Effectiveness of Traumatic Brain Injury Management Guideline Introduction in Hungary and Risk factors of External Ventricular Drain Infection

PhD Thesis

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ABBREVATIONS

AS - Abayomi Sorinola

SM - Stefania Mondello

EC - Endre Czeiter

ZV - Zoltan Vamos

CA - Krisztina Amrein

ACEP/CDC - American College of Emergency Physicians/ Centers for Disease Control and Prevention

APACHE - Acute Physiology And Chronic Health Evaluation

ASA – American Society of Anaesthesiologists

ASC/AST - Active Surveillance Culture/Testing

BM – Biomarker

BMI – Body Mass Index

CFR - Case Fatality Ratio

CINAHL - Cumulative Index to Nursing and Allied Health Literature

CNS - central nervous system

CNS – Central Nervous System

CSF - Cerebrospinal Fluid

CSF – Cerebrospinal Fluid

CT - Computed Tomography

CV - Coefficient of Variation

DSF - Depressed Cranial Fracture repairing surgery

DTI - Diffusion Tensor Imaging

ECI - Extracranial injury

ECLIA - Electrochemiluminescence Immunoassay

ED - Emergency Department

ELISA - Enzyme-Linked Immunosorbent Assay

EVD - External Ventricular Drain

EVD – External Ventricular drain

fcMRI - Functional Connectivity MRI

FDA - Food and Drug Administration

FITBIR - Federal Interagency Traumatic Brain Injury Research

FN - False Negative

FP - False Positive

GCS - Glasgow Coma Scale

GCS - Glasgow Coma Score

GFAP - Glial fibrillary acidic protein

GRADE - Grading of Recommendations Assessment Development and Evaluation

H - Hour

HDR - Hospital Discharge Records

ICD - International Classification of Diseases

ICH - Intracerebral Haemorrhage

ICP - Intracranial Pressure

ICPM – Intracranial Pressure Monitor

ICPM - Intracranial Pressure Monitoring

ICTRP - International Clinical Trials Registry Platform

IFMA - Immunofluorometric Assay

IgG - Immunoglobulin G

IgM - Immunoglobulin M

ILMA - Immunoluminometric Assay

IQR - Interquartile range

IVH - Intraventricular Haemorrhage

IVH - Intraventricular Haemorrhage

LIA - Luminescence Immunoassay

LLOD - Lower Limit of Detection

LLOQ - Lower Limit of Quantification

LOC - Loss of consciousness

LOD – Limit of Detection

LOS – length of stay

MHI - Mild Head Injury

MHT - Mild Head Trauma

Min - Minute

MRI – Magnetic Resonance Imaging

mTBI - Mild Traumatic Brain Injury

MVA - Motor Vehicle Accident

MW - Molecular Weight

NA - Not applicable

NHIF - National Health Insurance Fund

(N)ICU – (Neuro) Intensive Care Unit

NR - Not Reported

NSE - Neuron specific enolase

OR - Operating Room

PAI - Platelet Aggregation Inhibitor

POC - Point of Care

PRISMA - Preferred Reporting Items for Systematic Reviews and Meta-

PTA - Post-Traumatic Amnesia

Pts – Patients

RIA – Radioimmunoassay

(H)(S)ROC – (Hierarchical) (Summary) Receiver Operating Characteristic

S100 β - S100 calcium binding protein β

SAH - Sub-Arachnoid Haemorrhage

SAPS - Simplified Acute Physiology Score

SEM - Standard Error of the Mean

sTBI - Severe Traumatic Brain Injury

SWI - Susceptibility Weighted Imaging

TBI - Traumatic brain injury

TN - True Negative

TP - True Positive

UCH-L1 - Ubiquitin C-terminal hydrolase-L1

ULOQ - Upper Limit of Quantification

WHO - World Health Organization

I. INTRODUCTION: Background of Theses

1. Traumatic Brain Injury management guideline in Hungary

1.1. Epidemiology, economic implication and expected outcomes of proper guideline adherence

Traumatic brain injury (TBI) is a major cause of death and disability in the world harbouring significant public health and socio-economic importance. TBI is estimated to be the primary cause of death and disability among young individuals and associated with a cost of over $76 billion ($384,864,000/100,000/year) in the USA (1) and at least €33 billion (€ 77,550,000/100,000/year) in Europe (2). According to the Centers for Disease Control and Prevention (CDC) in 2016 about 823.7/100,000/year emergency department visits were associated with TBI - either alone or in combination with other injuries. Epidemiological data on TBI from the European Union are scarce, but do indicate an incidence of hospitalized TBI of approximately 235/100 000/year, although substantial variation exists between European countries (3).

In order to reduce the disability, mortality and socio-economic burden of TBI, guidelines (4) for managing TBI need to be adhered to. The implementation of guidelines produces improved efficiency and outcomes for healthcare professionals and patients beginning with pre-hospital phase and extending throughout long-term application of care. If all trauma centers in the U.S. adopted the guidelines, the CDC projects a $3.8 billion savings in associated cost (5). Although TBI management guidelines are widely published, their implication is seldom assessed and the guideline adherence is hardly documented (4, 6).

If TBI management guidelines are properly adhered to, the pre-hospital management of TBI should lead to correct identification of TBI, optimal treatment in the ambulance/emergency room and direct transfer to a TBI trauma center. The in-hospital management of TBI will produce reduced duration of ICU/hospital stay, reduced healthcare cost, decreased death and disability (7) by 30%-50% and improved neurological outcome (8) upon discharge by 30%-50%. The post-hospital management of TBI would lead to faster rehabilitation and timely re-integration of a patient into the society (9). Adherence to guideline possesses further great potential for managing TBI in terms of helping to standardize clinical management of TBI (hence, ensure quality control) and aid data collection for further audit/benchmarking and research purposes.

1.2. Incidence of Traumatic Brain Injury in Hungary

The disease burden of serious intracranial trauma is continuously high in Hungary especially among middle aged men representing the leading cause of death in the young, active population. The reported incidence of TBI patients in Hungary is 140/100,000/year. The proportions of mild, moderate, and severe cases are 67%, 23%, and 11%, respectively. The case fatality ratio (CFR) was extremely high in Hungary: the estimated CFR for hospitalized TBI cases was 45% in 2002 (10). To exploit the evidence based guidelines opportunities, the Hungarian Ministry of Health introduced the guideline of TBI care in 2006, which was established on recommendation of Brain Trauma Foundation (4). It focused on the prehospital and clinical management of patients, but it was not supported by reformulated financing protocols and establishment of quality monitoring.

2. Risk factors of External Ventricular Drain infection

2.1. Epidemiology and incidence of ventriculostomy

Ventriculostomy is frequently used in the management and monitoring of intracranial pressure (ICP) in severe TBI patients. In the US, an average of about 20,586–25,634 (24,380) patients per annum undergo ventriculostomy (11).

The application of external ventricular drain (EVD) is a crucial point in TBI protocols, EVD infections are among the complications for EVD application with high influence on the outcome of the underlying disease and are not well characterized.

Many conditions such as intracranial hemorrhage, intracranial tumor and hydrocephalus prompt the use of EVD. EVD application is frequently complicated by misplacement, hemorrhage, dislodgement, blockage and most importantly infection (12). EVD infection rate ranges from 0% to 22% (13, 14, 15) resulting in a significant increase in cost, hospital stay, morbidity and mortality (12, 16).

In order to avoid EVD infection and reduce cost, EVD application is often avoided despite the fact that EVD application can improve the prognosis of TBI patients. Unfortunately the avoidance EVD application can be supported by the EVD infection related uncertainties. The cases where the fear of infection is justified (and the intention of saving resources and reducing cost is acceptable) cannot be differentiated from cases where the risk of EVD infection is acceptably low (and the resource saving jeopardizes the patients' prognosis).

Due to the heterogeneous knowledge on the effectiveness of EVD, uncertainties of EVD application and the infection related complications, further research is required.

3. Blood based Diagnostic Protein Biomarkers in Adult Mild Traumatic Brain Injuries

Traumatic brain injury (TBI) is among the most common neurological disorders worldwide and globally its incidence continues to rise (17, 18). According to the Centres for Disease Control (CDC) in the US, over the past decade, rates of TBI-related emergency department (ED) visits has increased by 70%, of which most are classified as mild (MTBI), posing a substantial everyday workload. Clinical diagnosis remains a challenge and computed tomography (CT) is considered the diagnostic cornerstone used in the ED to rule out post-traumatic brain lesions and complement clinical assessment of patients with a possible MTBI (19). However, it is generally acknowledged that CT is not always available, implies patient radiation exposure, and is relatively costly in terms of ED logistical burden and health care expenditures owing to the small proportion of subjects (~10%) diagnosed as having actual traumatic intracranial lesion (19, 20).

The need to manage patients with possible mild TBI more effectively and efficiently — to reduce unnecessary CT scans and medical costs, while not compromising patient care and safety - has driven the quest for sensitive blood-based markers as objective parameters that can be easily and rapidly measured in the systemic circulation. Identification of biomarker signatures associated with distinct aspects of TBI pathophysiology may be also of clinical value for a more accurate characterization and risk stratification of TBI, thereby optimizing medical-decision making and facilitating individualized and targeted therapeutic intervention. As such, over the past decades, a focused effort has been made to identify novel blood biomarkers for TBI, and a growing number of candidates has been described and proposed (21, 22, 23, 24), leading to the recent incorporation of S100B into the Scandinavian Neurotrauma guidelines (25). Nonetheless at present,

the role of body fluid biomarkers in TBI is primarily relegated to research studies, and the provision of high quality evidence is paramount to meet regulatory requirements and support their adoption and routine use in clinical practice.

Meta-analysis can exploit the quantity of data collected in separate studies and provide the statistical power to assess more precise estimates of sensitivity and specificity, to determine influence of potential confounding factors on the biomarker diagnostic performance, and to detect differences in accuracy of different marker tests.

We focused on markers for which promising scientific evidence of analytical and clinical validity is available and, thus, are likely to be rapidly transferable to clinical practice, namely S100B, glial fibrillary acidic protein (GFAP), neuron specific enolase (NSE), ubiquitin C-terminal hydrolase-L1 (UCH-L1), and Tau and neurofilament proteins. As TBI biomarker research and technological and analytical advances are dynamic, we felt that a living systematic review - a high quality, online review that is updated as new research becomes available (26)- would best fit our purpose. The "*living*" nature of such work will permit, indeed, the potential inclusions and investigation of novel markers, marker combinations, and more refined diagnostic time windows for which relevant scientific literature/body of evidence will be gained.

II. AIMS

Traumatic Brain Injury management guideline in Hungary

1. The aim was to describe the impact of the guideline introduction on the degree of care centralization and the CFR for the Hungarian severe TBI patients, in order to describe the usefulness of guideline introduction without parallel introduction of audit system.

Risk factors of External Ventricular Drain infection

2. The aim of this review was to identify risk factors that can potentially affect the incidence of EVD infection and create a model, which can be used in future studies to determine the real risk factors with their real strengths in order to contribute to the elaborations on the guideline for EVD application among TBI patients.

Blood based Diagnostic Protein Biomarkers in Adult Mild Traumatic Brain Injuries

3. The aim of this systematic review and meta-analysis was to comprehensively summarize and critically evaluate the existing body of evidence for the use of blood protein biomarkers for diagnosis of brain injury as assessed by CT in adult patients presenting to the ED after mild head trauma.

III. EFFECTIVENESS OF TRAUMATIC BRAIN INJURY MANAGEMENT GUIDELINE INTRODUCTION IN HUNGARY

1. Materials and Methods

1.1. Case definition

National Health Insurance Fund (NHIF), the only institution responsible for financing the inpatient neuro-traumatology care in Hungary provided the data as hospital discharge records (HDR). NHIF HDR contains patients' data such as age, gender, zip code of residential address, date of admission, codes of interventions applied, International Classification of Diseases (ICD) codes of diagnosed main disorders, date of discharge, date of death (if it happened). Direct assessment of TBI severity was not possible in this studied dataset because the NHIF HDR does not contain the Glasgow Coma Score (GCS). Instead, the severe TBI cases (sTBI) were defined by ICD code and clinical intervention codes. Patients with S06 diagnosis of intracranial injury and with a code of external ventricular drainage application were considered as sTBI subjects. The HDR of sTBI patients admitted between 01/01/2004 and 31/12/2010 recorded in every inpatient institution of Hungary were included in the database analyzed by our investigation. The records were pseudonymized, and the pseudo-identifiers were used to link the episodes of care to patients. Severe TBI patients who died at the scene of trauma or before arrival to the hospitals were not included in the study population.

Age and sex specific incidence of sTBI was calculated for Hungary using demographic data of Hungarian population provided by the Hungarian Central Statistical Office.

1.2. Study center definition

The institutions that took part in the sTBI care were described by the number of patients first admitted by them. By evaluating the pathways of sTBI patients, the TBI centers and secondary institutions were differentiated. Hungary has a declared hierarchy of institutions devoted to TBI care. Unfortunately, this levelling system is neither enforced by health authorities nor adhered to in the practice. In fact, the patient pathways are determined by the traditional relationship between neurosurgeons and the emergency care providers in a certain catchment area, beside the geographical position of injury. Hence, centers had to be determined by a statistical approach in our analyses, instead of by the misleading official categorization. Centers and secondary institutions were distinguished by the number of patients admitted in the study period. A Lorenz curve like graph was constructed to show the level of centralization which plotted the cumulative percentage of the total number of patients in the function of the top percentage of institutions that treated the highest number of patients. The biggest institutions altogether treated 50% of the patients and were considered as centers while the rest of institutions as secondary.

1.3. Case Fatality Rate (CFR): Period, Age group, Gender and Center CFRs

The CFR was calculated for the period of one week, one month and six months after the first hospital admission of sTBI patients. Age group and gender specific CFRs for the whole country were also calculated for each studied year. The center and secondary institution specific CFRs were calculated, as well, and compared by chi-square test to check the change in time. The indicator for centralization of care (number of patients admitted in centers over number of patients admitted in

secondary institutions), the center and secondary institution specific CFRs were computed for the whole study interval (2004-2010), period before (2004-2006) and after (2007-2010) guideline introduction. The period specific results were compared by chi-square test.

1.4. Statistical analysis

To control for the potential confounding effect of patients demographic characteristics, the determinants of CFRs were investigated by multivariate logistic regression models where the sex and age of sTBI patients, the level of first admitting institution (classified as centers or secondary institutions), and time of the admittance (distinguishing before and after guideline introduction periods) were the explanatory variables. The results of statistical tests were considered as significant if the obtained P-value was less than 0.05. All the statistical computations were carried out by PASW Statistics 18.

1.5. Ethical permission

The database was processed anonymously. The processing of the data was a secondary analysis and according to the contemporary Hungarian legal requirement, ethical permission was not necessary to carry out analyses. The research protocol was reviewed, permitted and in concordance with the Internal Data Safety and Patient Rights Board of the Hungarian Health Insurance Fund.

2. Results

2.1. Patient population and high risk age group CFRs

The total number of discharge episodes during the study duration was 77,442 episodes of 7,230 patients. Male dominance was observed. (**Table 1)** The average age of males and females was not different. Among females the age group of 75-84 years was at highest risk. Among males, the highest risk period was wider. (**Figure 1**) There were 3,391 fatal outcomes detected in 6 months of the hospital admittance.

2.2. Total CFR post injury at one week, one month and six months

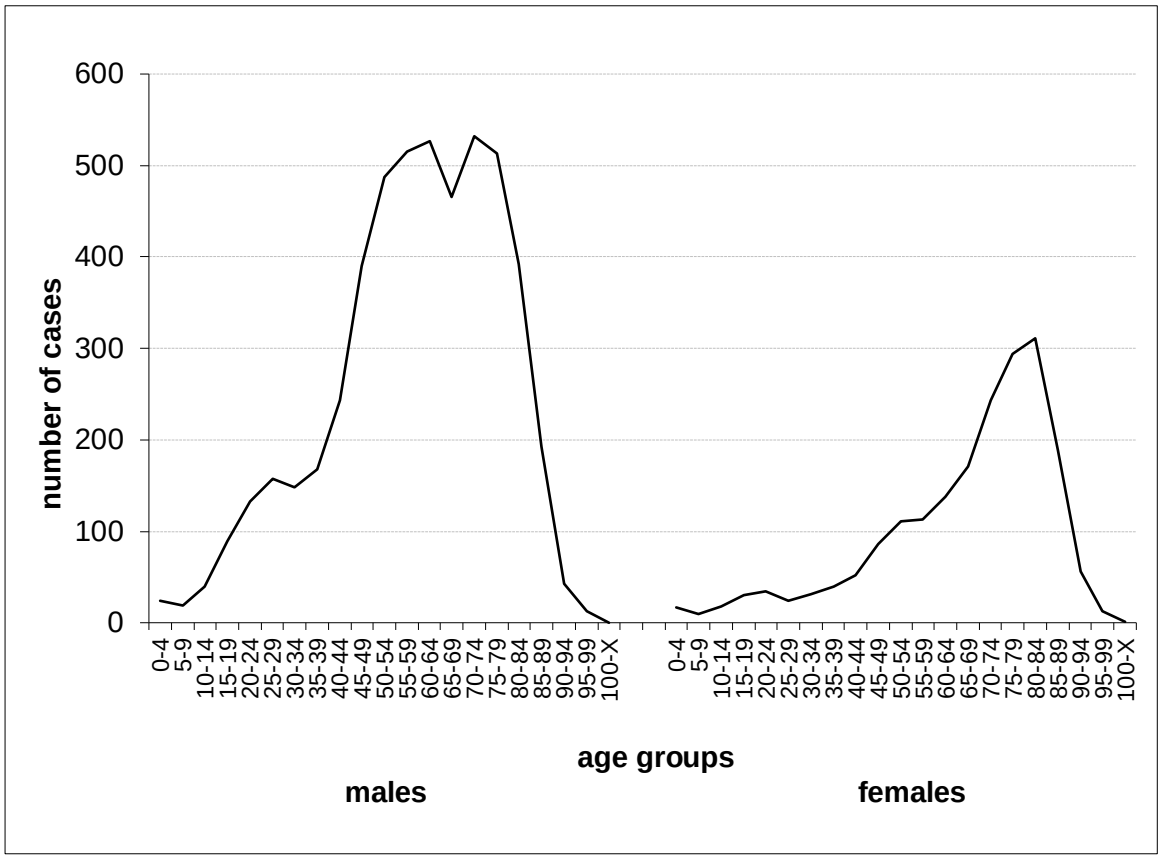
CFR at one week post-injury was 21.9% (21.2% among males and 23.6% among females), which was elevated up to 36.8% (36.1% and 38.8%) at one month, and up to 48.0% (47.0% and 50.4%) at six months.

Table 1 Influence of guideline introduction in 2006 on care centralization and case fatality ratios.

	Whole period (2004-2010)	Before guideline (2004-2006)	After guideline (2007-2010)	P-value*
Male/Female	2.58 (5211/2019)	2.77 (2387/861)	2.44 (2824/1158)	0.015
Age, Mean ± SD	60.89±19.23	59.01±19.30	62.41±19.04	<0.001
Centers/Secondary institutions, (N/N)	0.97 (3551/3679)	0.85 (1492/1756)	1.07 (2059/1923)	<0.001
CFR in 1 week in centers, N (%)	803 (22.6%)	349 (23.4%)	454 (22.1%)	0.454
CFR in 1 week in secondary institutions, N (%)	798 (21.7%)	377 (21.5%)	421 (21.9%)	0.803
CFR in 1 month in centers, N (%)	1369 (38.6%)	563 (37.7%)	806 (39.1%)	0.570
CFR in 1 month in secondary institutions, N (%)	1322 (35.9%)	611 (34.8%)	711 (37.0%)	0.345
CFR in 6 months in centers, N (%)	1736 (48.9%)	709 (47.5%)	1027 (50.0%)	0.416
CFR in 6 months in secondary institutions, N (%)	1753 (47.6%)	813 (46.3%)	940 (48.9%)	0.351

* for comparison of 2004-2006 and 2007-2010 periods

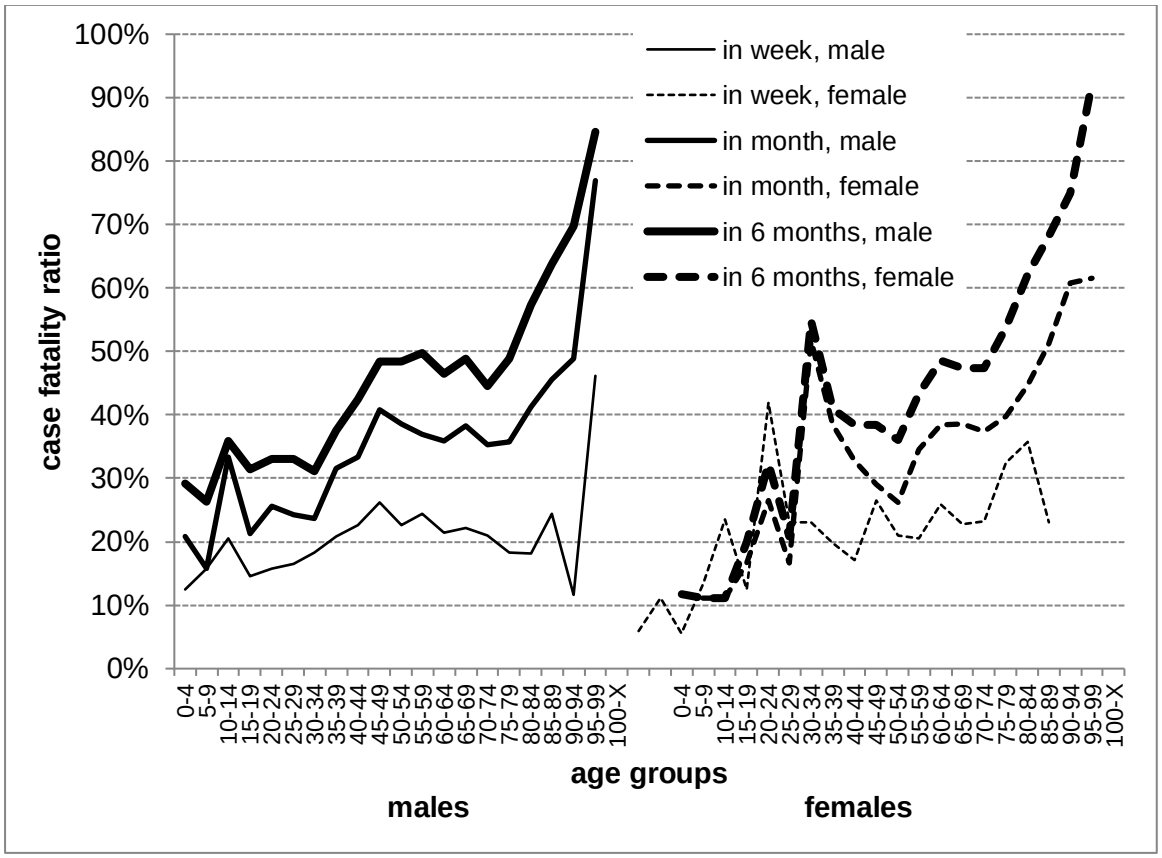
Figure 1 Number of age and sex specific cases of traumatic brain injuries in Hungary (2004-2010) according to the hospital discharge records of National Health Insurance Fund.



2.3. Age, gender and period based CFRs

At one week, in males, the highest CFRs were in the ninth and fourth decades (with CFRs of 46.2 % and 26.2 %). While in females, the highest CFRs were observed in third and ninth decades (with CFRs of 41.9 % and 35.7 %). At one month, in males, the highest CFRs were detected in age groups 95 and 90 (with CFRs of 76.9 % and 61.5 %) and in females, in age groups 95 and 90 (with CFRs of 61.5 % and 60.7 %). At six months, in males, the highest CFRs have been described in age groups 95 and 90 (with CFR of 84.6 % and 69.8 %) in females in age groups 95 and 90 (with CFRs of 92.3 % and 75.0 %). (**Figure 2**)

Figure 2 Age and sex specific case fatality ratio of traumatic brain injury in Hungary (2004-2010) for 1 week, 1 month, and 6 months according to the hospital discharge records of National Health Insurance Fund.



2.4. Total CFR post injury at one week, one month and 6 months before and after 2006

Throughout the study period, the CFR in one week, in one month and in 6 months remained the same for almost all age groups before and after 2006 when the guideline was introduced. (**Figure 3-5**)

Figure 3 Time trend of age specific case fatality ratio (CFR) of traumatic brain injury in Hungary (2004-2010) for 1 week.

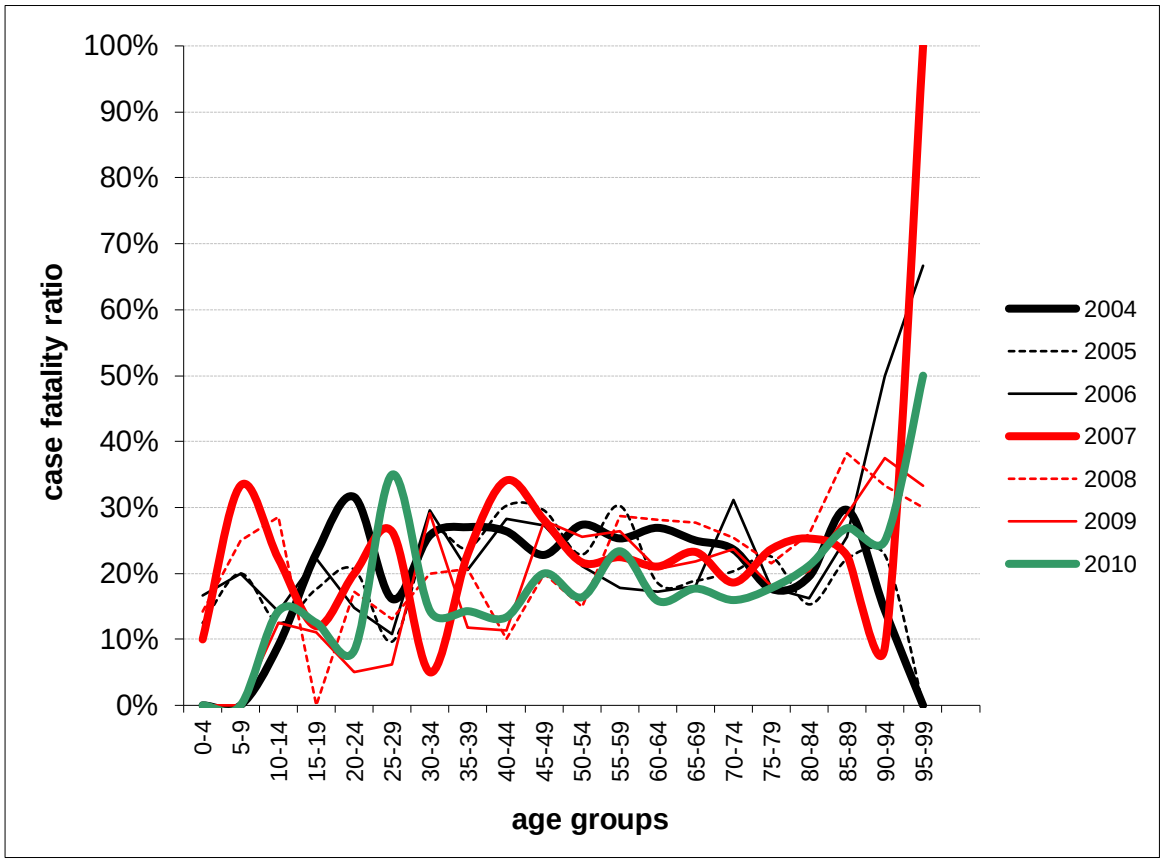


Figure 4 Time trend of age specific case fatality ratio (CFR) of traumatic brain injury in Hungary (2004-2010) for 1 month.

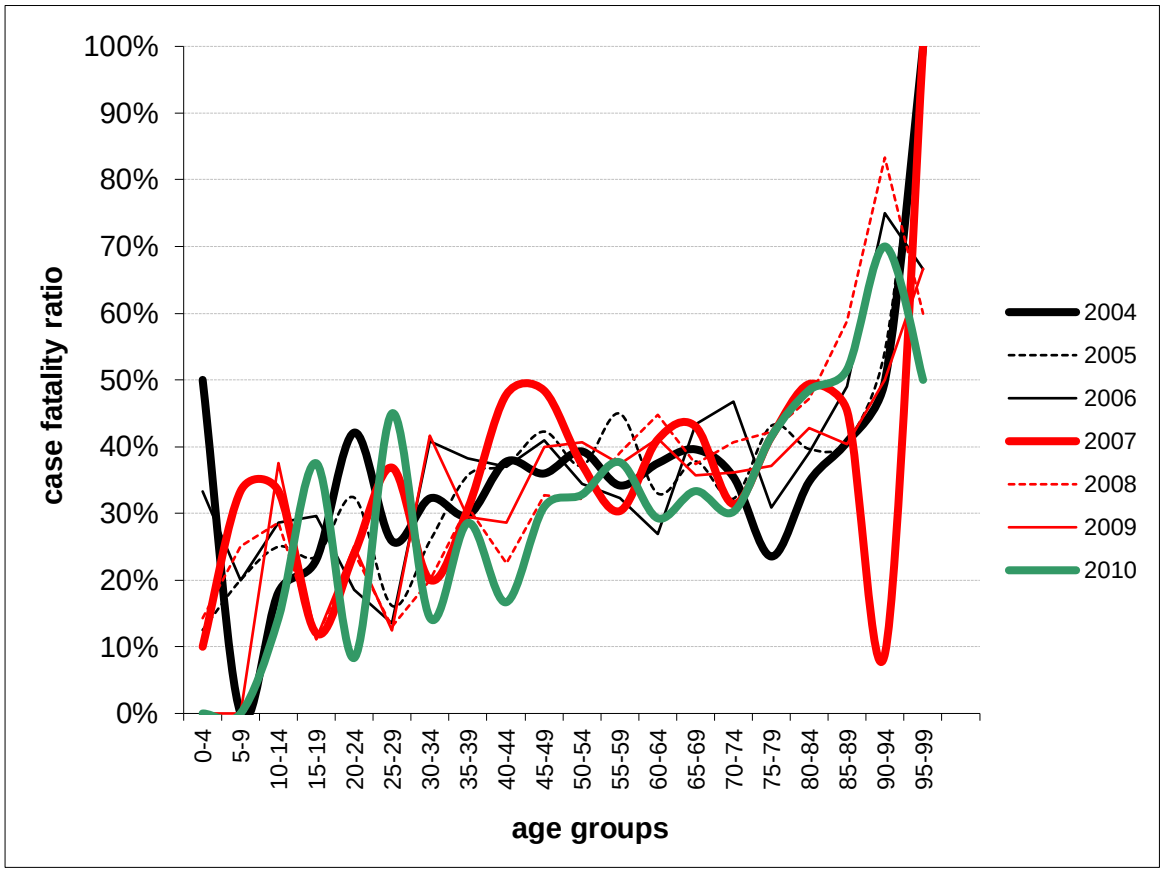
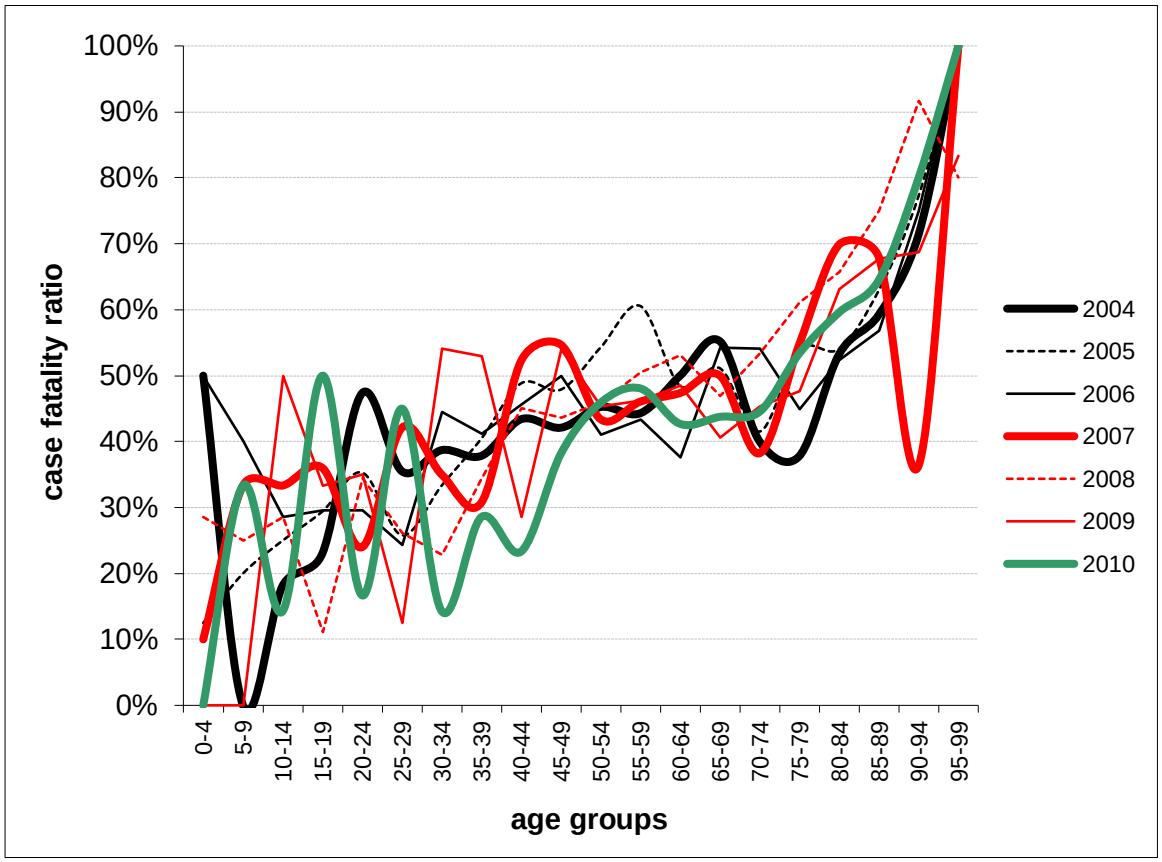


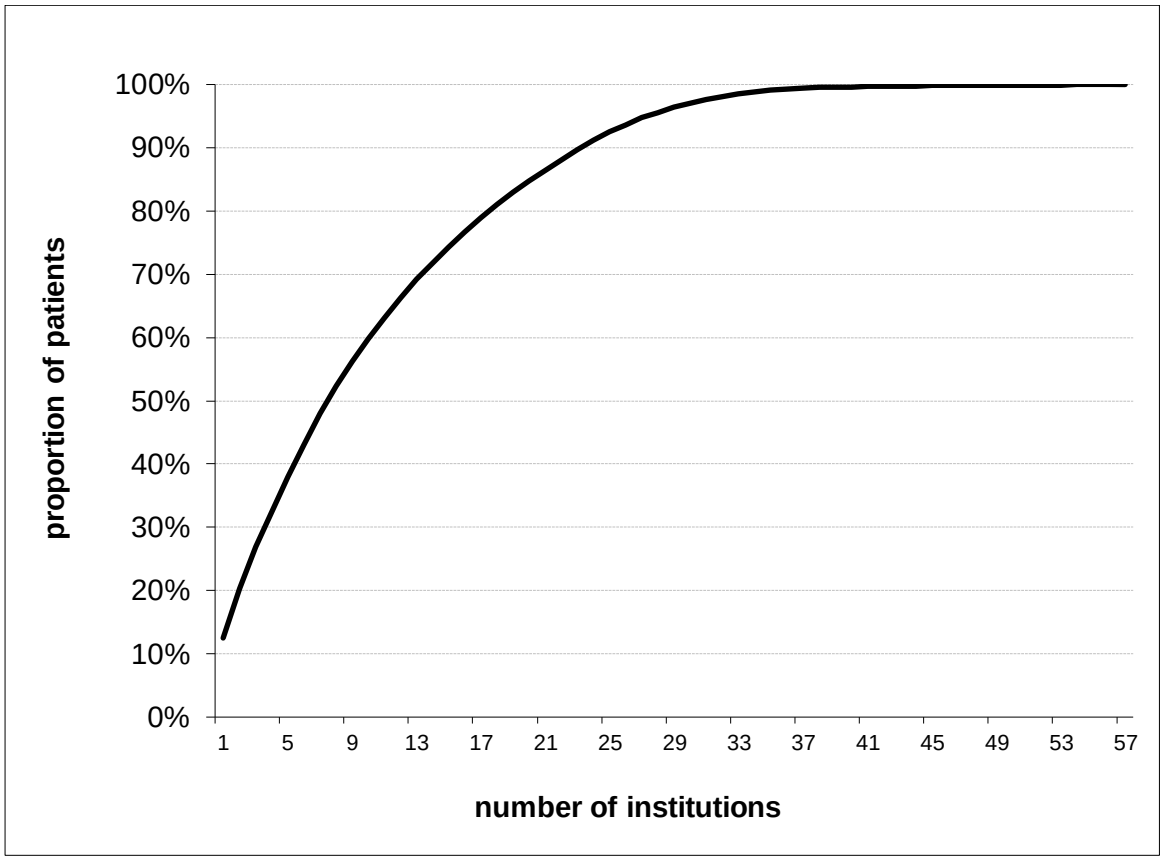
Figure 5 Time trend of age specific case fatality ratio (CFR) of traumatic brain injury in Hungary (2004-2010) for 6 months.



2.5. Study center classification

A total of 57 institutions took part in the study with 8 (referred as centers) providing 50 % of the care. (**Figure 6**) There was an increase in care centralization according to the ratio of the center to secondary institutions treated number of patients. (0.85 vs 1.07; $p<0.001$) (**Table 1**)

Figure 6 Cumulative proportion of severe traumatic brain injured patients in the function of the cumulative number of institutions providing the care in Hungary (2004-2010) according to the hospital discharge records of National Health Insurance Fund.



2.6. Study center CFR

The centers together at one week, one month and six months had CFRs of 22.6 %, 38.6 % and 48.9 % respectively. The secondary institutions together at one week, one month and six months had CFRs of 21.7 %, 35.9 % and 47.6 %. Differences were not significant for one week and for 6 months period (p=0.358, and p=0.267). The centers' CFR was significantly higher for 1 month (p=0.018). The centers and the secondary institutions specific CFR showed no change when the before and after guideline introduction periods were compared. (**Table 1**)

2.7. Factors influencing CFR

According to the multivariate statistical evaluation, sex was not a CFR influencing prognostic factor for any survival interval, but the higher age proved to be risk factor for each CFR studied. Neither the level of first admitting institution nor the time period of care had any significant influence on CFRs. **(Table 2)**

Table 2 Influence of guideline introduction on case fatality ratio of severe traumatic brain injured patients in Hungary according to multivariate logistic regression analysis controlled for age and sex of the patients and for the level of institution providing the care.

		OR	P-value
CFR in 1 week	sex (female/male)	1,110	0,099
	age (year)	1,004	0,018
	level of institution (center/secondary)	1,061	0,300
	guideline introduction (after/before)	0,962	0,502
CFR in 1 month	sex (female/male)	1,006	0,907
	age (year)	1,012	<0,001
	level of institution (center/secondary)	1,095	0,064
	guideline introduction (after/before)	1,042	0,405
CFR in 6 months	sex (female/male)	0,994	0,906
	age (year)	1,017	<0,001
	level of institution (center/secondary)	1,061	0,213
	guideline introduction (after/before)	1,045	0,359

3. Discussion

Main findings

In this study, data (HDRs) from the NHIF was analyzed. Our results demonstrated a steady, high case fatality in the Hungarian TBI population undergoing External Ventricular Drain (EVD) installation, and "no effect" of the introduction of scientific evidence-based practice guidelines in 2006 was revealed. Though, the guideline introduction coincided with moderate increase of centralization. The unreduced CFR in Hungary suggested that the existence of guidelines *"per se"* will not result in outcome improvement and additional measures (audit of care, enforcement of guideline compliance) should also be introduced (27).

Like in Hungary, TBI guidelines were introduced in other European countries and in the U.S. many years ago but in most of these countries, there is a long term tradition of external quality management in clinical care. Further, if there is negligence in medical practice, it can result in lawsuit and also there is competition between the medical institutions. These factors establish the guideline adherence which varies in countries remarkably (between 18- 100%), but contributes to significant reduction in mortality (28).

In the last decades several Eastern and Middle European countries introduced scientific evidence-based practice guidelines to comply with European legislations and regulations mandatory to participate in international multicenter studies and collaborations. Nevertheless, policy makers did not necessarily cope with these guidelines in terms of introducing novel approaches in health care financing and quality assurance.

Similarly to the majority of these countries such “mechanisms” do not exist in the Hungarian health care either and a desperate need for contemporary audit systems has long been voiced by clinical and scientific societies. Due to the lack of external pressure, the hospital managements neglect the internal resource allocation needed to improve the resource demanding guideline adherence. Former small scale studies as well as a cross sectional snapshot-like questionnaire based analysis of the care for traumatic brain injury in Hungary revealed similar results and led to the same conclusions about the reasons of high in-hospital mortality and limited adherence to the international guidelines. This nationwide survey however was unable to provide a trend analysis neither supplied data on long term outcome (10).

Strengths and limitations

The database provided by NHIF was nation-wide with complete national coverage ensuring the fairly high power in the statistical evaluations. However, there are some obvious limitations of such a health insurance data based study: (1) NHIF monitors the financing of care but not the quality of care; (2) the financial interest of the hospital may lead to bias of reported data; (3) data collection could not make distinction between severe and mild TBI cases by the usual GCS classification; (4) and there were no data on the process of clinical treatment apart from the EVD application. However, the case definition and the quality of data collection was not changed in the study period. Therefore, the time trend analysis yielded reliable results on change of care centralization and CFR in time.

TBI in Hungary

The average incidence of TBI in Europe is 235/100,000/year with a range of 150-300/100,000/year (29). The incidence of TBI in Hungary estimated as 140/100,000/year is only a bit less than this European reference (10). Our study

estimated 72.3/100,000/year the incidence of sTBI. Considering the former Hungarian observation on the severity of TBI cases in Hungary, the estimated number of TBI patients according to our present investigation is 957/100,000/year if it is supposed that all studied sTBI patients meet the severe TBI criteria. It seems impossibly high. If it is assumed that sTBI definition corresponds to severe and moderate TBI cases, then the TBI incidence estimated by our study dataset is 212/100,000/year which is in the European reference range. It is probable that our working case definition included severe and moderate TBI cases as well. On the other hand, our study underestimated both incidence rate and number of fatal outcomes. Presumably due to the excluded cases with lethal prehospital outcome, and cases which reached the hospitals but due to the very severe clinical status, the invasive surgical interventions were not performed before lethal outcome. Although, the study was not aimed to determine the exact incidence and case fatality for TBI or for severe TBI in Hungary, taking into consideration the above mentioned validity issues, the observed high CFRs for sTBI demonstrated that the Hungarian care for TBI patients was far less effective than it should be on the basis of the country's general development (30, 31, 32).

In Hungary, the highest CFRs in women at six months were found in the young adults (35 years old) and elderly (> 90 years old) while the highest CFRs in men at six months were found in the middle aged (50 years old) and elderly (> 90 years old). The combined CFRs for both sexes at six months were highest at age group 95 (with CFR of 88.5 %). A similar trend of high CFR in the elderly was reported in other European countries and the USA (31, 33, 34). The similarity between published articles and our study observed age dependence of CFR shows that our design is reliable in dealing with the time trend of CFR.

IV. RISK FACTORS OF EXTERNAL VENTRICULAR DRAIN (EVD) INFECTION: PROPOSING A MODEL FOR FUTURE STUDIES

1. Materials and Methods

1.1. Search strategy

We performed a systematic search on PubMed and Google Scholar databases (from 1966 - August 2017) for relevant studies related to ventricular drain infections. Keywords used in the search strategy include:

1. Infections (ventricular drain, ventriculostomy related, external ventricular drain, ventricular catheter and extra-ventricular drain) and
2. One of the following (traumatic brain injury, Intensive care (ICU) patients', neuro-intensive care (NICU) patients, head injury, brain injury, cerebral hemorrhage, sub-arachnoid hemorrhage).

1.2. Study selection

The combination of keywords generated 328 and 276 references on PubMed and Google Scholar, respectively. Of 604 references, 28 were found relevant after the title and abstract screening. In addition to these, the references of these 28 relevant articles were searched manually to find more related articles, which generated 4 new articles. The 32 relevant articles were screened; those that performed a *multivariate analysis* of suspected risk factors (making a difference between factors which proved to be a confounding factor in a study and factors which can have an influence on outcome according to the published reports) and had a positive culture as a mandatory component in diagnosis were selected for data

collection and analysis. Twenty articles (16 of these are as a result of the keyword-based literature search and 4 are as a result of manual reference search) were finally selected for our review. The cumulative sample size of the 20 studies was 5113 patients, with a median of 164.5.

1.3. Common terms and definitions

In our review, quantitative methods (meta-analysis) could not be applied due to the heterogeneity of the studies in respect to the risk factors identified i.e. the number of studies identifying a certain risk factor was small, making meta-analysis impossible.

As a result of the heterogeneity of the clinical terms used by the authors a common term was chosen for the varying terminologies. “Age” and “younger age” were simplified to ***age***; “co-infection”, “concurrent systemic infection”, “open infection source” and “skin colonization by pathogen” were simplified to ***co-infection***; “Depressed Cranial Fracture repairing surgery (DSF)”, “neurosurgical operation”, “length of tunneling (> 5cm)”,“craniotomy” and “two or more burr-holes” were simplified to ***neurosurgical operation***; “duration of catheterization”, “duration of catheterization (> 11 days)” and “intracranial pressure monitoring (ICPM) > 5 days” were simplified to ***duration of catheterization***; “standard catheter”, “conventional catheter” and “antibiotic coated catheter” were simplified to ***catheter type***; “neuro-trauma”, “multiple trauma”, “sub-arachnoid hemorrhage (SAH)”, “intraventricular hemorrhage (IVH)”, “intracerebral hemorrhage (ICH)” and “intraventricular hemorrhage (IVH)” were simplified to ***diagnosis***; “repeat insertion”, “patients with >1 ICPM”, “multiple catheter replacements” and “number of catheters” were simplified to ***multiple catheters***.

Duration of catheterization refers to the time period between post-catheter insertion and the detection of infection or discharge from ICU in the absence of infection.

1.4. Centers for Disease Control and Prevention criteria for Meningitis or ventriculitis

According to CDC, meningitis or ventriculitis must meet at least one of the following criteria:

1. Patient has organism(s) identified from cerebrospinal fluid (CSF) by a culture or non-culture based microbiologic testing method which is performed for purposes of clinical diagnosis or treatment for example, not Active Surveillance Culture/Testing (ASC/AST).

2. Patient has at least two of the following:

i. fever (>38.0°C) or headache (Note: Elements of "i" alone may not be used to meet the two required elements)

ii. meningeal sign(s)*

iii. cranial nerve sign(s)*

3. Patient ≤1 year of age has at least two of the following elements:

i. fever (>38.0°C), hypothermia, apnea*, bradycardia*, or irritability* (Note: Elements of "i" alone may not be used to meet the required two elements). ii. meningeal signs* iii. cranial nerve signs*

ii. ii. meningeal signs*

iii. iii. cranial nerve signs*

* With no other recognized cause

And at least one of the following for Number 2 and two of the following for Number 3 listed above:

a. increased white cells, elevated protein, and decreased glucose in CSF (per reporting laboratory's reference range)

b. organism(s) seen on Gram stain of CSF

c. organism(s) identified from blood by a culture or non-culture based microbiologic testing method which is performed for purposes of clinical diagnosis or treatment, for example, not Active Surveillance Culture/Testing (ASC/AST)

d. diagnostic single antibody titer (IgM) or 4-fold increase in paired sera (IgG) for organism

1.5. Other definitions

The definition of infection varied among authors. Some defined infection as a positive CSF culture from either the ventricular catheter or lumbar subarachnoid space, verified by growth on agar plates (35,36,37). Others defined infection by the CDC criteria for meningitis/ventriculitis (38,39). Some authors defined infection with a few criteria, e.g Mayhall et al defined infection as; no other detectable source of central nervous system (CNS) infection, negative cultures of CSF obtained at the time of catheter insertion, ventricular catheterization for 24 hours or longer and a positive CSF culture from either the ventricular catheter or lumbar subarachnoid space. Pople et al also defined infection by a few criteria which includes; CSF culture with no organisms identified on initial Gram stain that subsequently grew a positive culture on agar, or CSF culture negative, but with Gram stain showing either gram-positive or gram-negative organisms, or CSF leukocytosis with a white blood cell/ red blood cell CSF count >0.02. Omar et al

defined infection as a positive CSF culture and Gram stain and presence of other supportive CSF laboratory findings such as pleocytosis with microscopic examination showing presence of white blood cells of more than 11/mm^3, a decrease in the CSF glucose level and an increase in the CSF protein level.

Standard catheter refers to catheters without hydrogel or silver or antibiotic(s) coating or impregnation while antibiotic coated catheter refers to catheters coated with antibiotic(s).

2. Results

2.1. Classification of risk factors

Table 3 summarizes the factors that proved to be significant or nonsignificant in the univariate analyses of the studies included in this review. Out of the 20 articles selected for analysis, three studies reported no significant association between the risk factors evaluated and EVD infection (40,12,39) after multivariate analysis. Altogether 15 risk factors (10 patient-related and 5 catheter-related) were identified by our review. Risk factors found by most investigations were neurosurgical operation and duration of catheterization. In general, the reviewed studies dealt only with a narrow set of possible risk factors. The maximum number of risk factors identified by a study was 5. (**Table 4**)

Table 3 Variables identified by univariate analysis having no significant association with EVD infection and factors which showed significant association with EVD infection only in univariate analyses

Study	Significant variables identified in uni-variate analyses	Non-significant variables identified in uni-variate analyses
Arabi 2005	Prophylactic antibiotics.	APACHE II; SAPS II; Placement of EVD outside the OR; ICU - LOS; Hospital LOS and Mortality.
Bari 2017	-	-
Bota 2005	-	APACHE II; LOS; ICU mortality rate and In-hospital mortality rate.
Camacho 2011	Hospital – LOS; ASA I and Antibiotic prophylaxis.	Duration of surgery and Mortality.
Flibotte 2004	Hospital – LOS and NICU - LOS	Admission GCS ≤ 9; In-hospital mortality and VP shunt.
Gozal 2014	-	-
Hagel 2014	ICU – LOS and Hospital LOS.	BMI; ASA; Accommodation and In-hospital mortality.
Hoefnagel 2008	-	Operating time and Prophylactic antibiotic.
Holloway 1996	-	-
Kirmani 2015	Intraventricular antibiotic.	Steroid use.
Lo 2007	-	Presence of trauma.
Mayhall 1984	-	Underlying disease; Placement of EVD in ICU; Antibiotic prophylaxis; CSF drain disconnections; Previous ventriculostomy and Other CNS instrument.
Mounier 2015	-	Immunodeficiency; Recent neurosurgery; Antibiotics prophylaxis during EVD placement; Antibiotics administration during EVD drainage; EVD placement by resident; Emergency EVD placement; EVD exchange; Drainage lock and EVD disconnection.
Omar 2010	-	Venue of surgery and Surgeon's status.
Paramore 1994	-	Location of catheterization within the hospital.
Park 2004	-	-
Peter 2016	-	-
Pople 2012	-	-
Rebuck 2000	-	Skull fracture; Presence of multiple trauma; Penetrating head injury; Antibiotic prophylaxis and Location of catheter placement within the hospital
Wright 2013	-	-

EVD – External Ventricular drain; LOS – length of stay; (N)ICU – (Neuro) Intensive Care Unit; GCS – Glasgow Coma Scale; ASA – American Society of Anaesthesiologists; APACHE - Acute Physiology And Chronic Health Evaluation; SAPS - Simplified Acute Physiology Score; CSF – Cerebrospinal Fluid; CNS – Central Nervous System; ICPM – Intracranial Pressure Monitor; OR - Operating Room; BMI – Body Mass Index

2.2. Patients' factors

Age was identified by Flibotte et al [OR & 95%CI: 1.04, 1.01–.081; P-value: 0.03] and Wright et al [HR & 95%CI: 1.051, (1.01–1.09) P-value, 0.014] as a risk factor associated with ventricular catheter infection. Both studies measured age in years and found a 4% - 5.1% increase in risk of EVD infection per annum respectively (35,41).

Lo et al reported that females [OR & 95%CI: 3.4, (1.2–9.7); P-value, 0.02] were three times as likely to be infected as males (42).

Age & sex interaction [HR & 95%CI: 0.912, (0.85–0.98); P-value, 0.0112] was identified as a risk factor for EVD infection by Wright at al. They also reported that female patients were 6 times likely to have an EVD infection than male patients (23.7% vs 3.1%, OR: 6.4, $p < 0.003$) (41).

Co-infection, a risk factor with a higher incidence among the patient factors was identified by Bota et al [OR & 95%CI: 3.92, (0.66–7.84); P-value, 0.02], Holloway et al [P-value, 0.001], Kirmani et al [P-value, 0.002] and Mounier et al [OR & 95%CI: 11.8, (2.5–56.8); P-value, 0.002] and was found to be significantly associated with ventricular catheter infection (13,43,44,45). The EVD infection rates of 12%, 20.7%, 15% & 37.5% in patients who had a concurrent infection versus 7%, 8.6%, 6% & 4.7% in patients who did not were reported respectively (13,43,44,45).

Mounier et al reported cerebrospinal fluid (CSF) leakage at the site of insertion [OR & 95%CI: 10, (2.4–41.2); P-value, 0.001] as a risk factor and that among the infected patients, most of the catheter infection was as result of the colonization at the site of catheter insertion (45).

Diagnosis was identified by Bota et al [(SAH: OR & 95%CI: 2.95, (0.59–5.26); P-value, 0.02), (IVH: OR & 95%CI: 2.07, (0.65–4.87) P-value, 0.02), (neurotrauma: P-value, 0.03)], Holloway et al [P-value, 0.007] and Mayhall et al [P-value, 0.027] as a risk factor with a high prevalence among the patient factors with significant influence on the incidence of ventricular catheter infection (13,43,46).

According to Hoefnagel et al, increased frequency of CSF sampling [OR & 95%CI: 4.12, (1.84–9.22); P-value, 0.001] is a risk factor to EVD infection. The authors noted that CSF was not always sampled according to the institution's protocol that had been set which inevitably increased the frequency of catheter manipulation and consequently the risk of infection (36).

According to Mayhall et al, intracranial pressure (ICP) above 20 mmHg [P-value, 0.019] is a risk factor for ventricular catheter infection but they mention also the alternate explanation for their observation, that patients with high ICP may need ventricular catheter for longer periods which predisposes them to infection (46).

Neurosurgical operation was identified by Bota et al [OR & 95%CI: 4.74, (0.27–9.52); P-value, 0.03], Holloway et al [craniotomy: P-value, 0.005; DSF: P-value, 0.003], Mayhall et al [P-value, 0.016], Omar et al [OR & 95%CI: 10.46, (3.38–32.32); P-value, 0.001] and Peter et al [P-value, 0.047] as a risk factor (13,43,46,47,16). The reported infection rate varied between 15.2% and 82% in patients who underwent neurosurgical operation against 3.4% and 69% in patients who did not (13,43,46,47,16). Some authors reported that patients with one or more neurosurgical procedures were at a significantly higher risk for infection which may be due to immunosuppression or trauma associated with surgical procedures (44,46).

Gozal et al reported that there is a significant correlation between CSF glucose levels [OR & 95% CI: 4.87, (1.26–18.75); P-value, 0.021] drawn immediately after EVD placement associating less than 50% of serum glucose and subsequent risk of infection. Gozal et al pointed out that this should not be mistaken for a pre-existing systemic infection since similar association was not found in CSF pleocytosis or protein levels which would have been expected in an on-going infection (49).

2.3. Catheter factors

Wright et al reported catheter type [standard catheter vs antibiotic coated catheter: HR & 95% CI: 0.091, (0.02–0.41); P-value, 0.007] as a risk factor associated with ventricular catheter infection. In the study carried out by Wright et al, two types of catheters (standard and antibiotic coated) were used. They reported infection rate as 23.5% for standard catheters and as 4.3% for antibiotic coated catheters. This represents a risk ratio of 0.18 and an absolute risk reduction of 19.2% after changing from standard catheter to antibiotic coated catheter (41).

Park et al reported that patients that underwent catheter insertion outside the study center had a higher risk [HR & 95%CI: 3.42, (1.46–8.02); P-value, 0.005] of infection than patient that underwent catheter insertion in the study center and that there was limited information on the technique of catheter placement or specific location of the patient outside the study center at the time of catheter insertion. On the other hand, Park et al also reported that the location of catheter insertion (OR/ICU/ED) within the study center did not significantly influence the infection rate of patient with catheters inserted in the study center (49).

Duration of catheterization was identified by Camacho et al [OR & 95%CI: 1.08, (1.1–1.2); P-value, 0.036], Flibotte et al [OR & 95%CI: 1.2, (1.1–1.3); P-value, 0.001], Hoefnagel et al [OR & 95%CI: 4.12, (1.84–9.22); P-value, 0.001], Mayhall

et al [P-value, 0.017], Omar et al [OR & 95%CI: 3.61, (1.19–10.94); P-value, 0.024], Paramore et al [P-value, 0.016], Peter et al [P-value < 0.001] and Pople et al [RC & 95%CI: -0.048, (-0.092 to -0.003)] as a risk factor (38,35,36,46,47,37,16,50). The duration of catheterization ranged from 1- 44 days in these studies. Some authors suggest that the longer duration of catheterization may cause microbial infection of the catheter (43,46,37) while there is another opinion that the longer duration of catheterization is a consequence of the catheter infection rather than the cause (12).

Multiple catheters was identified Arabi et al [OR & 95%CI: 6.34, (1.36-29.64); P-value, 0.019], Lo et al [OR & 95%CI: 4.6, (2.3–9.2); P-value, < 0.0001], and Peter et al [P-value, < 0.01] as a risk factor for EVD infection (51,42,16). Arabi et al and Peter et al reported an infection rate of 42% & 84.5% in patients who received multiple catheters versus 3% & 18.3% in patients who did not respectively. Lo et al also reported that infected patients used almost twice the amount of ventricular catheters as their uninfected counterparts (51,42,16). Each additional catheter was reported to increase the risk of infection by four folds or 8% by some authors (42,14). Arabi et al found out that antibiotics were given more frequently with first insertion than with repeated insertions [68% vs 7%, P-value, 0.001] and it may be responsible for making multiple catheters a risk factor (1).

Mayhall et al who identified irrigation [P-value, 0.021] as a risk factor for EVD infection also hypothesized that infections are likely introduced into the ventricles by retrograde movement of microbes due the manipulation of the catheter system (46).

Table 4 Identified risk factors of extra-ventricular drain infections according to the published results from 1984.

Risk Factors	Arabi 2005	Bari 2017	Bota 2005	Camacho 2011	Flibotte 2004	Gozal 2014	Hagel 2014	Hoefnagel 2008	Holloway 1996	Kirmani 2015	Lo 2007	Mayhall 1984	Mounier 2015	Omar 2010	Paramore 1994	Park 2004	Peter 2016	Pople 2012	Rebuck 2000	Wright 2013	**Number of positive publications**
Patient factors/Sample size	84	256	638	119	311	498	218	228	584	130	199	172	101	87	161	595	100	434	215	144	
Sex		NS	NS	NS	NS		NS			NS	X			NS		NS				NS	**1**
Age		NS	NS		X		NS			NS						NS	NS	NS		X	**2**
Age & sex interaction																				X	**1**
Co-infection			X						X	X			X						NS		**4**
CSF leakage										NS			X			NS			NS		**1**
Neurosurgical operation			X				NS		X	X		X		X			X				**6**
CSF sampling frequency								X													**1**
ICP > 20mmHg												X							NS		**1**
Diagnosis			X		NS		NS		X			X				NS	NS	NS			**3**
Reduced CSF glucose						X															**1**
Catheter factors																					
Multiple catheters	X	NS							NS		X						X		NS		**3**
Catheter insertion outside the hospital	NS															X					**1**
Duration of catheterization	NS			X	X		NS	X			NS	X	NS	X	X		X	X	NS		**8**
Catheter type							NS											NS	NS	X	**1**
Irrigation												X									**1**
Number of identified risk factors	**1**	**_**	**3**	**1**	**2**	**1**	**_**	**2**	**3**	**2**	**2**	**5**	**2**	**2**	**1**	**1**	**2**	**1**	**_**	**3**	

X – Factors investigated and found to be significant

NS – Factors investigated but found to be non-significant

3. Discussion

The keywords and references used in our review were similar to the keywords and references in other reviews (52,14,15). Despite the sparse amount of articles on this topic, we were able to identify a total of 15 risk factors.

According to our review, distinct studies were able to identify only few risk factors, since the published studies were intentionally focused to a few specific causes of EVD infection with controlling for only few confounding factors, instead of testing a comprehensive set of causal factors representing the hypotheses on the etiological background. On the other hand, the sample size of reviewed studies was rather limited, preventing the effective identification of less dominant risk factors. Further, the terms of investigated clinical factors were highly variable, limiting the effectiveness of comparative evaluation. Therefore, the research on the risk of EVD infection is in its initial phase. Identification of factors which can play role in development of infective complications of EVD was the function of the till-now-published papers. The reviewed investigations could contribute to the building of research model to be tested in future, and cannot be considered as reliable quantification of the risk factors' role.

Although, only papers with multivariate models were analyzed in this review, as shown in **Table 4**, none of them could cover the majority of risk factors. Even the paper with the most extended model could not cover half of the identified risk factors. Consequently, the published measures of associations reflect both the strength of the risk factors and the confounding effects of factors that were not included in the model but were associated with the risk factors included in the studied models. Therefore, the relative importance of a risk factor cannot be evaluated by the published models. The whole set of suspected risk factors need to

be included in the model which will be tested in clinical practice in order to determine the risk factors with their strength and clinical importance.

There were studies that found some of the identified risk factors not significantly associated with EVD infection in their multivariate analysis. Age, sex, CSF leakage, catheter type and diagnosis were identified by more studies as not significantly associated with EVD infection (12,13,16,35,38-41,44,47,49,50) than studies that did. On the other hand, while more studies reported some risk factors to be significantly associated with EVD infection, a few studies did not find a similar association between these risk factors and EVD infection after multivariate analysis. Duration of catheterization, co-infection, and neurosurgical operation were found by more studies to be associated with EVD infection than a few studies that did not (13,16, 35-38,42-47,50,51). Catheter insertion outside the hospital, multiple catheters and ICP > 20mmHg were found to be either associated with EVD infection or not by equal number of studies (39-41,49-51). The varying results from the reviewed studies are possibly due to non-standardized research procedures (i.e. some risk factors were selectively analyzed while some risk factors were omitted, resulting in a possible causal or coincidental relationship or the lack thereof between EVD infection and these risk factors). Some of the factors presented in this review for later research may only be confounding factors without direct influence on ventricular catheter infection occurrence (e.g. the association between gender and drain infection; far more males were involved in severe injuries whereas females appeared to be a predictive factor).

This review has a few limitations. Firstly, not all the possible research papers were collected as only the PubMed and Google Scholar databases were used, also the research papers published in other languages other than English language were not included in this review. Secondly, as mentioned previously, the meta-analysis of

the risk factors could not be carried out. Thirdly, some of the selected papers were not focused on EVD exclusively (some included ICP monitors). Lastly, the explanatory power of the proposed study model could not be determined because the studies evaluated only a narrow set of influencing factors. Consequently, the proposed study model may include interrelated prognostic factors and it is not possible to predict whether factors omitted from our proposed research model (risk factors) have high impact on manifestation of EVD infection.

Application of ICP monitoring has recently become a major topic of discussions in the scientific community also leading to the conduct of major randomized clinical trial (BEST-TRIP) (7). Nevertheless, to much of our surprise, application of such a common monitoring and therapeutic tool is based on very limited knowledge of purported risk factors associated with its' utilization.

Being aware of such complications and their rate would be of ample importance to inform the relatives, train the care givers as well as enhance therapeutic efficacy. We hope that the present work not only focused attention at our above-detailed weaknesses but also highlighted those potential factors that should be considered when EVD-related infective complications are to be predicted.

V. BLOOD-BASED PROTEIN BIOMARKERS FOR THE MANAGEMENT OF TRAUMATIC BRAIN INJURIES IN ADULTS PRESENTING WITH MILD HEAD INJURY TO EMERGENCY DEPARTMENTS: A LIVING SYSTEMATIC REVIEW AND META-ANALYSIS

1. Materials and Methods

This review is being prepared as a 'living systematic review', initiated in the context of the CENTER-TBI project (www.center-tbi.eu) (26,28,54). Following a predefined protocol registered on the PROSPERO database (registration number CRD42016048154), we conducted a systematic review and meta-analysis according to the PRISMA guidelines (55).

1.1. Information sources

We searched Ovid MEDLINE® (1946 to October 2016), OVID Embase (1980 to October 2016), OVID EBM Reviews (October 2016) and Cochrane Library (October 2016) for relevant studies. The search strategies used can be found in the **Appendix**. For possible ongoing trials and studies we searched WHO International clinical trials registry platform (ICTRP) (searched November, 2016) and ClinicalTrials.gov registry (searched November, 2016).

Additional studies were identified by reviewing the reference lists of published clinical trials and relevant narratives as well as systematic reviews. Abstracts from relevant scientific meetings were also examined and experts in the field were consulted for any further studies.

Citations were uploaded into web-based systematic review program (Covidence, Alfred Health Melbourne, Australia) (http://www.covidence.org/).

1.2. Study selection

Two reviewers (AS and SM) independently reviewed the title and abstract of each citation identified by the search strategy. In the second stage, the full text was reviewed and eligible studies selected. Any disagreement between the two authors was resolved through discussion, or where necessary, arbitration by a third party (EC, ZV or CA). Studies were included if the article met the pre-specified list of eligibility criteria: studies enrolling adult patients presenting to the ED with a history of possible brain injury complying with any authors' definition of mild TBI; report of the admission head CT findings; at least one quantitative measurement of the circulating biomarkers of interest (S100B, GFAP, NSE, UCH-L1, tau and Neurofilament proteins) on admission, and relevant accuracy data.

We included studies containing mixed populations (i.e. participants with moderate and severe TBI [GCS<13] or pediatric populations). Studies were included irrespective of their geographic location and language of publication. We excluded studies using non-quantitative methods to assess biomarker concentrations (e.g. western blot or explorative proteomics). Studies with small cohorts (fewer than 50 participants) were excluded, given the high likelihood of being underpowered, thus, impacting the reliability of findings.

1.3. Data extraction and assessment of methodological quality

Two reviewers (AS and SM) independently extracted data using a standardized data abstraction form. We abstracted relevant information related to the study design, patient characteristics (demographic and clinical data, including indices of injury severity, presence of extracerebral injuries and polytrauma, and CT findings) and biomarker characteristics (concentrations, sampling time, cut-offs and statistical levels of diagnostic accuracy [sensitivity and specificity]), analytical aspects of biomarker testing, and study limitations. Details regarding the definition of mild TBI and CT abnormality were also extracted.

In the case of multiple studies from the same research group, authors were contacted to ensure there was no overlap in patient populations. We also contacted authors for clarification of study sample, missing data or ambiguity in the cutoffs used. If biomarker measurements were carried out at multiple timepoints, we used the sample on admission for analysis.

The methodological quality of the included studies was independently assessed by two reviewers (AS and SM) using a modified version of the tool for quality assessment of studies of diagnostic accuracy included in systematic reviews (QUADAS-2) (56), as recommended by the Cochrane Collaboration. Discrepancies were resolved through discussion or arbitration by a third reviewer (EC, ZV or CA).

1.4. Data synthesis

The analysis includes a structured narrative synthesis. We constructed evidentiary tables identifying the results pertinent to diagnostic capabilities of the different biomarkers (detection of intracranial lesions as assessed by CT) and study characteristics for all included studies. We conducted exploratory analyses by plotting estimates of sensitivity and specificity from each study on forest plots and in receiver operating characteristic (ROC) space.

Where adequate data were available, we performed meta-analyses for each biomarker to summarize data and obtain more precise estimates of diagnostic performance. For studies with diverse thresholds, we meta-analyzed pairs of sensitivity and specificity using the hierarchical summary ROC (HSROC) model which allows for the possibility of variation in threshold between studies, and also account for variation between studies and any potential correlation between sensitivity and specificity (57). For these analyses we used the NLMIXED procedure in SAS software (version 9.4; SAS Institute 2011, Cary, NC). For studies that reported data at common pre-specified cut-off values, we calculated the pooled estimates of sensitivity and specificity (clinically interpretable), by undertaking a random effects bivariate regression approach (58).

We explored heterogeneity through visual examination of the forest plot and the summary ROC (SROC) plot for each biomarker. However, as there were insufficient studies, lack of individual data and/or important variation across studies with simultaneous presence of factors with potentially diverging effects on biomarker accuracy estimates, we did not perform meta-regression (by including each potential source of heterogeneity as a covariate in the bivariate model) as planned.

Sensitivity analyses were performed to check the robustness of the results. We used Cook's distance to identify particularly influential studies and checked for outliers using scatter plots of the standardized predicted random effects. Then the robustness of the results was checked by refitting the model excluding any outliers and very influential studies. Sensitivity analyses were also conducted to investigate the impact on biomarker performance of studies including mixed populations, bias in the selection of participants, high prevalence of abnormal CT findings and different definitions of traumatic brain injury as assessed by CT.

Data processing and statistical analyses were conducted using Review Manager version 5.3 (Cochrane Collaboration, Copenhagen, Denmark) and STATA version 13.0 (StataCorp, Colleage Station, Texas, USA) including the user written commands METANDI and MIDAS.

1.5. Quality of evidence

The Grading of Recommendations Assessment, Development and Evaluation (GRADE) (59) approach was used to assess the overall quality of evidence of the included biomarker tests. The results were summarized using GRADEPro software (version Version 3.2, 2008).

2. Results

2.1. Description of studies

Our search strategy identified a total of 7260 citations. Removal of duplicates resulted in 5567 distinct citations, of which 90 full-text articles were assessed for eligibility, and 26 articles (19,60-84) were included in the systematic review (**Figure 7** - Flow diagram of search and eligibility results and **Table 5**). **Tables 6 and 7** show the main characteristics of the included publications.

Two of the 26 included papers reported biomarker results from the same patient cohort (76,77). All studies were published in 2000 or later. With the exception of one study published in French (63), and one in Italian (66), all studies were published in English.

The total number of patients with TBI in the included studies was 8127, ranging from 50 (70,79) to 1560 (84) per study (median 170, interquartile range 104-258). Of those, 865 had positive CT scans with an average prevalence of 17% (median 13%) (range 5% to 51%) (**Table 6**). In 9 papers the presence of a skull fracture was considered as a traumatic CT abnormality.

The reported mean or median age of the included patients ranged from 32 (80) to 83 years (81) with ten studies including children and/or adolescents (patient age <18yrs). The total subject pool was largely male (median 63% across the studies), with the exception of the study by Thaler et al, which comprised 68.7% of females (81). Two cohort studies included mild to severe TBI patients (GCS 3-15) (71, 80), and two other cohorts included mild to moderate TBI patients (GCS 9-15) (76-78,82). Six studies enrolled TBI patients with multiple trauma and/or extracranial injuries (**Table 6**). Nine of the included papers reported biomarker concentration

from different types of control cohorts, including healthy individuals, or non–head-injured trauma patients (See **Table 7** for details).

Most of the studies defined specific timeframe from injury to blood draw as an inclusion criterion, with the majority of the samples collected within 6 hours of injury (16 studies) and with mean or median time ranging from 24.3 minutes (75) to 5 hours (**Table 7**) (70). In one study samples were collected within 12 hours (73) and in 2 studies within 24 hours (71,80).

A single marker was evaluated in most of the studies (n= 21), while 1 study simultaneously assessed 3 markers (82). Of the eligible studies, 22 reported data on S100B (total number of TBI patients 7754), 4 on GFAP (total number of TBI patients 783), 3 on NSE (total number of TBI patients 314), and 2 on UCH-L1 (total number of TBI patients 347). Fewer data were available for tau (1 study which included only 50 patients) (70), while we found no studies evaluating neurofilament proteins that met our inclusion criteria.

Figure 7 Study flow diagram

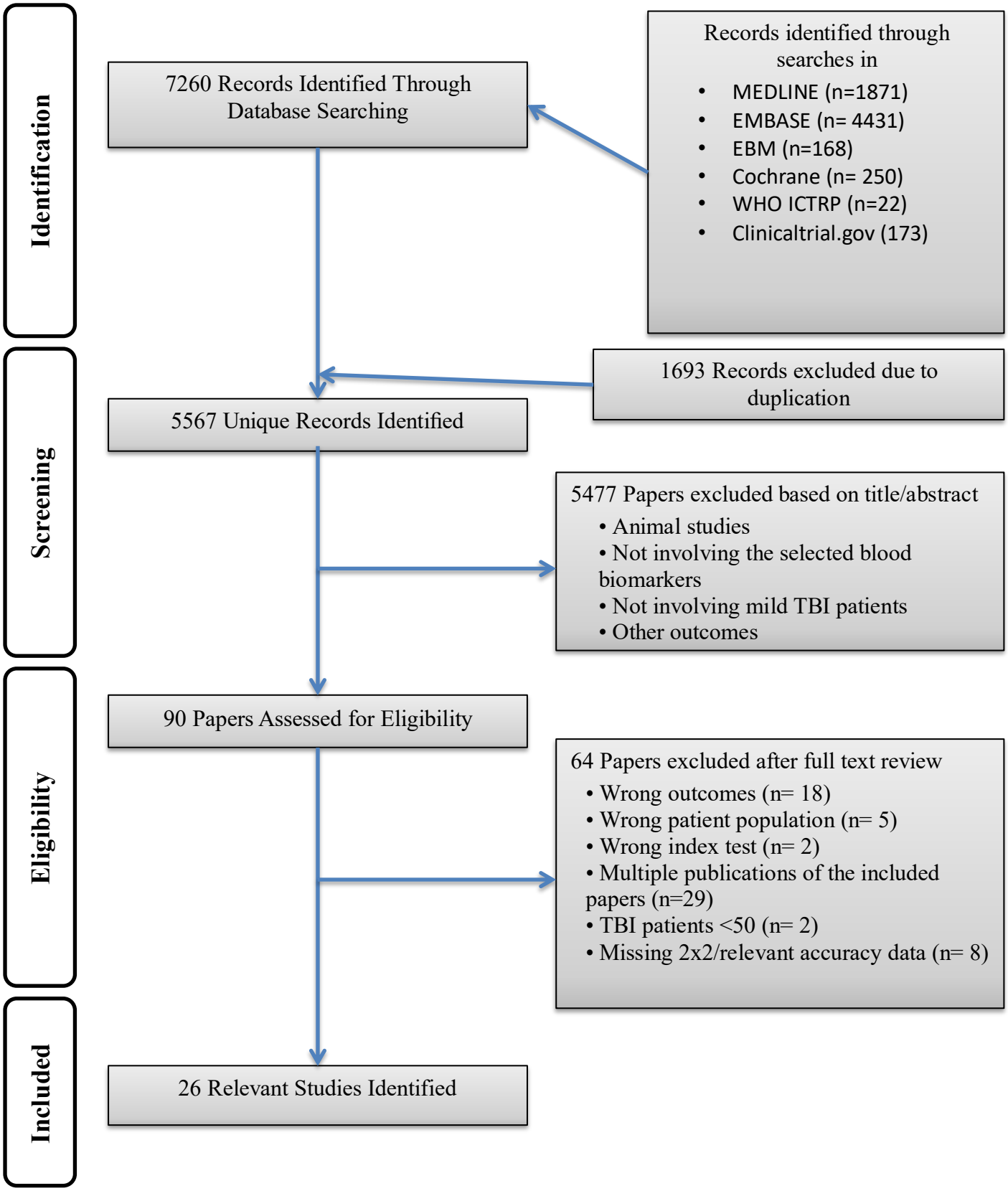


Table 5 Summary of the number and characteristics of primary articles identified for each biomarker

Marker	N of studies	N of participants	N of studies (%) by N of participants in each study		N of studies by GCS		N of studies with pre-defined cut-off	N of studies by sample type	Relevant Results (Range individual sensitivities and specificities)
S-100B	22	7754 (CT+=713; CT-=7041)	**50-100** **101-200** **201-500** **>500**	4 (18) 7 (32) 6 (27) 5 (23)	GCS 15: GCS 14-15: GCS 13-15: GCS 9-15: GCS 3-15:	1 3 15 2 1	16	Serum 21 Plasma 1	Sens 0.83-1.00 Spec 0.12-0.77
GFAP	4	783 (CT+=198; CT-=595)	**101-200** **201-500**	1 (25) 3 (75)	GCS 9-15: GCS 3-15:	3 1	0	Serum 3 Plasma 1	Sens 0.67-1.00 Spec 0.00-0.89
NSE	3	314 (CT+=55; CT-=259)	**50-100** **101-200**	1 (33) 2 (67)	GCS 14-15: GCS 13-15:	1 2	0	Serum 3	Sens 0.56-1.00 Spec 0.07-0.77
UCH-L1	2	347 (CT+=64; CT-=283)	**50-100** **201-500**	1 (50) 1 (50)	GCS 9-15:	2	0	Serum 2	Sens 1.00 Spec 0.21-0.39
Tau	1	50 (CT+=10; CT-=40)	**50-100**	1 (100)	GCS 13-15:	1	0	Serum 1	Sens 0.50 Spec 0.75

Table 6 Characteristics of the 26 included studies

Study ID	BM	No. TBI	GCS	Inclusion criteria	Prevalence of Positive CT Scan findings	Age (years)*	Sex (% female)	Poly trauma/ ECI
Asadollahi 2016 [60]	S100 B	158	13-15	History of isolated MTBI. Age ≥18yrs. Admission within 2h of injury.	50%	35.4 (15.8)	48 (30.4%)	No
Bazarian 2013 [61]	S100 B	787	13-15	GCS >13 measured 30' or more after injury. Patient age ≥ 1yr. Blood drawn within 6 h of injury. CT scan performed as part of the clinical care.	6%	38.2 (19.5) Children & adolescents included	287 (36.5%)	Yes
Biberthaler 2001 [62]	S100 B	52	13-15	History of isolated MHT. GCS 13-15. At least one of the following symptoms: amnesia, LOC, nausea, vomiting, vertigo, or severe headache.	29%	NR	14 (27%)	No
Biberthaler 2006 [19]	S100 B	130 9	13-15	History of isolated head trauma. Admission within 3 h. GCS 13-15 on admission. At least one of the following risk factor: LOC, PTA, nausea, vomiting, severe headache, dizziness, vertigo, intoxication, anticoagulation, age>60 yrs.	7%	Median (IQR) 47 (32-75)	454 (35%)	No
Bouvier 2009 [63]	S100 B	105	13-15	History of isolated head trauma and admission within 3 h. GCS 13-15 on admission. At least one of the following risk factor: LOC, PTA, nausea, vomiting, severe headache, dizziness, vertigo, intoxication, anticoagulation, age>60 yrs.	15%	53 (range 18-94; IQR 37)	40 (38%)	No
Calcagnile 2012 [64]	S100 B	512	14–15	History of head trauma. GCS 14–15 during examination and LOC< 5' and/or amnesia.	5%	42.2	198 (38.5%)	Unclear
Cervellin 2012 [65]	S100 B	60	14–15	History of MHI. GCS 14–15 on admission. Patients with chronic neurologic diseases, but not those with suspected/visible brain tumor.	33%	58 (range 14–80) Adolescents included	18 (32%)	No
Cervellin 2014 [66]	S100 B NSE	68	14–15	History of MHI, GCS 13-15 at admission. age>14 yrs	16%	55 (range 15–86) Adolescents included	24 (35%)	Unclear
Egea-Guerrero 2012 [67]	S100 B	143	15	Patient age ≥ 14yrs. GCS 15 at hospital admission and one or more of the following symptoms: transitory LOC; amnesia; persistent headache; nausea or vomiting; and vertigo.	10.5%	49 (20.6) Including pediatric population >14	54 (37.8%)	Yes

Study ID	BM	No. TBI	GCS	Inclusion criteria	Prevalence of Positive CT Scan findings	Age (years)*	Sex (% female)	Poly trauma/ ECI
Ingebrigtsen 2000 [68]	S100 B	182	13-15	Head injury with brief LOC. GCS 13-15 at admission. Age 15-80 yrs. Admission within 12h post-injury. CT performed within 24h after injury	5%	33 (range 15-78) Adolescents included	71 (39%)	Unclear
Laribi 2014 [69]	S100 B	431	13-15	History of isolated MHI. GCS 13-15 with one or more of the following: amnesia, LOC, nausea, vomiting, vertigo, anticoagulation before injury or severe headache on admission. Patient age ≥ 18yrs, admission within 3 h after injury.	6%	Median (IQR) 36 (24–54)	152 (35%)	No
Ma 2008 [70]	Tau	50	13-15	Patient age ≥ 18yrs. GCS 13-15 at admission. Admission within 12h of injury. CT performed as part of the clinical care. Blunt head trauma followed by LOC and/or PTA.	20%	40.3 (17.7)	12 (24%)	Unclear
McMahon 2015 [71]	GFAP	215	3-15	Admission within 24h of injury. Positive clinical screen for acute TBI necessitating a noncontrast head CT according to ACEP/CDC evidence-based joint practice guidelines.	51%	42.1 (18) (range 16–93)	54 (27%)	Yes
Morochovic 2009 [72]	S100 B	102	13-15	Patients with head injury. GCS 13–15 with or without risk factors	18%	42.0 (19.7) (range 12–84) Including pediatric population	31 (30.39%)	Yes
Muller 2007 [73]	S100 B	236	13-15	History of head injury. LOC or PTA. GCS 13-15 at admission. CT scan within 12h of trauma.	9%	39 (range 18–92)	58 (25.7%)	No
Muller 2011 [74]	S100 B	233	13-15	Adult patients (>16yrs). GCS 13-15.	9%	Median (IQR) 48.4 (24-72) (range 11-97) Adolescents included	90 (39%)	No
Mussack 2002 [75]	S100 B NSE	139	13-15	History of trauma, GCS 13–15, and at least one of the following symptoms: transient LOC (less than 5'), PTA, nausea, vomiting, or vertigo	14%	Median 36.0	33 (24%)	No
Papa 2012a [76]	GFAP	307	9-15	History of blunt head trauma followed by LOC, amnesia, or disorientation. GCS 9-15. Admission to the ED within 4h of injury. Patient age ≥ 18yrs.	30%	39 (15) (range 18–89)	38 (35%)	Unclear

Study ID	BM	No. TBI	GCS	Inclusion criteria	Prevalence of Positive CT Scan findings	Age (years)*	Sex (% female)	Poly trauma/ ECI
Papa 2012b [77]	UCH-L1	96	9-15	History of blunt head trauma followed by LOC, amnesia, or disorientation. GCS 9-15. Admission to the ED within 4h of injury. Patient age ≥ 18yrs.	29%	39 (15) (range 18–89)	36 (38%)	Unclear
Papa 2014 [78]	S100 B GFAP		9-15	History of blunt head trauma followed by LOC, amnesia, or disorientation. GCS 9-15. Admission to the ED within 4h of injury. Patient age ≥ 18yrs.	10%	40 (16)	78 (37%)	Yes
Poli-de-Figueiredo 2006 [79]	S100 B	50	13-15	Isolated MHI. GCS 13-15. At least one of the following symptoms: amnesia, LOC, nausea, vomiting, vertigo, or severe headache.	12%	NR	22 (44%)	No
Romner 2000 [80]	S100 B	278	3-15	Head injury with LOC, blood sample collected within 24 h after injury, and CT performed within 24 h after the injury. LOC was considered to have occurred when the patient had amnesia for the trauma event and if accompanying persons reported LOC.	9%	32 (range 1–84) Children & adolescents included	103 (37%)	Yes
Thaler 2015 [81]	S100 B	782	13-15	MHI (GCS Score 13–15) in patients on medication with PAI with age ≥ 18yrs, and MHI in patients with age ≥ 65yrs independent of PAI intake. Admission within 3h of injury.	6%	Median 83 (range 74–88)	537 (68.7%)	No
Welch 2016 [82]	S100 B GFAP UCH-L1	251	9–15	GCS 9-15 on admission. Patient age ≥ 18y <80yrs. Acceleration or deceleration closed injury to the head Admission within 4 h after injury. ED workup included a head CT scan.	14%	45.6 (18.4) (range 18–80)	100 (39.8%)	Unclear
Wolf 2013 [83]	S100 B NSE	107	13-15	GCS 13-15 at admission. Blunt head trauma. Admission to the ED within 3h of injury.	23%	59 (23) (range 18-97)	47 (44%)	No
Zongo 2012 [84]	S100 B	1560	13-15	Patient age ≥ 15yrs. GCS 13 -15. Admission to the ED within 6h of injury. At least one of the following risk factors: LOC, PTA, repeated vomiting, severe headache, dizziness, vertigo, alcohol intoxication, anticoagulation, and age>65 yrs.	7%	median (IQR) 57 (32- 82) Adolescents included	690 (44.2%)	No

Abbreviations: ACEP/CDC = American College of Emergency Physicians/ Centers for Disease Control and Prevention CT = Computed Tomography; ECI= extracranial injury; ED= emergency department; GCS= Glasgow Coma Scale; LOC = loss of consciousness; MHI = mild head injury; MHT = mild head trauma; MTBI = mild traumatic brain injury; NR = not reported; PAI= platelet aggregation inhibitor; PTA = post-traumatic amnesia; yrs = years.

*Mean (SD) unless stated otherwise.

Table 7 Biomarker Characteristics of the 26 included studies

Study ID	Sampling type	Assay Analyzer & Manufacturer/is	Timing of sample collection*	Assay Range/ CV	Cut-off	BM Levels in TBI patients‡	BM Levels in Patients with CT Positive‡	BM Levels in Patients with CT Negative‡	BM Levels in Controls‡
S-100B									
Asadollahi 2016 [60]	Serum (venous)	ECLIA Elecsys® Roche	Within 3 h post-injury	LOD 0.02µgL range 0.02-30 CV <10%	0.11 µg/L	NR	Mean (95%CI) 0.68 µgL (0.58-0.77)	Mean (95%CI) 0.10 µgL (0.07-0.11)	NA
Bazarian 2013 [61]	Serum (venous)	ECLIA Elecsys® Roche	Within 6 h post-injury	DT 0.005-39µgL	0.10 µg/L†	0.149µg L	0.292µg/L	0.144µg/L	0.071µg/L Negative Control Group
Biberthaler 2001[62]	Serum (venous)	ILMA LIA-mat, Sangtec 100	On admission 116' (18.8)	NR	0.10 µg/L	Mean (SD) 0.470 ng/ml (0.099)	NR	NR	0.05 ng/ml (0.01) Negative Control Group 7.16 ng/ml (3.77) Positive Control Group
Biberthaler 2006 [19]	Serum (venous)	ECLIA Elecsys® Roche	Within 3 h post-injury Median 60' (range 40-80')	LOD 0.005µgL range 0.005-39	0.10 µg/L	0.17 µg/L (0.10-0.37)	0.49 µg/L (0.25-1.46)	0.16 µg/L (0.09-0.33)	0.05 µg/L (0.03-0.06) Negative Control Group 0.45 µg/L (0.19-2.63) Positive Control Group
Bouvier 2009 [63]	Serum (venous)	ECLIA Elecsys® Roche	On admission Median 1h36'	LOD 0.005µgL range 0.005-39	0.10 µg/L†	Mean 0.37 µg/L (SD 0.76)	Mean 0.88 µg/L (SD 1.52)	Mean 0.28µg/L (SD 0.49)	Mean (SD) 0.05 µg/L (0.02) Negative Control Group

Study ID	Sampling type	Assay Analyzer & Manufacturer/is	Timing of sample collection*	Assay Range/ CV	Cut-off	BM Levels in TBI patients‡	BM Levels in Patients with CT Positive‡	BM Levels in Patients with CT Negative‡	BM Levels in Controls‡
Calcagnile 2012 [64]	Serum (venous)	ECLIA Elecsys® Roche	Within 3 h post-injury	LOD 0.005µgL range 0.005-39 Intra-assay CV <2.1%	0.10 µg/L	NR	NR	NR	NA
Cervellin 2012 [65]	Serum (venous)	ILMA LIAISON ® Diasorin	Within 3 h post-injury 62’	LOD 0.02- µg/L range 0.02-30 CV <10%	0.38 µg/L	NR	Geometric mean 1.35 µg/L (95% CI 0.73–1.97)	Geometric mean 0.48 µg/L (95% CI 0.33–0.63)	NA
Cervellin 2014 [66]	Serum (venous)	ILMA LIAISON ® Diasorin	Within 3 h post-injury 62’	LOD 0.02- µg/L range 0.02-30 CV <10%	0.56 µg/L	NR	1.5 µg/L (1.19-2.37)	0.22 µg/L (0.12-0.48)	NA
Egea-Guerrero 2012 [67]	Serum (venous)	ECLIA Elecsys® Roche	Within 6 h post-injury	LOD 0.005µgL range 0.005-39	0.105 µg/L†	Mean (95% CI) 0.392 µg/L (0.327–0.456)	Mean (95% CI) 0.585 µg/L (0.363–0.806) Median 0.350	Mean (95% CI) 0.369 µg/L (0.302–0.436) Median 0.220	NA
Ingebrigtsen 2000 [68]	Serum (venous)	RIA AB Sangtec	On admission 3h (range 0.5-12.0)	LOD 0.2 µg/L	0.2 µg/L	Mean 0.5 µg/L (range 0.2-1.9) Detectable in 69 (38%) pts, non-detectable in 113 (62%)	Mean 0.7 µg/L (range 0.2-1.9) 9 out 10 with detectable level	NR	NA
Laribi 2014 [69]	Serum (venous)	ECLIA Elecsys® Roche	Within 3 h post-injury Median (IQR) 115’ (75–150)	LOD 0.005µgL range 0.005-39 Intra-assay CV 2.1% Inter-assay CV 2.8%	0.10 µg/L	H0 - 0.14 µg/L (0.08–0.25) H+3 - TBI 0.10 µg/L (0.06–0.16)	H0 - 0.24 µg/L (0.15–0.34) H+3 - 0.13 µg/L (0.10–0.25)	H0 - 0.13 µg/L (0.08–0.25) H+3 - 0.10 µg/L (0.06–0.15)	NA

Study ID	Sampling type	Assay Analyzer & Manufacturer/is	Timing of sample collection*	Assay Range/ CV	Cut-off	BM Levels in TBI patients‡	BM Levels in Patients with CT Positive‡	BM Levels in Patients with CT Negative‡	BM Levels in Controls‡
Morochovic 2009 [72]	Serum (venous)	ECLIA Elecsys® Roche	Within 3 h post-injury 1.8h	LLOD 0.005µg/L Inter-assay CV 4.9%	0.10 µg/L	Mean (SD) GCS13 0.26 µg/L (0.34) GCS14 0.43 µg/L (0.56) GCS15 0.85 µg/L (3.11)	NR	NR	NA
Muller 2007 [73]	Serum (venous)	ILMA LIAISON ® Diasorin	Within 12h post-injury	LOD 0.013 µg/L Intra-assay CV<5% Inter-assay CV<10%	0.10 µg/L	Mean (95%CI) GCS13 0.32 µg/L (0.16–0.49) GCS14 0.22 µg/L (0.13–0.30) GCS15 0.18µg/L (0.16–0.21)	Mean (95%CI) 0.36 µg/L (0.21–0.50)	Mean (95%CI) 0.18 µg/L 0.16–0.20	NA
Muller 2011 [74]	Serum (venous)	ECLIA Elecsys® Roche	NR	NR	0.105 µg/L†	NR	NR	NR	NA
Mussack 2002 [75]	Serum (venous)	ILMA LIAISON ® Diasorin	On admission Median 24.3’	LLOD 0.02 ng/mL	0.21 ng/mL	0.24 ng/mL (0.15–0.49)	0.94 ng/mL (0.39–1.43)	0.22 ng/mL (0.14–0.39)	0.06 ng/mL (0.05–0.09) Negative Control Group
Papa 2014 [78]	Serum (venous)	ELISA Banyan	Within 4h post-injury 3.1 h (95% CI 2.8-3.3)	LLOQ 0.083 ng/mL LLOD 0.017 ng/mL	0.020 ng/mL	NR	NR	NR	NR

Study ID	Sampling type	Assay Analyzer & Manufacturer/is	Timing of sample collection*	Assay Range/ CV	Cut-off	BM Levels in TBI patients‡	BM Levels in Patients with CT Positive‡	BM Levels in Patients with CT Negative‡	BM Levels in Controls‡
Poli-de-Figueiredo 2006 [79]	Serum (venous)	ECLIA Elecsys® Roche	On admission Median (IQR) 82' (60-110)	NR	0.10 µg/L	0.29 µg/L (0.14–0.76)	0.75 µg/L (0.66–6.5)	0.26 µg/L (0.12–0.65)	0.04 µg/L (0.03–0.05) Negative Control Group
Romner 2000 [80]	Serum (venous)	RIA Sangtec	Within 24h post-injury 3.8h (range 0.5–24.0)	LOD 0.2 µg/L	0.2 µg/L (LOD)	Mean 0.6 m g/L (range 0.2–6.2) Detectable in 35% MHI	Mean 2.2 µg/L (range 0.2–12.5) Detectable in 23 (92%) mild-severe TBI pts	NR	Non detectable levels Negative Control Group
Thaler 2015 [81]	Serum (venous)	ECLIA Elecsys® Roche	Within 3 h post-injury Median (IQR) 2.05h (1.30–2.30)	DTs 0.005-39µgL	0.105 µg/L	MTBI 0.15µg/L (0.088–0.291) GCS 15 0.139 (0.085–0.267) GCS 14 0.178 (0.102–0.311) GCS 13 0.284 (0.130–0.652)	0.285 µg/L (0.185–0.532)	0.143 µg/L (0.085–0.274)	NA
Welch 2016 [82]	Serum (venous)	ECLIA Cobas 6000® Roche	Within 6h post-injury	NR	0.10 µg/L†	120 (70-230) All values in detectable range	NR	NR	NA
Wolf 2013 [83]	Serum (venous)	ECLIA Elecsys® Roche	Within 3h post-injury	NR	0.105 µg/L†	NR	Mean (SD) 0.7 µg/L (1.19)	Mean (SD) 0.21 µg/L (0.26)	NA
Zongo 2012 [84]	Plasma (venous)	ECLIA Elecsys® Roche	Within 6h post-injury	NR	0.10 µg/L†	0.23 µg/L (0.14–0.38)	0.46 µg/L (0.27-0.72)	0.22 µg/L (0.14-0.36)	NA

Study ID	Sampling type	Assay Analyzer & Manufacturer/is	Timing of sample collection*	Assay Range/ CV	Cut-off	BM Levels in TBI patients‡	BM Levels in Patients with CT Positive‡	BM Levels in Patients with CT Negative‡	BM Levels in Controls‡
GFAP									
McMahon 2015 [71]	Plasma (venous)	ELISA Banyan	Within 24h post-injury	LLOD 0.01ng/mL Intra-assay CV 4.3–7.8% Inter-assay CV 7.8–14.3%	0.6 ng/mL	NR	Mean (SD) 2.86 ng/ml (3.74)	Mean (SD) 0.26 ng/ml (0.41)	NA
Papa 2012a [76]	Serum (venous)	ELISA Banyan	Within 4h post-injury 2.6 h (95% CI 2.4-2.9)	LLOD 0.020 ng/mL Intra-assay CV 4.3-7.8%, Inter-assay CV 7.8-14.3%	0.035 ng/mL	0.316 ng/mL (IQR 0.60) Mean (SD) 0.893 (1.677) (95% CI 0.573 - 1.213)	NR	NR	0.010 ng/mL (0.050) Negative Control Group 0.216 ng/mL (0.275) Orthopedic control group 0.122 ng/mL (0.373) MVA control group 0.010 ng/mL (0.060) All controls
Papa 2014 [78]	Serum (venous)	ELISA Banyan	Within 4h post-injury 3.1 h (95% CI 2.8-3.3)	LLOQ 0.030ng/mL ULOQ 50.000ng/mL LLOD 0.008ng/mL	0.067 ng/mL	NR	NR	NR	NR

Study ID	Sampling type	Assay Analyzer & Manufacturer/is	Timing of sample collection*	Assay Range/ CV	Cut-off	BM Levels in TBI patients‡	BM Levels in Patients with CT Positive‡	BM Levels in Patients with CT Negative‡	BM Levels in Controls‡
Welch 2016 [82]	Serum (venous)	ELISA Banyan	Within 6h post-injury	NR	0 pg/mL	10.3 pg/mL (3.5, 37.4) 45 pts below LOD (4 with CT+)	NR	NR	NA
NSE									
Cervellin 2014 [66]	Serum (venous)	IFMA Kryptor (BRAHMS AG)	Within 3 h post-injury 62’	LOD 0.08µgL CV <6%	9.0 µg/L	NR	13.3 µg/L (12.1-20.3)	9.6 µg/L (8.2-12.3)	NA
Mussack 2002 [75]	Serum (venous)	ECLIA Elecsys® Roche	On admission Median 24.3’	LLOD 0.01 ng/mL	12.28 ng/mL	17.50 ng/mL (14.40–21.34)	18.43 ng/mL (15.31–26.03)	17.46 ng/mL (14.31–20.77)	15.55 ng/mL (14.90–17.00) Negative Control Group
Wolf 2013 [83]	Serum (venous)	ECLIA Elecsys® Roche	Within 3h post-injury	NR	14.7 µg/L†	Missing values in 47 pts (44%)	Mean (SD) 18.1 µg/L (10.84)	Mean (SD) 12.4 µg/L (4.82)	NA
UCH-L1									
Papa 2012b [77]	Serum (venous)	ELISA Banyan	Within 4h post-injury 2.7 h (95% CI 2.4-2.9)	LLOD 0.030 ng/mL	0.029 ng/mL	Mean (SEM) 0.955ng/mL (0.248) (range 0.015–19.25)	Mean (SEM) 1.618 ng/mL (0.474)	Mean (SEM) 0.620 ng/mL (0.254)	Mean (SEM) 0.083 ng/mL (0.005) (range 0.015–0.490) All controls (Negative, Orthopedic, MVA controls)

Study ID	Sampling type	Assay Analyzer & Manufacturer/is	Timing of sample collection*	Assay Range/ CV	Cut-off	BM Levels in TBI patients‡	BM Levels in Patients with CT Positive‡	BM Levels in Patients with CT Negative‡	BM Levels in Controls‡
Welch 2016 [82]	Serum (venous)	ELISA Banyan	Within 6h post-injury	NR	40 pg/mL	65.8 (39.6, 125.2) 2 pts below LOD (none with CT+)	NR	NR	NA
Tau									
Ma 2008 [70]	Serum (venous)	ELISA	On admission 5.0 h (2.8)	LOD 1.5 ng/mL	NR	Mean (SD) 5.0 ng/mL (2.98) 15 patients with detectable levels	NR	NR	NA

Abbreviations: BM = Biomarker**;** CV = coefficient of variation; ECLIA = electrochemiluminescence immunoassay; ELISA = enzyme-linked immunosorbent assay; H0 = within 3 h after the clinical event; H3 = 3 h after the first sampling; IFMA = immunofluorometric assay; ILMA= Immunoluminometric assay; LIA = luminescence immunoassay; LLOD = lower limit of detection; LLOQ = lower limit of quantification; LOD = limit of detection; MVA = motor vehicle accident; NA= Not Applicable; NR= Not reported; pts= Patients; RIA = radioimmunoassay; SEM = standard error of the mean; ULOQ = upper limit of quantification; yrs = years.

*Mean (SD) unless stated otherwise.

† Additional thresholds have been evaluated.

‡Median (IQR) unless stated otherwise.

[a] Control Group Definition:

- Negative Control Group=Healthy individuals (e.g. healthy volunteers, voluntary blood donators, outpatients for routine blood work) who were checked on their health and potential head trauma status. Positive control group—
- Positive Control Group = Patients with moderate to severe head injury.
- Orthopedic Control Group = non–head-injured patients presenting to the ED with either a single-limb orthopedic injury
- MVA Control Group patients presenting to the ED after a motor vehicle crash without blunt head trauma

2.2. Methodological quality

The assessments of the methodological quality and risk of bias of the included studies are presented in **Figure 8**. Participants neither consecutively nor randomly enrolled, the use of vague definitions of mild TBI or inclusion of a non-representative spectrum of patients (pediatric population or patients with GCS<13) may lead to incorporation bias, thus limiting the conclusions that can be drawn by affecting the accuracy estimates and compromising the applicability of the results.

In half of the studies, thresholds were not pre-specified and ROC analyses were used to determine optimal cut-offs, likely resulting in an overestimation of the diagnostic accuracy of the biomarker evaluated. In addition, the inclusion of skull fracture as a CT abnormality may cause inflation of the accuracy estimates of S100B, whereas, using a brain-specific marker as an index test may result in patients with skull fractures being misclassified as false negative. Finally, in different domains, a substantial number of studies were considered to be at unclear risk of bias due to substandard reporting. We investigated the effect of these factors in sensitivity and subgroup analyses.

2.3. S100 calcium binding protein B (S100 β)

The accuracy of S100B for detecting intracranial lesions on CT scan was evaluated in 22 studies (7754 patients) (19,60-69,72-75,78-84). The individual sensitivities and the specificities were between 72% and 100% and between 5% and 77%, respectively (**Figure 9**). All but six of the included studies used the same cut-off (0.10-0.11μg/L), which represents the 95th percentile of a healthy reference population and is conventionally considered to discriminate between physiological from pathophysiological serum concentrations (19). Seven studies reported multiple cut-offs (**Table 7**). The summary ROC curve showing the accuracy of

S100B across all the studies, regardless the threshold used, is presented in **Figure 10.**

In terms of the assays/platforms used, most of the studies (13/22) used an automated electrochemiluminescence immunoassay (ECLIA) on an Elecsys® analyzer (Roche Diagnostics) while one used the Cobas 6000 analyzer (Roche Diagnostics). There were four studies conducted using an automated immunoluminometric assay (ILMA) on a Liaison® analyzer (Diasorin), and one on LIA®-mat (Sangtec® 100); one study used a radioimmunoassay (Sangtec), and one an ELISA platform (Banyan Biomarkers, Inc.) (**Table 7**). In one study, the analytical performance of the two automated immunoassays (i.e. Diasorin and Roche Diagnostics assays) was compared and, though, not interchangeable, the two methods strongly correlated and appeared usable in a similar manner (69).

2.3.1. Performance of S100 β at 0.10 – 0.11 µg/L cut-off value

To obtain clinically relevant estimates of the performance of S100B, we pooled the results from the 16 studies using the cut-off value of 0.10-0.11µg/L. The individual sensitivities and the specificities for each study included in this meta-analysis were between 72% and 100% and between 5% and 77%, respectively (**Figure 11**). The following summary estimates were obtained: sensitivity 96% (95% CI 92% to 98%), specificity 31% (95% CI 27% to 36%), positive likelihood ratio 1.4 (1.3 to 1.5) and negative likelihood ratio 0.12 (0.06 to 0.25). **Figure 11** shows the pooled sensitivity and specificity (the solid red spot in the middle) and the 95% confidence and prediction regions (the inner and outer ellipses, respectively).

There was a significant level of heterogeneity in the results, greater for specificity than for sensitivity (**Figure 11**). The value for sensitivity was over 80% in all the studies but one (83). The value for specificity was mainly over 30%, though, in the

remaining studies the low specificity was accompanied by a very high sensitivity. However, due to important variation across studies with simultaneous presence of factors (time, presence of extracranial injuries, mixed populations) with potentially contrasting effects on the accuracy estimates and lack of individual data and/or insufficient number of studies, we were unable to compare patient characteristics and investigate the effect of the planned sources of heterogeneity. Poor reporting of patient and study information also contributed to unknown sources of heterogeneity.

One study was an outlier (Zongo et al, 2012) (84). Exclusion of this study made no change in sensitivity (96.3% vs 96.1%) but specificity increased from 31% to 33%. This could be explained by the fact that in this study including the greatest number of patients S100B levels were measured in plasma, thus increasing the probability of false positive results.

To explore the effect of risk of bias in the patient selection domain on the summary estimates, we excluded eight studies considered at high (n = 1) or unclear (n = 7) risk of bias. The exclusion of these studies slightly improved sensitivity (98%). A sensitivity analysis was also undertaken to assess the impact of studies containing mixed populations on our findings. We excluded one study (Welch 2016) (82), because the authors included patients with moderate TBI (GCS 9-12). There was no impact on our findings. Four studies enrolled a mixed pediatric and adult population. Exclusion of these studies as well as those where this information was unclearly reported made no difference to our results.

The prevalence of CT findings was relatively high (>11%) in seven studies. Excluding these studies resulted in a slight increase in sensitivity and a slight decrease in specificity (98% and 29% respectively). Finally, eight studies

considered skull fracture as a CT abnormality. To explore the impact of the type of reference standard on the summary estimates, we excluded these studies as well as those where this information was unclearly reported. The exclusion of these studies slightly impacted sensitivity and specificity (93% and 35%, respectively).

2.3.2. Quality of evidence of S100 β

The quality of the evidence for the use of blood S100B levels to diagnose brain injury as assessed by CT scan in patients with mild TBI was moderate (**Figure 12**).

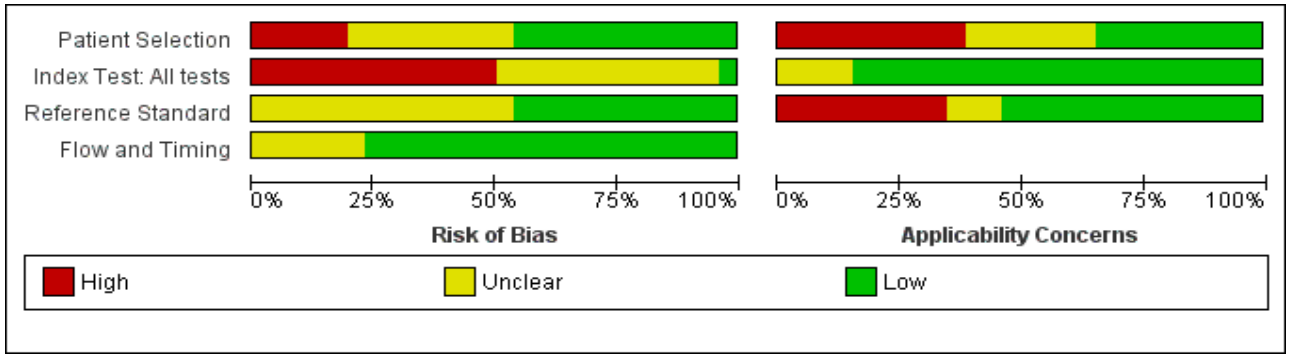
2.4. Glial Fibrillary Acidic Protein

Eligible studies reporting the accuracy of GFAP for detecting intracranial lesions on CT scan comprised 3 cohorts with mild to moderate TBI patients and one cohort with mild to severe TBI patients (783 patients) (**Figures 8 and 9**) (71,76,78,82). All studies were recent publications (2012 to 2016).

The individual sensitivities were between 67% and 100% while the specificities were between 0% and 89%. Sensitivities were sufficiently homogenous while specificities were clearly heterogeneous. The thresholds used, ranging from 0 ng/ml (82) to 0.6ng/ml (71), were not pre-specified and were determined from ROC analyses. The summary ROC curve of the accuracy of GFAP across all four studies, regardless of the threshold used, is shown in **Figure 9**.

The planned comparison between S100B and GFAP diagnostic performance was not possible due to the limited number of studies and different spectrum of patients available for GFAP.

A)



B)

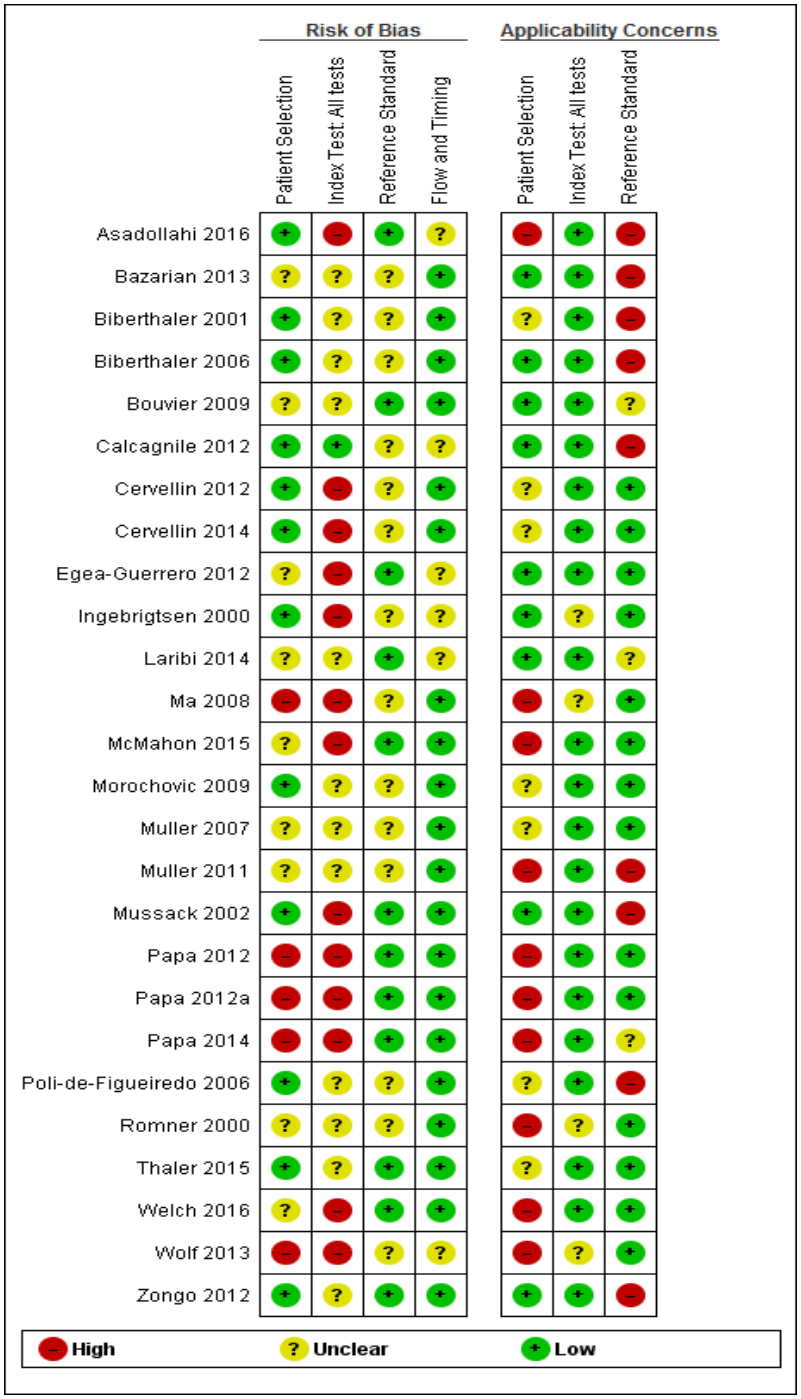


Figure 8

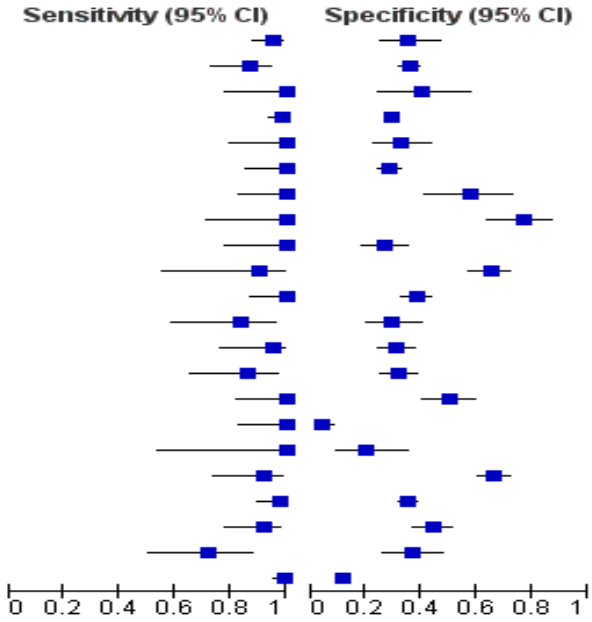
A) Risk of bias and applicability concerns graph: review authors' judgments about each domain presented as percentages across included studies.

B) Risk of bias and applicability concerns summary: review authors' judgments about each domain for each included study.

Figure 9 Forest plot of blood-based protein biomarkers for detection of CT abnormalities

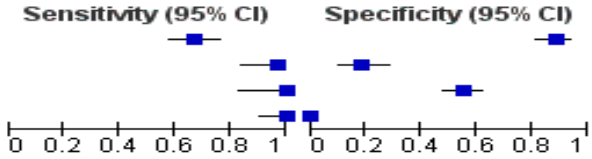
S100B

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Asadollahi 2016	75	51	4	28	0.95 [0.88, 0.99]	0.35 [0.25, 0.47]
Bazarian 2013	39	473	6	264	0.87 [0.73, 0.95]	0.36 [0.32, 0.39]
Biberthaler 2001	15	22	0	15	1.00 [0.78, 1.00]	0.41 [0.25, 0.58]
Biberthaler 2006	92	855	1	361	0.99 [0.94, 1.00]	0.30 [0.27, 0.32]
Bouvier 2009	16	60	0	29	1.00 [0.79, 1.00]	0.33 [0.23, 0.43]
Calcagnile 2012	24	350	0	138	1.00 [0.86, 1.00]	0.28 [0.24, 0.33]
Cervellin 2012	20	17	0	23	1.00 [0.83, 1.00]	0.57 [0.41, 0.73]
Cervellin 2014	11	13	0	44	1.00 [0.72, 1.00]	0.77 [0.64, 0.87]
Egea-Guerrero 2012	15	94	0	34	1.00 [0.78, 1.00]	0.27 [0.19, 0.35]
Ingebrigtsen 2000	9	60	1	112	0.90 [0.55, 1.00]	0.65 [0.57, 0.72]
Laribi 2014	26	231	0	143	1.00 [0.87, 1.00]	0.38 [0.33, 0.43]
Morochovic 2009	15	59	3	25	0.83 [0.59, 0.96]	0.30 [0.20, 0.41]
Muller 2007	20	141	1	64	0.95 [0.76, 1.00]	0.31 [0.25, 0.38]
Muller 2011	19	144	3	67	0.86 [0.65, 0.97]	0.32 [0.26, 0.38]
Mussack 2002	19	60	0	60	1.00 [0.82, 1.00]	0.50 [0.41, 0.59]
Papa 2014	20	180	0	9	1.00 [0.83, 1.00]	0.05 [0.02, 0.09]
Poli-de-Figueiredo 2006	6	35	0	9	1.00 [0.54, 1.00]	0.20 [0.10, 0.35]
Romner 2000	23	85	2	168	0.92 [0.74, 0.99]	0.66 [0.60, 0.72]
Thaler 2015	49	474	1	258	0.98 [0.89, 1.00]	0.35 [0.32, 0.39]
Welch 2016	33	120	3	95	0.92 [0.78, 0.98]	0.44 [0.37, 0.51]
Wolf 2013	18	52	7	30	0.72 [0.51, 0.88]	0.37 [0.26, 0.48]
Zongo 2012	110	1272	1	177	0.99 [0.95, 1.00]	0.12 [0.11, 0.14]



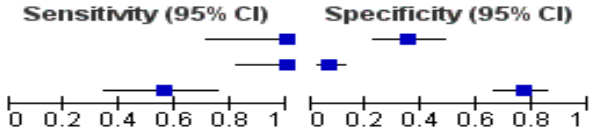
GFAP

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
McMahon 2015	74	12	36	93	0.67 [0.58, 0.76]	0.89 [0.81, 0.94]
Papa 2012	31	62	1	14	0.97 [0.84, 1.00]	0.18 [0.10, 0.29]
Papa 2014	20	85	0	104	1.00 [0.83, 1.00]	0.55 [0.48, 0.62]
Welch 2016	36	215	0	0	1.00 [0.90, 1.00]	0.00 [0.00, 0.02]



NSE

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Cervellin 2014	11	37	0	20	1.00 [0.72, 1.00]	0.35 [0.23, 0.49]
Mussack 2002	19	112	0	8	1.00 [0.82, 1.00]	0.07 [0.03, 0.13]
Wolf 2013	14	19	11	63	0.56 [0.35, 0.76]	0.77 [0.66, 0.85]



UCH-L1

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Papa 2012a	28	54	0	14	1.00 [0.88, 1.00]	0.21 [0.12, 0.32]
Welch 2016	36	131	0	84	1.00 [0.90, 1.00]	0.39 [0.33, 0.46]

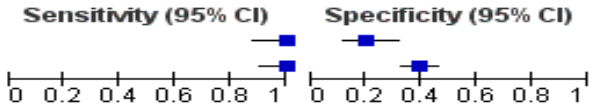
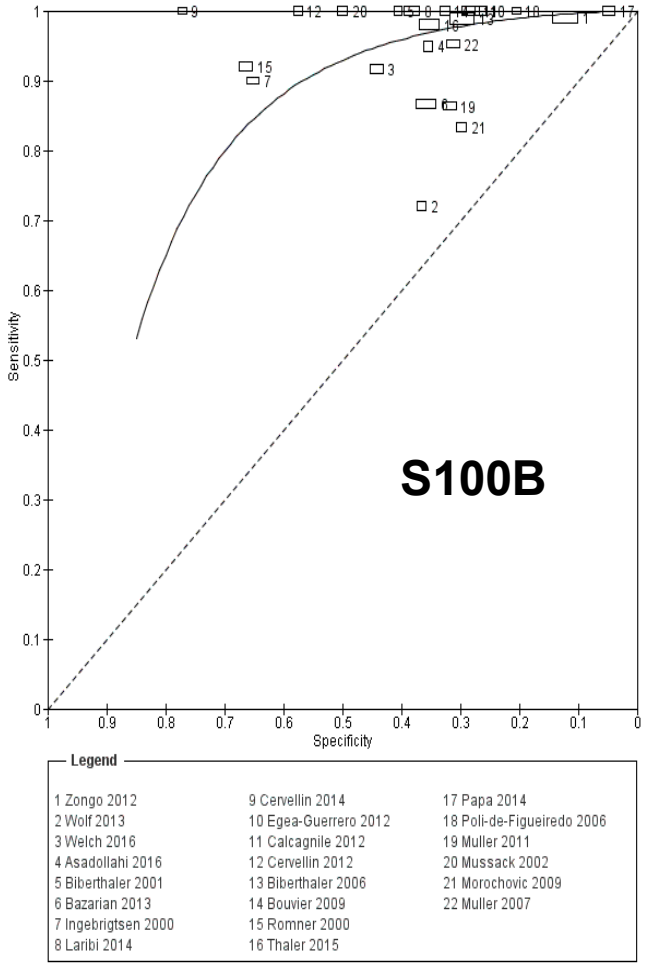


Figure 10 (A, B) Summary ROC plots for S100B and GFAP for detection of CT abnormalities
The width of the symbol is proportional to the number of patients (sample size) in each study. The HSROC model was used to estimate a summary curve using Proc NLMIXED in SAS.

A



B

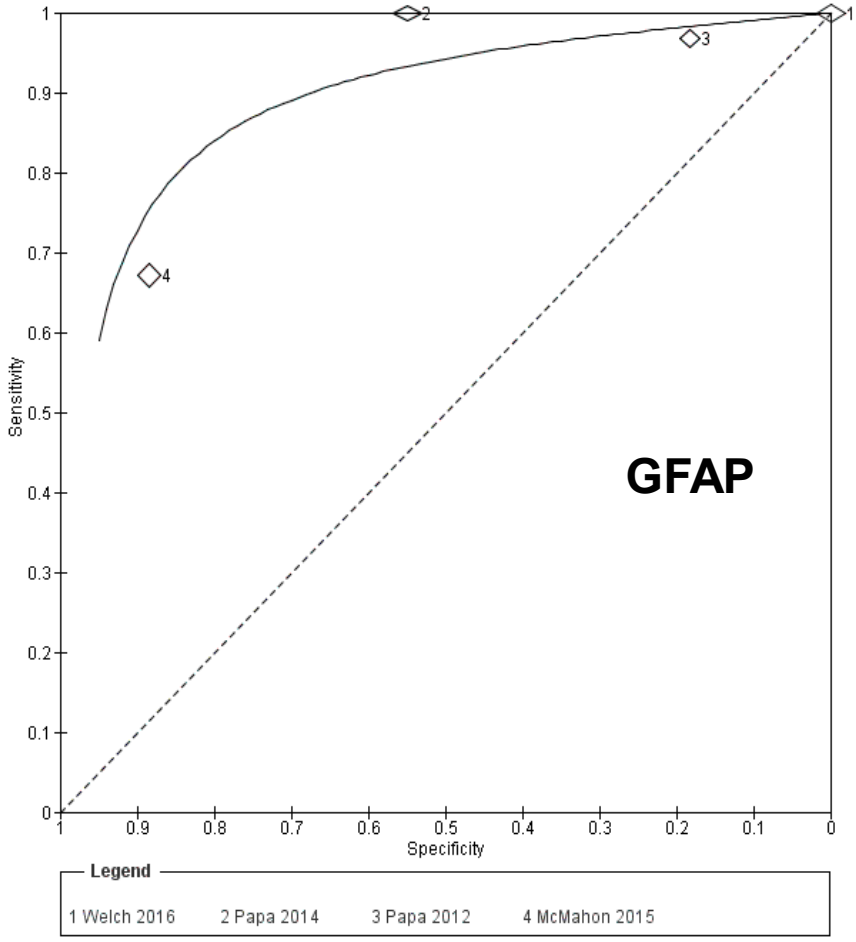
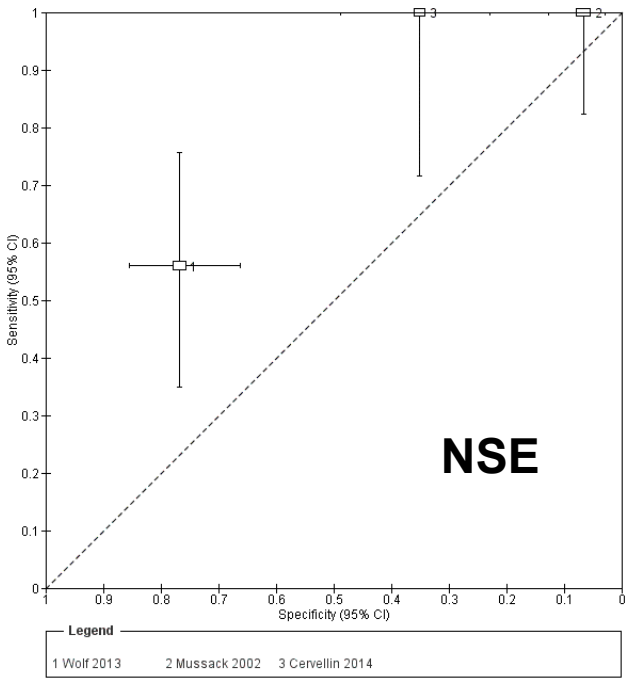


Figure 10 (C, D) Study estimates of sensitivity and specificity with 95% confidence intervals plotted in ROC space for NSE and UCH-L1 for detection of CT abnormalities

The width of the symbol is proportional to the number of patients (sample size) in each study. The HSROC model was used to estimate a summary curve using Proc NLMIXED in SAS.

C



D

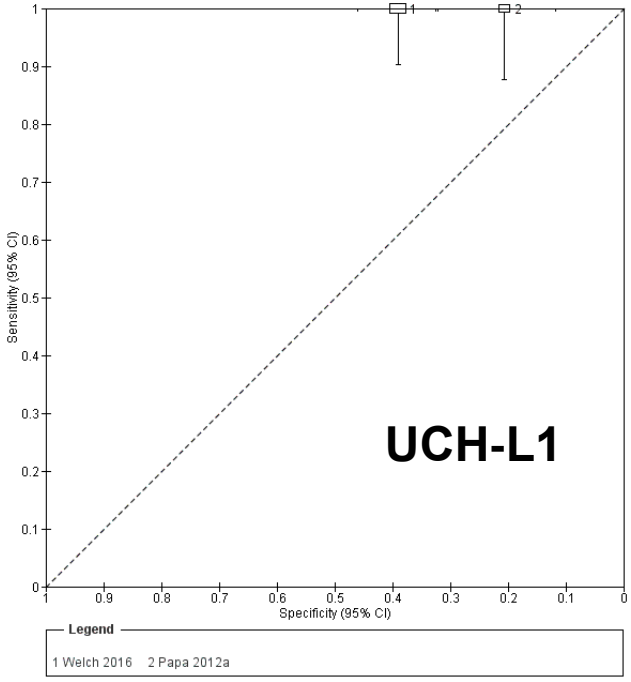
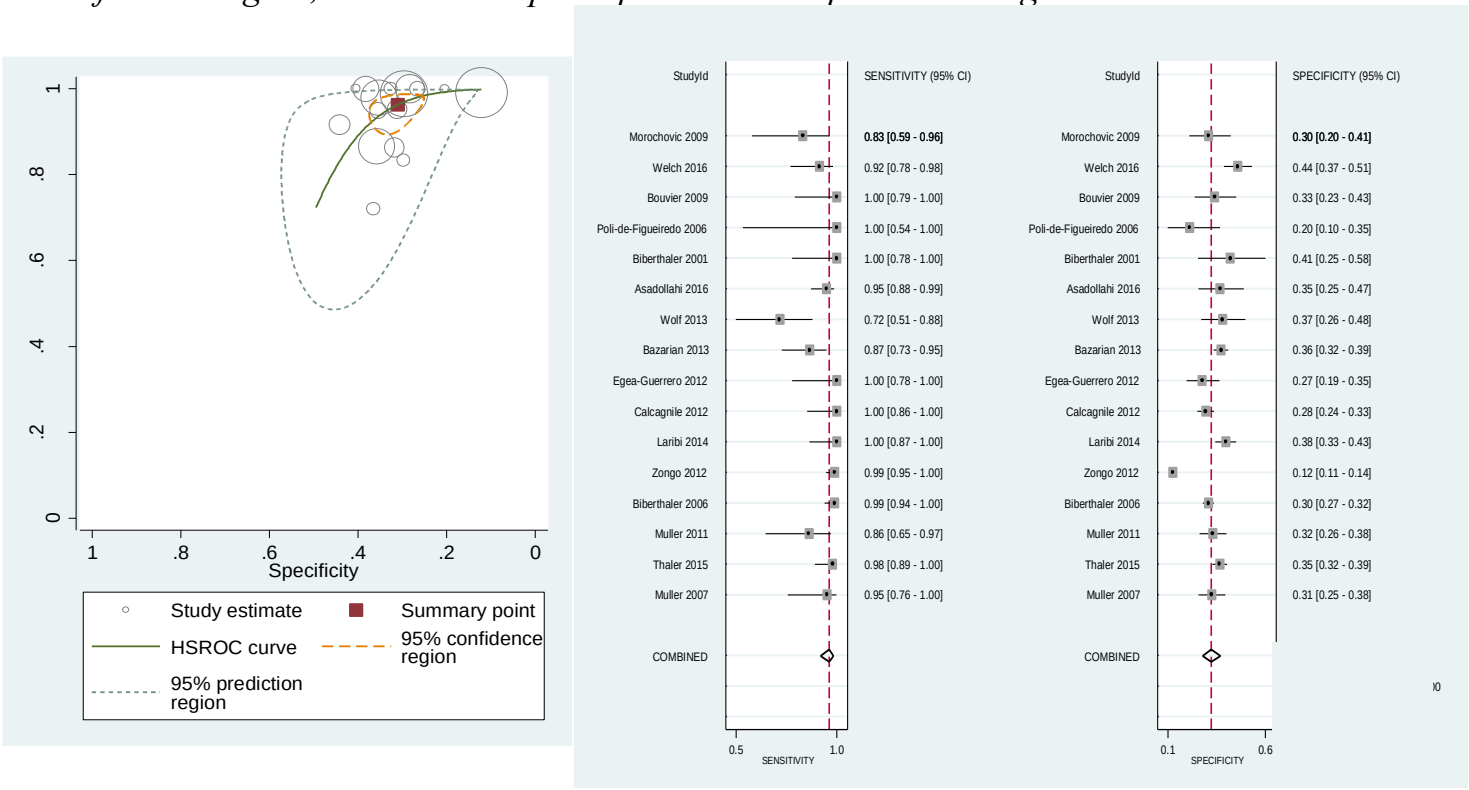


Figure 11 Summary receiver operating characteristics plot of sensitivity and specificity of S100B at 0.1-0.105 µg/L cut-off value. *Each circle represents an individual study; size of symbol reflects number of patients in the studies; solid spot in middle is summary sensitivity and specificity; inner ellipse represents 95% confidence region, and outer ellipse represents 95% prediction region*



Alternative figure with No individual study reported & Likelihood ratio scattergram

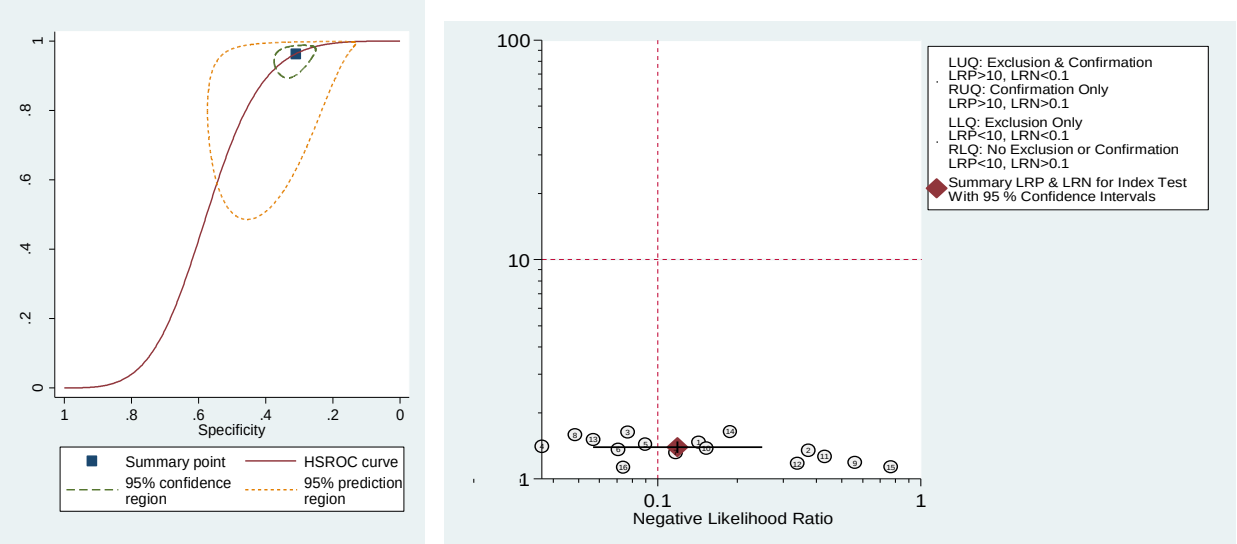
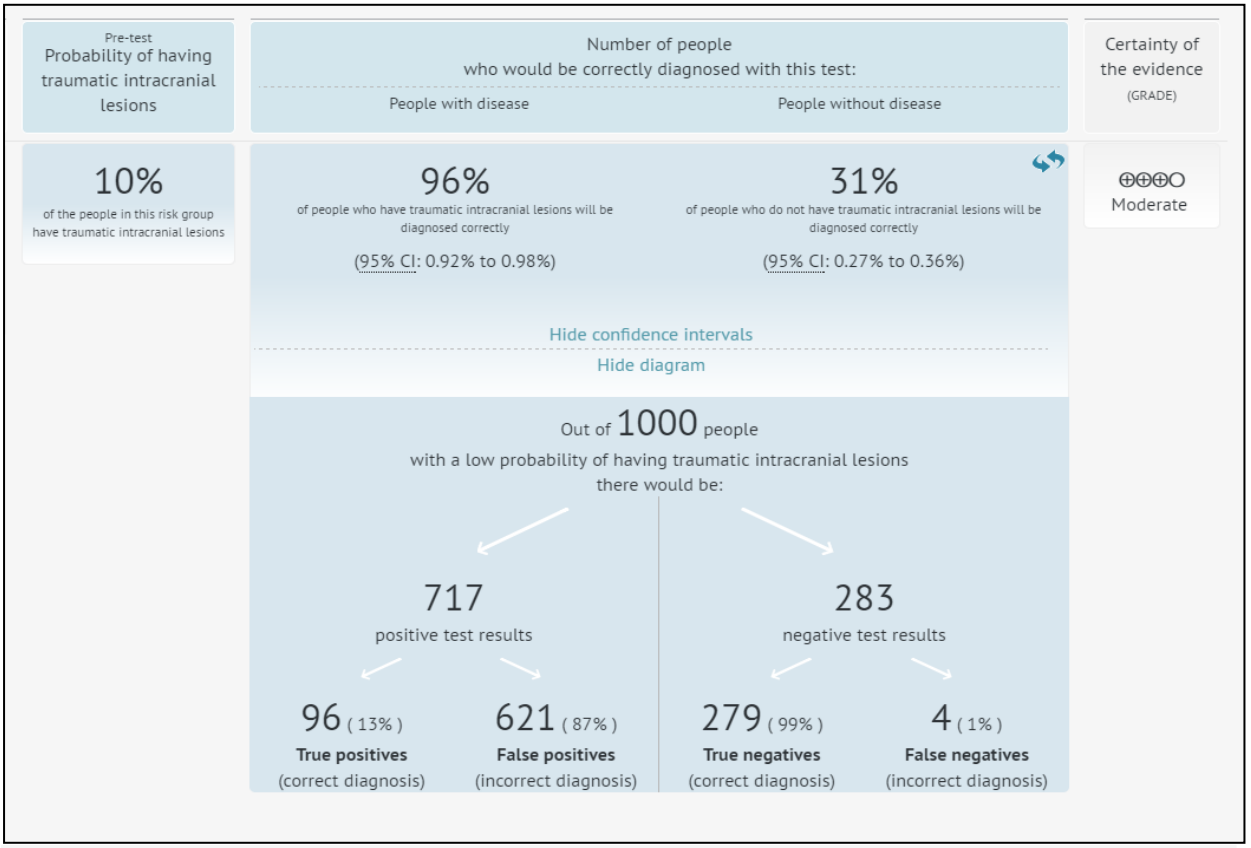
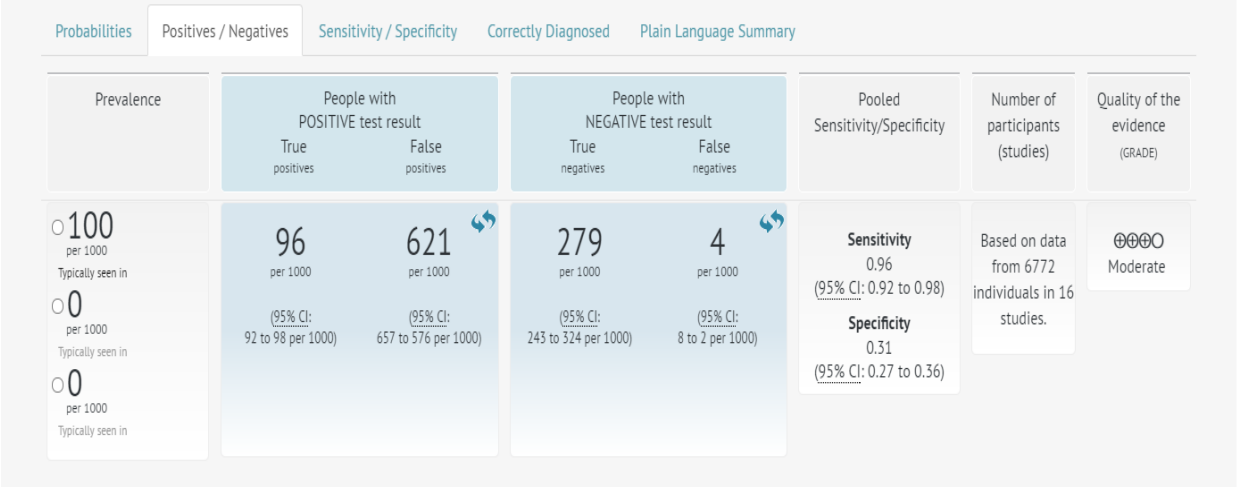


Figure 12 Summary of evidence for the use of blood S100B protein concentrations (0.1-0.105 μg/L cut-off) to diagnose brain injury as assessed by CT scan in patients with mild TBI.





2.5. Neuron Specific Enolase

The accuracy of NSE for discriminating between TBI patients with intracranial lesions on CT scanning from those without lesions was evaluated in 3 studies (314 patients) (75,83). **Figure 8** shows a forest plot of the individual study estimates of sensitivity and specificity. The sensitivities were between 56% and 100% while the specificities were between 7% and 77%. The studies reported a considerable variation in the threshold adopted, ranging from 9-14.7 μg/L (**Table 7**).

2.6. Ubiquitin C-terminal hydrolase-L1

The accuracy of the initial circulating UCH-L1 levels for detection of intracranial lesion on CT was evaluated in two very recent studies (96 and 251 patients respectively) (77,82) including both mild to moderate adult TBI patients (GCS 9-15). The 2 studies yielded the same sensitivity of 100% (95% CI 88 to 100) and specificities of 21% (95% CI 12 to 32) and 39% (95% CI 33 to 46) (**Figure 8**). They reported similar thresholds (0.029 to 0.04ng/ml) and used the same assay (**Table 7**).

2.7. Tau

The accuracy of circulating tau (cleaved tau [C-tau]) for diagnosis of CT abnormalities was evaluated only in one small study (50 patients) (70). The sensitivity was 50% while the specificity was 75%. Among the 10 patients with abnormal findings on CT enrolled in this study, 5 (50%) had no detectable C-tau levels.

3. Discussion

In this systematic review, we have provided a comprehensive and thorough examination of the literature on protein biomarker diagnostic signatures for traumatic brain lesions to define how to best take advantage of these tests in ED daily patient care. We found that of the six biomarkers explored, current evidence only supports the measurement of S100B to help informed decision-making in patients presenting to the ED with suspected intracranial lesion following mild TBI, possibly reducing resource use. There is as yet insufficient evidence that GFAP, NSE and UCH-L1 are ready for clinical application, despite their unequivocal association with TBI. Furthermore, tau and neurofilament proteins were analyzed in too few studies to draw any meaningful conclusions. Importantly, serious problems were observed in many of the studies, ranging from unfocused design and inappropriate target groups to biased reporting and inadequate analysis. These points are further elaborated in the discussions below.

S100B

Our findings demonstrate the clinical utility of S100B for the intended use of allowing physicians to be more selective in their use of CT without compromising care of patients with mild TBI. More specifically, the 16 studies applying the same pre-specified cutoff of 0.10-0.11μg/L yielded a pooled sensitivity of 96% (95% CI 92%–98%) and specificities of 31% (95% CI 27%–36%). Assuming a pre-test probability of 10% (85), would mean that, overall, 100 of 1000 tested patients will have a final diagnosis of intracranial lesion. The pooled results obtained for sensitivity and specificity would mean that, of these, between 92 and 98 will test positive (true positives) and 2 to 8 will test negative (false negatives). Of the 900 with negative CT, between 243 and 324 will test negative (true negatives) and between 576 and 657 will test positive (false positives) (**Figure 12**).

Even though this high sensitivity and excellent negative predictive value looks promising, information regarding which lesions could be missed and the associated consequences - if left untreated - is particularly relevant to the broad acceptance and adoption of S100B by the medical community. Accordingly, there is an ongoing debate about the risk of sending home a misdiagnosed patient with a potentially life-threatening condition such as an epidural hemorrhage. From the available data (19,61,72,74,81,84), we were unable to identify specific types of injury that were systematically missed, albeit, subdural hematomas were slightly more frequently misclassified as false-negatives. We speculate that this may be due to the brain lesion location and/or extension as well as the pathoanotomic and neurovascular features of the different injuries that cause an altered or delayed leakage of S100B into the circulation. Importantly, one study (72) demonstrated that lesions requiring surgery (Subdural hematoma and 1 epidural hematoma) were missed by S100 B, thereby indicating that this marker – if used alone as a diagnostic tool – is not completely reliable. Given that distinct patterns of injury are linked to patient-specific variability, efforts must be made to develop advanced multiparameter-based solutions integrating marker signature and patient features. Such multimodal prediction models could be more suitable for an accurate diagnosis, characterization of injury types and risk stratification of MTBI patients (86).

It will be also critical to estimate the independent and complementary value of biomarkers and determine whether this strategy provides added diagnostic utility when combined with a careful clinical assessment or when integrated into existing clinical decision rules for the selective use of CT, such as the CT in Head Injury Patients (CHIP) model (87), the New Orleans criteria (20), or the Canadian Head CT rule (88). Unless a biomarker-based approach yields an incremental diagnostic

value and clearly demonstrates its superiority over standard, readily available patient characteristics, the broad acceptance in medical practice is unlikely (89).

Reliability and reproducibility of S100B results also requires a critical consideration of the comparability and potential variability in biomarker measurements when using assays from different manufacturers. We found the adoption of a relatively uniform and standardized approach for S100B determination, with fourteen studies using the ECLIA Elecsys® Roche and 2 studies using the ILMA LIA-mat Sangtec 100. These 2 automated immunometric assays have been demonstrated to have a good correlation, with almost identical diagnostic capability (69), therefore, excluding that this factor could have influenced our conclusions. A comparable level of consistency in analytical methods and assays used is not available for any of the other biomarkers considered in this review.

Our review showed that the results across S100B studies using the pre-specified cut-off were consistent in terms of sensitivities and specificities, with only one outlier showing an exceptionally low specificity (12%) (84). A plausible explanation for this anomaly is that in this study plasma samples were used to measure S100B. This interpretation fits well with evidence from previous literature demonstrating how the interference of the anticoagulant on the immunoreactivity for S100B can alter its levels relative to serum (values higher by ~20%) (90). Consequently, in the study of Zongo and colleagues the use of the pre-specified cut-off for serum has inevitably resulted in a systematic increase of false positive results (84). This observation, while complicating the analysis of S100B blood levels, points to the need for a more exhaustive knowledge and understanding of pre-analytical factors as potential confounders and sources of variability, and supports the adoption of different cut-off values depending on the sample type

used. Intriguingly, this observation suggests that plasma could be more suitable and possibly desirable for measuring S100B levels in mild TBI patients owing to the very low concentrations in this population. However, even after removing the outlier, a considerable heterogeneity remained, necessitating caution when interpreting analysis results.

Investigations from multiple research groups provided evidence that a series of factors other than the brain injury may influence levels of biomarkers in the circulation and, therefore, the diagnostic accuracies. Such factors encompass biomarker characteristics such as molecular weight; injury-specific release mechanisms and clearance, (91,92) patient features including presence of extracranial injuries or polytrauma, intoxication, location of the injury, and even genetic, pre-analytical and laboratory-dependent procedures including all steps from management of equipment to execution of assays manufacturing processes; and post-analytical data handling (61,93-95).

We were not able, though, to systematically investigate these potential sources of heterogeneity due to a substantial variation across studies, the suboptimal reporting of patient and study information and the coexistence in the same study of factors with contrasting or controversial effects on the accuracy estimates. Taken together, these findings demonstrate that future research must be refined by improvements in study design as well as standards and characterization of patient selection.

In this regard, surprisingly, we noted that to date no attempt has been made to specifically investigate the effect of comorbidities and sex on the diagnostic performance of S100B or any other marker. Sex is recognized as a primary determinant of biologic variability, responsible for anatomical, neurochemical and functional brain connectivity differences, heavily influencing neurobiological and

neuropathophysiological response (93). It is also associated with important differences in hormones, metabolism, and immunological system, which in turn may interfere with the determination of circulating TBI biomarker (94). Factoring sex into research designs and analyses is a theme of active debate and is considered fundamental to rigorous and relevant biomedical research. Hence, we emphasize that this is a critical knowledge gap for future investigation, especially in light of the mounting evidence of the changing gender pattern due to the shift in the TBI population towards older age, also at risk of multiple comorbid conditions (see Thaler et. al) (81). Systematic reviews and meta-analyses of individual participant data (IPD) may represent a powerful approach to overcome some of these gaps and limitations (98), also supported by the current initiatives to share clinical data and establishment of common repositories, such as the Federal Interagency Traumatic Brain Injury Research (FITBIR) database (https://fitbir.nih.gov/) (99).

Clinical application of S100B implies that choosing the right assessment time point (time between injury and sampling) (100) is an integral part of the test. Based on the results of S100B kinetics studies, guidelines have specifically indicated a time window within 3 (25,101) to 6 (25) hours post-injury for S100B to detect intracranial lesions. A recent study supported a 3-hour window for safe rule-out of acute intracranial lesion in clinical practice showing that a second blood sampling 3 hours after the first one is not informative and resulted in a non-trivial loss of sensitivity of about 6% (e.g. eight patients with positive CT would have been missed) (69). We were unable to further address this specific issue in this review because of the heterogeneity in study design. Besides post-injury delays in sampling, the delay from obtaining samples to processing and analysis, and the storage conditions during this delay could both be important modulators of S100B stability and assay results. Age, gender and comorbidities or their combination can

also importantly affect kinetics of S100B (102). Future studies should inform whether these variables should be considered and what the potential influence on biomarker results and interpretation is.

The results of our study expand and corroborate those from previous systematic reviews and meta-analyses (103,105) and confirm that the implementation of S100B might allow a reduction of the number of CT scans by approximately 30% (19). These considerations also have broad financial implications for healthcare costs. However, none of the studies in our review explored the cost effectiveness of the use of biomarkers, and the few economic studies and data in the literature are controversial.

An earlier study by Ruan et al. (106) reported a limited effect of S100B on health care resources and a potential economic impact only in specific clinical scenarios (i.e. CT scanning rate >78% or a faster turnaround time of biomarker results of at least 96 minutes compared with CT scan results).

Conversely, in a more recent cost analysis conducted in a Swedish regional hospital, the clinical use of S100B incorporated into the Scandinavian guidelines substantially reduced healthcare costs, especially in cases of strict adherence to management recommendations (71€ per patient) (107). These results are not generalizable, and must be carefully interpreted according to their specific contexts, because of the differences across countries, healthcare systems, hospital settings, and ensuing care patterns. To refine cost calculations, future studies should take these factors into consideration, as well as CT overutilization and the socioeconomic costs associated with increased cancer risks from CT scans. Clear demonstration of cost saving and added benefits beyond those obtained by current management strategies for mTBI are essential for TBI biomarkers to be adopted and widely used by the medical community.

GFAP

Recent narrative reviews have outlined the potential of GFAP for identifying patients with intracranial lesions after head trauma (23), but none of these used systematic review methods or meta-analyses. In the meta-analysis reported here, we included four studies, in which diagnostic accuracy of GFAP ranged from sensitivities of 67% (71) to 100% (78,82) and specificities from 0% (82) to 100% (71). While promising, these results must be approached with caution since the studies included patients with severe and moderate TBI not representative of the target population of the test (the median prevalence of abnormal CT findings across the studies was 22%) and thresholds were not pre-specified, all factors that may have inflated the accuracy estimates (108). For diagnostic validation, it will be fundamental to establish reliable and valid thresholds. Also, GFAP needs be tested in larger clinical studies with a focus on the *intended use* (109,110). To this end, it has been argued that studies investigating the implementation of biomarker measurements in guidelines for mild head injury management - to avoid use of unnecessary CT - should be limited to patients currently recommended for such examination (GCS 14 to 15), therefore excluding patients with GCS score of 13 for whom biomarker assessment would not add to clinical examination (25). As mentioned earlier, the definition of these setting-specific characteristics is also critical to perform reliable cost analyses and determine the primary economic advantage of using blood biomarkers as a pre-head CT screening tool.

A meaningful comparison between GFAP and S100B diagnostic performances was precluded by a substantial difference in study populations. In this context, we note that TBI biomarkers discussed in this review are usually considered individually. Further work should more consistently explore simultaneous assessment of

multiple biomarkers providing the framework for comparing the accuracy of tests which have directly been compared in individual studies.

NSE and UCH-L1

The relative dearth of studies evaluating the diagnostic accuracy of NSE, UCH-L1 and Tau in the ED for identifying patients with intracranial lesions following mild head injury hampered the possibility of performing meta-analyses. The diagnostic value of NSE remains uncertain, with studies showing remarkable variations and inconsistency. In contrast, the accuracy of UCH-L1 for detecting intracranial lesions on CT scan was evaluated in 2 studies which yielded an optimal sensitivity (100%) but modest specificities (21% to 39%). Similar to GFAP, the thresholds used were not pre-specified and the studies included patients with mild to moderate TBI (GCS 9-15). Hence, further studies are required to confirm the reproducibility of these findings and to determine clinical utility in daily bedside care.

Tau and Neurofilament Proteins

There is insufficient evidence to support the clinical validity of initial circulating c-Tau or Neurofilament protein concentrations for the management of patients with mild TBI.

Implications for Research and Practice - Strengths and Weakness of the Review

Our current insight appreciates the complexity of the pathobiology of TBI most probably requiring multifaceted, multimodal approaches, integrating biomarkers and traditional clinical characteristics to allow a more powerful and accurate characterization and risk stratification of MTBI (86,111) –a premise currently insufficiently reflected in the literature. In addition, if the different biomarkers do indeed reflect different pathophysiological processes (92) with independent

information about imaging abnormality, outcome impact and different diagnostic windows, it is possible that the use of a panel of biomarkers may substantially increase the diagnostic specificity for the endpoint of interest (112,113). Unfortunately, to date, only a few such studies are available. More data are needed to evaluate whether a multimarker approach based on a panel of biomarkers with distinct time-dependent discriminatory accuracy provides a better performance for the detection and characterization of TBI.

Further, we should be cautious in using CT as a gold standard to judge the performance of circulating biomarkers. When compared with MRI, there is increasing recognition that X-ray CT provides poor sensitivity for structural lesions in TBI such as microbleeds and diffuse axonal injury (114,115). It follows that we cannot assume that false positivity in detection of CT-visible abnormality equates to false positivity in detection of structural injury, because some of these false positives may be associated with abnormalities on MRI or other advanced neuroimaging, persistent post-concussive symptoms, or long-term neurological, cognitive, and/or neuropsychiatric complications (116-119). On the other hand, these considerations suggest a broader clinical application of a biomarker-based strategy for diagnosis and management of mTBI. Biomarkers could be used to provide guidance for prognostic groupings, to refine risk stratification, and to inform and guide different management and treatment decisions including indications for advanced MRI techniques (diffusion tensor imaging [DTI], susceptibility weighted imaging [SWI], functional connectivity MRI [fcMRI]), enrollment into clinical trials, and closer monitoring and follow-up of mTBI patients.

From a clinical perspective, biomarkers are not useful if they do not provide real-time decision support for diagnosis of mTBI at the bedside in the ED. A successful

approach to the rapid incorporation into routine patient care will be to develop an automated multiplex point of care (POC) device, capable of providing accurate measurements to the clinician at a reasonable cost and with short turnaround times (~15–20 min) (93,94).

The studies discussed in this review focus primarily on adult patients. There is, however, a growing interest in using biomarkers to optimize diagnosis and management of pediatric mTBI, because of the high risk of TBI in children ≤ 4 years of age, the difficult functional assessments, and the radiation exposure at a young age with ensuing increased cancer risk (116,120,121). Future studies and systematic reviews taking current and new evidence into account are urgently needed to elucidate the role of biomarkers and establish their clinical utility in this special and vulnerable population.

Several potential limitations merit consideration. Patient selection is a critical aspect in reviews of test accuracy, as it can alter the spectrum of disease and non-disease and the prevalence in the population, strongly impacting test accuracy (108). Given the heterogeneous and polymorphous nature of TBI, in particular at the milder end of the spectrum, there has been an inconsistent, sometime controversial, definition of mTBI adopted in the included studies. For example, focal neurological deficit has been considered either as an inclusion or as an exclusion criterion. This diagnostic uncertainty may possibly have introduced different biases. Although this is an issue that we cannot solve in this review as we had to rely on the criteria that were listed in the included studies; nonetheless, we were able to assess the robustness of the findings using sensitivity analysis, which even demonstrated an improvement in S100B performance.

However, with respect to selection of patients and study design, our group endorses the importance of methodological rigor, and advocates the use of standardized protocols and a prespecified set of data analysis both as a means to reduce related biases and inadequate reporting, and as a mandatory prerequisite to ensure successful validation and implementation of TBI diagnostic biomarkers. Also critical consideration for sample size planning based on assay precision, clinical significance, and regulatory considerations is necessary. Involvement of regulatory bodies in driving forward harmonization and standardization is considered essential. A major step forward in this direction is the recently established collaboration between researchers and the United States Food and Drug Administration (FDA) in the context of the TBI Endpoints Development (https://tbiendpoints.ucsf.edu/).

Further, despite the broad adoption by the scientific community of the STARD statement (Standards for Reporting of Diagnostic Accuracy studies) (122), we found a number of studies with poor or inconsistent reporting of important information, including patient and specimen characteristics, assay methods, handling of missing data, and statistical analysis methods, in addition to suboptimal descriptions of study findings, which hampered our assessment of potential for bias and interpretation of the results. Our observations are important in raising awareness of key reporting issues in many of the TBI diagnostic studies. The STARDdem Initiative recently proposed an implementation of the STARD statement with guidance pertinent to studies of cognitive disorders, which is expected to contribute to the development of Alzheimer biomarkers (123). A similar initiative for TBI biomarker studies could increase transparency and the quality of information provided by such studies, enabling evaluation of internal

and external validity and, consequently, a more effective translation and application of their findings to clinical practice.

Harmonization and standardization of biomarker assays that can reliably quantify biomarkers with high analytical precision is critical to ensure that measurements are reproducible and consistent across different analytical platforms and multiple laboratories.

VI. SUMMARY OF THESES

1. Traumatic Brain Injury management guideline in Hungary

1. The total CFR at one week, one month and six month post injury were 21.9%, 36.8% and 48.0% respectively.
2. The CFR at one week, one month and six month post injury were higher in females than in males.
3. The centers together at one week, one month and six months had CFRs of 22.6 %, 38.6 % and 48.9 % respectively.
4. The secondary institutions together at one week, one month and six months had CFRs of 21.7 %, 35.9 % and 47.6 %.
5. The centers and the secondary institutions specific CFR showed no change when the before and after guideline introduction periods were compared.
6. Sex was not a CFR influencing prognostic factor for any survival interval.
7. Higher age proved to be risk factor for each CFR period studied.
8. Neither the level of first admitting institution nor the time period of care had any significant influence on CFRs.
9. The guideline introduction without supportive financing and external auditing cannot achieve the quality improvement in countries having similar legal environment and economic development like Hungary.

2. Risk factors of External Ventricular Drain infection

10. The studies published on risk factors of EVD infection till 2017 have serious limitations and can be considered only as preliminary investigations which yielded a set of variables (patients and EVD related factors) that should be covered by future observational epidemiological investigations.
11. Despite the huge cumulative sample size 5113 patients of these studies, the comparability of the results was seriously hampered by the non-standard assessment of investigated clinical factors.
12. The outcome of our review is a recommendation that former approaches should be replaced by a design able to determine the clinical importance of factors related to EVD infection and able to prepare a formal quantitative meta-analysis.
13. According to our results, the set of the parameters in the study model should be used at least – besides other factors depending on the tested hypothesis in the etiology of EVD infection. These variables are: age, sex, age & sex interactions, coinfection, catheter insertion outside the hospital, catheter type, CSF leakage, CSF sampling frequency, diagnosis, duration of catheterization, ICP > 20 mmHg, irrigation, multiple catheter, neurosurgical operation, and reduced CSF glucose at insertion of ventricular catheter.

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1. Articles grounding the thesis

- **Sorinola A,** Buki A, Sandor J, Czeiter E. Effectiveness of Traumatic Brain Injury Management Guideline Introduction in Hungary. Turk Neurosurg. 2018;28(3):410-415. doi: 10.5137/1019-5149.JTN.19396-16.1.
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2. Presentation and poster grounding the thesis

- Limitations of Guidelines in Neurotrauma: Lessons learned from recent studies. **Sorinola A,** Buki A, Sándor J, Czeiter E. 23rd Annual EMN Congress (Euroacademia Multidisciplinaria Neurotraumatologica), 8-10 May 2018, Pécs, Hungary (Presentation)

3. Article not grounding the thesis

- Mondello S, **Sorinola A,** Czeiter E, Vámos Z, Amrein K, Synnot A, Donoghue E, Sándor J, Wang KKW, Diaz-Arrastia R, Steyerberg EW, Menon DK, Maas AIR, Buki A. Blood-Based Protein Biomarkers for the Management of Traumatic Brain Injuries in Adults Presenting to Emergency

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IF.: 5.002 (2017); Scimago: Neurology (clinical): D1.

4. Presentation and poster not grounding the thesis

- Harmonization and Systemic Approach to the Sophisticated Use of Biomarkers in Field of Traumatic Brain Injury (TBI). Mondello S, **Sorinola A,** Czeiter E, Vámos Z, Amrein K, Synnot A, Donoghue EL, Sándor J, Wang KKW, Diaz-Arrastia R, Steyerberg EW, Menon D, Maas A, Buki A. 7th CNS (Pannonian Symposium on CNS Injury), 30th August – 1st of September 2017, Pécs, Hungary (Presentation)

Sum IF.:
Articles grounding the thesis: 4.283
Article not grounding the thesis: 5.002
Total IF.: 9.285

X. APPENDIX: Search Strategy

MEDLINE (Ovid) 1946 to October Week 2 2016

1. Brain Injuries/

2. Craniocerebral Trauma/

3. head*.ti,ab.

4. brain*.ti,ab.

5. injur*.ti,ab.

6. trauma*.ti,ab.

7. 3 or 4

8. 5 or 6

9. 7 and 8

10. or/1-2,9

11. Biological markers/

12. biomarker.ti,ab.

13. marker*.ti,ab.

14. biomarker*.ti,ab.

15. or/11-14

16. S-100*.ti,ab.

17. S100*.ti,ab.

18. S100 proteins.ti,ab.

19. S100 Proteins/

20. or/16-19

21. GFAP.ti,ab.

22. glial protein*.ti,ab.

23. glial fibrillary acidic protein*.ti,ab.

24. glial intermediate filament protein*.ti,ab.

25. astroprotein*.ti,ab.

26. GFA-protein*.ti,ab.

27. glial fibrillary acidic protein/

28. or/21-27

29. C-tau.ti,ab.

30. cleaved-tau.ti,ab.

31. tau protein*.ti,ab.

32. p-tau.ti,ab.

33. tau Proteins/

34. or/29-33

35. NSE.ti,ab.

36. neuron specific enolase*.ti,ab.

37. gamma-enolase*.ti,ab.

38. enolase 2.ti,ab.

39. nervous system specific enolase*.ti,ab.

40. phosphopyruvate hydratase*.ti,ab.

41. phosphopyruvate hydratase/

42. or/35-41

43. UCH-L1.ti,ab.

44. UCHL1.ti,ab.

45. Ubiquitin Carboxyl-Terminal Hydrolase L-1.ti,ab.

46. ubiquitin c-terminal hydrolase*.ti,ab.

47. ubiquitin carboxy- terminal esterase*.ti,ab.

48. ubiquitin thiolesterase*.ti,ab.

49. Ubiquitin Carboxyl-Terminal Hydrolase L-1, human.ti,ab.

50. UCHL1 Protein.ti,ab.

51. ubiquitin/

52. ubiquitin thiolesterase/

53. or/43-52

54. NF-H.ti,ab.

55. NFH.ti,ab.

56. NFP-200.ti,ab.

57. NFP200.ti,ab.

58. hyperphosphorylated neurofilament*.ti,ab.

59. neurofilament protein*.ti,ab.

60. neurofilament H protein*.ti,ab.

61. neurofilament triplet protein*.ti,ab.

62. Neurofilament Protein H.ti,ab.

63. phosphorylated neurofilament.ti,ab.

64. Neurofilament Proteins/

65. or/54-64

66. blood.ti,ab.

67. serum.ti,ab.

68. plasma.ti,ab.

69. or/66-68

70. or/15,20,28,34,42,53,65

71. and/10,69-70

72. 71 not (animals/ not humans.sh.)

Embase (OVID) 1980 to 2016 Week 43

1. exp brain injury/

2. Craniocerebral Trauma/

3. (head* and injur*).ti,ab.

4. (brain* and injur*).ti,ab.

5. ((head* or brain*) and trauma*).ti,ab.

6. or/1-5

7. exp biological marker/

8. biomarker.ti,ab.

9. (marker* or biomarker*).ti,ab.

10. or/7-9

11. (blood or serum or plasma).ti,ab.

12. exp blood/

13. exp serum/

14. exp plasma/

15. or/11-14

16. exp prognosis/

17. prognos*.ti,ab.

18. exp diagnostic procedure/

19. diagnos*.ti,ab.

20. di.fs.

21. or/16-20

22. and/6,10,15,21

23. animal/ not human/

24. 22 not 23

Cochrane Library (searched 19 October 2016)

#1 MeSH descriptor: [Brain Injuries] explode all trees

#2 MeSH descriptor: [Craniocerebral Trauma] explode all trees

#3 (head* or brain*) and (injur* or trauma*):ti,ab,kw (Word variations have been searched)

#4 (#1 OR #2 OR #3)

#5 MeSH descriptor: [Biomarkers] explode all trees

#6 (biomarker* or marker*):ti,ab,kw (Word variations have been searched)

#7 (#5 OR #6)

#8 MeSH descriptor: [Blood] explode all trees

#9 MeSH descriptor: [Serum] explode all trees

#10 MeSH descriptor: [Plasma] explode all trees

#11 (blood OR serum OR plasma):ti,ab,kw (Word variations have been searched)

#12 (#8 OR #9 OR #10 OR #11)

#13 (#4 AND #7 AND #12)

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Original Investigation

Effectiveness of Traumatic Brain Injury Management Guideline Introduction in Hungary

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ABSTRACT

AIM: To describe the impact of the Traumatic Brain Injury management guideline introduction in Hungary.

MATERIAL and METHODS: Hospital discharge records (HDR) including age, gender, codes of interventions applied, ICD codes of diagnosed disorders of patients admitted between 01/01/2004 and 31/12/2010 with the diagnosis of intracranial injury (S06 by ICD10) from every inpatient institution in Hungary were collected from the database of National Health Insurance Fund (NHIF). The Case Fatality Ratios (CFR) for one week, one month and six months were calculated for the periods before and after the guideline introduction. The change of CFRs was applied as indicators for change of clinical quality elicited by the guideline.

RESULTS: The centers together at one week, one month and six months had pre-guideline introduction CFRs of 23.4%, 37.7% and 47.5% and post-guideline introduction CFRs of 22.1%, 39.1%, and 50.0% respectively. The secondary institutions together at one week, one month and six months had pre-guideline introduction CFRs of 21.5%, 34.8% and 46.3% and post-guideline introduction CFRs of 21.9%, 37.0%, and 48.9% respectively. None of the CFRs showed significant change.

CONCLUSION: The effectiveness of TBI management guideline adaptation in Hungary is poor. Without supportive financing and external auditing system, guideline introduction alone cannot achieve standard clinical practice and a reduction in CFR.

KEYWORDS: Case fatality ratio, Guideline, Guideline introduction, Hungary, Traumatic brain injury

■ INTRODUCTION

Traumatic brain injury (TBI) is a major cause of death and disability in the world harboring significant public health and socio-economic importance. TBI is estimated to be the primary cause of mortality and disability among young individuals and is associated with a cost of over $76 billion ($384,864,000/100,000/year) in the USA (12), and at least €33 billion (€ 77,550,000/100,000/year) in Europe (7). According to the Center for Disease Control and Prevention (CDC) in 2016, about 823.7/100,000/year emergency department visits were associated with TBI - either alone or in combination with other injuries. Epidemiological data on TBI from the European Union are scarce, but do indicate an incidence of hospitalized TBI of approximately 235/100,000/year, although substantial variation exists between European countries (11).

In order to reduce the disability, mortality and socio-economic burden of TBI, guidelines (1) for managing TBI need to be adhered to. The implementation of guidelines produces improved efficiency and outcomes for healthcare professionals and patients beginning with pre-hospital phase and extending throughout long-term application of care. If all trauma centers in the USA adopted the guidelines, the CDC projects a $ 3.8 billion savings in associated cost (2). Although TBI management guidelines are widely published, their

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implication is seldom assessed and the guideline adherence is hardly documented (1,10).

If TBI management guidelines are properly adhered to, the pre-hospital management of TBI should lead to correct identification of TBI, optimal treatment in the ambulance/emergency room and direct transfer to a TBI trauma center. The in-hospital management of TBI will produce reduced duration of intensive care unit/hospital stay, reduced healthcare cost, decreased death and disability (6) by 30%-50% and improved neurological outcome (5) upon discharge by 30%-50%. The post-hospital management of TBI would lead to faster rehabilitation and timely re-integration of a patient into the society (15). Adherence to guideline possesses further great potential for managing TBI in terms of helping to standardize clinical management of TBI (hence, ensure quality control) and aid data collection for further audit/benchmarking and research purposes.

The disease burden of serious intracranial trauma is continuously high in Hungary, especially among middle aged men representing the leading cause of death in the young, active population. The reported incidence of TBI patients in Hungary is 140/100,000/year. The proportions of mild, moderate, and severe cases are 67%, 23%, and 11%, respectively. The case fatality ratio (CFR) was extremely high in Hungary: the estimated CFR for hospitalized TBI cases was 45% in 2002 (4). To exploit the evidence based guidelines opportunities, the Hungarian Ministry of Health introduced the guideline of TBI care in 2006, which was established on recommendation of Brain Trauma Foundation (1). It focused on the pre-hospital and clinical management of patients, but it was not supported by reformulated financing protocols and establishment of quality monitoring.

The study aimed to describe the impact of the guideline introduction on the degree of care centralization and the CFR for the Hungarian severe TBI patients, in order to describe the usefulness of guideline introduction without parallel introduction of an audit system.

MATERIAL and METHODS

National Health Insurance Fund (NHIF), the only institution responsible for financing the inpatient neuro-traumatology care in Hungary, provided the data as hospital discharge records (HDR). NHIF HDR contains patients' data such as age, gender, zip code of residential address, date of admission, codes of interventions applied, International Classification of Diseases (ICD) codes of diagnosed main disorders, date of discharge, date of death (if it happened). Direct assessment of TBI severity was not possible in this studied dataset because the NHIF HDR does not contain the Glasgow Coma Score (GCS). Instead, the severe TBI cases (sTBI) were defined by ICD code and clinical intervention codes. Patients with S06 diagnosis of intracranial injury and with a code of external ventricular drainage application were considered as sTBI subjects. The HDR of sTBI patients admitted between 01/01/2004 and 31/12/2010 recorded in every inpatient institution of Hungary were included in the database analyzed by our investigation. The records were pseudonymized, and the pseudo-identifiers were used to link the episodes of care to patients. Severe TBI patients who died at the scene of trauma or before arrival to the hospitals were not included in the study population.

Age and sex specific incidence of sTBI was calculated for Hungary using demographic data of the Hungarian population provided by the Hungarian Central Statistical Office.

The institutions that took part in the sTBI care were described by the number of patients first admitted by them. By evaluating the pathways of sTBI patients, the TBI centers and secondary institutions were differentiated. Hungary has a declared hierarchy of institutions devoted to TBI care. Unfortunately, this levelling system is neither enforced by health authorities nor adhered to in the practice. In fact, the patient pathways are determined by the traditional relationship between neurosurgeons and the emergency care providers in a certain catchment area, beside the geographical position of injury. Hence, centers had to be determined by a statistical approach in our analyses, instead of by the misleading official categorization. Centers and secondary institutions were distinguished by the number of patients admitted in the study period. A Lorenz curve like graph was constructed to show the level of centralization which plotted the cumulative percentage of the total number of patients in the function of the top percentage of institutions that treated the highest number of patients. The biggest institutions altogether treated 50% of the patients and were considered as centers while the rest of institutions as secondary.

The CFR was calculated for the period of one week, one month and six months after the first hospital admission of sTBI patients. Age group and gender specific CFRs for the whole country were also calculated for each studied year. The center and secondary institution specific CFRs were calculated, as well, and compared by chi-square test to check the change in time.

The indicator for centralization of care (number of patients admitted in centers over number of patients admitted in secondary institutions), the center and secondary institution specific CFRs were computed for the whole study interval (2004-2010), period before (2004-2006) and after (2007-2010) guideline introduction. The period specific results were compared by chi-square test.

To control for the potential confounding effect of patients demographic characteristics, the determinants of CFRs were investigated by multivariate logistic regression models where the sex and age of sTBI patients, the level of first admitting institution (classified as centers or secondary institutions), and time of the admittance (distinguishing before and after guideline introduction periods) were the explanatory variables.

The results of statistical tests were considered as significant if the p-value was less than 0.05. All the statistical computations were carried out by PASW Statistics 18.

The database was processed anonymously. The processing of the data was a secondary analysis and according to the contemporary Hungarian legal requirement, ethical permission

was not necessary to carry out analyses. The research protocol was reviewed, permitted and in concordance with the Internal Data Safety and Patient Rights Board of the Hungarian Health Insurance Fund.

■ RESULTS

The total number of discharge episodes during the study duration was 77,442 episodes of 7,230 patients. Male dominance was observed (Table I). The average age of males and females was not different. Among females, the age group of 75-84 years was at highest risk. Among males, the highest risk period was wider (Figure 1). There were 3,391 fatal outcomes detected in 6 months of the hospital admittance. CFR at one week post-injury was 21.9% (21.2% among males and 23.6% among females), which was elevated up to 36.8% (36.1% and 38.8%) at one month, and up to 48.0% (47.0% and 50.4%) at six months.

At one week, in males, the highest CFRs were in the ninth and fourth decades (with CFRs of 46.2% and 26.2%). While in females, the highest CFRs were observed in third and ninth decades (with CFRs of 41.9% and 35.7%). At one month, in males, the highest CFRs were detected in age groups 95 and 90 (with CFRs of 76.9% and 61.5%) and in females, in age groups 95 and 90 (with CFRs of 61.5% and 60.7%). At six months, in males, the highest CFRs have been described in age groups 95 and 90 (with CFR of 84.6% and 69.8%) in females in age groups 95 and 90 (with CFRs of 92.3% and 75.0%) (Figure 2).

Throughout the study period, the CFR in one week, in one month and in 6 months remained the same for almost all age groups before and after 2006 when the guideline was introduced (Figures 3-5).

A total of 57 institutions took part in the study with 8 (referred as centers) providing 50 % of the care (Figure 6). There was

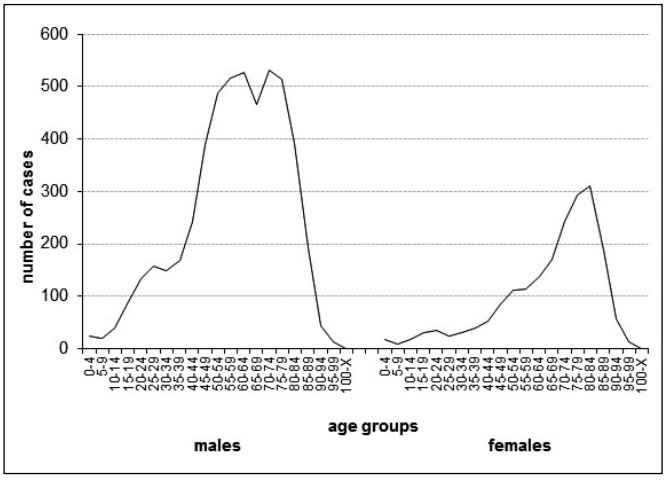

Figure 1: Number of age- and sex-specific cases of traumatic brain injuries in Hungary (2004-2010) according to the hospital discharge records of the National Health Insurance Fund.

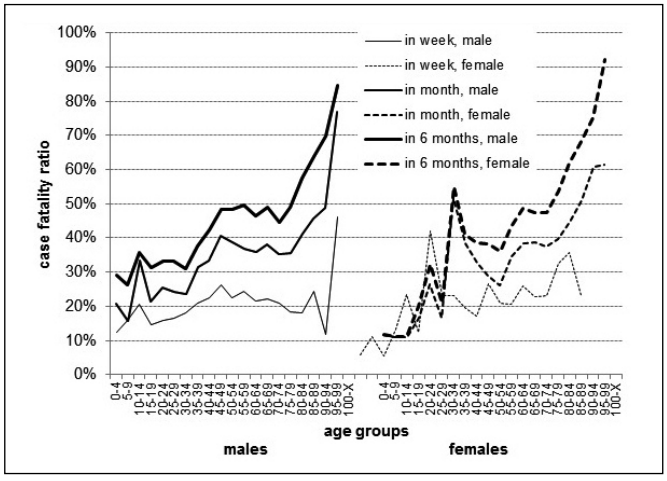

Figure 2: Age- and sex-specific case fatality ratio of traumatic brain injury in Hungary (2004-2010) for 1 week, 1 month, and 6 months according to the hospital discharge records of the National Health Insurance Fund.

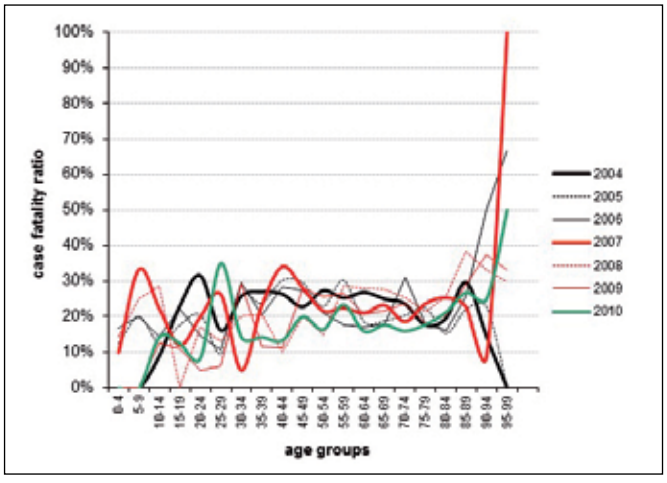

Figure 3: Time trend of age-specific case fatality ratio (CFR) of traumatic brain injury in Hungary (2004-2010) for 1 **week**.

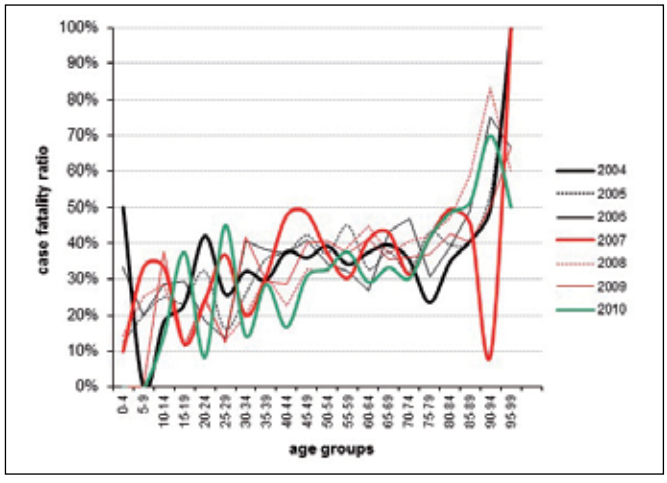

Figure 4: Time trend of age-specific case fatality ratio (CFR) of traumatic brain injury in Hungary (2004-2010) for 1 **month**.

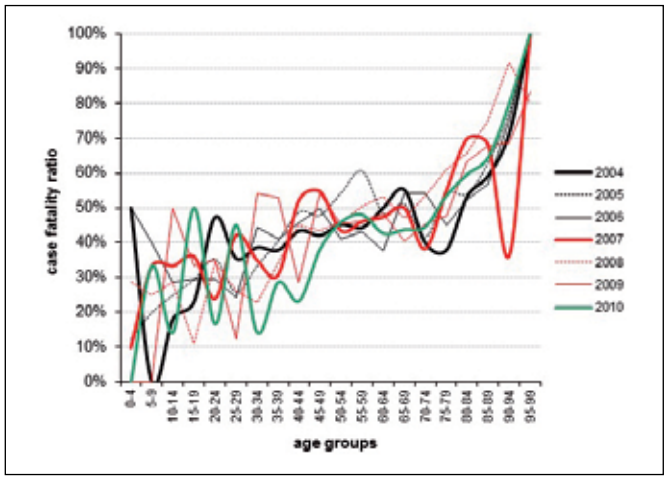

Figure 5: Time trend of age-specific case fatality ratio (CFR) of traumatic brain injury in Hungary (2004-2010) for 6 **months**.

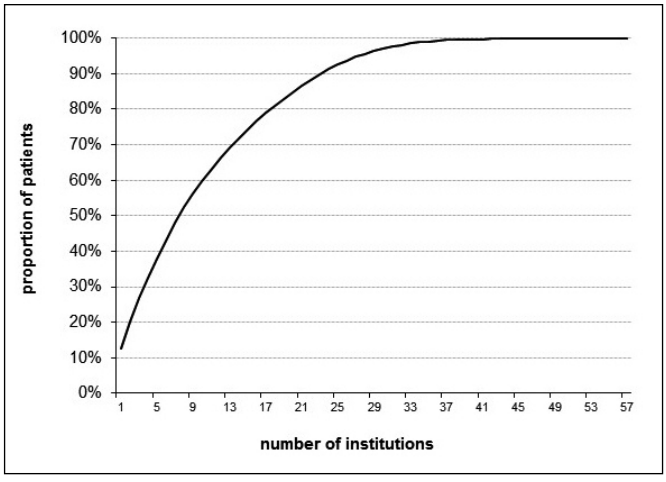

Figure 6: Cumulative proportion of severe traumatic brain injured patients in the function of the cumulative number of institutions providing the care in Hungary (2004-2010) according to the hospital discharge records of the National Health Insurance Fund.

an increase in care centralization according to the ratio of the center to secondary institutions treated number of patients. (0.85 vs 1.07; p<0.001) (Table I).

The centers together at one week, one month and six months had CFRs of 22.6%, 38.6% and 48.9% respectively. The secondary institutions together at one week, one month and six months had CFRs of 21.7%, 35.9% and 47.6%. Differences were not significant for the one week and for the 6 months period (p=0.358, and p=0.267). The centers' CFR was significantly higher for 1 month (p=0.018). The centers and the secondary institutions specific CFR showed no change when the before and after guideline introduction periods were compared (Table I).

According to the multivariate statistical evaluation, sex was not a CFR influencing prognostic factor for any survival interval, but the higher age proved to be risk factor for each CFR studied. Neither the level of first admitting institution nor the time period of care had any significant influence on CFRs (Table II).

DISCUSSION

Main Findings

In this study, data (HDRs) from the NHIF was analyzed. Our results demonstrated a steady, high case fatality in the Hungarian TBI population undergoing external ventricular drain (EVD) installation, and "no effect" of the introduction of scientific evidence-based practice guidelines in 2006 was revealed. Though, the guideline introduction coincided with moderate increase of centralization. The unreduced CFR in Hungary suggested that the existence of guidelines *"per se"* will not result in outcome improvement and additional measures (audit of care, enforcement of guideline compliance) should also be introduced (8).

Like in Hungary, TBI guidelines were introduced in other European countries and in the USA many years ago, but in most of these countries, there is a long term tradition of external quality management in clinical care. Further, if there is negligence in medical practice, it can result in lawsuit and also there is competition between the medical institutions. These factors establish the guideline adherence which varies in countries remarkably (between 18-100%), but contributes to significant reduction in mortality (3).

In the last few decades, several Eastern and Middle European countries introduced scientific evidence-based practice guidelines to comply with European legislations and regulations mandatory to participate in international multicenter studies and collaborations. Nevertheless, policy makers did not necessarily cope with these guidelines in terms of introducing novel approaches in health care financing and quality assurance.

Similarly to the majority of these countries, such "mechanisms" do not exist in the Hungarian health care either and a desperate need for contemporary audit systems has long been voiced by clinical and scientific societies. Due to the lack of external pressure, the hospital managements neglect the internal resource allocation needed to improve the resource demanding guideline adherence. Former small scale studies as well as a cross sectional snapshot-like questionnaire based analysis of the care for TBI in Hungary revealed similar results and led to the same conclusions about the reasons of high in-hospital mortality and limited adherence to the international guidelines. This nationwide survey, however, was unable to provide a trend analysis neither supplied data on long term outcome (4).

Strengths and Limitations

The database provided by NHIF was nation-wide with complete national coverage ensuring the fairly high power in the statistical evaluations. However, there are some obvious limitations of such a health insurance data based study: (1) NHIF monitors the financing of care but not the quality of care; (2) the financial interest of the hospital may lead to bias of

Table I: Influence of Guideline Introduction in 2006 on Care Centralization and Case Fatality Ratios

	Whole period (2004-2010)	Before guideline (2004-2006)	After guideline (2007-2010)	p-value*
Male/Female	2.58 (5211/2019)	2.77 (2387/861)	2.44 (2824/1158)	0.015
Age (years), Mean ± SD	60.89±19.23	59.01±19.30	62.41±19.04	<0.001
Centers/Secondary institutions, (N/N)	0.97 (3551/3679)	0.85 (1492/1756)	1.07 (2059/1923)	<0.001
CFR in 1 week in centers, N (%)	803 (22.6%)	349 (23.4%)	454 (22.1%)	0.454
CFR in 1 week in secondary institutions, N (%)	798 (21.7%)	377 (21.5%)	421 (21.9%)	0.803
CFR in 1 month in centers, N (%)	1369 (38.6%)	563 (37.7%)	806 (39.1%)	0.570
CFR in 1 month in secondary institutions, N (%)	1322 (35.9%)	611 (34.8%)	711 (37.0%)	0.345
CFR in 6 months in centers, N (%)	1736 (48.9%)	709 (47.5%)	1027 (50.0%)	0.416
CFR in 6 months in secondary institutions, N (%)	1753 (47.6%)	813 (46.3%)	940 (48.9%)	0.351

for comparison of 2004-2006 and 2007-2010 periods.* ***CFR:* *Case fatality rate.*

Table II: Influence of Guideline Introduction on Case Fatality Ratio of Severe Traumatic Brain Injured Patients in Hungary according to Multivariate Logistic Regression Analysis Controlled for Age and Sex of the Patients and for the Level of Institution Providing the Care

		OR	p-value
CFR in 1 week	sex (female/male)	1.110	0.099
	age (year)	1.004	0.018
	level of institution (center/secondary)	1.061	0.300
	guideline introduction (after/before)	0.962	0.502
CFR in 1 month	sex (female/male)	1.006	0.907
	age (year)	1.012	<0.001
	level of institution (center/secondary)	1.095	0.064
	guideline introduction (after/before)	1.042	0.405
CFR in 6 months	sex (female/male)	0.994	0.906
	age (year)	1.017	<0.001
	level of institution (center/secondary)	1.061	0.213
	guideline introduction (after/before)	1.045	0.359

CFR: *Case fatality rate.*

reported data; (3) data collection could not make distinction between severe and mild TBI cases by the usual GCS classification; (4) and there were no data on the process of clinical treatment apart from the EVD application. However, the case definition and the quality of data collection was not changed in the study period. Therefore, the time trend analysis yielded reliable results on change of care centralization and CFR in time.

The average incidence of TBI in Europe is 235/100,000/year with a range of 150-300/100,000/year (18). The incidence of TBI in Hungary estimated as 140/100,000/year is only a bit less than this European reference (4). Our study estimated 72.3/100,000/year the incidence of sTBI. Considering the former Hungarian observation on the severity of TBI cases in Hungary, the estimated number of TBI patients according to our present investigation is 957/100,000/year if it is supposed that all studied sTBI patients meet the severe TBI criteria. It seems impossibly high. If it is assumed that sTBI definition corresponds to severe and moderate TBI cases, then the TBI incidence estimated by our study dataset is 212/100,000/year which is in the European reference range. It is probable that our working case definition included severe and moderate TBI cases as well. On the other hand, our study underestimated both incidence rate and number of fatal outcomes. Presumably

due to the excluded cases with lethal pre-hospital outcome, and cases which reached the hospitals but due to the very severe clinical status, the invasive surgical interventions were not perfomed before lethal outcome. Although, the study was not aimed to determine the exact incidence and case fatality for TBI or for severe TBI in Hungary, taking into consideration the above mentioned validity issues, the observed high CFRs for sTBI demonstrated that the Hungarian care for TBI patients was far less effective than it should be on the basis of the country's general development (9,13,14).

In Hungary, the highest CFRs in women at six months were found in the young adults (35 years old) and the elderly (>90 years old) while the highest CFRs in men at six months were found in the middle aged (50 years old) and elderly (>90 years old) groups. The combined CFRs for both sexes at six months were highest at the age group of 95 (with CFR of 88.5 %). A similar trend of high CFR in the elderly was reported in other European countries and the USA (13,16,17). The similarity between published articles and our observed age dependence of CFR shows that our design is reliable in dealing with the time trend of CFR.

CONCLUSION

On the basis of our study which utilized hospital discharge records by which the severe traumatic brain injury incidence was slightly underestimated but rigorous case definition was applied, the lethal outcome was not reduced after the introduction of evidence based guideline in the TBI patients who did not die at the scene of the trauma or during transport to hospital, and whose clinical statuses at admission were not too serious to prevent the neurosurgical invasive intervention, but whose brain trauma were serious enough to indicate EVD application. The guideline introduction without supportive financing and external auditing cannot achieve the desired quality improvement in countries with a legal environment and economic development similar to Hungary.

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frontiers in Neurology

REVIEW
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Risk Factors of External Ventricular Drain Infection: Proposing a Model for Future Studies

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Background: External ventricular drain (EVD) has a major role in the management and monitoring of intracranial pressure (ICP) and its major complication is EVD infection. The risk factors for EVD infection are still a major topic of controversy, hence the need for further research.

Objective: The objective of this review was to identify risk factors that affect the incidence of EVD infection and create a model, which can be used in future studies in order to contribute to elaborations on guideline for EVD.

Methods: A PubMed and Google Scholar literature search was performed and data were extracted from studies published from 1966 through 2017. The search of the databases generated 604 articles and 28 articles of these were found to be relevant. A manual search of the 28 relevant papers generated 4 new articles. Of the 32 relevant articles, 20 articles that performed a multivariate analysis of the suspected risk factors of EVD infection and had a positive culture as a mandatory component in diagnosis were selected for data collection and analysis.

Results: Because reviewed papers investigated only a few influencing factors, and could not determine convincingly the real risk factors of EVD infection and their real strengths. A total of 15 supposed influencing factors which includes: age, age & sex interactions, coinfection, catheter insertion outside the hospital, catheter type, CSF leakage, CSF sampling frequency, diagnosis, duration of catheterization, ICP > 20 mmHg, irrigation, multiple catheter, neurosurgical operation, reduced CSF glucose at catheter insertion and sex were identified.

Conclusion: This review summarizes a set of variables which have to be covered by future clinical epidemiological investigations in order to describe the etiological background of EVD infection.

Keywords: (EVD) external ventricular drain, EVD infection, ventricular catheter (VC), ventricular catheter infection, risk factors

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INTRODUCTION

Traumatic brain injury (TBI) is a major cause of death and disability in the world harboring significant public health and socio-economic importance. TBI is estimated to be the primary cause of mortality and disability among young individuals. In 2013, in the United States, TBI was a diagnosis in more than 2.5 million emergency department visits and 282,000 hospitalizations.

According to the Center for Disease Control and Prevention (CDC), TBI contributes to 30% of all injury-related deaths in the United States (1). Epidemiological data on TBI from the European Union are scarce, but do indicate an incidence of hospitalized TBI of approximately 235/100,000/year, although substantial variation exists between European countries (2). TBI is associated with a cost of over $76 billion in the USA (3) and least €33 billion in Europe (4) and severe TBI accounts for 90% total medical cost of TBI (1).

Ventriculostomy is frequently used in the management and monitoring of intracranial pressure (ICP) in severe TBI patients. In the US, an average of about 20,586–25,634 (24,380) patients per annum undergo ventriculostomy (5).

Although TBI management is our main interest and the application of external ventricular drain (EVD) is a crucial point in TBI protocols, EVD infections are among the complications for EVD application with high influence on the outcome of the underlying disease and are not well-characterized.

Many conditions such as intracranial hemorrhage, intracranial tumor and hydrocephalus prompt the use of EVD. EVD application is frequently complicated by misplacement, hemorrhage, dislodgement, blockage, and most importantly infection (6). EVD infection rate ranges from 0 to 22% (7–9) resulting in a significant increase in cost, hospital stay, morbidity, and mortality (6, 10).

Due to the heterogeneous knowledge on the effectiveness of EVD, uncertainties of EVD application and the infection related complications, further research is required. The aim of this qualitative review was to identify risk factors that can potentially affect the incidence of EVD infection and create a model, which can be used in future studies to determine the real risk factors with their real strengths in order to contribute to the elaborations on the guideline for EVD application among TBI patients.

METHODS

We performed a systematic search on PubMed and Google Scholar databases (from 1966 to August 2017) for relevant studies related to ventricular drain infections. Keywords used in the search strategy include:

1. Infections (ventricular drain, ventriculostomy related, external ventricular drain, ventricular catheter, and extra-ventricular drain) and
2. One of the following [traumatic brain injury, Intensive care (ICU) patients', neuro-intensive care (NICU) patients, head injury, brain injury, cerebral hemorrhage, sub-arachnoid hemorrhage].

The combination of keywords generated 328 and 276 references on PubMed and Google Scholar, respectively. Of 604 references, 28 were found relevant after the title and abstract screening. In addition to these, the references of these 28 relevant articles were searched manually to find more related articles, which generated 4 new articles.

The 32 relevant articles were screened; those that performed a *multivariate analysis* of suspected risk factors (making a difference between factors which proved to be a confounding factor in a study and factors which can have an influence on outcome according to the published reports) and had a positive culture as a mandatory component in diagnosis were selected for data collection and analysis. Twenty articles (16 of these are as a result of the keyword-based literature search and 4 are as a result of manual reference search) were finally selected for our review. The cumulative sample size of the 20 studies was 5,113 patients, with a median of 164.5.

In our review, quantitative methods (meta-analysis) could not be applied due to the heterogeneity of the studies in respect to the risk factors identified i.e., the number of studies identifying a certain risk factor was small, making meta-analysis impossible.

As a result of the heterogeneity of the clinical terms used by the authors a common term was chosen for the varying terminologies. "Age" and "younger age" were simplified to ***age***; "co-infection," "concurrent systemic infection," "open infection source," and "skin colonization by pathogen" were simplified to ***co-infection***; "Depressed Cranial Fracture repairing surgery (DSF)," "neurosurgical operation," "length of tunneling (> 5cm)," "craniotomy," and "two or more burr-holes" were simplified to ***neurosurgical operation***; "duration of catheterization," "duration of catheterization (>11 days)" and "intracranial pressure monitoring (ICPM) > 5 days" were simplified to ***duration of catheterization***; "standard catheter," "conventional catheter" and "antibiotic coated catheter" were simplified to ***catheter type***; "neuro-trauma," "multiple trauma," "sub-arachnoid hemorrhage (SAH)," "intraventricular hemorrhage (IVH)," "intracerebral hemorrhage (ICH)," and "intraventricular hemorrhage (IVH)" were simplified to ***diagnosis***; "repeat insertion," "patients with >1 ICPM," "multiple catheter replacements," and "number of catheters" were simplified to ***multiple catheters***.

Duration of catheterization refers to the time period between post-catheter insertion and the detection of infection or discharge from ICU in the absence of infection.

According to CDC, meningitis or ventriculitis must meet at least one of the following criteria:

1. Patient has organism(s) identified from cerebrospinal fluid (CSF) by a culture or non-culture based microbiologic testing method which is performed for purposes of clinical diagnosis or treatment for example, not Active Surveillance Culture/Testing (ASC/AST).
2. Patient has at least two of the following:
   i. fever (>38.0°C) or headache (Note: Elements of "i" alone may not be used to meet the two required elements)
   ii. meningeal sign(s)*
   iii. cranial nerve sign(s)*
3. Patient ≤1 year of age has at least two of the following elements: i. fever (>38.0°C), hypothermia, apnea*, bradycardia*, or irritability* (Note: Elements of "i" alone may not be used to meet the required two elements).
   ii. meningeal signs*
   iii. cranial nerve signs*

* With no other recognized cause

And at least one of the following for Number 2 and two of the following for Number 3 listed above:

a. increased white cells, elevated protein, and decreased glucose in CSF (per reporting laboratory's reference range)
b. organism(s) seen on Gram stain of CSF
c. organism(s) identified from blood by a culture or non-culture based microbiologic testing method which is performed for purposes of clinical diagnosis or treatment, for example, not Active Surveillance Culture/Testing (ASC/AST)
d. diagnostic single antibody titer (IgM) or 4-fold increase in paired sera (IgG) for organism

The definition of infection varied among authors. Some defined infection as a positive CSF culture from either the ventricular catheter or lumbar subarachnoid space, verified by growth on agar plates (11–13). Others defined infection by the CDC criteria for meningitis/ventriculitis (14, 15). Some authors defined infection with a few criteria, e.g., Mayhall et al. defined infection as; no other detectable source of central nervous system (CNS) infection, negative cultures of CSF obtained at the time of catheter insertion, ventricular catheterization for 24 h or longer and a positive CSF culture from either the ventricular catheter or lumbar subarachnoid space. Pople et al. also defined infection by a few criteria which includes; CSF culture with no organisms identified on initial Gram stain that subsequently grew a positive culture on agar, or CSF culture negative, but with Gram stain showing either gram-positive or gram-negative organisms, or CSF leukocytosis with a white blood cell/ red blood cell CSF count >0.02. Omar et al. defined infection as a positive CSF culture and Gram stain and presence of other supportive CSF laboratory findings such as pleocytosis with microscopic examination showing presence of white blood cells of more than 11/mm^3, a decrease in the CSF glucose level and an increase in the CSF protein level. Standard catheter refers to catheters without hydrogel or silver or antibiotic(s) coating or impregnation while antibiotic coated catheter refers to catheters coated with antibiotic(s).

RESULTS

Table 1 summarizes the factors that proved to be significant or non-significant in the univariate analyses of the studies included in this review. Out of the 20 articles selected for analysis, three studies reported no significant association between the risk factors evaluated and EVD infection (6, 15, 17) after multivariate analysis. Altogether 15 risk factors (10 patient-related and 5 catheter-related) were identified by our review. Risk factors found by most investigations were neurosurgical operation and duration of catheterization. In general, the reviewed studies dealt only with a narrow set of possible risk factors. The maximum number of risk factors identified by a study was 5 (**Table 2**).

Patient Factors

Age was identified by Flibotte et al. [OR & 95%CI: 1.04, 1.01–0.081; *P*-value: 0.03] and Wright et al. [HR & 95%CI: 1.051, (1.01–1.09) *P*-value, 0.014] as a risk factor associated with ventricular catheter infection. Both studies measured age in years and found a 4–5.1% increase in risk of EVD infection per annum respectively (11, 27).

Lo et al. reported that females [OR & 95%CI: 3.4, (1.2–9.7); *P*-value, 0.02] were three times as likely to be infected as males (21).

Age & sex interaction [HR & 95%CI: 0.912, (0.85–0.98); *P*-value, 0.0112] was identified as a risk factor for EVD infection by Wright at al. They also reported that female patients were 6 times likely to have an EVD infection than male patients (23.7% vs. 3.1%, OR: 6.4, $p < 0.003$) (27).

Co-infection, a risk factor with a higher incidence among the patient factors was identified by Bota et al. [OR & 95%CI: 3.92, (0.66–7.84); *P*-value, 0.02], Holloway et al. [*P*-value, 0.001], Kirmani et al. [*P*-value, 0.002], and Mounier et al. [OR & 95%CI: 11.8, (2.5–56.8); *P*-value, 0.002] and was found to be significantly associated with ventricular catheter infection (7, 19, 20, 23). The EVD infection rates of 12, 20.7, 15, & 37.5% in patients who had a concurrent infection vs. 7, 8.6, 6 & 4.7% in patients who did not were reported respectively (7, 19, 20, 23).

Mounier et al. reported cerebrospinal fluid (CSF) leakage at the site of insertion [OR & 95%CI: 10, (2.4–41.2); *P*-value, 0.001] as a risk factor and that among the infected patients, most of the catheter infection was as result of the colonization at the site of catheter insertion (23).

Diagnosis was identified by Bota et al. [(SAH: OR & 95%CI: 2.95, (0.59–5.26); *P*-value, 0.02), (IVH: OR & 95%CI: 2.07, (0.65–4.87) *P*-value, 0.02), (neurotrauma: *P*-value, 0.03)], Holloway et al. [*P*-value, 0.007], and Mayhall et al. [*P*-value, 0.027] as a risk factor with a high prevalence among the patient factors with significant influence on the incidence of ventricular catheter infection (7, 19, 22).

According to Hoefnagel et al. increased frequency of CSF sampling [OR & 95%CI: 4.12, (1.84–9.22); *P*-value, 0.001] is a risk factor to EVD infection. The authors noted that CSF was not always sampled according to the institution's protocol that had been set which inevitably increased the frequency of catheter manipulation and consequently the risk of infection (12).

According to Mayhall et al. intracranial pressure (ICP) above 20 mmHg [*P*-value, 0.019] is a risk factor for ventricular catheter infection but they mention also the alternate explanation for their observation, that patients with high ICP may need ventricular catheter for longer periods which predisposes them to infection (22).

Neurosurgical operation was identified by Bota et al. [OR & 95%CI: 4.74, (0.27–9.52); *P*-value, 0.03], Holloway et al. [craniotomy: *P*-value, 0.005; DSF: *P*-value, 0.003], Mayhall et al. [*P*-value, 0.016], Omar et al. [OR & 95%CI: 10.46, (3.38–32.32); *P*-value, 0.001], and Peter et al. [*P*-value, 0.047] as a risk factor (7, 10, 19, 22, 24). The reported infection rate varied between 15.2 and 82% in patients who underwent neurosurgical operation against 3.4 and 69% in patients who did not (7, 10, 19, 22, 24). Some authors reported that patients with one or more neurosurgical procedures were at a significantly higher risk for infection which may be due to immunosuppression or trauma associated with surgical procedures (20, 22).

Gozal et al. reported that there is a significant correlation between CSF glucose levels [OR & 95% CI: 4.87, (1.26–18.75);

TABLE 1 | Variables identified by univariate analysis having no significant association with EVD infection and factors which showed significant association with EVD infection only in univariate analyses.

Study	Significant variables identified in uni-variate analyses	Non-significant variables identified in uni-variate analyses
Arabi et al. (16)	Prophylactic antibiotics.	APACHE II; SAPS II; Placement of EVD outside the OR; ICU–LOS; Hospital LOS and Mortality.
Bari et al. (17)	–	–
Bota et al. (7)	–	APACHE II; LOS; ICU mortality rate and In-hospital mortality rate.
Camacho et al. (14)	Hospital–LOS; ASA I and Antibiotic prophylaxis.	Duration of surgery and Mortality.
Flibotte et al. (11)	Hospital – LOS and NICU - LOS	Admission GCS $\leq$ 9; In-hospital mortality and VP shunt.
Gozal et al. (18)	–	–
Hagel et al. (6)	ICU–LOS and Hospital LOS.	BMI; ASA; Accommodation and In-hospital mortality.
Hoefnagel et al. (12)	–	Operating time and Prophylactic antibiotic.
Holloway et al. (19)	–	–
Kirmani et al. (20)	Intraventricular antibiotic.	Steroid use.
Lo et al. (21)	–	Presence of trauma.
Mayhall et al. (22)	–	Underlying disease; Placement of EVD in ICU; Antibiotic prophylaxis; CSF drain Disconnections; Previous ventriculostomy and Other CNS instrument.
Mounier et al. (23)	–	Immunodeficiency; Recent neurosurgery; Antibiotics prophylaxis during EVD placement; Antibiotics administration during EVD drainage; EVD placement by resident; Emergency EVD placement; EVD exchange; Drainage lock and EVD disconnection.
Omar et al. (24)	–	Venue of surgery and Surgeon's status.
Paramore et al. (13)	–	Location of catheterization within the hospital.
Park et al. (25)	–	–
Peter et al. (10)	–	–
Pople et al. (26)	–	–
Rebuck et al. (15)	–	Skull fracture; Presence of multiple trauma; Penetrating head injury; Antibiotic prophylaxis and Location of catheter placement within the hospital
Wright et al. (27)	–	–

EVD, External Ventricular drain; LOS, length of stay; (N)ICU, (Neuro) Intensive Care Unit; GCS, Glasgow Coma Scale; ASA, American Society of Anaesthesiologists; APACHE, Acute Physiology And Chronic Health Evaluation; SAPS, Simplified Acute Physiology Score; CSF, Cerebrospinal Fluid; CNS, Central Nervous System; ICPM, Intracranial Pressure Monitor; OR, Operating Room; BMI, Body Mass Index.

P-value, 0.021] drawn immediately after EVD placement associating <50% of serum glucose and subsequent risk of infection. Gozal et al. pointed out that this should not be mistaken for a pre-existing systemic infection since similar association was not found in CSF pleocytosis or protein levels which would have been expected in an on-going infection (18).

Catheter Factors

Wright et al. reported catheter type [standard catheter vs. antibiotic coated catheter: HR & 95% CI: 0.091, (0.02–0.41); *P*-value, 0.007] as a risk factor associated with ventricular catheter infection. In the study carried out by Wright et al. two types of catheters (standard and antibiotic coated) were used. They reported infection rate as 23.5% for standard catheters and as 4.3% for antibiotic coated catheters. This represents a risk ratio of 0.18 and an absolute risk reduction of 19.2% after changing from standard catheter to antibiotic coated catheter (27).

Park et al. reported that patients that underwent catheter insertion outside the study center had a higher risk [HR & 95%CI: 3.42, (1.46–8.02); *P*-value, 0.005] of infection than patient that underwent catheter insertion in the study center and that there was limited information on the technique of catheter placement or specific location of the patient outside the study center at the time of catheter insertion. On the other hand, Park et al. also reported that the location of catheter insertion (OR/ICU/ED) within the study center did not significantly influence the infection rate of patient with catheters inserted in the study center (25).

Duration of catheterization was identified by Camacho et al. [OR & 95%CI: 1.08, (1.1–1.2); *P*-value, 0.036], Flibotte et al. [OR & 95%CI: 1.2, (1.1–1.3); *P*-value, 0.001], Hoefnagel et al. [OR & 95%CI: 4.12, (1.84–9.22); *P*-value, 0.001], Mayhall et al. [*P*-value, 0.017], Omar et al. [OR & 95%CI: 3.61, (1.19–10.94); *P*-value, 0.024], Paramore et al. [*P*-value, 0.016], Peter et al. [*P*-value < 0.001], and Pople et al. [RC & 95%CI: −0.048, (−0.092 to −0.003)] as a risk factor (10–14, 22, 24, 26). The duration of catheterization ranged from 1 to 44 days in these studies. Some authors suggest that the longer duration of catheterization may cause microbial infection of the catheter (13, 19, 22) while there is another opinion that the longer duration of catheterization is a consequence of the catheter infection rather than the cause (6).

Multiple catheters was identified Arabi et al. [OR & 95%CI: 6.34, (1.36–29.64); *P*-value, 0.019], Lo et al. [OR & 95%CI: 4.6, (2.3–9.2); *P*-value, < 0.0001], and Peter et al. [*P*-value, < 0.01] as a risk factor for EVD infection (10, 16, 21). Arabi et al. and Peter et al. reported an infection rate of 42 and 84.5% in patients

TABLE 2 | Identified risk factors of extra-ventricular drain infections according to the published results from 1984.

Risk Factors	Arabi et al. (16)	Bari et al. (17)	Bota et al. (7)	Camacho et al. (14)	Flibotte et al. (11)	Gozal et al. (18)	Hagel et al. (6)	Hoefnagel et al. (12)	Holloway et al. (19)	Kirmani et al. (20)	Lo et al. (21)	Mayhall et al. (22)	Mounier et al. (23)	Omar et al. (24)	Paramore et al. (13)	Park et al. (25)	Peter et al. (10)	Pople et al. (26)	Rebuck et al. (15)	Wright et al. (27)	Number of positive publications
Patient factors/ Sample size	84	256	638	119	311	498	218	228	584	130	199	172	101	87	161	595	100	434	215	144	
Sex		NS	NS	NS	NS		NS			NS	X			NS		NS				NS	1
Age		NS	NS		X		NS			NS						NS	NS	NS		X	2
Age & sex interaction																				X	1
Co-infection			X						X	X			X						NS		4
CSF leakage										NS			X			NS			NS		1
Neurosurgical operation			X				NS		X	X		X		X			X				6
CSF sampling frequency								X													1
ICP > 20mmHg												X							NS		1
Diagnosis			X		NS		NS		X			X				NS	NS	NS			3
Reduced CSF glucose						X															1
Catheter factors																					
Multiple catheters	X	NS							NS		X						X		NS		3
Catheter insertion outside the hospital	NS															X					1
Duration of catheterization	NS			X	X		NS	X			NS	X	NS	X	X		X	X	NS		8
Catheter type							NS											NS	NS	X	1
Irrigation												X									1
Number of identified risk factors	1	_	3	1	2	1	_	2	3	2	2	5	2	2	1	1	2	1	_	3	

X, factors investigated and found to be significant; NS, factors investigated but found to be non-significant.

who received multiple catheters vs. 3 and 18.3% in patients who did not respectively. Lo et al. also reported that infected patients used almost twice the amount of ventricular catheters as their uninfected counterparts (10, 16, 21). Each additional catheter was reported to increase the risk of infection by 4-fold or 8% by some authors (8, 21). Arabi et al. found out that antibiotics were given more frequently with first insertion than with repeated insertions [68 vs. 7%, *P*-value, 0.001] and it may be responsible for making multiple catheters a risk factor (16).

Mayhall et al. who identified irrigation [*P*-value, 0.021] as a risk factor for EVD infection also hypothesized that infections are likely introduced into the ventricles by retrograde movement of microbes due the manipulation of the catheter system (22).

DISCUSSION

The keywords and references used in our review were similar to the keywords and references in other reviews (8, 9, 28). Despite the sparse amount of articles on this topic, we were able to identify a total of 15 risk factors.

According to our review, distinct studies were able to identify only few risk factors, since the published studies were intentionally focused to a few specific causes of EVD infection with controlling for only few confounding factors, instead of testing a comprehensive set of causal factors representing the hypotheses on the etiological background. On the other hand, the sample size of reviewed studies was rather limited, preventing the effective identification of less dominant risk factors. Further, the terms of investigated clinical factors were highly variable, limiting the effectiveness of comparative evaluation. Therefore, the research on the risk factors of EVD infection is in its initial phase. Identification of factors which can play role in development of infective complications of EVD was the function of the till-now-published papers. The reviewed investigations could contribute to the building of research model to be tested in future, and cannot be considered as reliable quantification of the risk factors' role.

Although, only papers with multivariate models were analyzed in this review, as shown in **Table 2**, none of them could cover the majority of risk factors. Even the paper with the most extended model could not cover half of the identified risk factors. Consequently, the published measures of associations reflect both the strength of the risk factors and the confounding effects of factors that were not included in the model but were associated with the risk factors included in the studied models. Therefore, the relative importance of a risk factor cannot be evaluated by the published models. The whole set of suspected risk factors need to be included in the model which will be tested in clinical practice in order to determine the risk factors with their strength and clinical importance.

There were studies that found some of the identified risk factors not significantly associated with EVD infection in their multivariate analysis. Age, sex, CSF leakage, catheter type, and diagnosis were identified by more studies as not significantly associated with EVD infection (6, 7, 10, 11, 14, 15, 17, 20, 24–27) than studies that did. On the other hand, while more studies reported some risk factors to be significantly associated with EVD infection, a few studies did not find a similar association between these risk factors and EVD infection after multivariate analysis. Duration of catheterization, co-infection, and neurosurgical operation were found by more studies to be associated with EVD infection than a few studies that did not (7, 10–14, 16, 19–24, 26). Catheter insertion outside the hospital, multiple catheters, and ICP $>$ 20 mmHg were found to be either associated with EVD infection or not by equal number of studies (15–17, 25–27). The varying results from the reviewed studies are possibly due to non-standardized research procedures (i.e., some risk factors were selectively analyzed while some risk factors were omitted, resulting in a possible causal or coincidental relationship or the lack thereof between EVD infection and these risk factors). Some of the factors presented in this review for later research may only be confounding factors without direct influence on ventricular catheter infection occurrence (e.g., the association between gender and drain infection; far more males were involved in severe injuries whereas females appeared to be a predictive factor).

This review has a few limitations. Firstly, not all the possible research papers were collected as only the PubMed and Google Scholar databases were used, also the research papers published in other languages other than English language were not included in this review. Secondly, as mentioned previously, the meta-analysis of the risk factors could not be carried out. Thirdly, some of the selected papers were not focused on EVD exclusively (some included ICP monitors). Lastly, the explanatory power of the proposed study model could not be determined because the studies evaluated only a narrow set of influencing factors. Consequently, the proposed study model may include interrelated prognostic factors and it is not possible to predict whether factors omitted from our proposed research model (risk factors) have high impact on manifestation of EVD infection.

Application of ICP monitoring has recently become a major topic of discussions in the scientific community also leading to the conduct of major randomized clinical trial (BEST-TRIP) (29). Nevertheless, to much of our surprise, application of such a common monitoring and therapeutic tool is based on very limited knowledge of purported risk factors associated with its' utilization.

Being aware of such complications and their rate would be of ample importance to inform the relatives, train the care givers as well as enhance therapeutic efficacy. We hope that the present work not only focused attention at our above-detailed weaknesses but also highlighted those potential factors that should be considered when EVD-related infective complications are to be predicted.

CONCLUSION

Studies published on risk factors of EVD infection till 2017 have serious limitations and can be considered only as preliminary investigations which yielded a set of variables

(patients and EVD related factors) that should be covered by future observational epidemiological investigations. Despite the huge cumulative sample size 5,113 patients of these studies, the comparability of the results was seriously hampered by the non-standard assessment of investigated clinical factors. The outcome of our review is a recommendation that former approaches should be replaced by a design able to determine the clinical importance of factors related to EVD infection and able to prepare a formal quantitative meta-analysis. According to our results, the set of the parameters in the study model should be used at a minimum–besides other factors depending on the tested hypothesis in the etiology of EVD infection. These variables are: age, sex, age & sex interactions, co-infection, catheter insertion outside the hospital, catheter type, CSF leakage, CSF sampling frequency, diagnosis, duration of catheterization, ICP > 20 mmHg, irrigation, multiple catheter, neurosurgical operation, and reduced CSF glucose at insertion of ventricular catheter.

AUTHOR CONTRIBUTIONS

AS was responsible for the search of articles. JS was responsible for the structure of the content in the manuscript. AS, JS, and AB were responsible for the writing of the manuscript. AB, JS, and EC were responsible for the editing, proof-reading and finalization of the manuscript

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Living Systematic Review

Blood-Based Protein Biomarkers for the Management of Traumatic Brain Injuries in Adults Presenting to Emergency Departments with Mild Brain Injury: A Living Systematic Review and Meta-Analysis

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Abstract

Accurate diagnosis of traumatic brain injury (TBI) is critical to effective management and intervention, but can be challenging in patients with mild TBI. A substantial number of studies have reported the use of circulating biomarkers as signatures for TBI, capable of improving diagnostic accuracy and clinical decision making beyond current practice standards. We performed a systematic review and meta-analysis to comprehensively and critically evaluate the existing body of evidence for the use of blood protein biomarkers (S100 calcium binding protein B [S100B], glial fibrillary acidic protein [GFAP], neuron specific enolase [NSE], ubiquitin C-terminal hydrolase-L1 [UCH-L1]. tau, and neurofilament proteins) for diagnosis of intracranial lesions on CT following mild TBI. Effects of potential confounding factors and differential diagnostic performance of the included markers were explored. Further, appropriateness of study design, analysis, quality, and demonstration of clinical utility were assessed. Studies published up to October 2016 were identified through a MEDLINE®, Embase, and Cumulative Index to Nursing and Allied Health Literature (CINAHL) search. Following screening of the identified articles, 26 were selected as relevant. We found that measurement of S100B can help informed decision making in the emergency department, possibly reducing resource use; however, there is insufficient evidence that any of the other markers is ready for clinical application. Our work pointed out serious problems in the design, analysis, and reporting of many of the studies, and identified substantial heterogeneity and research gaps. These findings emphasize the importance of methodologically rigorous studies focused on a biomarker's intended use, and defining standardized, validated, and reproducible approaches. The living nature of this systematic review, which will summarize key updated information as it becomes available, can inform and guide future implementation of biomarkers in the clinical arena.

Keywords: biomarkers; diagnosis; living systematic review; meta-analysis; TBI

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Introduction

Traumatic brain injury (TBI) is among the most common neurological disorders worldwide, and globally, its incidence continues to rise.[1,2] According to the Centers for Disease Control (CDC) in the United States, over the past decade, rates of TBI-related emergency department (ED) visits have increased by 70%. Most of these TBIs are classified as mild (mTBI), posing a substantial everyday workload. Clinical diagnosis remains a challenge, and CT is considered the diagnostic cornerstone used in the ED to rule out post-traumatic brain lesions and complement clinical assessment of patients with a possible mTBI.[3] However, it is generally acknowledged that CT is not always available, implies patient radiation exposure, and is relatively costly in terms of ED logistical burden and healthcare expenditures because of the small proportion of subjects ($\sim$10%) diagnosed as having actual traumatic intracranial lesions.[3,4]

The need to manage patients with possible mTBI more effectively and efficiently–to reduce unnecessary CT scans and medical costs, while not compromising patient care and safety–has driven the quest for sensitive blood-based markers as objective parameters that can be easily and rapidly measured in the systemic circulation. Identification of biomarker signatures associated with distinct aspects of TBI pathophysiology may be also of clinical value for a more accurate characterization and risk stratification of TBI, thereby optimizing medical decision making and facilitating individualized and targeted therapeutic intervention. As such, over the past decades, a focused effort has been made to identify novel blood biomarkers for TBI, and a growing number of candidates has been described and proposed,[5–8] leading to the recent incorporation of S100B into the Scandinavian Neurotrauma Guidelines.[9] Nonetheless at present, the role of body fluid biomarkers in TBI is primarily relegated to research studies, and the provision of high quality evidence is paramount to meet regulatory requirements and support their adoption and routine use in clinical practice.

Meta-analysis can exploit the quantity of data collected in separate studies and provide the statistical power to assess more precise estimates of sensitivity and specificity, to determine influence of potential confounding factors on the biomarker diagnostic performance, and to detect differences in the accuracy of different marker tests. Hence, we conducted a systematic review and meta-analysis to comprehensively summarize and critically evaluate the existing body of evidence for the use of blood protein biomarkers for diagnosis of brain injury as assessed by CT in adult patients presenting to the ED after mild head trauma.

We focused on markers for which promising scientific evidence of analytical and clinical validity is available and which therefore, are likely to be rapidly transferable to clinical practice; namely, S100 calcium binding protein B (S100B), glial fibrillary acidic protein (GFAP), neuron specific enolase (NSE), ubiquitin C-terminal hydrolase-L1 (UCH-L1), and tau and neurofilament proteins. As TBI biomarker research and technological and analytical advances are dynamic, we felt that a living systematic review–a high quality, online review that is updated as new research becomes available[10]–would best fit our purpose. The ''*living*'' nature of such work will permit the potential inclusions and investigation of novel markers, marker combinations, and more refined diagnostic time windows for which relevant scientific literature/body of evidence will be gained.

Methods

This review is being prepared as a ''living systematic review,'' initiated in the context of the CENTER-TBI project (www.center-tbi.eu).[10–12] Following a predefined protocol registered on the PROSPERO database (registration number CRD42016048154), we conducted a systematic review and meta-analysis according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines.[13]

Information sources

We searched Ovid MEDLINE® (1946 to October 2016), OVID Embase (1980 to October 2016), OVID Evidence-Based Medicine (EBM) Reviews (October 2016) and Cochrane Library (October 2016) for relevant studies. The search strategies used can be found in the supplementary Appendix (see online supplementary material at http://www.liebertpub.com).

For possible ongoing trials and studies, we searched the World Health Organization (WHO) International Clinical Trials Registry Platform (ICTRP) (searched November 2016) and ClinicalTrials.gov registry (searched November 2016). Update searches will be run every 3 months after publication, to identify new studies for inclusion in this living systematic review.

Additional studies were identified by reviewing the reference lists of published clinical trials and relevant narratives as well as systematic reviews. Abstracts from relevant scientific meetings were also examined, and experts in the field were consulted for any further studies.

Citations were uploaded into a web-based systematic review program (Covidence, Alfred Health Melbourne, Australia) (http://www.covidence.org/).

Study selection

Two reviewers independently reviewed the title and abstract of each citation identified by the search strategy. In the second stage, the full text was reviewed and eligible studies selected. Any disagreement between the two authors was resolved through discussion, or where necessary, arbitration by a third party. Studies were included if the article met the prespecified list of eligibility criteria: studies enrolling adult patients presenting to the ED with a history of possible brain injury complying with any authors' definition of mTBI; report of the admission head CT findings; at least one quantitative measurement of the circulating biomarkers of interest (S100B, GFAP, NSE, UCH-L1, tau, and neurofilament proteins) on admission; and relevant accuracy data.

We included studies containing mixed populations; that is, participants with moderate and severe TBI (Glasgow Coma Score [GCS] <13) or pediatric populations. Studies were included irrespective of their geographic location and language of publication. We excluded studies using non-quantitative methods to assess biomarker concentrations (e.g., Western blot or explorative proteomics). Studies with small cohorts (< 50 participants) were excluded, given the high likelihood of their being underpowered and therefore impacting the reliability of findings.

Data extraction and assessment of methodological quality

Two reviewers independently extracted data using a standardized data abstraction form. We abstracted relevant information related to the study design, patient characteristics (demographic and clinical data, including indices of injury severity, presence of extracerebral injuries and polytrauma, and CT findings) and biomarker characteristics (concentrations, sampling time, cutoffs, and statistical levels of diagnostic accuracy [sensitivity and specificity]), analytical aspects of biomarker testing, and study limitations. Details regarding the definition of mTBI and CT abnormality were also extracted.

In the case of multiple studies from the same research group, authors were contacted to ensure that there was no overlap in patient populations. We also contacted authors for clarification of study sample, missing data, or ambiguity in the cutoffs used. If biomarker measurements were taken at multiple time points, we used the sample on admission for analysis.

The methodological quality of the included studies was independently assessed by two reviewers using a modified version of the tool for quality assessment of studies of diagnostic accuracy included in systematic reviews (QUADAS-2),[14] as recommended by the Cochrane Collaboration. Discrepancies were resolved through discussion or arbitration by a third reviewer.

Statistical analysis and data synthesis

The analysis includes a structured narrative synthesis. We constructed evidentiary tables identifying the results pertinent to diagnostic capabilities of the different biomarkers (detection of intracranial lesions as assessed by CT) and study characteristics for all included studies. We conducted exploratory analyses by plotting estimates of sensitivity and specificity from each study on forest plots and in receiver operating characteristic (ROC) space.

Where adequate data were available, we performed meta-analyses for each biomarker, to summarize data and obtain more precise estimates of diagnostic performance. For studies with diverse thresholds, we meta-analyzed pairs of sensitivity and specificity using the hierarchical summary ROC (HSROC) model, which allows for the possibility of variation in threshold between studies, and also accounts for variation among studies and any potential correlation between sensitivity and specificity.[15] For these analyses, we used the NLMIXED procedure in SAS software (version 9.4; SAS Institute 2011, Cary, NC). For studies that reported data at common prespecified cutoff values, we calculated the pooled estimates of sensitivity and specificity (clinically interpretable), by undertaking a random effects bivariate regression approach.[16]

We explored heterogeneity through visual examination of the forest plot and the SROC plot for each biomarker. However, as there were insufficient studies, lack of individual data, and/or important variation across studies with simultaneous presence of factors with potentially diverging effects on biomarker accuracy estimates, we did not perform meta-regression (by including each potential source of heterogeneity as a covariate in the bivariate model) as planned.

Sensitivity analyses were performed to check the robustness of the results. We used Cook's distance to identify particularly influential studies, and checked for outliers using scatter plots of the standardized predicted random effects. Then, the robustness of the results was checked by refitting the model excluding any outliers and very influential studies. Sensitivity analyses were also conducted to investigate the impact on biomarker performance of studies including mixed populations, bias in the selection of participants, high prevalence of abnormal CT findings, and different definitions of TBI as assessed by CT.

Data processing and statistical analyses were conducted using Review Manager version 5.3 (Cochrane Collaboration, Copenhagen, Denmark) and STATA version 13.0 (StataCorp, Colleage Station, TX) including the user written commands METANDI and MIDAS.

Quality of the evidence

The Grading of Recommendations Assessment, Development, and Evaluation (GRADE)[17] approach was used to assess the overall quality of evidence of the included biomarker tests. The results were summarized using GRADEPro software (Version 3.2, 2008).

Results

Description of studies

Our search strategy identified a total of 7260 citations. Removal of duplicates resulted in 5567 distinct citations, of which 90 full-text articles were assessed for eligibility, and 26 articles[3,18–42] were included in the systematic review (Fig. 1, flow diagram of search and eligibility results, and Table 1). Tables 2 and 3 show the main characteristics of the included publications, and additional details are provided in Tables S1 and S2(see online supplementary material at http://www.liebertpub.com).

Two of the 26 included articles reported biomarker results from the same patient cohort.[34,43] All studies were published in 2000 or later. With the exception of one study published in French,[21] and one published in Italian,[24] all studies were published in English.

The total number of patients with TBI in the included studies was 8127, ranging from 50[28,37] to 1560[42] per study (median 170, interquartile range 104–258). Of those, 865 had positive CT scans, with an average prevalence of 17% (median 13%) (range 5–51%) (Table 2). Table S2 shows the criteria used for the definition of TBI/mTBI and positive CT scans (reference standard) in the different studies. In nine articles, the presence of a skull fracture was considered as a traumatic CT abnormality.

The reported mean or median age of the included patients ranged from 32[38] to 83 years,[39] with 10 studies including children and/or adolescents (patient age <18years). The total subject pool was largely male (median 63% across the studies), with the exception of the study by Thaler and colleagues, which was 68.7% female.[39] Two cohort studies included mild to severe TBI patients (GCS 3–15),[29,38] and two other cohorts included mild to moderate TBI patients (GCS 9–15).[34–36,40] Six studies enrolled TBI patients with multiple trauma and/or extracranial injuries (Table 2). Nine of the included articles reported biomarker concentration from different types of control cohorts, including healthy individuals, or non–brain-injured trauma patients (See Table 3 for details).

Most of the studies defined the specific time frame from injury to blood draw as an inclusion criterion, with the majority of the samples collected within 6 h of injury (16 studies) and with mean or median time ranging from 24.3 min[33] to 5 h (Table 3).[28] In one study, samples were collected within 12 h,[31] and in two studies, they were collected within 24 h.[29,38]

A single marker was evaluated in most of the studies ($n=21$), while one study simultaneously assessed three markers.[40] Of the eligible studies, 22 reported data on S100B (total number of TBI patients 7754), 4 reported data on GFAP (total number of TBI patients 783), 3 reported data on NSE (total number of TBI patients 314), and 2 reported data on UCH-L1 (total number of TBI patients 347). Fewer data were available for tau (one study that included only 50 patients),[28] and we found no studies evaluating neurofilament proteins that met our inclusion criteria.

Methodological quality

The assessments of the methodological quality and risk of bias of the included studies are presented in Figure 2 and Figure S1(see online supplementary material at http://www.liebertpub.com). Participants neither consecutively nor randomly enrolled, the use of vague definitions of mTBI, or inclusion of an unrepresentative spectrum of patients (pediatric population or patients with GCS <13) may lead to incorporation bias, thus limiting the conclusions that can be drawn by affecting the accuracy estimates and compromising the applicability of the results.

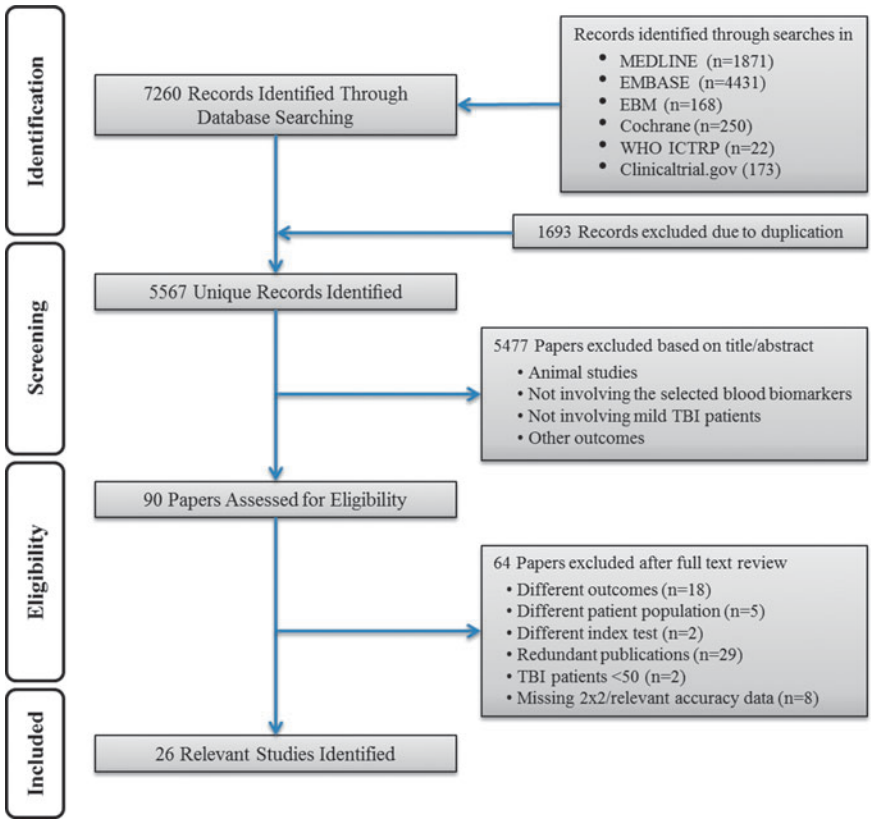

FIG. 1. Study flow diagram.

In half of the studies, thresholds were not prespecified, and ROC analyses were used to determine optimal cutoffs, likely resulting in an overestimation of the diagnostic accuracy of the biomarker evaluated. In addition, the inclusion of skull fracture as a CT abnormality may cause inflation of the accuracy estimates of S100B, whereas, using a brain-specific marker as an index test may result in patients with skull fractures being misclassified as false negative. Finally, in different domains, a substantial number of studies were considered to be at unclear risk of bias because of substandard reporting. We investigated the effect of these factors in sensitivity and subgroup analyses.

Table 1. Summary of the Number and Characteristics of Primary Articles Identified for Each Biomarker

Marker	*No. of studies*	*No. of participants*	*No. of studies (%) by no. of participants in each study*		*No. of studies by GCS*		*No. of studies with predefined cutoff*	*No of studies by sample type*	*Relevant results (Range individual sensitivities and specificities)*
S100B	22	7754 (CT+=713; CT-=7041)	50–100 101–200 201–500 >500	4 (18) 7 (32) 6 (27) 5 (23)	GCS 15: GCS 14–15: GCS 13–15: GCS 9–15: GCS 3–15:	1 3 15 2 1	16	Serum 21 Plasma 1	Sens 0.83–1.00 Spec 0.12–0.77
GFAP	4	783 (CT+=198; CT-=595)	101–200 201–500	1 (25) 3 (75)	GCS 9–15: GCS 3–15:	3 1	0	Serum 3 Plasma 1	Sens 0.67–1.00 Spec 0.00–0.89
NSE	3	314 (CT+=55; CT-=259)	50–100 101–200	1 (33) 2 (67)	GCS 14–15: GCS 13–15:	1 2	0	Serum 3	Sens 0.56–1.00 Spec 0.07–0.77
UCH-L1	2	347 (CT+=64; CT-=283)	50–100 201–500	1 (50) 1 (50)	GCS 9–15:	2	0	Serum 2	Sens 1.00 Spec 0.21–0.39
Tau	1	50 (CT+=10; CT-=40)	50–100	1 (100)	GCS 13–15:	1	0	Serum 1	Sens 0.50 Spec 0.75

GCS, Glasgow Coma Scale; S100B, S100 calcium binding protein B; GFAP, glial fibrillary acidic protein; NSE, neuron specific enolase; UCH-L1, ubiquitin C-terminal hydrolase-L1.

Table 2. Characteristics of the 26 Included Studies

Study ID	BM	No TBI	GCS	Inclusion criteria	Prevalence of positive CT scan findings	Age (years)*	Sex (% female)	Polytrauma/ ECI
Asadollahi 2016[18]	S100B	158	13–15	History of isolated mTBI, age ≥18 yr., admission within 2 h of injury	50%	35.4 (15.8)	48 (30.4%)	No
Bazarian 2013[19]	S100B	787	13–15	GCS >13 measured 30' or more after injury, patient age ≥1 yr., blood drawn within 6 h of injury, CT scan performed as part of the clinical care	6%	38.2 (19.5) Children & adolescents included	287 (36.5%)	Yes
Biberthaler 2001[20]	S100B	52	13–15	History of isolated MHT, GCS 13–15, at least one of the following symptoms: amnesia, LOC, nausea, vomiting, vertigo, or severe headache	29%	NR	14 (27%)	No
Biberthaler 2006 [3]	S100B	1309	13–15	History of isolated head trauma, admission within 3 h, GCS 13–15 on admission, at least one of the following risk factors: LOC, PTA, nausea, vomiting, severe headache, dizziness, vertigo, intoxication, anticoagulation, age >60 yr.	7%	Median (IQR) 47 (32–75)	454 (35%)	No
Bouvier 2009[21]	S100B	105	13–15	History of isolated head trauma and admission within 3 h, GCS 13–15 on admission, at least one of the following risk factors: LOC, PTA, nausea, vomiting, severe headache, dizziness, vertigo, intoxication, anticoagulation, age >60 yr.	15%	53 (range 18–94; IQR 37)	40 (38%)	No
Calcagnile 2012[22]	S100B	512	14–15	History of head trauma, GCS 14–15 during examination and LOC <5' and/or amnesia	5%	42.2	198 (38.5%)	Unclear
Cervellin 2012[23]	S100B	60	14–15	History of MHI, GCS 14–15 on admission, patients with chronic neurological diseases, but not those with suspected/visible brain tumor	33%	58 (range 14–80) Adolescents included	18 (32%)	No
Cervellin 2014[24]	S100B NSE	68	14–15	History of MHI, GCS 13–15 at admission, age >14 yr.	16%	55 (range 15–86) Adolescents included	24 (35%)	Unclear
Egea-Guerrero 2012[25]	S100B	143	15	Patient age ≥14 yr., GCS 15 at hospital admission and one or more of the following symptoms: transitory LOC; amnesia; persistent headache; nausea or vomiting; and vertigo	10.5%	49 (20.6) Including pediatric population >14	54 (37.8%)	Yes
Ingebrigtsen 2000[26]	S100B	182	13–15	Brain injury with brief LOC, GCS 13–15 at admission, age 15–80 yr., admission within 12 h post-injury, CT performed within 24 h after injury	5%	33 (range 15–78) Adolescents included	71 (39%)	Unclear
Laribi 2014[27]	S100B	431	13–15	History of isolated MHI; GCS 13–15 with one or more of the following: amnesia, LOC, nausea, vomiting, vertigo, anticoagulation before injury or severe headache on admission. Patient age ≥18 yr, admission within 3 h after injury	6%	Median (IQR) 36 (24–54)	152 (35%)	No
Ma 2008[28]	Tau	50	13–15	Patient age ≥18 yr., GCS 13–15 at admission, admission within 12 h of injury, CT performed as part of the clinical care, blunt head trauma followed by LOC and/or PTA	20%	40.3 (17.7)	12 (24%)	Unclear
McMahon 2015[29]	GFAP	215	3–15	Admission within 24 h of injury, positive clinical screen for acute TBI necessitating a noncontrast head CT according to ACEP/CDC evidence-based joint practice guidelines	51%	42.1 (18) (range 16–93)	54 (27%)	Yes

(*continued*)

Table 2. (Continued)

Study ID	BM	No TBI	GCS	Inclusion criteria	Prevalence of positive CT scan findings	Age (years)*	Sex (% female)	Polytrauma/ ECI
Morochovic 2009[30]	S100B	102	13–15	Patients with brain injury, GCS 13–15 with or without risk factors	18%	42.0 (19.7) (range 12–84) Including pediatric population	31 (30.39%)	Yes
Muller 2007[31]	S100B	236	13–15	History of brain injury; LOC or PTA; GCS 13–15 at admission; CT scan within 12 h of trauma	9%	39 (range 18–92)	58 (25.7%)	No
Muller 2011[32]	S100B	233	13–15	Adult patients (>16yr.), GCS 13–15	9%	Median (IQR) 48.4 (24–72) (range 11–97) Adolescents included	90 (39%)	No
Mussack 2002[33]	S100B NSE	139	13–15	History of trauma, GCS 13–15, and at least one of the following symptoms: transient LOC (less than 5'), PTA, nausea, vomiting, or vertigo	14%	Median 36.0	33 (24%)	No
Papa 2012[34]	GFAP	307	9–15	History of blunt head trauma followed by LOC, amnesia, or disorientation; GCS 9–15; admission to the ED within 4 h of injury; patient age ≥18 yr.	30%	39 (15) (range 18–89)	38 (35%)	Unclear
Papa 2012[35]	UCH-L1	96	9–15	History of blunt head trauma followed by LOC, amnesia, or disorientation; GCS 9–15; admission to the ED within 4 h of injury; patient age ≥18 yr.	29%	39 (15) (range 18–89)	36 (38%)	Unclear
Papa 2014[36]	S100B GFAP		9–15	History of blunt head trauma followed by LOC, amnesia, or disorientation; GCS 9–15; admission to the ED within 4 h of injury; patient age ≥18 yr.	10%	40 (16)	78 (37%)	Yes
Poli-de-Figueiredo 2006[37]	S100B	50	13–15	Isolated MHI, GCS 13–15, at least one of the following symptoms: amnesia, LOC, nausea, vomiting, vertigo, or severe headache	12%	NR	22 (44%)	No
Romner 2000[38]	S100B	278	3–15	Brain injury with LOC, blood sample collected within 24 h after injury, and CT performed within 24 h after the injury. LOC was considered to have occurred when the patient had amnesia for the trauma event and if accompanying persons reported LOC.	9%	32 (range 1–84) Children & adolescents included	103 (37%)	Yes
Thaler 2015[39]	S100B	782	13–15	MHI (GCS Score 13–15) in patients on medication with PAI with age ≥18 yr., and MHI in patients with age ≥65 yr. independent of PAI intake; admission within 3 h of injury	6%	Median 83 (range 74–88)	537 (68.7%)	No
Welch 2016[40]	S100B GFAP UCH-L1	251	9–15	GCS 9–15 on admission, patient age ≥18 <80 yr.; acceleration or deceleration closed injury to the head; admission within 4 h after injury; ED workup included a head CT scan.	14%	45.6 (18.4) (range 18–80)	100 (39.8%)	Unclear
Wolf 2013[41]	S100B NSE	107	13–15	GCS 13–15 at admission, blunt head trauma, admission to the ED within 3 h of injury	23%	59 (23) (range 18–97)	47 (44%)	No
Zongo 2012[42]	S100B	1560	13–15	Patient age ≥15 yr., GCS 13–15, admission to the ED within 6 h of injury, at least one of the following risk factors: LOC, PTA, repeated vomiting, severe headache, dizziness, vertigo, alcohol intoxication, anticoagulation, and age >65 yrs.	7%	median (IQR) 57 (32–82) Adolescents included	690 (44.2%)	No

*Mean (SD) unless stated otherwise.

ACEP/CDC, American College of Emergency Physicians/ Centers for Disease Control and Prevention; BM, biomarker; ECI, extracranial injury; ED, emergency department; GCS, Glasgow Coma Scale; GFAP, glial fibrillary acidic protein; IQR, interquartile range; LOC, loss of consciousness; MHI, mild head injury; MHT, mild head trauma; mTBI, mild traumatic brain injury; NR, not reported; NSE, neuron specific enolase; PAI, platelet aggregation inhibitor; PTA, post-traumatic amnesia; S100B, S100 calcium binding protein B; UCH-L1, ubiquitin C-terminal hydrolase-L1.

TABLE 3. BIOMARKER CHARACTERISTICS OF THE 26 INCLUDED STUDIES

Study ID	*Sampling type*	*Assay analyzer & manufacturer/is*	*Timing of sample collection*[a]	*Assay range/ CV*	*Cutoff*	*BM levels in TBI patients*[c]	*BM levels in patients with CT positive*[c]	*BM levels in patients with CT negative*[c]	*BM levels in controls*[c]
S-100B									
Asadollahi 2016[18]	Serum (venous)	ECLIA Elecsys® Roche	Within 3 h post-injury	LOD 0.02μgL range 0.02–30 CV <10%	0.11 μg/L	NR	Mean (95% CI) 0.68 μgL (0.58–0.77)	Mean (95% CI) 0.10 μgL (0.07–0.11)	NA
Bazarian 2013[19]	Serum (venous)	ECLIA Elecsys® Roche	Within 6 h post-injury	Range 0.005-39 μgL	0.10 μg/L[b]	0.149 μg L	0.292 μg/L	0.144 μg/L	0.071 μg/L Negative control group
Biberthaler 2001[20]	Serum (venous)	ILMA LIA-mat, Sangtec 100	On admission 116' (18.8)	NR	0.10 μg/L	Mean (SD) 0.470 ng/mL (0.099)	NR	NR	0.05 ng/mL (0.01) Negative control group 7.16 ng/mL (3.77) Positive control group
Biberthaler 2006 [3]	Serum (venous)	ECLIA Elecsys® Roche	Within 3 h post-injury Median 60' (range 40–80')	LOD 0.005 μgL range 0.005–39	0.10 μg/L	0.17 μg/L (0.10–0.37)	0.49 μg/L (0.25–1.46)	0.16 μg/L (0.09–0.33)	0.05 μg/L (0.03–0.06) Negative control group 0.45 μg/L (0.19–2.63) Positive control group
Bouvier 2009[21]	Serum (venous)	ECLIA Elecsys® Roche	On admission Median 1 h 36'	LOD 0.005 μgL range 0.005–39	0.10 μg/L[b]	Mean 0.37 μg/L (SD 0.76)	Mean 0.88 μg/L (SD 1.52)	Mean 0.28 μg/L (SD 0.49)	Mean (SD) 0.05 μg/L (0.02) Negative control group
Calcagnile 2012 [22]	Serum (venous)	ECLIA Elecsys® Roche	Within 3 h post-injury	LOD 0.005 μgL range 0.005–39 Intra-assay CV <2.1%	0.10 μg/L	NR	NR	NR	NA
Cervellin 2012 [23]	Serum (venous)	ILMA Liaison® Diasorin	Within 3 h post-injury 62'	LOD 0.02- μg/L range 0.02–30 CV <10%	0.38 μg/L	NR	Geometric mean 1.35 μg/L (95% CI 0.73–1.97)	Geometric mean 0.48 μg/L (95% CI 0.33–0.63)	NA
Cervellin 2014 [24]	Serum (venous)	ILMA Liaison® Diasorin	Within 3 h post-injury 62'	LOD 0.02- μg/L range 0.02–30 CV <10%	0.56 μg/L	NR	1.5 μg/L (1.19–2.37)	0.22 μg/L (0.12–0.48)	NA
Egea-Guerrero 2012[25]	Serum (venous)	ECLIA Elecsys® Roche	Within 6 h post-injury	LOD 0.005μgL range 0.005–39	0.105μg/L[b]	Mean (95% CI) 0.392 μg/L (0.327–0.456)	Mean (95% CI) 0.585 μg/L (0.363–0.806) Median 0.350	Mean (95% CI) 0.369 μg/L (0.302–0.436) Median 0.220	NA
Ingebrigtsen 2000[26]	Serum (venous)	RIA AB Sangtec	On admission 3 h (range 0.5–12.0)	LOD 0.2 μg/L	0.2 μg/L	Mean 0.5 μg/L (range 0.2–1.9) Detectable in 69 (38%) pts, undetectable in 113 (62%)	Mean 0.7 μg/L (range 0.2–1.9) 9/10 with detectable level	NR	NA
Laribi 2014[27]	Serum (venous)	ECLIA Elecsys® Roche	Within 3 h post-injury Median (IQR) 115' (75–150)	LOD 0.005 μgL range 0.005–39 Intra-assay CV 2.1% Inter-assay CV 2.8%	0.10 μg/L	H0 - 0.14 μg/L (0.08–0.25) H+3 - TBI 0.10 μg/L (0.06–0.16)	H0 - 0.24 μg/L (0.15–0.34) H+3 - 0.13 μg/L (0.10–0.25)	H0 - 0.13 μg/L (0.08–0.25) H+3 - 0.10 μg/L (0.06–0.15)	NA
Morochovic 2009[30]	Serum (venous)	ECLIA Elecsys® Roche	Within 3 h post-injury 1.8 h	LLOD 0.005 μg/L Inter-assay CV 4.9%	0.10 μg/L	Mean (SD) GCS 13 0.26 μg/L (0.34) GCS 14 0.43 μg/L (0.56) GCS 15 0.85 μg/L (3.11)	NR	NR	NA

(*continued*)

TABLE 3. (CONTINUED)

Study ID	*Sampling type*	*Assay analyzer & manufacturer/is*	*Timing of sample collection*[a]	*Assay range/ CV*	*Cutoff*	*BM levels in TBI patients*[c]	*BM levels in patients with CT positive*[c]	*BM levels in patients with CT negative*[c]	*BM levels in controls*[c]
Muller 2007[31]	Serum (venous)	ILMA Liaison® Diasorin	Within 12 h post-injury	LOD 0.013 μg/L Intra-assay CV <5% Inter-assay CV <10%	0.10 μg/L	Mean (95% CI) GCS 13 0.32 μg/L (0.16–0.49) GCS 14 0.22 μg/L (0.13–0.30) GCS 15 0.18μg/L (0.16–0.21)	Mean (95% CI) 0.36 μg/L (0.21–0.50)	Mean (95% CI) 0.18 μg/L 0.16–0.20	NA
Muller 2011[32]	Serum (venous)	ECLIA Elecsys® Roche	NR	NR	0.105 μg/L[b]	NR	NR	NR	NA
Mussack 2002[33]	Serum (venous)	ILMA Liaison® Diasorin	On admission Median 24.3'	LLOD 0.02 ng/mL	0.21 ng/mL	0.24 ng/mL (0.15–0.49)	0.94 ng/mL (0.39–1.43)	0.22 ng/mL (0.14–0.39)	0.06 ng/mL (0.05–0.09) Negative control group
Papa 2014[36]	Serum (venous)	ELISA Banyan	Within 4 h post-injury 3.1 h (95% CI 2.8–3.3)	LLOQ 0.083 ng/mL LLOD 0.017 ng/mL	0.020 ng/mL	NR	NR	NR	NR
Poli-de-Figueiredo 2006[37]	Serum (venous)	ECLIA Elecsys® Roche	On admission Median (IQR) 82' (60–110)	NR	0.10 μg/L	0.29 μg/L (0.14–0.76)	0.75 μg/L (0.66–6.5)	0.26 μg/L (0.12–0.65)	0.04 μg/L (0.03–0.05) Negative control group
Romner 2000[38]	Serum (venous)	RIA Sangtec	Within 24h post-injury 3.8 h (range 0.5–24.0)	LOD 0.2 μg/L	0.2 μg/L (LOD)	Mean 0.6 m g/L (range 0.2–6.2) Detectable in 35% MHI	Mean 2.2 μg/L (range 0.2–12.5) Detectable in 23 (92%) mild-severe TBI pts	NR	Non detectable levels Negative control group
Thaler 2015[39]	Serum (venous)	ECLIA Elecsys® Roche	Within 3 h post-injury Median (IQR) 2.05h (1.30–2.30)	Range 0.005–39 μgL	0.105 μg/L	mTBI 0.15μg/L (0.088–0.291) GCS 15 0.139 (0.085–0.267) GCS 14 0.178 (0.102–0.311) GCS 13 0.284 (0.130–0.652)	0.285 μg/L (0.185–0.532)	0.143 μg/L (0.085–0.274)	NA
Welch 2016[40]	Serum (venous)	ECLIA Cobas 6000® Roche	Within 6 h post-injury	NR	0.10 μg/L[b]	120 (70–230) All values in detectable range	NR	NR	NA
Wolf 2013[41]	Serum (venous)	ECLIA Elecsys® Roche	Within 3 h post-injury	NR	0.105 μg/L[b]	NR	Mean (SD) 0.7 μg/L (1.19)	Mean (SD) 0.21 μg/L (0.26)	NA
Zongo 2012[42]	Plasma (venous)	ECLIA Elecsys® Roche	Within 6 h post-injury	NR	0.10 μg/L[b]	0.23 μg/L (0.14–0.38)	0.46 μg/L (0.27–0.72)	0.22 μg/L (0.14–0.36)	NA
GFAP									
McMahon 2015[29]	Plasma (venous)	ELISA Banyan	Within 24 h post-injury	LLOD 0.01 ng/mL Intra-assay CV 4.3–7.8% Inter-assay CV 7.8–14.3%	0.6 ng/mL	NR	Mean (SD) 2.86 ng/mL (3.74)	Mean (SD) 0.26 ng/mL (0.41)	NA
Papa 2012[34]	Serum (venous)	ELISA Banyan	Within 4 h post-injury 2.6 h (95% CI 2.4–2.9)	LLOD 0.020 ng/mL Intra-assay CV 4.3–7.8%, Inter-assay CV 7.8–14.3%	0.035 ng/mL	0.316 ng/mL (IQR 0.60) Mean (SD) 0.893 (1.677) (95% CI 0.573 – 1.213)	NR	NR	0.010 ng/mL (0.050) Negative control group 0.216 ng/mL (0.275) Orthopedic control group 0.122 ng/mL (0.373) MVA control group 0.010 ng/mL (0.060) All controls
Papa 2014[36]	Serum (venous)	ELISA Banyan	Within 4 h post-injury 3.1 h (95% CI 2.8–3.3)	LLOQ 0.030 ng/mL ULOQ 50.000 ng/mL LLOD 0.008 ng/mL	0.067 ng/mL	NR	NR	NR	NR
Welch 2016[40]	Serum (venous)	ELISA Banyan	Within 6 h post-injury	NR	0 pg/mL	10.3 pg/mL (3.5, 37.4) 45 pts below LOD	NR	NR	NA

(*continued*)

TABLE 3. (CONTINUED)

Study ID	Sampling type	Assay analyzer & manufacturer/is	Timing of sample collection[a]	Assay range/ CV	Cutoff	BM levels in TBI patients[c]	BM levels in patients with CT positive[c]	BM levels in patients with CT negative[c]	BM levels in controls[c]
						(4 with CT+)			
NSE									
Cervellin 2014 [24]	Serum (venous)	IFMA Kryptor (BRAHMS AG)	Within 3 h post-injury 62'	LOD 0.08 μgL CV <6%	9.0 μg/L	NR	13.3 μg/L (12.1–20.3)	9.6 μg/L (8.2–12.3)	NA
Mussack 2002[33]	Serum (venous)	ECLIA Elecsys® Roche	On admission Median 24.3'	LLOD 0.01 ng/mL	12.28 ng/mL	17.50 ng/mL (14.40–21.34)	18.43 ng/mL (15.31–26.03)	17.46 ng/mL (14.31–20.77)	15.55 ng/mL (14.90–17.00) Negative control group
Wolf 2013[41]	Serum (venous)	ECLIA Elecsys® Roche	Within 3 h post-injury	NR	14.7 μg/L[b]	Missing values in 47 pts (44%)	Mean (SD) 18.1 μg/L (10.84)	Mean (SD) 12.4 μg/L (4.82)	NA
UCH-L1									
Papa 2012 [35]	Serum (venous)	ELISA Banyan	Within 4 h post-injury 2.7 h (95% CI 2.4–2.9)	LLOD 0.030 ng/mL	0.029 ng/mL	Mean (SEM) 0.955ng/mL (0.248) (range 0.015–19.25)	Mean (SEM) 1.618 ng/mL (0.474)	Mean (SEM) 0.620 ng/mL (0.254)	Mean (SEM) 0.083 ng/mL (0.005) (range 0.015–0.490) All controls (Negative, orthopedic, MVA controls)
Welch 2016[40]	Serum (venous)	ELISA Banyan	Within 6 h post-injury	NR	40 pg/mL	65.8 (39.6, 125.2) 2 pts below LOD (none with CT+)	NR	NR	NA
Tau									
Ma 2008 [28]	Serum (venous)	ELISA	On admission 5.0 h (2.8)	LOD 1.5 ng/mL	NR	Mean (SD) 5.0 ng/mL (2.98) 15 pts with detectable levels	NR	NR	NA

[a]Mean (SD) unless stated otherwise.

[b]Additional thresholds have been evaluated.

[c]Median (IQR) unless stated otherwise.

Control group definition:

- Negative Control Group: healthy individuals (e.g., healthy volunteers, voluntary blood donors, outpatients for routine blood work) who were checked on their health and potential head trauma status.
- Positive Control Group: patients with moderate to severe brain injury.
- Orthopedic Control Group: non–brain-injured patients presenting to the ED with a single-limb orthopedic injury without blunt head trauma.
- MVA Control Group: patients presenting to the ED after a motor vehicle crash without blunt head trauma

BM, biomarker; CV, coefficient of variation; ECLIA, electrochemiluminescence immunoassay; ED, emergency department; ELISA, enzyme-linked immunosorbent assay; GCS, Glasgow Coma Score; GFAP, glial fibrillary acidic protein; H0, within 3 h after the clinical event; H+3, 3 h after the first sampling; IFMA, immunofluorometric assay; ILMA, immunoluminometric assay; IQR, interquartile range; LIA, luminescence immunoassay; LLOD, lower limit of detection; LLOQ, lower limit of quantification; LOD, limit of detection; mTBI, mild traumatic brain injury; MVA, motor vehicle accident; NA, not applicable; NR, not reported; NSE, neuron specific enolase; pts, patients; RIA, radioimmunoassay; S100B, S100 calcium binding protein B; SEM, standard error of the mean; TBI, traumatic brain injury; UCH-L1, ubiquitin C-terminal hydrolase-L1; ULOQ, upper limit of quantification.

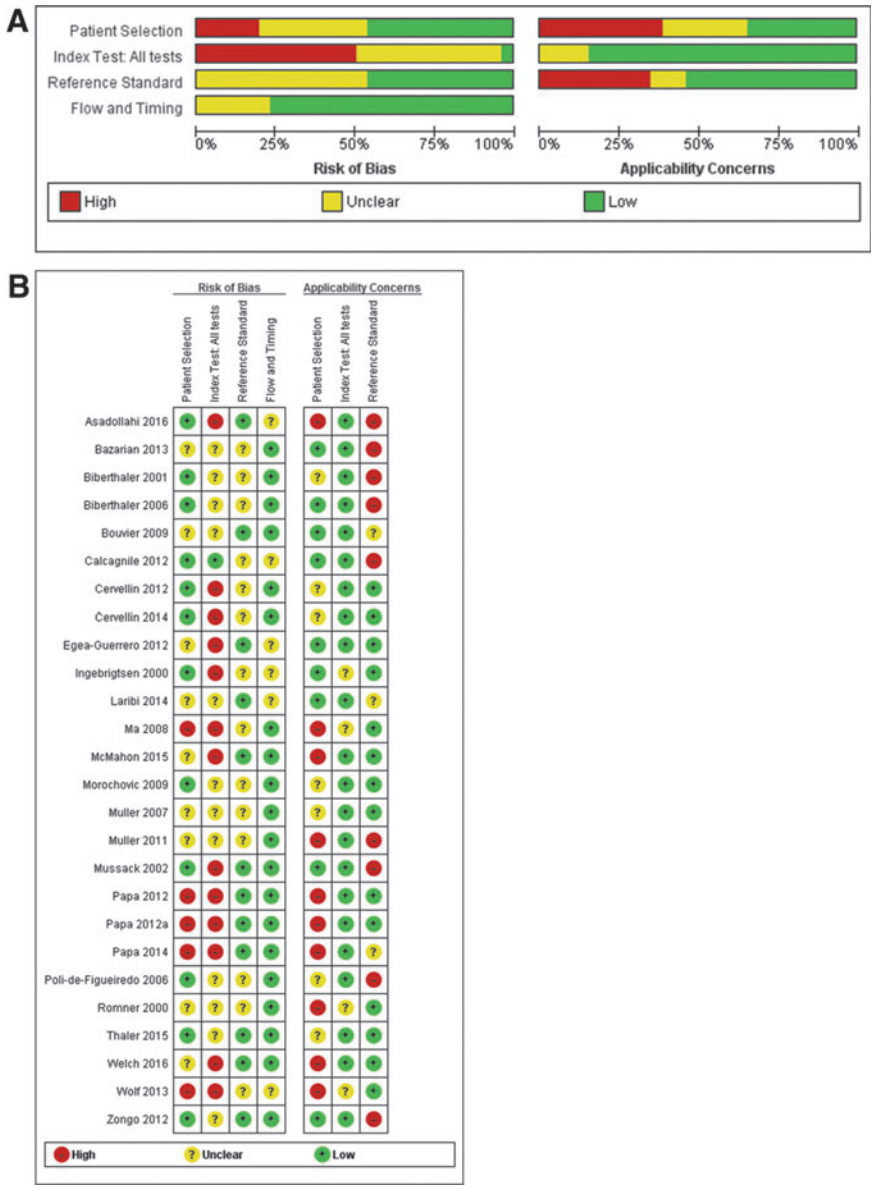

FIG. 2. **(A)** Risk of bias and applicability concerns graph. Review authors' judgments about each domain presented as percentages across included studies. **(B)** Risk of bias and applicability concerns summary. Review authors' judgments about each domain for each included study.

S100B

The accuracy of S100B for detecting intracranial lesions on CT scan was evaluated in 22 studies (7754 patients).[3,18–27,30–33,36–42] The individual sensitivities and the specificities were between 72% and 100% and between 5% and 77%, respectively (Fig. 3). All but six of the included studies used the same cutoff (0.10–0.11μg/L), which represents the 95th percentile of a healthy reference population and is conventionally considered to distinguish physiological from pathophysiological serum concentrations.[3] Seven studies reported multiple cutoffs (Table 3). The summary ROC curve showing the accuracy of S100B across all the studies, regardless the threshold used, is presented in Figure 4.

In terms of the assays/platforms used, most of the studies (13/22) used an automated electrochemiluminescence immunoassay (ECLIA) on an Elecsys® analyzer (Roche Diagnostics), while one used the Cobas 6000 analyzer (Roche Diagnostics). There were four studies conducted using an automated immunoluminometric assay (ILMA) on a Liaison® analyzer (Diasorin), and one was conducted on LIA®-mat (Sangtec® 100); one study used a radioimmunoassay (Sangtec), and one used an enzyme-linked immunosorbent assay (ELISA) platform (Banyan Biomarkers, Inc.) (Table 3). In one study, the analytical performance of the two automated immunoassays (i.e., Diasorin and Roche Diagnostics assays) was compared and, although not interchangeable, the two methods strongly correlated and appeared usable in a similar manner.[27]

Performance of S100B at a 0.10–0.11μg/L cutoff value

To obtain clinically relevant estimates of the performance of S100B, we pooled the results from the 16 studies using the cutoff

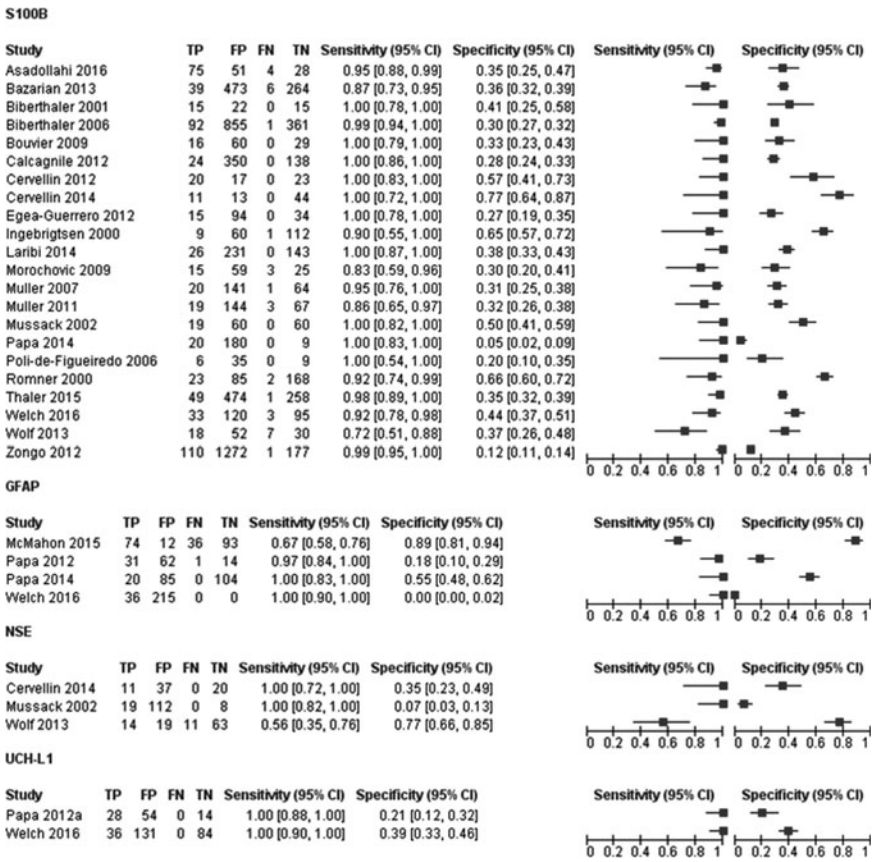

FIG. 3. Forest plot showing individual sensitivity and specificity of circulating S100 calcium binding protein B (S100B), glial fibrillary acidic protein (GFAP), neuron specific enolase (NSE), and ubiquitin C-terminal hydrolase-L1 (UCH-L1) for detection of intracranial lesions on CT. Horizontal lines represent 95% confidence intervals. TP, true positive; FP, false positive; FN, false negative; TN, true negative.

value of 0.10–0.11 μg/L. The individual sensitivities and the specificities for each study included in this meta-analysis were between 72% and 100% and between 5% and 77%, respectively (Fig. 5). The following summary estimates were obtained: sensitivity 96% (95% CI 92–98%), specificity 31% (95% CI 27–36%), positive likelihood ratio 1.4 (1.3–1.5) and negative likelihood ratio 0.12 (0.06–0.25). Figure 5 shows the pooled sensitivity and specificity (the solid red spot in the middle) and the 95% confidence and prediction regions (the inner and outer ellipses, respectively).

There was a significant level of heterogeneity in the results, greater for specificity than for sensitivity (Fig. 5). The value for sensitivity was >80% in all the studies but one.[41] The value for specificity was mainly >30%; however, in the remaining studies, the low specificity was accompanied by a very high sensitivity. However, because of important variation across studies with simultaneous presence of factors (time, presence of extracranial injuries, mixed populations) (Fig. S2) with potentially contrasting effects on the accuracy estimates and lack of individual data and/or insufficient number of studies, we were unable to compare patient characteristics and investigate the effect of the planned sources of heterogeneity (see online supplementary material at http://www.liebertpub.com). Poor reporting of patient and study information also contributed to unknown sources of heterogeneity.

One study was an outlier (Zongo and colleagues).[42] Exclusion of this study made no change in sensitivity (96.3% vs 96.1%); however, specificity increased from 31% to 33%. This could be explained by the fact that in this study, including the greatest number of patients, S100B levels were measured in plasma, thus increasing the probability of false positive results (Fig. S3) (see online supplementary material at http://www.liebertpub.com).

To explore the effect of risk of bias in the patient selection domain on the summary estimates, we excluded eight studies considered at high ($n=1$) or unclear ($n=7$) risk of bias. The exclusion of these studies slightly improved sensitivity (98%) (Fig. S4) (see online supplementary material at http://www.liebertpub.com). A sensitivity analysis was also undertaken to assess the impact of studies containing mixed populations on our findings. We excluded one study (Welch and colleagues),[40] because the authors included patients with moderate TBI (GCS 9–12). There was no impact on our findings. Four studies enrolled a mixed pediatric and adult population. Exclusion of these studies as well as those in which this information was unclearly reported made no difference to our results (Fig. S4).

The prevalence of CT findings was relatively high (> 11%) in seven studies. Excluding these studies resulted in a slight increase in sensitivity and a slight decrease in specificity (98% and 29%, respectively). Finally, eight studies considered skull fracture as a CT abnormality. To explore the impact of the type of reference standard on the summary estimates, we excluded these studies as well as those in which this information was unclearly reported. The exclusion of these studies slightly impacted sensitivity and specificity (93% and 35%, respectively) (Fig. S4).

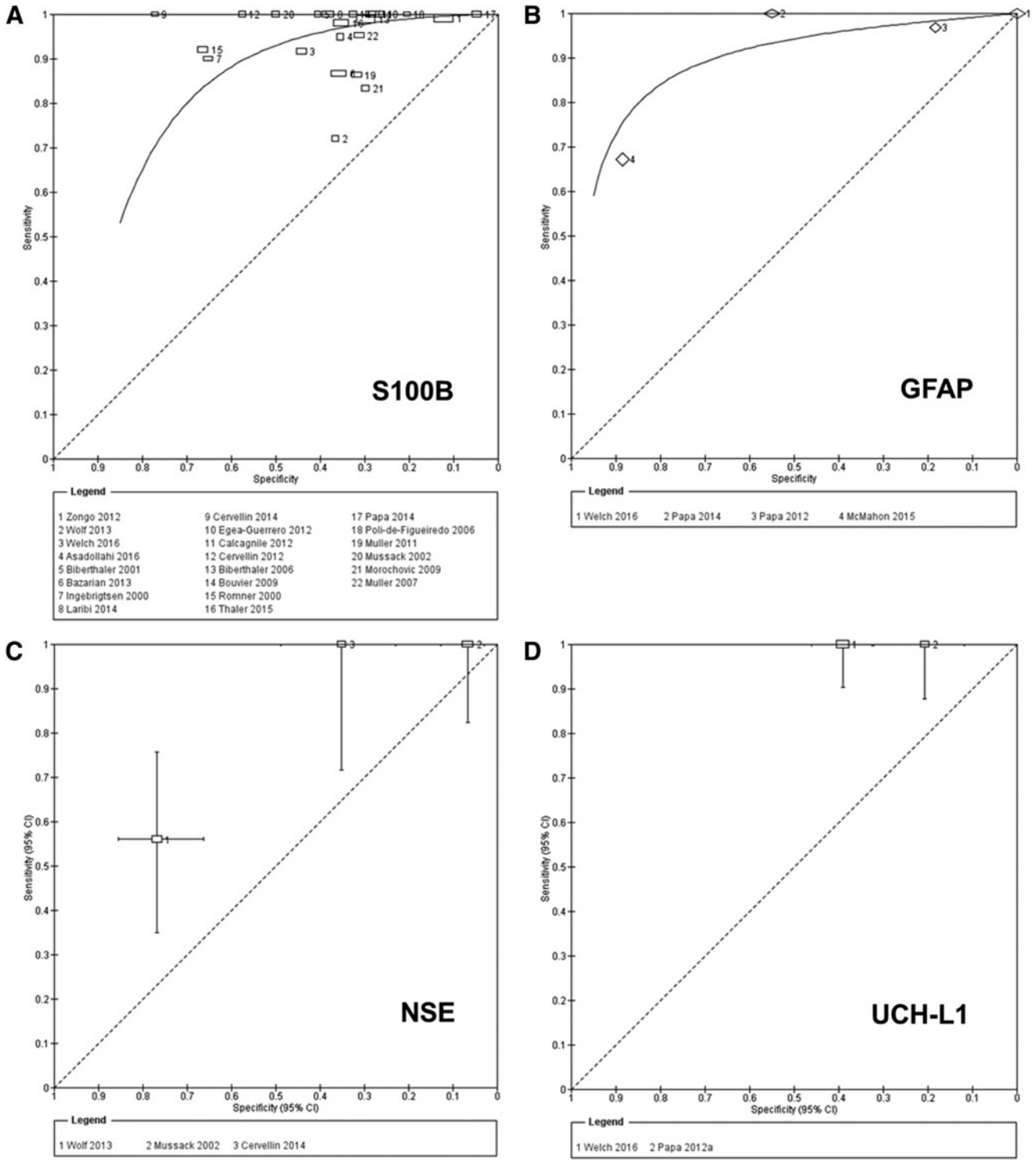

FIG. 4. **(A, B)** Summary receiver operating characteristic (ROC) plots for S100 calcium binding protein B (S100B) and glial fibrillary acidic protein (GFAP) for detection of CT abnormalities. **(C, D)** Study estimates of sensitivity and specificity with 95% confidence intervals plotted in ROC space for neuron specific enolase (NSE), and ubiquitin C-terminal hydrolase-L1 (UCH-L1) for detection of CT abnormalities. Each square represents an individual study; the size of the symbol is proportional to the number of patients in each study. The hierarchical summary ROC (HSROC) model was used to estimate a summary curve using Proc NLMIXED in SAS.

Quality of evidence of S100B

The quality of the evidence for the use of blood S100B levels to diagnose brain injury as assessed by CT scan in patients with mild TBI was moderate (Fig. 6).

GFAP

Eligible studies reporting the accuracy of GFAP for detecting intracranial lesions on CT scan comprised three cohorts with mild to moderate TBI patients and one cohort with mild to severe TBI

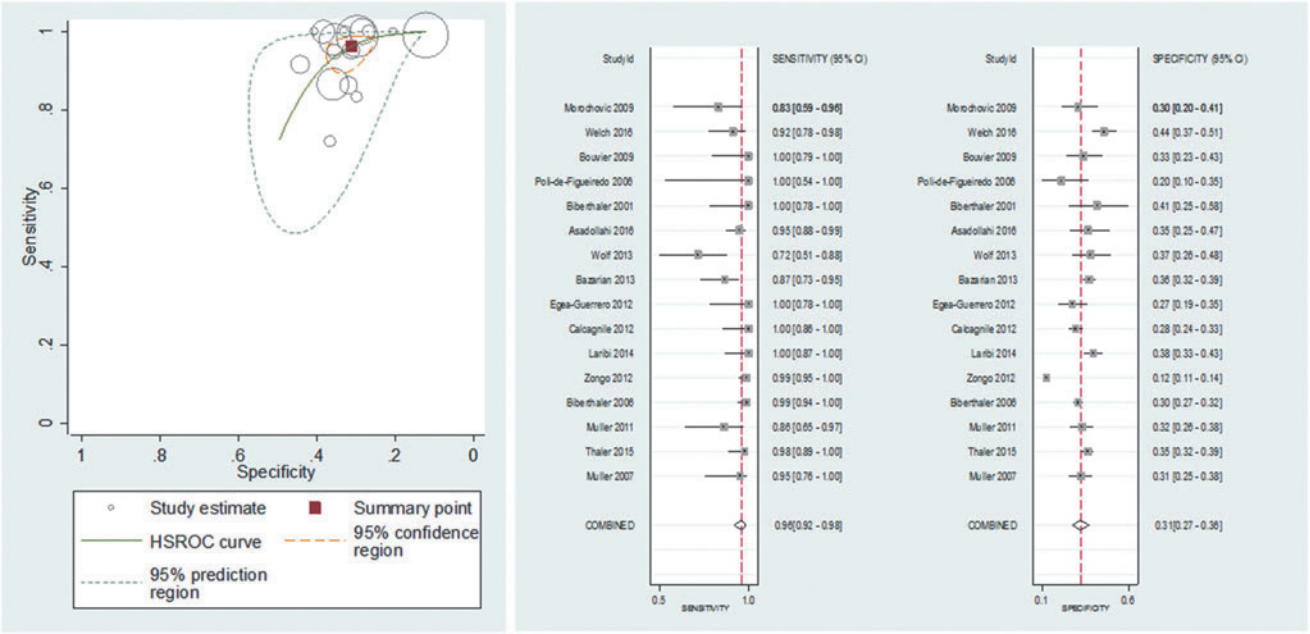

FIG. 5. Summary receiver operating characteristics plot of sensitivity and specificity of S100 calcium binding protein B (S100B) at a 0.10–0.11μg/L cutoff value for detecting intracranial lesions on CT. Each circle represents an individual study; size of the symbol reflects the number of patients in the studies; red solid spot in the middle is summary sensitivity and specificity; inner ellipse represents 95% confidence region, and outer ellipse represents 95% prediction region.

FIG. 6. Summary of evidence for the use of blood S100 calcium binding protein B (S100B) protein concentrations (0.10–0.11μg/L cutoff) to diagnose brain injury as assessed by CT scan in patients with mild traumatic brain injury (mTBI).

patients (783 patients) (Figs. 2 and 3).[29,34,36,40] All studies were recent publications (2012–2016).

The individual sensitivities were between 67% and 100%, whereas the specificities were between 0% and 89%. Sensitivities were sufficiently homogenous, whereas specificities were clearly heterogeneous. The thresholds used, ranging from 0 ng/mL[40] to 0.6ng/mL[29] were not pre-specified, and were determined from ROC analyses. The summary ROC curve of the accuracy of GFAP across all four studies, regardless of the threshold used, is shown in Figure 3.

The planned comparison between S100B and GFAP diagnostic performance was not possible, because of the limited number of studies and different spectrum of patients available for GFAP.

NSE

The accuracy of NSE for discriminating between TBI patients with intracranial lesions on CT scanning from those without lesions was evaluated in three studies (314 patients).[33,41] Figure 2 shows a forest plot of the individual study estimates of sensitivity and specificity. The sensitivities were between 56% and 100%, whereas the specificities were between 7% and 77%. The studies reported a considerable variation in the threshold adopted, ranging from 9 to 14.7 μg/L (Table 3).

UCH-L1

The accuracy of the initial circulating UCH-L1 levels for detection of intracranial lesion on CT was evaluated in two very recent studies (96 and 251 patients respectively)[35,40] including both mild to moderate adult TBI patients (GCS 9–15). The two studies yielded the same sensitivity of 100% (95% CI 88–100) and specificities of 21% (95% CI 12–32) and 39% (95% CI 33–46) (Fig. 2). They reported similar thresholds (0.029–0.04 ng/ml) and used the same assay (Table 3).

Tau

The accuracy of circulating tau (cleaved tau [C-tau]) for diagnosis of CT abnormalities was evaluated only in one small study (50 patients).[28] The sensitivity was 50%, whereas the specificity was 75%. Among the 10 patients with abnormal findings on CT enrolled in this study, 5 (50%) had no detectable C-tau levels.

Discussion

In this systematic review, we have provided a comprehensive and thorough examination of the literature on protein biomarker diagnostic signatures for traumatic brain lesions to define how to best take advantage of these tests in ED daily patient care. We found that of the six biomarkers explored, current evidence only supports the measurement of S100B to help informed decision making in patients presenting to the ED with suspected intracranial lesion following mild TBI, possibly reducing resource use. There is as yet insufficient evidence that GFAP, NSE, and UCH-L1 are ready for clinical application, despite their unequivocal association with TBI. Further, tau and neurofilament proteins were analyzed in too few studies to draw any meaningful conclusions. Importantly, serious problems were observed in many of the studies, ranging from unfocused design and inappropriate target groups to biased reporting and inadequate analysis. These points are further elaborated in the subsequent discussions.

S100B

Our findings demonstrate the clinical utility of S100B for the intended use of allowing physicians to be more selective in their use of CT without compromising care of patients with mTBI. More specifically, the 16 studies applying the same prespecified cutoff of 0.10–0.11 μg/L yielded a pooled sensitivity of 96% (95% CI 92–98%) and specificities of 31% (95% CI 27–36%). Assuming a pretest probability of 10%[44] would mean that, overall, 100 of 1000 tested patients will have a final diagnosis of intracranial lesion. The pooled results obtained for sensitivity and specificity would mean that, of these, between 92 and 98 will test positive (true positives) and 2–8 will test negative (false negatives). Of the 900 with negative CT, between 243 and 324 will test negative (true negatives) and between 576 and 657 will test positive (false positives) (Fig. 6).

Even though this high sensitivity and excellent negative predictive value looks promising, information regarding which lesions could be missed and the associated consequences—if left untreated—is particularly relevant to the broad acceptance and adoption of S100B by the medical community. Accordingly, there is an ongoing debate about the risk of sending home a misdiagnosed patient with a potentially life-threatening condition such as an epidural hemorrhage. From the available data,[3,19,30,32,39,42] we were unable to identify specific types of injury that were systematically missed, albeit subdural hematomas were slightly more frequently misclassified as false negatives. We speculate that this may be because of the brain lesion location and/or extension as well as the pathoanotomical and neurovascular features of the different injuries that cause an altered or delayed leakage of S100B into the circulation. Importantly, one study[30] demonstrated that lesions requiring surgery (one subdural hematoma and one epidural hematoma) were missed by S100B, thereby indicating that this marker—if used alone as a diagnostic tool—is not completely reliable. Given that distinct patterns of injury are linked to patient-specific variability, efforts must to be made to develop advanced multiparameter-based solutions integrating marker signature and patient features. Such multimodal prediction models could be more suitable for an accurate diagnosis, characterization of injury types, and risk stratification of mTBI patients.[45]

It will be also critical to estimate the independent and complementary value of biomarkers and determine whether this strategy provides added diagnostic utility when combined with a careful clinical assessment or when integrated into existing clinical decision rules for the selective use of CT, such as the CT in Head Injury Patients (CHIP) model,[46] the New Orleans criteria,[4] or the Canadian Head CT rule.[47] Unless a biomarker-based approach yields an incremental diagnostic value and clearly demonstrates its superiority over standard, readily available patient characteristics, the broad acceptance in medical practice is unlikely.[48]

Reliability and reproducibility of S100B results also requires a critical consideration of the comparability and potential variability in biomarker measurements when using assays from different manufacturers. We found the adoption of a relatively uniform and standardized approach for S100B determination, with 14 studies using the ECLIA Elecsys® Roche and 2 studies using the ILMA LIA-mat Sangtec 100. These two automated immunometric assays have been demonstrated to have a good correlation, with almost identical diagnostic capability,[27] therefore excluding that this factor could have influenced our conclusions. A comparable level of consistency in analytical methods and assays used is not available for any of the other biomarkers considered in this review.

Our review showed that the results across S100B studies using the prespecified cutoff were consistent in terms of sensitivities and specificities, with only one outlier showing an exceptionally low specificity (12%).[42] A plausible explanation for this anomaly is that in this study, plasma samples were used to measure S100B. This

interpretation fits well with evidence from previous literature demonstrating how the interference of the anticoagulant on the immunoreactivity for S100B can alter its levels relative to serum (values higher by ~20%).[49] Consequently, in the study of Zongo and colleagues, the use of the prespecified cutoff for serum inevitably resulted in a systematic increase of false positive results.[42] This observation, while complicating the analysis of S100B blood levels, points to the need for a more exhaustive knowledge and understanding of pre-analytical factors as potential confounders and sources of variability, and supports the adoption of different cutoff values, depending on the sample type used. Intriguingly, this observation suggests that plasma could be more suitable and possibly desirable for measuring S100B levels in mild TBI patients, because of very low concentrations in this population. However, even after removing the outlier, a considerable heterogeneity remained, necessitating caution when interpreting analysis results.

Investigations from multiple research groups provided evidence that a series of factors other than the brain injury may influence levels of biomarkers in the circulation and, therefore, the diagnostic accuracies. Such factors encompass biomarker characteristics such as molecular weight; injury-specific release mechanisms and clearance (Table S1);[50,51] patient features including presence of extracranial injuries or polytrauma, intoxication, location of the injury, and even genetic, pre-analytical and laboratory-dependent procedures including all steps from management of equipment to execution of assays manufacturing processes; and post-analytical data handling.[19,52–54] We were not able, however, to systematically investigate these potential sources of heterogeneity, because of a substantial variation across studies, the suboptimal reporting of patient and study information, and the coexistence in the same study of factors with contrasting or controversial effects on the accuracy estimates. Taken together, these findings demonstrate that future research must be refined by improvements in study design as well as standards and characterization of patient selection (See box on page 17) .

In this regard, surprisingly, we noted that to date no attempt has been made to specifically investigate the effect of comorbidities and sex on the diagnostic performance of S100B or any other marker. Sex is recognized as a primary determinant of biological variability, responsible for anatomical, neurochemical, and functional brain connectivity differences, heavily influencing neurobiological and neuropathophysiological response.[55] It is also associated with important differences in hormones, metabolism, and the immunological system, which in turn may interfere with the determination of circulating TBI biomarker.[56] Factoring sex into research designs and analyses is a theme under active debate, and is considered fundamental to rigorous and relevant biomedical research. Hence, we emphasize that this is a critical knowledge gap for future investigation, especially in light of the mounting evidence of the changing gender pattern caused by the shift in the TBI population toward older age, also at risk of multiple comorbid conditions (see Thaler and colleagues).[39] Systematic reviews and meta-analyses of individual participant data (IPD) may represent a powerful approach to overcome some of these gaps and limitations,[57] also supported by the current initiatives to share clinical data and the establishment of common repositories, such as the Federal Interagency Traumatic Brain Injury Research (FITBIR) database (https://fitbir.nih.gov/).[58]

Clinical application of S100B implies that choosing the right assessment time point (time between injury and sampling)[59] is an integral part of the test. Based on the results of S100B kinetics studies, guidelines have specifically indicated a time window within $3^{9,60}$ to 6^{9} h post-injury for S100B to detect intracranial lesions. A recent study supported a 3 h window for safe rule-out of acute intracranial lesion in clinical practice, showing that a second blood sampling 3 h after the first one is not informative and resulted in a non-trivial loss of sensitivity of ~6% (e.g., eight patients with positive CT would have been missed).[27] We were unable to further address this specific issue in this review because of the heterogeneity in study design. In addition to post-injury delays in sampling, the delay from obtaining samples to processing and analysis, and the storage conditions during this delay could both be important modulators of S100B stability and assay results. Age, gender, and comorbidities or their combination can also importantly affect the kinetics of S100B.[61] Future studies should inform whether these variables should be considered, and what the potential influence on biomarker results and interpretation is.

The results of our study expand and corroborate those from previous systematic reviews and meta-analyses,[62–64] and confirm that the implementation of S100B might allow a reduction of the number of CT scans by ~30%.[3] These considerations also have broad financial implications for healthcare costs. However, none of the studies in our review explored the cost effectiveness of the use of biomarkers, and the few economic studies and data in the literature are controversial. An earlier study by Ruan and colleagues[65] reported a limited effect of S100B on healthcare resources and a potential economic impact only in specific clinical scenarios (i.e., CT scanning rate >78% or a faster turnaround time of biomarker results of at least 96 min compared with CT scan results). Conversely, in a more recent cost analysis conducted in a Swedish regional hospital, the clinical use of S100B incorporated into the Scandinavian guidelines substantially reduced healthcare costs, especially in cases of strict adherence to management recommendations (71€ per patient).[66] These results are not generalizable, and must be carefully interpreted according to their specific contexts, because of the differences across countries, healthcare systems, hospital settings, and ensuing care patterns. To refine cost calculations, future studies should take these factors into consideration, as well as CT overutilization and the socioeconomic costs associated with increased cancer risks from CT scans. Clear demonstration of cost saving and added benefits beyond those obtained by current management strategies for mTBI are essential for TBI biomarkers to be adopted and widely used by the medical community.

GFAP

Recent narrative reviews have outlined the potential of GFAP for identifying patients with intracranial lesions after head trauma,[7] but none of these used systematic review methods or meta-analyses. In the meta-analysis reported here, we included four studies, in which the diagnostic accuracy of GFAP reflected sensitivities of 67^{29}–$100\%^{36,40}$ and specificities of 0^{40}–100%.[29] Although promising, these results must be approached with caution, because the studies included patients with severe and moderate TBI not representative of the target population of the test (the median prevalence of abnormal CT findings across the studies was 22%), and thresholds were not prespecified, factors that may have inflated the accuracy estimates.[67] For diagnostic validation, it will be fundamental to establish reliable and valid thresholds. Also, GFAP needs be tested in larger clinical studies with a focus on the *intended use*.[68,69] To this end, it has been argued that studies investigating the implementation of biomarker measurements in guidelines for mTBI management—to avoid use of unnecessary CT—should be limited to patients currently recommended for such examination (GCS 14–15), therefore excluding patients with GCS score of 13 for whom biomarker assessment would not add to

clinical examination.[9] As mentioned earlier, the definition of these setting-specific characteristics is also critical for performing reliable cost analyses and determining the primary economic advantage of using blood biomarkers as a pre-head CT screening tool.

A meaningful comparison between GFAP and S100B diagnostic performances was precluded by a substantial difference in study populations. In this context, we note that TBI biomarkers discussed in this review are usually considered individually. Further work should more consistently explore simultaneous assessment of multiple biomarkers providing the framework for comparing the accuracy of tests that have directly been compared in individual studies.

NSE and UCH-L1

The relative dearth of studies evaluating the diagnostic accuracy of NSE, UCH-L1, and Tau in the ED for identifying patients with intracranial lesions following mTBI hampered the possibility of performing meta-analyses. The diagnostic value of NSE remains uncertain, with studies showing remarkable variations and inconsistency. In contrast, the accuracy of UCH-L1 for detecting intracranial lesions on CT scan was evaluated in two studies that yielded an optimal sensitivity (100%) but modest specificities (21–39%). Similar to GFAP, the thresholds used were not prespecified, and the studies included patients with mild to moderate TBI (GCS 9–15). Hence, further studies are required to confirm the reproducibility of these findings and to determine clinical utility in daily bedside care.

Tau and neurofilament proteins

There is insufficient evidence to support the clinical validity of initial circulating c-Tau or neurofilament protein concentrations for the management of patients with mTBI.

Implications for research and practice: Strengths and weakness of the review

Our current insight appreciates the complexity of the pathobiology of TBI most probably requiring multifaceted, multimodal approaches, integrating biomarkers and traditional clinical characteristics to allow a more powerful and accurate characterization and risk stratification of mTBI,[45,70] a premise currently insufficiently reflected in the literature. In addition, if the different biomarkers do indeed reflect different pathophysiological processes[51] with independent information about imaging abnormality, outcome impact, and different diagnostic windows, it is possible that the use of a panel of biomarkers may substantially increase the diagnostic specificity for the end-point of interest.[71,72] Unfortunately, to date, only a few such studies are available. More data are needed to evaluate whether a multi-marker approach based on a panel of biomarkers with distinct time-dependent discriminatory accuracy provides a better performance for the detection and characterization of TBI.

Further, we should be cautious in using CT as a gold standard to judge the performance of circulating biomarkers. When compared with MRI, there is increasing recognition that X-ray CT provides poor sensitivity for structural lesions in TBI such as microbleeds and diffuse axonal injury.[73,74] It follows that we cannot assume that false positivity in detection of CT-visible abnormality equates to false positivity in detection of structural injury, because some of these false positives may be associated with abnormalities on MRI or other advanced neuroimaging, persistent post-concussive symptoms, or long-term neurological, cognitive, and/or neuropsychiatric complications.[75–78] On the other hand, these considerations suggest a broader clinical application of a biomarker-based strategy for diagnosis and management of mTBI. Biomarkers could be used to provide guidance for prognostic groupings, to refine risk stratification, and to inform and guide different management and treatment decisions including indications for advanced MRI techniques (diffusion tensor imaging [DTI], susceptibility weighted imaging [SWI], functional connectivity MRI [fcMRI]), enrollment into clinical trials, and closer monitoring and follow-up of mTBI patients.

From a clinical perspective, biomarkers are not useful if they do not provide real-time decision support for diagnosis of mTBI at the bedside in the ED. A successful approach to the rapid incorporation into routine patient care will be to develop an automated multiplex point of care (POC) device, capable of providing accurate measurements to the clinician at a reasonable cost and with short turnaround times ($\sim$15–20 min).[52,53]

The studies discussed in this review focus primarily on adult patients. There is, however, a growing interest in using biomarkers to optimize diagnosis and management of pediatric mTBI, because of the high risk of TBI in children $\leq$4 years of age, the difficult functional assessments, and the radiation exposure at a young age with ensuing increased cancer risk.[75,79,80] Future studies and systematic reviews taking current and new evidence into account are urgently needed to elucidate the role of biomarkers and establish their clinical utility in this special and vulnerable population.

Several potential limitations merit consideration. Patient selection is a critical aspect in reviews of test accuracy, as it can alter the spectrum of disease and non-disease and the prevalence in the population, strongly impacting test accuracy.[67] Given the heterogeneous and polymorphous nature of TBI, in particular at the milder end of the spectrum, there has been an inconsistent, sometime controversial, definition of mTBI adopted in the included studies. For example, focal neurological deficit has been considered either as an inclusion or as an exclusion criterion (Table S2). This diagnostic uncertainty may possibly have introduced different biases. Although this is an issue that we cannot solve in this review as we had to rely on the criteria that were listed in the included studies; nonetheless, we were able to assess the robustness of the findings using sensitivity analysis, which even demonstrated an improvement in S100B performance (Fig. S4).

However, with respect to selection of patients and study design, our group endorses the importance of methodological rigor, and advocates the use of standardized protocols and a prespecified set of data analysis both as a means to reduce related biases and inadequate reporting, and as a mandatory prerequisite to ensure successful validation and implementation of TBI diagnostic biomarkers. Also critical consideration for sample size planning based on assay precision, clinical significance, and regulatory considerations is necessary. Involvement of regulatory bodies in driving forward harmonization and standardization is considered essential. A major step forward in this direction is the recently established collaboration between researchers and the United States Food and Drug Administration (FDA) in the context of the TBI Endpoints Development (https://tbiendpoints.ucsf.edu/).

Further, despite the broad adoption by the scientific community of the STARD statement (Standards for Reporting of Diagnostic Accuracy studies),[81] we found a number of studies with poor or inconsistent reporting of important information, including patient and specimen characteristics, assay methods, handling of missing data, and statistical analysis methods, in addition to suboptimal descriptions of study findings, which hampered our assessment of potential for bias and interpretation of the results. Our observations are important in raising awareness of key reporting issues in many of the

TBI diagnostic studies. The STARDdem Initiative recently proposed an implementation of the STARD statement with guidance pertinent to studies of cognitive disorders, which is expected to contribute to the development of Alzheimer biomarkers.[82] A similar initiative for TBI biomarker studies could increase transparency and the quality of information provided by such studies, enabling evaluation of internal and external validity and, consequently, a more effective translation and application of their findings to clinical practice.

Harmonization and standardization of biomarker assays that can reliably quantify biomarkers with high analytical precision is critical to ensure that measurements are reproducible and consistent across different analytical platforms and multiple laboratories.

Conclusion

Based on this review, we found that measurement of S100B can help informed decision making in the ED with respect to the selection of adults with a mTBI for CT scan, possibly safely reducing resource use. Conversely, there is little evidence for clinical application of GFAP, UCH-L1, NSE, tau or neurofilaments. However, much work remains to evaluate factors that may influence biomarker levels, and a critical confrontation is required with the implications for actual management, clinical impact, and health economic implications. We also found serious problems in the design, reporting, and analysis of many of the studies, emphasizing the importance for the research community to establish methodological standards and acquire extensive high-quality data for TBI biomarker validation. This is an essential prerequisite for drawing firm conclusions about the performance of tests based on these biomarkers and their clinical utility.

Finally, through the extensive and critical review of the current TBI biomarker existing literature, and state-of-the-science discussions with key opinion leaders and subject matter experts, members of our work group collaborated to evaluate the evidence necessary to demonstrate clinical utility of TBI biomarkers, to identify critical gaps for advancing the field, and to lay the foundation for a ''*living*'' TBI biomarker registry capable of providing an up-to-date list and information on biomarker studies and their results (see Box). Such a strategy, helping to foster collaboration, developing the high levels of evidence needed to support analytical validity and clinical utility, and improving the quality of assessments of novel candidate biomarkers, should establish the solid ground needed for changing biomarker research from data that informs into data that transforms, turning knowledge into a new medical practice.

PANEL: CONSENSUS-BASED RECOMMENDATIONS TO ENHANCE ADOPTION OF DIAGNOSTIC TRAUMATIC BRAIN INJURY BIOMARKERS IN THE CLINIC

1. Standardized Study and Analysis Protocols and Methodological Rigor

- Focus on ''real-world'' clinical questions (appropriate target populations) to optimize clinical translation effectiveness and measure of healthcare economic implication.
- Increase transparency and quality of reporting by calling on investigators to adopt optimal/consolidated guidelines for reporting biomarker work (http://www.stard-statement.org/).
- Reduce biases by implementing critical appraisal tools for evaluating the quality of research (http://www.quadas.org/).
- Develop internationally accepted common reference standards and reference methods to reduce the variability while permitting reliability of biomarker results, reproducibility, and comparability across analytical platforms/laboratories and clinical studies, and the establishment of general exact diagnostic cutoffs.

2. Additional Knowledge Needed to Improve Reliability in the Use of Blood Biomarkers and to Ensure a Successful Validation and Implementation in Clinical Practice

- Assess relationships between specific types and patterns of injury and biomarker kinetics.
- Factor primary biological and clinical variables, including sex and comorbidities, into research design and analyses to exhaustively understand their influence on biomarker pathophysiology and levels.
- Separate and systematically explore special populations (e.g., geriatric and pediatric traumatic brain injury [TBI]).
- Take a thorough investigative approach accounting for pre-analytical factors and adoption of different cutoff values and alternative/complementary time points.

3. Exploration of Novel Opportunities and Strategies for Expanding and Informing Biomarker Clinical Research as a Basis for Developing Multimodal Multidimensional Models to Diagnose Mild TBI

- Simultaneous assessment of multiple biomarkers to compare accuracy and evaluate the performance of multi-marker panels for the detection and characterization of TBI.
- Sharing of clinical data and establishment of common repositories to support individual participant data meta-analyses (IPDMAs) for more robust development of diagnostic models tailored to specific (sub)populations or settings, and testing their generalizability and usefulness.
- Systematic and rigorous evaluation, quantification, and demonstration of the incremental diagnostic value of TBI biomarkers over standard, readily available patient characteristics, and existing prediction rules for the selective use of CT.
- Combination of brain injury biomarkers and patient characteristics yielding independent and incremental diagnostic information toward a powerful multi-parameter platform to assist and enhance clinical decision making (triage for CT scanning) in patients with mTBI at the bedside in the emergency department.

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Author Disclosure Statement

Dr. Wang is a former employee of Banyan Biomarkers Inc. and owns stock. Dr. Wang also receives royalties from licensing fees, and as such, Dr. Wang may benefit financially as a result of the outcomes of this research or work reported in this publication. There are no other disclosures to report.

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Appendix: Search Strategy

MEDLINE® (Ovid) 1946 to October Week 2 2016

1. brain injuries/
2. craniocerebral trauma/
3. head*.ti,ab.
4. brain*.ti,ab.
5. injur*.ti,ab.
6. trauma*.ti,ab.
7. 3 or 4
8. 5 or 6
9. 7 and 8
10. or/1-2,9
11. biological markers/
12. biomarker.ti,ab.
13. marker*.ti,ab.
14. biomarker*.ti,ab.
15. or/11-14
16. S-100*.ti,ab.
17. S100*.ti,ab.
18. S100 proteins.ti,ab.
19. S100 Proteins/
20. or/16-19
21. GFAP.ti,ab.
22. glial protein*.ti,ab.
23. glial fibrillary acidic protein*.ti,ab.
24. glial intermediate filament protein*.ti,ab.
25. astroprotein*.ti,ab.
26. GFA-protein*.ti,ab.
27. glial fibrillary acidic protein/
28. or/21-27
29. C-tau.ti,ab.
30. cleaved-tau.ti,ab.
31. tau protein*.ti,ab.
32. p-tau.ti,ab.
33. tau proteins/
34. or/29-33
35. NSE.ti,ab.
36. neuron specific enolase*.ti,ab.
37. gamma-enolase*.ti,ab.
38. enolase 2.ti,ab.
39. nervous system specific enolase*.ti,ab.

40. phosphopyruvate hydratase*.ti,ab.
41. phosphopyruvate hydratase/
42. or/35-41
43. UCH-L1.ti,ab.
44. UCHL1.ti,ab.
45. ubiquitin carboxyl-terminal hydrolase L-1.ti,ab.
46. ubiquitin c-terminal hydrolase*.ti,ab.
47. ubiquitin carboxy- terminal esterase*.ti,ab.
48. ubiquitin thiolesterase*.ti,ab.
49. ubiquitin carboxyl-terminal hydrolase L-1, human.ti,ab.
50. UCHL1 protein.ti,ab.
51. ubiquitin/
52. ubiquitin thiolesterase/
53. or/43-52
54. NF-H.ti,ab.
55. NFH.ti,ab.
56. NFP-200.ti,ab.
57. NFP200.ti,ab.
58. hyperphosphorylated neurofilament*.ti,ab.
59. neurofilament protein*.ti,ab.
60. neurofilament H protein*.ti,ab.
61. neurofilament triplet protein*.ti,ab.
62. neurofilament protein H.ti,ab.
63. phosphorylated neurofilament.ti,ab.
64. neurofilament proteins/
65. or/54-64
66. blood.ti,ab.
67. serum.ti,ab.
68. plasma.ti,ab.
69. or/66-68
70. or/15,20,28,34,42,53,65
71. and/10,69-70
72. 71 not (animals/ not humans.sh.)

Embase (OVID) 1980 to 2016 Week 43

1. exp brain injury/
2. craniocerebral trauma/
3. (head* and injur*).ti,ab.
4. (brain* and injur*).ti,ab.
5. ((head* or brain*) and trauma*).ti,ab.
6. or/1-5
7. exp biological marker/
8. biomarker.ti,ab.
9. (marker* or biomarker*).ti,ab.
10. or/7-9
11. (blood or serum or plasma).ti,ab.
12. exp blood/
13. exp serum/
14. exp plasma/
15. or/11-14
16. exp prognosis/
17. prognos*.ti,ab.
18. exp diagnostic procedure/
19. diagnos*.ti,ab.
20. di.fs.
21. or/16-20
22. and/6,10,15,21
23. animal/ not human/
24. 22 not 23

Cochrane Library (searched 19 October 2016)

#1 MeSH descriptor: [brain injuries] explode all trees
#2 MeSH descriptor: [craniocerebral trauma] explode all trees
#3 (head* or brain*) and (injur* or trauma*):ti,ab,kw (word variations have been searched)
#4 (#1 OR #2 OR #3)
#5 MeSH descriptor: [biomarkers] explode all trees
#6 (biomarker* or marker*):ti,ab,kw (word variations have been searched)
#7 (#5 OR #6)
#8 MeSH descriptor: [blood] explode all trees
#9 MeSH descriptor: [serum] explode all trees
#10 MeSH descriptor: [plasma] explode all trees
#11 (blood OR serum OR plasma):ti,ab,kw (word variations have been searched)
#12 (#8 OR #9 OR #10 OR #11)
#13 (#4 AND #7 AND #12)