-STAT Alinity and ARCHITECT are two highly correlated assays, and ARCHITECT values were converted to i-STAT equivalents using previously derived equations



Study	Population	Cut-offs GFAP and UCH-L1 (pg/ml)	Median time measured from injury in hours	Measured within 'hours'	Analyser (provider)	N	CT+ %	Sensitivity % (95% CI)	Specificity % (95% CI)	LR-
<i>Kopcinovic 2025 65+years</i>	Adults aged ≥65 GCS: 13–15	35 and 400	-	12	Alinity i platform in plasma/serum (Abbott)	403	16.1	96.2 (89.3; 99.6)	5.6 (3.4; 8.6)	0.06 (0.01; 0.22)
<i>Ladang 2025⁷³</i>	Adults. Median age: 64 GCS: 13–15	35 and 400	-	12	Alinity i platform in plasma (Abbott)	362	31.2	99.1 (95.2-99.98)	40.6 (34.4; 46.9)	0.02 (0.00; 0.15)
<i>Ladang 2025 <65 years</i>	Adults aged <65 GCS: 13–15	35 and 400	-	12	Alinity i platform in plasma (Abbott)	191	33.0	98 (91-100)	65 (56-73)	0.02 (0.00; 0.17)
<i>Ladang 2025 65+ years</i>	Adults aged <65. GCS: 13–15	35 and 400	-	12	Alinity i platform in plasma (Abbott)	171	29.2	100 (93-100)	15 (9-22)	0
<i>Ladang 2025 overall, modified cut-offs in 65+</i>	Adults. Median age: 64. GCS: 13–15	Cut-offs in 65+: 115 and 335	-	12	Alinity i platform in plasma (Abbott)	362	31.2	99.1	48.2	0.02 (0.00; 0.13)
<i>Lagares 2024⁶⁹</i>	Adults ≥18y in FR and ≥15y in Sp. Median age 69. GCS: 13–15	22 and 327	4.5	12	VIDAS in serum (Biomérieux)	1438	12.2	98.3 (95; 99.7)	24.9 (22.6; 27.4)	0.07 (0.02; 0.21)
<i>Lagares 2024 <65 years</i>	Adults <65 years	22 and 327	4.5	12	VIDAS in serum (Biomérieux)	645	10.1	95.4 (86.8; 98.9)	42.1 (38.1; 46.1)	0.11 (0.04; 0.33)
<i>Lagares 2024 65+ years</i>	Adults ≥65 years	22 and 327	4.5	12	VIDAS in serum (Biomérieux)	793	14.38	100 (96.1; 100)	10.3 (8.2; 12.8)	0
<i>Lapić 2024⁷⁹</i>	Adults. Median age: 62. GCS 14-15.	35 and 400	2.5	12	Alinity i platform in plasma (Abbott)	62	11.3	100 (59.0; 100)	30.9 (19.1; 44.8)	0
<i>Legramante 2024⁸⁰</i>	Adults. Mean age: 54. Incl. GCS 13–15 (but all patients in the end had GCS 14-15)	35 and 400	2	12	Alinity i platform in serum (Abbott)	130	5.4	100 (59; 100)	27.6 (20.0; 36.4)	0



Study	Population	Cut-offs GFAP and UCH-L1 (pg/ml)	Median time measured from injury in hours	Measured within 'hours'	Analyser (provider)	N	CT+ %	Sensitivity % (95% CI)	Specificity % (95% CI)	LR-
<i>Li 2023</i> ⁸¹	Adults. Mean age: 50.8. GCS 13-15 (72% GCS: 15)	22 and 327	1	6	ELISA in plasma/serum ^b (Banyan)	663	27	100 (98; 100)	13 (10; 16)	0
<i>Oris 2023 (<3 hours)</i> ⁸²	Adults. Mean age: 59.1. Moderate risk within the mTBI according to French guidelines: GCS 14-15	30 and 360	1.7	3	i-STAT Alinity in plasma (Abbott)	192	9.8	100 (66.4; 100).	29 (22.5; 36.1)	0
<i>Oris 2023 (<12 hours)</i> ⁸²	Adults. Mean age: 59.1. Moderate risk within the mTBI according to French guidelines: GCS 14-15	30 and 360	-	12	i-STAT Alinity in plasma (Abbott)	239	4.6	100 (73.5; 100)	31.7 (25.7; 38.2)	0
<i>Oris 2024</i> ^{c83}	Adults. Mean age: 66.2. mTBI with intermediate risk as per French guidelines. GCS 13-15, (95.7% GCS 15)	35 and 400	1.7	12	Alinity i platform in plasma (Abbott)	230	4.8	100 (75.1; 100)	28.8 (22.9; 35.3)	0

^b Results are available in the article for serum and plasma. As these results are highly correlated, we report here merged results.

^c Oris 2023 and Oris 2024 share the same population. Oris 2024 compares results with i-STAT Alinity and Alinity i platform. Only the latter is presented here as results with i-STAT Alinity is presented in Oris 2023.



Study	Population	Cut-offs GFAP and UCH-L1 (pg/ml)	Median time measured from injury in hours	Measured within 'hours'	Analyser (provider)	N	CT+ %	Sensitivity % (95% CI)	Specificity % (95% CI)	LR-
<i>Papa 2022</i> ³⁴	Adults. Mean age 40. GCS 13-15 (90% GCSS 15).	30 and 327 ^d	3	4	ELISA in serum (Banyan)	349	6.6	91 (70; 98)	20 (16; 24)	0.44 (0.12; 1.70) ^e
	Adults. Mean age 40. GCS 13-15 (90% GCSS 15).	67 and 189	3	4	ELISA in serum (Banyan)	349	6.6	100 (82; 100)	25 (20; 30)	0
<i>Puravet 2025</i> ⁶⁸	Aged ≤16. Median age: 4.7y. GCSS: 15 requiring hospitalisation or CT scan according to French guidelines	By age group ^f	2.4	3	Alinity i platform in serum (Abbott)	68	20.6	100 (75; 100)	42 (29; 56)	0
<i>Trivedi 2024</i> ⁸⁴	Adults. Median age: 47 GCS: 14-15 (88.7% GCS 15)	AUC	-	6	Simoa digital immunoassay in serum (Quanterix)	531	24.9	76 (66, 83)	83 (71, 90)	0.29 (0.22; 0.40)
	NA ^g	AUC	-	6-9	Simoa digital immunoassay in serum (Quanterix)	248	47.6	80 (62, 86)	80 (60, 87)	0.25 (0.18; 0.37)

^d Two sets of prespecified biomarker cutoff values were used in the analysis based on previous studies. 30ng/L GFAP and 327 ng/L UCH-L1 were the cut-offs used in the meta-analysis because they were the closest to cut-offs used in the assays for clinical use (i-STAT Alinity, Alinity i platform, VIDAS).

^e A LR- greater than 1 means the test is worse than random guessing and behaves in the opposite way of a useful diagnostic, i.e. the probability of having a CT scan positive is greater after a negative test than before any test (probability pre-test).

^f Cut-off GFAP and UCH-L1 by age group <2y 180 pg/mL and 373 pg/mL; 2-4y 118 pg/mL and 272 pg/mL; >4y 73 pg/mL and 217 pg/mL based on the 95th percentile for the mean of a control group of children. For S100B 0–9 months, greater than 0.35 µg/L; 10–24 months, greater than 0.23 µg/L; and older than 24 months, greater than 0.18 µg/L. The result combines the ages groups together.

^g The characteristics of the patients are only available for the full sample



Study	Population	Cut-offs GFAP and UCH-L1 (pg/ml)	Median time measured from injury in hours	Measured within 'hours'	Analyser (provider)	N	CT+ %	Sensitivity % (95% CI)	Specificity % (95% CI)	LR-
	NA ^g	AUC	-	9-12	Simoa digital immunoassay in serum (Quanterix)	154	43.5	73 (46, 82)	83 (57, 91)	0.32 (0.22; 0.49)
Welch 2025⁸⁵	Adults. Mean age: 49.1 GCS 13-15	35 and 400	3.2	12	Alinity i platform in plasma (Abbott)	1899	6.3	96.7 (91.7; 98.7)	40.1 (37.8; 42.4)	0.08 (0.03; 0.22)



Table 5 – Main results of studies assessing the performance of GFAP to rule out a cerebral injury

Study ID		Cut-off value GFAP (pg/ml)	Median time measured from injury (hrs)	Measured within (hrs)	Analyser (provider)	N	CT+ %	Sensitivity % (95% CI)	Specificity % (95% CI)	LR-
Bazarian 2018⁷⁴	Adults. Mean age 48,8. 80% GCSS 14–15 (n=1920), 20% GCS 9-13 (n=39), not included here.	22	3.2	12	Chemiluminescent sandwich ELISA in serum (Banyan)	1780	6.0	96.3 (91.0; 99.0)	51.0 (48.6; 53.4)	0.07 (0.03; 0.19)
Chayoua 2024⁷⁶	Adults. Median age: 48. GCSS 13-15 (60%)	30	Most within 3h	24	i-STAT Alinity in plasma (Abbott)	253	23.3	95 (86; 98)	28 (22; 35)	0.18 (0.06; 0.55)
Chiollaz 2024⁶⁷	Children. Aged ≤16. Mean age: 8. GCS 14-15	204 AUC	4	6	ELISA in serum (Meso Scale Diagnostics)	43	16.3	100h	38.9 (23.1; 56.5)	0.0
	Children. Aged ≤16. Mean age: 8. GCS 14-15	78 AUC	-	24	ELISA in serum (Meso Scale Diagnostics)	72		100h	22.2 (12.0; 35.6)	0.0
Forouzan 2021⁸⁶	Aged ≥16. Mean age: 36.4. GCSS: 14-15	1350 AUC	1	6	CLIA sandwich assay in plasma (Biotech) ⁱ	176	4.5	50.0 (15.7; 84.3)	44.1 (36.4; 51.9)	1.14 (0.56; 2.32) ^j
Gardner 2018 overall⁸⁷	Aged ≥16. Weighted mean for age (by age category) 41.9. GCS 13-15 (82% GCS 15)	430 AUC	-	24	ELISA (Banyan)	169	33.1	80 (68; 90)	80 (71; 87)	0.25 (0.14; 0.42)

^h Sensitivity was set at 100% by the authors to determine the cut-off allowing the best specificity on the ROC curve

ⁱ CLIA: chemiluminescent immunoassay sandwich method

^j A LR- greater than 1 means the test is worse than random guessing and behaves in the opposite way of a useful diagnostic, i.e. the probability of having a CT scan positive is greater after a negative test than before any test (probability pre-test).



Study ID		Cut-off value GFAP (pg/ml)	Median time measured from injury (hrs)	Measured within (hrs)	Analyser (provider)	N	CT+ %	Sensitivity % (95% CI)	Specificity % (95% CI)	LR-
Gardner 2018 <40 years	Aged ≥16. Mean age: 25.8. GCS 13-15 (77% GCS 15)	430 AUC	-	24	ELISA (Banyan)	79	22.8	83.3 (59; 96)	83.6 (72; 92)	0.20 (0.07; 0.56)
Gardner 2018 40-59 years	Aged ≥16. Mean age: 50. GCS 13-15 (83% GCS 15)	430 AUC	-	24	ELISA (Banyan)	60	33.33	90.0 (68; 99)	77.5 (62; 89)	0.13 (0.03; 0.49)
Gardner 2018 ≥60 years	Aged ≥16. (Mean age: 68. GCS 13-15 (93% GCS 15)	430 AUC	-	24	ELISA (Banyan)	30	60	66.7 (35; 90)	66.7 (35; 90)	0.50 (0.23; 1.08)
Harris 2025⁷⁸	Patients aged ≥17, GCS 3-15 (but subgroup analysis for GCS 13-15)	30	-	24	i-STAT Alinity in plasma (Abbott) ARCHITECT platform (Abbott)a	599	NA	97 (94; 99)	28 (24; 33)	0.10 (0.05; 0.23)
Iverson 2022⁷¹	Aged ≥60. Mean age 79. GCS of 13-15.	323 AUC	3.1	12	Simoa digital immunoassay in serum (Quanterix)	83	26.5	86.4 (65.1; 97.1)	60.7 (47.3; 72.9)	0.22 (0.08; 0.66)
Keski-Pukkila 2024⁸⁸	Adults. Mean age: 61.1. GCS 13-15	140		24	GFAP Discovery kit (Quanterix)	36	11.1	100 (39.8; 100)	34.4 (18.6; 53.2)	0
Kopcinovic 2025⁷²	Adults. Median age: 64. GCS 13-15	35	-	12	Alinity i platform in plasma/serum (Abbott)	822	13.6	89.3 (82.0; 94.3)	38.3 (34.7; 42.0)	0.28 (0.16; 0.48)
Kopcinovic 2025 <65 years	Subgroup analysis for adults aged <65. GCS 13-15	35	-	12	Alinity i platform in plasma/serum (Abbott)	418	11.2	80.9 (66.7; 90.9)	62.8 (57.7; 67.7)	0.30 (0.17; 0.55)
Kopcinovic 2025 ≥65 years	Subgroup analysis for adults aged ≥65. GCS 13-15	35	-	12	Alinity i platform in plasma/serum (Abbott)	403	16.1	95.4 (87.1; 99.0)	11.2 (8.1; 15.1)	0.41 (0.13; 1.29)
Ladang 2025⁷³	Adults. Median age: 64. GCS 13-15	35	-	12	Alinity i platform in plasma (Abbott)	362	35.9	96.5 (91.2; 99.0)	42.6 (36.4; 49.0)	0.08 (0.03; 0.22)



Study ID		Cut-off value GFAP (pg/ml)	Median time measured from injury (hrs)	Measured within (hrs)	Analyser (provider)	N	CT+ %	Sensitivity % (95% CI)	Specificity % (95% CI)	LR-
<i>Ladang 2025 <65 years</i>	Adults aged <65 GCS: 13–15	35	-	12	Alinity i platform in plasma (Abbott)	191	33.0	93.6 (84.5; 98.2)	68 (59.1; 75.9)	0.09 (0.04; 0.24)
<i>Ladang 2025 65+ years</i>	Adults aged <65. GCS: 13–15	35	-	12	Alinity i platform in plasma (Abbott)	171	29.2	100 (92.9; 100)	15.7 (9.7; 23.4)	0
<i>Lagares 2024⁶⁹</i>	Adults in FR and ≥15 in Sp. Median age 69. GCSS 13–15	22	4.5	12	VIDAS in serum (Biomérieux)	1436	12.2	98.3 (94.9; 99.7)	31.3 (28.8; 33.9)	0.05 (0.02; 0.17)
<i>Lagerstedt 2018⁷⁰</i>	Aged > 14 years. Weighted mean age (by CT result): 48.7 GCS 15 .	97 AUC	3	6	Electrochemiluminescence (F211M kit) in serum/plasma (Meso Scale Diagnosis)	132	15.9	100	30.6 (22.0; 39.6)	0
<i>Lapić 2024⁷⁹</i>	Adults. Median age: 62. GCS 14–15.	35	2.5	12	Alinity i platform in plasma (Abbott)	62	11.3	85.7 (42.1; 99.6)	41.8 (28.7; 55.9)	0.34 (0.05; 2.15)
<i>Li 2023</i>	Adults. Mean age: 50.8. GCS 13–15. (72% GCS: 15),	22	-	6	ELISA in plasmak (Banyan)	440	27	96.6 (91.6; 99.1)	50.5 (44.9; 56.1)	0.07 (0.03; 0.18)
<i>Okonkwo 2020⁸⁹</i>	Adults. Mean age 40.1. Mixed severity. Most GCS 13–15: (89%). Here only results for GCS 13–15	13 AUC		24	i-STAT Alinity in plasma (Abbott)	1137	31.5	99.4 (98.6; 100)	15.7 (13.1; 18.2)	0.04 (0.01; 0.14)
	id	37 AUC		id	id	id	id	96.4 (94.4; 98.0)	30.3 (27.1; 34.0)	0.12 (0.07; 0.21)

^k Results are available in the article also for serum.



Study ID		Cut-off value GFAP (pg/ml)	Median time measured from injury (hrs)	Measured within (hrs)	Analyser (provider)	N	CT+ %	Sensitivity % (95% CI)	Specificity % (95% CI)	LR-
	id	113 AUC		id	id	id	id	90.2 (86.9; 93.3)	49.8 (46.6; 53.0)	0.20 (0.14; 0.27)
	id	190 AUC		id	id	id	id	84.6 (81.0; 88.3)	59.4 (56.1; 63.0)	0.26 (0.20; 0.33)
Oris 2023⁸²	Adults. Mean age: 59.1. Moderate risk within the mTBI according to French guidelines: GCS 14-15	30	1.7	3	i-STAT Alinity in plasma (Abbott)	192	9.8	100 (66.4; 100)	39.3 (32.2; 46.8)	0
	id	id		12	id	239	4.6	91.7 (61.5; 99.8)	40.1 (33.7; 46.8)	0.21 (0.03; 1.37)
Papa 2022³⁴	Adults. Mean age 40. GCS 13-15 (90% GCSS 15).	30	3	4	ELISA in serum (Banyan)	349	6.6	91 (70; 98)	33 (28; 38)	0.26 (0.07; 1.01)
	id	67	id	id	ELISA in serum (Banyan)	id	6.6	87 (66; 97)	65 (60; 70)	0.20 (0.07; 0.58)
Polat 2022⁹⁰	Adults. Median age: 40.5. GCS 14-15	1560 AUC		3	ELISA in serum (FineTest)	60	33.3	50 (27.2; 72.8)	97.5 (86.8; 99.9)	0.51 (0.33; 0.80)
Posti 2019⁹¹	Adults. Mean age: 42.8. All severities (n=160) but here only GCS 13-15 (n=93). Mean GCS for the 13-15: 14.65	67 AUC	15.2	24	Neurology 4-Plex A assay (N4PA) in plasma (Quanterix)	93	39.8	100 (91; 100)	16.1 (8; 28)	0
	id	132 AUC	id	id	id	id	39.8	97.3	32.1	0.08 (0.01; 0.60)



Study ID		Cut-off value GFAP (pg/ml)	Median time measured from injury (hrs)	Measured within (hrs)	Analyser (provider)	N	CT+ %	Sensitivity % (95% CI)	Specificity % (95% CI)	LR-
Puravet 2025⁶⁸	Aged ≤16. Median age: 4.67. GCSS: 15 requiring hospitalisation or CT scan according to French guidelines	By age group ¹	2.4	3	Alinity i platform in serum (Abbott)	68	20.6	100 (75; 100)	27 (16; 41)	0
Trivedi 2024⁸⁴	Adults. Median age 47 (ICI present), 58 (no ICI present) GCS 14-15 (88.7% GCS 15)	AUC	-	6	Simoa digital immunoassay in serum (Quanterix)	531	24.9	71 (61; 78)	87 (76, 93)	0.33 (0.25; 0.43)
	NAg	AUC	-	6-9	Simoa digital immunoassay in serum (Quanterix)	248	47.6	79 (61, 86)	81 (61, 88)	0.26 (0.18; 0.38)
	NAg	AUC	-	9-12	Simoa digital immunoassay in serum (Quanterix)	154	43.5	73 (43, 82)	82 (54, 90)	0.33 (0.22; 0.49)
Whitehouse 2025⁹²	Aged ≥16. Median age: 59. Overall sample larger. Here only results for GCS 13-15	145 AUC	-	6	Neurology 4-Plex A assay (N4PA) in serum (Quanterix)	127	18.1	95.7 (78.1; 99.9)	18.3 (11.4; 27.1)	0.24 (0.03; 1.69)
	Aged ≥16. Median age: 59. Overall sample larger. Here only results for GCS 13-15	300 AUC	10	24	Neurology 4-Plex A assay (N4PA) in serum (Quanterix)	385	36	95.0 (90.0; 98.0)	33.1 (27.2; 39.3)	0.15 (0.07; 0.32)

¹ Cut-off GFAP and UCH-L1 by age group <2y 180 pg/mL and 373 pg/mL; 2-4y 118 pg/mL and 272 pg/mL; >4y 73 pg/mL and 217 pg/mL based on the 95th percentile for the mean of a control group of children. For S100B 0–9 months, greater than 0.35 µg/L; 10–24 months, greater than 0.23 µg/L; and older than 24 months, greater than 0.18 µg/L. The result combines the ages groups together.

Table 6 – Main results of studies assessing the performance of UCH-L1 to rule out a cerebral injury

Study ID	Population	Cut-off UCH-L1 (pg/ml)	Median time measured from injury in hours	Measured within 'hours'	Analyser (provider)	N	CT+ %	Sensitivity (95% CI)	Specificity (95% CI)	LR-
<i>Bazarian 2018</i> ⁷⁴	Adults. Mean age 48.8. 80% GCSS 14–15 (n=1920), 20% GCS 9-13 (n=39), not included here.	327		12	Chemiluminescent sandwich ELISA in serum (Banyan)	1780	6.0	70.1 (61.0; 79.0)	51.5 (49.3; 53.7)	0.58 (0.43; 0.78)
<i>Chayoua 2024</i> ⁷⁶	Adults. Median age: 48. GCSS 13-15 (60%)	360	Most within 3h	24	i-STAT Alinity in plasma (Abbott)	235	23.3	71 (58; 82) ¹	46 (39; 53)	0.63 (0.41; 0.96)
<i>Harris 2025</i> ⁷⁸	Patients aged ≥17, GCS 3-15 (but subgroup analysis for GCS 13-15)	360	NA	24	i-STAT Alinity in plasma (Abbott) ARCHITECT platform (Abbott) ^a	599	NA	19 (13; 25)	79 (75; 83)	1.03 (0.95; 1.12) ^m
<i>Iverson 2022</i> ⁷¹	Aged ≥60. Mean age 79. GCS of 13-15.	42 AUC	3.1	12	Simoa digital immunoassay in serum (Quanterix)	83	26.5	72.7 (49.8; 89.3)	50.8 (37.7; 63.9)	0.54 (0.26; 1.11)
<i>Kopcinovic 2025</i> ⁷²	Adults. Median age: 64. GCS 13–15	400	-	12	Alinity i platform in plasma/serum (Abbott)	822	13.6	80.4 (71.8; 87.3)	48.9 (45.1; 52.6)	0.40 (0.27; 0.59)
<i>Kopcinovic 2025 <65 years</i>	Subgroup analysis for adults aged <65. GCS 13–15	400		12	Alinity i platform in plasma/serum (Abbott)	418	11.2	70.2 (55.1; 82.7)	60.4 (55.2; 65.4)	0.49 (0.32; 0.77)
<i>Kopcinovic 2025 ≥65 years</i>	Subgroup analysis for adults aged ≥65. GCS 13–15	400	-	12	Alinity i platform in plasma/serum (Abbott)	403	16.1	87.7 (77.2; 94.5)	36.1 (31.0; 41.5)	0.34 (0.18; 0.66)
<i>Ladang 2025</i> ⁷³	Adults. Median age: 64. GCS 13–15	400	-	12	Alinity i platform in plasma (Abbott)	362	35.9	82.3 (74.0; 88.8)	74.3 (68.4; 79.6)	0.24 (0.16; 0.36)

^m An LR- greater than 1 means the test is worse than random guessing and behaves in the opposite way of a useful diagnostic, i.e. the probability of having a CT scan positive is greater after a negative test than before any test (probability post-test).



Study ID	Population	Cut-off UCH-L1 (pg/ml)	Median time measured from injury in hours	Measured within 'hours'	Analyser (provider)	N	CT+ %	Sensitivity (95% CI) %	Specificity (95% CI) %	LR-
<i>Lagares 2024</i> ⁶⁹	Adults in FR and ≥15 in Sp. Median age 69. GCSS 13–15	327	4.5	12	VIDAS in serum (Biomérieux)	1437	12.2	57.0 (49.7; 64.0)	63.0 (60.3; 65.7)	0.68 (0.57; 0.81)
<i>Lapić 2024</i> ⁷⁹	Adults. Median age: 62. GCS 14–15.	400	2.5	12	Alinity i platform in plasma (Abbott)	62	11.3	57.1 (18.4; 90.1)	56.4 (42.3; 69.7)	0.76 (0.31; 1.85)
<i>Li 2023</i> ⁸¹	Adults. Mean age: 50.8. GCS 13–15. (72% GCS: 15)	327	-	6	ELISA in plasman (Banyan)	440	26.9	95.0 (89.4; 98.1)	17.7 (13.7; 22.3)	0.29 (0.13; 0.65)
<i>Oris 2023 (3hours)</i> ⁸²	Adults. Mean age: 59.1. Moderate risk within the mTBI according to French guidelines: GCS 14–15	360	1.7	3	i-STAT Alinity in plasma (Abbott)	192	9.8	33.3 (7.5; 70.1)	59.0 (51.5; 66.2)	1.13 (0.70; 1.8)
<i>Oris 2023 (12 hours)</i> ⁸²	id	id		12	id	239	4.6	41.7 (15.2; 72.3)	64.3 (57.7; 70.6)	0.91 (0.56; 1.48)
<i>Papa 2022</i> ³⁴	Adults. Mean age 40. GCS 13–15 (90% GCSS 15).	327	3	4	ELISA in serum (Banyan)	349	6.6	78 (56; 92)	44 (38; 49)	0.50 (0.23; 1.09)
	Adults. Mean age 40. GCS 13–15 (90% GCSS 15).	189	3	4	ELISA in serum (Banyan)	349	6.6	96 (76; 100)	29 (24; 34)	0.15 (0.02; 1.03)
<i>Puravet 2025</i> ⁶⁸	Aged ≤16. Median age: 4.67. GCSS: 15 requiring	By age group	2.4	3	Alinity i platform in serum (Abbott)	68	20.6	100 (75; 100)	27 (16; 41)	0

ⁿ Results are available in the article also for serum.

^o Cutt-off GFAP and UCH-L1 by age group <2y 180 pg/mL and 373 pg/mL; 2–4y 118 pg/mL and 272 pg/mL; >4y 73 pg/mL and 217 pg/mL based on the 95th percentile for the mean of a control group of children. For S100B 0–9 months, greater than 0.35 µg/L; 10–24 months, greater than 0.23 µg/L; and older than 24 months, greater than 0.18 µg/L. The result combines the ages groups together.



Study ID	Population	Cut-off UCH-L1 (pg/ml)	Median time measured from injury in hours	Measured within 'hours'	Analyser (provider)	N	CT+ %	Sensitivity (95% CI) %	Specificity (95% CI) %	LR-
	hospitalisation or CT scan according to French guidelines									
<i>Trivedi 2024</i> ⁸⁴	Adults. Median age 47 (ICI present), 58 (no ICI present) GCS 14-15 (88.7% GCS 15)	AUC	-	6	Simoa digital immunoassay in serum (Quanterix)	531	24.9	73 (64, 81)	77 (67, 84)	0.35 (0.27; 0.47)
	NAp	AUC	-	6-9	Simoa digital immunoassay in serum (Quanterix)	248	47.6	74 (54, 82)	75 (56, 82)	0.35 (0.26; 0.48)
	NAp	AUC	-	9-12	Simoa digital immunoassay in serum (Quanterix)	154	43.5	70 (46, 81)	66 (40, 76)	0.46 (0.31; 0.68)
<i>Whitehouse 2025</i> ⁹²	Aged ≥16. Median age: 59. Overall sample larger. Here only results for GCS 13-15	31.6 AUC	-	6	Neurology 4-Plex A assay (N4PA) in serum (Quanterix)	127	18.1	95.7 (78.1; 99.9)	32.7 (23.8; 42.6)	0.13 (0.02; 0.92)
	Aged ≥16. Median age: 59. Overall sample larger. Here only results for GCS 13-15	17.9 AUC	10	24	Neurology 4-Plex A assay (N4PA) in serum (Quanterix)	385	36.4	95.0 (90.0; 97.97)	18.4 (13.7; 23.8)	0.27 (0.13; 0.59)

^p The characteristics of the patients are only available for the full sample

Table 7 – Main results of studies assessing the performance of protein S100 β to rule out a cerebral injury

Study ID		Cut-off values	Median time measured from injury in hours	Measured within (hrs)	Analyser (provider)	N	CT+ %	Sensitivity % (95% CI)	Specificity % (95% CI)	LR-
Chiollaz 2024⁶⁷	Children. Aged ≤ 16 . Mean age: 8. GCS 14-15	43 pg/mL	4	6	ELISA in serum (Meso Scale Diagnostics)	39	?	100	34.4 (18.6; 51.0)	0.0
Lagares 2024⁶⁹	Adults in FR and ≥ 15 in Sp. Median age 69. GCSS 13–15	0.1 $\mu\text{g/L}$		6	Electrochemiluminescence immunoassay (ECLIA) in serum (Cobas®, Roche)	846	11.7	87.8 (79.8; 93.6)	34.8 (31.4; 38.3)	0.35 (0.20; 0.60)
Lagerstedt 2018⁷⁰	Aged > 14 years. Weighted mean age (by CT result): 48.7 GCS 15.	0.06 $\mu\text{g/L}$	3	6	Electrochemiluminescence immunoassay (ECLIA) in serum (Cobas®, Roche) EZHS100 β -33K (Millipore)	132	15.9	100	10.8 (5.4; 17.1)	0
Li 2023⁸¹	Adults. Mean age: 50.8. GCS 13-15. (72% GCS: 15)	105 pg/mL	-	6	Electrochemiluminescence immunoassay (ECLIA) in serum (Cobas®, Roche)	189	23.3	93.2 (81.3; 98.6)	17.2 (11.5; 24.4)	0.40 (0.13; 1.25)
Oris 2023⁸²	Adults. Mean age: 59.1. Moderate risk within the mTBI according to French guidelines: GCS 14-15	0.1 $\mu\text{g/L}$	1.7	3	Electrochemiluminescence immunoassay (ECLIA) in serum (Cobas®, Roche)	192	9.8	100 (66.4; 100)	25.7 (19.5; 32.6)	0
Posti 2019⁹¹	Adults. Mean age: 42.8. All severities (n=160) but here only GCS 13-15 (n=93). Mean GCS for the 13-15: 14.65	179 pg/mL AUC	15.2	24	EZHS100 β -33K (Millipore)	93	39.8	91.9	12.5	0.65 (0.18; 2.35)



Study ID		Cut-off values	Median time measured from injury in hours	Measured within (hrs)	Analyser (provider)	N	CT+ %	Sensitivity (95% CI) %	Specificity (95% CI) %	LR-
Puravet 2025	Aged ≤16. Median age: 4.67. GCSS: 15 requiring hospitalisation or CT scan according to French guidelines	By age group ^q	2.4	3	Electrochemiluminescence immunoassay (ECLIA) in serum (Cobas®, Roche)	68	20.6	92 (64; 100)	51.9 (37.8; 65.7)	0.15 (0.02; 1.01)
Trivedi 2024	Adults. Median age 47 (ICI present), 58 (no ICI present) GCS 14-15 (88.7% GCS 15).	AUC	-	6	Electrochemiluminescence immunoassay (ECLIA) in serum (Cobas®, Roche)	531	24.9	78 (68; 83)	61 (56; 66)	0.36 (0.26; 0.50)
Whitehouse 2025	Aged ≥16. Median age: 59. Overall sample larger. Here only results for GCS 13-15	0.1µg/L	-	6	Electrochemiluminescence immunoassay (ECLIA) in serum (Cobas®, Roche)	127	18.1	95.7 (78.1; 99.9)	31.7 (23; 41.6)	0.14 (0.02; 0.95)

^q Cutt-off GFAP and UCH-L1 by age group <2y 180 pg/mL and 373 pg/mL; 2-4y 118 pg/mL and 272 pg/mL; >4y 73 pg/mL and 217 pg/mL based on the 95th percentile for the mean of a control group of children. For S100B 0–9 months, greater than 0.35 µg/L; 10–24 months, greater than 0.23 µg/L; and older than 24 months, greater than 0.18 µg/L. The result combines the ages groups together.



3.2.2 Quality appraisal of included studies

Results of the quality appraisal are presented in Table 8 and in Figure 2. Overall, study bias for patient selection was highly present; often this was a result of retrospective analysis of banked samples (Table 8). Patient selection was of high concern due to non-consecutive enrolment in 17 studies, as recommended by the QUADAS-2 methodology.^{34, 70, 71, 74, 75, 77-79, 81, 84-90, 92} In three papers the patients were selected on a raised S100 β marker.^{82, 83, 88} The Scandinavian rule was applied in one study,⁸⁸ and the French guidelines in two papers (both with consecutive enrolment).^{82, 83} Having a high risk of patient selection because the recruitment was not consecutive did not automatically result in an overall high risk of bias, because other domains weighed against this one, particularly the correct blinding of both clinicians and laboratory technicians.

The index test was considered of some concern for bias whenever a research platform was used which was the case in 8 studies.^{67, 70, 71, 86, 87, 90-92} Further, the index test was also considered of some concern for bias whenever the AUC defined the cutoffs which was the case in 11 studies.^{67, 70, 71, 78, 84, 86, 87, 89-92} A concern of bias for the index test due to non-blinding or insufficient description of blinding was present in 8 studies.^{68, 70, 73, 77, 79, 80, 82, 83}

The reference standard was clearly described or defined in most studies, except for 5 studies.^{70, 79, 80, 84, 91} Concern of bias for the reference test due to non-blinding or insufficient description of blinding was present in 11 studies.^{68, 70, 72, 79, 80, 82-84, 86, 90, 91}

High concern or some concern of bias for the flow and timing was present in 7 studies due to not including all patients in the analysis.^{67, 70, 71, 77, 87-89}

As can be seen in Figure 2, the majority of studies presented some concerns regarding a risk of bias. Applicability was overall considered of low concern, whether a formal head rule had been adopted to select patients eligible for a CT scan or not (see Table 9).



Table 8 – Summary QUADAS-2 quality appraisal primary studies

Study ID	Bias: Patient selection	Bias: Index test	Bias: Reference standard	Bias: Timing and flow	Overall score	Applicability: Patient selection	Applicability: Index test	Applicability: Reference standard
<i>Bazarian 2018</i> ⁷⁴	Secondary analysis of banked venous plasma samples; not consecutive, not randomised: high concern	Clearly described; blinded; low concern	Clearly described; blinded; Low concern	Low concern	Some concerns	Low concern	Low concern	Low concern
<i>Bazarian 2021</i> ⁷⁵	Secondary analysis of banked venous plasma samples; not consecutive, not randomised: high concern	Clearly described; blinded; low concern	Clearly described; blinded; Low concern	Low concern	Some concerns	Low concern	Low concern	Low concern
<i>Chayoua 2024</i> ⁷⁶	All consecutive patients were assessed; low concern	Clearly described; blinded; low concern	Clearly described; blinded; Low concern	Low concern	Low concern	Low concern	Low concern	Low concern
<i>Chiollaz 2024</i> ⁸⁷	All consecutive patients were assessed; low concern	Research platform; AUC to define cutoffs; Blinded; unclear concern	Clearly described; blinded; Low concern	Low concern	Low concern	Low concern	Research platform; High concern	Low concern
<i>Curran 2025</i> ⁷⁷	Not consecutive, not randomised: high concern	Test described; Blinding ; low concern	Clearly described; blinded; Low concern	Not all patients were included in the analysis (insufficient sample volume, lack of test calibration material); unclear concern	Some concerns	Low concern	Low concern	Low concern
<i>Forouzan 2021</i> ⁸⁶	Not consecutive, not randomised: high concern	Research platform; AUC to define cutoffs; Blinded; Unclear concern	Described; Blinding unclear; Unclear concern	Low concern	Some concerns	Low concern	Research platform; High concern	Low concern
<i>Gardner 2018</i> ⁸⁷	Secondary analysis of banked samples; not consecutive, not randomised: high concern	Research platform; AUC to define cutoffs; blinded; Unclear concern	Clearly described; blinded; Low concern	Not all patients included in the analysis; High concern	High concern	Low concern	Research platform; High concern	Low concern
<i>Harris 2025</i> ⁷⁸	Secondary analysis of banked samples; not consecutive, not randomised: high concern	AUC to define cutoffs; Two platforms used; Blinded; unclear concern	Clearly described; blinded; Low concern	Low concern	Some concerns	Analysis by alcohol status resulted in exclusion of 69% of samples for this review; High concern	Low concern	Low concern
<i>Iverson 2022</i> ⁷¹	Secondary analysis of banked samples; not	Research platform; AUC to define cutoffs;	Clearly described; blinded; Low concern	Not all patients were included in	High concern	Low concern	Research platform;	Low concern



Study ID	Bias: Patient selection	Bias: Index test	Bias: Reference standard	Bias: Timing and flow	Overall score	Applicability: Patient selection	Applicability: Index test	Applicability: Reference standard
	consecutive, not randomised: high concern	Blinded; Unclear concern		the analysis; High concern			High concern	
Keski-Pukkila 2024⁸⁸	Secondary analysis of banked samples; not consecutive, not randomised: Exclusion when S100 β not raised; High concern	Clearly described; blinded; low concern	Clearly described; blinded; Low concern	Not all patients were included in the analysis; High concern	High concern	The Scandinavian rules were followed: S100 β raised AND mild (low risk) group; High concern	Research platform; High concern	Low concern
Kopcinovic 2025(Kopcinovic Milevoy 2025)	Not stated that enrolment was consecutive; Unclear concern	Clearly described; blinding unclear; Unclear concern	Defined; Blinding unclear; Unclear concern	Low concern	Some concerns	Low concern	Low concern	Low concern
Ladang 2025⁷³	Retrospective study on banked samples; consecutive; Low concern	Clearly described; Blinding unclear; Unclear concern	Clearly described; Blinded; Low concern	Not all patients were included in the analysis; Unclear concern	Low concern	Low concern	Low concern	Low concern
Lagares 2024⁶⁹	Not stated that enrolment was consecutive; Unclear concern	Clearly described; blinded; low concern	Clearly described; blinded; Low concern	Low concern	Low concern	Low concern	Low concern	Low concern
Lagerstedt 2018⁷⁰	Not consecutive, not randomised: High concern	Research platform; AUC to define cutoffs; Blinding unclear; High concern	No definition except for results in table; Blinding unclear; Unclear concern	Not all patients were included in the analysis; unclear concern	High concern	Low concern	Research platform; High concern	Low concern
Lapić 2024⁷⁹	Not consecutive, not randomised: High concern	Clearly described; blinding unclear; unclear concern	No definition except for results in table; Blinding unclear; Unclear concern	Low concern	Some concern	Low concern	Low concern	Low concern
Legramante 2024⁸⁰	Retrospective study on banked samples; consecutive enrolled based on the ED registration; Unclear concern	Clearly described; blinding unclear; Unclear concern	No definition except for results in table; Blinding unclear; Unclear concern	Low concern	Some concerns	Low concern	Low concern	Low concern
Li 2023⁸¹	Retrospective study on banked samples; not consecutive; Unclear concern	Clearly described; blinded; low concern	Clearly described; blinded; low concern	Low concern	Low concern	Only patients admitted by helicopter or ambulance on Mondays and Tuesdays; High concern	Research platform; High concern	Low concern
Okonkwo 2020⁸⁹	Secondary analysis of banked samples; not consecutive, not randomised: High concern	AUC to define cutoffs; Blinded; unclear concern	Clearly described; blinded; low concern	Not all patients included in the analysis due to missing data; high concern	High concern	Low concern	Low concern	Low concern



Study ID	Bias: Patient selection	Bias: Index test	Bias: Reference standard	Bias: Timing and flow	Overall score	Applicability: Patient selection	Applicability: Index test	Applicability: Reference standard
<i>Oris 2023</i> ⁸²	Retrospective study on banked samples; exclusion when S100 not raised; consecutive; High concern	Clearly described; blinding unclear; unclear concern	Clearly described; blinding unclear; unclear concern	Low concern	Some concern	The guidelines were followed: S100β raised; concern	French were S100β High	Low concern
<i>Oris 2024</i> ⁸³	Retrospective study on banked samples; exclusion when S100 not raised; consecutive; unclear concern	Clearly described; blinding unclear; Unclear concern	Clearly described; blinding unclear; Unclear concern	Low concern	Some concern	The guidelines were followed: S100β raised; concern	French were S100β High	Low concern
<i>Papa 2022</i> ³⁴	Cross-sectional study; not consecutive, not randomised; High concern	Clearly described; blinded; Low concern	Clearly described; blinded; Low concern	Low concern	Low concern	Low concern	Low concern	Low concern
<i>Polat 2022</i> ⁹⁰	Cross-sectional study; not consecutive, not randomised; Exclusion when > 3 hours after injury; High concern	Research platform; AUC to define cut-offs; unclear concern	Clearly described; blinding unclear; Unclear concern	Low concern	Some concern	Low concern	Research platform; High concern	Low concern
<i>Posti 2019</i> ⁹¹	Consecutive enrolment; Low concern	Research platform; AUC to define cut-offs; Blinded; unclear concern	Clearly described; blinding unclear; Unclear concern	Low concern	Some concern	Low concern	Research platform; High concern	Low concern
<i>Puravet 2025</i> ⁶⁸	Retrospective analysis of banked samples; consecutive not randomised; Low concern	Pre-specified cutoffs from a reference population of children applied; Blinding unclear; unclear concern	Clearly described; blinding unclear; Unclear concern	Low concern	Low concern	Low concern	Low concern	Low concern
<i>Trivedi 2024</i> ⁸⁴	Retrospective analysis of banked samples; not consecutive, not randomised; High concern	AUC to define cutoffs; Blinded; unclear concern	No definition except for results in table; Blinding unclear; Unclear concern	Low concern	Some concerns	Low concern	Low concern	Low concern
<i>Welch 2025</i> ⁸⁵	Retrospective analysis of banked samples; not consecutive, not randomised; High concern	Clearly described; blinded; Low concern	Clearly described; blinded; Low concern	Low concern	Low	Low concern	Low concern	Low concern
<i>Whitehouse 2025</i> ⁹²	Retrospective analysis of banked samples; not consecutive, not randomised; High concern	Research platform; AUC to define cutoffs; Blinded; unclear concern	Clearly described; blinded; Low concern	Low concern	Some concern	Low concern	Research platform; High concern	Low concern



Figure 2 – Visual summary of QUADAS-2 quality appraisal of all included studies

	Risk of bias domains				Overall
	D1	D2	D3	D4	
Bazarian 2018{Bazarian, 2018 #27}	⊗	⊕	⊕	⊕	⊖
Bazarian 2021{Bazarian, 2021 #26}	⊗	⊕	⊕	⊕	⊖
Chayoua 2024{Chayoua, 2024 #4}	⊕	⊕	⊕	⊕	⊕
Chiollaz 2024{Chiollaz, 2024 #23}	⊕	⊖	⊕	⊕	⊕
Curran 2025{Curran, 2025 #11}	⊗	⊕	⊕	⊖	⊖
Forouzan 2021{Forouzan, 2021 #17}	⊗	⊖	⊖	⊕	⊖
Gardner 2018{Gardner, 2018 #16}	⊗	⊖	⊕	⊗	⊗
Harris 2024{Harris, 2025 #39}	⊗	⊖	⊕	⊕	⊖
Iverson 2022{Iverson, 2022 #15}	⊗	⊖	⊕	⊗	⊗
Keski-Pukkila2024{Keski-Pukkila, 2024 #38}	⊗	⊕	⊕	⊗	⊗
Kopcinovic 2025{Kopcinovic Milevoy 2025}	⊖	⊖	⊖	⊕	⊖
Ladang 2024{Ladang, 2025 #5}	⊕	⊖	⊕	⊖	⊕
Lagares 2024{Lagares, 2024 #6}	⊖	⊕	⊕	⊕	⊕
Lagerstedt 2018{Lagerstedt, 2018 #19}	⊗	⊗	⊖	⊖	⊗
Lapić 2024{Lapić, 2024 #7}	⊗	⊖	⊖	⊕	⊖
Legramante 2024{Legramante, 2024 #8}	⊖	⊖	⊖	⊕	⊖
Li 2023{Li, 2023 #3}	⊖	⊕	⊕	⊕	⊕
Okonkwo 2020 {Okonkwo, 2020 #56}	⊗	⊖	⊕	⊗	⊗
Oris 2023{Oris, 2024 #2}	⊗	⊖	⊖	⊕	⊖
Oris 2024{Oris, 2024 #9}	⊖	⊖	⊖	⊕	⊖
Papa 2022{Papa, 2022 #1}	⊗	⊕	⊕	⊕	⊕
Polat 2022{Polat, 2022 #28}	⊗	⊖	⊖	⊕	⊖
Posti 2019{Posti, 2019 #18}	⊕	⊖	⊖	⊕	⊖
Puravet 2025{Puravet, 2025 #14}	⊕	⊖	⊖	⊕	⊖
Trivedi 2024{Trivedi, 2024 #10}	⊗	⊖	⊖	⊕	⊖
Welch 2025{Welch, 2025 #13}	⊗	⊕	⊕	⊕	⊕
Whitehouse 2025{Whitehouse, 2025 #22}	⊗	⊖	⊕	⊕	⊖

Study

Domains:
D1: Patient selection.
D2: Index test.
D3: Reference standard.
D4: Flow & timing.

Judgement
⊗ High
⊖ Some concerns
⊕ Low

3.2.3 Meta-analysis

3.2.3.1 GFAP+UCH-L1 combined

Of the 18 studies retrieved (Table 4), three were excluded from the meta-analysis:

- a. Trivedi 2024 was excluded because no cut-offs of biomarkers concentration were provided^r.⁸⁴ Each biomarker's sensitivity, specificity, and accuracy were reported at the cut-off that maximized the Youden's index for each model. Such an approach does not serve the clinical purpose of excluding an intracerebral lesion based on a negative test. The reported sensitivity was 83% (95%CI: 71-90%), an unacceptably low level for excluding any serious health condition.⁸⁴
- b. Harris 2025 could not be meta-analysed because the proportion of GCS 13-15 patients with a positive CT scan was not reported, and therefore deriving crude data to fit the meta-analysis was not possible.⁷⁸ The main author and co-authors were contacted three times but no response was received. However, it should be noted that the sensitivity was high, from 99% in those with non-elevated Blood Alcohol Level (BAL) to 100% in patients with significantly or modestly elevated BAL (97% in those with unknown BAL).⁷⁸ Strikingly, the same results were observed in the patients group with GCS 3-15 which were the focus of the presented paper and analysis (2 320 patients were GCS 3-15, among whom 1 832 were GCS 13-15).
- c. Puravet 2025 was not included in the meta-analysis because it included children only (<16 years), with various GFAP and UCH-L1 cut-offs according to age ranges^s, whereas all the other studies were in adults.⁶⁸ The sensitivity was 100% (95%CI: 75-100), and specificity 42% (95%CI: 29-56).⁶⁸ Of note, all patients had a GCS 15 and had a medium risk according to the PECARN guidelines. A minority of children had a head CT scan (13%, 68/531).

Fifteen studies were thus included in the meta-analysis.^{34, 69, 71-77, 79-83, 85} Characteristics of these studies are reported in Table 4 and in another table in the supplement. A validated head rule to select patients eligible for a CT scan was used in only 5 studies (Table 9). Study populations overlapped in two occurrences. First, Oris 2024 included the same population as Oris 2023, although some slight unexplained numerical differences between the 2 papers were noted.^{82, 83} Oris 2023 compared results obtained with i-STAT Alinity handheld within 3 hours vs 12 hours after the trauma⁸² whereas Oris 2024 compared the results obtained by using the Alinity i platform versus the i-STAT Alinity handheld (the latter being also reported in Oris 2023).⁸³ Second, Bazarian 2018 and Bazarian 2021 also shared the same population.^{74, 75} Bazarian 2021 applied the i-STAT Alinity on banked plasma samples from Bazarian 2018 (ALERT-TBI). Welch 2025 also used blood samples from patients enrolled for the ALERT-TBI trial to test the performance of the Alinity i platform.⁸⁵ Studies with overlapping populations were all included in the meta-analysis because their results related to different lab techniques, i.e. the counting unit in the meta-analysis is at the test level, not at the individual level.

In Ladang 2025, the percentage of patients with a positive CT scan in adults <65 years and adults ≥ 65 years was provided by the authors at the request of KCE, which allowed the inclusion of their results

^r Serum GFAP and UCH-L1 were analyzed with an ultrasensitive immunoassay using digital array technology (Single Molecule Arrays, SiMoA)-based Human Neurology 4-Plex B assay (N4PB) run on the SR-X Benchtop assay platform (Quanterix Corp., Lexington, MA). The ability of these biomarkers to detect any ICI was evaluated based on the area under the receiver operating characteristic curve (AUC) for a logistic regression model with the biomarker as the predictor and ICI as the response variable. The combined predictive ability of GFAP and UCH-L1 was also assessed by including both as predictors in a logistic regression model with ICI as the response variable.

^s Cut-offs: GFAP+UCH-L1: <2 yrs: GFAP: 179.54 ng/L; UCH-L1: 372.94 ng/L; ≥2 and <4 yrs: GFAP: 117.52 ng/L; UCH-L1: 272.02 ng/L; ≥4 and <16 yrs: GFAP: 73.45 ng/L; UCH-L1: 217.45 ng/L



in the meta-analysis stratified by age group.⁹³ In Li 2023, ELISA assays on plasma or serum were merged as results were very similar.⁸¹

The cut-offs defining a positive test were predefined in studies using the i-STAT Alinity (Abbott), the Alinity i platform (Abbott), or the VIDAS (Biomérieux) (see Table 4 for details on the cut-offs). In other studies, the cut-offs could be either close to pre-defined Abbott cut-offs or very different. In case more than one set of cut-offs was tested, we included in the primary meta-analysis the set yielding the lowest LR-, and tested the alternative set in a sensitivity analysis.

Table 9 – Use of validated head rules in included studies

Study ID	Proportion of tests with manageable lesions	false negative neurosurgically	Validated head rules
Bazarian 2018 ⁷⁴	0/3 cases*		Not mentioned
Bazarian 2021 ⁷⁵	0/5 cases		Not mentioned
Chayoua 2024 ⁷⁶	0/2 cases		CT in Head Injury Patients (CHIP) decision rule
Curran 2025 ⁷⁷	0 false negative case		Not mentioned
Iverson 2022 ⁷¹	0 false negative case		Scandinavian guidelines
Kopcinovic 2025 ⁷²	?/5, diagnoses not reported		Not mentioned
Ladang 2025 ⁷³	0/1 cases		Not mentioned
Lagares 2024 ⁶⁹	0/3 cases**		Canadian CT Head Injury Rule (CCHR)
Lapić 2024 ⁷⁹	0 false negative case		Not mentioned
Legramante 2024 ⁸⁰	0 false negative case		Not mentioned
Li 2023 ⁸¹	0 false negative case		Not mentioned
Oris 2023 ⁸²	0 false negative case		Société Française de Médecine d'Urgence (SFMU) criteria
Oris 2024 ⁸³	0 false negative case		Société Française de Médecine d'Urgence (SFMU) criteria
Papa 2022 ³⁴	0 false negative case with cut-offs GFAP 67 pg/ml, UCH-L1 189 pg/ml ?/2 with cut-offs GFAP 30 pg/ml, UCH-L1 327 pg/ml, diagnoses not reported		Not mentioned
Welch 2025 ⁸⁵	?/4, diagnoses not reported		Not mentioned

*: 1 case might have been false CT positive (cavernous malformation); **: 2 cases might have been false CT positive (ruptured intracerebral aneurysm might have been the cause of the fall)

The pooled sensitivity and specificity over the 15 studies (n=10 440 tests in adults) were 98% (95%CI: 96-99) and 29% (95%CI: 25-34), respectively (Figure 3 and Figure 4). As it can be visualised on the forest plot (Figure 4), the inter-study variability was very low for sensitivity ($I^2=4.3\%$), but substantial for specificity ($I^2=85.6\%$). The reasons for such variation in specificity are unclear. For example, Curran 2025 and Kopcinovic 2025 reported a specificity around 25%, while in Ladang 2025 the specificity was 41% (95%CI: 0.34; 0.47) although the 3 studies used the Alinity i platform (Abbott) with identical cut-offs and the median age of participants was similar.^{72, 73, 77} The reason of the great heterogeneity of specificity across studies could be explained partly by the proportion of aged participant populations (see below). For some studies, the confidence interval around the sensitivity was wide, e.g. the lowest bound was 74% in Curran 2025 and 59% in Lapić 2024. This imprecision was primarily due to small sample sizes (e.g. 89 in Curran 2025, 62 in Lapić 2024) and therefore the

impact on the pooled estimate and I^2 was limited.^{77, 79} **The negative likelihood ratio was 0.08 (95%CI: 0.05-0.12), i.e. the power of a negative result to rule out a brain lesion was very high. However, the reduction in CT scan was very much dependent on the specificity, and this one was very variable across studies.**

It is also important to note that false negative cases were generally not linked to lesions requiring immediate neurosurgery, i.e. the consequence of a false negative test did not jeopardize the survival of the patients (Table 9). However, the diagnoses of false negative tests were not reported in 3 studies.^{34, 72, 85}

Various types of analysers were used: the handheld i-STAT Alinity (Abbott) in 3 studies,^{75, 76, 83} the Alinity i platform (Abbott) in 7 studies,^{72, 73, 77, 79, 80, 82, 85} and the VIDAS (Biomérieux) in only one study.⁶⁹ The remaining 4 studies used various ELISA assays or digital immunoassays.^{34, 71, 74, 81} We assessed if the performance of GFAP+UCHL-1 varied by device. As can be seen on the corresponding forest plot, no major differences in pooled sensitivity and specificity were apparent (Figure 5).

Of note, although the i-STAT Alinity handheld is supposed to serve as a point-of-care with whole blood, all studies using this device actually used serum or plasma, i.e. not as a point of care test. Similarly to the stratification by devices, there was little difference when results were stratified by the incidence of brain lesions (Figure 6). This incidence was highly variable across studies (from 5.9% to more than 30%; 17% on average), most probably as a result of various inclusion criteria for mTBI. For example, in three papers the patients were selected on a raised S100 β marker.^{82, 83, 88}

We also tested if the performance of the test varied with the age of participants. Results stratified by age were provided in 3 studies.^{69, 72, 73} The forest plot (Figure 7) shows that sensitivity was barely affected by age. In contrast, the specificity was much lower in adults aged ≥ 65 years (9%; 95%CI: 6-14) than in adults aged < 65 years (50%; 95%CI: 38-61). In Iverson 2022, only adults aged ≥ 60 years were included. While sensitivity was also high (100%), the specificity was greater than in the three studies with adults aged ≥ 65 years (36%; 95%CI: 24.2-49.4). The reason for this higher specificity is unclear, but could be linked to the use of a different assay (Simoa digital immunoassay) and different cut-offs (323 ng/L GFAP, 42 ng/L UCH-L1) than the ones recommended by the manufacturer for the Alinity i platform, used in the other three studies.⁷¹



Figure 3 – SROC curve of GFAP+UCH-L1 in adults

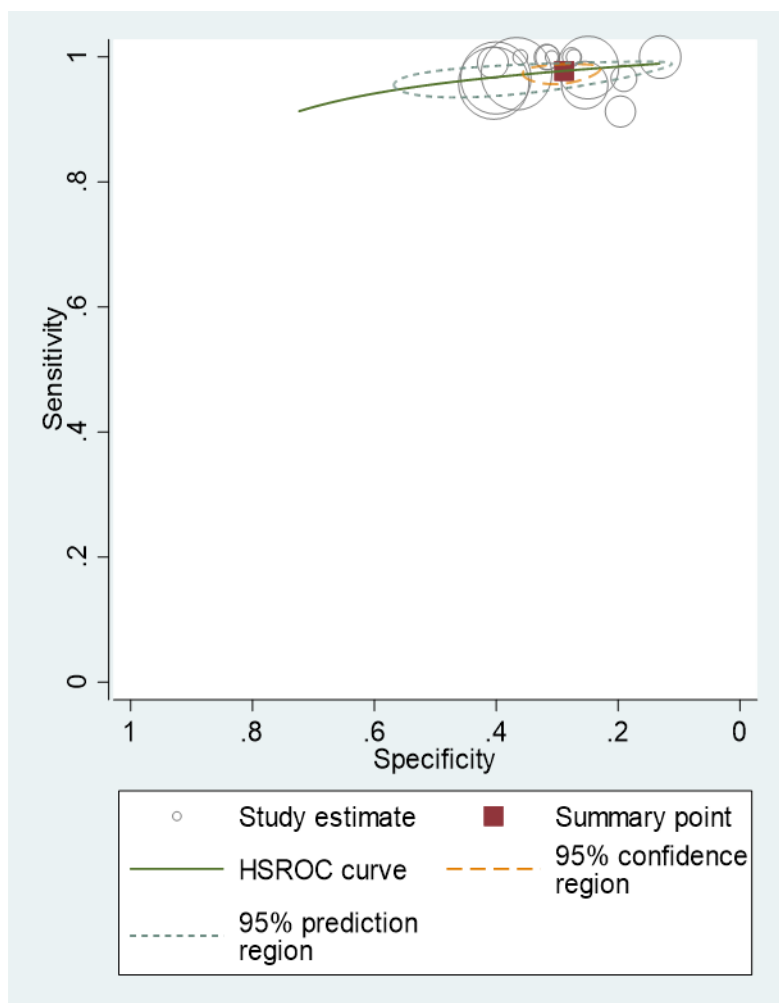


Figure 4 – Meta-analysis of sensitivity and specificity of GFAP+UCH-L1 in adults (n=10 440)

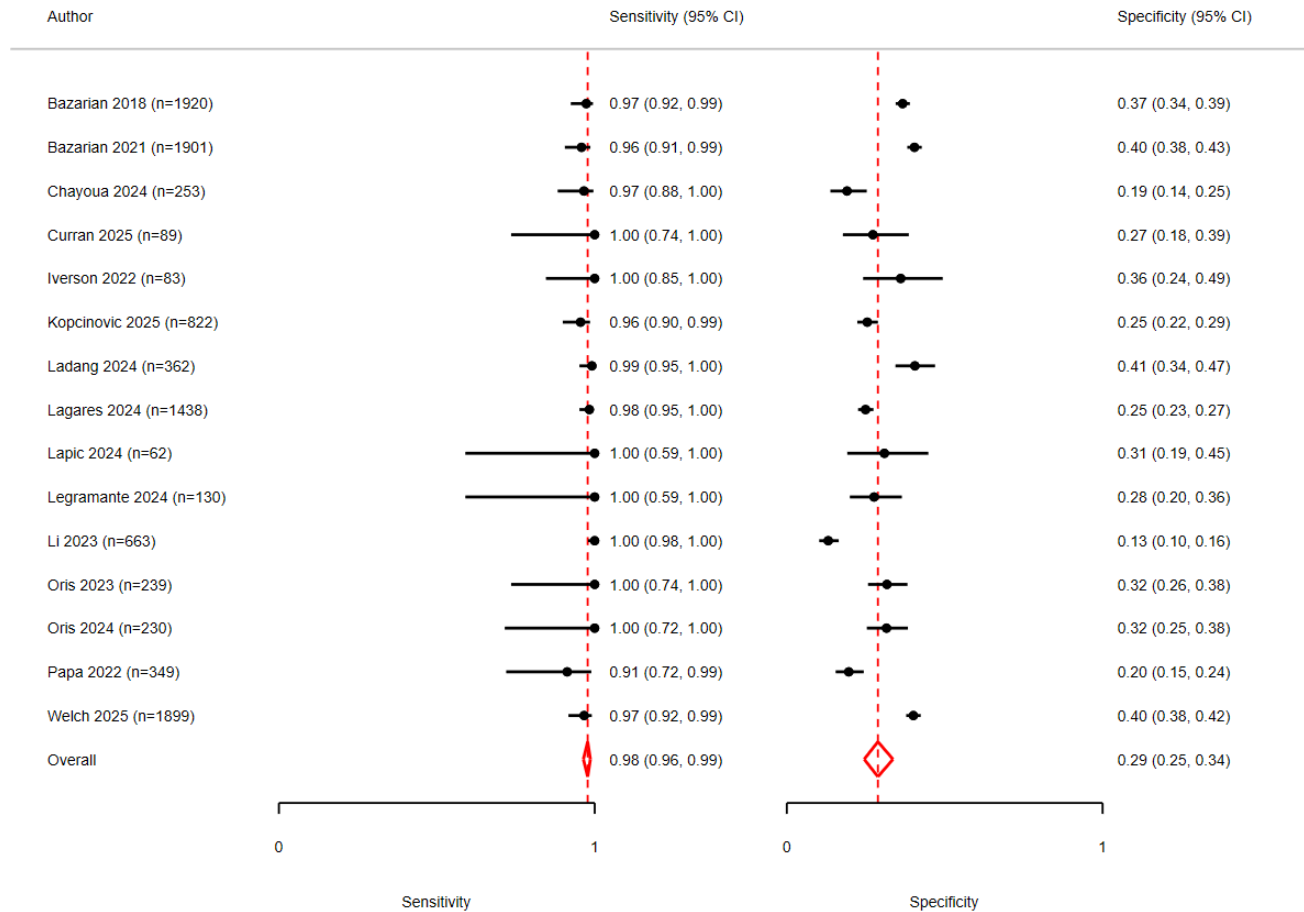




Figure 5 – Meta-analysis of sensitivity and specificity of GFAP+UCH-L1 in adults, by analyser type (n=10 440)

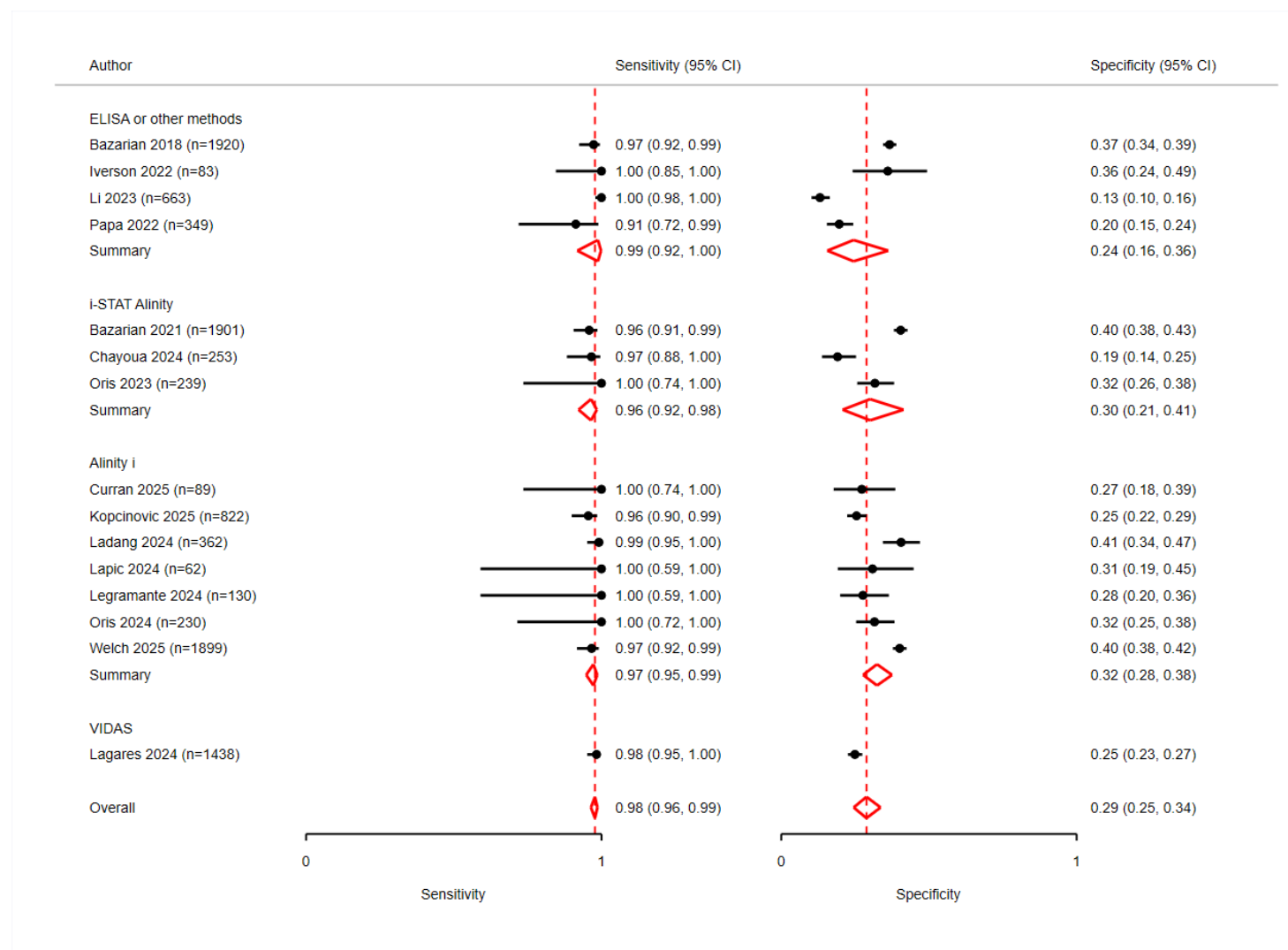


Figure 6 – Meta-analysis of sensitivity and specificity of GFAP+UCH-L1 in adults, by incidence level of brain lesions confirmed by CT scan (n=10 440)

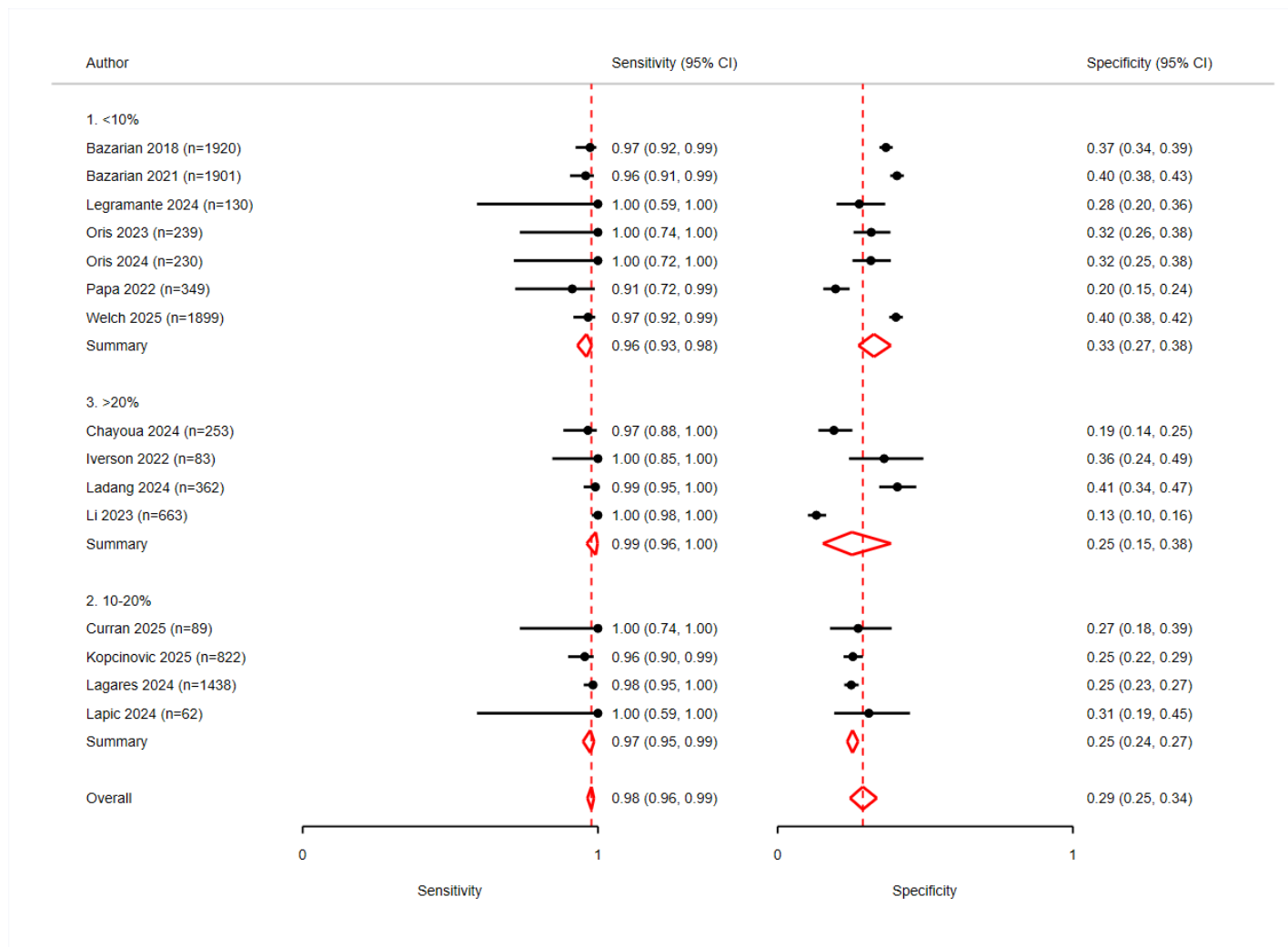
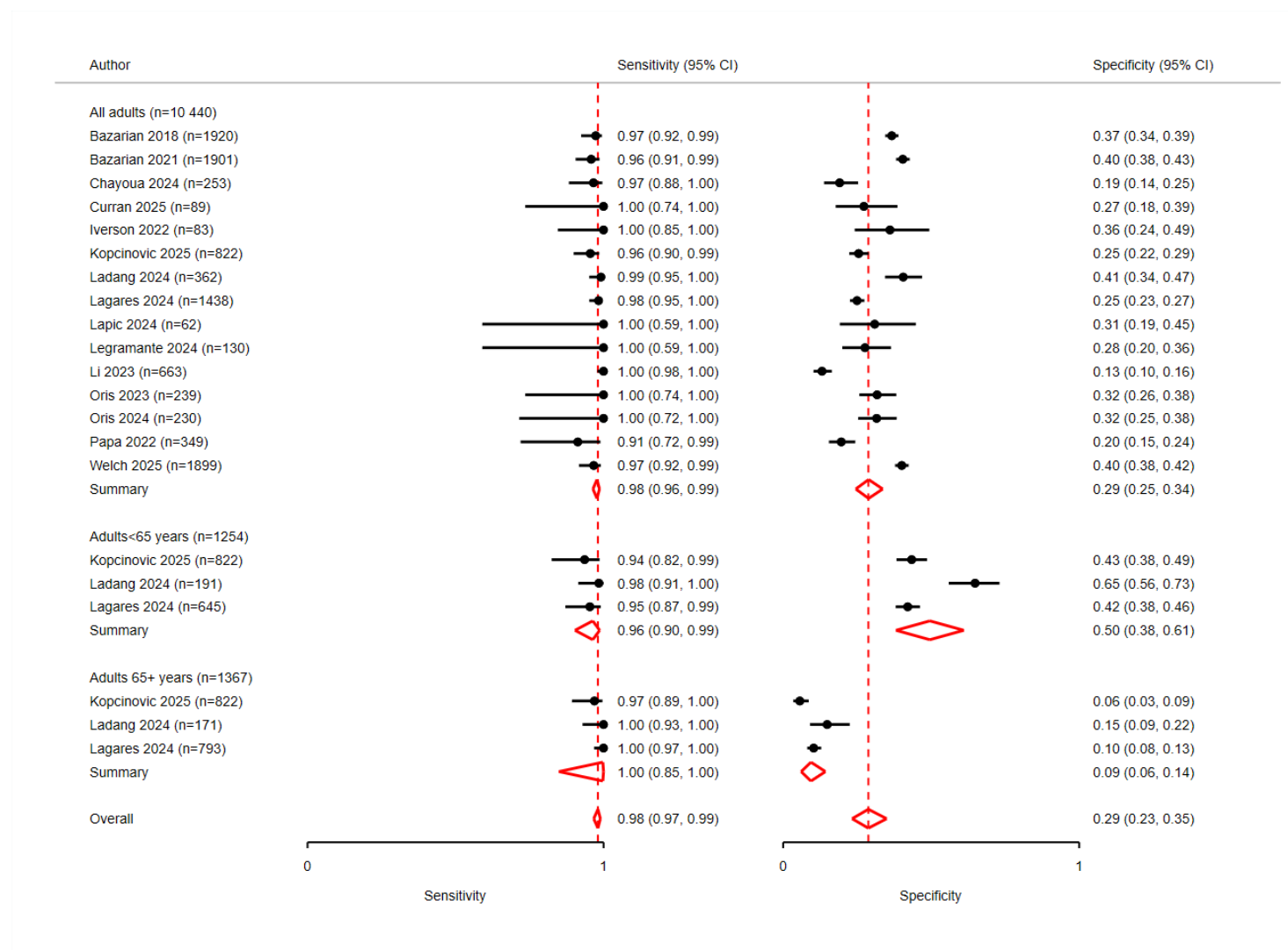




Figure 7 – Meta-analysis of sensitivity and specificity of GFAP+UCH-L1 in adults, by age (n=10 440)



Some studies investigated how changing the cut-offs could improve the performance of the test. Ladang et al. 2024 compared the cut-offs recommended by the manufacturer for the Alinity i platform (35 ng/L for GFAP, 400 ng/L for UCH-L1) to age-dependent cut-offs, i.e. in adults ≥ 65 years, the new cut-offs were 115 ng/L for GFAP and 335 ng/L for UCH-L1, whereas the usual cut-offs were maintained in adults < 65 years.⁷³ In adults ≥ 65 years the specificity increased from initial 15% to 31%, and in the whole group (all adults), specificity improved from 41% to 48%, without decreasing sensitivity. The number of CT scans avoided was doubled in adults ≥ 65 years.⁷³ In Papa 2022, cut-offs of 30 ng/L for GFAP and 327 ng/L for UCH-L1 were used for their ELISA assay, i.e. close to the cut-offs recommended for the i-STAT Alinity (Abbott). They yielded a sensitivity of 91% (95%CI: 72-99) and a specificity of 20% (95%CI: 15-24).³⁴ When the cut-offs of 67 ng/L GFAP and 189 ng/L UCH-L1 were used, the sensitivity went up to 100% (95%CI: 82-100) and the specificity up to 25% (95%CI: 20-30).³⁴ These two examples showed that the specificity of the test can be improved by increasing the cut-off for GFAP while preserving the sensitivity by a reduction of the cut-off of UCH-L1.

Regarding the time elapsed between trauma and blood sampling, the vast majority of the studies aimed at a maximum time of 12 hours, and when the actual median time was reported it was in majority less than 6 hours (Table 4). Because of this relative homogeneity across studies, assessing the effect of this parameter on the performance of the test was not possible. In Oris 2023, the sensitivity (around 100%) and the specificity (around 30%) remained very similar when the blood was sampled within 3 or 12 hours after the trauma, not an unexpected result as 80% of tests within 12 hours were also within 3 hours.⁸²

The quality of the studies was also quite homogeneous, and no stratification of results could be carried out. Over the 15 studies included, six studies were considered at low risk of bias, 8 studies presented some concerns, and only one was considered globally at high risk of bias (Figure 8).⁷¹ No publication bias was apparent (Figure 9).



Figure 8 – Visual summary of QUADAS-2 quality appraisal of studies on GFAP+UCH-L1

	Risk of bias domains				Overall
	D1	D2	D3	D4	
Bazarian 2018{Bazarian, 2018 #27}	⊗	⊕	⊕	⊕	⊖
Bazarian 2021{Bazarian, 2021 #26}	⊗	⊕	⊕	⊕	⊖
Chayoua 2024{Chayoua, 2024 #4}	⊕	⊕	⊕	⊕	⊕
Curran 2025{Curran, 2025 #11}	⊗	⊕	⊕	⊖	⊖
Iverson 2022{Iverson, 2022 #15}	⊗	⊖	⊕	⊗	⊗
Kopcinovic 2025{Kopcinovic Milevoy 2025}	⊖	⊖	⊖	⊕	⊖
Ladang 2024{Ladang, 2025 #5}	⊕	⊖	⊕	⊖	⊕
Lagares 2024{Lagares, 2024 #6}	⊖	⊕	⊕	⊕	⊕
Lapić 2024{Lapić, 2024 #7}	⊗	⊖	⊖	⊕	⊖
Legramante 2024{Legramante, 2024 #8}	⊖	⊖	⊖	⊕	⊖
Li 2023{Li, 2023 #3}	⊖	⊕	⊕	⊕	⊕
Oris 2023{Oris, 2024 #2}	⊗	⊖	⊖	⊕	⊖
Oris 2024{Oris, 2024 #9}	⊖	⊖	⊖	⊕	⊖
Papa 2022{Papa, 2022 #1}	⊗	⊕	⊕	⊕	⊕
Welch 2025{Welch, 2025 #13}	⊗	⊕	⊕	⊕	⊕

Study

Domains:
D1: Patient selection.
D2: Index test.
D3: Reference standard.
D4: Flow & timing.

Judgement
⊗ High
⊖ Some concerns
⊕ Low

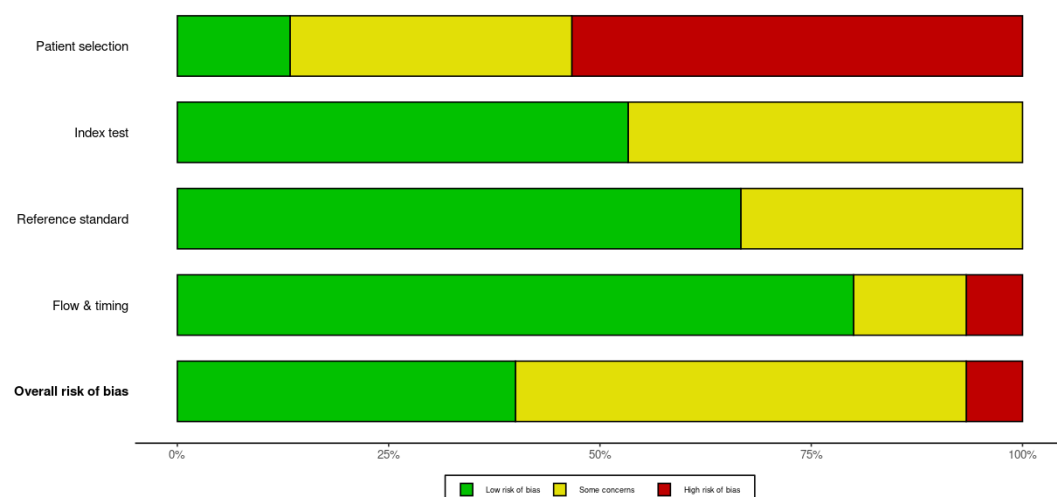
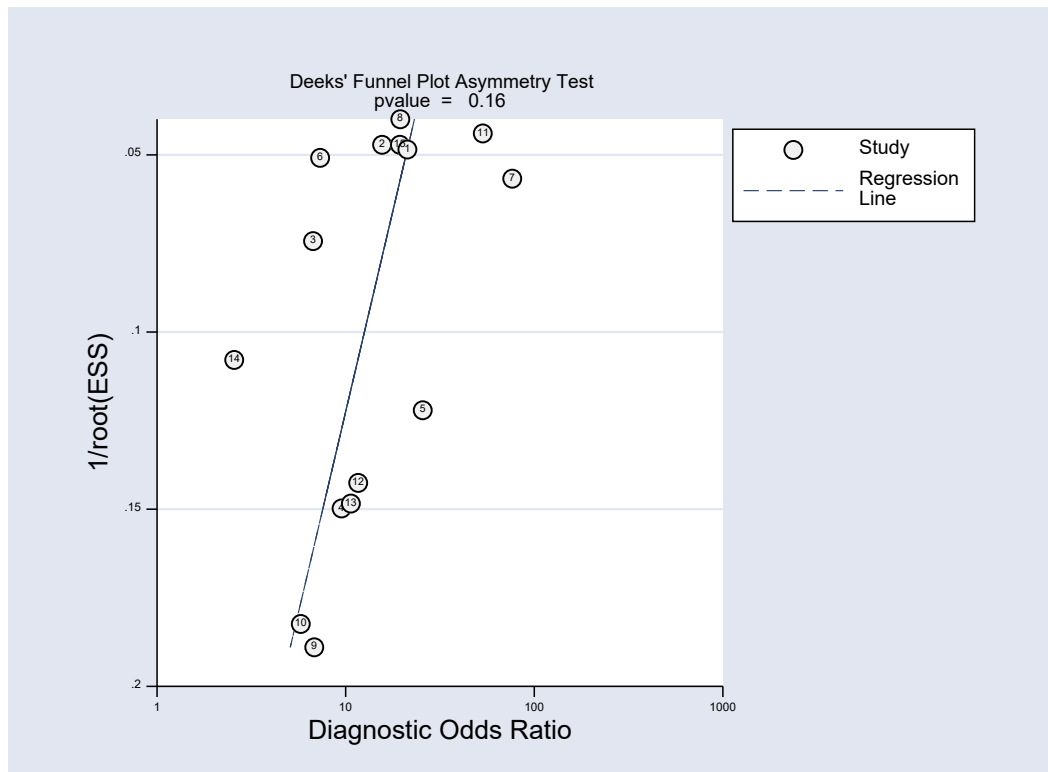


Figure 9 – Deeks' funnel plot of studies on performance of GFAP+UCH-L1 test



Based on the **GRADE criteria**, we state that:

- **There is moderate to high quality evidence that the combination GFAP+UCH-L1 can rule out a brain injury reliably in patients with mTBI received in ED within 12 hours** (sensitivity: some concerns for risk of bias, no indirectness, no heterogeneity, no imprecision, no apparent publication bias)
- **There is low quality evidence regarding the performance of these biomarkers in real world practice** (some concerns for risk of bias, indirectness ++ as all tests were performed on frozen samples, no heterogeneity, no imprecision, no apparent publication bias).
- There is insufficient evidence that the point of care device i-STAT Alinity (Abbott) presents an acceptable risk-benefit balance in real world practice.
- **There is low quality evidence that these biomarkers may reduce unnecessary CT scans by approximately 30%** (specificity: some concerns for risk of bias, no indirectness, heterogeneity +, no imprecision, no apparent publication bias).
- **There is very low quality evidence that these biomarkers may reduce unnecessary CT scans by approximately 30% in real life practice** (some concerns for risk of bias, indirectness ++, heterogeneity +, no imprecision, no apparent publication bias).
- There is insufficient evidence that these biomarkers generate other benefits such a reduction in observation time or a gain in QALYs.



3.2.3.2 GFAP alone

Twenty-one studies were retrieved, of which the study by Harris et al. 2025 could not be included for the reasons explained above (Table 5).^{34, 41, 67, 69-74, 76, 78, 79, 81-84, 86-92} Eighteen studies were carried out in adults, and only 2 in children.^{67, 68} Our meta-analysis included only studies in adults (n=8 119). Of these 18 studies, 9 were in common with the studies on GFAP+UCH-L1 combined, and the other half were specific studies.^{67, 70, 86-92} We followed the same analysis strategy as for GFAP+UCH-L1.

The pooled sensitivity across the 18 studies included was 94% (95%CI: 90-97) and the pooled specificity was 43% (95%CI: 33-54) (Figure 10 and Figure 11). In contrast with the combined test, the heterogeneity across studies was also elevated for sensitivity ($I^2=41\%$). When looking at the forest plot, 2 studies displayed more extreme values with a sensitivity at 50% for Forouzan 2021 and Polat 2022 (Figure 11).^{86, 90} This can be explained by the high cut-offs applied in these studies (1350 ng/L and 1560 ng/L, respectively). When these two studies were removed from the meta-analysis, the heterogeneity of sensitivity results dropped substantially ($I^2=24\%$). The heterogeneity of specificity across studies was extremely high ($I^2=95\%$). Figure 13, Figure 14 and Figure 15 showed that this high variability could be due to the use of different devices and different cut-offs.

The overall LR+ and LR- were 1.64 (95%CI: 1.39-1.94) and 0.14 (95%CI: 0.09-0.21), respectively. The excluding power of a negative test was considered good. On the Fagan's nomogram in Figure 12, the post-test probability of observing a brain lesion was less than 2% when the pre-test probability was 10%.

A greater variety of assays were used than for assessing GFAP+UCH-L1 and this could also have contributed to the higher heterogeneity observed (Figure 13). In particular, studies with an ELISA assay had a lower sensitivity (87%; 95%CI: 72-94) and a higher specificity (63%; 95%CI: 42-80), but this was mainly driven by the 2 studies mentioned above, i.e. Forouzan 2021 and Polat 2022.^{86, 90} The LR- for ELISA test was 0.21 (95%CI: 0.11-0.40), i.e. the excluding power of the test was moderate at best.

Results stratified by age showed again a very low specificity in adults aged ≥ 65 years (Figure 14). The pooled estimate of the specificity in the studies by Kopcinovic 2025 and Ladang 2025 was 12% (95%CI: 10-16).^{72, 73} In Iverson 2022, only adults with age ≥ 60 years were included. The sensitivity was intermediate (86%; 95%CI: 65%; 97%) and the specificity much higher than in the other studies (61%; 95%CI: 47-73). The reason for this better specificity is unclear, and could be linked to a different assay (Simoa digital immunoassay) and different cut-offs (323 ng/L GFAP, 42 ng/L UCH-L1) than the ones recommended by the manufacturer for Alinity i platform used in the 2 other studies.⁷¹

There was substantial variation of sensitivity and specificity when results were stratified by the incidence of CT-confirmed ICI (Figure 15). Again the studies by Forouzan 2021 and Polat 2022 were important factors of variation.^{86, 90} The quality appraisal of studies can be found in Figure 17. Five studies were at low risk of bias, 8 studies had some concerns, and 5 were at high risk of bias. No publication bias was detected (Figure 16).

Figure 10– SROC curve of GFAP in adults

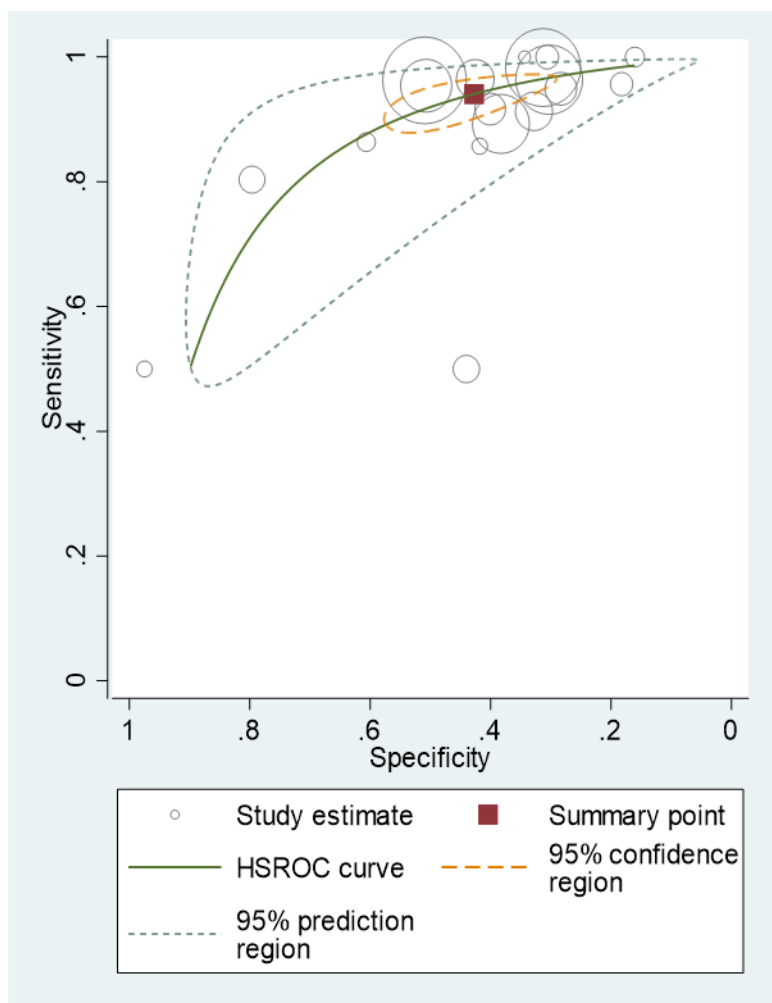




Figure 11 – Meta-analysis of sensitivity and specificity of GFAP in adults (n=8119)

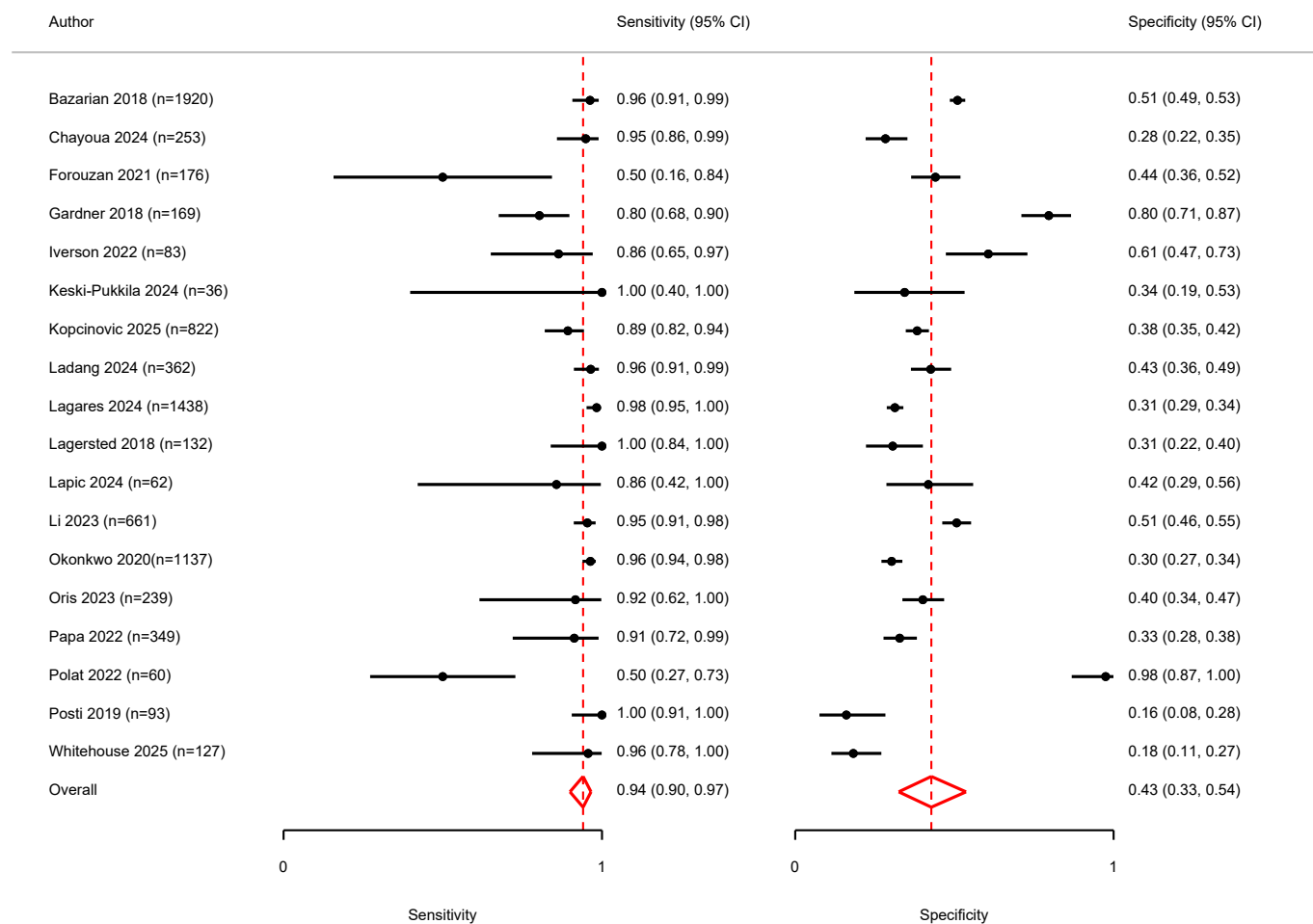


Figure 12 – Fagan's nomogram for a 10% pre-test probability of brain lesion

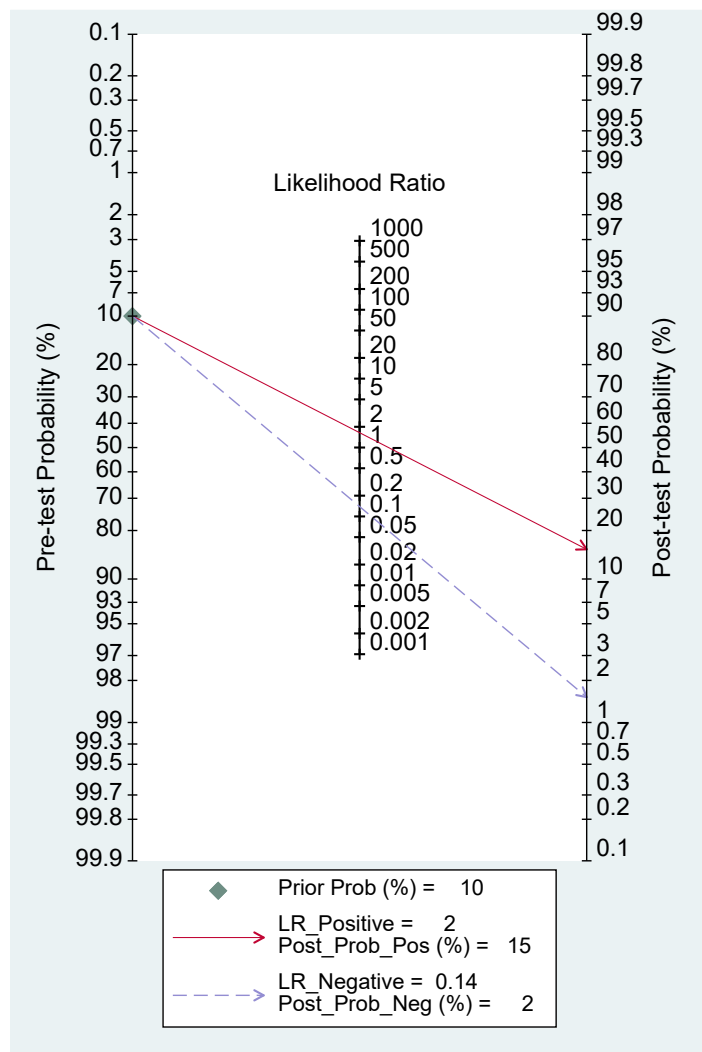




Figure 13 – Meta-analysis of sensitivity and specificity of GFAP in adults, by analyser type (n=8119)

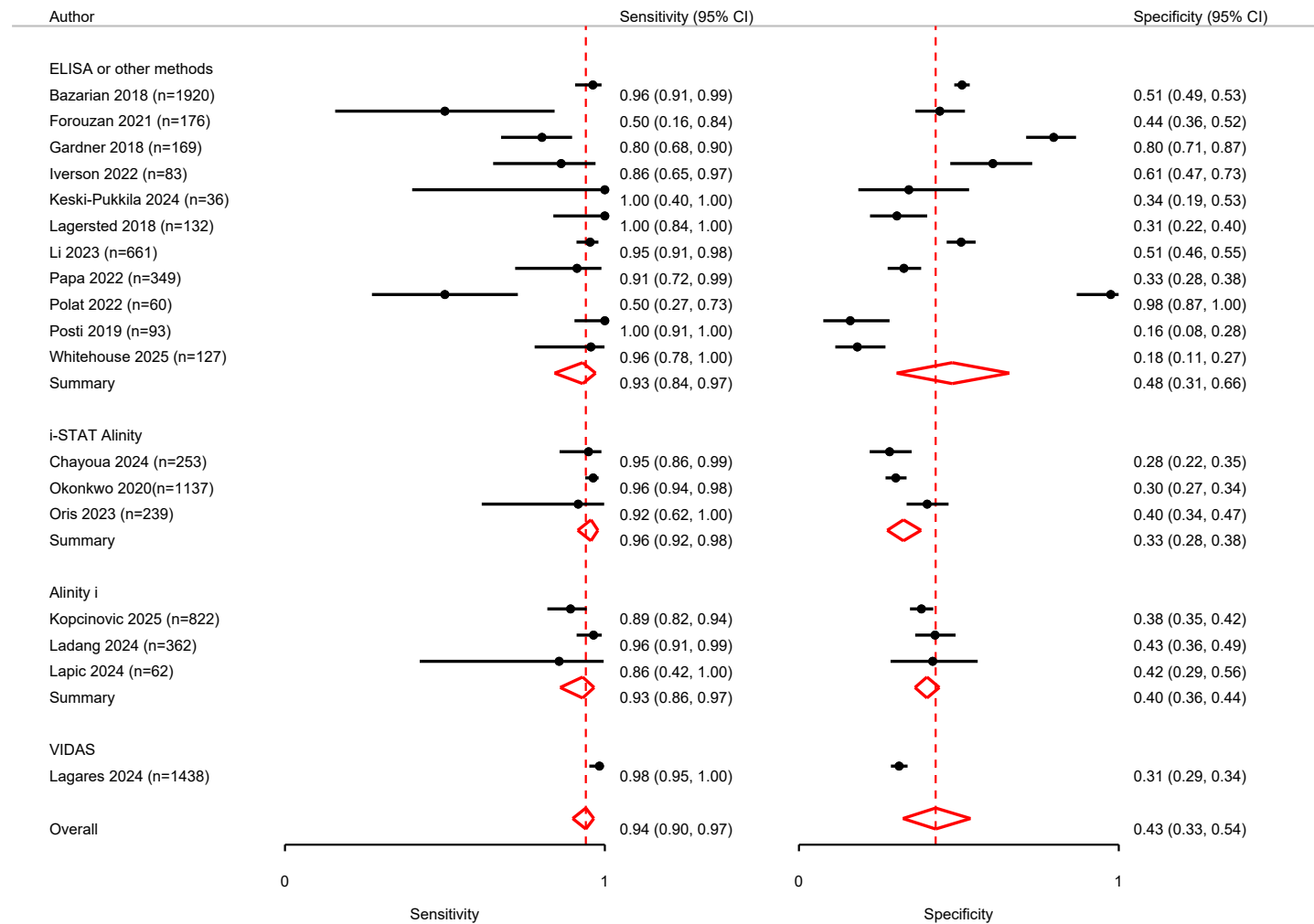


Figure 14 – Meta-analysis of sensitivity and specificity of GFAP in adults, by age (n=8119)

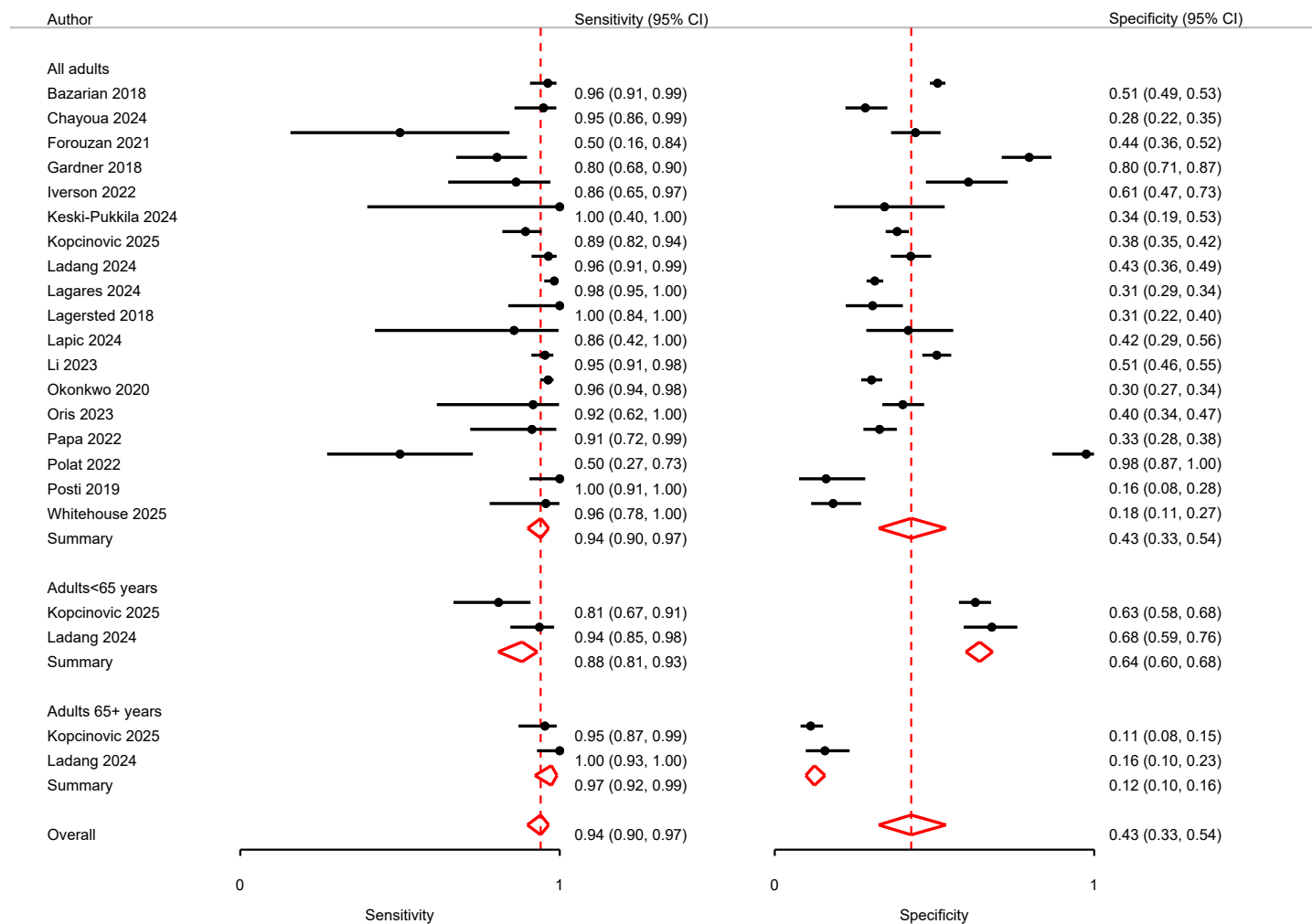




Figure 15 – Meta-analysis of sensitivity and specificity of GFAP in adults, by incidence level of brain lesions confirmed by CT scan (n=8119)

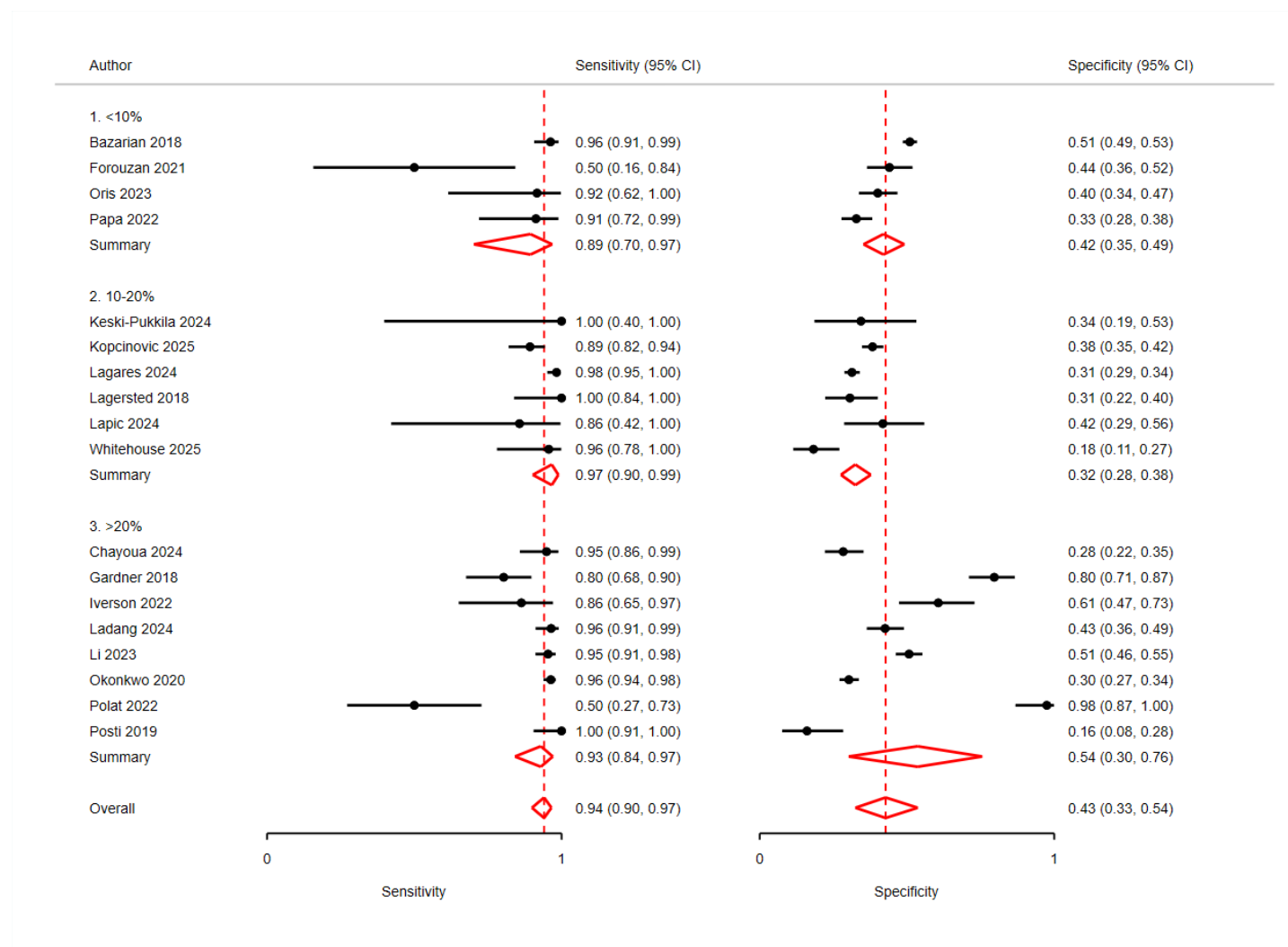




Figure 16 – Deeks' funnel plot of studies on GFAP performance

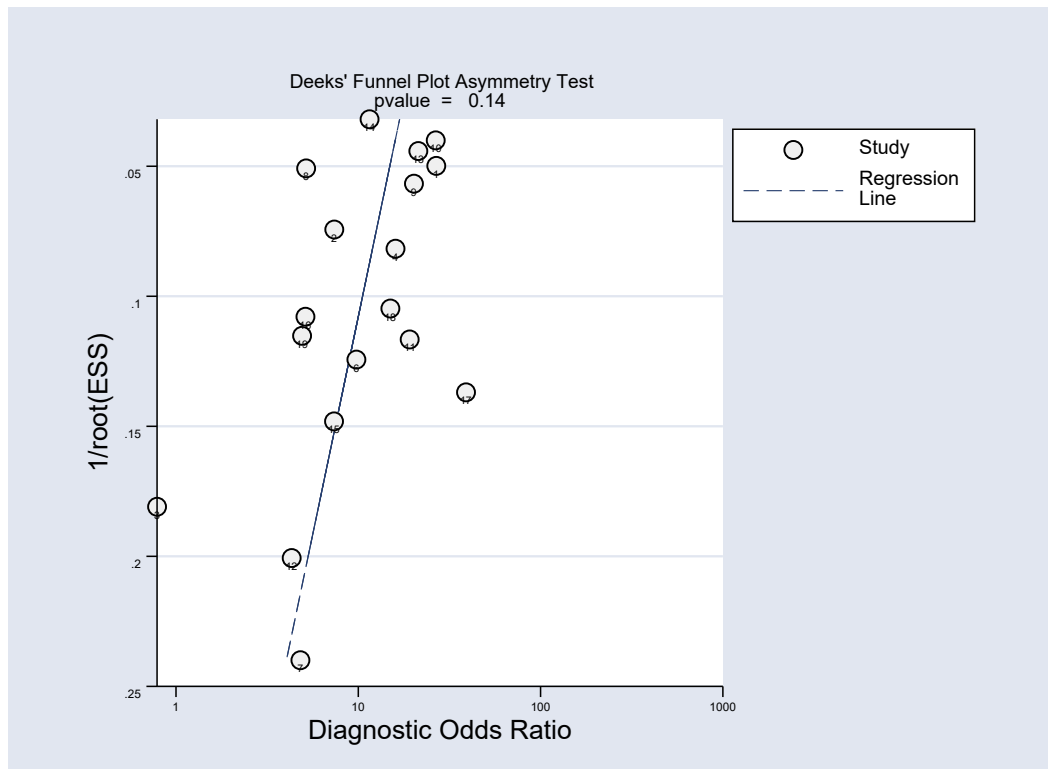




Figure 17 – QUADAS-2 quality appraisal of studies on GFAP performance

	Risk of bias domains				Overall
	D1	D2	D3	D4	
Bazarian 2018{Bazarian, 2018 #27}	⊗	⊕	⊕	⊕	⊖
Chayoua 2024{Chayoua, 2024 #4}	⊕	⊕	⊕	⊕	⊕
Forouzan 2021{Forouzan, 2021 #17}	⊗	⊖	⊖	⊕	⊖
Gardner 2018{Gardner, 2018 #16}	⊗	⊖	⊕	⊗	⊗
Iverson 2022{Iverson, 2022 #15}	⊗	⊖	⊕	⊗	⊗
Keski-Pukkila 2024{Keski-Pukkila, 2024 #38}	⊗	⊕	⊕	⊗	⊗
Kopcinovic 2025{Kopcinovic Milevoy 2025}	⊖	⊖	⊖	⊕	⊖
Ladang 2024{Ladang, 2025 #5}	⊕	⊖	⊕	⊖	⊕
Lagares 2024{Lagares, 2024 #6}	⊖	⊕	⊕	⊕	⊕
Lagerstedt 2018{Lagerstedt, 2018 #19}	⊗	⊗	⊖	⊖	⊗
Lapić 2024{Lapić, 2024 #7}	⊗	⊖	⊖	⊕	⊖
Li 2023{Li, 2023 #3}	⊖	⊕	⊕	⊕	⊕
Okonkwo 2020 {Okonkwo, 2020 #56}	⊗	⊖	⊕	⊗	⊗
Oris 2023{Oris, 2024 #2}	⊗	⊖	⊖	⊕	⊖
Papa 2022{Papa, 2022 #1}	⊗	⊕	⊕	⊕	⊕
Polat 2022{Polat, 2022 #28}	⊗	⊖	⊖	⊕	⊖
Posti 2019{Posti, 2019 #18}	⊕	⊖	⊖	⊕	⊖
Whitehouse 2025{Whitehouse, 2025 #22}	⊗	⊖	⊕	⊕	⊖

Study

Domains:
D1: Patient selection.
D2: Index test.
D3: Reference standard.
D4: Flow & timing.

Judgement
⊗ High
⊖ Some concerns
⊕ Low

3.2.3.3 UCH-L1 alone

Fourteen studies assessed the performance of UCH-L1 alone to rule out brain lesions in patients with a mTBI, 13 of which were in adults^{34, 69, 71-74, 76, 78, 79, 81, 82, 84, 92} and one in children⁴¹ (Table 6). Of these 13 studies, all but one were also included in the analysis of GFAP+UCH-L1 combined.⁹²

Most of the studies reported a sensitivity lower than 80% with LR- between 0.20 and 0.60, i.e. with a moderate to low exclusion power of the test. There were three notable exceptions: Li 2023 reported a sensitivity of 95% (95%CI: 89.4-98.1). However, it had a low specificity (18%) and the LR- was 0.29 (95%CI: 0.13-0.65), i.e. a low power of exclusion.⁸¹ In Papa 2022, the sensitivity was 96% (95%CI: 76-100) when the cut-off was set at 189 ng/L and the test done within 4 hours after the trauma. Nevertheless, the result was quite imprecise.³⁴ Finally, in Whitehouse et al. 2025, the optimal cut-off

was defined based on the ROC curve, and a sensitivity of 95% could be reached with a cut-off set at 31.6 and 17.9 ng/L for tests measured within 6 and 24 hours, respectively. However, the LR- were very imprecise.⁹²

Given these globally poor results, in addition to the lack of clearance from the FDA and CE labelling, we did not perform a meta-analysis.

3.2.3.4 Protein S100 β

The performance of the protein S100 β was assessed in 9 studies (Table 7).^{67-70, 81, 82, 84, 91, 92} Seven studies were in adults and two in children.^{41, 67} The results were extremely heterogeneous with a sensitivity going from 100% in three studies to a low 87.8% in Lagares 2024,⁶⁹ although in the latter the recommended cut-off (0.1 μ g/L) was applied in the Roche well-known device.^{69, 84} Such variations could be linked to various assays, time elapsed between injury and biomarker dosage, and cut-offs defining a positive test. Given these variations and the low number of studies, no attempt to meta-analyse data was made and we refer the reader to a more extensive meta-analysis published by NICE in 2023.⁵⁷

A comparison with the combination GFAP+UCH-L1 was provided in 5 of these studies (a table is presented in the supplement).^{68, 69, 81, 82, 84} Puravet 2025 was carried out in children and Trivedi 2024 determined the cut-offs to maximise the Younden's index.^{68, 84} In two of the three remaining studies (i.e. Lagares 2024⁶⁹ and Li 2023⁸¹), the protein S100 β (measured within 6 hours) had a lower sensitivity than GFAP+UCH-L1. The sensitivity of protein S100 β (measured within 3 hours) and that of GFAP+UCH-L1 was similar in the last study by Oris 2023,⁸² but the specificity was lower for the protein S100 β (26% vs. 32%), although this difference was not statistically significant.

3.2.4 On-going studies

We searched the study register ClinicalTrials.gov for ongoing studies with query ((GFAP OR UCH-L1) AND Mild TBI) on 31 October 2025. Results are presented in Table 10. We retrieved 10 on-going studies, some of which are already in the analysis phase, notably the BRAINI-2 study in elderly and the BRAINI-2 study in children.^{94, 95} We can thus expect soon more data on children, with 2 other studies planned for completion in December 2027 (NCT06940232) and December 2029 (NCT05964764). There should also be more data available soon on the performance of the VIDAS device (the 2 BRAINI-2 studies, NCT05108909, NCT06449183). Only one of these planned studies is interventional, and it aims at testing the ability of the Abbott i-STAT TBI to decrease the number of head CT scan (NCT06766435).



Table 10 – On-going studies on GFAP and UCH-L1 combined or separated referenced in ClinicalTrials.gov (30/11/2025)

NCT	Title	Population	Completion date
NCT05425251	BRAINI-2 Elderly MTBI European Study ⁹⁴	OLDER_ADULT	Mar-25
NCT05413499	Blood Biomarkers to Improve Management of Children With TBI (BRAINI-2) ⁹⁵	CHILD	Apr-25
NCT05108909	Rapid Diagnosis and Prognosis Recognition of Imaging and Biomarkers in Mild to Moderate TBI	ADULT, OLDER_ADULT	Aug-25
NCT06766435	Evaluation of the Abbott i-STAT TBI Biomarker Test	ADULT, OLDER_ADULT	Oct-25
NCT06449183	VIDAS® TBI Real Life Performance in Subjects with Mild TBI (mTBI)	ADULT, OLDER_ADULT	Nov-25
NCT05885529	Glial Fibrillary Acidic Protein (GFAP) and Ubiquitin Carboxy-terminal Hydrolase L1 (UCH-L1) to Exclude Lesions Linked to Significant Traumatic Brain Injuries	ADULT, OLDER_ADULT	Mar-26
NCT06940232	Validating a Blood Test for the Detection of TBI in Children	CHILD	Dec-27
NCT05588115	Rapid, Accurate, Cost-effective Assessment of Blood Biomarkers for Diagnosis of Concussion	ADULT, OLDER_ADULT	Dec-27
NCT05964764	Validation of the Scandinavian Guidelines for Minor and Moderate Head Trauma in Children	CHILD	Dec-29
NCT04602806	Transforming Research and Clinical Knowledge in TBI (TRACK-TBI) Precision Medicine Phase 2 Option 1	ADULT, OLDER_ADULT	Apr-30

3.3 Discussion

- The priority of the biomarkers-based test is to rule out cerebral lesions. To reach this aim the highest sensitivity and the lowest LR- are required. Available studies reported an excellent performance of GFAP+UCH-L1, without big variations in sub-group analysis. The close to 100% sensitivity is due to the different and complementary half-lives of the 2 proteins. UCH-L1 allows for early detection (peak concentration immediately after mTBI) of patients with ICIs, whereas GFAP identifies patients after 8 hours, hence the recommendation of measurement within 12 hours of trauma.⁴¹ It should be noted that most of the studies were carried out in adults, and more research is needed in the child population in whom avoiding useless CT scan is particularly important. As mentioned in section 3.2.4, new studies are under way.
- In contrast to sensitivity, the specificity of the test was overall quite low (29%; 95%CI: 25-34), which will result in a substantial proportion of the test being falsely positive, particularly if the probability pre-test of a cerebral lesion is low. The specificity is even lower in adults 65+ years (3 studies). This could be explained by chronic low-grade astrogliosis, even in the absence of trauma, or other comorbidities.⁹⁶ Age-stratified reference ranges are therefore needed for accurate interpretation, as demonstrated in the study by Ladang et al. 2024 where the number of CT scans avoided in adults aged ≥ 65 years doubled when the cut-off for that sub-population were modified.⁷³ Additionally, patients with systemic trauma or multi-traumas present a challenge as physical injuries outside of the central nervous system can also contribute to elevated serum

levels, complicating the specificity of GFAP and UCH-L1 for brain injury.⁵¹ Further research should propose means to improve the specificity of the GFAP+UCH-L1 test. For example, it has been proposed that considering the test positive only when both GFAP and UCH-L1 levels exceed their respective cut-off values could improve the specificity,⁴¹ but that would be at the expense of the sensitivity.

- The performance of the combination of GFAP and UCH-L1 is similar to that of protein S100 β as reported in the recent meta-analysis by NICE.⁵⁷ GFAP+UCH-L1 present two possible advantages over protein S100 β . First, their concentrations are not affected by skin pigmentation, facilitating their use in routine practice. Second, the sampling window can be extended up to 12 hours post-mTBI, or even 24h for the i-STAT Alinity (as reported in FDA clearance, see Table 3), whereas, for S100 β the window is 3 to 6 hours.^{21, 22}
- One additional advantage of GFAP+UCH-L1 over CT scan could be discharging patients from ED department more rapidly and improving patients flow. This could be particularly the case with the handheld point-of-care i-STAT Alinity (Abbott) which could deliver results in 15 minutes. However, all the studies included in our review that assessed the performance of that device used frozen plasma samples. Hence, performance with whole blood must still be documented in good quality studies. Moreover, the idea that measuring GFAP+UCH-L1 saves time is not straightforward. For example, in Curran 2025 the median time from request to CT report availability was 89 minutes (IQR: 53; 136) and from venesection to biomarker result availability 88 minutes (IQR: 75; 109)^{t, 77}. Furthermore, for the great proportion of patients with a false positive test who will have a CT scan, the ED length of stay may be significantly increased.
- Even more important is to test the added value of biomarkers dosage in real-life conditions. Randomized (multicenter) interventional trials to assess the benefits of biomarkers (improved patients flow, number of CT scans avoided) in current practice are needed.
- GFAP alone had a sensitivity similar to that of the combined test and a better specificity, the latter probably because it indicates structural glial injuries. However, FDA issued a clearance only for the combined test, and this approach has been endorsed in many scientific and professional guidelines. GFAP-only testing is being explored mainly for follow-up, research, or delayed assessment purposes, rather than for initial emergency triage. More good-quality studies are needed to define cut-offs and time windows for safe standalone use.
- It should also be noted that research so far focused on the ability of biomarkers to rule out brain injury that would be detectable by a CT scan. In that context, an elevated dosage with a negative CT scan is considered a failure of the test (false positive). It has been suggested that blood biomarkers are more objective and sensitive than head CT and they might be able to detect subtler ICIs, not visible with standard neuroimaging techniques, but detectable using MRI. The inadequacy of head CT as a reference method in studies assessing the diagnostic accuracy of blood biomarkers might directly influence their low specificity values. A positive test may reflect microstructural lesions which are not detectable at a macrostructural level with the CT scan. Detecting such microstructural lesions could serve as a guide to more structured follow-up, aiming to reduce long term (persisting) symptoms.^{5, 74, 76} The absence of CT-related intracranial lesions does not guarantee a lack of persistent symptoms post-TBI.⁹⁷

^t As the samples were frozen, 'time to result' was estimated as time from venepuncture to sample reception in the biochemistry laboratory plus 60 min (the time taken for sample preparation, testing and issuing of result).



4 ECONOMIC STUDIES EVALUATING THE USE OF BIOMARKERS IN MTBI

KEY POINTS

Key findings:

- All three studies identified via our search support the potential for biomarker-guided strategies (GFAP+UCH-L1) to reduce unnecessary CT scans and improve resource use in mTBI management.
- All studies used the same source for their biomarker accuracy data (i.e. the ALERT-TBI study).
- European evaluations (France & Spain) showed potential cost savings, though with differing magnitudes due to model assumptions and healthcare costs.
- Biomarkers are recognized to be most useful in cases where CT decisions are uncertain, (mTBI patients with an intermediate risk) and should complement, not replace, clinical decision rules.

Limitations:

- Limited economic evidence and reliance on assumptions not well backed up by the evidence, especially about clinical practice patterns and biomarker implementation.
- Only one study incorporated clinical head rules (CCHR); others used oversimplified models.
- Real-world challenges (e.g. workflow integration) not addressed in the models.
- Transferability to Belgium remains to this date uncertain due to differences in healthcare systems and lack of national data.

This chapter provides an overview of economic studies evaluating the use of GFAP and UCH-L1 used individually or in combination to rule out ICIs in patients with mTBI. The aim is to review the literature on the economic value of these tests as an alternative to current practice, where more expensive and potentially harmful CT scans are often performed.

4.1 Methods

4.1.1 Search strategy

A systematic search for relevant publications was carried out on the 14th of July 2025 with the consultation of the following electronic reference databases:

- Medline,
- EMBASE,
- Econlit
- and the Cochrane library database.

These were searched to retrieve primary full economic evaluations (studies comparing both costs and outcomes) and reviews of economic evaluations (i.e. secondary economic evaluations). Our search strategy is provided in an appendix.

Furthermore, the websites of Health Technology Assessment institutes listed on the INAHTA website (International Network of Agencies for Health Technology Assessment) were also be checked to capture reports in this field of interest.

No restrictions were imposed for language but the time period was limited to studies published after the release of the review by NICE in 2023,²⁰ during which the authors could not identify any economic studies.

4.1.2 Selection procedure

To identify potentially relevant evaluations for our analysis we first went through all titles and abstracts in order to exclude any obvious studies that did not match our research question. All articles that appeared to be interesting, or for which there were some doubts, were read in full in order to select those relevant for inclusion in our review. Reference lists of the selected primary and secondary studies found via our search were checked for additional references worth adding to our analysis. Study selection was completed by one researcher but any doubts that came up during the exercise were discussed and solved in collaboration with a second reviewer. Studies finally included in our review were critically appraised by using an in-house structured data extraction sheet based on the CHEERS 2022 checklist.^u

4.1.3 Selection criteria

All economic evaluations looking at any GFAP and UCH-L1 (in combination or individually) as diagnostic tests for ruling out ICI in patients presenting at the ED with mTBI were included in our review. No specific terms for “mild” were included in our search and the selection of relevant papers on mTBI was done by KCE researchers on the basis of the GCS score, which had to be from 13 to 15, with some of the included studies focusing purely on GCS 14-15. Cost descriptive analyses or cost or pricing comparisons that did not consider the effectiveness of the test in accurately identifying ICI were excluded. Similarly, publications in the form of letters, editorials or notes and abstracts were excluded from our review, since these would not offer enough information to critically appraise their findings. An overview of the inclusion/exclusion criteria is given in Table 11.

Table 11 – Selection criteria for economic evaluations

Selection criteria	Inclusion criteria	Exclusion criteria
Population	Patients presenting at the ED with mTBI (i.e. GCS 13-15) following a trauma accident.	Moderate or severe TBIs.
Intervention	GFAP, UCH-L1 OR the combination of both	Any other biomarkers.
Comparator	Current practice including CT scans.	Clinical practice not considering CT scans.
Design	Analyses comparing GFAP and UCH-L1 (individually or in combination) versus head CT from both a cost and an accuracy perspective.	Pricing comparisons, or cost descriptions no taking into consideration test accuracy.
Type of publication	Primary studies or reviews.	Letters, editorials, notes, abstracts, posters.

CT: Computed Tomography; ED: Emergency Department; GFAP: Glial Fibrillary Acidic Protein; ICI: Intracranial Injuries; mTBI: mild Traumatic Brain Injuries; TBI: Traumatic Brain Injuries; UCH-L1: Ubiquitin Carboxy-terminal Hydrolase L1.

^u <https://www.equator-network.org/wp-content/uploads/2013/04/CHEERS-2022-checklist-1.pdf>



4.1.4 Data analysis:

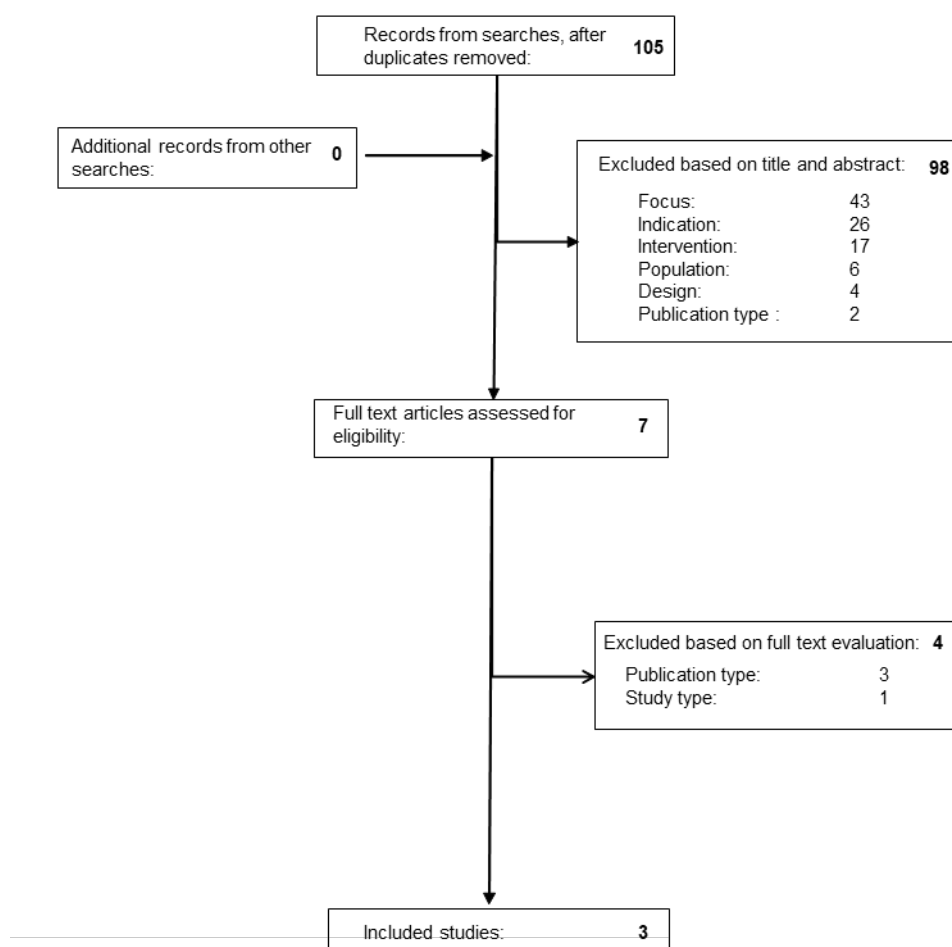
No attempt to perform a full cost-effectiveness study was done due to the limitations of the available Belgian data that would have resulted in great uncertainty (eg. no clear and accurate identification of mTBIs is possible in existing, accessible administrative databases). Instead, (limited) data analyses looking at the number of CT scans currently performed after mTBIs in Belgium, based on our survey of Belgian experts (see chapter 6) and the number of these that could be (theoretically) spared, based on the pooling of sensitivity and specificity results extracted from the clinical literature (see chapter 3 on the performance of biomarkers to exclude cerebral lesions) are presented in a subsequent chapter (i.e. chapter 7).

4.2 Results

Our search returned 105 citations, after eliminating duplicates. Of those, 96 did not meet our inclusion criteria based on a review of their title and/or abstract, mainly because of their focus (45) or the indication investigated (26). Other reasons for exclusion included the intervention/biomarker under research (17), the population (6), the study design (4) and the publication type. Seven studies in total were reviewed in full. From these, 4 were excluded. Three because they were abstracts and therefore did not offer enough information to allow for their critical review.⁹⁸⁻¹⁰⁰ The remaining consisted of a review of studies and was therefore only used for reference-checking purposes.¹⁰¹ A review of the grey literature did not identify further economic evaluations that could be of interest for the purpose of our review. This left us with 3 relevant studies to include in our review.

Our literature selection process is illustrated in a flow chart in Figure 18.

Figure 18 – Flow chart selection of costing studies



4.2.1 Overview of economic evaluations

From the three studies, one was performed in the USA,¹⁰² one in France¹⁰³ and one in Spain.¹⁰⁴

The US study dates from 2019, while the French and Spanish evaluations were published more recently, in 2023 and 2025 respectively. The three studies were model-based (decision-tree models), but presented important differences that will be explored in the following sections of this chapter.

4.2.2 Type of economic evaluation

Two studies performed cost-utility analyses (CUA),^{102, 103} while the remaining, the Spanish publication, presented a budget impact analysis (BIA).¹⁰⁴ Nevertheless, the accuracy of the biomarkers to detect ICIs was also considered in the latter. The three studies looked at the combination of GFAP and UCH-L1 versus standard practice, including CT scans and did not include the individual biomarkers in their models.

4.2.3 Time frame of analyses and discounting

Despite the acute nature of the indication here reviewed, and the fact that symptoms linked to mTBI tend to develop within the first days after the trauma accident,¹⁰⁵⁻¹⁰⁷ the two cost-utility studies looked at costs and outcomes over a lifetime, considering also less frequent, but longer-term harms that can be experienced by these patients.^{102, 103} Discounting differed from one study to the other. Thus, while the US evaluation¹⁰² followed the recommendations of the Panel on Cost-effectiveness in Health and Medicine,¹⁰⁸ and applied a constant annual discount rate of 3% for both cost and outcomes, the French study¹⁰³ applied an annual discount rate of 2,5% up to 30 years and 1,5% thereafter (also for both costs and outcomes), aligned with the directives of the Haute Autorité de Santé.¹⁰⁹ The authors of the BIA limited its timeframe to one year, and as a consequence, it did not require discounting.¹⁰⁴

4.2.4 Perspective and population

The included studies differed in their perspective. Moya Torrecilla,¹⁰⁴ completed their BIA from a hospital perspective. The US cost-utility study by Su et al.¹⁰² was carried out from a societal perspective, while the study by Zimmer et al.¹⁰³ followed a health care system perspective.

With regards to the population studied, Moya Torrecilla et al.¹⁰⁴ and Zimmer et al.¹⁰³ modelled a population of adults presenting to the ED with mTBI, defined by a Glasgow Coma Score (GCS) of 13–15. In both studies, all patients were assumed to present to the ED within 12 hours of injury.

In 2019, Su et al.¹⁰² modelled a population of patients with mild or moderate TBI presenting to the ED within 12 hours from injury, but in this case, they defined “mild” as a GCS of 14–15, with a GCS 13 already considered by the authors as “moderate”. Their base case model was set for a 20-year-old male, with additional analyses performed for older male and female patients (aged 40, 60, and 80).

Table 12 – Overview of cost evaluations on GFAP+UCH-L1 in mTBI

Study (Yr)	Country	Type of analysis	Perspective	Time horizon	Discount rate
Sue (2019) ¹⁰²	USA	CUA	Societal	Life-time	3% (both costs and outcomes).
Zimmer (2023) ¹⁰³	France	CUA	Healthcare system	Life-time	2.5% up to 30 years; 1.5% thereafter (both costs and outcomes).
Moya Torrecilla (2025) ¹⁰⁴	Spain	BIA	Hospital	1 year	NA.

BIA: Budget Impact Analysis; CUA: Cost-Utility Analysis. NA: Not Applicable.



4.2.5 Intervention and comparator

All evaluations identified via our search focused on the potential gains that could be derived from the use of GFAP in combination with UCH-L1 at the ED, as a screening tool to discard ICIs in adults presenting mTBI and thus, on the potential that such biomarker would have to reduce the need for CT scans. It is important to note that these biomarkers are currently recommended for use in adults, which explains the more limited focus on paediatric populations that was already seen in our review and MA of the clinical evidence.

The Spanish BIA presented “GFAP+UCH-L1 for all” mTBI patients, versus “CT scans for all.

The French cost-utility analysis¹⁰³ included the following comparisons:

1. GFAP+UCH-L1 compared to CT scanning within 12 h of injury.
2. GFAP+UCH-L1 versus S100 β within 6 h of injury.
3. GFAP+UCH-L1 versus a combination of S100 β and CT scans, assuming different proportions of patients arriving within three hours and getting S100 β , (reflecting the timeframe recommended in the French guidelines) and the rest getting a CT scan (i.e. 30%, 50% and 70% of mTBI cases).

The USA analysis by Su et al.¹⁰² compared “GFAP+UCH-L1 for all”, and a combination of CCTH rules (pre or post GFAP+UCH-L1 testing) versus their “standard of care” which was defined as the use of CCTH rules followed by a CT scan when recommended. As we will see later on in this report, according to a survey amongst Belgian experts, the CCTH rules appear to be the most commonly used clinical rules in Belgium at present.

4.2.6 Cost and outcome inputs

Costs used in the two cost-utility analyses included in this review,^{102, 103} were primarily based on reimbursement rates derived from administrative data. While, the US study¹⁰² derived costs (updated to the year 2018) from Medicare and Medicaid reimbursement codes, the more recent French cost-utility study,¹⁰³ gathered tariffs from the social security, the ministry of health and official hospital tariffs (for the year 2020). The remaining, the BIA,¹⁰⁴ used hospital tariffs of the year 2023 from the most representative autonomous regions in Spain, as well as other official national sources, such as the Minimum Basic Data Set of the Ministry of Health^v and the Spanish Network of Hospital Costs.^w

Assumptions made for the cost of the GFAP+UCH-L1 test in the two studies where this was specified, were similar, with Moya Torrecilla et al.¹⁰⁴ quoting €32.00 per test based on real-world use in a Spanish hospital over two years, and Zimmer et al assuming parity with S100 β at a cost of €32.40 per test.¹⁰³ The remaining study, did not assume a cost for the biomarker and estimated instead the maximum cost that such test would need to have in order for it to be cost-effective at common US willingness to pay thresholds (i.e. US\$50 000 per QUALY).¹⁰²

Factors taken in consideration for the costing side of the two European evaluations here described^{103, 104} included ED visits, CT scans, biomarkers, observation, hospitalisation, or physicians' time, neurosurgery, and subsequent visits to the ED. In addition to this, the study of Zimmer et al.¹⁰³ also included outpatient visits, reflecting its wider health care system perspective, versus the hospital perspective used in the Spanish BIA.¹⁰⁴

The study by Su et al.¹⁰² did not provide a direct description of their costing considerations and sources and referred instead to an older publication dating from 2012¹¹⁰ focusing on severe TBI. Thus their

^v Registro de Actividad de Atención Especializada. RAE-CMBD. Ministry of Health: <https://www.sanidad.gob.es/estadEstudios/estadisticas/cmbdhome.htm>

^w Red Española de Costes Hospitalarios: <https://www.rechosp.org/faces/es/jsf/index.jsp>

costs covered mainly hospitalisation and long-term rehabilitation costs. In addition to these, the authors also considered indirect cost (productivity costs), given its societal perspective.

Regarding outcome inputs, both cost-utility studies, included probabilities on the incidence of ICIs, the need for surgery, the probability of prolonged symptoms, utilities (by Glasgow outcome score - GOS^x) and the accuracy of the biomarker. The estimates were derived in both cases from the literature, but not surprisingly, differed in their sources, given the time elapsed from one publication to the other. Nevertheless, a crucial estimate was common to both CUAs. Thus, the accuracy of the GFAP+UCH-L1 was extracted in both cases from the FDA market authorisation report.¹¹¹ This market authorisation based the accuracy of GFAP+UCH-L1 in the ALERT-TBI study by Bazarian et al.,⁷⁴ which studied a population of 1947 patients and reported a sensitivity of 97,5% and a NPV of 99,6%. Regarding the prevalence of ICIs, the French study,¹⁰³ also used estimates from the ALER-TBI study, while Su et al.¹⁰² estimated this from different studies. Other sources from the literature were used for their sensitivity analysis.

The BIA published in 2025, based their probabilities primarily on estimates made by a group of experts from various specialties (emergency medicine, radiology, clinical laboratory, and clinical biochemistry), with practical experience with the GFAP + UCH-L1 test in Spanish hospitals. Probabilities on the prevalence of ICIs, observation, need for surgical treatment, hospitalisations, readmissions, necessary follow-up or subsequent CT scans were considered. In the few cases in which estimates could not be provided by the experts, data was taken from a US cost-effectiveness study of the S100 β protein by Ruan et al. published in 2009.¹¹² Sensitivity and specificity of the biomarker test were estimated at 98,96% and 32,93% respectively. The NPV for these estimates corresponds to a 99,68%. These estimates are therefore higher (only slightly in the case of the NPV) than those used in the CUAs and quoted by Bazarian et al.⁷⁴

Table 13 – Costs and outcomes included in the economic evaluation on GFAP+UCH-L1 in mTBI

Study (Yr)	Costs included	Cost sources	Outcomes included	Outcome sources
Sue (2019)¹⁰²	Direct costs: CT, biomarkers: GFAP-UCH-L1, surgery, hospitalisation, rehabilitation, Indirect costs: productivity.	Medicare and Medicare tariffs, literature (2018 US\$).	ICIs, surgery, GFAP-UCH-L1 accuracy, utilities linked to GOS scores, GOS specific mortality rates.	Meta-analyses, Social Security data, literature.
Zimmer (2023)¹⁰³	ED visits, CT, biomarkers: GFAP+UCH-L1 and S100 β , hospitalisations, physician resources, subsequent ED visits surgery, GOS health state costs.	French tariffs, INSEE, literature, (in 2020€).	Neurosurgical and non-neurosurgical lesions, surgery, GFAP+UCH-L1 and S100 β accuracy, utilities, cancer risk and mortality from CT scan exposure.	ALERT-TBI, ⁷⁴ CHIP study, ¹¹³ literature, expert opinion.
Moya Torrecilla (2025)¹⁰⁴	ED visits, CT, follow-up CT, observation, neurosurgery, ICU, biomarker: GFAP+UCH-L1, outpatient, operating room, readmissions.	Hospital tariffs from autonomous regions, Spanish Network of Hospital Costs (RECH), Ministry of Health data, expert panel (in 2023€).	ICIs, observations, surgery, hospitalisations, readmissions, physicians time, number of CTs.	Expert panel, literature (Ruan et al. 2009 ¹¹²).

INSEE: Institut national de la statistique et des études économiques; ED: Emergency Department; GOS: Glasgow Outcome Score; ICU: Intensive Care Unit; LYG: Life Years Gained; QALY: Quality Adjusted Life Year;

^x Clinical tool to assess the functional outcome of patients after brain injury. It categorises recovery into five levels: Death, persistent vegetative state, severe disability, moderate disability, and good recovery.



4.2.7 Modelling

The studies here reviewed consisted of decision-tree modelling exercises. However, their general structure and specific branches differed from one study to another.

The simplest structure was displayed in the BIA by Moya Torrecilla et al.,¹⁰⁴ where only two scenarios are compared: “CT scan for all patients”, defined as routine clinical practice by which 7% of patients (those with GCS 15 and no risk factors or symptoms) are discharged without a head CT scan and the remaining 93% undergo a CT scan) versus “GFAP+UCH-L1 test for all” described as biomarker testing in all those patients who would get a CT scan under routine clinical practice, arriving to the ED within 12 hours from injury (~88% of all mTBI patients), followed by CT scan if the result of the biomarker is positive.

Patients start at the initial ED visit where 93% of them will either get a CT scan or a biomarker test, depending on the scenario tested. It is assumed that the remaining 7% are patients would present a GCS of 15 and no symptoms and thus, these are discharged from the hospital without imaging or testing.

Positive biomarker testing determines if a patient would need a scan (all those with a positive test get a scan), and the results of the CT scans (positive/negative) determine if patients are discharged, left under observation or require hospitalisation or surgery. Follow-up CTs for symptomatic patients are considered, as well as readmissions for false negatives.

Su et al.¹⁰² compared four different management strategies: Canadian CT head rules (CCTH), followed by CT scan when medium or high-risk; GFAP+UCH-L1 test for all, followed by CT scan if test is positive; CCTH rules then GFAP+UCH-L1 test in medium or high-risk patients; and GFAP+UCH-L1 test followed by either CCTH rules (if the test is negative), or CT scan (if the test is positive).

Initial biomarker or clinical head rules are followed by CT scans if indicated. The results from the CT scan will indicate if there is a lesion and if so, if it requires surgery or other “appropriate treatment”. Glasgow outcome scores (GOS) are considered as well as the impact that delayed surgery due to false negatives could have on such outcomes. Readmissions for all patients with a non-surgical injury are possible.

Finally, the French evaluation,¹⁰³ included three comparisons: GFAP+UCH-L1 versus CT for all; GFAP+UCH-L1 versus S100 β protein within 6 hours from injury, followed by a CT scan only in biomarker positive cases, and GFAP+UCH-L1, followed by CT scan if biomarker positive versus different combinations of S100 β and CT strategies (i.e. S100 β only performed within 3 hours from injury in 30% 50% or 80% of patients with the remaining undergoing a CT scan).

In the initial ED visit the patient either gets a biomarker test (S100 β or GFAP+UCH-L1) or a CT scan. Those in which a biomarker test is performed, will be referred to a CT scan if the test is positive. CT results would indicate the presence or not of a lesion, and its type (i.e. non-neurosurgical or neurosurgical) and appropriate management (discharge, observation or surgery) would be decided on that basis. This model includes long-term GOS health states (1-5), based on the literature. In their base-case scenario, an assumption was made that GOS remains constant over the lifetime of a patient. This assumption was tested in their sensitivity analysis, where transitions between health states were allowed after 6 years (consistent with a study by Pandor et al.¹¹⁴ This study also considered radiation-induced cancer risk and mortality. Utility estimated were extracted from the literature.

4.2.8 Reductions in CT scans and potential savings

The study by Moya Torrecilla¹⁰⁴ modelled potential savings for a population of 3 500 patients with mTBI. They reported a reduction of 28.50% in the proportion of patients undergoing at least one CT scan, from 93% for the CT arm to 66,5% under the GFAP+UCH-L1 testing scenario. The reduction in overall CT scans (including follow-up scans) was higher and reached 35.04% (from 4 177 to 2 713). The BIA estimated a mean cost per patient of €899 for routine care (CT scan) versus €700 for the biomarker strategy, resulting in a per-patient saving of €199. The authors highlighted that the

biomarker pathway also reduced mean patient management time by 111 minutes, mainly by reducing the time linked to patient observation.

The analysis by Su et al.¹⁰² found that a combined strategy, biomarkers first, followed by CCTH was the most effective, resulting in 28.2952 (SD: 0.0011) quality adjusted life years (QALYs). However, expected QALYs were highly similar across all scenarios, with the CCTH rules first, followed by the biomarker showing the lowest QALYs per patient: 28.2898 (SD: 0.0359). The authors do not present the costs in their article but referred to an older publication (from 2012) in which these are detailed instead. Nevertheless, that publication focused on severe TBI and thus, the focus for direct medical costs was on costs associated with the first hospitalisation, surgery and rehabilitation of medical needs linked to the GOS after surgery. The authors did not estimate ICERs. Instead, they stated that the biomarkers first, followed by the CCTH rules, would be considered cost-effective at a willingness to pay (WTP) threshold of US\$50 000, if the cost per test is maximum US\$308.96. It is important to highlight that these estimations include indirect costs (i.e. productivity), and that the authors did not present separate estimations with and without such costs. The results offer a societal perspective only, different to the recommended public and private payer perspective recommended as reference case in the current Belgian guidelines for economic evaluation.¹¹⁵ It is also important to mention, that the use of these biomarkers is at present recommended after clinical assessment (most often by means of clinical head rules) and not prior to these. Therefore, the most cost-effective alternative presented in Su et al.¹⁰² appears not to be as relevant as others, with slightly less QALYs (i.e. CCTH rules followed by GFAP+UCH-L1 in medium or high-risk patients), for which no detailed results (maximum cost per test at current WTP thresholds) are offered.

Finally, the study by Zimmer et al.¹⁰³ reported that when GFAP+UCH-L1 was used instead of CT for all, CT scan utilisation dropped by 29.68% (from 1096 to 771 per 1000 patients). The reduction in patients in which at least one scan was performed was higher, reaching 32.32%. In contrast to the results shown in the BIA by Moya Torrecilla,¹⁰⁴ Zimmer et al. presented little difference between follow-up CT scans between the GFAP+UCH-L1 and the CT for all alternatives, with a reduction in follow-up scans of just 2.29%, from 96.3 to 94.09, while the reduction in follow-up scans in Moya et al. was of 58% (from 922 to 386 for a population of 3 500 patients). This is likely to reflect differences in national protocols/practice regarding follow-up scanning versus observation.

In Zimmer et al.,¹⁰³ the overall savings associated to the use of GFAP+UCH-L1 instead of the current strategy of CT for all remained modest, at €4.15 per patient, despite the reduction of CT scan use previously mentioned of almost 30%.

When compared directly to S100 β , GFAP+UCH-L1 led to 5.6% fewer CT scans per 1000 patients (dropping from 817 with S100 β to 771 with GFAP+UCH-L1) and a cost reduction of €4.73 per patient. Outcomes measured in QALYs and life years were practically identical across the three screening strategies (biomarker testing with GFAP-UCH-L1 or with S100 β , or CT scan for all). GFAP+UCH-L1 also showed to be less costly with similar QALYs and LYGs when compared with a strategy in which only mTBI patients in which the S100 β test could be performed within 3 hours of injury were tested with the protein, while the rest had a CT scan. Such result was obtained independently of the assumptions made regarding the proportion of patients tested with S100 β (30%, 50% or 70%).

Since at the time of the analysis, the GFAP+UCH-L1 test was not yet marketed in France and its price was unknown, the authors assumed the same cost as the S100 β test, but also performed a break-even analysis in which they showed that in order to remain cost-neutral, the cost of GFAP+UCH-L1 per test would need to be equal or below €36.55.



4.2.9 Sensitivity analysis

Uncertainty is intrinsic to any economic evaluation and should therefore always be accounted for. All evaluations here included, performed some kind of sensitivity analysis to assess the robustness of their results.

Moya Torrecilla et al.¹⁰⁴ carried out a univariate sensitivity analysis in which they varied all parameters by $\pm 20\%$. Their overall results showed to be most sensitive to the proportion of positive biomarker tests and the percentage of patients tested but remained robust with GFAP+UCH-L1 offering savings under all estimations pursued.

Su et al.¹⁰² undertook one, two, and three-way sensitivity analyses. Their three-way analyses showed how the cost-effectiveness threshold for the biomarker test varies with both the probability of ICIs and the probability that a lesion requires surgery. The model explored a wide range of plausible values and found the main findings to be robust. The authors also performed a Monte Carlo simulation but only for effectiveness, since appropriate costing data was missing for the biomarker.

Zimmer et al.¹⁰³ also performed one-way, scenario and probabilistic sensitivity analyses during which variation in key parameters were tested. Their one-way sensitivity analysis showed the prevalence of no lesions, the specificity of the biomarkers or the proportion of patients discharged immediately after a negative biomarker test to be the most influential factors. Nevertheless, GFAP-UCH-L1 facilitated the reduction of the overall number of CT scans, when compared to CT all or S100 β , for all plausible tested values. With regards to the results from their probabilistic analysis, no information is given in the main article. However, a table is provided by the authors as an appendix detailing mean values, SD and 5th and 95th percentage intervals for costs and number of CT scans. While the mean cost difference was negative implying that GFAP-UCH-L1 offers savings, and the 5th percentile was also negative, the 95th percentile was positive, which means that some simulations did not favor GFAP+UCH-L1. No additional information or further interpretation of these findings are offered by the authors.

Table 14 – Results of economic evaluations on GFAP+UCH-L1 in mTBI

Study (Yr)	Costs	Outcomes	ICERs OR alternative summary results	Sensitivity/Robustness
Sue (2019)¹⁰²	Not explicitly reported for each strategy.	CCTH only: 28.29 QALYs GFAP+UCH-L1: 28.29 QALYs. CCTH first, then GFAP+UCH-L1: 28.29 QALYs. GFAP+UCH-L1 first, then CCTH: 28.30 QALYs.	ICERs not calculated (missing cost for biomarker test). GFAP+UCH-L1 CE at WTP US\$50 000, if cost/test ≤ US\$308.96.	One-/two-/three-way. Monte Carlo simulation for effectiveness only. Overall robust results.
Zimmer (2023)¹⁰³	GFAP+UCH-L1 vs CT for all: –€4.15/patient - CT scan: €568.43/patient. - GFAP+UCH-L1: €564.28/patient. GFAP+UCH-L1 vs S100β: –€4.73/patient - S100β: €569.01/patient. - GFAP+UCH-L1: €564.28/patient.	GFAP+UCH-L1 vs CT for all: no diff. in QALYs/patient: - GFAP+UCH-L1: 30.70. - CT scan: 30.70. ↓ in CT scans: 29.7% (from 1 096 to 771). GFAP+UCH-L1 vs S100β: no diff in QALYs/patient: - GFAP+UCH-L1: 30.70. - S100β: 30.70. ↓ in CT scans: 5.6% (from 817 to 771).	GFAP+UCH-L1 less costly for equivalent* QALYs/patient .	One-way, scenarios, probabilistic; results robust for one way and scenarios. No details available on probabilistic.
Moya Torrecilla (2025)¹⁰⁴	–€199/patient - Standard care (CT): €899 /patient. - GFAP+UCH-L1: €700/patient.	Care time/patient: -111 min ↓ in patients with at least one CT scan: 28.5% (-927, from 3255 to 2328). ↓ in overall number of CT scans: 35.04% (-1464 from 4 177 to 2 713).	NA	Univariate SA: ±20%. Robust results, most sensitive to % positive biomarker and % of patients tested.

*Minimal differences in QALYs per patient in favour of the GFAP+UCH-L1 management strategy versus the CT scan for all (+0,00003 QALYs/patient) or the S100β (+0,00001 QALYs/patient)



4.2.10 Conflict of interest

The study by Zimmer et al¹⁰³ was sponsored by BioMérieux, manufacturers of GFAP+UCH-L1 tests. The authors of the Spanish BIA¹⁰⁴ reported no conflict of interest but declare that Abbott facilitated the participation of an independent external consulting team to coordinate the different phases of the study. The remaining, the US cost-utility study¹⁰² reported not to have received any specific funding from manufacturers or external organisations and not to have any conflict of interest. The existence of conflicts of interest may introduce a bias which could affect the validity of the study results, although there is, up to date, no hard evidence on this.

4.3 Conclusion

Our review identified three economic evaluations assessing the use of GFAP+UCH-L1 biomarkers in the management of mTBI. One of these was a BIA, which relied on limited expert opinion and estimated costs over a one-year horizon.¹⁰⁴ The other two were CUAs that explored alternative screening strategies for detecting ICIs in mTBI populations, each based on distinct modelling frameworks and assumptions.^{102, 103}

All three studies, despite differences in scope, methodology, country and publication date, relied on the same source of evidence for biomarker accuracy: the ALERT-TBI study by Bazarian et al.⁷⁴ This was the case even though additional data on GFAP+UCH-L1 had become available between the publication of the first study by Su et al.¹⁰² and the other, more recent evaluations. Despite the fact that the sensitivity in this specific study seems to be aligned with the findings of our review and MA, this is not the case with the specificity, which is lower in our pooled analysis.

Collectively, the studies support the value of the GFAP+UCH-L1 biomarker in facilitating the exclusion of ICIs in patients with mTBI. Thus, each analysis demonstrated that biomarker-guided strategies can lead to a reduction in unnecessary CT scans, improving resource allocation, and, in the CUAs at comparable or slightly improved QALYs when compared to standard care.

However, despite the consistency in general findings, it is important to note that these evaluations rely on multiple assumptions, some of which are not strongly supported by empirical evidence. These is particularly the case for assumptions about clinical practice patterns. As such, important uncertainties remain, and the transferability of these results to other healthcare settings, such as Belgium, should be approached with caution.

4.4 Discussion

Important discussion points should be raised regarding the limited economic evidence to this date in this field.

4.4.1 Perspective of the evaluations

First, the US-based study does not provide detailed estimations on their costs and is performed from a societal perspective, which impedes the separation of productivity costs from direct costs to the health care payer and thus, an assessment of the weight associated to costs that are more relevant for the Belgian situation, where the current guidelines for economic evaluation¹¹⁵ recommend to use a health care payer perspective for their case base. This makes it a less informative study for our purposes.

4.4.2 Target population for biomarker use in mTBI

Second, it should be noted that the use of blood-based biomarkers such as GFAP + UCH-L1 in the assessment of mTBI should not replace clinical evaluation. These biomarkers are best employed as adjuncts to established clinical decision rules, such as the CCHR, which guide the initial triage and imaging decisions. Relying solely on biomarkers, especially given their limited specificity, may lead to increased CT utilisation, contrary to their intended purpose.

Clinical head rules enable stratification of patients into those who can be safely discharged without imaging and those who warrant further investigation due to elevated risk of ICI. Nevertheless, their low specificity, coupled with the fear of missing ICIs often leads clinicians to perform CT scans that might be avoided with additional diagnostic support from .

Despite this potential, only one CUA specifically incorporated the use of head rules, specifically the CCHR, into its modelling.

4.4.3 Challenges in biomarker use implementation

Challenges with real-world implementation such as test availability, physicians knowledge, turnaround time, or integration with clinical workflows may have a significant impact on the adoption of these types of biomarkers. These aspects, were not considered in the scope of the studies here analysed, since these are challenging to incorporate and fully cover in economic evaluations. Nevertheless, some of these points were specifically raised during our survey to the Belgian experts that we will cover in a subsequent chapter as reasons why they do not use biomarkers in their hospitals.

4.4.4 Effectiveness versus CT scans in economic evaluations

The magnitude of QALY gains in the CUAs was, as expected, minimal. The assumption that the gold standard (i.e. CT scan) has 100% accuracy in identifying ICIs makes the main objective for these biomarkers not to be an improvement in performance but rather a reduction in the use (and costs) of CT scans, and thus, in radiation, at a similar performance. On this regard, only one of the CUAs, the CUA by Zimmer et al.,¹⁰³ included radiation harms in their model, and the authors recognised the weight of such harms in the overall results to remain modest, since the increase in radiation linked to one single CT scan is considered relatively limited.

4.4.5 Comparison between the two European countries

Focusing on the two European studies, both evaluations reached consistent conclusions regarding the potential for cost savings through the adoption of GFAP+UCH-L1 biomarkers in clinical practice for mTBI. Despite this alignment, the magnitude of the estimated savings differed substantially, even though both models used the same biomarker accuracy data from the ALERT-TBI study by Bazarian et al.⁷⁴

This divergence can be partly attributed to the distinct nature of the studies: one was a short-term BIA conducted in Spain, while the other was a CUA performed in France with a lifetime horizon. A comparison of their assumptions and input parameters reveals several key differences.

4.4.6 Healthcare Tariffs and Cost Inputs

The Spanish model applied higher unit costs across multiple healthcare services:

- CT scan: €184 in Spain versus. €102.61 in France.
- Emergency department (ED) visit: €215 in Spain versus €25.42 in France.
- ICU admission: €857 in Spain versus €323.27 in France.
- ED observation: €410 in Spain versus €326.00 in France.

Although the difference in observation costs was less pronounced, reductions in observation-related expenditure were the main driver of overall cost savings in the Spanish BIA. This could potentially reflect variations in clinical practice between the two countries.

It is important to note that none of these analyses looked at the POC test, and that such a test could potentially bring in further gains in time.



4.4.7 Biomarker Test Costs

Despite the absence of an official tariff, both studies assumed a similar cost for the GFAP+UCH-L1 test: approximately €32. The French study based its estimate on the cost of the S100 β test, whereas the Spanish study derived its figure from operational data collected over two years from a single hospital, taking into account calibration frequency, daily control levels and test volumes. The Spanish authors noted that real-world costs may vary depending on usage patterns, staffing expertise, and laboratory protocols. Additionally, it is important to highlight that integrating new platforms into clinical practice also incurs costs that were not considered in the reviewed research.

4.4.8 Impact on CT Scan Utilisation.

The Spanish study reported a greater reduction in CT scan use.

- Overall CT scans: 35.04% reduction in Spain versus 29.68% in France.
- Patients receiving at least one CT scan: 28.5% reduction in Spain versus 32.32% in France.
- Follow-up CT scans: 58% reduction in Spain versus minimal reduction in France.

These differences suggest that follow-up imaging practices may be more prevalent in Spain, contributing further to the observed cost savings.

4.4.9 Relevance to Belgian Practice

Whether these findings are applicable to the Belgian healthcare context remains uncertain, given the lack of national studies on this topic. However, our search identified a Belgian cost-effectiveness evaluation,⁹⁸ currently available only as an abstract. Correspondance with the corresponding author confirmed that a full publication of this study is planned for the near future. Although this study is limited to practices in a single hospital, it will be the first economic evaluation to incorporate Belgian-specific data and may provide valuable insights to complement the existing evidence base.

In the meantime, the chapter presenting results from our national survey on mTBI management will discuss how these international findings may align with Belgian clinical practice. A key issue to be raised is the lack of explicit mention of, and consideration for, clinical head rules in the two European models identified via our review. While the models describe their approaches as 'CT for all' or 'biomarker for all', the extent to which these approaches already reflect the results of a first step involving a clinical assessment, with or without head rules, remains unclear.

5 GUIDELINES AND RECOMMENDATIONS CONSIDERING BLOOD-BASED BIOMARKERS IN EUROPE

KEY POINTS

- Overall, 5 different relevant European guidelines, consensus papers or recommendations were identified.
- The NICE guidelines do not recommend using biomarkers in this field due to the evidence being "insufficiently strong".
- All other existing European recommendations identified during our research, agree on the fact that biomarkers can help reduce unnecessary CT scans in mTBI, but emphasise the need for these to be used alongside clinical assessment and head rules and not in isolation. In these guidelines:
 - GFAP + UCH-L1 is consistently recommended to be used within 12 hours after injury.
 - S100 β is more selectively recommended than GFAP+UCH-L1, and the timing of sampling differs: French guidelines within 3 hours for intermediate-risk, Scandinavian within 6 hours for low-risk only.
 - Target population and scope vary: Spanish recommendations are more inclusive (all mTBI), Scandinavian most cautious (limited use), others fall in between with risk-based approaches.

5.1 Introduction

In recent years, research and discussions regarding the potential value of integrating blood-based biomarkers into the clinical decision-making process for mTBI have gained momentum. As a consequence, several national and international publications have begun to incorporate recommendations for using biomarkers to rule out ICIs in patients presenting with mTBI. While the guidelines by NICE published in 2023 concluded that it was premature to recommend the use of these biomarkers based on the existing evidence at the time,²⁰ all others recommend their use in certain populations. Notably, the French national guidelines,²² and the European multidisciplinary consensus⁴² explicitly endorse the use of S100 β and the combination of GFAP and UCH-L1 in specific patient groups. A Spanish consensus paper¹¹⁶ supported the combination of GFAP+UCH-L1, while the Scandinavian guidelines²¹ recommend S100 β only in narrowly defined low-risk scenarios and explicitly exclude GFAP+UCH-L1 to this date, pending further validation. The authors of these guidelines were contacted in October 2025 and confirmed that they are updating them at present and that the new version, which will address amongst other topics biomarker use, should be available by early 2026.

These publications reflect growing interest in, and recognition of, the potential clinical utility of biomarkers in this field, particularly in cases where clinical assessment rules alone may be insufficient to guide imaging decisions.

This chapter provides a comparative overview of these publications, focusing on the rationale, target populations, timing and evidence-base for recommendations on biomarker use in mTBI. It does not offer a full critical analysis or quality assessment of the sources here included and aims instead at offering a narrative description of where the different European countries are in this field.



5.1.1 Clinical Context and Rationale for Biomarker Use

All the publications identified recognise the challenge of managing mTBI, particularly in accurately identifying patients at risk of ICI, while avoiding unnecessary CT scans. They emphasise the low prevalence of ICIs in mTBI and the need for risk stratification tools to help optimise the use of resources while reducing radiation exposure. Despite their differences in scope, focus, timing, and target population for these publications, there is a high level of consensus among all on the importance of the clinical assessment guidelines in this field. Thus, even those endorsing biomarker use highlight that these should always be used alongside clinical assessment and not as standalone tools. They should, therefore, not replace clinical decision-making rules, but instead provide physicians with an additional tool to assist them in making clinical decisions. Despite the promising results, it is acknowledged that uncertainties remain regarding real-world implementation, cost-effectiveness, and clinical impact, and that further research and national-level evaluations would be valuable.

5.2 Existing guidelines considering biomarker use in mTBI

5.2.1 The NICE guidelines

The latest update of the guidelines on the assessment and early management of head injuries by NICE was published in May 2023.²⁰ It included a chapter presenting a systematic review on the evidence of biomarkers used for diagnostic purposes. The methods used reflected those of NICE's standard guidelines.^y It included cross-sectional and cohort studies (prospective and retrospective), as well as systematic reviews/meta-analyses, when available. The QUADAS tool was used to assess risk of bias, while GRADE was applied to evaluate the overall quality of the evidence.

Studies were stratified by age groups (infants, children, adults) and biomarker types. Meta-analyses were conducted only when at least three studies used comparable thresholds and timing, which was only the case for a limited number of studies on the S100 β (6 studies on a total of 3 676 patients).

Both sensitivity and specificity were judged equally important, and thresholds for these outcomes were set at 0.90 (above which a test would be recommended) and 0.60 (below which a test was considered of no clinical use).

Overall, based on 64 cross sectional prospective and retrospective studies (most on the S100 β), the committee recognised the potential promise of biomarkers in this field but concluded that no recommendation could be made for their use in predicting acute post-injury complications in mTBI and that decisions on CT scans in the NHS should be based on clinical decision rules, while more research on biomarker testing became available.

The authors of this review justify their lack of endorsement based on the insufficient and inconsistent evidence identified in their review. As previously stated, equal weight and importance were given to sensitivity and specificity, but while sensitivity was recognised to be high for some biomarkers at certain thresholds, specificity values were not considered high enough across the evidence.

5.2.2 The French guidelines

These guidelines were developed by the French Society of Emergency Medicine (SFMU) and the French Society of Anaesthesia and Intensive Care (SFAR), with contributions from the French Society of Clinical Biology (SFBC), the French Society of Radiology (SFR), and the French Society of Physical Medicine and Rehabilitation (SOFMER).²²

To draft the recommendations, a panel of 22 experts in emergency medicine, anaesthesia, radiology, clinical biochemistry and rehabilitation was convened. Reviews of the available evidence were pursued, but no description of the searches performed are provided by the authors. GRADE (Grading

^y <https://www.nice.org.uk/process/pmg20/chapter/introduction-and-overview>

of Recommendations, Assessment, Development and Evaluation) methodology was used to assess the quality of the evidence found. However, the authors highlight the limited availability of high-quality evidence, reason why they opted for the development of recommendations for professional practice (RPPs).

Each recommendation was rated by experts on a scale from 1 to 9 (1 = complete disagreement, 9 = complete agreement). These were validated if $\geq 50\%$ of experts expressed a convergent opinion and $< 20\%$ expressed a divergent opinion. A strong recommendation required $\geq 70\%$ agreement. Two rounds of ratings were conducted to reach a consensus.

Scope and structure:

The guidelines addressed 11 key clinical questions across three domains: pre-hospital assessment; emergency room management; and discharge modalities. Two recommendations were made with strong agreement in favour of using biomarkers (GFAP+UCH-L1 within 12 hours of injury or S100 β within three hours of injury) as complementary tools in risk stratification and imaging decision-making, specifically for adults who do not clearly fall into low- or high-risk categories. The target population in France for these biomarkers was described as follows:

- Age ≥ 65 years with mono antiplatelet therapy.
- GCS < 15 at 2 hours with intoxication.
- High-energy trauma (e.g. ejection from a vehicle, falling from a height of over 2 meters, overturning of a vehicle).
- Amnesia of the events having occurred more than 30 minutes after the traumatic event.

The recommendations for the use of GFAP+UCH-L1 were based on the findings of the Bazarian et al. ALERT-TBI study.⁷⁴

For S100 β , the recommendations were mainly based on two systematic reviews and meta-analyses.^{117, 118}

5.2.3 The Scandinavian Neurotrauma Committee guidelines

These guidelines were developed and published in 2013 by the Scandinavian Neurotrauma Committee (SNC), using the AGREE II tool^z (Appraisal of Guidelines for Research and Evaluation).

The process included setting up a task force and working group, led by the SNC, including neurosurgeons, anesthesiologists, and stakeholders from emergency medicine, general surgery, and orthopaedics.

A systematic review was performed, and the search strategy was provided. Studies were selected based on predefined inclusion criteria and quality thresholds, and reporting followed the PRISMA guidelines (Preferred Reporting Items for Systematic Reviews and Meta-Analyses).

The quality of the included studies was rated using the QUADAS tool (Quality Assessment of Diagnostic Accuracy Studies) and recommendations were graded as strong, weak, or uncertain using GRADE (Grading of Recommendations Assessment, Development and Evaluation).

A modified Delphi method was used in two rounds to reach consensus. Final recommendations were approved by the working group and external stakeholders.

S100 β is the only biomarker included in these guidelines. Whether this reflects the fact that at the time of their publication, evidence was limited to the protein S100 β should become clear after publication

^z <https://www.agreetrust.org/wp-content/uploads/2017/12/AGREE-II-Users-Manual-and-23-item-Instrument-2009-Update-2017.pdf>



of the ongoing update of these guidelines. Direct communication with the SNC representatives confirmed that the update should be published in early 2026 and that biomarker use is part of the update. In the current version from 2013, the target Population for S100 β use is described as follows:

Patients with GCS 14 and no other risk factors, or GCS 15 with loss of consciousness or ≥ 2 episodes of vomiting and no other high-risk features. In contrast to what we saw in the French guidelines, that recommend sampling for S100 β within 3 hours, the Scandinavian guidelines recommend sampling within 6 hours after trauma. It is important to note that the use of these SNC guidelines in clinical practice has been validated in a number of studies, which assessed their sensitivity and specificity and concluded that no cases requiring neurosurgery were missed by implementing these guidelines.¹¹⁹⁻¹²²

5.3 Consensus recommendations endorsing biomarker use in mTBI

5.3.1 *The Spanish recommendations*

The Spanish recommendations were developed by a multidisciplinary panel of experts from various specialties, including: emergency medicine, neurosurgery, radiology, clinical biochemistry and prehospital emergency services. The document was endorsed by five national societies: the Spanish Society of Emergency Medicine (SEMES); the Spanish Society of Neurosurgery (SENEC); the Spanish Society of Medical Radiology (SERAM); the Spanish Society of Emergency Radiology (SERAU); and the Spanish Society of Laboratory Medicine (SEQC-ML).

An independent consulting team coordinated the study externally, which included a narrative review of the literature on the diagnostic performance of biomarkers such as GFAP, UCH-L1 and S100 β . Regarding GFAP+UCH-L1, the ALERT-TBI study⁷⁴ is cited as a key reference supporting the high sensitivity and negative predictive value of this biomarker in ruling out ICIs.

The document does not explicitly employ a formal grading system such as GRADE.

Biomarker testing (specifically GFAP+UCH-L1) is recommended for:

Adults (≥ 18 years) with mTBI (GCS 13-15) presenting to the ED within 12 hours of trauma.

More specifically:

GCS 13-14, regardless of symptoms, or GCS 15 with symptoms and/or risk factors. The latter are defined as having one or more of the following: neurological deficit, coagulopathy, or use of anticoagulants or antiplatelets (excluding aspirin monotherapy), age ≥ 65 , intoxication (alcohol or drugs), vomiting (≥ 2 episodes), headache, post-traumatic seizures, amnesia or short-term memory loss, evidence of head/neck trauma, prior brain injury or neurosurgery, or a dangerous mechanism of injury (i.e. ejection from a vehicle, being struck by a vehicle, falling from a height greater than one's own height, or falling down more than five stairs).

5.3.2 *European consensus paper*

Published in 2024, this consensus paper aimed to provide practical, harmonised recommendations for assessing adult patients (defined in this case as aged ≥ 16 years) with mTBI (GCS 13-15) in EDs across Europe. The focus was on patients who did not require immediate hospital admission, with the paper addressing topics such as acute assessment, imaging decisions, biomarker use, discharge and follow-up.

To develop these recommendations, a multidisciplinary panel of experts from various specialties was established, including: emergency medicine, radiology, trauma surgery, biochemistry, physical therapy, and neurology. Contributors represented institutions from Finland, France, Germany, Netherlands, Norway, Spain and the UK.

The panel included clinicians and researchers with experience in TBI care and biomarker development.

It is important to note that this study offers a consensus-based expert review, rather than a formal guideline, and did not use the GRADE or Delphi method to reach their conclusions.

It synthesizes recent scientific literature, clinical experience, and existing national guidelines. The recommendations are pragmatic and are designed for real-world ED settings rather than being systematically graded or evidence-ranked.

The evidence reviewed regarding GFAP+UCH-L1 includes the ALERT-TBI study⁷⁴ as well as two CENTER-TBI studies,^{76, 84} while the quoted evidence for the protein S100 β referred to one recent CENTER-TBI study⁸⁴ and an older study dating from 2006.¹²³

The paper discusses the limitations of clinical decision rules, particularly when applied to elderly and intoxicated patients, and emphasizes the importance of integrating biomarkers into clinical assessment practices to enhance diagnostic accuracy.

The paper includes a recommendation on using blood biomarkers in ED mTBI assessments, including both GFAP+UCH-L1 and S100 β , although the latter is recommended to be used with caution. Such caution is justified by the authors by the fact that it is not brain-specific; it can react to skin pigmentation and presents a short time window in which it can be (accurately) performed. The target population for biomarker testing is described as follows:

Adults (aged ≥ 16 years) with GCS 13-15, not requiring immediate hospital admission. Immediate admission was recommended in cases with GCS < 13 over 30 minutes from injury, severe/worsening of symptoms, seizures or multiple trauma.

Biomarker use is recommended to be considered in patients with risk factors identified during clinical assessment which could indicate the need for a CT scan. These risk factors may include: prolonged LOC and/or amnesia, seizure after injury, suspected skull fracture, or intoxication. The authors highlight that at the time of their publication, there was not sufficient evidence to show that the threshold values of biomarkers for CT applied for patients on anticoagulants and therefore, biomarkers should not be used in those patients.

Table 15 offers a summary of the target populations for biomarker use according to the sources described in this chapter that recommend the use of biomarkers.



Table 15 – Summary of studies recommending the use of biomarkers in mild TBI

Author Yr	Country	Type of document	Biomarker recommended	Target population for biomarker use*
Undén 2013	Scandinavian countries	Guidelines	S100 β (≤ 6 hrs)	Mild head injury patients with GCS 14 and no other risk factors* OR GCS 15 with LOC or repeated vomiting, and no other risk factors*. Should be sampled within 6 hours of trauma.
Gil-Jardiné 2023	France	Guidelines	S100 β (≤ 3 hrs), GFAP + UCH-L1 (≤ 12 hrs)	Intermediate-risk mTBI (GCS 13-15) adults with: • Age ≥ 65 years with mono antiplatelet therapy • GCS < 15 at 2 hours post-trauma with intoxication • High-energy trauma • Amnesia > 30 minutes post trauma.
Backus 2024	Europe	Consensus paper	S100 β ($\leq 3-6$ hrs), GFAP + UCH-L1 (≤ 12 hrs)	Presented as optional when the following factors are present: • Prolonged LOC and/or amnesia • Seizure after injury • Suspected skull fracture • Intoxication. Not recommended in patients on anticoagulants.
Temboury Ruiz 2024	Spain	Recommendations	GFAP + UCH-L1 (≤ 12 hrs)	GCS 13-14 adults regardless of symptoms GCS 15 adults with presence of symptoms: • Neurological deficit • Coagulopathy or anticoagulants/antiplatelets therapy (except • Age ≥ 65 years • Alcohol or drug intoxication • ≥ 2 episodes of vomiting • Headache • Post-traumatic seizures • Amnesia/short-term memory loss • Evidence of head/neck trauma • History of brain injury or neurosurgery • Dangerous trauma mechanism.

GCS: Glasgow Coma Scale; LOS: Loss of consciousness; * Repeated vomiting (≥ 2 episodes), anticoagulant therapy or coagulation disorders, clinical signs of depressed or basal skull fracture, post-traumatic seizures, focal neurological deficits, shunt-treated hydrocephalus, aged ≥ 65 combined with antiplatelet medication

5.4 Impact of guidelines and recommendations on use of biomarkers in mTBI in France and Spain

Following the publication of their guidelines/recommendations, experts in Spain and France were contacted to check the situation regarding pricing and reimbursement for biomarkers in mTBI. Questions about the uptake of their use were also posed. As the Scandinavian guidelines are currently being updated, no discussion was held on the current situation in these countries, as this could change after publication of the update.

In France, neither S100 β protein nor the GFAP+UCH-L1 panel is currently reimbursed (there is no listing under the Nomenclature of Medical Biology Procedures), so no official price exists to date. When used, this is typically within pilot studies or under laboratory-defined pricing.

The 2022 national recommendations reinforced existing practices in some centres and boosted the use of S100 β nationally. They have also facilitated the introduction of GFAP+UCH-L1. Nevertheless, the lack of reimbursement remains the main obstacle to wider adoption.

A similar situation exists in Spain, where there is no general coverage and therefore no official price. Instead, costs are absorbed by hospital laboratory budgets.

Use of biomarkers, particularly GFAP+UCH-L1, has increased across Spanish public hospitals, though adoption varies significantly by autonomous region. One large public hospital's extensive experience showed that using biomarkers led to a mean 38% reduction in CT scans in the general mTBI population. However, in elderly patients (defined as those aged 75 years or over), the proportion of negative biomarker results was notably lower (5–7%), which limits its utility in this patient subgroup.

5.5 Conclusion

Overall, two guidelines (one under revision) and two consensus or recommendation papers currently support the consideration of biomarkers in mTBI, although differences exist with regards to both the biomarkers included and the populations in which these are recommended to be used. A further guideline, the one produced by NICE, does not recommend the use of biomarkers within the NHS, since its authors believed the evidence base at the time of their publication (i.e. 2023) was not robust enough.

5.6 Discussion

Regarding the publications identified that support the adoption of biomarkers in the diagnosis of mTBI, differences are found in scope, timing, and clinical integration:

The Spanish consensus offers the amplest endorsement of GFAP+UCH-L1, positioning them as a potentially useful adjunct in all mTBI patients (GCS 13-15), including those with symptoms or risk factors, to guide early decision-making and reduce unnecessary CT scans.

The French guidelines provide a structured, risk-stratified approach, recommending biomarkers only for intermediate-risk patients, defined by specific clinical and anamnestic criteria. Both S100 β ($\leq 3h$) and GFAP+UCH-L1 ($\leq 12h$) are endorsed.

The European consensus supports the use of GFAP+UCH-L1 as part of a stepwise ED algorithm, particularly in cases of diagnostic uncertainty or intermediate risk, though it does not formally define this category. S100 β is discussed but recommended to be used with caution due to its limitations in specificity, timing, and confounding factors.

The Scandinavian guidelines remain the most conservative, recommending S100 β only in low-risk patients (GCS 14-15) with isolated symptoms such as vomiting or loss of consciousness. The combination of GFAP and UCH-L1 is not included, pending further validation.

Across all four, there is strong consensus that biomarkers should be used in conjunction with clinical assessment and decision rules, not as standalone tools. There is also agreement regarding the time



of sampling for GFAP+UCH-L1 which is in all cases recommended to be used within 12 hours from injury, reflecting the current evidence-based recommendations by manufacturers. The timing for sampling of the S100 β differed from the French to the Scandinavian guidelines, with the former being more restrictive and recommending its use within 3 hours from injury.

Despite promising diagnostic performance, the authors of these recommendations recognize that uncertainties remain regarding real-world implementation, cost-effectiveness, and clinical impact. Further research, validation, and national-level evaluations, such as the ongoing update of the Scandinavian guidelines (expected to be published in early 2026), would be of value in this field.

Despite this existing endorsement in France and Spain, the lack of reimbursement and variability across regions and/or centers continue to influence clinical uptake.

6 MANAGEMENT OF MILD TBI IN BELGIUM

KEY POINTS

CT use in mTBI patients in Belgium:

- CT scan use is high in adults: over 60% of adult mTBI patients receive CT scans in most hospitals.
- In children, CT use is much lower (<20%) in the majority of cases.
- Most hospitals make CT decisions within 2 hours after admission, and results are typically available within 1 hour.

Potential for biomarker use in Belgium:

- Respondents stated that biomarkers could be most valuable in “grey zone” cases following clinical assessment.
- In particular, the following cases were mentioned as potentially interesting:
 - GCS 13-15, with the following symptoms:
 - Retrograde amnesia
 - Suspected LOC
 - Repeated vomiting
 - Anticoagulant use
 - Age ≥ 65 .

Limited experience and adoption to this date in Belgium:

- Only one from the hospitals that responded to our survey reported actual biomarker use (i.e. use of S100 β).
- Awareness is low: 39% of respondents could not answer any of the questions about biomarkers.
- S100 β is the most recognised; with others being less known.

Current barriers to biomarker use:

- Lack of training/knowledge.
- No reimbursement.
- Concerns about test accuracy and timing.

- Preference for CT due to fast access and workflow integration.

Mixed views on which patient groups would benefit most, and some skepticism about added value in current ED settings.

This section aims at describing clinical practice specifically in Belgium.

6.1 Methods

The survey had two aims:

Firstly, to understand current practice in Belgium in diagnosing mTBI in the ED.

Secondly, to evaluate the potential introduction of blood-based diagnostic biomarkers into clinical practice to reduce the number of unnecessary CT scans performed on patients with mTBI. With this in mind, the survey was structured into four sections.

The first is related to the type of hospital/facility. The second section captured data on the number of patients with mTBI, in order to address the lack of administrative data in this regard. Section 3 covered the current management of mTBIs in the ED. The final section asked questions about the use or interest in using blood-based biomarkers to diagnose mTBI patients.

The survey was tested during three video conferences with Belgian experts in the field and adapted as necessary to make the questions easier for the relevant respondents to understand, while keeping the survey as short as possible. The focus was on key questions that could not be answered by administrative data analysis or literature reviews.

The survey (LimeSurvey) was available in both French and Dutch and was shared with medical hospital directors of all Belgian hospitals with the help of the FPS Public Health, Safety of the Food Chain and Environment. Medical directors were asked to send the link to the survey to the head of the ED department in their hospital. Instructions were given to only send the link to the head of the ED of the main hospital site, if the medical director had responsibility for more than one site. Participation was voluntary, with participants having two months to respond. A copy of the survey is presented in the supplement.

The survey responses were expected to provide valuable insights into practices, perceptions and acceptance/defiance of clinicians towards biomarkers, as well as their willingness to use them. Responses to the survey were anonymous, and the analysis and reporting were conducted on an aggregated basis and are presented here.

6.2 Results

A total of 103 hospital medical directors were contacted, and 33 responses were obtained from the heads of EDs at their respective hospitals, yielding a response rate of 32%.

Of these, six came from university hospitals, 20 from general hospitals, and seven from general hospitals with academic beds. Most (23) hospitals had CT scanning facilities within the ED. The remaining 10 hospitals reported having access to a CT scan within one hour. Twenty hospitals had the necessary technical staff to perform CT scans on site 24 hours a day, while the remaining thirteen explained that the staff were either on site or available on call.



6.2.1 Target population:

Respondents were asked to report the exact number of TBI cases in their ED in 2023 and 2024, as well as the number classified as mild. To ensure the figures could be interpreted correctly, respondents were asked to specify the basis on which they classified patients as 'mild'. In this regard, all but five (85%) respondents mentioned that they followed the ACRM 1993 definition,¹²⁴ recognised and adapted by the WHO, that defines mild head trauma as: “A *Glasgow Coma Scale (GCS) score of 13 to 15, 30 minutes after the trauma, and ≥ 1 of the following symptoms: loss of consciousness <30 min; post-traumatic amnesia <24 hours; altered mental state at the time of the accident (confusion, disorientation, etc.); and/or transient neurological deficit*”. One respondent mentioned that they only partly followed this definition. The remaining respondents either mentioned the Manchester Triage System^{aa}, the PECARN guidelines,¹²⁵ or were unable to answer the question.

Only four (12%) respondents were able to provide reliable data, as they have their own registration system. A national registry does not exist at present. A further 15 (45%) respondents provided estimates based on their knowledge, while 14 respondents specified that they did not have any data and were unable to provide reliable estimates.

6.2.1.1 TBI in children

The reported number of children (under 18s) presenting to the ED after a head injury varied greatly, ranging from a minimum of 20 TBI cases in both 2023 and 2024, to a maximum of 2111 in 2023 and 1818 in 2024. The average was 392 in 2023 and 380 in 2024. Of these, it was estimated that 91% were classified as mTBI.

6.2.1.2 TBI in adults

The number of adults presenting to the ED with a head injury also varied greatly, ranging from a minimum of 100 cases in both 2023 and 2024 to a maximum of 3,000 in both years. The average was 889 in 2023 and 882 in 2024. Of these, an estimated 78% were classified as mTBI in 2023, rising to 79% in 2024.

6.2.2 Current diagnosis of mTBI in the ED in Belgium

When asked about the clinical rules used to assess the need for a CT scan when a child arrived in the ED, over 65% of respondents (17/26) who could answer this question used the PECARN guidelines for paediatric patients. Four respondents reported that they do not use any guidelines, and five used more than one. Seven respondents used the NEXUS-II guidelines, and three mentioned the NICE head rules.

Regarding adults, the most commonly used head rule amongst those who could respond to this question was the CCTH,¹⁸ mentioned by 14/27 respondents, followed by the SFMU French rules,²² which was used in five hospitals. Two respondents used the NICE rules,⁵⁷ and two the New Orleans rules.¹⁹ One respondent mentioned the Dutch guidelines.²⁴

Six respondents stated that they did not use any specific guidelines and based their decisions on clinical assessments that considered aspects such as the GCS two hours after injury, patient age, anticoagulant consumption, signs of open or depressed skull fracture, repeated vomiting and whether the mechanism of the injury was considered dangerous.

^{aa} https://www.triagenet.net/content/MTS_ET1.html

6.2.2.1 CT scan use in mTBI cases

As expected, CT scans were much more common in adults than in the paediatric population. Of those able to offer estimates, 23/29 (79%) stated that they perform CT scans in less than 20% of children with mTBI. A further three do them in 20–30% of such patients, and one performs them in around one-third of paediatric mTBI cases. Two respondents appear to be outliers, reporting that they perform CT scans on most of these patients (70–89% of cases).

Although CT scans in adults were more common, there was variation across those hospitals that responded to the question. Thus, more than half (16/29) of the respondents reported performing CT scans on over 60% of their adult mTBI patients, with one third using them in most or practically all of these patients (70–100%). Conversely, four respondents stated that they use CT scans in fewer than 30% of their adult mTBI cases. The remaining nine used them in 30–60% of cases.

Figure 19 and Figure 20 summarise the responses on the proportion of paediatric and adult cases in which the respondents reported CT use.

Figure 19 – Proportion of PAEDIATRIC mTBI cases on which CT scans are performed

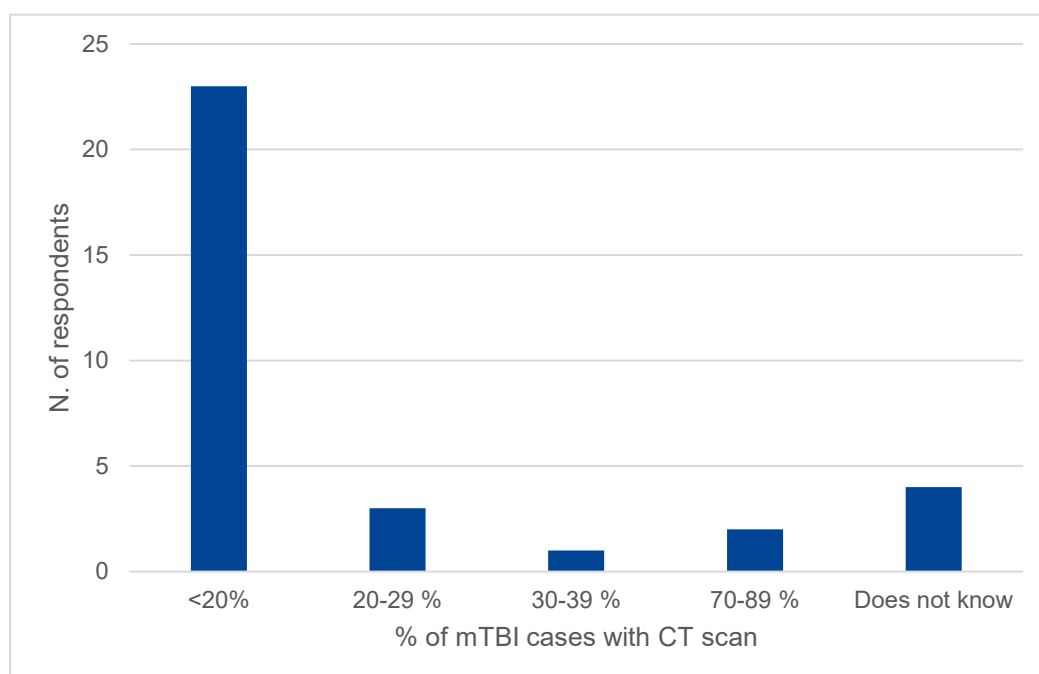
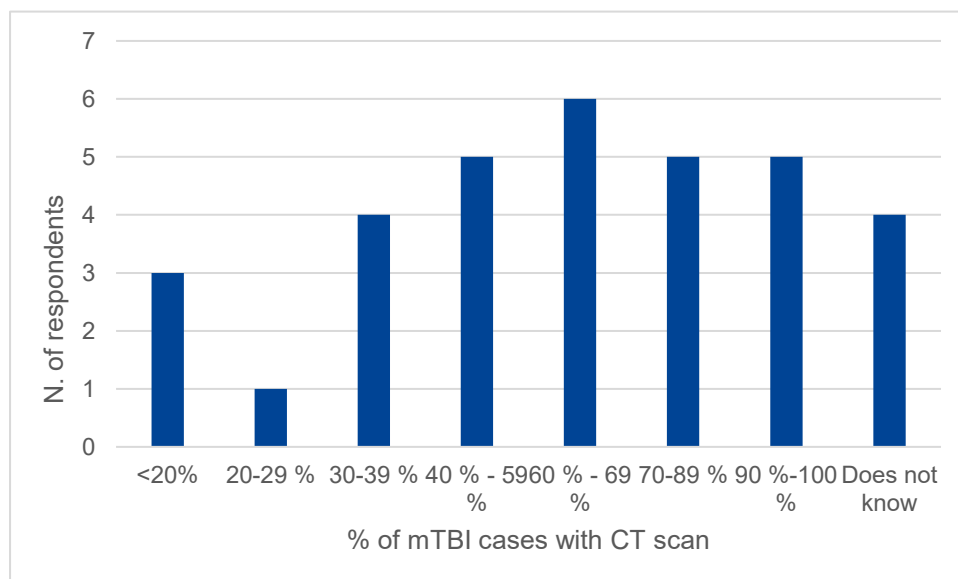




Figure 20 – Proportion of ADULT mTBI cases on which CT scans are performed



Over 80% of respondents said that there are certain patient categories for which they would always recommend a CT scan, meaning they would not be a target for biomarker use. These categories included patients with repeated vomiting (mentioned by 89%), older patients (≥ 65 years) on antiplatelet or anticoagulant treatment (85%), patients on antiplatelet or anticoagulant treatment regardless of age (74%), a GCS of 13 (70%), retrograde amnesia within 30 minutes of injury (56%), suspected or confirmed loss of consciousness (48%), and advanced age (≥ 65) (41%). Six respondents mentioned other cases, including intoxicated patients and patients with signs of a fracture. One respondent stated that they follow the recommendations of the guidelines for Dutch specialists.²³

Information was sought on the average time taken from arrival in the ED until a clinical decision is made regarding the need for a CT scan, hospitalisation, observation or discharge. Of those who could answer this question, the majority (26/32; 81%) reported a maximum of two hours, while amongst those, 12/32 (38%) reported less than one hour. The remaining six respondents reported 2–4 hours. In addition to this, 63% of respondents stated that, when a CT scan was requested, results were obtained in less than one hour, while 94% reported waiting for less than two hours. Only two respondents said that it often took 2–4 hours.

6.2.2.2 Discharge from the ED without a CT scan

The proportion of mTBI patients discharged directly following triage in the ED without a CT scan or observation varied greatly between hospitals and depended on whether the patient was a child or adult. Thus, 38% (11/29) of respondents stated that at least 70% of children with mTBI are discharged home directly, while 59% (17/29) of respondents discharged a similar proportion of adults with mTBI. Conversely, 31% (9/29) of respondents reported sending home only a small proportion (10–30%) of paediatric mTBI patients without a CT scan or observation period, compared to 7% (2/29) for adults. Finally, 5/29 and 6/29 of respondents reported discharging around half of their paediatric and adult mTBI patients home without a CT scan, respectively, while 4/29 of hospitals mentioned that they would not discharge any mTBI patient directly.

6.2.2.3 Observation (without CT scan)

Observation for six hours was uncommon for adults with mTBI in which a CT scan is not performed, with 18/28 respondents (64%) reporting that they leave fewer than 10% of these cases under observation, and only two respondents (7%) mentioning that they leave 70% or more of their adult patients under observation.

Among children, a smaller proportion of respondents (13/28) reported leaving 10% or fewer of their paediatric patients under observation, while 5/28 (18%) left at least 70% of their paediatric mTBI patients under observation.

6.2.3 Perceptions and (potential) use of blood-based biomarkers

Regarding blood-based biomarkers that are considered valuable for ruling out ICIs, it is important to note that a significant proportion of respondents were unable to answer this question (13/33; 39%). Of those who could answer, most considered the S100 β biomarker to be potentially valuable for their practices (12/20; 60%). Fewer respondents recognised the value of other biomarkers, such as GFAP (4/20; 20%), the combination of GFAP and UCH-L1 (3/20; 15%), or UCH-L1 individually (2/20; 10%).

Around 30% (6/20) of those who could answer said that they did not think these blood-based biomarkers would be valuable in the ED. When asked about their reasons, two respondents did not provide a justification; two respondents thought that the evidence regarding the biomarkers' accuracy was insufficient; one respondent was from a paediatric hospital and thought that the PECARN clinical rules were sufficient for their patients; one remaining considered that the combination of clinical assessment, the option to observe patients, and the high availability of CT scans left little room for biomarkers in this field.

Of all the respondents, only one reported using biomarkers (i.e. S100 β) and explained that they limit their use according to a risk-structured clinical algorithm in patients with a GCS of 14-15 and at least one of the following symptoms: suspected or confirmed loss of consciousness (no more than 15 minutes), or retrograde amnesia for at least 30 minutes prior to the trauma and no further risk factors. In these situations, a CT scan would only be performed if the biomarker result was positive or if the biomarker was negative but other symptoms of possible brain damage appeared (e.g. after a period of observation). They do not use it on patients with a GCS of 13 or less, those taking antiplatelet or anticoagulant drugs, the elderly (aged >60), or those who have experienced two or more episodes of vomiting. In such cases, they perform a CT scan directly. A copy (in English) of the full protocol is provided in appendix. It is important to note that the protocol foresees observation for 6 hours for patients in which the S100 β test is negative, before discharging the patient, so while the number of CT scans was reduced after the introduction of this protocol, observation increased.

A question was addressed to non-users regarding the main reasons why they did not use biomarkers in the ED. The following reasons were given: lack of training or knowledge (reported by 44% of respondents); lack of reimbursement (38%); insufficient test accuracy (25%); fear of missing some complications (19%); not possible/available in lab (16%); and risk of having to wait a long time for a CT scan to be requested (16%). Additional justifications were reported by individual respondents. These included the need to perform the test within a short time after head injury for it to be accurate (e.g. S100 β must be performed within six hours of injury), fast and efficient access to the scanner to enable better management of patient flow, the need to take a venous blood sample (unnecessary invasiveness for some patients), and unclear evidence or validation of such tests. One respondent from a paediatric hospital explained that, since fewer CT scans are performed on children and more observation is carried out, the value of biomarkers is perceived as being more limited.

When asked about the three most important factors for a mTBI biomarker to add value to their ED department, respondents mentioned the following: high sensitivity (25/33; 76%), which half of the respondents defined as a minimum of 95% and half as a minimum of 99%; rapid results (24/33; 73%); high specificity (14/33; 42%); an acceptable price/cost (8/33; 24%); compatibility with existing laboratory instruments (8/33; 24%); a long time window to perform the test, so there is no need to do it within a short time after head injury (7/33; 21%); and availability as a point-of-care test (6/33; 18%).

Two more respondents mentioned that it would be crucial for the biomarkers to help reduce the number of CT scans (and thus, radiation). One of these respondents referred specifically to cases involving children, where a low number of CT scans are already performed in mild cases.



Regarding the maximum time the respondents would consider waiting for the results of the blood-based biomarker tests to be reasonable, more than 65% of respondents (17/26) said that it should be less than one hour. 19% (5/26) of respondents thought that waiting times of up to two hours would be reasonable, while 8% (2/26) thought that waiting times of up to half an hour would be reasonable.

When asked about acceptable waiting times for test results, some differences emerged depending on whether the test was point-of-care or laboratory-based. Thus, while acceptable waiting times for POC test results were reported as being less than 30 minutes, most respondents stated that an hour was acceptable for laboratory-based tests.

Respondents were asked about the types of patients/cases in which they would consider using blood-based biomarkers. Most respondents (22/32, 69%) said they would consider using them for patients with a GCS of 13-15 (while the remaining 10/32, 31%, would limit their use to patients with a GCS of 14-15). They would also consider using them if the patient presented with some of the following symptoms: Retrograde amnesia 30 minutes before the trauma (23/27; 85%), suspected or confirmed loss of consciousness (22/27; 81%), repeated vomiting (21/27; 78%), patients taking antiplatelet or anticoagulant medication (18/27; 67%), and patients aged 65 years or over (12/27; 44%).

When asked to explain why they would use them in these patients, multiple responses were offered. These included the fact that the current clinical criteria are sometimes unclear for some patients, and that CT scans are often negative but still performed due to uncertainty.

Some people mentioned that they would use it more with younger patients, as it is more important to avoid radiation at a younger age.

Some thought it would be helpful for testing elderly patients or those on antiplatelet or anticoagulant therapy. Others thought they would avoid using it for these patient groups and go straight to the CT scan.

When asked about patients for whom they would not find biomarkers informative, the following responses were given:

- Patients who require observation or treatment for other traumatic reasons (2).
- Elderly patients (2).
- Those with an absence of any red flags according to clinical rules (3);
- On anticoagulants (5).
- When there is a decrease in GCS and clinical neurological signs (e.g. vomiting, amnesia, loss of consciousness).
- Patients with a history of head injuries
- Children with confirmed loss of consciousness, retrograde amnesia or repeated vomiting.
- If you have an indication for CT anyway (2).
- GCS >14
- Patients with highly suggestive symptoms for which the pre-test probability is already very high.
- Those who, according to the Canadian head CT guidelines, should still receive a CT scan.

6.3 Conclusion

Our survey revealed that in Belgium, CT scans are widely used for adult patients with mTBI, while usage in children is much lower. Most hospitals who responded to our survey reported to make decisions regarding the need or not for a CT scan within 2 hours and results are obtained quickly once requested. The experts consulted recognised the potential value of biomarkers particularly in patients

where doubts remain after a clinical assessment. However, actual biomarker use is rare, with limited awareness, no reimbursement, and concerns about accuracy and workflow integration.

Despite the modest response rate, the information captured in our survey appear to indicate the existence of great heterogeneity in clinical practice regarding the diagnosis of mTBI in Belgium in general and the proportion of these patients undergoing CT scanning in particular. Such differences may be (partly) explained by the combination of different factors, such as patient demographics and the adoption (or lack of) different clinical head rules/protocols in the different hospitals.

6.4 Discussion

This survey provides a valuable overview of the current practices and perceptions surrounding the diagnosis and management of mTBI in Belgian EDs. However, only a small number of respondents were able to provide reliable estimates of TBI and mTBI cases in their hospitals. This reflects the absence of a national registry and the lack of detailed, standardised data collection in EDs.

When clinical decision rules were used, there was a high level of consistency in the rules adopted, with the PECARN guideline being the most commonly used for paediatric patients and the CCH guideline for adults. However, CT scan usage varied significantly across institutions, particularly for adult patients. While over half of respondents reported performing CT scans in more than 60% of adult mTBI cases, others used them far less frequently. Despite this variation, the overall proportion of adults receiving CT scans remained high. Experts consulted after our survey attributed these differences to several factors, including hospital-specific patient characteristics such as age, medication use or language barriers; the degree of guideline implementation; and other factors such as medico-legal pressures. One expert emphasised that healthcare and legal systems tend to reward extensive testing.

In contrast, CT scan use in paediatric mTBI cases was generally lower, with most respondents performing scans in fewer than 20% of cases. Observation was more commonly used in children than in adults, and discharge practices varied widely, further highlighting the heterogeneity in clinical management.

The survey also explored perceptions of blood-based biomarkers, revealing their limited current use and awareness. Of the respondents, only one reported using the S100 β biomarker, applying it selectively to patients with a GCS of 14–15 and specific symptoms such as retrograde amnesia or loss of consciousness. Most respondents had not adopted biomarkers, citing barriers such as a lack of training, insufficient reimbursement, concerns about test accuracy and logistical constraints. Nevertheless, there was a broad consensus on the attributes that a clinically valuable biomarker should possess: high sensitivity (defined by most as ≥ 95 –99%), which matches the sensitivity found via our MAs, as well as factors such as a rapid turnaround time. Despite the lower interest in specificity in this field, given the aim of these biomarkers is to rule out ICIs, some experts still recognised the value of higher specificity. However, when consulted about this, they explained that they would not be willing to sacrifice sensitivity for greater specificity, since missing an ICI could have “catastrophic” consequences. Additional desirable features include affordability and compatibility with existing laboratory infrastructure.

Respondents expressed interest in using biomarkers, particularly for patients with ambiguous clinical presentations, for younger individuals where radiation avoidance is critical and for cases where CT scans are frequently negative, but are still performed due to diagnostic uncertainty. However, opinions diverged regarding their use in elderly patients or those on anticoagulants, with some clinicians preferring direct CT imaging in these groups.

Experts also noted that, even with a negative biomarker result, they might still opt for observation. It is important to note that observation after a negative CT scan is also sometimes performed.

Given the lack of detailed data, the potential impact of adopting these biomarkers in Belgian hospitals remains unknown. Nevertheless, the next chapter presents an attempt to provide some estimations based on this limited data.



7 CT SCAN REDUCTION IN THE BELGIAN CONTEXT

KEY POINTS

- Biomarker adoption (GFAP+UCH-L1) could reduce head CT scans in mTBI patients by around 26%:
 - The resulting savings in radiology may offset the increased laboratory costs, provided that the test is priced close to its break-even point.
 - The specificity of the test was the main factor influencing the magnitude of CT reduction and economic impact in univariate sensitivity analysis.
- Limitations:
 - High uncertainty due to lack of detailed Belgian data on ED practices and follow-up protocols.
 - Results rely on simplifying assumptions that may not reflect real-world variability (e.g., CT use in biomarker-negative cases).
 - Our cost estimations exclude downstream consequences such as missed lesions or follow-up care.
 - Hospital-level differences in clinical decision-making could substantially alter the results presented.

7.1 Introduction

This section provides estimates of the potential reduction in head CT scans in Belgium if mTBI biomarkers were implemented in emergency departments. Because robust national data on the management of mTBI are lacking, we did not attempt to quantify other downstream implications such as observation periods, hospital admissions, surgeries, or follow-up care.

We had initially planned to consult the CENTER-TBI data registry to validate the information gathered through our Belgian survey. However, the survey yielded limited data, and additional validation was judged unlikely to meaningfully reduce uncertainty. For this reason, we based our estimations directly on the results of the survey, our meta-analysis, and the available literature.

The figures presented here provide a simplified illustration of the potential impact of biomarker implementation on CT scan volumes. They rely on the current number of CT scans performed in this population and on the diagnostic accuracy of the biomarkers, as established through the meta-analyses described earlier. The intention is to offer an approximate indication of the likely reductions and to highlight key factors that may influence these outcomes. This information can support future discussions regarding the integration of such tests into clinical practice.

As shown in the chapter reporting our survey of Belgian hospitals, CT scanning is frequently used to rule out intracranial injuries in patients with mTBI. Although hospitals reported an average CT rate of around 60%, substantial variation was observed, potentially reflecting differences in hospital demographics, adherence to clinical head-injury rules, or local practice patterns.

7.2 Methods

A hypothetical cohort of 10 000 TBI patients was used as our starting point. No registration on the number of TBI patients presenting to the ED exists to this date in our country. Such data are only available in cases of hospitalisation.¹¹ However, focusing solely on hospitalised patients would not accurately represent the target population for the biomarkers under study (i.e., mTBI patients).

Estimates of mTBI in the European literature report that 70-90% of all TBI patients are classified as mild.^{2, 13, 126} This aligns with our survey of Belgian experts, which estimated 78% for adults and 90% for pediatric patients. For these estimations, we focused on adults due to limited evidence on biomarkers in children to this date.

We compared the survey estimates to those available in the literature whenever this was possible.

Our MA results informed the following:

- Sensitivity of GFAP+UCH-L1.
- Specificity of GFAP+UCH-L1.
- Prevalence of ICIs (mean across the 15 studies included in our MA).

Estimates from the survey (and the literature) were used for the following variables:

- Proportion of mTBI.
- Proportion of mTBI cases for which at least one head CT scan is performed under current practice (without biomarker use).

Several important assumptions had to be made. These are oversimplifications for the purpose of our illustration and include the following:

- The target for biomarker testing would include all patients who, under current practice, would undergo a CT scan following an initial clinical assessment. In real life, some patients may still receive a CT scan despite the availability of biomarkers.
- All biomarker-positive patients get a CT scan.
- From the biomarker-negative patients, only false negative cases would eventually receive a CT scan. In clinical practice, it is possible that some patients with a (true) negative biomarker result may still receive a CT scan.
- No follow-up after the first CT scan, whether positive or negative, is included in the estimations. Some patients are likely to undergo a follow-up scan. Our estimations focus on reducing the number of patients who would get at least one CT scan.

We performed a simple, univariate sensitivity analysis. Minimum and maximum values were derived from our MA or the literature.

7.3 Estimations for base case scenario

From a hypothetical population of 10 000 patients presenting to the ED with TBI, 78% were considered mTBI patients (n= 7 800). This figure was reported by Belgian experts via our survey. Of these mTBI cases, 60% (n=4 680) would receive a head CT scan under standard care, according to our survey. The mean prevalence for ICIs as determined by our MA was 10.28% (median 11.29%). It is important to note that although this figure appears to be relatively high when compared to the general estimates given in the literature for ICIs in the general mTBI population,^{127, 128} some of the studies included in our review of the clinical literature found significantly higher rates.^{73, 76, 81} However, this estimate will be examined in our univariate sensitivity analysis to determine its impact on the overall CT scan reduction.



The alternative arm was described as follows:

Following testing in the population that would have received a CT scan under standard care and based on the sensitivity and specificity found in our MA (i.e. 98% and 29%, respectively), 73.78% patients would test positive for the biomarker (n= 3 453). According to current recommendations, all of these biomarker-positive patients would undergo a head CT scan. The remaining 1 227 patients would be biomarker-negative in which the scan would be avoided. Of these, based on our MA, 0.78% (n= 10) would be false negatives. It is assumed that these patients eventually receive a CT scan.

Of the biomarker-positive patients 13.66% (n=471) would have a positive scan, reflecting true positives. The false positives would represent 86.34% of those tested (2 981) and would have negative CT scans.

7.4 Base Case Results

Under the current practice scenario, 4 680 CT scans are performed. With the biomarker strategy, 3 463 CT scans are carried out. Assuming GFAP+UCH-L1 is used for patients currently undergoing head CT scans, it could potentially spare 1 217 scans, representing a 26% reduction. This figure appears to be similar to the reduction reported by Moya et al. in their BIA,¹⁰⁴ who found a reduction in patients in which at least one CT scan was performed of 28.50%. The CUA by Zimmer et al.¹⁰³ presented higher reductions, reaching 32.32%.

Table 16 – Comparison of CT scans under standard practice vs biomarker adoption scenarios

	Assumptions	Patients	Sources
Population of TBI patients	Hypothetical cohort	10000	
mTBI Patients	78.00%	7800	Survey to Belgian experts and literature
mTBI patients undergoing a CT scan under standard practice scenario	60.00%	4680	Survey to Belgian experts and literature
mTBI population undergoing GFAP+UCH-L1 testing under biomarker scenario	60.00%	4680	Assumed 100% of those getting a CT scan under standard practice
Biomarker positive	73.78%	3453	MA of 15 primary studies (n=10 440)
% of biomarker positive in which CT scan performed	100.00%	3453	Assumes 100% of biomarker positive as recommended
CT negative after biomarker positive	86.34%	2981	From MA of 15 primary studies (n=10 440)
CT positive after biomarker positive	13.66%	471	
Biomarker negative	26.22%	1227	
True negatives	99.22%	1218	
False negatives	0.78%	10	
mTBI patients undergoing a CT scan under biomarker scenario	44.39%	3463	
Total reduction of head CT scans with biomarker scenario	26.00%	1217	

7.5 Costs linked to the adoption of GFAP+UCH-L1 in the ED in Belgium

At this stage, comprehensive cost estimations remain premature due to the lack of data on current clinical practices and follow-up procedures following head CT scans or biomarker testing in patients with mTBI in Belgium.

Therefore, the cost analysis presented here offers only a partial perspective, focusing specifically on the potential savings associated with a reduction in the number of patients undergoing at least one head CT scan. Follow-up costs and downstream healthcare expenditures (e.g. from missing out on a clinically important lesion) are not included in this estimation.

Based on current reimbursement rates for head CT scans in Belgium (nomenclature codes: 458673, 461016, and 460670), the estimated cost per scan would be €194.84. Of this amount, €184.92 is reimbursed by the National Institute for Health and Disability Insurance (NIHDI), while €9.92 represents out-of-pocket expenses for patients.^{bb}

Assuming a 26% reduction in CT scan utilisation due to the implementation of GFAP+UCH-L1 biomarker testing for mTBI in the ED for the hypothetical cohort here studied, the projected total savings amount to €237 259. This includes €225 179 in reduced expenditures from the radiology budget the NIHDI and €12 080 in patient out-of-pocket savings.

Currently, no official pricing is available for the GFAP+UCH-L1 test. However, considering the estimated savings and the target population for biomarker testing in our base-case scenario (i.e. the 60 % of mTBI patients who would otherwise receive a CT scan under current practice), a test cost of €50.70 would represent a break-even point, where the additional costs of biomarker testing are offset by the savings in radiology.

7.6 Sensitivity Analysis

A one-way sensitivity analysis was conducted to assess the impact of changes in key variables on CT reduction and cost outcomes.

The minimum and maximum values applied in this analysis were:

- Sensitivity and specificity of GFAP+UCH-L1 in adults were varied across the 95% CIs from our MA of 15 primary studies.
- Prevalence of ICIs was varied using minimum and maximum values reported in the primary studies included in the MA.
- The minimum and maximum ranges for the proportion of cases undergoing head CT under standard practice (representing the biomarker target population) were derived from our survey of Belgian hospitals and European literature. Thus, similar rates to those we saw in our survey were mentioned in a recent transnational survey in Southern Europe¹²⁹ and a recent prospective multicenter cross-sectional study from France.¹³⁰

Variations in sensitivity values across the 95% CI of our MA (i.e. 96%-99%) did not have an impact on the overall results.

On the contrary, variations in specificity values across the 95% CI of our MA (i.e. 25%-34%) did affect the overall results. Thus, the minimum value of 25% resulted in a reduction of 22.43% of CT scans, overall savings of €204 533 and a break-even cost for the test of €43.70, while the maximum value of 34% led to a reduction in CT scans of 30.51%, overall savings of €278 165 and a break-even cost per test of €59.44.

^{bb} Based on a non-preferential patient. The out of pocket costs for a preferential patients would be €2.97.



A prevalence for ICIs of 4.78% had a small impact on CT scan reduction (-27.61%), overall savings of €251 797 and break-even cost per test (€53.80). A higher prevalence rate (i.e. 31.22%) limited the reduction in CT scans to 19.95%, the overall savings to €181 880, and the break-even cost for the test to €38.86. A higher prevalence of ICIs would lead to a greater number of patients requiring CT scans following biomarker testing, thereby reducing the potential cost savings.

7.7 Conclusions

Our estimations based on the limited data gathered via the survey to Belgian experts show there is potential for reducing CT scans in the mTBI population in Belgium by approximately 26%. Nevertheless, great uncertainties exist and should be borne in mind when discussing the potential adoption of biomarkers in clinical practice, since the reality of each hospital is likely to differ from the general assumptions used here for the purpose of this illustrative exercise.

7.8 Discussion

The estimations presented in this analysis are subject to several important limitations, primarily due to the lack of robust and granular data on current clinical practice in Belgium. While the base-case scenario provides a simplified view of potential reductions in CT scan utilisation through GFAP+UCH-L1 biomarker testing, it does not account for the full spectrum of clinical decision-making and hospital-level variation.

Clinical practice regarding the management of patients with mTBI remains largely undocumented. Notably, there is significant variation between hospitals, and even between physicians within hospitals, in terms of the application of clinical head rules and as a consequence, on CT scan utilisation, but also patient observation protocols, and follow-up procedures. These differences can substantially influence the results linked to the implementation of biomarker-based strategies. For example, some hospitals may choose to leave patients under observation even when biomarker results are negative or may still perform CT scans in some biomarker-negative cases, while others may opt for direct patient discharge. These divergent practices were partially captured in our survey of Belgian experts, but not to an extent that would allow us to account for them in our estimations. As highlighted by Moya Torrecilla et al.(2025),¹⁰⁴ factors other than CT reduction can also have an important budgetary impact, which stresses the importance of including such variables in future analyses.

8 DISCUSSION AND CONCLUSION

8.1 Diagnostic Performance

Our meta-analyses demonstrated a high sensitivity for the combination of GFAP+UCH-L1 (i.e. 98%) and a great capacity for ruling out ICIs after mTBI. This performance reflects the complementary kinetics of both markers: UCH-L1 peaks soon after trauma, while GFAP rises later, extending the diagnostic window up to 12 hours. This evidence has granted the test FDA approval and appears to be gaining growing clinical endorsement across Europe, as shown in our chapter on the European recommendations.

Nevertheless, the test's low specificity (29%) results in numerous false positives, limiting CT scan reduction. Many patients may still undergo unnecessary imaging and radiation, while increased laboratory testing could add organisational complexity and budgetary impact. Adjusting cut-offs by age, as shown by Ladang et al. (2024), may significantly improve accuracy and reduce unnecessary CT scans in this population. Nevertheless, further validation is still needed in this regard.

Paediatric studies remain scarce, though the high sensitivity observed suggests potential utility if restricted to cases where CT would otherwise be performed. New studies in this specific population are underway (e.g. The BRAINI-2 study^{cc}).

Further research should validate whole-blood assays and assess real-world effectiveness comparing standard head-rule based care with biomarker-guided strategies.

Comparative and Practical Considerations

The diagnostic performance of GFAP+UCH-L1 is comparable to S100 β , yet its longer sampling window and independence from skin pigmentation offer practical advantages.

Although GFAP alone shows slightly higher specificity, no CE-marked test is currently available, and most experts favour the combined test due to its superior sensitivity, an essential feature in a field where missing ICIs must be avoided.

The added value of the GFAP+UCH-L1 combination lies beyond sensitivity in its extended diagnostic window and temporal complementarity. While GFAP is effective in detecting brain injury, it can produce false negatives if measured too soon after trauma, as its concentration only rises several hours post-injury. Conversely, UCH-L1 peaks almost immediately, covering the early time gap and ensuring high accuracy across a broader post-injury interval. Similarly, while S100 β is reliable within six hours of trauma, its effectiveness decreases sharply thereafter. Therefore, the combination of GFAP and UCH-L1 provides EDs with a more reliable and flexible tool, particularly when the exact timing of injury or sample collection is uncertain.

8.2 Economic and Implementation Evidence

The existing economic evaluations are limited as they rely on simplified, assumption-driven models that do not accurately reflect real-world clinical pathways or system-level differences. As such, cost-effectiveness in the Belgian context remains uncertain, highlighting the need for an economic assessment informed by local data. It is important to note that our review identified an abstract of a Belgian economic evaluation in this field, and the first author has confirmed that it will be published in full very soon.⁹⁸

^{cc} <https://clinicaltrials.gov/study/NCT05413499>



8.3 European recommendations

Recommendations that support the use of biomarkers in this field emphasise that they should complement, rather than replace, clinical decision rules. They also acknowledge that, despite promising evidence, uncertainties remain regarding clinical integration, cost and implementation.

8.4 Insights from Belgian Practice

While the Belgian expert survey provided useful insight into the current management of mTBI, it must be interpreted with caution. Participation was voluntary and self-reported, which introduced potential selection and reporting biases. The response rate was modest at 32%, with fewer than half of respondents able to provide quantitative data on mTBI management due mainly to the absence of national or local registries and standardised reporting systems. Consequently, the findings may not fully reflect real clinical practices.

The absence of mTBI case registration in Belgian administrative databases prevented a data-driven analysis, necessitating reliance on the survey despite its limitations. Nevertheless, the results highlight significant variations in practices, particularly with regard to CT use, influenced by internal protocols, differences in the adoption of clinical head rules, patient characteristics, and medico-legal factors. The use of biomarkers appears to remain negligible, mainly due to limited awareness, reimbursement issues and workflow constraints. Clinicians primarily valued biomarkers that were highly sensitive, had a rapid turnaround time and were affordable, considering them most useful in ambiguous cases or when access to CT or radiation exposure were a concern.

8.5 Outlook and Future Directions

GFAP+UCH-L1 testing should be considered an additional tool for clinical decision-making, particularly when a decision about the need for a CT scan is uncertain, delayed or undesirable. Further research should refine population-specific thresholds, validate whole-blood point-of-care testing and evaluate real-world cost-effectiveness. These biomarkers are also being studied as prognostic tools beyond triage, but such use is beyond the scope of this report.

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