KCE REPORT 261

Federaal Kenniscentrum voor de Gezondheidszorg
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Belgian Health Care Knowledge Centre

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HEALTH TECHNOLOGY ASSESSMENT

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COLOPHON

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LIST OF ABBREVIATIONS

ABBREVIATION	DEFINITION
AMSTAR	A Measurement Tool to Assess systematic Reviews
AUC	Area Under the Curve
CCHR	Canadian Computer Tomography Head Rule
CHALICE	Children's Head Injury Algorithm for the Prediction of Important Clinical Events
CK-BB	BB isozyme of creatine kinase
CT	Computerised Tomography
C-Tau	Serum cleaved tau
ED	Emergency Department
GCS	Glasgow Coma Score
GFAP	Astroglial protein (glial fibrillary acidic protein)
GRADE	The Grading of Recommendations Assessment, Development and Evaluation
HTA	Health Technology Assessment
ICI	Intracranial Injury
INAHTA	International Network of Agencies for Health Technology Assessment
Klk6	Kallikrein-6
LOC	Loss of Consciousness
LR+/-	Positive/Negative Likelihood Ratio
MA	Meta-Analysis
MBP	Myelin basic protein
mCT	Mild cranial trauma (most commonly referred to as mild traumatic brain injury (mTBI)
miRNA	Serum microRNA
MRI	Magnetic Resonance Imaging
mTBI	Mild Traumatic Brain Injury (also known as mild cranial trauma mCT)
NEXUS II	National Emergency X-Radiography Utilization Study II
NFL-H	Neurofilament-H
NFM	Neurofilament medium polypeptide protein
NHS	National Health Service

NICE	National Institute for Health and Care Excellence
NSE	Neuron-specific enolase
PECARN	Paediatric Emergency Care Applied Research Network
PrPC	Plasma soluble cellular prion protein
QUADAS	Quality Assessment of Diagnostic Accuracy Studies
S100B	S100 calcium-binding protein B
SBDPs	Spectrin breakdown products
SNTF	Spectrin N-terminal fragment
SR	Systematic Review
STARD	Standards for Reporting of Diagnostic Accuracy Studies
suPAR	Soluble urokinase plasminogen activator receptor
TBI	Traumatic Brain Injuries (also known as cranial trauma)
TNF-α	Tumor necrosis factor-alpha
UCD	University of California‑Davis rule
UCH-L1	Ubiquitin carboxy-terminal hydrolase L1
UREG	Enregistrement urgence (Registry of Emergencies)
WHO	World Health Organisation

SCIENTIFIC REPORT

1 THE ROLE OF BIOMARKERS IN RULING OUT CEREBRAL LESIONS IN MILD CRANIAL TRAUMA BACKGROUND

Cranial trauma, is most commonly referred to in the scientific literature as traumatic brain injuries (TBI), and we will subsequently use that terminology throughout this document.

1.1 Epidemiology of cranial trauma

TBI are non-degenerative, non-congenital insults to the brain from an external mechanical force, which may lead to a temporary or permanent impairment of cognitive, physical, and psychosocial functions. They represent one of the most critical public health problems around the world.[1]

Definitions in this field present some inconsistencies and data capturing varies from one country to another, with most registries focusing on hospitalized patients. As a consequence, accurate incidence estimations remain, to this date, challenging. Nevertheless, a comprehensive review on the epidemiology of TBI in Europe including 23 studies from different countries[a] was published in 2006 and estimated an aggregated annual incidence both hospitalized and fatal TBI of 235 per 100 000 population.[2] Despite important variations in country specific published incidence rates, most studies covered by the European review reported 150–300 new cases per 100 000 population per year, in line with previous estimations from a WHO study.[3] The estimated mortality rate was 15 per 100 000 population per year.[2] Given the lack of Belgian data in this regard the authors of this review used the estimates from the European literature to offer an approximation to the size of the problem. Thus, taking into consideration the latest statistics (2014) on the Belgian population,[4] the above mentioned aggregated figures would translate into approximately 26 000 new TBI cases and 1664 TBI deaths per year. Frequent causes of TBI include falls, vehicle related collisions, violence, alcohol consumption and sports injuries. Populations most at risk include children aged ≤ 4 and the elderly (both

[a] Denmark, Finland, France, Germany, Ireland, Italy, Norway, Portugal, Spain, Sweden, Switzerland and the UK

populations at high risk of falls); and young adults (most at risk of vehicle related collisions, violence or sports injuries).

1.2 TBI severity classification and prognosis

There is a distinction between mild (or light), moderate and severe brain lesions. Such distinction is most often based on the Glasgow Coma Scale (GCS): a 3 to 15-point scale used to assess patients' level of consciousness and neurologic functioning after a head injury.5 Scoring by means of this scale is based on motor, verbal, and eye opening responses at admission to the emergency department (**Table 1**). Scores from 3 to 8 represent severe brain lesions; 9 to 12: moderate and 13 to 15 mild. Mild TBI (mTBI) are by far, the most frequent, accounting for as many as 79 to 90% of cases3 and are the subject of interest of this review. The prognosis of TBI depends primarily on the severity of the injury, but other factors can also play a role such as the location of the injury, or the age and general health condition of the individual.

Table 1 – Glasgow Coma Scale

Glasgow Coma Scale values (GCS)	Value
I. MOTOR RESPONSE	
Obeys commands fully	6
Localizes to noxious stimuli	5
Withdraws from noxious stimuli	4
Abnormal flexion, i.e. decorticate posturing	3
Extensor response, i.e. decerebrate posturing	2
No response	1
II. VERBAL RESPONSE	
Alert and Oriented	5
Confused, yet coherent, speech	4
Inappropriate words and jumbled phrases consisting of words	3
Incomprehensible sounds	2
No sounds	1
III. EYE OPENING	
Spontaneous eye opening	4
Eyes open to speech	3
Eyes open to pain	2
No eye opening	1
OVERALL SCORE	Addition of values for I+II+III

1.3 The potential role of biomarkers in ruling out cerebral lesions in mTBI

Patients with mTBI, described as a GCS score 13 to 15, have a small but significant risk of intracranial injury (ICI) that requires early identification and neurosurgical treatment.6 Computerised tomography (CT) scanning is recognised as the gold standard in this field because of its high sensitivity at detecting complications linked to TBI.7, 8 Nevertheless, it is an expensive procedure that exposes patients to the risk of radiation, making it important to carefully assess the need for it in mTBI patients, in order to reduce unnecessary radiation, while still avoid missing ICI. Currently, clinicians identify patients at risk of ICI and thus in need of CT scan and/or hospital admission using clinical decision rules and unstructured assessments.9Amongst the clinical decision rules developed over the last years, the Canadian CT Head rule (CCHR) is at present, the most widely validated tool for making decisions regarding the need for a CT scan in the adult TBI population.9 Published in 2001, the CCHR consists of 5 high-risk factors10 (Table 2). Additional medium-risk factors include retrograde amnesia of more than 30 minutes before injury and dangerous mechanism of injury, such as high-speed road accidents or falls from a height greater than 3 metres.11NICE guidance published in 200312 and revisited in 200713, adapted the CCHR for use in the NHS and recommended it for adults. The French Society of Emergency Medicine "Société française de médecine d'urgence" used NICE's guidance as the basis of their recommendations for the same population.8 Although no official Belgian guidelines have been published to this date and thus, variability in clinical approaches is likely, a discussion with the experts consulted during this project revealed that very similar factors to the ones used in the CCHR are often considered in practice.

Table 2 – The Canadian CT Health Rule (CCHR)

If answer is YES to any of the points below, a CT should be considered
GCS<15 two hours after trauma
Suspected open or depressed skull fracture
Any signs of basilar skull fracture
≥ two episodes of vomiting
Age ≥ 65
Retrograde amnesia to the event ≥ 30min
"Dangerous mechanism" (struck by motor vehicle; ejected from motor vehicle; fall from > 3 feet or > 5 stairs)

A further rule "The New Orleans Criteria" for CT scanning, which includes headache, vomiting, patient age > 60 years, drug or alcohol intoxication, deficits in short-term memory, physical evidence of trauma, and seizure, has also been studied with good results in the adult population[11].

Clinical decision CT rules for children have been validated to a lesser extent. Nevertheless a number of them have been tested in at least two patient cohorts. These include: The Paediatric Emergency Care Applied Research Network (PECARN) rule, considered the most consistent with a sensitivity of 97% and a specificity between 58% and 60%.[9] This rule divides the

paediatric population in two groups: children under 2 years and children above that age and consider a number of age-specific factors to decide whether a CT scan is needed or observation is recommended instead. Amongst other factors, these rules consider GCS, the existence or not of a palpable skull fracture or signs of altered mental status (agitation, somnolence, repetitive questioning, or slow response to verbal communication). The current recommendations of the French Society of Emergency Medicine "Société française de médecine d'urgence" for CT scanning in children are based on this rule. Other clinical decision rules for children include: the CHALICE rule with high sensitivity (98%), but highly variable specificity (5% - 87%); which was recommended by NICE in its 2007 clinical guidline[13].

- the UCD rule with a very high sensitivity (99% -100%), but variable, low specificity (12% - 43%).[9]

Finally, the NEXUS II rule has high sensitivity for both neurosurgical and any injury, but variable specificity and very limited validation. Biomarkers in combination with clinical decision tools could help to rule out ICI without CT investigations, which would result in minimising unnecessary radiation exposure and resource use[9]. Nevertheless, to this date only the Scandinavian clinical guidelines on CT use in mild and moderate head injuries have formally incorporated the use of one of these biomarkers: the protein S100B, for patients with low-risk mild TBI (described as presenting GCS14, or GCS 15 and one of the following symptoms: suspected/confirmed loss of consciousness or repeated vomiting)[14]. The protein S100B, released into the blood from the cytosol of astroglial and Schwann cells due to a traumatic lesion to the cephalous and the hematoencephalic barrier[9] is to this date the most documented biomarker for this utilization. A meta-analysis of nine diagnosis studies, reported the performance of a protein S100B concentration below a given cut-off against the results of head CT scan[9]. The pooled sensitivity was 96.8% (95% CI: 93.8; 98.6) and the specificity was 42.5% (95% CI: 31.0; 54.2). A high sensitivity is necessary to rule out a condition. Such high sensitivity can be obtained with protein S100B at the usual cut-off 0.105µg/L.

However, it is important to bear in mind that the protein S100B is not the only biomarker that could be useful in this endeavour, and many other have been proposed including neurofilament medium polypeptide protein; neurofilament-H; neuron-specific enolase; ubiquitin carboxy-terminal hydrolase L1; glial fibrillary acidic protein; TNF-α; plasma soluble cellular prion protein; soluble urokinase plasminogen activator receptor; Serum microRNA; spectrin N-terminal fragment; kallikrein-6; "troponin I"; myelin basic protein; BB isozyme of creatine kinase; serum cleaved tau or C and spectrin breakdown products.

1.4 Objectives

This rapid review aims at assessing the value of biomarkers to rule out the presence of ICI in case of mTBI, and thus, their potential to reduce the utilization of CT scans. This is a diagnostic test accuracy review where the index test is expected to have a similar sensitivity as the reference test, but at lower costs and with less irradiation[15]. Biomarkers are not used in replacement of clinical rules, but in addition to them, i.e. in those remaining at risk after application of the clinical rule. Only in cases with a positive biomarker will a CTscan perform.

2 REVIEW OF THE CLINICAL LITERATURE

2.1 Background

A systematic review was performed to assess the predictive value of biomarkers compared to CT scan or magnetic resonance imaging (MRI), for ICI in adults and children with mTBI. The search strategy focused on:

Population: Patients with minor/mild/minimal head/cerebral/brain traumatic injury (mTBI)

Intervention: Brain injury assessed by biomarkers including:

- S100B protein;
- Neurofilament medium polypeptide protein(NFM) ;
- Neurofilament-H (NFL-H);
- Neuron-specific enolase (NSE);
- Ubiquitin carboxy-terminal hydrolase L1 (UCH-L1);
- Astroglial protein (glial fibrillary acidic protein; GFAP);
- Tumor necrosis factor-alpha (TNF-α);
- Plasma soluble cellular prion protein (PrPC);
- Soluble urokinase plasminogen activator receptor (suPAR);
- Serum microRNA (miRNA);
- Spectrin N-terminal fragment (SNTF);
- Kallikrein-6 (Klk6);
- Troponin I;
- BB isozyme of creatine kinase (CK-BB);
- Myelin basic protein (MBP);
- Serum cleaved tau (C-tau);
- Spectrin breakdown products (SBDPs)

Control: Brain injury assessed by CT scan or MRI

Outcomes: Sensitivity, negative likelihood ratio, negative predictive value

For ruling out a condition, a high sensitivity of the test is requested, i.e. if the test result is negative the likelihood that the patient is condition-free is high as there is no or very few false negatives. [16]. However, the power of a test to rule a diagnosis out does not depend exclusively of its sensitivity but is reduced by low specificity. These two dimensions are integrated in the negative likelihood ratio as well as in the negative predictive value.Methods. PUBMED, EMBASE, Cochrane, CRD HTA databases, Open Grey, GIN, and the National Guideline Clearing House were consulted for studies published up to May 2015. Full search strategy is available in Appendix 1. In view of the rapid nature of our review, we applied a step-wedge strategy. [17] we first identified all recently published systematic reviews (SR) or meta-analyses (MA) and applied the AMSTAR quality checklist (http://amstar.ca) to identify the most recent high quality SR which could be used as a starting point of our review. In a second step, we updated the selected SR by searching all primary studies published after the search date reported in the selected SR. Such step-wedge approach is efficient as it avoids re-doing previously validated research and focuses instead on updating it and checking its current validity. References of other reviews retrieved via our search, were used to check that no relevant clinical studies had been missed from our review, and references from included studies were also screened with that purpose. For the update based on primary studies, we applied no restriction to study design. Only papers written in English, French, Dutch, or Spanish were eligible. Any original study combining the four following criteria was considered eligible:

- Reference standard: CT scan or MRI.
- Index test: biomarker (none excluded a priori).
- Target condition: any intracranial abnormality detected on CT scan or MRI.
- Reported outcomes: sensitivity and specificity, likelihood ratios or predictive values were either reported or could be computed based on the information available in the primary study. If this was not the case, the authors were contacted to get the information.

Exclusion criteria were defined as:

- Studies focused on clinical prognosis of mTBI
- Studies on patients with moderate or severe head injuries (defined as patients with a GCS of ≤12 at presentation) or studies with mixed severities, where results for patients with mTBI could not be isolated.
- Studies of patients with no injuries (e.g. degenerative disease).

Standardized forms for data extraction were used to capture information on the characteristics of the studies as well as all relevant outcomes.

The quality appraisal of all included studies was performed using QUADAS—2.

QUADAS—2 is a tool specifically designed to assess the quality of primary diagnostic accuracy studies. It includes four different domains:

- Patient selection
- Index test(s)
- Reference standard
- Flow of patients through the study and timing of the test (index and reference standard).

Each of these domains is assessed in terms of risk of bias. Applicability of results is also assessed for the 3 first domains[18]. More details on the QUADAS—2 tool is available at www.quadas.org. Data were exported into Stata 12.0 (College Station, TX 77845) for meta-analysis and plotting Summary Receiver-Operator (SROC) Curves. A sensitivity analysis was performed to test the influence of study quality (risk of selection bias), type of device for biomarker measurement and time between trauma and biomarker measurement.

The strength of overall evidence was evaluated following the GRADE methods[15, 19, 20] on criteria of study limitations (risk of bias), indirectness, inconsistency in study results, imprecision and probability of publication bias.

2.2 Results

After eliminating duplicates, we found 915 studies, from which eight SR were identified. From these, three were excluded based on language[21-23], one because it was not a systematic review[24], one because it did not relate to a biomarker[25] and a last one because it was a conference abstract[26]. Two additional reviews were identified by hand searching references,[27, 28] but both resulted to be descriptive reviews and not systematic and thus were excluded from the critical appraisal. From the two remaining SR[9, 29], Pandor et al.[9] was the most recent and had the higher quality score (see AMSTAR quality appraisal in appendix). It was therefore selected as the starting point of our review. It is worth mentioning that a new systematic review[30] was published in June 2015, i.e. after the completion of our own literature search. This new SR rated 7/11 on the AMSTAR scale. This publication is not further discussed in this report for two main reasons: on the one hand, the paper does not provide additional evidence to our systematic search and on the other hand, a number of flaws were detected in study selection, reporting, and data pooling (see appendix for details on quality appraisal). For updating the SR by Pandor et al.[9], our search strategy yielded 545 potential relevant primary studies published from 2009 (search year used in Pandor et al.) to May 2015 (See Figure 1). Three additional citations were found by hand search. Five hundred and four citations were rejected on the basis of their title and/or abstract, and 28 were further excluded after a full-text examination (reasons for exclusion are presented in Table 4). Finally, 16 primary studies published after 2009 were included in our review, most of which referred to the protein S100B in adults (see Table 3 for an overview). As there is no compulsory registers of diagnosis accuracy studies, we could not formally assess the presence of a publication bias. However, medical experts consulted during our study confirmed that they were not aware of any other studies than those we retrieved through our search strategy.

Figure 1 – Flow chart of primary study selection

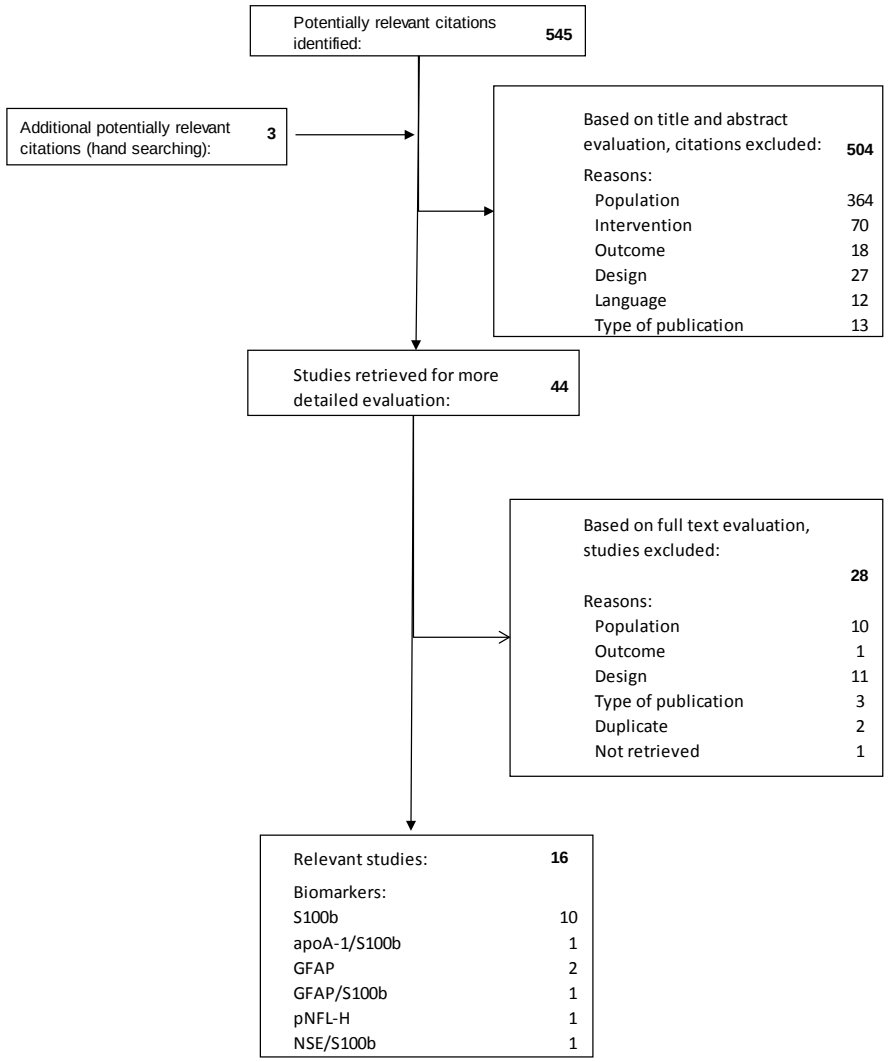

Table 3 – Overview of the included studies

	Study ID	Population		Biomarker			
		Adults	Children	S100B	GFAP	NSE	pNFL-H
1	Badock 2012[31, 32]		x	x			
2	Bazarian 2013[33]	x	x	x			
3	Bouvier 2012[34]		x	x			
4	Calgagnile 2013[35]	x		x			
5	Castellani 2009[36]		x	x			
6	Cervellin 2012[37]	x		x			
7	Egea-Guerrero 2012[38]	x		x			
8	Gatson 2014[39]	x					x
9	Kotlyar 2011[40]	x		x			
10	Laribi 2014[41]	x		x			
11	Metting 2012[42]	x			x		
12	Müller 2011[43]	x		x			
13	Papa 2012[44]	x			x		
14	Papa 2015[45]		x	x	x		
15	Wolf 2013[11]	x		x		x	
16	Zongo 2012[46]	x		x			

2.2.1 Protein S100B in adults

The SR by Pandor et al.[9] included 9 studies published before 2009 (2.442 adults) and reported a sensitivity of S100B to detect brain damages on CT scan or MRI of 96.9% (95%CI: 91.4%; 98.9%) and a LR- of 0.07 (95%CI: 0.03; 0.21)[b]. Pandor et al. mentioned that although the patient selection criteria were consistent across all studies, no study met all of the QUADAS criteria. It is worth mentioning that 2 studies used a specific sandwich immunoluminometric assay (Sangtec 100 (Sangtec Medical , Bromma, Sweden) which detection limit is less optimal than the other tests currently available and reported a delay of up to 24 hours between injury and blood sampling, in spite of the short half-life of protein S100B (30 to 90 minutes[47]). Removing these two studies improved slightly the pooled sensitivity but increased uncertainty around the point estimate (Se=99.0% (95%CI: 83.1%; 99.9%) and LR-=0.03 (95%CI: 0.00; 0.60)[c]. For more details on this SR, please consult the original publication (p102-p116)[9] (available at http://www.ncbi.nlm.nih.gov/pubmedhealth/PMH0015186/pdf/PubMedHealth_PMH0015186.pdf).

Our literature update included 10 additional studies published since 2009, totalling 3,795 individuals. The characteristics of the studies are presented in Table 5 and their quality appraisal in Table 6. Further details on the included studies are presented in and Appendix. The raw figures for the study by Calganile et al.[35] were obtained from the authors. The study by Bazarian et al.[33] mixed children and adults, and the authors provided us with raw data stratified by age range. None but one of the studies (Zongo 2012[46]) met all the QUADAS criteria. In the vast majority of the studies, the Standards for Reporting of Diagnostic Accuracy Studies (STARD)[48] were only partially followed, making the quality difficult to ascertain on a number of points. Although there was no uniform definition of mTBI across studies, the inclusion criteria were most often a GCS between 13 and 15 with at least one symptom, most generally post-injury amnesia and/or loss of consciousness.

The great variation of the proportion of CT scan found positive is an indication of heterogeneity in study populations and/or methods (Table 7). Overall, the sensitivity of S100B was high at 95.9% (95%CI: 88.2%; 98.7%), although slightly lower than in Pandor et al. The LR- was 0.16 (95% CI: 0.06; 0.43) vs. 0.07 (95% CI: 0.03; 0.21) in Pandor et al. Although quite close to the results reported in the SR by Pandor et al., the results of these more recent studies display a higher level of heterogeneity, as it can also be appraised visually in the SROC curves (Figure 2). This was mainly driven by the results of the large study by Bazarian et al.[33]. In this study, the long delay (6 hours) between trauma and the dosage of the protein S100B and the heterogeneous study population (16.4% Black or Hispanic people) could have contributed to the lower sensitivity of the test. Also, Müller et al.[43] reported that 2 of the 3 false negatives observed in their study had the test for protein S100B performed more than 6 hours after injury. Pooling the results of our review with those included in the review by Pandor et al., yielded an overall sensitivity of 96.6% (95% CI: 92.3%; 98.5%) and a LR- of 0.10 (95%: 0.04; 0.23) (Table 8). In a sensitivity analysis, we assessed the effect of some potential modifying factors on the results of the meta-analysis. Stratifying the meta-analysis by time between injury and sampling protein S100B (less than 3 hours vs. more than 3 hours), by manufacturer (Roche vs. DiaSorin), and by selection bias (yes/uncertain vs. no) did not result in statistically significant differences (Table 9). Given the low impact of studies presenting a high risk of bias on the overall results, given that the overall precision, consistency and directness[d] of results were considered high, and given that a significant publication bias is unlikely, we rated the overall quality of evidence as high following the terminology of GRADE (i.e. further research is very unlikely to change our confidence in the test performance).

[b] These results are slightly different from those reported in the original publication by Pandor et al. The difference originates probably in the use of hierarchical logistic regression models in Stata.

[c] Computations done by KCE

[d] The methodology of GRADE for diagnosis accuracy studies is not yet fully standardized. One could argue that the criterion of directness is not met as the accuracy of protein S100B was tested against CT scan findings and not clinical outcomes.

Table 4 – Reasons of exclusion for primary studies during the updating process

Stydy ID	Reason of exclusion
Abbasi 2014	Population: not separated results for mild and moderate
Beaudeux 2009	Design: narrative review
Bechtel 2009	Population : children among those 10% had GCS<12
Bouvier 2009	Population: uninjured control
Buonora 2015	Population: 2 studies were reported (1st included mild-moderate TBI compared with healthy volunteers and the 2nd one included moderate-severe TBI)
Di Battista 2013	Design : narrative review
Diaz-Arrastia 2013	Type of publication : conference abstract
El-Maraghi 2013	Duplicate
Forde et al. 2014	Population: inclusion of severe TBI
Jeter CB 2013	Population : inclusion of moderate and severe GCS
Kovesdi 2010	Design: narrative review
Mondello 2011	Design: narrative review
Mondello 2014	Design: narrative review
Morochovic 2009	Design: narrative review
Papa. 2012	Not retrieved: we received previous paper from the same author with same title but in other journal probably duplicate
Papa 2014	Population: inclusion of moderate and severe TBI
Pickering 2011	Type of publication: congress abstract
Rice 2012	Design: narrative review
Ruan 2009	Design: economic evaluation
Sharma 2012	Design: narrative review
Siman 2013	Outcome: 1) uninjured control 2) no outcome regarding the prediction of CT
Unden 2009	Design: narrative review
Unden 2015 (unpublished paper)	Duplicate: retrospective analysis of patients sample reported in Bazarian 2013
Wiesmann 2010	Population: inclusion of mild (10/60), moderate and severe TBI
Wolf 2013	Type of publication: Conference abstract
Yokobori 2013	Design: narrative review
Zurek 2010	Population: inclusion of severe TBI only
Zurek 2011	Population: inclusion of severe TBI only

TBI: Trauma Brain Injury

Table 5 – Characteristics of studies related to S100B in adults with mild TBI

Characteristics of included studies								
Study ID	**Country**	**Setting**	**Designe**	**Targeted population**	**GCS**	**Symptoms**	**Endpoint**	**Exclusion criteria**
Bazarian 2013[33]	USA	ED	Unclear	Adults / children > 1y	13-15	At least LOC<30 minutes Posttraumatic amnesia ≤ 24h Neuropsychological abnormality Neurological abnormality	Cerebral contusion, subarachnoidhemorrhage, subduralhematoma, linear skull fracture, epidural hematoma, intraventricular haemorrhage, pneumocephalus, edema, depressed skull fracture	History of brain tumour, melanoma, Alzheimer's history of concussion, bone fracture or stroke in previous months or patients who had undergone surgery the previous month
Calgagnile 2013[35]	Sweden	ED	Consecutive serie	Adults > 18y	14-15	LOC < 5 minutes or amnesia	Any signs of cranial (skull fracture) or intracranial pathology (hematoma, air or contusion)	Focal neurological deficit, therapeutic anticoagulation or haemophilia, radiographically demonstrated skull fracture, clinical signs of depressed skull fracture or skull base fracture, posttraumatic seizure, shunt-treated hydrocephalus, multiple organ trauma and patients where serum sampling for S100B was taken more than 3 hours post-injury
Cervellin 2012[37]	Italy	E	Consecutive serie	Adults	14-15	LOC AND/OR Amnesia AND (previous neurosurgical procedures) Inherited coagulopathy or anticoagulant therapy Vomit (more than 1 episode) Epilepsy or post-traumatic seizures or Worsening headache	Acute subdural, epidural or parenchymal hematoma, traumatic subarachnoid haemorrhage,cerebral contusion, brain swelling	Unknown time of injury, acute non-traumatic intra cerebral lesions, suspected/visible brain tumour, extracranial traumatic lesions

[e] consecutive series, random sample, case-control, other, NR ED= Emergency Department; LOC = loss of consciousness

Egea-Guerrero 2012[38]	Spain	Unclear	Other	Adults > 14y	15	Transitory LOC amnesia, persistent headache, nausea or vomiting, vertigo	Cerebral contusion, traumatic subarachnoid haemorrhage, epidural haematoma and subdural haematoma	(possible) pregnancy, history of drug/alcohol abuse, renal failure, drug interference in GCS evaluation, hospital admission after 6 hours post-trauma, history of syncope or seizure before head trauma, other previous concurrent nervous system disorders, hospital discharge < 24 hours post-TBI and ICU admission/transfer due to associated severe extracranial lesions
Kotlyar 2011[40]	US	ED	Nested case control	Adults	13-15	Non-focal neurologic examination, intent of the treating physician to obtain CT imaging due to trauma	Hemorrhage, Diffuse brain edema, Diffuse axonal injury, Skull fracture	Major trauma, non-English-speaking patients, head trauma occurring > 6 h before ED presentation, altered mental status of unclear aetiology, known prior intracranial pathology (surgery, tumour, prior haemorrhage)
Laribi, 2014[41]	France	ED	Unclear	Adults from 18 to 80 y	13-15	Amnesia, LOC, nausea, vomiting, vertigo, anticoagulation before injury, severe headache on admission	"Trauma-relevant cerebral lesion"	LOC>10 min, concomitant injuries, no need for a CT scan as decided by the treating physician, renal failure, malignant melanoma, focal neurological deficit; pregnant women
Metting 2012[42]	Netherlands	ER	Consecutive series	Adults from 18 to 65 y	13-15	Presence of posttraumatic amnesia	Marshall classification of CT abnormalities in brain trauma	Neurologic or psychiatric disease, mental retardation, addiction to alcohol or drugs, inability for long-term follow-up
Müller 2011[43]	Switzerland	ED	Consecutive series	Adults ≥16y	13-15	Some with clinical signs (undefined)	Skull fractures, localised epidural, subdural haematomas, isolated subarachnoid haematomas	Cancer, stroke or other neurological diseases, presenting with intracranial bleeds with a diameter > 5 mm or > 1 bleed, inherited coagulopathy or

									anticoagulant therapy, platelet aggregation inhibitor therapy or intoxication, patients with late admissions to the ER and/or multiple associated injuries
Wolf 2013[11]	Austria	ED	Other	Adults	13-15	Amnesia LOC<5min	AND/OR	Epidural, subdural, subarachnoid, intracerebral haemorrhages including contusions	Penetrating head injury, severe TBI, unstable vital signs; acute focal neurological deficit, pregnancy, significant extra cerebral injury including, for example, fractures of the long bones, soft-tissue injuries, and hematomas, malignant melanoma, polytrauma, inherited coagulopathy, cancer; and multiple sclerosis
Zongo 2012[46]	France	ED	Consecutive series	Adults ≥15 years	13-15	LOC, posttraumatic amnesia, repeated vomiting, severe headache, dizziness, vertigo, alcohol intoxication, anticoagulation		Subdural, epidural, or intracerebral haemorrhages bland contusion; oedema; pneumocephalus, skull fracture	Severe injury including open fracture, large open wounds, and intrathoracic or abdominal contusion, non-traumatic neurologic disease (e.g., cerebral ischemia, chronic subdural hematoma), known history of motor neuron disease

Table 6 – Quality appraisal of studies related to protein S100b protein in adults with mild TBI

	Risk of bias				Comments	Applicability			Comments
Author, year	**Patient selection**	**Index test**	**Reference standard**	**Flow and timing**		**Patient selection**	**Index test**	**Reference standard**	
Bazarian 2013[33]	Unclear	Low	low	low	Type of recruitment not clearly stated. Not clear if the lab was blinded to results	Unclear	low	low	Population with ethnic heterogeneity. Large sample size but most were GCS 15. Presentation within 6 hrs. None needed neurosurgical intervention
Calgagnile 2013[35]	High	Low	High	High	A pre-specified threshold was set for index test. Radiologist were not blinded to S100B results. Only 56.4% of the patients received the CT scan.	Low	Low	Low	All patients including elderly and alcohol intoxicated patients were eligible for the study
Cervellin 2012[37]	Unclear	High	Low	Low	Consecutive patients. Exclusion criterion "uncertain time of injury" may have led to inappropriate exclusion. It is unclear if the clinical signs leading to performing a scan where applied (see table 1). It is unknown if the index test was done without knowing results of standard test. Moreover the cut-off was defined ad hoc during analysis	Unclear	low	low	Small sample size of patients fulfilling criteria for CT scanning (n=60). It is unclear if the clinical signs leading to performing a scan where applied (see table 1). These signs include focal neurological deficit and fracture of the skull, i.e. not mTBI

	Risk of bias				Comments	Applicability			Comments
Author, year	**Patient selection**	**Index test**	**Reference standard**	**Flow and timing**		**Patient selection**	**Index test**	**Reference standard**	
Egea-Guerrero 2012[38]	High	Low	Low	Low	Patient selection: 143 mild TBI met the inclusion criteria (no other details). Two exclusion criteria (absence of post-trauma head CT scan, hospital discharge before the first 24 hours post-TBI) indicate a potential selection bias. No details provided on numbers excluded on these criteria	Unclear	Low	Low	12/143 patients were under hypo coagulation therapy at the time of injury; No patient suffered neurological deterioration and none required emergency neurosurgery
Kotlyar 2011[40]	High	Low	Low	High	835 were eligible but 346 were enrolled unclear reasons; random selection of HCT- not described; nested case control study; no a priori cut-off; whether the results of the CT	Unclear	Low	Low	no reason given for non-enrolment of eligible patients (58%); no a priori cut-off
Laribi, 2014[41]	Unclear	Low	Unclear	Unclear	It is not described whether inclusion was consecutive. Selection possible (only 431 patients in 7 hospitals during 2 years). 4/27 CT+ were reclassified CT- by the senior radiologist. No report on how these FP standards were managed in the analysis. Not all patients included in the analysis (n=400/431!!!)	Unclear	Low	Low	Patients with other injuries and injury>3hours were excluded. The vast majority of patients (87%) had a GCS=15.
Müller 2011[43]	High	Unclear	Unclear	Unclear	Intracranial bleeding>5mm and cases with >1 intracranial bleeding were excluded. No information regarding the timing of interpretation of results for the index test and the reference test. It is unclear how many S100B- in the group with clinical signs did indeed underwent CT scan	Unclear	Unclear	Unclear	The study population was a mix of GCS 13-15 with and without clinical signs, and proportions are not reported. The index test was done rapidly after admission (77 min) but we do not have information of the time between index test and injury.

Wolf 2013[11]	High	Low	Unclear	Low	No consecutive enrolment; the high % of ICH (23%) indicates that some sort of patient selection occurred; no threshold pre-specified; reading of CT without knowledge of biomarkers is not described;	High	Low	Low	23% of patients with ICH; 8/107 requiring neurosurgery; seven patients from the CT-positive group were on anticoagulants
Zongo 2012[46]	Low	Low	Low	Low	Although 387 patients and 86 patients of the initial sample did not receive S100B and CT scan, respectively, the reasons are well explained and the characteristics of excluded ones do not differ much	Low	Low	Low	

Table 7 – Main results of the included studies

Author	Cut-off value (µg/L)	Provider	Analyser	N	CT+	CT-	%CT +	%S100B+	%Scan avoided	TP	FP	FN	TN	Sensitivity	Specificity
Studies included in SR by Pandor et al.[9]															
Biberthaler 2001	0.12	Sangtec	LIA-mat	52	15	37	28.8	71.2	28.8	15	22	0	15	1.00 (0.78, 1.00)	0.41 (0.25, 0.58)
Biberthaler 2002	0.12	DiaSorin	Liaison	104	24	80	23.1	64.4	35.6	24	43	0	37	1.00 (0.86, 1.00)	0.46 (0.35, 0.58)
Biberthaler 2006	0.1	Roche	Elecsys	1309	93	1216	7.1	72.3	27.6	92	855	1	361	0.99 (0.94, 1.00)	0.30 (0.27, 0.32)
Ingebrigtsen 2000	0.2	Sangtec	Sangtec 100	182	10	172	5.5	37.9	62.1	9	60	1	112	0.90 (0.55, 1.00)	0.65 (0.57, 0.72)
Morochovic 2009	0.1	Roche	Elecsys	102	18	84	17.6	72.5	27.5	15	59	3	25	0.83 (0.59, 0.96)	0.30 (0.20, 0.41)
Muller 2007	0.1	DiaSorin	Liaison	226	21	205	9.0	71.2	28.8	20	141	1	64	0.95 (0.76, 1.00)	0.31 (0.25, 0.38)
Mussack 2002	0.21	DiaSorin	Liaison	139	19	120	13.7	56.8	43.2	19	60	0	60	1.00 (0.82, 1.00)	0.50 (0.41, 0.59)
Poli-de-Figueiredo 2006	0.1	Roche	Elecsys	50	6	44	12.0	82.0	18.0	6	35	0	9	1.00 (0.54; 1.00)	0.20 (0.10, 0.35)
Romner 2000	0.2	Sangtec	Sangtec 100	278	25	253	9.0	38.8	61.1	23	85	2	168	0.92 (0.74, 0.99)	0.66 (0.60, 0.72)
Studies included in KCE update 2015															
Bazarian 2013[33]	0.1	Roche	Elecsys S100	689[f]	40	649	5.8	65.9	34.0	34	420	6	229	85.0 (70.2; 94.3)	35.3 (31.6; 39.1)
Calganile 2013[35]	0.10	Roche	Elecsys S100	351[g]	29	322	8.3	91.7	8.0	29	293	0	29	100 (88.1; 100)	9.0 (6.1; 12.7)
Cervellin 2012[37]	0.38	DiaSorin	Liaison	60	20	40	3.3	61.7	38.3	20	17	0	23	100 (83.2; 100)	57.5 (40.9; 73.0)
Egea-Guerrero 2012[38]	0.105	Roche	Elecsys 2010	143	15	128	10.5	76.2	23.8	15	94	0	34	100 (78.2; 100)	26.6 (19.1; 35.1)
Kotlyar 2011[40]	0.24	Fujirebio	CanAg S100 EIA	158	22	136	13.9	88.0	12.0	21	118	1	18	95.4 (77.2; 99.9)	13.2 (8.0; 20.1)

[f] The study by Bazarian et al.[33] mixed children and adults, and the authors provided us with raw data stratified by age range.

[g] The raw figures for the study by Calganile et al.[35] were obtained from the authors.

Laribi 2014[41]	0.1	Roche	Elecsys	400	26	374	6.5	64.3	35.8	26	231	0	143	100 (86.8; 100)	38.2 (33.3;43.1)
Metting 2012[42]	0.15	DiaSorin	Laison	94	19	75	25.3	75.5	24.0	17	54	2	21	89.4 (66.9; 98.7)	28.0 (18.2; 39.6)
Müller 2011[43]	0.1	Roche	Elecsys S101	233	22	211	9.4	70.0	30.0	19	144	3	67	86.3 (65.1; 97.1)	31.7 (25.5; 38.5)
Wolf 2013[11]	0.105	Roche	Elecsys	107	25	82	23.4	65.4	34.6	18	52	7	30	72.0 (50.6; 87.9)	36.6 (26.2; 47.9)
Zongo 2012[46]	0.1	Roche	Elecsys	1560	111	1449	7.1	88.6	11.4	110	1272	1	177	99.1 (95.0; 100)	12.2 (10.6; 14.0)

CT: Computed Tomography Scan; FN: False Negative; FP: False Positive; TN: True Negative; TP: True Positive;

Figure 2 – SROC curves

Pandor et al.[9] (9 studies, 2,442 individuals)	KCE update (10 studies, 3,795 adults)	All (19 studies, 6,237 adults)

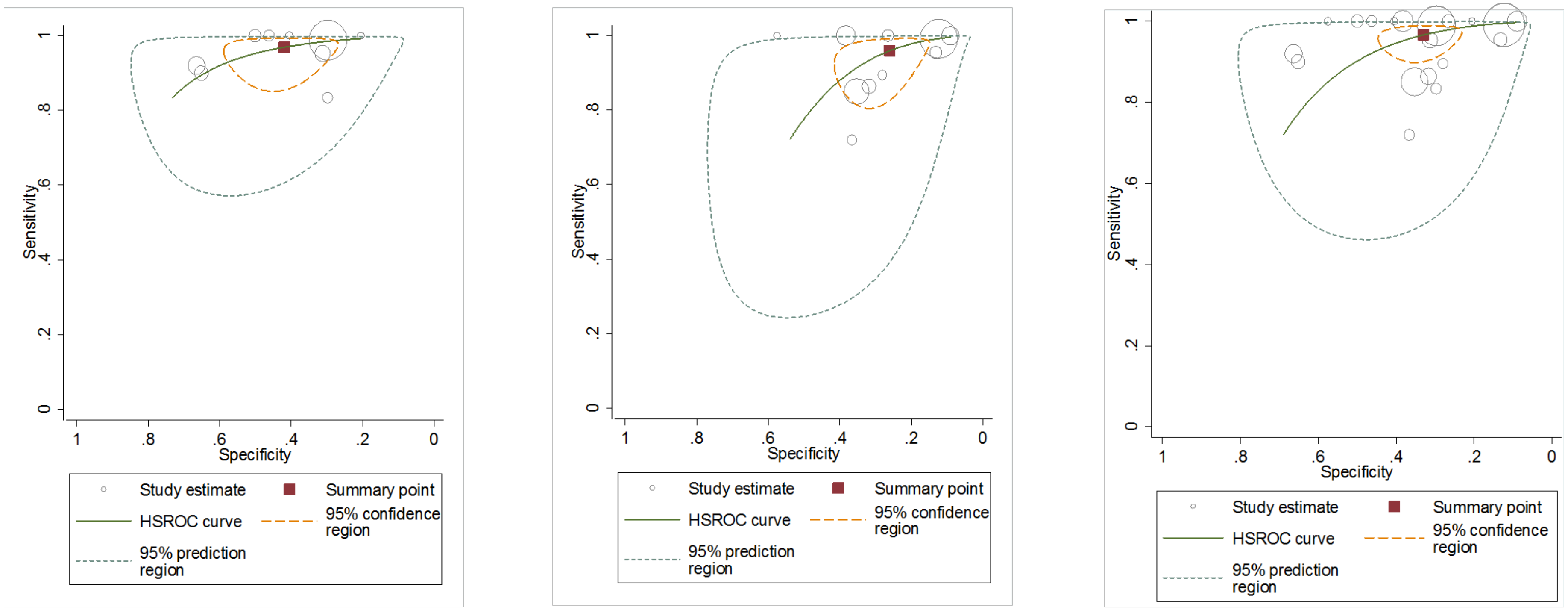

Table 8 – Meta-analysis

	Studies	Participants	Se	Sp	LR+	LR-
PANDOR 2011	9	2442	96.9 (91.4; 98.9)	42.0 (31.9; 52.8)	1.67 (1.40; 2.00)	0.07 (0.03; 0.21)
UPDATE KCE 2015	10	3795	95.9 (88.2; 98.7)	26.1 (18.2; 35.8)	1.30 (1.16; 1.45)	0.16 (0.06; 0.43)
ALL	19	6237	96.6 (92.3; 98.5)	33.0 (25.6; 41.4)	1.44 (1.28; 1.62)	0.10 (0.04; 0.23)

LR+: Positive Likelihood ratio; LR-: Negative Likelihood Ratio; Se: Sensitivity; Sp: Specificity

Table 9 – Sensitivity analysis

Parameters		Studies	Se (95%CI)	Sp (95%CI)	LR+ (95%CI)	LR-(95%CI)
Sampling <3hrs after injury	Yes	12	98.1 (85.6; 99.8)	36.5 (31.3; 42.02)	1.54 (1.39; 1.72)	0.05 (0.01; 0.46)
	No	7	95.3 (88.8; 98.1)	33.1(19.3; 50.7)	1.42 (1.14; 1.78)	0.14 (0.07; 0.30)
Manufacturer	DiaSorin	5	99.0 (80.8; 99.9)	41.4 (31.8; 51.8)	1.69 (1.40; 2.04)	0.02 (0.00; 0.61)
	Roche	10	96.7 (88.4; 99.1)	25.6 (19.0; 33.5)	1.30 (1.20; 1.41)	0.13 (0.04; 0.41)
Selection bias	Yes/Uncertain	5	94.8 (76.2; 99.0)	21.4 (13.0; 33.2)	1.21 (1.10; 1.32)	0.24 (0.06; 0.94)
	No	14	97.0 (92.7; 98.8)	38.0 (29.5; 47.3)	1.56 (1.35; 1.81)	0.08 (0.03; 0.19)

LR+: Positive Likelihood ratio; LR-: Negative Likelihood Ratio; Se: Sensitivity; Sp: Specificity

2.2.2 Protein S100B in children

We did not retrieve any SR specifically focused on the paediatric population. Pandor et al.[9] had included only one paediatric study in their systematic review (Castellani et al. 2009 [36]). We retrieved four additional primary studies published since 2009[33,34,32, 45] , totalling 469 individuals (Table 10). For the study by Bazarian et al.[33], in which adults and children were involved, the authors provided us with the data relating specifically to children. Further details on study population and methods are provided in Appendix 3.3.

The risk of selection bias was high in 3 of the 5 studies, and unclear in the remainders (Table 11). There was also great variation in the definition of the cut-off of protein S100B (Table 12).

The sensitivity of protein S100B was 99.1 (95% CI: 68.7; 100) and the LR- was 0.02 (95%CI: 0.00; 0.30) (Table 13).

Given the risk of bias and given the lack of precision around the point estimate, we assessed the quality of overall evidence as low.

Table 10 – Characteristics of studies related to S100B protein in children with mild TBI

Characteristics of included studies							
Study ID	Country	Setting	Design*	Targeted population	GCS	Symptoms	Exclusion criteria
Babcock 2012[32]	USA	ED	Convenience sample	Children <19 y	All	LOC <30 min Amnesia <24 hrs Any mental alteration at the time of injury	Pre-existing medical or psycho condition associated with high levels of protein S100B (Alzheimer, Down's syndrome, and schizophrenia. Those having run more than 10 miles in the last 12 hours); >6 hours after trauma
Bazarian 2013[33]	USA	ED	Unclear	Children <18y	13-15	At least LOC<30 minutes Posttraumatic amnesia ≤ 24h Neuropsychological abnormality Neurological abnormality	Bone fracture in previous months or patients who had undergone surgery the previous month
Bouvier 2012[34]	France	ED	Unclear	Children <16 y	13-15	Brief LOC, Posttraumatic amnesia, Nausea, vomiting, Severe or progressive headache, dizziness, vertigo, seizure, Intoxication, anticoagulation, Skull fracture	TBI>3 h before presentation; multiple injuries
Castellani 2009[36]	Austria	ED	Consecutive series	Children < 18 y	13-15	Vomiting, LOC, in patients > 4y persisting headache, retrograde amnesia and vertigo	Open head trauma
Papa 2015[45]	USA	ED	Convenience sample	Children ≤ 21 y	9-15	With or without TBI symptoms	Syncope or seizure prior to their head trauma; Known chronic psychosis, neurological disorder, or active central nervous system pathology; Pregnant; Incarcerated; Spinal cord injury; Hemodynamic instability.

**consecutive series, random sample, case-control, other, NR LOC = loss of consciousness*

Table 11 – Quality appraisal of studies related to protein S100b protein in children with mild TBI

	Risk of bias				Comments	Applicability			Comments
Author, year	Patient selection	Index test	Reference standard	Flow and timing		Patient selection	Index test	Reference standard	
Babcock 2012[32]	High	High	Low	Low	Methods for convenience sample not described. Of the 679 children in the TBI registry, 360 underwent a cranial CT and 155 had serum S100B levels measured. A total of 109 children had both a cranial CT and serum S100B measured. S100B was measured a long time after CT scan and blinding is not reported. No pre-specified cut-off for S100B	High	Low	Low	15% had GCS<13. Only children receiving a CT scan included. Likely to be relatively "high risk" patients, considering CT scans are limited in case of children due to radiation risks. Children within the study cohort were older and more likely to be involved in a severe mechanism of injury.
Bazarian 2013[33]	Unclear	Low	low	low	Type of recruitment not clearly stated. Not clear if the lab was blinded to results	Unclear	low	low	Population with ethnic heterogeneity. Most individuals were GCS 15. Presentation within 6 hrs. None needed neurosurgical intervention
Bouvier 2012[34]	Unclear	Low	Low	Unclear	Patients seem to be included consecutively but not really stated. CT decided by the physician according to the classical management rule for these patients. Only 65/241 received CT scan. All those with a CT scan included in the comparative analysis CT scan versus S100B	High	Low	Low	Children only

Castellani 2009[36]	High	Unclear	High	High	Of the 928 patients presented to the study centre with MTBI, 109 were selected if they had a S100B determined within 6 hours and CCT performed. % who got S100B and CT scan are not reported.	Low	Unclear	Unclear	Additional injuries were documented in 55%
Papa 2015[45]	High	High	Low	Unclear	Convenience sample, 91 of the 114 head trauma received CT scan Blinded lab personnel Blinded radiologist Blood sampling within 6h but no info regarding timing of CT scan. Cut-off derived from study data	Unclear	Low	Low	2% of moderate TBI (GCS 9-12)

Table 12 – Main results of the included studies

Author	Cut-off value (µg/L)	Provider	Analyser	N	CT+	CT-	%CT+	%S100B+	%Scan avoided*	TP	FP	FN	TN	Sensitivity	Specificity
Castellani2009[49]	0.16	Roche	E170	109	36	73	33	70.0	28.0	36	42	0	31	100 (90.3; 100)	42.5 (31.0; 54.6)
Bouvier2012[34]	*	Roche	E170	65	23	42	35.4	78.5	21.5	23	28	0	14	100 (85.2; 100)	33.3 (19.6; 49.5)
Babcock2012[32]	0.006	Nanogen	-	109	19	90	17.0	72.5	27.5	17	62	2	28	89.5 (66.9; 98.7)	31.1 (21.8; 41.7)
Papa2015[45]	0.02	Banyan	Elisa	91	8	83	8.8	76.9	24.0	9	60	0	22	100 (66.4; 100)	26.5 (17.4; 37.3)
Bazarian 2013[33]	0.1	Roche	Elecsys S100	95	6	89	6.7	63.2	37.0	6	54	0	35	100 (54.1; 100)	39.3 (29.1; 50.2)

**: 0-9 m: 0.35 µg/L; 10-24 m: 0.23 µg/L; >24 m: 0.18 µg/L CT: Computed Tomography Scan; FN: False Negative; FP: False Positive; TN: True Negative; TP: True Positive*

Table 13 – Meta-analysis

	Studies	Participants	Se	Sp	LR+	LR-
ALL	5	469	99.1 (68.7; 100)	34.7 (29.3; 40.5)	1.52 (1.38; 1.67)	0.02 (0.00; 0.30)

2.2.3 Other biomarkers

Six studies were identified via our search (see Table 14 for their characteristics). The study by Bazarian et al. published in 2013[33] assessed, in addition to the protein S100B, a further biomarker: apoA-1. However, no significant difference in mean apoA-I levels between subjects with mTBI with and without head CT scan abnormalities was retrieved, and thus, no further analysis was performed by the authors of the study. Three studies assessed the diagnostic accuracy of GFAP[42, 44, 45], two[42, 44] of which were in adults (n=202) and one in children[45] (n=91). The diagnosis accuracy of pNHFL-H and NSE were each assessed in 1 study (Table 14 & Table 15). In the review by Pandor et al., one study on NSE in adults[50] and one in children[51] were also included. The evidence base is currently insufficient to elaborate any policy recommendation regarding these other biomarkers. Therefore, we stopped our rapid review at this stage for these biomarkers. It is worth mentioning that some publications have reported that GFAP may out-perform protein S100b in detecting traumatic intracranial lesions on CT scan[44, 45]. However, this statement of superiority is based on the comparison of the AUC area between the 2 biomarkers. This is irrelevant for our research question as the current expectation for using biomarkers in the assessment of mTBI is to rule out ICI, not to detect ICI. At high sensitivity level, the specificity of GFAP and protein S100B are not significantly different.

Table 14 – Characteristics of studies related to other biomarkers in patients with mild TBI

Characteristics of included studies							
apoA-1							
Study ID	**Country**	**Setting**	**Design***	**Targeted population**	**GCS**	**Symptoms**	**Exclusion criteria**
Bazarian 2013[33]	USA	ED	Unclear	Adults / children > 1y	13-15	At least LOC<30 minutes Posttraumatic amnesia ≤ 24h Neuropsychological abnormality Neurological abnormality	History of brain tumour, melanoma, Alzheimer's history of concussion, bone fracture or stroke in previous months or patients who had undergone surgery the previous month
GFAP							
Study ID	**Country**	**Setting**	**Design***	**Targeted population**	**GCS**	**Symptoms**	**Exclusion criteria**
Metting 2012[42]	Netherlands	ER	Consecutive series	Adults from 18 to 65 y	13-15	Presence of posttraumatic amnesia	See above
Papa 2012[44]	USA	ED	Consecutive series	Adults > 18 y	9-15	LOC Amnesia or disorientation	Dementia, chronic psychosis or active CNS pathology. Pregnant women
Papa 2015[45]	USA	ED	convenience sample	Children ≤ 21 y	9-15	With or without TBI symptoms	See above
pNHFL-H							
Study ID	**Country**	**Setting**	**Design***	**Targeted population**	**GCS**	**Symptoms**	**Exclusion criteria**
Gatson 2014[39]	USA	Unclear	Unclear	Adults from 18 to 50 y	13-15	LOC, loss of memory before or after the event, altered mental status, and/or, Acute or chronic neurological deficits	Penetration injuries, inclusion in an interventional trial
NSE							
Study ID	**Country**	**Setting**	**Design***	**Targeted population**	**GCS**	**Symptoms**	**Exclusion criteria**
Wolf 2013[11]	Austria	ED	Other	Adults	13-15	Amnesia AND/OR LOC<5min	See above

**consecutive series, random sample, case-control, other, NR LOC = loss of consciousness*

Table 15 – Quality appraisal of studies on other biomarkers in patients with mild TBI

	Risk of bias				Comments	Applicability			Comments
Author, year	**Patient selection**	**Index test**	**Reference standard**	**Flow and timing**		**Patient selection**	**Index test**	**Reference standard**	
Bazarian 2013[33]	Unclear	Low	Low	Low	Type of recruitment not clearly stated. Not clear if the lab was blinded to results	Unclear	Low	Low	Mixed population including children. Large sample size but most GCS 15 although with symptoms. Presentation within 6 hrs. None needed neurosurgical intervention
Metting 2012[42]	Low	Low	Low	Low	-	Low	Low	Low	
Papa 2012[44]	Low	Low	Low	Low	Consecutive, when researchers on duty	Unclear	Low	Low	Small sample size most GCS 13-15 and with symptoms. Presentation within 4 hrs. 9 trauma patients (not brain trauma) received a CT scan and were included in the analysis (all negative results)
Papa 2015[45]	High	Low	Low	Unclear	Convenience sample Blinded lab personnel Blinded radiologist Blood sampling within 6h but no info regarding timing of CT scan	High	Low	Low	2% of moderate TBI + 1 patient without TBI
Gatson 2014[39]	Low	Low	low	Unclear	Patients consecutive when researchers on duty	Low	Low	Low	Small sample size for TBI. Patients presenting in the ED within 4 hours of injury
Wolf 2013[11]	High	Low	Unclear	Low	No consecutive enrolment; the high % of ICH (23%) indicates that some sort of patient selection occurred; no threshold pre-specified; reading of CT without knowledge of biomarkers is not described;	High	Low	Low	23% of patients with ICH; 8/107 requiring neurosurgery; seven patients from the CT-positive group were on anticoagulants

2.3 Conclusion

There is high quality evidence that protein S100B can reliably rule out the presence of intracranial injury after an mTBI in adults. Points of attention for deciding whether protein S100B should be used in case of mTBI are discussed in section 5. There is insufficient evidence to conclude about ruling out epidural haematoma, one of the most feared complication of mTBI. Epidural haematoma is the result of arterial haemorrhage leading to a rapid expanding extradural mass. If left untreated, brain damage arises rapidly due to the pressure exerted by the haematoma. Because epidural haematoma occurs prior to brain injuries, the potential for biomarkers for brain damage to identify this complication effectively and timely becomes crucial. Although there is evidence that protein S100B levels increase in case of epidural hematomas[29, 32, 33, 38, 46], it is still unclear if this is due to brain compression or an indication that protein S100B is also a biomarker of blood-brain barrier disruption. [52]. There is low quality evidence that protein S100B could rule out the presence of intracranial injury after an mTBI in children. There is insufficient evidence to assess the diagnosis accuracy of other biomarkers.

3 REVIEW OF THE ECONOMIC LITERATURE

3.1 Introduction

This chapter provides an overview of economic studies evaluating the use of different biomarkers in the identification of mild mTBI, in patients presenting to the emergency department (ED) following a trauma accident. The aim is to review the literature on the potential cost-consequences of these tests as an alternative to current practice, where more expensive and potentially harmful CT scans are often performed in order to detect ICI.

3.2 Methods

3.2.1 Search strategy

A systematic search for relevant publications was carried out with the consultation of electronic reference databases up to 31/03/2015.

Medline (through OVID), EMBASE, Econlit (through OVID), NHSEED (CRD) and NHSHTA (CRD) were searched to retrieve primary full economic evaluations (studies comparing both costs and outcomes) and reviews of economic evaluations (i.e. secondary economic evaluations). Search strategy is given in 0.

Furthermore, the websites of Health Technology Assessment (HTA) institutes listed on the INAHTA website (International Network of Agencies for Health Technology Assessment) and NICE (National Institute for Health and Care Excellence) were consulted to capture reports on the use of biomarkers in patients with mTBI. No restrictions were imposed for language or time period.

A search for grey literature was performed using google as search engine. In addition to this, the experts consulted throughout this project were asked to let us know of any unpublished studies that could be relevant for our review.

3.2.2 Selection procedure

To identify potentially relevant studies 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.

3.2.3 Selection criteria

All economic evaluations looking at any biomarker test as diagnostic tools for identifying those patients presenting at the ED with mTBI after a trauma accident were included in our review. All cost studies, including cost descriptive analyses or cost comparisons that did not look at the effectiveness of the test in accurately identifying mTBI were discarded. Similarly, publications in the form of letters, editorials or notes and abstracts were excluded, since these would not offer enough information to include them in our analysis and critically appraise their findings. An overview of the inclusion/exclusion criteria is given in Table 16.

Table 16 – Selection criteria for economic evaluations

Selection criteria	Inclusion criteria	Exclusion criteria
Population	Patients presenting at the ED with potential mTBI following a trauma accident	Other injuries or potential moderate and severe TBIs
Intervention	Biomarkers as screening tests to diagnose intracranial injuries	No biomarkers specifically excluded
Comparator	Current practice incl. CT scans	Clinical practice not considering CT scans
Design	Analyses comparing any biomarker with CT from both a cost and an accuracy perspective	Pricing comparisons, or cost comparisons no taking into consideration test accuracy
Type of publication	Articles or reviews	Letters, editorials, notes, abstracts

CT – Computed tomography; ED – Emergency department; mTBI – mild traumatic brain injuries; TBI –traumatic brain injuries

Our search returned 38 citations, after eliminating duplicates. Of those, 37 did not meet our inclusion criteria based on a review of their title and/or abstract, mainly because of the population/indication (32). Other reasons for exclusion included the intervention under research (4) and the type of publication (1). A review of the grey literature identified a further study, a full copy of which was shared by its author with the KCE research team. No further analyses in this field were found, which left us with 2 relevant studies to be included in our review.

Our literature selection process is illustrated in a flow chart in an Appendix.

3.3 Overview of economic evaluations

From the two studies, one was performed in the USA[53], and the other in Belgium.[54]

The US study was published in 2009, while the Belgian analysis remains to this date unpublished. The scarcity of studies in the field reflects the novelty of the topic. Both studies selected were model-based (decision-tree models).

3.3.1 Type of economic evaluation

Both studies performed cost analyses taking into consideration the accuracy of both the protein S100B and current practice (CT scan or observation as considered appropriate), and expressed their outcomes in cost per patient/case.

3.3.2 Time frame of analyses and discounting

Given the acute nature of the indication here reviewed, both studies looked at costs and outcomes within a limited time period. The study of Ruan et al.[53] looked at costs and outcomes over two days following the presentation at the ED of a patient with a potential mTBI, while the study by Annemans et al.[54] looked at a longer time horizon of 3 days. Despite the short time frames used, these appear to be justified given the acute nature of the indication and the fact that most complications linked to TBI develop within the first days after the trauma accident.[55-58]

3.3.3 Perspective and population

The US study was completed from a hospital perspective, while the Belgian analysis was conducted from two different perspectives, a hospital's perspective and a payer's perspective.

Both studies focused in similar populations: adults with potential isolated mild head injuries, but while the study by Ruan et al.[53] was centred around patients with a Glasgow Coma Scale (GCS) score of 15, the analysis by Annemans et al.[54] used data from patients with a GCS score of 13-15.

3.3.4 Intervention and comparator

The only two evaluations identified via our search aimed at computing the potential cost savings that could be derived from the use of S100B protein at the ED, as a screening tool to discard ICI and thus, to reduce the need for CT scans in adults presenting isolated potential mTBI and a high GCS (of 15 for Ruan et al. and 13-15 for Annemans et al.). The comparator was defined in both cases as "current practice" where an important number of CT scans are performed for liability concerns (61% in Ruan et al. and 50% in Anneman's et al. for their baseline calculations respectively).

3.3.5 Cost and outcome inputs

Costs used in both analyses were primarily based on reimbursement rates. While the US study derived costs from Medicare reimbursement codes from the year 2007, the Belgian study extracted costs from the RIZIV – INAMI reimbursement database and the minimal hospital data (FOD Health DRGs). For the cost of S100B one of the studies mentioned a personal communication with the manufacturers of this specific test (i.e. Roche) and gave a cost of €40,[54] while the remaining made assumptions on that regard, and used US $20 in their estimations[53]. Factors taken in consideration for the costing size of the analysis undertaken from a payer perspective included S100B testing, CT scans, visits to the ED, examination costs, costs of observation at the ED, hospitalisation for surgery and surgery costs and hospitalisation with medical treatment. For the analysis from a hospital perspective, the costs of surgery and hospitalisation were set to zero as they were considered not to be a cost for the hospital (remunerated by the INAMI – NIHDI). In addition to all costs previously mentioned, Ruan et al.[53] also included the cost of one hour waiting time at the ED, priced at US$380.

Inputs reflecting clinical practice, including the percentage of mTBI adults receiving a first scan, proportion of patients left under observation and surgery and hospitalisation rates, were extracted from the published literature for the US study[53], and based on a retrospective case review of Gent and VUB university hospitals ED data patients (n=640) for the Belgian study.[54] With regard to S100B test accuracy, inputs were taken from the published literature, with Ruan et al.[53] referencing studies published between 2000 and 2006, and Annemans et al.[54] referring to an analysis recently published.[41] The estimates used by the former were more

conservative (sensitivity: 97.3%; specificity: 30.2%) than those used by the later in their baseline scenario (i.e. sensitivity: 100%; specificity: 38.2%). Similarly, the estimates by Annemans et al. were also more positive towards S100B compared to the results obtained via our meta-analysis (i.e. sensitivity: 96.6%; specificity: 33.0%, see section 2). However, their baseline assumptions were tested to a certain extent during a one-way sensitivity and scenario analyses (see section 3.3.7). Finally, a further assumption taken from the literature by Annemans et al. regarding the specificity of CT scans (51%) appears to use low estimates. Ruan et al.[53] did not explicitly used accuracy figures for the CT scan but did include instead assumptions, from the literature, regarding the expected proportion of positive CT scans (11%). Such a factor could play an important role on the overall results from the cost-analysis, since the lower the specificity of a test, the higher the number of "false positives", which would result in unnecessary follow-ups (by means of a 2nd scan, observation, hospitalisation, etc) and a higher waste of resources. Thus, in the Belgian evaluation this low estimate could have resulted in higher costs differences between the CT and the S100B arms. The studies included in our review did not provide us with an estimate for the specificity of the CT scan, since they simply measured as their "final outcome" the number of positive scans as a proxy for ICIs. Nevertheless, the 19 studies included in our MA, gave a weighted mean proportion of patients with CT positive results (true positives + false positives) of 12.59% (slightly above the assumption of Ruan et al.), compared to a weighted mean proportion of S100B positive results of 69.18%. This implies that only 12.59% of patients which received a CT scan would be likely to benefit from a follow-up (unless they present other symptoms, or their condition deteriorates).

3.3.6 Modelling

The studies here reviewed consisted of decision tree modelling exercises, with Annemans et al.[54] using the model structure originally designed by Ruan et al. in 2009[53] as their starting point. Both decision trees departed from two branches: one in which current practice (i.e. no S100B testing) was followed and patients were divided between different potential routes: discharge without CT scan, or CT scan and following its results, discharge, observation or hospital admission. An alternative parallel branch foresaw S100B use as a pre-CT diagnostic tool, which could spare scanners to those who do not need it, saving both time and resources while avoiding unnecessary radiation.

3.3.7 Results

The original study by Ruan et al.[53] compared two possible intervention routes at the ED, for adult patients presenting potential isolated mTBI: the first, which could be referred to as "current practice" or "usual care" in which the decision to perform a CT scan was made primarily on the basis of symptoms (mean baseline proportion of CT scans: 61%) and an alternative route in which testing for S100B was performed in all patients and only those with S100B levels >0,1mcg/L received a CT scan, while the remaining were discharged home. Overall cost estimates were of US $281 per patient for the S100B arm versus US $160 for the "current practice" arm. A difference in favour of current practice of US $121 per patient case. In contrast with the findings previously described, the study by Annemans et al.[54], when undertaken from a third party payer perspective, found that the use of S100B in the ED could lead to cost savings of €365 per patient, under the baseline assumptions of limiting its use to patients with GCS 15 in which CT scans would have been performed under current practice (50% of patient cases). This implied perfect replacement of one technique for another in the first instance, as opposed to the approach taken in the US study in which the blood test was performed to every patient as a first step to aid treatment/intervention decisions. The perspective form which the analysis was performed appeared to play an important role on the overall results. Thus, when Annemans et al.[54] completed the same analysis under the same baseline assumptions but from a hospital perspective, measuring hospital revenues, the Belgian study found that the introduction of S100B testing pre-CT scan resulted in lower revenues to the hospital, primarily due to the lower number of CT scans performed, of around €7.78 per patient for their base case scenario (GCS 15 population and 50% CT-scan).

3.3.8 Sensitivity analysis

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

Ruan et al.[53] undertook one and two-way sensitivity analyses, during which they tested the following parameters of their model: CT scan rate, time difference associated with obtaining the results of a CT scan versus those of a S100B test and the hourly cost of keeping a patient in the ED. Their univariate sensitivity analysis showed that a strategy to test all patients via S100B would be cost saving only if:

- the CT scanning rate was over 78% or
- the time difference for obtaining results from a CT scan versus a S100B test was of at least 96 minutes (at a scan rate of 77%).

At the baseline-assumed scan rate of 61%, the cost per hour at the ED would need to be over US $600 for S100B to be cost saving.

For the two-way sensitivity analysis: the greater the CT scan rate, the lower the time differential and time costs of a "test all" via S100B would need to be in order to become the preferred option. Annemans et al.[54] undertook as part of their research a one- way sensitivity analysis as well as a scenario analysis. In their sensitivity analysis, the authors varied the percentage of intra and extra cranial injury in mTBI patients as well as all their cost inputs. Their one-way sensitivity analysis showed results to be most sensitive to the percentage of extra cerebral trauma, CT scan costs, proportion of mTBI with ICI and costs of S100B.

As part of their scenario analysis, four scenarios were tested:

- Scenario 1: data from GCS 13-14;
- Scenario 2: 75% CT scan use in GCS 15 patients;
- Scenario 3: best and worst cases tested for: GCS 15 and 50% CT; GCS 13-14 and 100% CT scan and GCS 15 with 75% CT scan.
  - o best case: sensitivity 97% and specificity 40%
  - o worst case: sensitivity 100% and specificity 28%
- Scenario 4: proportion of S100B testing varied between 50% and 100% for: GCS 15 and 50% CT (base case); and for GCS 15 and 75% CT.

Results reported for these scenarios showed that S100B was cost saving with the only exception of the last (varying the proportion of S100B use from 50%-100% while CT use is kept constant at 50%). Under the latter, S100B is cost saving if used in 80% or less of mTBI.

3.3.9 Conflict of interest

The Belgian study by Annemans et al.[54] was sponsored by Roche, manufacturers of a S100B test. The study undertaken in the US reported no competing financial interests. 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.

3.4 Conclusion

Our review of the economic evidence identified only two studies, one of which remains to this date unpublished[54], with apparent conflicting results. While Ruan et al.[53] concluded that using S100B would result in additional costs, Annemans et al.[54] found that the protein could offer some savings from a payers perspective by reducing the overall number of CT scans performed. These results highlight the importance of defining well the appropriate target group for the protein. Testing everyone, as assumed by Ruan's et al. is unlikely to help reducing the overall number of CT scans due to the low specificity of the protein, and could even cause an increase in the use of imagery due to the false "need" to test all patients with a positive S100B result. The Belgian study, on the other hand, assumed in their baseline estimations that only those who would have received a CT scan would be considered the target for S100B testing. Although positive in its results, this Belgian study rests on a number of assumptions for which good data is still lacking to this date.

4 BELGIAN DATA

In Belgium good databases exist for hospitalised patients with TBI. However, for ambulatory patients visiting the ED, data are still very limited and unavailable for analysis at the time of our review. An online registry (UREG) specifically on ED health care use is currently being piloted (since January 2015) in six hospitals[h] before registration is extended to the remaining of Belgium. Thus, a detailed estimation of the size of the problem in Belgium as well as the proportion of mTBI patients who pursue a CT scan will only be possible in the near future. Nevertheless, we decided to look at data available for hospitalised patients form recorded by the technical cell (https://tct.fgov.be/), using records from 2010 and 2011 and the following ICD-9-CM diagnosis, (after consultation with experts in the field), which were considered the most adequate to capture mTBI:

- 850.0 With no loss of consciousness

  Concussion with mental confusion or disorientation, without loss of consciousness
- 850.1 With brief loss of consciousness

  Loss of consciousness for less than one hour
  - 850.11With loss of consciousness of 30 minutes or less
  - 850.12With loss of consciousness from 31 to 59 minutes.

According to these data, most of the stays are referred from the ED (95.7%, n=14 174). When looking at the relevant codes as primary diagnosis (by selecting ICD-9-CM with the values 850.0, 850.11 and 850.12), it can be noted that a big proportion of stays (87%) present a "minor" severity of illness (SOI 1). Despite the fact that limiting the analysis to hospitalized patients would be expected to result in a biased, "more at risk" patient selection, the data shows an overall CT scan rate of 33% of patients (Table 17). Such proportion is noticeably lower than the one found by Annemans et al. (50%) in their retrospective review of medical records of 640 adults with GCS 13-15 TBI, from two Belgian university hospitals (UGent and UZBrussel).[54]

Table 17 – Number of scanners performed in hospitalised TBI patients in Belgium

Number of scanners	Frequency	Percent	Cumulative Frequency	Cumulative Percent
0	9464	66.76	9464	66.76
1	4405	31.07	13869	97.83
2	284	2.00	14153	99.84
3	20	0.14	14173	99.98
4	1	0.01	14174	99.99
5	1	0.01	14175	99.99
8	1	0.01	14176	100.00

Bearing in mind that the elderly are considered a population at a higher risk of complications[59, 60], we looked at the same data for patients ≥ 65 (n=1338) and found a higher proportion of CT scans being performed, with 57% of stays for patients 65 and over, having at least one CT scan versus only 31% of patients under 65. The severity of illness in this group also reflected the higher risk they present, with 53% of patients having a SOI ≥2, versus less than 10% of those under 65 years. Regarding the paediatric population (described as patients aged ≤ 15, n=7788), they were exposed to less than half of the scanners performed in the adult population.

4.1 Conclusions

Given the current lack of detailed data registration in Belgium for patients presenting to the ED, the study by Annemans et al. (unpublished to date)[54] represents despite its limitations (i.e. retrospective review of patient records in two university hospitals) the best attempt to date to measure CT scan utilisation in our country.

[h] ZNA, UZ Gent, AZ St-Lucas, CHU Tivoli La Louvière, CHU Charleroi, CHC Liège and CH St Vincent in Rocourt

5 DISCUSSION

The bulk of evidence on biomarkers for ruling out ICI in case of mTBI relates to protein S100B.The potential value of S100B depends on its ability to safely reduce unnecessary CT scans and as a consequence:

- Minimise the risk of radiation in populations not likely to benefit from a CT scan
- Reduce the waste of limited medical resources by saving on scans, hospitalisation and observation periods.

Its full potential could only be realised if the marker is used in combination (as part of) current clinical decision rules as opposed to replacing these, since the sensitivity of clinical decision rules based primarily on symptoms and history of trauma is already relatively high and can help to rule out a number of cases, after which the protein could play a secondary role in better defining and limiting the target population for CT scans. There is high quality evidence that protein S100B could be a valid tool to rule out ICI in cases of mTBI. Introducing protein S100B in the management of mTBI may facilitate a reduction of a substantial over-triage to CT scans (between 80–99.5% of CT scans after mTBI would be normal)[61]. As a result, clinical guidelines have been modified accordingly in some countries, such as Sweden[61], where the use of the protein is recommended under certain circumstances.

Nevertheless, despite its potential clinical value, some points of attention must be considered before deciding whether the dosage of protein S100B in the management of mTBI is appropriate in a given setting. The first one is the applicability of the study findings to a consulting population. It is worth mentioning that the study populations were not representative of all mTBI cases presenting in the ED, but rather a subset of patients. Use of anticoagulants was an exclusion criterion in the majority of studies, except in the study by Müller 2011[43]. Subjects with multiple injuries were excluded from most of the studies, as the specificity of protein S100B is even lower is such patients. A further crucial factor of exclusion from studies was a too long delay between occurrence of trauma and time for measuring protein S100B as the half-life of the protein is quite short. It is unknown how much these factors affect the proportion of real-life patients with a mTBI in whom protein S100B cannot be used. Second, the sensitivity of protein S100B is not 100%, the proportion of false negative lies between 1.5% and 7.7%, and the LR- between 0.04 and 0.23. Whether this is an acceptable level of uncertainty given the potential severity of the condition must be examined cautiously. One important element of the variability of the test performance is the incidence of ICI in mTBI patients, or the pre-test odds of ICI, in a given setting. The exact incidence of ICI in mCT Belgian patients is unknown to this date, but it is likely to approximate 5% as reported in the international literature[61, 62], which corresponds to the probability of ICI before testing protein S100B. With a LR- of 0.10 (95%CI: 0.04; 0.23) (see results of our systematic review), the probability of ICI in case of a negative test will downsize to 0.5% (95%CI: 0.2%; 1.2%). This seems to be quite a safe margin of certainty for discharging patients with a negative protein S100B, particularly if a rapid return to hospital is possible in case of appearance of alarming clinical signs. It is worth noting that ICI pre-test probability is even lower than 5 % when validated clinical rules are used (e.g. New Orleans Criteria or Canadian CT Head Rule) and all the items are negative[63]. In contrast, if the pre-test probability is clearly higher than 5%, as appraised by the medical doctor on the basis of the anamnesis and clinical examination, the value of testing protein S100B can be questioned given the uncertainty around the absence of ICI.

Third, the benefit of protein S100B in terms of resource utilization is also variable, going from 11% of CT scan possibly avoided in the study by Zongo 2012[46] to 38% in Cervellin et al.[37]. Again, the incidence of ICI is an important contributing factor. If the incidence of ICI is low among people identified as suffering from a mTBI, the proportion of false positive among all tested will increase, and thus the proportion of patients getting both protein S100B and confirmatory CT scan. It should also be noted that CT scan is considered the gold standard and that clinicians may be reluctant to rely only on a negative S100B test to discharge a patient. This fact is well illustrated in a Swedish study where 31.8% (44/138) of the patients with a negative protein S100B test were still either hospitalized or received a CT scan or both[61]. However, this evaluation was done only a few months after protein S100B had been integrated in the clinical protocol, and may also be a reflection of the time lag to adapt to new recommendations.

On another note, the conclusions drawn by the authors of the only two economic evaluations identified via our search appear at first sight, contradictory.

On the one hand, Annemans et al.[54] assumed that the biomarker would only be used in those patients who would have undergone CT scanning under current practice. On the other hand, Ruan et al.[53] assumed all patients presenting in the ED after a cranial trauma accident, meeting the definition of mBIT would be tested for S100B protein levels.

Given the low specificity of the test, its use in all (or a very high proportion of) patients could result in a relatively high level of "false positives" which would then undergo an expensive CT scan to rule out ICI.

The sensitivity analysis performed by Annemans et al.[54], found that the biomarker test would be cost saving, for as long as it was provided to a maximum of 80% of those patients presenting to the ED after a mTBI. Although such proportion may appear to be very high, it is important to note that an assumption was used in the model under which those patients with a negative S100B test were discharged home, while a small proportion of those with a negative CT scan were assumed to remain under observation. While avoiding some "observations" by using protein S100B as opposed to CT scans could bring in additional savings (on top of pure "testing" costs), it remains unclear whether immediate discharge after a negative protein test would be widely accepted by the clinical community[61]. Factors such as the size of the target population, its correct identification and the clinical consequences of a negative S100B will all have an important weight on the overall economic value of the protein S100B and should be carefully considered if wanting to ensure some savings will be realised with the introduction of the protein in clinical practice. Appropriate targeting and use of biomarker tests has proved to be a challenge in other fields and thus, should be carefully considered. This was for example the case of troponin, a biomarker used to evaluate patients suspected of suffering from acute coronary syndrome (ACS) for which guidelines recommended limited use. Despite the clear recommendations, the biomarker was misused in cases where troponin elevation could not add any clinical diagnostic value.[64, 65] Nevertheless, monitoring concordance between use and guidelines of biomarker testing has proven effective in reducing unnecessary use and wastage.[65] Although MRI could be another imaging option to assess mTBI, CT scanning is more appropriate for this purpose because it is more widely accessible, faster, and less expensive; furthermore, acute bleeding and skull fractures are better outlined. [66] Hence it can be stated that CT scanning is still the imaging method to be chosen to disclose traumatic conditions requiring neurosurgical intervention. The only exceptions may be cases of children or young women where ionizing irradiation has to be avoided. Then MRI may be considered as the preferred first-line imaging tool. However, if the question is the presence of more subtle injuries such as small contusions or microscopic bleeding (haemorrhagic axonal injury), routine MRI is the preferable tool because it is far more sensitive to such lesions.[67] Unfortunately, to date, the clinical value of these focal lesions is debated; no general conclusions can be drawn on how these lesions can be attributed to injury severity within the spectrum of mTBI or the outcome.[68] Although MRI is not yet considered cost beneficial for mTBI, it may become so in the future by developing cheaper magnetic resonance instruments (e.g., head-only MRI).[66]

Our review of the economic literature highlighted some additional factors also worthwhile discussing:

Regarding data limitations: not all data inputs used in the model developed by Ruan et al.[53], referred to GCS 15 since data for GCS was not always available. In addition to that, data was not always limited to isolated mTBI, which would be the specific indication for the test. A further weakness is given by the way in which the assumptions were based purely on mean values taken from the literature without taking into consideration the quality of the different studies considered (e.g. some studies presented very low sample sizes). Regarding the generalisability of the findings specifically linked to the Belgian study, it is important to highlight that due to the general lack of data on current management, the authors of the study based their assumptions on that regard on a retrospective review of ED data from two large university hospitals. Whether such data could be representative of the overall Belgian situation is unclear, although the figure quoted appears to be in line with the results mentioned in a recent SR on CT scanning rates following the use of different clinical rules.[69] There is on the other hand, no prospective data on S100B use which could facilitate a selection of the appropriate target population for the test.

APPENDICES

APPENDIX 1. SEARCH STRATEGIES

Appendix 1.1. Search of clinical literature — focused search

Date	18/05/2015	
Database	MEDLINE (via PUBMED)	
1	Head OR cerebral OR brain OR cortical OR cranial	2 424 877
2	injury OR trauma OR traumatic	1 277 738
3	#1 AND #2	244 600
4	concussion	6945
5	#3 OR #4	245 261
6	Minor OR mild OR minimal OR severity	1 013 916
7	#5 AND #6	31 488
8	biomarker	697 598
9	(S100B OR S100B OR s100beta OR S100Beta) AND protein	3064
10	neurofilament medium polypeptide protein OR NFM	508
11	neurofilament-H OR NFL-H	154
12	neuron-specific enolase OR NSE	11 095
13	ubiquitin carboxy-terminal hydrolase L1 OR UCH-L1	403
14	astroglial protein OR glial fibrillary acidic protein OR GFAP	24 072
15	((tumor OR tumour) AND necrosis factor-alpha) OR TNF-α	181 110
16	plasma soluble cellular prion protein	24
17	Soluble urokinase plasminogen activator receptor OR suPAR	630
18	Serum microRNA OR miRNA	37 493
19	spectrin N-terminal fragment OR SNTF	30
20	kallikrein-6 OR Klk6	219
21	neuron-specific enolase OR NSE	11 095
22	“troponin I”	8317
23	myelin basic protein OR MBP	15 904
24	BB isozyme of creatine kinase OR CK-BB	1126
25	serum cleaved tau OR C-tau	97
26	spectrin breakdown products OR SBDPs	241
27	#8 OR #9 OR #10 OR #11 OR #12 OR #13 OR #14 OR #15 OR #16 OR #17 OR #18 OR #19 OR #20 OR #21 OR #22 OR #23 OR #24 OR #25 OR #26	918 675
28	Computed Tomography	424 226

29	Magnetic Resonance Imaging	384 844
30	Scan*	620 401
31	#28 OR #29 #30	1 212 297
32	sensitivity OR specificity OR "Predictive Value of Tests"[Mesh] OR predict* OR prognosis[Mesh] OR prognos* OR likelihood OR classification OR stratification OR diagnosis	10 802 668
33	#7 AND #27 AND #31 AND #32	295

Date	**18/05/2015**	
Database	EMBASE	
1	'head'/exp OR head OR cerebral OR 'brain'/exp OR brain OR cortical OR cranial	2 811 765
2	'injury'/exp OR injury OR 'trauma'/exp OR trauma OR traumatic	1 963 893
3	#1 AND #2	399 810
4	'concussion'/exp OR concussion	8117
5	#3 OR #4	401 031
6	Minor OR mild OR minimal OR severity	1 409 539
7	#5 AND #6	56 934
8	'biomarker'/exp OR biomarker	188 376
9	(S100B OR S100B OR s100beta OR S100Beta) AND protein	4008
10	neurofilament medium polypeptide protein OR NFM	353
11	neurofilament-H OR NFL-H	266
12	neuron-specific enolase OR NSE	11 610
13	ubiquitin carboxy-terminal hydrolase L1 OR UCH-L1	520
14	astroglial protein OR glial fibrillary acidic protein OR GFAP	26 720
15	((tumor OR tumour) AND necrosis factor-alpha) OR TNF-α	212 280
16	plasma soluble cellular prion protein	26
17	Soluble urokinase plasminogen activator receptor OR suPAR	769
18	Serum microRNA OR miRNA	32 397
19	spectrin N-terminal fragment OR SNTF	35
20	kallikrein-6 OR Klk6	437
21	neuron-specific enolase OR NSE	11 610
22	‘troponin I’	15 748
23	myelin basic protein OR MBP	19 672
24	BB isozyme creatine kinase OR CK-BB	610
25	serum cleaved tau OR C-tau	90
26	spectrin breakdown products OR SBDPs	174

27	#8 OR #9 OR #10 OR #11 OR #12 OR #13 OR #14 OR #15 OR #16 OR #17 OR #18 OR #19 OR #20 OR #21 OR #22 OR #23 OR #24 OR #25 OR #26	492 357
28	'computed tomography'/exp OR 'computed tomography'	678 457
29	'magnetic resonance imaging'	579 595
30	Scan*	788 559
31	#28 OR #29 #30	1 667 384
32	sensitivity OR specificity OR 'Predictive Value'/exp OR predict* OR 'prognosis'/de OR prognos* OR likelihood OR classification OR stratification OR diagnosis	6 989 640
33	#7 AND #27 AND #31 AND #32	481

Date	**18/05/2015**	
Database	Cochrane	
1	biomarker	2558
2	(brain or head or cerebral or cortical) and (concussion or injury or trauma)	5872
3	#1 AND #2	69

Date	**19/05/2015**	
Database	CRD-HTAdb	
1	(brain or head or cerebral or cortical) AND (concussion or injury or trauma)	72

Date	**19/05/2015**	
Database	Open Grey	
1	Protein S100B	11
2	mild head injury	9
3	#1 OR #2	20

Date	**19/05/2015**	
Database	GIN	
1	mild brain injury	5

Date	**National Guideline Clearinghouse**	
Database	19/05/2015	
1	mild brain injury	40

Appendix 1.2. Search for economic literature

Appendix 1.2.1. Medline

Database: Ovid MEDLINE(R) <1946 to March Week 5 2015>

Search Strategy:

1 (head or cerebral or brain or cortical).mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier] (1 504 721)

2 (injury or trauma or traumatic).mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier] (654091)

3 1 and 2 (119051)

4 exp Brain Concussion/ (5057)

5 3 or 4 (120658)

6 (minor or ,ild or minimal or severity).mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier] (742690)

7 5 and 6 (16880)

8 exp Biological Markers/ (651 390)

9 exp S100 Proteins/ or S100B protein.mp. (17 189)

10 (neurofilament medium polypeptide protein or NFM).mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier] (199)

11 (neurofilament-H or NFL-H).mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier] (146)

12 (neuron-specific enolase or NSE).mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier] (6856)

13 (ubiquitin caboxy-terminal hydrolase L1 or UCH-L1).mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier] (281)

14 (astroglial protein or glial fibrillary acidic protein or GFAP).mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier] (20575)

15 (((tumor or tumour) and necrosis factor-alpha) or TNF).mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier] (158046)

16 (serum microRNA or miRNA).mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier] (15354)

17 (spectrin N-terminal fragment or SNTF).mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier] (1)

18 (kallikrein-6 or Klk6).mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier] (197)

19 (neuron-specific enolase or NSE).mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier] (6856)

20 exp Troponin I/ (5372)

21 (myelin basic protein or MBP).mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier] (14 599)

22 (BB isozyme of creatine kinase or CK-BB).mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier] (497)

23 (serum cleaved tau or C-tau).mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier] (88)

24 (spectrin breakdown products or SBDPs).mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier] (89)

25 plasma soluble cellular prion protein.mp. (0)

26 8 or 9 or 10 or 11 or 12 or 13 or 14 or 15 or 16 or 17 or 18 or 19 or 20 or 21 or 22 or 23 or 24 or 25 (837 996)

27 exp Tomography, X-Ray Computed/ (313 646)

28 exp Magnetic Resonance Imaging/ (319 829)

29 27 or 28 (573 435)

30 (sensitivity or predict* or prognos* or likelihood or classification or stratification or diagnosis).mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier] (3 294 582)

31 exp Economics/ (500 729)

32 exp Health Care Costs/ (48 217)

33 (cost and cost analysis).mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier] (44 466)

34 exp Economics, Pharmaceutical/ or exp Economics, Medical/ or exp Economics, Hospital/ or exp Economics, Nursing/ (39 399)

35 exp "Value of Life"/ (5423)

36 exp Cost-Benefit Analysis/ (61 664)

37 (cost effectiveness or cost-effectiveness).mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier] (33 653)

38 (cost utility or cost-utility).mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier] (2386)

39 exp Quality-Adjusted Life Years/ (7380)

40 exp Health Expenditures/ (15 947)

41 budget*.mp. (23 479)

42 (price or prices or pricing).mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier] (22 806)

43 exp "Costs and Cost Analysis"/ (185 787)

44 31 or 32 or 33 or 34 or 35 or 36 or 37 or 38 or 39 or 40 or 41 or 42 or 43 (534 372)

45 7 and 26 and 29 and 30 and 44 (1).

Appendix 1.2.2. Embase

SEARCH QUERY

((((('head'/exp or head or cerebral or 'brain'/exp or brain or cortical and [embase]/lim)

or ('injury'/exp or injury or 'trauma'/exp or trauma or traumatic and [embase]/lim))

or ('brain concussion'/exp and [embase]/lim)) and ('minor'/exp or minor or mild or minimal

or severity and [embase]/lim)) and (('biological marker'/exp and [embase]/lim)

or ('protein s100b'/exp and [embase]/lim) or ('neurofilament'/exp or neurofilament and medium

and ('polypeptide'/exp or polypeptide) and ('protein'/exp or protein) or nfm and [embase]/lim)

or ('neurofilament h' or 'nfl h' and [embase]/lim) or ('neuron specific' and ('enolase'/exp or enolase)

or nse and [embase]/lim) or ('ubiquitin'/exp or ubiquitin and 'carboxy terminal'

and ('hydrolase'/exp or hydrolase) and l1 or 'uch l1' and [embase]/lim) or (astroglial

and ('protein'/exp or protein) or glial and fibrillary and acidic and ('protein'/exp or protein)

or gfap and [embase]/lim) or ('tumor'/exp or tumor or 'tumour'/exp or tumour and ('necrosis'/exp or necrosis)

and 'factor alpha' or tnf and [embase]/lim) or ('plasma'/exp or plasma and soluble and cellular

and ('prion'/exp or prion) and ptrotein and [embase]/lim) or ('soluble urokinase plasminogen activator receptor'

or supar and [embase]/lim) or ('serum'/exp or serum and ('microrna'/exp or microrna) or 'mirna'/exp or mirna

and [embase]/lim) or ('spectrin'/exp or spectrin and 'n terminal' and fragment or sntf and [embase]/lim)

or ('kallikrein 6'/exp or 'kallikrein 6' or klk6 and [embase]/lim) or ('neuron specific'

and ('enolase'/exp or enolase) or nse and [embase]/lim) or ('troponin i'/exp or 'troponin i' and [embase]/lim)

or ('myelin'/exp or myelin and basic and ('protein'/exp or protein) or mbp and [embase]/lim)

or (bb and ('isozyme'/exp or isozyme) and of and ('creatine'/exp or creatine) and ('kinase'/exp or kinase)

or 'ck bb' and [embase]/lim) or ('serum'/exp or serum and cleaved and tau or 'c tau' and [embase]/lim)

or ('spectrin'/exp or spectrin and breakdown and products or sbdps and [embase]/lim))

and (('computed tomography scanner'/exp and [embase]/lim)

or ('nuclear magnetic resonance imaging'/exp and [embase]/lim))

and (sensitivity or predict* or prognos* or likelihood or 'classification'/exp or classification

or 'stratification'/exp or stratification or 'diagnosis'/exp or diagnosis and [embase]/lim)

and (('economics'/exp and [embase]/lim) or ('health care cost'/exp and [embase]/lim)

or ('health economics'/exp and [embase]/lim) or ('health care financing'/exp and [embase]/lim)

or ('cost benefit analysis'/exp and [embase]/lim) or ('cost effectiveness analysis'/exp and [embase]/lim)

or ('cost of illness'/exp and [embase]/lim) or ('cost control'/exp and [embase]/lim)

or ('hospital cost'/exp and [embase]/lim) or ('cost utility analysis'/exp and [embase]/lim)

or ('cost minimization analysis'/exp and [embase]/lim) or (price or prices or pricing and [embase]/lim)

or ('budget impact analysis' and [embase]/lim) or (budget* and [embase]/lim)) (37)

Appendix 1.2.3. EconLit

1. head.mp. [mp=heading words, abstract, title, country as subject] (2536)
2. cerebral.mp. [mp=heading words, abstract, title, country as subject] (22)
3. brain.mp. [mp=heading words, abstract, title, country as subject] (1112)
4. cortical.mp. [mp=heading words, abstract, title, country as subject] (15)
5. #1 or #2 or #3 or #4 (3656)
6. injury.mp. [mp=heading words, abstract, title, country as subject] (1041)
7. trauma.mp. [mp=heading words, abstract, title, country as subject] (139)
8. traumatic.mp. [mp=heading words, abstract, title, country as subject] (109)
9. #6 or #7 or #8 (1264)
10. #5 and #9 (23)
11. (brain adj concussion).mp. [mp=heading words, abstract, title, country as subject] (0)
12. #10 or #11 (23)

13. (S100B or S100B).mp. [mp=heading words, abstract, title, country as subject] (0)
14. (biological adj marker*).mp. [mp=heading words, abstract, title, country as subject] (10)
15. marker*.mp. [mp=heading words, abstract, title, country as subject] (408)
16. #14 or #15
17. (computed and tomography).mp. [mp=heading words, abstract, title, country as subject] (20)
18. (magnetic and resonance and imaging).mp. [mp=heading words, abstract, title, country as subject] (68)
19. #17 or #18 (86)
20. #12 and #16 and #19 (0)

Appendix 1.2.4. CRS (HTA & EED)

1. (head OR cerebral OR brain OR cortical) AND (injury OR trauma OR traumatic) IN NHSEED, HTA (202)
2. MeSH DESCRIPTOR Brain Concussion EXPLODE ALL TREES (7)
3. (S100B) OR (S100B) IN NHSEED, HTA (2)
4. (biological) AND (marker*) IN NHSEED, HTA (270)
5. (marker*) IN NHSEED, HTA (499)
6. (computed) AND (tomography) IN NHSEED, HTA (886)
7. (magnetic) AND (resonance) AND (imaging) IN NHSEED, HTA (507)
8. #1 OR #2 (208)
9. #3 OR #4 OR #5 (499)
10. #6 OR #7 (1236)
11. #8 AND #9 AND #10 (1)

APPENDIX 2. QUALITY APPRAISAL OF SYSTEMATIC REVIEWS: AMSTAR

Appendix 2.1. Amstar for Pandor 2011[9]

Item	Score	Justification
1. Was an 'a priori' design provided?	Yes	
2. Was there duplicate study selection and data extraction?	Yes	
3. Was a comprehensive literature search performed?	Yes	
4. Was the status of publication (i.e. grey literature) used as an inclusion criterion?	No	English only. Grey literature searched
5. Was a list of studies (included and excluded) provided?	Yes	
6. Were the characteristics of the included studies provided?	Yes	

7. Was the scientific quality of the included studies assessed and documented?	Yes	
8. Was the scientific quality of the included studies used appropriately in formulating conclusions?	Yes	
9. Were the methods used to combine the findings of studies appropriate?	Yes	
10. Was the likelihood of bias assessed?	No	No graphical or statistical analysis provided
11. Was the conflict of interest included?	Yes	

Appendix 2.2. Amstar for Unden 2010[29]

Item	Score	Justification
1. Was an 'a priori' design provided?	?	Unclear if established before conduct of review
2. Was there duplicate study selection and data extraction?	?	Unclear for both study selection and data extraction
3. Was a comprehensive literature search performed?	No	Only in Medline. “Less comprehensive” searches in TripDatabase and Clinical Queries also undertaken
4. Was the status of publication (i.e. grey literature) used as an inclusion criterion?	?	Not reported
5. Was a list of studies (included and excluded) provided?	No	No list of excluded studies
6. Were the characteristics of the included studies provided?	Yes	
7. Was the scientific quality of the included studies assessed and documented?	Yes	
8. Was the scientific quality of the included studies used appropriately in formulating conclusions?	Yes	
9. Were the methods used to combine the findings of studies appropriate?	Yes	
10. Was the likelihood of bias assessed?	No	
11. Was the conflict of interest included?	No	

Appendix 2.3. Comparison of included studies Pandor 2011[9] and Unden 2010[29]

S100B			
	Pandor 2011	**Unden 2010**	**Comments**
Biberthaler 2006[70]	v	v	
Müller 2007[71]	v	v	
Biberthaler 2002[72]	v	v	
Castellani 2009[73]	v		Out-of-scope in Unden 2010 - limited to adults
Ingebrigtsen 2000[74]	v	v	
Morochovic 2009[75]	v	v	
Poli-de-Figuerredo 2006[76]	v	v	
Romner 2000[77]	v		Out-of-scope in Unden 2010 - limited to minor TBI
Biberthaler 2001[78]	v	v	
Nygren De Boussard 2004[79]		v	Comparators are orthopaedic injuries and non-injured controls
Ingebrigtsen 1999[80]		v	No clear reason but probably same data analysed in Ingebrigtsen 2000
Bazarian 2006[81]		v	Excluded by Pandor 2011 because no useable diagnostic data
Bouvier 2009[82]		v	Excluded by Pandor 2011 because in French but retrieved by our search
NSE			
	Pandor 2011	**Unden 2010**	**Comments**
Fridriksson 2000[51]	v		Out-of-scope in Unden 2010 - limited to S100B
S100B + NSE			
	Pandor 2011	**Unden 2010**	**Comments**
Mussack 2002[50]	v	v	
Creatine kinase isozyme (CK-BB), noradrenaline, adrenaline, dopamine, amylase and total catecholamines			
	Pandor 2011	**Unden 2010**	**Comments**
Levitt 1995[83]	v		Out-of-scope in Unden 2010 - limited to S100B

Appendix 2.4. Amstar for Heidari 2015

Item	Score	Justification
1. Was an 'a priori' design provided?	Yes	
2. Was there duplicate study selection and data extraction?	Yes	
3. Was a comprehensive literature search performed?	No	No search date or search strings provided
4. Was the status of publication (i.e. grey literature) used as an inclusion criterion?	Yes	Not reported
5. Was a list of studies (included and excluded) provided?	Yes	Reasons for exclusion not mentioned
6. Were the characteristics of the included studies provided?	Yes	
7. Was the scientific quality of the included studies assessed and documented?	Yes	
8. Was the scientific quality of the included studies used appropriately in formulating conclusions?	Yes	
9. Were the methods used to combine the findings of studies appropriate?	No	Studies including children and those including adults were mixed. One study (Hermann 2001) was included despite it does not fit the inclusion criteria (median sampling time 27 hours after the trauma). The authors reported a cut-off point of 0.50 μgL^{-1} for one study (Savola 2003) and pooled this study in the group of cut-off points between 0.16-0.2 μgL^{-1}. The authors did not provide data about imaging in table 1 for one study (Stranjalis 2004) and still used it in the pooling. Mussack 2000 does not report sensitivity and specificity. Source of data in table 1 reported is not mentioned. A cut-off point category of > 0.2 μgL^{-1} lead to a higher sensitivity than lower concentrations cut-off points (0.1-0.15μgL^{-1} or 0.16-0.2 μgL^{-1}). This result is challenging.

Item	Score	Justification
		Bazarian 2012 and Calcagnile 2009 were not mentioned in the included studies but were reported in the pooling (typo?). Calcagnile 2012 was included despite the blood sampling was provided only for CTscan positive cases. One study (Egea-Guerrero 2012) was included twice in the pooling.
10. Was the likelihood of bias assessed?	Yes	
11. Was the conflict of interest included?	Yes	

APPENDIX 3. CLINICAL EVIDENCE

Appendix 3.1. Systematic review Pandor 2011[9]

Pandor 2011[9]	
Methods	
Design	Systematic review and meta-analysis
Source of funding and competing interest	Funding: The National Institute for Health Research Health Technology Declared competing interests of authors: none
Search date	April 2009 (March 2010 for MEDLINE only)
Searched databases	MEDLINE, MEDLINE In-Process & Other Non-Indexed Citations, CINAHL, EMBASE and The Cochrane Library
Included study designs	RCT, controlled clinical trials, controlled before/after studies
Number of included studies	12 studies (S100B[36, 70-72, 74-78], NSE[51], S100B and NSE[50], other multiple markers[83])
Statistical analysis	Bayesian analysis for (positive and negative) likelihood ratio
Patient characteristics	
Eligibility criteria	Study design: Prospective or retrospective diagnostic cohort studies including minimum 20 patients with any intracranial injury (defined as any intracranial abnormality detected on CT or MRI scan due to trauma that require or not neurosurgery). Data reporting: Numbers of true-positives, true negatives, false positives and false negatives or sufficient data to allow their calculation
Exclusion criteria	Study design: Case–control studies, reviews of primary studies. Publication type: Animal studies, narrative reviews, editorials, opinions, non-English-language papers and reports in which study quality cannot be appraised because a lack of methodological details. Population:

	Patients with moderate or severe head injury (defined as patients with a GCS of ≤ 12 at presentation) or no history of injury.
Patient & disease characteristics	Adults and children (of any age) with mild head injury (MHI defined as patients with a blunt head injury and a GCS of 13–15 at presentation) or with at least 50% of included patients had MHI
Interventions	
Intervention group	Any biomarker
Control group	CT scan or MRI scan or combination of CT scan and follow-up for those with no CT scan
Results	
Protein S100B in adults (9 studies)[50, 70-72, 74, 76-78, 84]	
Sensitivity (95% HDR)	96.8% (93.8%-98.6%)
Specificity (95% HDR)	42.5% (31.0%-54.2%)
Negative likelihood ratio (95% HDR)	0.076 (0.031-0.156)
Positive likelihood ratio (95% HDR)	1.68 (1.40-2.11)
Protein S100B in children (1 study)[36]	
Sensitivity (95% CI)	100% (92%-100%)
Specificity (95% HDR)	42% (38%-43%)
Negative likelihood ratio	NR
Positive likelihood ratio	NR
Negative predictive value (95%CI)	100% (90%-100%)
Protein NSE in adults (1 study)[50]	
Sensitivity	100%
Specificity	6.9%
Negative likelihood ratio	NR
Positive likelihood ratio	NR

Protein NSE in children (1 study)[51]	
Sensitivity	77%
Specificity	52%
Negative likelihood ratio	NR
Positive likelihood ratio	NR
Other biomarkers in adults (1 study)[83]	
Sensitivity (95% CI)	*CK-BB, noradrenaline, amylase and total catecholamine were not associated with positive CT findings*. Adrenaline (104pg/ml): 100% (66%-100%) Dopamine (116pg/ml): 100% (66%-100%)
Specificity (95% CI)	Adrenaline (104pg/ml): 57% (47%-67%) Dopamine (116pg/ml): 58% (48%-68%)
Negative likelihood ratio	NR
Positive likelihood ratio	NR
Limitations and other comments	
Limitations	Limitations of included studies Inconsistencies between studies in terms of timing for sampling (<3h ?) and analyser used Selection bias: only symptomatic patients at presentation were included No studies focus on neurosurgical injury All studies are unclear whether there were any uninterpretable imaging or patients withdrawal Limitations of the review Only studies in English were included No assessment of publication bias No reporting of potential conflict of interest for the included studies

Appendix 3.2. Meta analysis Heidari 2015

Study ID	Method	Patient characteristics	Intervention(s)	Results primary outcome	Results secondary and other outcomes	Critical appraisal of review quality
Heidari 2015	Design: meta-analysis. Source of funding: not mentioned Search date: not mentioned Searched databases: Medline, Embase, Cochrane, Google scholar, CINAHL, SUMSearch, Bandolier and Trip databases Included study designs: observational studies Included studies: Ingebrigtsen 1999 Mussack 2000 Romner 2000 Ingebrigtsen 2000 Biberthaler 2001 de Kurij 2001 Herrman 2001 Mussack 2002 de Kurij 2002 Savola 2003	Eligibility: All cohort and case-control studies published in English Patient characteristics: patients with mild traumatic brain injury with Glasgow Coma Scale score of 13-15	Index test(s): serum or plasma concentration of S100B protein within 6 hours of the initial closed head injury Reference test: Post-traumatic head CT scanning or MRI	Sensitivity (95% CI; I^2) Cut point: ** 0.10-0.15 μgL^{-1}* 93.83 (88.99-98.67; 56.8%) ** 0.16-0.20 μgL^{-1}* 98.65 (95.53-101.77; 0.0%) ** >0.20 μgL^{-1}* 99.63 (96.00-103.25; 0.0%) Specificity (95% CI; I^2) Cut point: ** 0.10-0.15 μgL^{-1}* 37.06 (29.67-44.44; 94.6%) ** 0.16-0.20 μgL^{-1}* 50.69 (40.69-60.69; 76.3%) ** >0.20 μgL^{-1}* 46.94 (39.01-54.87; 95.5%)	S100B level difference between absence and presence of trauma-related findings on CT scan *Standardized mean (95% CI; I^2;pvalue)* All age groups Number of studies: 22 1.92 (1.39-2.45; 100%;p<0.001) Age < 18 years Number of studies: 4 0.70 (0.44-0.95; 98%;p<0.001) Age ≥ 18 years Number of studies: 18 2.19 (1.58-2.80; 100%;p<0.001)	Results critical appraisal: Adequate search, however error in reporting and in selection criteria were questionable Quality appraisal performed and level of evidence was reported for all included studies Heterogeneity and publication bias is clearly reported Quality of reporting was low Pooling of data was not done properly Children and adults were pooled together Unexplained discrepancies in studies inclusion in comparison of older systematic reviews as Pandor 2011 and Unden 2010

	De Boussard 2004
	Stapert 2005
	Stranjalis 2004
	Muller 2007
	Bechtel 2009
	Castellani 2009
	Schultke 2009
	Geyer 2009
	Muller 2011
	Bouvier 2012
	Calcagnile 2012
	Egea-Guerrero 2012
	Cervellin 2012
	Zongo 2012
	Wolf 2013
	Bazarian 2013

Appendix 3.3. Primary clinical studies

Table 18 – Patients' characteristics

PATIENTS CHARACTERISTICS						
Study ID	**Biomarker**	**n**	**Age***	**Male, %**	**GCS (%)**	**MHI°, %**
Babcock 2012	S100B	109	14.6 ±4	57.0	GCS 13-15 (86.2%) GCS≤12 (13.8%)	85
Bazarian 2013	S100B	787	38.2 ±19.5	63.5	GCS 15 (89.2%) GCS 14 (6.5%) GCS 13 (1.3%) 13≤GCS≥ 15 (2.5%)	100
Bouvier 2012	S100B	65	5.2 (range 2.1- 9.9)	63	GCS 13-15 (100%)	100
Calgagnile 2013	S100B	621	NR	NR	GCS 14-15 (100%)	100
Castellani 2009	S100B	109	9.5 ±4.7	67.0	GCS 15 (78.9%) GCS 14 (11.9%) GCS 13 (9.2%)	100
Cervellin 2012	S100B	60	58 (range 14-80)	68	GCS 14-15 (100%)	100
Egea-Guerrero 2012	S100B	143	49.20 ± 20.60	62.2	GCS-15 (100%)	100
Gatson 2014	pNFL-H	34	CT- 33.4 ± 9.8 CT+ 35.1 ± 1.6	61.8	GCS 13 (35.3%) GCS 14 (20.6%) GCS 15 (44.2%)	100
Kotlyar 2011	S100B	346	48 (NR)	62.0	GCS 15 (89%) 13≤GCS> 15(10 %)	100
Laribi, 2014	S100B	431	36 (IQR 24-54)	65.0	GCS 15 (87%) GCS 14 (11%) GCS 13 (2%)	100

PATIENTS CHARACTERISTICS						
Study ID	**Biomarker**	**n**	**Age***	**Male, %**	**GCS (%)**	**MHI°, %**
Metting 2012	S100B - GFAP	94	34.3 ± 13.9	NR	GCS 15 (26.6%) GCS 14 (61.7%) GCS 13 (11.7%)	100
Müller 2011	S100B	233	48.4 (IQR 24-72)	61.4	NR	100
Papa 2012	GFAP-BDP	108	39 ±15	65.0	13≤GCS≥ 15 (89.8%) 9≤GCS≥ 12 (10.2%)	90
Papa 2015	S100B - GFAP	114	13± 7	67.0	GCS 15 (89 %) GCS 14 (8%) GCS 13 (1%) GCS 9-12 (2%)	98
Wolf 2013	S100B - NSE	107	59± 23	56.0	NR	100
Zongo 2012	S100B	1560	57 (IQR 32-82)	55.8	GCS15 (75.9 %) GCS14 (21.6%) GCS13 (2.5%)	100

NR not reported, IQR interquartile range
** age in years - mean±SD or median (IQR) or median (range), ° MHI: Mild head injuries*

Table 19 – Patients characteristics

INDEX AND REFERENCE TESTS CHARACTERISTICS									
Study ID	**Index test**							**Reference test**	
	Biomarker	**Cut-off value**	**Analyser**	**TIS°**	**NT**	**RF (%)**	**TRF**	**Endpoint**	**Validation**
Babcock 2012	S100B	0.006 µg/L	Nanogen (San Diego CA)	< 6h	CT scan	100	NR	Any intracranial injury, including subdural haematomas, epidural haematomas and cerebral contusions, as well as the presence of skull fractures.	NR
Bazarian 2013	S100B	Different levels tested. For high sensitivity >0.060 µg/L >0,097 µg/L >0,10 µg/L	Elecsys S100	< 6h	CT scan	99.4	NR	Cerebral contusion , subarachnoidhemorrhage , subduralhematoma , linear skull fracture , epidural hematoma, intraventricular haemorrhage, pneumocephalus, edema, and depressed skull fracture	Interpretation of scan by board certified radiologists blinded to lab results
Bouvier 2012	S100B	0-9 months: 0.35µg/L; 10-24 months: 0.23µg/L; >24 months: 0.18µg/L	Roche diagnostics modular analytics system	2,05h (range 1.30-2.45)	CT scan	27 GCS 13-15	NR	Epidural haemorrhage, hemorrhagic contusion, bone fracture, subdural haemorrhage, nonhemorrhagic contusion, subarachnoidal hemorrhage, othematoma	NR
Calgagnile 2013	S100B	0.10 µg/l	Elecsys S100B (Roche AB)	≤ 6h	CT scan	56.5	NR	Any signs of cranial (skull fracture) or intracranial pathology (hematoma, air or contusion)	Radiologists were not blinded to S100B results

Castellani 2009	S100B	0.16 µg/l	Modular analytics intrument (E170) (Roche diagnotic, Mannheim, Germany)	≤ 3h	CT scan	100.0	NR	Skull fractures, intracranial haemorrhage	NR
Cervellin 2012	S100B	0.38 µg/L	Liaison	< 3h	CT scan	100	30 min post blood sample	Acute subdural, epidural or parenchymal hematoma, traumatic subarachnoid haemorrhage, cerebral contusion and brain swelling	Reliability of first scan checked by a senior neuroradiologist blinded from the conclusion of previous reading
Egea-Guerrero 2012	S100B	0.105 µg/L	Elecsys 2010	3 to 6h	CT scan	100	≤ 24 h	Cerebral contusion, traumatic subarachnoid haemorrhage, epidural haematoma and subdural haematoma	Imaging was assessed by a neuroradiologist blinded
Gatson 2014	pNFL-H	1071 pg/ml	ELISA (EMD Millipore)	18 to 24h	CT scan	100	NR	Since a majority of TBI victims do not present immediately to ED, NFL-H can used to decide which patients shoud undergo a CT	Broad-certificated neuroradiologist documented all CT scans
Kotlyar 2011	S100B	0.42µg/L	EIA	≤6 h	CT scan	100	≤ 3h	Hemorrhage, Diffuse brain edema, Diffuse axonal injury, Skull fracture	CT+ confirmed by a neuroradiologist
Laribi, 2014	S100B	0.10µg/l	Elecsys	2h	CT scan	92.8	3hours (median)	"Trauma-relevant cerebral lesion"	CT+ confirmed by a board-certified radiologist

Metting 2012	S100B GFAP	0.005 µg/L 0.045ng/mL	ELISA (Biovendor GmbH, Heidelberg, Germany)	2.4h (± 2.1)	CT Scan OR MRI		on admission	concentration of GFAP in patients with CT+ and CT-	Blinding review by an experienced neuroradiologist
Müller 2011	S100B	0.105 µg/l	Elecsys S-100	1.25 (IQR: 1-2)	Ct scan	100	NR	Skull fractures, localised epidural, subdural haemotomas, isolated subarachoid haematomas	NR
Papa 2012	GFAP-BDP	≥0.035ng/ml	GFAP-BDP	< 4h	CT scan	100	NR	Diagnostic accuracy compared to CT scans	CT scans interpreted by board certified radiologists, blinded to study protocol.
Papa 2015	S100B GFAP	0.020 µg/l 0.15ng/l	ELISA (Banyan Biomarkers Inc)	≤ 6h	CT scan	63.2	NR	Haemorrhages (epidural, subdural, subarachnoid, ventricular and parenchymal), contusions, edema, pneumocephalus, facial fractures skull fractures	Blinded radiologists and blinded lab personnel
Wolf 2013	S100B NSE	0.105 µg/l 16.4 µg/L	Elecsys	≤ 3h	CT scan	100	≤ 30 minutes after the first medical examination	Epidural, subdural, subarachnoid, or intracerebral hemorrhage, including contusions	No cross check of the CT protocol
Zongo 2012	S100B	0.1	Elecsys	CT+ 138 (range 105-210) CT- 150 (range 100-211)			<6hrs	Subdural, epidural, or intracerebral hemorrhages; bland contusion; edema; pneumocephalus; and skull fracture	Confirmed by a board certified radiologist, blinded to the S100B level

TIS time from injury to sample; NT neuroimaging type; RF Patients getting the reference test; TRT time to reference test; NR not reported, IQR interquartile range
° mediane (range), median (IQR) or mean (± SD)

REFERENCES

1. Peeters W, van den Brande R, Polinder S, Brazinova A, Steyerberg EW, Lingsma HF, et al. Epidemiology of traumatic brain injury in Europe. Acta Neurochir (Wien). 2015;157(10):1683-96.
2. Tagliaferri F, Compagnone C, Korsic M, Servadei F, Kraus J. A systematic review of brain injury epidemiology in Europe. Acta Neurochir (Wien). 2006;148(3):255-68; discussion 68.
3. Cassidy JD, Carroll LJ, Peloso PM, Borg J, von Holst H, Holm L, et al. Incidence, risk factors and prevention of mild traumatic brain injury: results of the WHO Collaborating Centre Task Force on Mild Traumatic Brain Injury. J Rehabil Med. 2004(43 Suppl):28-60.
4. Economie; S. Statistics Belgium Available at: http://statbel.fgov.be/en/statistiques/. 2014.
5. Teasdale G, Jennett B. Assessment of coma and impaired consciousness. A practical scale. Lancet. 1974;2(7872):81-4.
6. L AGJ. Mild Head Injury. Acta Neurochir 2003;145:843-50.
7. S.C S. Minor head injury,. Ann Emerg Med. 1993;22:1193-6.
8. Jehlé EH, D. Grasleguen, C. et al. Minor head injury (Glasgow Coma Score 13 to 15): triage, assessment, investigation and early management of minor head injury in infants, children and adults. Ann. Fr. Med. Urgence 2012;2:199-214.
9. Pandor A, Goodacre S, Harnan S, Holmes M, Pickering A, Fitzgerald P, et al. Diagnostic management strategies for adults and children with minor head injury: A systematic review and an economic evaluation. Health Technology Assessment. 2011;15(27):1-202.
10. Stiell IG, Wells GA, Vandemheen K, Clement C, Lesiuk H, Laupacis A, et al. The Canadian CT Head Rule for patients with minor head injury. Lancet. 2001;357(9266):1391-6.
11. Wolf H, Frantal S, Pajenda GS, Salameh O, Widhalm H, Hajdu S, et al. Predictive value of neuromarkers supported by a set of clinical criteria in patients with mild traumatic brain injury: S100B protein and neuron-specific enolase on trial. Journal of Neurosurgery. 2013;118(6):1298-303.

12. (NICE) NIfHaCE. Head injury – triage, assessment, investigation and early management of head injury in infants, children and adults. NICE Clinical Guideline No. 4. London: NICE. 2003.
13. (NICE) NIfHaCE. Head injury – triage, assessment, investigation and early management of head injury in infants, children and adults. NICE Clinical Guideline no. 56. London: NICE. 2007.
14. Unden J, Bellander BM, Romner B. Scandinavian guidelines for initial management of minimal, mild and moderate head injuries in adults: an evidence and consensus-based update BMC Medicine. 2013;11:50.
15. Kisser A, Zechmeister-Koss I. Procedural guidance for the systematic evaluation of biomarker tests. Vienna: Ludwig Boltzmann Institute for Health Technology Assessment; 2014. 77
16. Pewsner D, Battaglia M, Minder C, Marx A, Bucher HC, Egger M. Ruling a diagnosis in or out with "SpPIn" and "SnNOut": a note of caution. BMJ. 2004;329(7459):209-13.
17. Robinson KA, Chou R, Berkman ND, Newberry SJ, Fu R, Hartling L, et al. Twelve recommendations for integrating existing systematic reviews into new reviews: EPC guidance. J Clin Epidemiol. 2015.
18. Whiting PF, Rutjes AW, Westwood ME, Mallett S, Deeks JJ, Reitsma JB, et al. QUADAS-2: a revised tool for the quality assessment of diagnostic accuracy studies. Ann Intern Med. 2011;155(8):529-36.
19. Singh S, Chang SM, Matchar DB, Bass EB. Chapter 7: grading a body of evidence on diagnostic tests. J Gen Intern Med. 2012;27 Suppl 1:S47-55.
20. Schunemann HJ, Oxman AD, Brozek J, Glasziou P, Jaeschke R, Vist GE, et al. Grading quality of evidence and strength of recommendations for diagnostic tests and strategies. BMJ. 2008;336(7653):1106-10.
21. Zock M, Leidel BA, Biberthaler P, Mutschler W, Kanz KG. Emergency diagnostics for minor head trauma. Importance of neuroglial protein S100B. Notfall und Rettungsmedizin. 2011;14(4):261-7.
22. Sundstrom T, Wester K, Enger M, Melhuus K, Ingebrigtsen T, Romner B, et al. [Scandinavian guidelines for the acute management of adult patients with minimal, mild, or moderate head injuries]. Tidsskrift for den Norske lægeforening : tidsskrift for praktisk medicin, ny række. 2013;133(22):E1-6.
23. Lippi G, Cervellin G. Diagnostic approach to the mild head trauma of the adult in Emergency Medicine: Between biomarkers and imaging. Recenti Progressi in Medicina. 2013;104(3):120-32.
24. Tavarez MM, Atabaki SM, Teach SJ. Acute evaluation of pediatric patients with minor traumatic brain injury. Current Opinion in Pediatrics. 2012;24(3):307-13.
25. Barbosa RR, Jawa R, Watters JM, Knight JC, Kerwin AJ, Winston ES, et al. Evaluation and management of mild traumatic brain injury: An eastern association for the surgery of trauma practice management guideline. Journal of Trauma and Acute Care Surgery. 2012;73(5 SUPPL.4):S307-S14.
26. Pickering A, Fitzgerald P, Harnan SE, Pandor A, Goodacre SW. Biochemical markers for the identification of intracranial injury following minor head injury: A systematic review and meta-analysis. Value in Health. 2011;14(7):A244.
27. Yokobori S, Hosein K, Burks S, Sharma I, Gajavelli S, Bullock R. Biomarkers for the clinical differential diagnosis in traumatic brain injury--a systematic review. CNS Neurosci Ther. 2013;19(8):556-65.
28. Forde CT, Karri SK, Young AM, Ogilvy CS. Predictive markers in traumatic brain injury: opportunities for a serum biosignature. Br J Neurosurg. 2014;28(1):8-15.
29. Unden J, Romner B. Can low serum levels of S100B predict normal CT findings after minor head injury in adults?: An evidence-based review and meta-analysis. Journal of Head Trauma Rehabilitation. 2010;25(4):228-40.
30. Heidari K, Asadollahi S, Jamshidian M, Abrishamchi S.N, Nouroozi M. Prediction of neuropsychological outcome after mild traumatic brain injury using clinical parameters, serum S100B protein and findings on computed tomography. Brain Inj. 2015;29(1):33-40.

31. Babcock L, Byczkowski T, Mookerjee S, Bazarian JJ. Ability of S100B to predict severity and cranial CT results in children with TBI. Brain Inj. 2012;26(11):1372-80.
32. Babcock L, Byczkowski T, Mookerjee S, Bazarian JJ. Ability of S100B to predict severity and cranial CT results in children with TBI. Brain Injury. 2012;26(11):1372-80.
33. Bazarian JJ, Blyth BJ, He H, Mookerjee S, Jones C, Kiechle K, et al. Classification accuracy of serum apo a-i and s100b for the diagnosis of mild traumatic brain injury and prediction of abnormal initial head computed tomography scan. Journal of Neurotrauma. 2013;30(20):1747-54.
34. Bouvier D, Fournier M, Dauphin JB, Amat F, Ughetto S, Labbe A, et al. Serum S100B determination in the management of pediatric mild traumatic brain injury. Clinical Chemistry. 2012;58(7):1116-22.
35. Calcagnile O, Holmen A, Chew M, Unden J. S100B levels are affected by older age but not by alcohol intoxication following mild traumatic brain injury. Scandinavian journal of trauma, resuscitation and emergency medicine. 2013;21((Calcagnile O.; Holmen A.; Chew M.; Unden J.)):52.
36. Castellani C, Bimbashi P, Ruttenstock E, Sacherer P, Stojakovic T, Weinberg AM. Neuroprotein s-100B - A useful parameter in paediatric patients with mild traumatic brain injury? Acta Paediatrica, International Journal of Paediatrics. 2009;98(10):1607-12.
37. Cervellin G, Benatti M, Carbucicchio A, Mattei L, Cerasti D, Aloe R, et al. Serum levels of protein S100B predict intracranial lesions in mild head injury. Clinical Biochemistry. 2012;45(6):408-11.
38. Egea-Guerrero JJ, Revuelto-Rey J, Murillo-Cabezas F, Munoz-Sanchez MA, Vilches-Arenas A, Sanchez-Linares P, et al. Accuracy of the S100beta protein as a marker of brain damage in traumatic brain injury. Brain Inj. 2012;26(1):76-82.
39. Gatson JW, Barillas J, Hynan LS, Diaz-Arrastia R, Wolf SE, Minei JP. Detection of neurofilament-H in serum as a diagnostic tool to predict injury severity in patients who have suffered mild traumatic brain injury. Journal of neurosurgery. 2014;121(5):1232-8.
40. Kotlyar S, Larkin GL, Moore CL, D'Onofrio G. S100b immunoassay: An assessment of diagnostic utility in minor head trauma. Journal of Emergency Medicine. 2011;41(3):285-93.
41. Laribi S, Kansao J, Borderie D, Collet C, Deschamps P, Ababsa R, et al. S100B blood level measurement to exclude cerebral lesions after minor head injury: The multicenter STIC-S100 French study. Clinical Chemistry and Laboratory Medicine. 2014;52(4):527-36.
42. Metting Z, Wilczak N, Rodiger LA, Schaaf JM, Van Der Naalt J. GFAP and S100B in the acute phase of mild traumatic brain injury. Neurology. 2012;78(18):1428-33.
43. Muller B, Evangelopoulos DS, Bias K, Wildisen A, Zimmermann H, Exadaktylos AK. Can S-100B serum protein help to save cranial CT resources in a peripheral trauma centre? A study and consensus paper. Emergency Medicine Journal. 2011;28(11):938-40.
44. Papa L, Lewis LM, Falk JL, Zhang Z, Silvestri S, Giordano P, et al. Elevated levels of serum glial fibrillary acidic protein breakdown products in mild and moderate traumatic brain injury are associated with intracranial lesions and neurosurgical intervention. Ann Emerg Med. 2012;59(6):471-83.
45. Papa L, Mittal MK, Ramirez J, Ramia M, Kirby S, Silvestri S, et al. In Children and Youth With Mild and Moderate Traumatic Brain Injury GFAP Out-performs S100beta In Detecting Traumatic Intracranial Lesions On CT. J Neurotrauma. 2015.
46. Zongo D, Ribereau-Gayon R, Masson F, Laborey M, Contrand B, Salmi LR, et al. S100-B protein as a screening tool for the early assessment of minor head injury. Annals of Emergency Medicine. 2012;59(3):209-18.
47. Jonsson H, Johnsson P, Hoglund P, Alling C, Blomquist S. Elimination of S100B and renal function after cardiac surgery. J Cardiothorac Vasc Anesth. 2000;14(6):698-701.
48. Bossuyt PM, Reitsma JB, Bruns DE, Gatsonis CA, Glasziou PP, Irwig LM, et al. Towards complete and accurate reporting of studies of diagnostic accuracy: the STARD initiative. BMJ. 2003;326(7379):41-4.

49. Castellani C, Bimbashi P, Ruttenstock E, Sacherer P, Stojakovic T, Weinberg AM. Neuroprotein s-100B -- a useful parameter in paediatric patients with mild traumatic brain injury? Acta Paediatr. 2009;98(10):1607-12.
50. Mussack T, Biberthaler P, Kanz K, Heckl U, Gruber R, Linsenmaier U, et al. Immediate S-100B and neuron-specific enolase plasma measurements for rapid evaluation of primary brain damage in alcohol-intoxicated, minor head-injured patients. Shock. 2002 18(5):395-400.
51. Fridriksson T, Kini N, Walsh-Kelly C, Hennes H. Serum neuron-specific enolase as a predictor of intracranial lesions in children with head trauma: A pilot study. Acad Emerg Med. 2000;7:816–20.
52. Kleindienst A, Schmidt C, Parsch H, Emtmann I, Xu Y, Buchfelder M. The Passage of S100B from Brain to Blood Is Not Specifically Related to the Blood-Brain Barrier Integrity. Cardiovasc Psychiatry Neurol. 2010;2010:801295.
53. Ruan S, Noyes K, Bazarian JJ. The economic impact of S-100B as a pre-head CT screening test on emergency department management of adult patients with mild traumatic brain injury. Journal of Neurotrauma. 2009;26(10):1655-64.
54. Annemans L, Verhaeghe N, Tack P, Hachimi-Idrissi S, Hubloue I, Lauwaert D. Health Economic Evaluation of the use of S-100B as a screening test in the emergency department management of patients with mild traumatic brain injury. Unpublished manuscript; University of Ghent, Belgium 2015.
55. Voss M, Knottenbelt JD, Peden MM. Patients who reattend after head injury: a high risk group. BMJ. 1995;311(7017):1395-8.
56. Fabbri A, Servadei F, Marchesini G, Dente M, Iervese T, Spada M, et al. Clinical performance of NICE recommendations versus NCWFNS proposal in patients with mild head injury. J Neurotrauma. 2005;22(12):1419-27.
57. af Geijerstam JL, Britton M. Mild head injury: reliability of early computed tomographic findings in triage for admission. Emerg Med J. 2005;22(2):103-7.
58. af Geijerstam JL, Britton M. Mild head injury: observation or computed tomography? Economic aspects by literature review and decision analysis. Emerg Med J. 2004;21(1):54-8.
59. Papa L, Mendes M.E, Braga C.F. Mild Traumatic Brain Injury Among the Geriatric Population. Curr. Transl. Geriatr. Gerontol. Rep. 2012;1(3):135-42.
60. Thompson HJ, McCormick WC, Kagan SH. Traumatic brain injury in older adults: epidemiology, outcomes, and future implications. J Am Geriatr Soc. 2006;54(10):1590-5.
61. Calcagnile O, Unden L, Unden J. Clinical validation of S100B use in management of mild head injury. BMC Emergency Medicine. 2012;12((Calcagnile O., olga.calcagnile@regionhalland.se) Dept of Paediatric Medicine, Halmstad Regional Hospital, Halmstad, Sweden).
62. Albers CE, von Allmen M, Evangelopoulos DS, Zisakis AK, Zimmermann H, Exadaktylos AK. What is the incidence of intracranial bleeding in patients with mild traumatic brain injury? A retrospective study in 3088 Canadian CT head rule patients. Biomed Res Int. 2013;2013:453978.
63. Easter JS, Haukoos JS, Meehan WP, Novack V, Edlow JA. Will Neuroimaging Reveal a Severe Intracranial Injury in This Adult With Minor Head Trauma?: The Rational Clinical Examination Systematic Review. Jama. 2015;314(24):2672-81.
64. Nallet O, Arbaoui S, Grenier A, Michaud P, Safrano G, Sergent J. 324 Troponin in Emergency Department: an overused test for patient screening without clinical suspicion of acute coronary syndrome? Archives of Cardiovascular Diseases Supplements. 2011;3(1):109.
65. Larochelle MR, Knight AM, Pantle H, Riedel S, Trost JC. Reducing excess cardiac biomarker testing at an academic medical center. J Gen Intern Med. 2014;29(11):1468-74.
66. Toth A. Magnetic Resonance Imaging Application in the Area of Mild and Acute Traumatic Brain Injury: Implications for Diagnostic Markers? In: Kobeissy FH, editor. Brain Neurotrauma: Molecular, Neuropsychological, and Rehabilitation Aspects. Boca Raton (FL);

2015. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26269902

67. Yue JK, Vassar MJ, Lingsma HF, Cooper SR, Okonkwo DO, Valadka AB, et al. Transforming research and clinical knowledge in traumatic brain injury pilot: multicenter implementation of the common data elements for traumatic brain injury. J Neurotrauma. 2013;30(22):1831-44.

68. Hughes DG, Jackson A, Mason DL, Berry E, Hollis S, Yates DW. Abnormalities on magnetic resonance imaging seen acutely following mild traumatic brain injury: correlation with neuropsychological tests and delayed recovery. Neuroradiology. 2004;46(7):550-8.

69. Stein SC, Fabbri A, Servadei F, Glick HA. A critical comparison of clinical decision instruments for computed tomographic scanning in mild closed traumatic brain injury in adolescents and adults. Ann Emerg Med. 2009;53(2):180-8.

70. Biberthaler P, Linsenmeier U, Pfeifer K, Kroetz M, Mussack T, Kanz K, et al. Serum S-100B concentration provides additional information for the indication of computed tomography in patients after minor head injury a prospective multicenter study. Shock 2006;25:446–53.

71. Müller K, Townend W, Biasca N, Undén J, Waterloo K, Romner B, et al. S100B serum level predicts computed tomography findings after minor head injury. ;:. J Trauma Acute Care Surg. 2007;62:1452–6.

72. Biberthaler P, Mussack T, Wiedemann E, Kanz K, Mutschler W, Linsenmaier U, et al. Rapid identification of high-risk patients after minor head trauma (MHT) by assessment of S-100B: ascertainment of a cut-off level. Eur J Med Res. 2002;7(4):164-70.

73. Castellani C, Bimbashi P, Ruttenstock E, Sacherer P, Stojakovic T, Weinberg A. Neuroprotein S-100B: a useful parameter in paediatric patients with mild traumatic brain injury? Acta Pediatr 2009;98(10):1607–12.

74. Ingebrigtsen T, Romner B, Marup-Jensen S, Dons M, Lundqvist C, Bellner J, et al. The clinical value of serum S-100 protein measurements in minor head injury: a Scandinavian multicentre study. Brain Injury 2000;14:1047–55.

75. Morochovi R, Rácz O, Kitka M, Pingorová S, Cibur P, Tomková D, et al. Serum S100B protein in early management of patients after mild traumatic brain injury. Eur J Neurol. 2009;16(10):1112–17.

76. Poli-de-Figueiredo L, Biberthaler P, Simao Filho C, Hauser C, Mutschler W, Jochum M. Measurement of S-100B for risk classification of victims sustaining minor head injury--first pilot study in Brazil. Clinics (Sao Paulo). 2006;61(1):41-6.

77. Romner B, Ingebrigtsen T, Kongstad P, Borgesen S. Traumatic brain damage: Serum S-100 protein measurements related to neuroradiological findings. J Neurotrauma. 2000;17(8):641–7.

78. Biberthaler P, Mussack T, Wiedemann E, Kanz K, Koelsch M, Gippner-Steppert C, et al. Evaluation of S-100b as a specific marker for neuronal damage due to minor head trauma. World J Surg. 2001;25(1):93-7.

79. Nygren De Boussard C, Fredman P, Lundin A, Andersson K, Edman G, Borg J. S100 in mild traumatic brain injury. Brain Inj. 2004;18(7):671–83.

80. Ingebrigtsen T, Romner B, Kongstad P, Langbakk B. Increased serum concentrations of S-100 after minor head injury. A biochemical marker with prognostic value? J Neurol Neurosurg Psychiatry. 1995;59:103–4.

81. Bazarian J, Beck C, Blyth B, von Ahsen N, Hasselblatt M. Impact of creatine kinase correction on the predictive value of S-100B after mild traumatic brain injury. Restor Neurol Neurosci. 2006;24(3):163–72.

82. Bouvier D, Oddoze C, Ben Haim D, Moustafa F, Legrand A, Alazia M, et al. Interest of S100B protein blood level determination for the management of patients with minor head trauma. Ann Biol Clin (Paris). 2009;67(4):425–31.

83. Levitt MA, Cook LA, Simon BC, Williams V. Biochemical markers of cerebral injury in patients with minor head trauma and ethanol intoxication. Acad Emerg Med. 1995;2:675–80.

84. Morochovic R, Racz O, Kitka M, Pingorova S, Cibur P, Tomkova D, et al. Serum S100B protein in early management of patients after mild traumatic brain injury. European Journal of Neurology. 2009;16(10):1112-7.