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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

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Running Title: Blood Protein Biomarkers for the Diagnosis of TBI

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 (S100B, GFAP, NSE, 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. Furthermore, 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 CINHAL 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, but 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 a 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.

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Key Words: Biomarkers; Traumatic Brain Injury; Diagnosis; Living Systematic review; Meta-analysis

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 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.[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 health care expenditures owing to the small proportion of subjects (~10%) diagnosed as having actual traumatic intracranial lesion.[3, 4]

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,[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 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, 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 [10]- 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.

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 PRISMA guidelines.[13]

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 Appendix 1. 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). 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 web-based systematic review program (Covidence, Alfred Health Melbourne, Australia) (http://www.covidence.org/).

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.

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),[14] as recommended by the Cochrane Collaboration. Discrepancies were resolved through discussion or arbitration by a third reviewer (EC, ZV or CA).

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.[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 pre-specified cut-off 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 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.

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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 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 (Figure 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 supplementary Tables 1S and 2S.

Two of the 26 included papers 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 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% to 51%) (Table 2). Supplementary Table 2S shows the criteria used for definition of TBI/MTBI and positive CT scans (reference standard) in the different studies. 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 [38] to 83 years [39] 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.[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 papers reported biomarker concentration from different types of control cohorts, including healthy individuals, or non–head-injured trauma patients (See Table 3 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[33] to 5 hours (Table 3).[28] In one study samples were collected within 12 hours[31] and in 2 studies within 24 hours.[29, 38]

A single marker was evaluated in most of the studies (n= 21), while 1 study simultaneously assessed 3 markers.[40] 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),[28] while 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 1S. 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.

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 (Figure 3). 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.[3] Seven studies reported multiple cut-offs (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 on LIA®-mat (Sangtec® 100); one study used a

radioimmunoassay (Sangtec), and one an 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, though, not interchangeable, the two methods strongly correlated and appeared usable in a similar manner.[27]

Performance of S100B at 0.10-0.11µg/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 5). 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 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 over 80% in all the studies but one.[41] 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) (Suppl Fig. 2S) 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).[31] 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 (Supplemental Figure 3S).

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%) (Suppl Fig. 4S). A sensitivity analysis was also undertaken to assess the impact of studies containing mixed populations on our findings. We excluded one study (Welch 2016), 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 (Suppl Fig. 4S).

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) (Suppl Fig. 4S).

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 (Figure 6).

GFAP

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 2 and 3).[29, 34, 36, 40] 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[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 due to 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 3 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% while 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 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 2). They reported similar thresholds (0.029 to 0.04ng/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% 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.

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%[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 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 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 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[30] demonstrated that lesions requiring surgery (1 subdural hematoma and 1 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 CHIP model,[46] the New Orleans criteria[4] or the Canadian Head CT rule.[47] Indeed, 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 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,[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 pre-specified cut-off 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 pre-specified cut-off for serum has 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 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 (Supplemental Table 1),[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, 76-78]. 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 (See Panel).

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.[52] 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.[53] 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).[39] Systematic reviews and meta-analyses of individual participant data (IPD) may represent a powerful approach to overcome some of these gaps and limitations,[54] 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/).[55]

Clinical application of S100B implies that choosing the right assessment time point (time between injury and sampling)[56] 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, 57] to 6[9] 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).[27] 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.[58] 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[59-61] and confirm that the implementation of S100B might allow a reduction of the number of CT scans by approximately 30%.[3] These considerations also have broad financial implications for health care costs. However, none of the studies in our review explored 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.[62] 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 in the Scandinavian guidelines substantially reduced health care costs, especially in case of strict adherence to management recommendations (71€ per patient).[63] These results are not generalizable and must be carefully interpreted according to their specific contexts, owing to 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 over-utilization and 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 diagnostic accuracy of GFAP ranged from sensitivities of 67%[29] to 100%[36, 40] and specificities from 0%[40] to 100%.[29] 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.[64] 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*.[65, 66] 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.[9] 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

A multimarker risk stratification strategy based on a combination of biomarkers reflecting diverse pathophysiological pathways involved in heart failure is a promising approach that could serve as signature of disease and can greatly enhance accuracy of risk prediction

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 [45, 67]–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 endpoint of interest.[68, 69] 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.

Furthermore, we should be cautious in using CT as a gold standard to judge the performance of circulating biomarkers. When compared to magnetic resonance imaging, there is increasing recognition that X-ray CT provides poor sensitivity for structural lesions in TBI such as microbleeds and diffuse axonal injury.[70, 71] It follows that we cannot assume that false positivity in detection of CT-visible abnormality equates to false positivity in detection of structural injury, since some of these false positives may be associated with abnormalities on MR imaging or other advanced neuroimaging, persistent post-concussive

symptoms or long-term neurologic, cognitive and/or neuropsychiatric complications.[72-75] 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, in refining risk stratification, and to inform and guide different management and treatment decisions including indications for advanced MRI techniques (DTI, SWI, 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 emergency department. A successful approach to the rapid incorporation into routine patient care will be to develop 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).[76, 77]

The studies discussed in this review focus primarily on adult patients. There is, though, a growing interest in using biomarkers to optimize diagnosis and management of pediatric MTBI, owing to the high risk of TBI in children 4 years or younger, the difficult functional assessments, and the radiation exposure at a young age with ensuing increased cancer risk.[78-80] 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.[64] 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 mild TBI adopted in the included studies. For instance, focal neurologic deficit has been considered either as an inclusion or as an exclusion criterion (Supplemental Table 2). This diagnostic uncertainty may possibly have introduced different biases. While, 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 (Supplemental Figure 4S).

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 pre-specified set of data analysis as a means to reduce related biases and inadequate reporting as well as a mandatory prerequisite to ensure a 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 FDA in the context of the TBI Endpoints Development (https://tbiendpoints.ucsf.edu/).

Furthermore, 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, beside suboptimal descriptions of study findings, that 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 that 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.

Conclusions

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 mild TBI 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 Workgroup 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 to provide an up-to-date list and information of biomarker studies and their results (see Panel). Such a strategy, helping foster collaboration and developing high levels of evidence needed to support the 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 TBI BIOMARKERS INTO 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 health care economic implication.

■ Increase transparency and quality of reporting by calling upon 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.

■ Assessment of the 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 systematic exploration of special populations (e.g., geriatric and pediatric TBI).

■ Thorough investigative approach accounting for pre-analytical factors and adoption of different cut-off values and alternative/complementary timepoints.

Journal of Neurotrauma
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 (DOI: 10.1089/neu.2017.5182)
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3. Exploration of novel opportunities and strategies for expanding and informing biomarker clinical research as a basis for developing multimodal multidimensional models to diagnosis of mild TBI.

■ Simultaneous assessment of multiple biomarkers to compare accuracy and evaluate the performance of multimarker panels for the detection and characterization of TBI.

■ Sharing clinical data and establishment of common repositories to support individual participant data meta-analyses (IPD-MAs) 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 ED.

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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 all of these individuals 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.

References

1. Roozenbeek, B., Maas, A.I. and Menon, D.K. (2013). Changing patterns in the epidemiology of traumatic brain injury. Nature reviews. Neurology 9, 231-236.

2. Katz, D.I., Cohen, S.I. and Alexander, M.P. (2015). Mild traumatic brain injury. Handb Clin Neurol 127, 131-156.

3. Biberthaler, P., Linsenmeier, U., Pfeifer, K.J., Kroetz, M., Mussack, T., Kanz, K.G., Hoecherl, E.F., Jonas, F., Marzi, I., Leucht, P., Jochum, M. and Mutschler, W. (2006). Serum S-100B concentration provides additional information fot the indication of computed tomography in patients after minor head injury: a prospective multicenter study. Shock 25, 446-453.

4. Haydel, M.J., Preston, C.A., Mills, T.J., Luber, S., Blaudeau, E. and DeBlieux, P.M. (2000). Indications for computed tomography in patients with minor head injury. N Engl J Med 343, 100-105.

5. Mondello, S., Muller, U., Jeromin, A., Streeter, J., Hayes, R.L. and Wang, K.K. (2011). Blood-based diagnostics of traumatic brain injuries. Expert Rev Mol Diagn 11, 65-78.

6. Mondello, S., Schmid, K., Berger, R.P., Kobeissy, F., Italiano, D., Jeromin, A., Hayes, R.L., Tortella, F.C. and Buki, A. (2014). The challenge of mild traumatic brain injury: role of biochemical markers in diagnosis of brain damage. Med Res Rev 34, 503-531.

7. Zetterberg, H., Smith, D.H. and Blennow, K. (2013). Biomarkers of mild traumatic brain injury in cerebrospinal fluid and blood. Nature Reviews Neurology 9, 201-210.

8. Mondello, S., Buki, A., Italiano, D. and Jeromin, A. (2013). alpha-Synuclein in CSF of patients with severe traumatic brain injury. Neurology 80, 1662-1668.

9. Unden, J., Ingebrigtsen, T., Romner, B. and Scandinavian Neurotrauma, C. (2013). Scandinavian guidelines for initial management of minimal, mild and moderate head injuries in adults: an evidence and consensus-based update. BMC medicine 11, 50.

10. Synnot, A., Gruen, R.L., Menon, D., Steyerberg, E.W., Buki, A., Peul, W.C., Elliott, J.H. and Maas, A. (2016). A New Approach to Evidence Synthesis in Traumatic Brain Injury: A Living

Systematic Review. J Neurotrauma.

11. Cnossen, M.C., Scholten, A.C., Lingsma, H.F., Synnot, A., Tavender, E., Gantner, D., Lecky, F., Steyerberg, E.W. and Polinder, S. (2016). Adherence to Guidelines in Adult Patients with Traumatic Brain Injury: A Living Systematic Review. J Neurotrauma.

12. Brazinova, A., Rehorcikova, V., Taylor, M.S., Buckova, V., Majdan, M., Psota, M., Peeters, W., Feigin, V., Theadom, A., Holkovic, L. and Synnot, A. (2016). Epidemiology of Traumatic Brain Injury in Europe: A Living Systematic Review. J Neurotrauma.

13. Moher, D., Liberati, A., Tetzlaff, J., Altman, D.G. and Group, P. (2009). Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. PLoS Med 6, e1000097.

14. Whiting, P.F., Rutjes, A.W., Westwood, M.E., Mallett, S., Deeks, J.J., Reitsma, J.B., Leeflang, M.M., Sterne, J.A., Bossuyt, P.M. and Group, Q.-. (2011). QUADAS-2: a revised tool for the quality assessment of diagnostic accuracy studies. Ann Intern Med 155, 529-536.

15. Rutter, C.M. and Gatsonis, C.A. (2001). A hierarchical regression approach to meta-analysis of diagnostic test accuracy evaluations. Stat Med 20, 2865-2884.

16. Reitsma, J.B., Glas, A.S., Rutjes, A.W., Scholten, R.J., Bossuyt, P.M. and Zwinderman, A.H. (2005). Bivariate analysis of sensitivity and specificity produces informative summary measures in diagnostic reviews. J Clin Epidemiol 58, 982-990.

17. Atkins, D., Best, D., Briss, P.A., Eccles, M., Falck-Ytter, Y., Flottorp, S., Guyatt, G.H., Harbour, R.T., Haugh, M.C., Henry, D., Hill, S., Jaeschke, R., Leng, G., Liberati, A., Magrini, N., Mason, J., Middleton, P., Mrukowicz, J., O'Connell, D., Oxman, A.D., Phillips, B., Schunemann, H.J., Edejer, T., Varonen, H., Vist, G.E., Williams, J.W., Jr., Zaza, S. and Group, G.W. (2004). Grading quality of evidence and strength of recommendations. BMJ (Clinical research ed.) 328, 1490.

18. Asadollahi, S., Heidari, K., Taghizadeh, M., Seidabadi, A.M., Jamshidian, M., Vafaee, A., Manoochehri, M., Shojaee, A.H. and Hatamabadi, H.R. (2016). Reducing head computed

tomography after mild traumatic brain injury: Screening value of clinical findings and S100B protein levels. Brain Inj 30, 172-178.

19. Bazarian, J.J., Blyth, B.J., He, H., Mookerjee, S., Jones, C., Kiechle, K., Moynihan, R., Wojcik, S.M., Grant, W.D., Secreti, L.M., Triner, W., Moscati, R., Leinhart, A., Ellis, G.L. and Khan, J. (2013). Classification accuracy of serum apo a-i and s100b for the diagnosis of mild traumatic brain injury and prediction of abnormal initial head computed tomography scan. Journal of Neurotrauma 30, 1747-1754.

20. Biberthaler, P., Mussack, T., Wiedemann, E., Kanz, K.G., Koelsch, M., Gippner-Steppert, C. and Jochum, M. (2001). Evaluation of S-100b as a specific marker for neuronal damage due to minor head trauma. World Journal of Surgery 25, 93-97.

21. Bouvier, D., Oddoze, C., Ben Haim, D., Moustafa, F., Legrand, A., Alazia, M., Jehle, E., Schmidt, J. and Sapin, V. (2009). Interest of S100B protein blood level determination for the management of patients with minor head trauma. [French]

Interet du dosage serique de la proteine S100B dans la prise en charge du patient apres traumatisme cranien leger. Annales de biologie clinique 67, 425-431.

22. Calcagnile, O., Unden, L. and Unden, J. (2012). Clinical validation of S100B use in management of mild head injury. BMC Emergency Medicine 12.

23. Cervellin, G., Benatti, M., Carbucicchio, A., Mattei, L., Cerasti, D., Aloe, R. and Lippi, G. (2012). Serum levels of protein S100B predict intracranial lesions in mild head injury. Clinical Biochemistry 45, 408-411.

24. Cervellin, G., Benatti, M., Carbucicchio, A., Aloe, R. and Lippi, G. (2014). Protein S100B and neuron-specific enolase (NSE) for the initial evaluation of mild head trauma in adults: Ready for prime time? Biochimica clinica 38, 7.

25. Egea-Guerrero, J.J., Revuelto-Rey, J., Murillo-Cabezas, F., Munoz-Sanchez, M.A., Vilches-Arenas, A., Sanchez-Linares, P., Dominguez-Roldan, J.M. and Leon-Carrion, J. (2012). Accuracy of the S100beta protein as a marker of brain damage in traumatic brain injury. Brain Injury 26, 76-82.

26. Ingebrigtsen, T., Romner, B., Marup-Jensen, S., Dons, M., Lundqvist, C., Bellner, J., Alling, C. and Borgesen, S.E. (2000). The clinical value of serum S-100 protein measurements in minor head injury: A Scandinavian multicentre study. Brain Injury 14, 1047-1055.

27. Laribi, S., Kansao, J., Borderie, D., Collet, C., Deschamps, P., Ababsa, R., Mouniam, L., Got, L., Leon, A., Thoannes, H., Santin, A., Kouyoumdjian, J.C., Dahyot-Fizelier, C., Millet, C., Golmard, J.L. and Beaudeux, J.L. (2014). S100B blood level measurement to exclude cerebral lesions after minor head injury: The multicenter STIC-S100 French study. Clinical Chemistry and Laboratory Medicine 52, 527-536.

28. Ma, M., Lindsell, C.J., Rosenberry, C.M., Shaw, G.J. and Zemlan, F.P. (2008). Serum cleaved tau does not predict postconcussion syndrome after mild traumatic brain injury. Am J Emerg Med 26, 763-768.

29. McMahon, P.J., Panczykowski, D.M., Yue, J.K., Puccio, A.M., Inoue, T., Sorani, M.D., Lingsma, H.F., Maas, A.I., Valadka, A.B., Yuh, E.L., Mukherjee, P., Manley, G.T., Okonkwo, D.O. and Investigators, T.-T. (2015). Measurement of the glial fibrillary acidic protein and its breakdown products GFAP-BDP biomarker for the detection of traumatic brain injury compared to computed tomography and magnetic resonance imaging. J Neurotrauma 32, 527-533.

30. Morochovic, R., Racz, O., Kitka, M., Pingorova, S., Cibur, P., Tomkova, D. and Lenartova, R. (2009). Serum S100B protein in early management of patients after mild traumatic brain injury. European Journal of Neurology 16, 1112-1117.

31. Muller, K., Townend, W., Biasca, N., Unden, J., Waterloo, K., Romner, B. and Ingebrigtsen, T. (2007). S100B serum level predicts computed tomography findings after minor head injury. Journal of Trauma - Injury, Infection and Critical Care 62, 1452-1456.

32. Muller, B., Evangelopoulos, D.S., Bias, K., Wildisen, A., Zimmermann, H. and Exadaktylos, A.K. (2011). Can S-100B serum protein help to save cranial CT resources in a peripheral trauma centre? A study and consensus paper. Emergency Medicine Journal 28, 938-940.

33. Mussack, T., Biberthaler, P., Kanz, K.G., Heckl, U., Gruber, R., Linsenmaier, U., Mutschler, W. and Jochum, M. (2002). Immediate S-100B and neuron-specific enolase plasma measurements for rapid evaluation of primary brain damage in alcohol-intoxicated, minor head-injured patients. Shock 18, 395-400.

34. Papa, L., Lewis, L.M., Falk, J.L., Zhang, Z., Silvestri, S., Giordano, P., Brophy, G.M., Demery, J.A., Dixit, N.K., Ferguson, I., Liu, M.C., Mo, J., Akinyi, L., Schmid, K., Mondello, S., Robertson, C.S., Tortella, F.C., Hayes, R.L. and Wang, K.K. (2012). Elevated levels of serum glial fibrillary acidic protein breakdown products in mild and moderate traumatic brain injury are associated with intracranial lesions and neurosurgical intervention. Ann Emerg Med 59, 471-483.

35. Papa, L., Lewis, L.M., Silvestri, S., Falk, J.L., Giordano, P., Brophy, G.M., Demery, J.A., Liu, M.C., Mo, J., Akinyi, L., Mondello, S., Schmid, K., Robertson, C.S., Tortella, F.C., Hayes, R.L. and Wang, K.K. (2012). Serum levels of ubiquitin C-terminal hydrolase distinguish mild traumatic brain injury from trauma controls and are elevated in mild and moderate traumatic brain injury patients with intracranial lesions and neurosurgical intervention. J Trauma Acute Care Surg 72, 1335-1344.

36. Papa, L., Silvestri, S., Brophy, G.M., Giordano, P., Falk, J.L., Braga, C.F., Tan, C.N., Ameli, N.J., Demery, J.A., Dixit, N.K., Mendes, M.E., Hayes, R.L., Wang, K.K. and Robertson, C.S. (2014). GFAP out-performs S100beta in detecting traumatic intracranial lesions on computed tomography in trauma patients with mild traumatic brain injury and those with extracranial lesions. J Neurotrauma 31, 1815-1822.

37. Poli-de-Figueiredo, L.F., Biberthaler, P., Simao Filho, C., Hauser, C., Mutschler, W. and Jochum, M. (2006). Measurement of S-100B for risk classification of victims sustaining minor head injury - First pilot study in Brazil. Clinics 61, 41-46.

38. Romner, B., Ingebrigtsen, T., Kongstad, P. and Borgesen, S.E. (2000). Traumatic brain damage: serum S-100 protein measurements related to neuroradiological findings. J Neurotrauma 17, 641-647.

39. Thaler, H.W., Schmidsfeld, J., Pusch, M., Pienaar, S., Wunderer, J., Pittermann, P.,

Valenta, R., Gleiss, A., Fialka, C. and Mousavi, M. (2015). Evaluation of S100B in the diagnosis of suspected intracranial hemorrhage after minor head injury in patients who are receiving platelet aggregation inhibitors and in patients 65 years of age and older. J Neurosurg 123, 1202-1208.

40. Welch, R.D., Ayaz, S.I., Lewis, L.M., Unden, J., Chen, J.Y., Mika, V.H., Saville, B., Tyndall, J.A., Nash, M., Buki, A., Barzo, P., Hack, D., Tortella, F.C., Schmid, K., Hayes, R.L., Vossough, A., Sweriduk, S.T. and Bazarian, J.J. (2016). Ability of Serum Glial Fibrillary Acidic Protein, Ubiquitin C-Terminal Hydrolase-L1, and S100B To Differentiate Normal and Abnormal Head Computed Tomography Findings in Patients with Suspected Mild or Moderate Traumatic Brain Injury. J Neurotrauma 33, 203-214.

41. Wolf, H., Frantal, S., Pajenda, G.S., Salameh, O., Widhalm, H., Hajdu, S. and Sarahrudi, K. (2013). Predictive value of neuromarkers supported by a set of clinical criteria in patients with mild traumatic brain injury: S100B protein and neuron-specific enolase on trial. Journal of Neurosurgery 118, 1298-1303.

42. Zongo, D., Ribereau-Gayon, R., Masson, F., Laborey, M., Contrand, B., Salmi, L.R., Montaudon, D., Beaudeux, J.L., Meurin, A., Dousset, V., Loiseau, H. and Lagarde, E. (2012). S100-B protein as a screening tool for the early assessment of minor head injury. Annals of Emergency Medicine 59, 209-218.

43. Papa, L., Lewis, L.M., Silvestri, S., Falk, J.L., Giordano, P., Brophy, G.M., Demery, J.A., Liu, M.C., Mo, J., Akinyi, L., Mondello, S., Schmid, K., Robertson, C.S., Tortella, F.C., Hayes, R.L. and Wang, K.K.W. (2012). Serum levels of ubiquitin C-terminal hydrolase distinguish mild traumatic brain injury from trauma controls and are elevated in mild and moderate traumatic brain injury patients with intracranial lesions and neurosurgical intervention. Journal of Trauma and Acute Care Surgery 72, 1335-1344.

44. Kisat, M., Zafar, S.N., Latif, A., Villegas, C.V., Efron, D.T., Stevens, K.A., Haut, E.R., Schneider, E.B., Zafar, H. and Haider, A.H. (2012). Predictors of positive head CT scan and neurosurgical procedures after minor head trauma. The Journal of surgical research 173, 31-37.

45. Steyerberg, E.W., Moons, K.G., van der Windt, D.A., Hayden, J.A., Perel, P., Schroter, S., Riley, R.D., Hemingway, H., Altman, D.G. and Group, P. (2013). Prognosis Research Strategy (PROGRESS) 3: prognostic model research. PLoS Med 10, e1001381.

46. Smits, M., Dippel, D.W., Steyerberg, E.W., de Haan, G.G., Dekker, H.M., Vos, P.E., Kool, D.R., Nederkoorn, P.J., Hofman, P.A., Twijnstra, A., Tanghe, H.L. and Hunink, M.G. (2007). Predicting intracranial traumatic findings on computed tomography in patients with minor head injury: the CHIP prediction rule. Ann Intern Med 146, 397-405.

47. Stiell, I.G., Wells, G.A., Vandemheen, K., Clement, C., Lesiuk, H., Laupacis, A., McKnight, R.D., Verbeek, R., Brison, R., Cass, D., Eisenhauer, M.E., Greenberg, G. and Worthington, J. (2001). The Canadian CT Head Rule for patients with minor head injury. Lancet 357, 1391-1396.

48. Steyerberg, E.W., Pencina, M.J., Lingsma, H.F., Kattan, M.W., Vickers, A.J. and Van Calster, B. (2012). Assessing the incremental value of diagnostic and prognostic markers: a review and illustration. Eur J Clin Invest 42, 216-228.

49. Tort, A.B., Dietrich, M.O., Goncalves, C.A., Souza, D.O. and Portela, L.V. (2003). Influence of anticoagulants on the measurement of S100B protein in blood. Clinical biochemistry 36, 629-632.

50. Thelin, E.P., Zeiler, F.A., Ercole, A., Mondello, S., Buki, A., Bellander, B.M., Helmy, A., Menon, D.K. and Nelson, D.W. (2017). Serial Sampling of Serum Protein Biomarkers for Monitoring Human Traumatic Brain Injury Dynamics: A Systematic Review. Front Neurol 8, 300.

51. Halford, J., Shen, S., Itamura, K., Levine, J., Chong, A.C., Czerwieniec, G., Glenn, T.C., Hovda, D.A., Vespa, P., Bullock, R., Dietrich, W.D., Mondello, S., Loo, J.A. and Wanner, I.B. (2017). New astroglial injury-defined biomarkers for neurotrauma assessment. Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism, 271678X17724681.

52. Brooks, C.E. and Clayton, J.A. (2017). Sex/gender influences on the nervous system: Basic steps toward clinical progress. Journal of neuroscience research 95, 14-16.

53. Clayton, J.A. and Tannenbaum, C. (2016). Reporting Sex, Gender, or Both in Clinical Research? JAMA 316, 1863-1864.

54. Tudur Smith, C., Marcucci, M., Nolan, S.J., Iorio, A., Sudell, M., Riley, R., Rovers, M.M. and Williamson, P.R. (2016). Individual participant data meta-analyses compared with meta-analyses based on aggregate data. Cochrane Database Syst Rev 9, MR000007.

55. Manley, G.T. and Maas, A.I. (2013). Traumatic brain injury: an international knowledge-based approach. JAMA 310, 473-474.

56. Brophy, G.M., Mondello, S., Papa, L., Robicsek, S.A., Gabrielli, A., Tepas, J., 3rd, Buki, A., Robertson, C., Tortella, F.C., Hayes, R.L. and Wang, K.K. (2011). Biokinetic analysis of ubiquitin C-terminal hydrolase-L1 (UCH-L1) in severe traumatic brain injury patient biofluids. J Neurotrauma 28, 861-870.

57. Jagoda, A.S., Bazarian, J.J., Bruns, J.J., Jr., Cantrill, S.V., Gean, A.D., Howard, P.K., Ghajar, J., Riggio, S., Wright, D.W., Wears, R.L., Bakshy, A., Burgess, P., Wald, M.M., Whitson, R.R., American College of Emergency, P., Centers for Disease, C. and Prevention (2008). Clinical policy: neuroimaging and decisionmaking in adult mild traumatic brain injury in the acute setting. Ann Emerg Med 52, 714-748.

58. Calcagnile, O., Holmen, A., Chew, M. and Unden, J. (2013). S100B levels are affected by older age but not by alcohol intoxication following mild traumatic brain injury. Scandinavian journal of trauma, resuscitation and emergency medicine 21, 52.

59. Unden, J. and Romner, B. (2010). Can low serum levels of S100B predict normal CT findings after minor head injury in adults?: An evidence-based review and meta-analysis. Journal of Head Trauma Rehabilitation 25, 228-240.

60. Heidari, K., Vafaee, A., Rastekenari, A.M., Taghizadeh, M., Shad, E.G., Eley, R., Sinnott, M. and Asadollahi, S. (2015). S100B protein as a screening tool for computed tomography findings after mild traumatic brain injury: Systematic review and meta-analysis. Brain Inj, 1-12.

61. Thelin, E.P., Nelson, D.W. and Bellander, B.M. (2017). A review of the clinical utility of

serum S100B protein levels in the assessment of traumatic brain injury. Acta Neurochirurgica 159, 209-225.

62. Ruan, S., Noyes, K. and Bazarian, J.J. (2009). The economic impact of S-100B as a pre-head CT screening test on emergency department management of adult patients with mild traumatic brain injury. Journal of Neurotrauma 26, 1655-1664.

63. Calcagnile, O., Anell, A. and Unden, J. (2016). The addition of S100B to guidelines for management of mild head injury is potentially cost saving. BMC Neurology 16 (1) (no pagination).

64. Cochrane_Collaboration Diagnostic Test Accuracy Working Group: handbook for DTA reviews. http://srdta.cochrane.org/handbook-dta-review.

65. (2016). In: *BEST (Biomarkers, EndpointS, and other Tools) Resource*: Maryland.

66. Amur, S., LaVange, L., Zineh, I., Buckman-Garner, S. and Woodcock, J. (2015). Biomarker Qualification: Toward a Multiple Stakeholder Framework for Biomarker Development, Regulatory Acceptance, and Utilization. Clinical pharmacology and therapeutics 98, 34-46.

67. Jeromin, A., Maas, A.I., Buki, A. and Mondello, S. (2015). Developing a molecular taxonomy for traumatic brain injury: a perspective to enable the development of diagnostics and therapeutics. Biomark Med 9, 619-621.

68. Mondello, S., Gabrielli, A., Catani, S., D'Ippolito, M., Jeromin, A., Ciaramella, A., Bossu, P., Schmid, K., Tortella, F., Wang, K.K., Hayes, R.L. and Formisano, R. (2012). Increased levels of serum MAP-2 at 6-months correlate with improved outcome in survivors of severe traumatic brain injury. Brain Inj 26, 1629-1635.

69. Mondello, S., Galuppo, M., Mazzon, E., Italiano, D., Mondello, P., Aloisi, C. and Cuzzocrea, S. (2011). Glutamine treatment attenuates the development of organ injury induced by zymosan administration in mice. Eur J Pharmacol 658, 28-40.

70. Yuh, E.L., Cooper, S.R., Mukherjee, P., Yue, J.K., Lingsma, H.F., Gordon, W.A., Valadka, A.B., Okonkwo, D.O., Schnyer, D.M., Vassar, M.J., Maas, A.I., Manley, G.T. and Track-Tbi, I. (2014). Diffusion tensor imaging for outcome prediction in mild traumatic brain injury: a

TRACK-TBI study. J Neurotrauma 31, 1457-1477.

71. Yuh, E.L., Mukherjee, P., Lingsma, H.F., Yue, J.K., Ferguson, A.R., Gordon, W.A., Valadka, A.B., Schnyer, D.M., Okonkwo, D.O., Maas, A.I., Manley, G.T. and Investigators, T.-T. (2013). Magnetic resonance imaging improves 3-month outcome prediction in mild traumatic brain injury. Annals of neurology 73, 224-235.

72. Cnossen, M.C., Winkler, E.A., Yue, J.K., Okonkwo, D.O., Valadka, A., Steyerberg, E.W., Lingsma, H. and Manley, G.T.M.D.P.D. (2017). Development of a Prediction Model for Post-Concussive Symptoms following Mild Traumatic Brain Injury: A TRACK-TBI Pilot Study. J Neurotrauma.

73. Ling, H., Morris, H.R., Neal, J.W., Lees, A.J., Hardy, J., Holton, J.L., Revesz, T. and Williams, D.D. (2017). Mixed pathologies including chronic traumatic encephalopathy account for dementia in retired association football (soccer) players. Acta Neuropathol 133, 337-352.

74. Mac Donald, C.L., Barber, J., Andre, J., Evans, N., Panks, C., Sun, S., Zalewski, K., Elizabeth Sanders, R. and Temkin, N. (2017). 5-Year imaging sequelae of concussive blast injury and relation to early clinical outcome. Neuroimage Clin 14, 371-378.

75. Kou, Z., Gattu, R., Kobeissy, F., Welch, R.D., O'Neil, B.J., Woodard, J.L., Ayaz, S.I., Kulek, A., Kas-Shamoun, R., Mika, V., Zuk, C., Tomasello, F. and Mondello, S. (2013). Combining biochemical and imaging markers to improve diagnosis and characterization of mild traumatic brain injury in the acute setting: results from a pilot study. PLoS One 8, e80296.

76. Arruda, D.L., Wilson, W.C., Nguyen, C., Yao, Q.W., Caiazzo, R.J., Talpasanu, I., Dow, D.E. and Liu, B.C. (2009). Microelectrical sensors as emerging platforms for protein biomarker detection in point-of-care diagnostics. Expert Rev Mol Diagn 9, 749-755.

77. Morrow, D.A. and de Lemos, J.A. (2007). Benchmarks for the assessment of novel cardiovascular biomarkers. Circulation 115, 949-952.

78. Levin, H.S. and Diaz-Arrastia, R.R. (2015). Diagnosis, prognosis, and clinical management of mild traumatic brain injury. Lancet neurology 14, 506-517.

79. Hayes, R.L., Mondello, S. and Wang, K. (2011). Glial fibrillary acidic protein: a promising biomarker in pediatric brain injury. Pediatr Crit Care Med 12, 603-604.

80. Brenner, D.J. and Hall, E.J. (2007). Computed tomography--an increasing source of radiation exposure. N Engl J Med 357, 2277-2284.

81. Bossuyt, P.M., Reitsma, J.B., Bruns, D.E., Gatsonis, C.A., Glasziou, P.P., Irwig, L.M., Lijmer, J.G., Moher, D., Rennie, D., de Vet, H.C. and Standards for Reporting of Diagnostic, A. (2003). Towards complete and accurate reporting of studies of diagnostic accuracy: the STARD initiative. Clinical biochemistry 36, 2-7.

82. Noel-Storr, A.H., McCleery, J.M., Richard, E., Ritchie, C.W., Flicker, L., Cullum, S.J., Davis, D., Quinn, T.J., Hyde, C., Rutjes, A.W., Smailagic, N., Marcus, S., Black, S., Blennow, K., Brayne, C., Fiorivanti, M., Johnson, J.K., Kopke, S., Schneider, L.S., Simmons, A., Mattsson, N., Zetterberg, H., Bossuyt, P.M., Wilcock, G. and McShane, R. (2014). Reporting standards for studies of diagnostic test accuracy in dementia: The STARDdem Initiative. Neurology 83, 364-373.

Table 1. 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

Abbreviations: CT= Computed tomography; GCS= Glasgow Coma Scale; N= Number.

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)	Poly trauma / ECI
Asadollahi 2016 [18]	S100B	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 [19]	S100B	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 [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 factor: LOC, PTA, nausea, vomiting, severe headache, dizziness,	7%	Median (IQR) 47 (32-75)	454 (35%)	No

				vertigo, intoxication, anticoagulation, age>60 yrs.				
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 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 [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 neurologic diseases, but not those with suspected/visible brain tumor.	33%	58 (range 14–80) Adolescents included	18 (32%)	No
Cervellin 2014 [24]	S100BNS	68	14–15	History of MHI, GCS 13-15 at admission. age>14 yrs	16%	55 (range 15–86) Adolescents included	24 (35%)	Unclear

	E							
Egea-Guerrero 2012 [25]	S100B	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
Ingebrigtsen 2000 [26]	S100B	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 [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 ≥ 18yrs, admission within 3 h after injury.	6%	Median (IQR) 36 (24–54)	152 (35%)	No
Ma 2008 [28]	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	20%	40.3 (17.7)	12 (24%)	Unclear

				followed by LOC and/or PTA.				
McMahon 2015 [29]	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 [30]	S100B	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 [31]	S100B	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 [32]	S100B	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	S1	139	13-15	History of trauma, GCS 13–15, and at least	14%	Median	33 (24%)	No

2002 [33]	00BNSE			one of the following symptoms: transient LOC (less than 5'), PTA, nausea, vomiting, or vertigo		36.0		
Papa 2012a [34]	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
Papa 2012b [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 4h of injury. Patient age ≥ 18yrs.	29%	39 (15) (range 18–89)	36 (38%)	Unclear
Papa 2014 [36]	S100BGFAP		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-Figueire	S100	50	13-15	Isolated MHI. GCS 13-15. At least one of the following symptoms: amnesia, LOC, nausea,	12%	NR	22 (44%)	No

do 2006 [37]	B			vomiting, vertigo, or severe headache.				
Romner 2000 [38]	S100B	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 [39]	S100B	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 [40]	S100B GFAP UC	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

	H-L1							
Wolf 2013 [41]	S100B 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 [42]	S100B	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 3. 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 [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	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[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 0.005µgL 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 [21]	Serum (venous)	ECLIA Elecsys® Roche	On admission Median	LOD 0.005µgL range 0.005-	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)

			1h36’	39					Negative Control Group
Calcagnile 2012 [22]	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 [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†	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 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 [27]	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%	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

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reviewed and accepted for publication, but has yet to undergo copyediting and proof correction. The final published version may differ from this proof.

				Inter-assay CV 2.8%					
Morochovic 2009 [30]	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 [31]	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	Mean (95%CI) 0.36 µg/L (0.21– 0.50)	Mean (95%CI) 0.18 µg/L 0.16–0.20	NA

						0.18µg/L (0.16–0.21)			
Muller 2011 [32]	Serum (venous)	ECLIA Elecsys® Roche	NR	NR	0.105 µg/L†	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 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
Poli-de-Figueire	Serum (venous)	ECLIA Elecsys®	On admission	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–

do 2006 [37]		Roche	Median (IQR) 82' (60-110)						0.05) Negative Control Group
Romner 2000 [38]	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 [39]	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 [40]	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 [41]	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 [42]	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
GFAP									
McMahon 2015 [29]	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	Serum	ELISA	Within 4h	LLOD 0.020	0.035	0.316 ng/mL	NR	NR	0.010

2012a [34]	(venous)	Banyan	post-injury 2.6 h (95% CI 2.4-2.9)	ng/mL Intra-assay CV 4.3-7.8%, Inter-assay CV 7.8-14.3%	ng/mL	(IQR 0.60) Mean (SD) 0.893 (1.677) (95% CI 0.573 - 1.213)			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 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
Welch 2016 [40]	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									
Cervelli n 2014 [24]	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

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Mussack 2002 [4133]	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 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 [35]	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)
Welch 2016 [40]	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 [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 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

Figure Legend

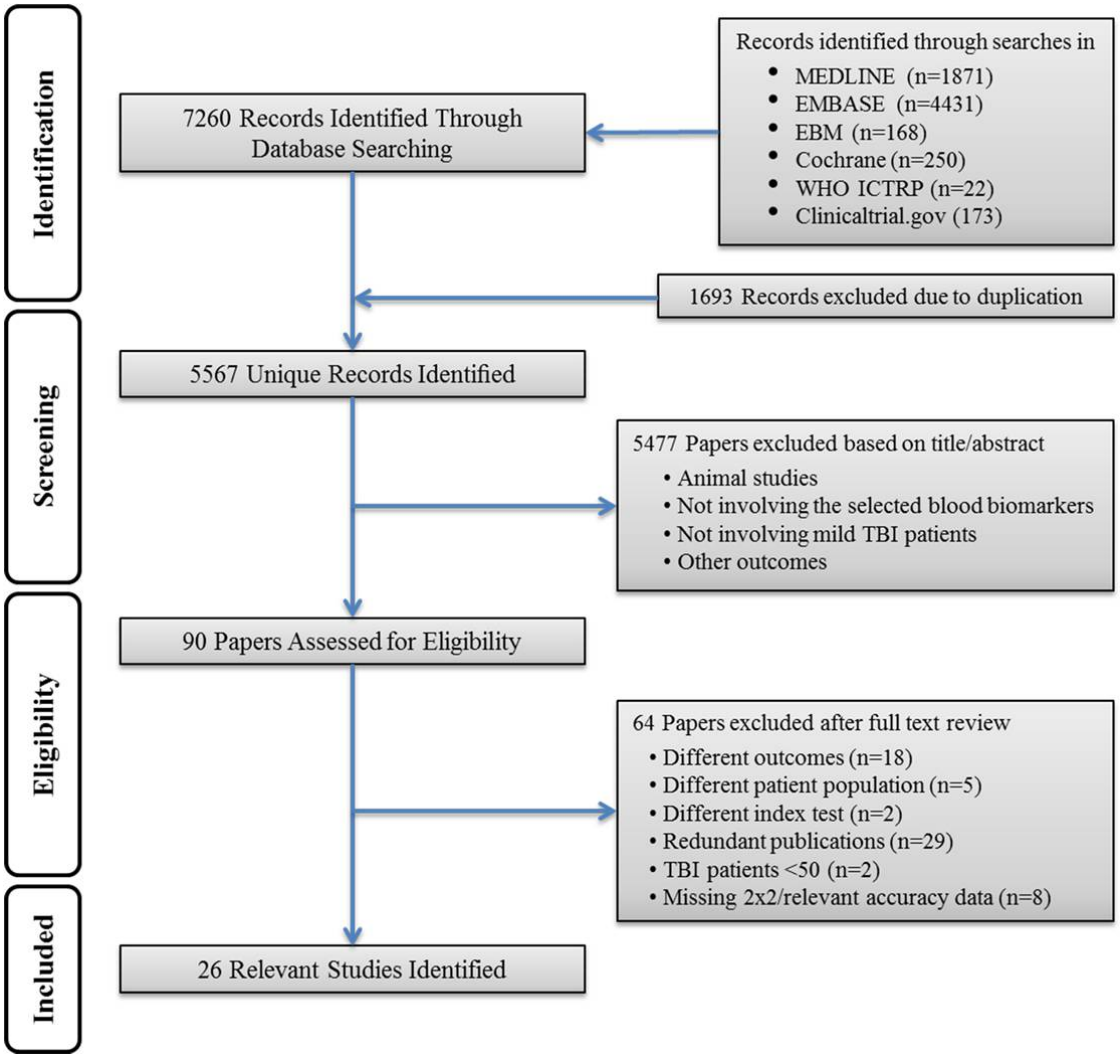

Figure 1. Study flow diagram.

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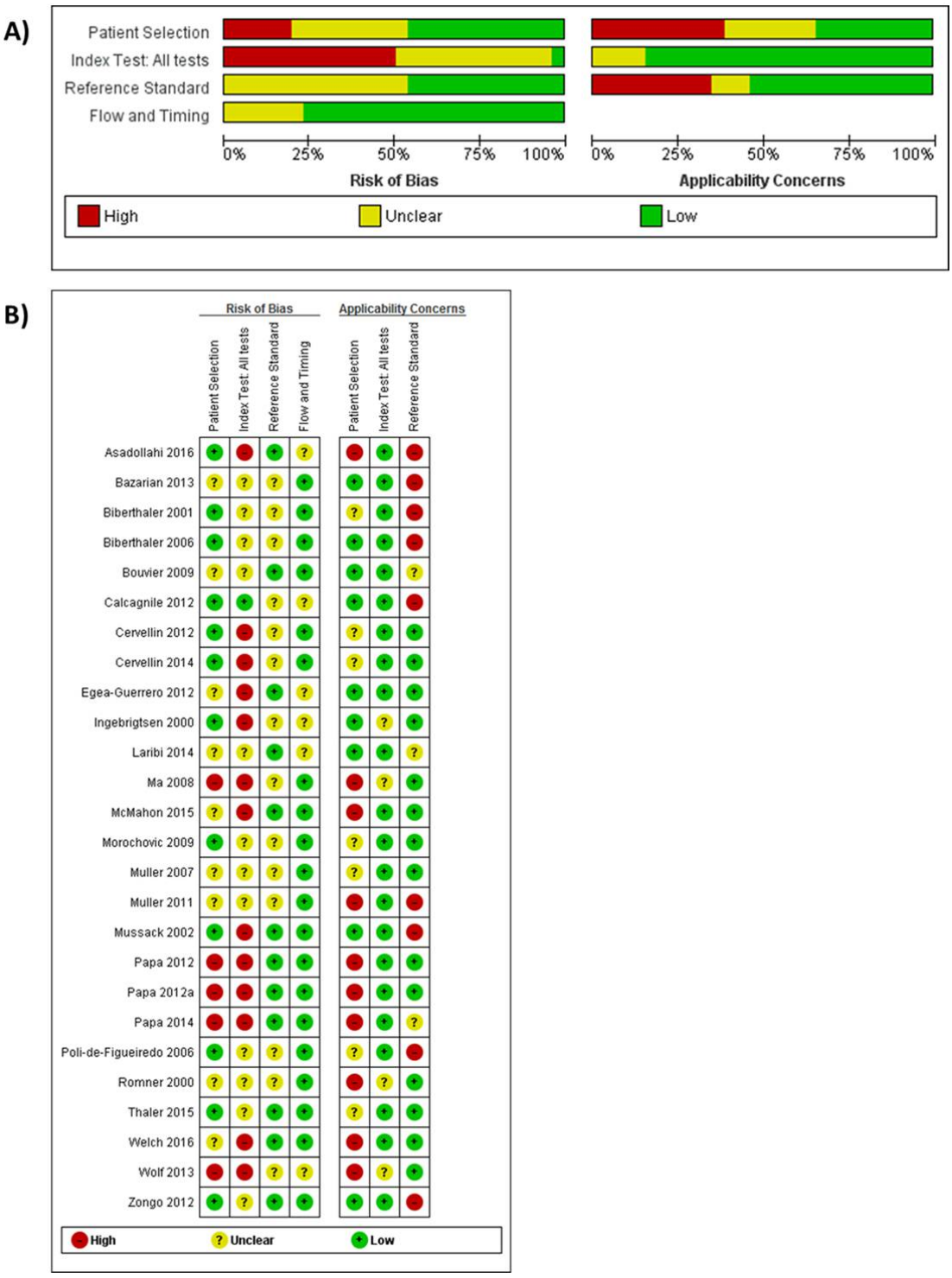

Figure 2. (A) Risk of bias and applicability concerns graph. Review authors' judgments about each domain presented as percentages across included studies. **(B) Risk of bias and applicability concerns summary.** Review authors' judgments about each domain for each included study.

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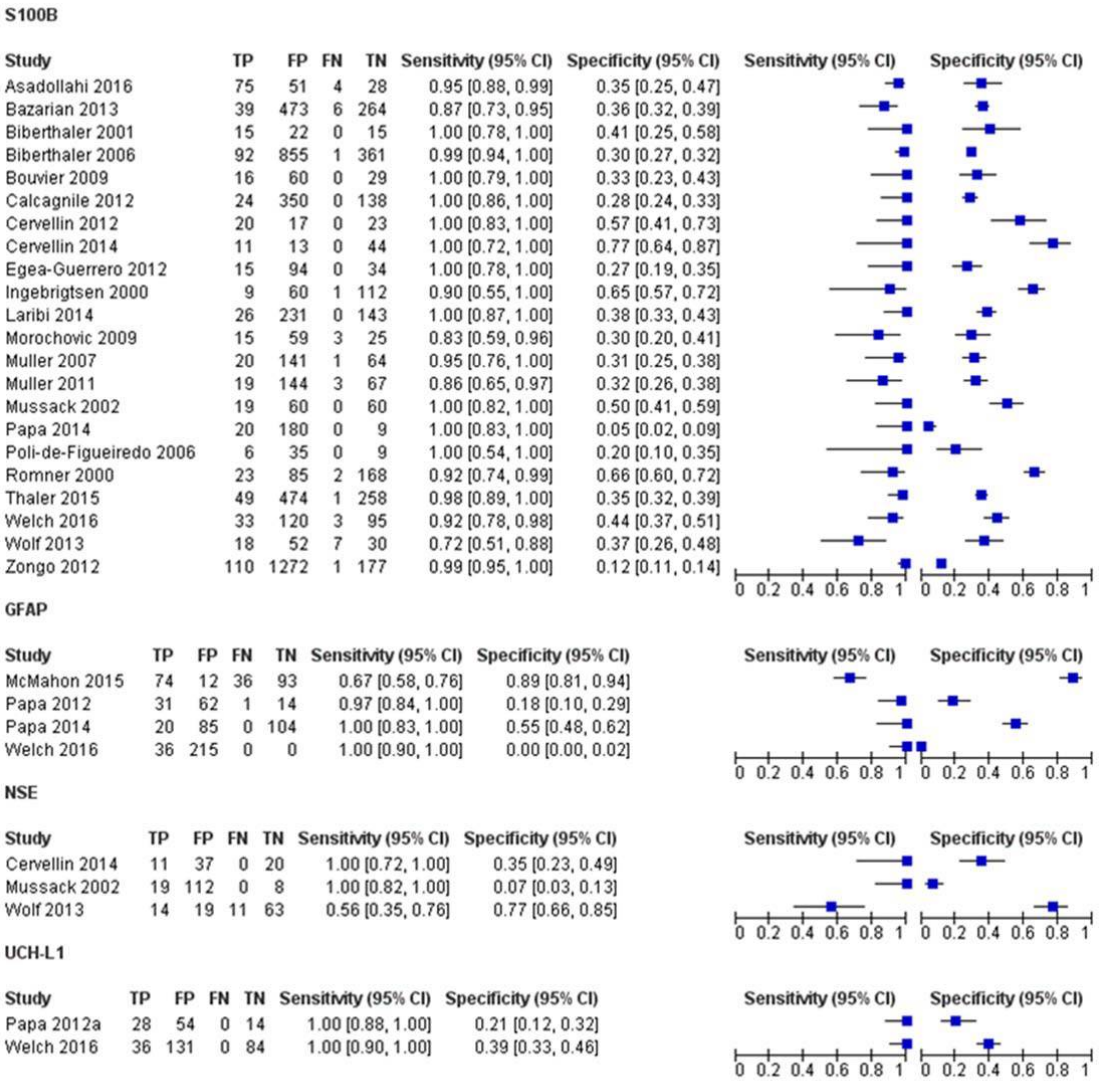

Figure 3. Forest plot showing individual sensitivity and specificity of circulating S100B, GFAP, NSE and UCH-L1 for detection of intracranial lesions on CT. Horizontal lines represent 95% confidence intervals. TP=true positive; FP=false positive; FN=false negative; TN=true negative.

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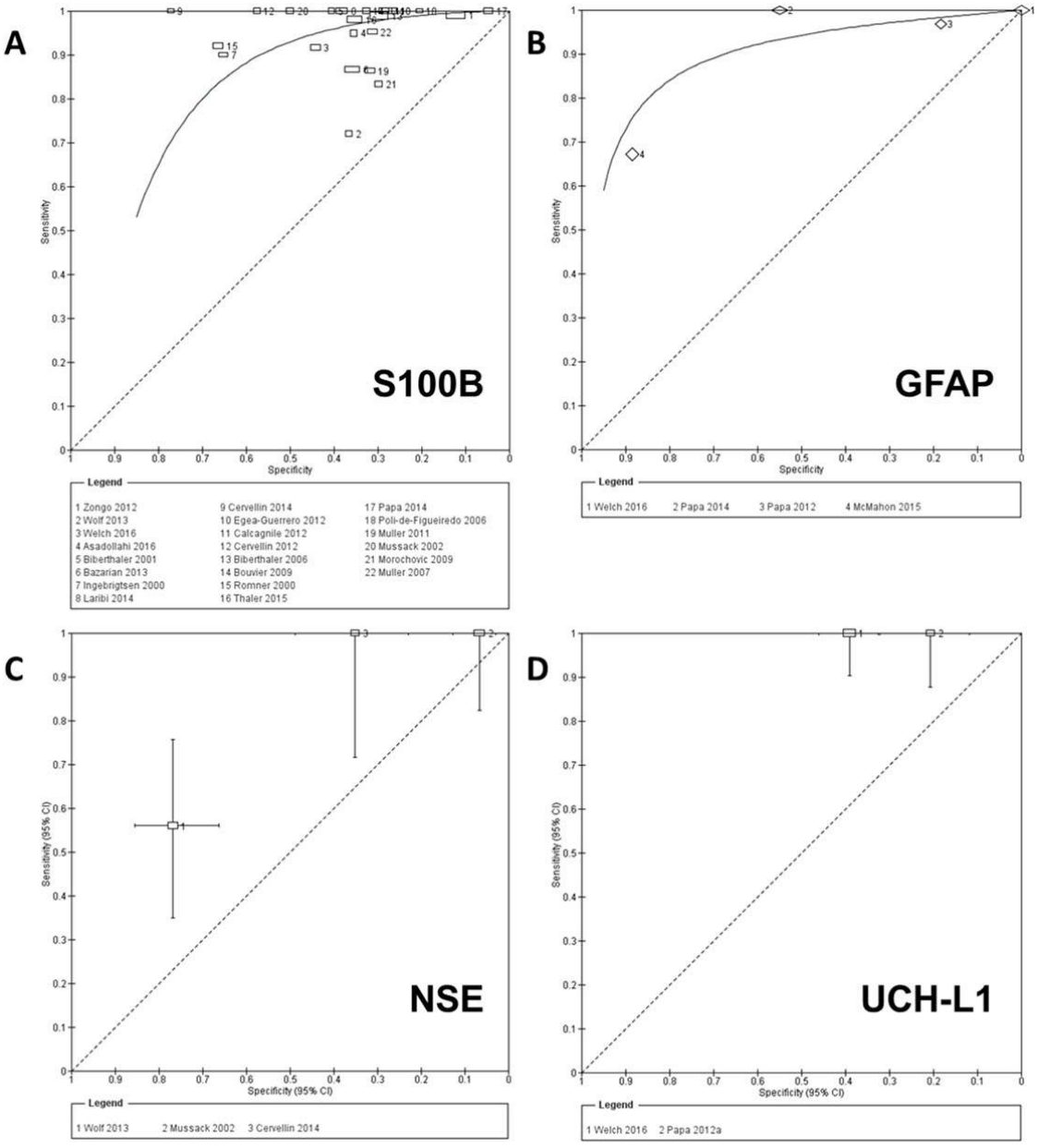

Figure 4. (A, B) Summary ROC plots for S100B and GFAP for detection of CT abnormalities. (C, D) Study estimates of sensitivity and specificity with 95% confidence intervals plotted in ROC space for NSE and UCH-L1 for detection of CT abnormalities. Each square represents an individual study; the size of the symbol is proportional to the number of patients in each study.

The HSROC model was used to estimate a summary curve using Proc NLMIXED in SAS.

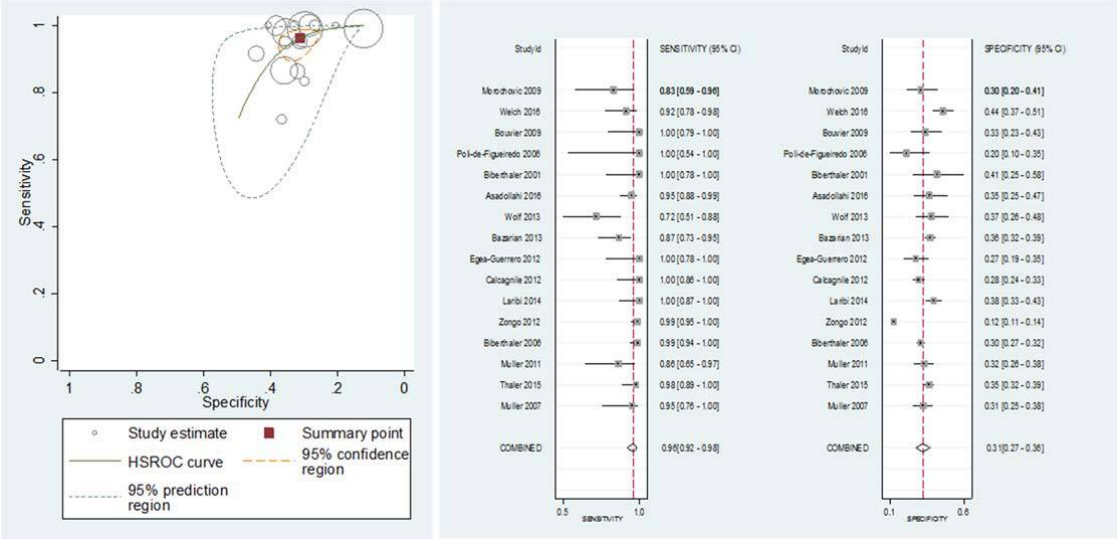

Figure 5. Summary receiver operating characteristics plot of sensitivity and specificity of S100B at 0.1-0.11µg/L cut-off value for detecting intracranial lesions on CT. Each circle represents an individual study; size of symbol reflects number of patients in the studies; red solid spot in middle is summary sensitivity and specificity; inner ellipse represents 95% confidence region, and outer ellipse represents 95% prediction region.

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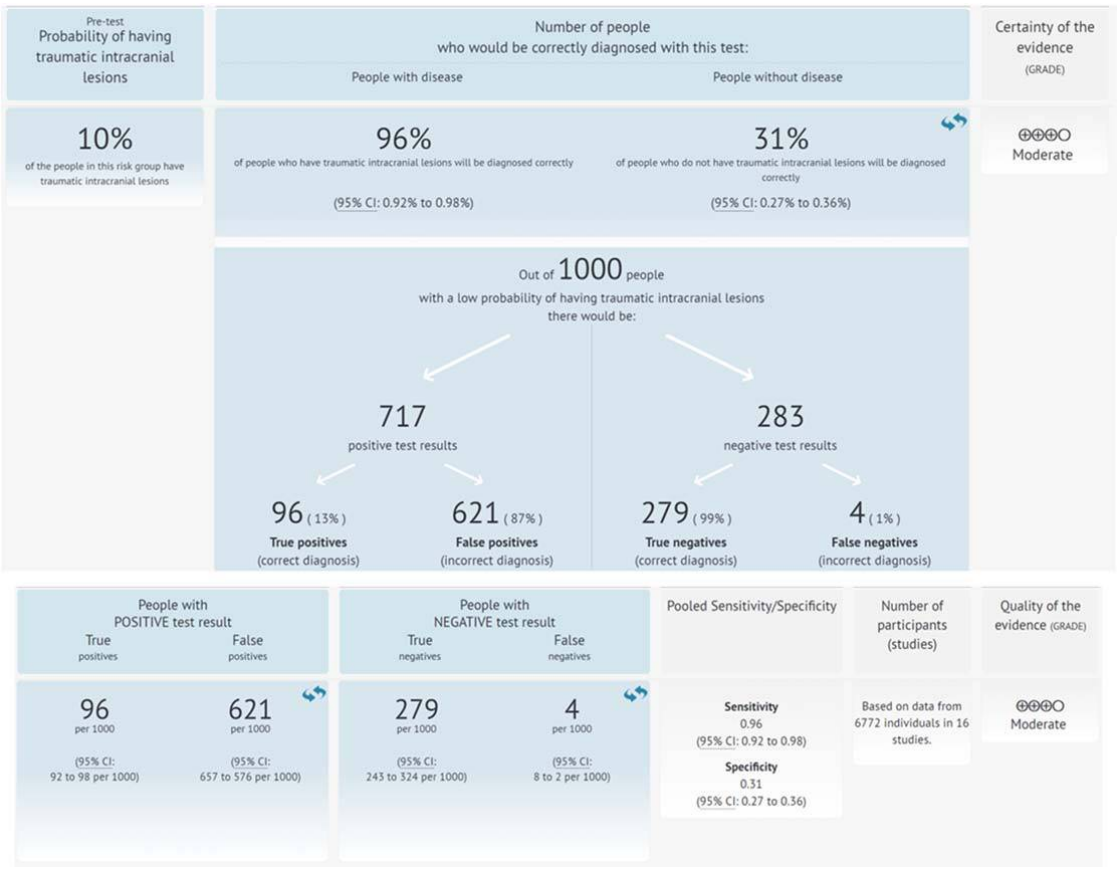

Figure 6. Summary of evidence for the use of blood S100B protein concentrations (0.1-0.11µg/L cut-off) to diagnose brain injury as assessed by CT scan in patients with mild TBI.

Supplemental Material

Supplemental Table. 1S. Summary of the characteristics of the protein biomarkers evaluated

Biomarker	Location/ Protein attributes	Function/ Pathogenic process	Comment	Release Kinetics in serum
Glial Markers				
S100B	Astroglial cells MW 21 kDa bb homodimer	- Calcium-binding protein - Involved in signal transduction and regulation of cell morphology, with neurotrophic properties	- Release from extracerebral tissues - 100% renal clearance - levels potentially affected by renal insufficiency - High normative concentrations in young children	Short half-life (~ 90-120 min) Peak <6 h after injury
GFAP	Major constituent of glial filaments in astrocytes Highly stable	Cytoskeleton support	- Almost exclusively found in glial, negligible contribution of non-CNS derived protein - Raised expression in response to stimuli and injury - “ reactive astrogliosis ” after TBI	The exact half-life is not yet understood Peak at ~16–24 h following injury Rapid appearance in

	MW 49.8 kDa		- Pathology-dependent generation of breakdown products (BDPs) of 38, 25, 20 and 18 kDa - Presence of anti-GFAP auto-antibodies	serum post-injury, with levels detectable within 1 h
Neuronal and Axonal Markers				
NSE / γ–enolase	Prominently in the cytoplasm of neurons Two γ-subunits (γγ) MW 78 kDa	Involved with regulating intraneuronal chloride levels during neural activity	- Present in erythrocytes and platelets - Hemolysis increases blood levels representing a significant artifact and source of error	Biological half-life of 48 h
UCH-L1	Neuronal cell Body (perikarya) Globular shape MW 24kDa	Protein de-ubiquitinization, playing a critical role in removal of damaged, misfolded, or overexpressed proteins both under normal and pathologic conditions	- Resistant to degradation - Implicated in familial Parkinsonism - Elevated serum levels have been associated with abnormal BBB function after TBI - High normative concentrations in	Peak ~8 h after injury Rapid appearance in serum (< 1 h post-injury) with rapid decrease following TBI

			young children and elderly subjects (≥ 65 years)	
Tau	Highly enriched in thin, nonmyelinated axons of cortical interneurons Six isoforms MW 48 to 68 kDa	Involved with assembling axonal microtubule bundles and participating in anterograde axoplasmic transport	- Possibly indicative of axonal damage in gray matter neurons - Upon cellular injury, proteolytically cleaved into fragments of 10–18kDa and 30–50 kDa (c-tau) - Not completely specific for the CNS - Injuries lead to the phosphorylation of tau, which can aggregate (tau tangles)	Levels peak 4–10 days after injury
Neurofilaments (NF)	Predominantly in axons Heteropolymeric components Three main	Main component of the axonal cytoskeleton providing structural support and regulating axon diameter	- Potential specific measure of axonal injury - Subcategory (Type IV) of intermediate filaments - Phosphorylated heavy-chain neurofilament (pNF-H) is the	Unlike other markers, NF-L and pNF-H tend to continuously increase over time during the first 1–2 weeks and remain

	subunits: - light (NF-L) MW 68–70 kDa - medium (NF-M) MW 145–160 kDa - heavy (NF-H) MW 200-220kDa		extensively phosphorylated, axon-specific form of the NF-H subunit of neurofilament and represents one of the most abundantly distributed axonal proteins	elevated 1 year after injury

Abbreviations: h= hour; kDa= kilodaltons; min= minute**;** MW = molecular weight.

Supplemental Table. 2S. MTBI and CT abnormality definition used in the 26 included studies

Study ID	Patients/population Definition	Reference standard (CT Abnormality) Definition	Are skull fractures considered as a CT abnormality?
Asadollahi 2016 [18]	MTBI: GCS score 13–15, LOC < 30' and PTA <1h. Exclusion criterion: Focal neurologic deficit.	EDH, SDH, SAH, ICH, cerebral contusion, brain edema, depressed skull fracture	Yes, Depressed skull fracture
Bazarian 2013 [19]	CDC and Prevention's definition: a blow to the head or rapid acceleration/deceleration resulting in at least one of the following: LOC < 30', PTA <24h, neuropsychological abnormality (any transient period of confusion, disorientation, or impaired consciousness; in children <2 yrs: irritability, lethargy, or vomiting post-injury), or neurological abnormality (seizure acutely after injury, hemiplegia, or diplopia). GCS score of ≥13 within 30' of the injury.	EDH, SDH, SAH, edema, skull fracture, and cerebral contusions.	Yes, Skull fracture
Biberthaler 2001 [20]	GCS score 13–15 at admission, and at least one of the following symptoms: amnesia, LOC, nausea, vomiting, vertigo, or severe headache. Exclusion criterion: Focal	Hemorrhage, diffuse brain swelling, skull fracture	Yes, Skull fracture

	neurologic deficit.		
Biberthaler 2006 [3]	History of isolated head trauma; GCS score of 13 to 15 upon admission; and one or more of 10 clinical risk factors: brief LOC, PTA, nausea, vomiting, severe headache, dizziness, vertigo, intoxication, anticoagulation, and age of above 60yrs	Hemorrhage: EDH, SDH, SAH, ICH, Ventricular, Cerebellar, Brainstem/ Cortex contusion: Hemorrhagic, Non-Hemorrhagic / Fractures: skull cap, skull base, Mastoid/ ICP: Focal and generalized brain edema	Yes, Skull cap, skull base, Mastoid
Bouvier 2009 [21]	History of isolated head trauma; GCS score of 13 to 15 upon admission; and at least one of the following symptom: headache, nausea, vomiting, amnesia, LOC, focal neurologic deficit, seizure, intoxication, age >60 yrs, anticoagulation, clinical signs of skull cap or skull base fractures.	EDH, SDH, SAH, skull fracture, cerebral contusions, petechial hemorrhage, pneumocephalus.	Unclear
Calcagnile 2012 [22]	History of head trauma. GCS 14–15 during examination and LOC< 5' and/or amnesia. Subjects with neurological deficits and additional risk factors from the SNC guidelines (therapeutic anticoagulation or hemophilia, clinical signs of depressed skull fracture or skull base fracture, posttraumatic seizures, shunt-treated hydrocephalus and multiple injuries)	Any signs of cranial (skull fracture) or intracranial pathology (hematoma, air or contusion)	Yes, Skull fracture

	were excluded.		
Cervellin 2012 [23]	MHI requiring CT scanning according to the local guideline: - GCS 14–15; - History: LOS or PTA associated with at least one of the following: previous neurosurgical procedures, inherited coagulopathy or anticoagulant therapy, vomit (more than 1 episode), epilepsy or post-traumatic seizures, worsening headache; - Clinical Findings: drug or alcohol intoxication (even suspected), clinical signs of depressed or basilar skull fracture, focal neurological deficits.	EDH, SDH, SAH, ICH, cerebral contusion, brain swelling	No
Cervellin 2014 [24]	MHI, GCS 14–15 on admission. Exclusion criteria: clinical signs of depressed or basilar skull fracture, focal neurological deficits.	EDH, SDH, SAH, ICH, cerebral contusion, brain swelling (brain edema)	No
Egea-Guerrero 2012 [25]	GCS 15 at hospital admission and one or more of the following symptoms: (1) transitory LOC; (2) amnesia; (3) persistent headache; (4) nausea or vomiting; and (5) vertigo.	EDH, SDH, SAH, cerebral contusion	No
Ingebrigtsen 2000 [26]	Head injury with brief (<10') LOC. GCS 13-15 at admission. LOC was considered to have occurred also when the patient had amnesia for the trauma.	Brain contusion, EDH, SAH	No
Laribi 2014	MHI: GCS 13–15 with one or more of the following risk	NR	Unclear

[27]	factors: amnesia, LOC, nausea, vomiting, vertigo, anticoagulation before injury or severe headache on admission. Exclusion criteria: Focal neurologic deficit, LOC > 10'.		
Ma 2008 [28]	MTBI: LOC and/or PTA, and admitted within 12 h of trauma with a GCS score 13-15. Exclusion criteria: Focal neurologic deficit, penetrating injury to the skull.	EDH, SDH, SAH, ICH, cerebral contusion, hemorrhagic shear injury, intraventricular hemorrhage, pneumocephalus	No
McMahon 2015 [29]	Positive clinical screen for acute TBI necessitating a noncontrast head CT according to ACEP/CDC evidence-based joint practice guidelines.	Recommendations of the TBICDE Neuroimaging WG: Cisternal effacement, mid-line shift, EDH, SAH, and intraventricular hemorrhage.	No
Morochovic 2009 [30]	MTBI: categories 1–3 according to EFNS Category 1: GCS = 15, LOC < 30 min, PTA < 1 h, no risk factors Category 2: GCS = 15 and risk factors present Category 3: GCS = 13–14, LOC < 30 min, PTA < 1 h, with/without risk factors -Risk factors: Unclear or ambiguous accident history, continued post-traumatic amnesia, retrograde amnesia	Acute EDH, SDH, SAH, parenchymal hematoma, cerebral contusion and brain swelling	No

	longer than 30 min, trauma above the clavicules, severe headache, vomiting, focal neurological deficit, seizure, coagulation disorder, high energy accident, intoxication with alcohol/drugs.		
Muller 2007 [31]	History of head injury, LOC or retrograde amnesia, GCS 13–15 on admission. Exclusion criterion: Focal neurologic deficit.	Brain contusion, SDH, EDH	No
Muller 2011 [32]	Mild head trauma, GCS 13–15 on admission	Localized EDH, SDH, isolated SAH, skull fracture	Yes, Skull fracture
Mussack 2002 [33]	History of trauma, GCS 13–15 and at least one of the following symptoms: transient LOC (less than 5 min), antero- or retrograde amnesia, nausea, vomiting, or vertigo.	EDH, SDH, SAH, intracerebral hemorrhage, diffuse brain edema, skull fracture	Yes, Skull fracture
Papa 2012a [34]	Suspected mild TBI: History of blunt head trauma followed by LOC, amnesia, or disorientation. GCS 9-15.	EDH, SDH, SAH, contusion, intracerebral hemorrhage, pneumocephalus, combination of lesions	No
Papa 2012b [35]	Suspected mild TBI: History of blunt head trauma followed by LOC, amnesia, or disorientation. GCS 9-15.	EDH, SDH, SAH, contusion, intracerebral hemorrhage, pneumocephalus, combination of	No

		lesions	
Papa 2014 [36]	Suspected mild or moderate TBI (MMTBI): History of blunt head trauma followed by LOC, amnesia, or disorientation. GCS 9-15.	NR	Unclear
Poli-de-Figueiredo 2006 [37]	Isolated MHI: GCS 13-15 and at least one of the following symptoms: amnesia, LOC, nausea, vomiting, vertigo, or severe headache. Exclusion criterion: Focal neurologic deficit.	Intracranial hemorrhage, skull fracture, and/or diffuse brain swelling (edema)	Yes, Skull fracture
Romner 2000 [38]	Mild head injury: GCS 14–15, LOC for<20 min, absence of focal neurological deficits, and no signs of acute intracranial abnormality revealed by a CT scan.	EDH, SDH, SAH, brain contusion, brain edema	No
Thaler 2015 [39]	MHI: a score of 13–15 on the GCS.	EDH, SDH, SAH, intracerebral bleeding	No
Welch 2016 [40]	Blunt closed head injury, GCS 9-15.	Acute intracranial lesion is defined as any trauma induced or related finding and includes extra-axial lesions (acute EDH, SDH), cortical contusion, ventricular compression or trapping, brain herniation, intraventricular hemorrhage, hydrocephalus, SAH,	No

		petechial hemorrhagic or bland sheer injury, brain edema, post-traumatic ischemia, intracerebral hematoma, dural venous sinus injury and/or thrombosis	
Wolf 2013 [41]	Blunt head trauma, GCS 13-15.	EDH, SDH, SAH, intracerebral hemorrhage, brain contusion	No
Zongo 2012 [42]	Isolated head trauma, GCS 13-15 determined by the attending physician, and with one or more of the following risk factors: LOC, PTA, repeated vomiting, severe headache, dizziness, vertigo, alcohol intoxication, anticoagulation, and age > 65 yrs.	EDH, SDH, intracerebral hemorrhages, bland contusion, edema, pneumocephalus, skull fracture	Yes, Skull fracture

Abbreviations: ACEP/CDC = American College of Emergency Physicians/Centers for Disease Control and Prevention; CT = Computed Tomography; ECI = extracranial injury; ED = emergency department; EDH= epidural mass lesion; EFNS = European Federation of Neurological Societies; GCS= Glasgow Coma Scale; LOC = loss of consciousness; MHI = mild head injury; MHT = mild head trauma; MTBI = mild traumatic brain injury; NR = not reported; PTA = post-traumatic amnesia; SAH subarachnoid hemorrhage; SDH = subdural hematomas; SNC = Scandinavian Neurotrauma Committee; yrs = years.

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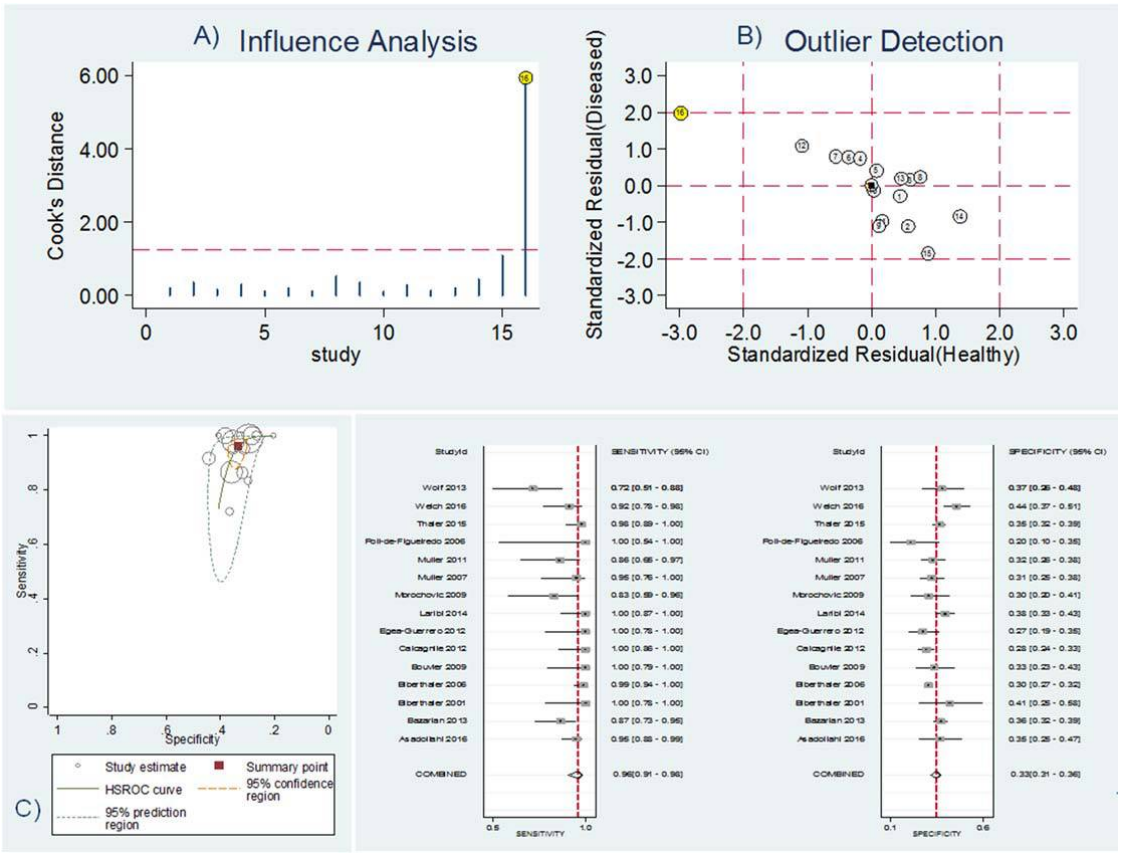

Figure 1S: Graphical depiction of (A) influence and (B) outlier detection analyses of S100B 0.1-0.11µg/Lcut-off value studies. (C) Summary receiver operating characteristics plot of sensitivity and specificity of S100B at 0.1-0.11µg/L cut-off value after removing the influential study (Zongo 2012).

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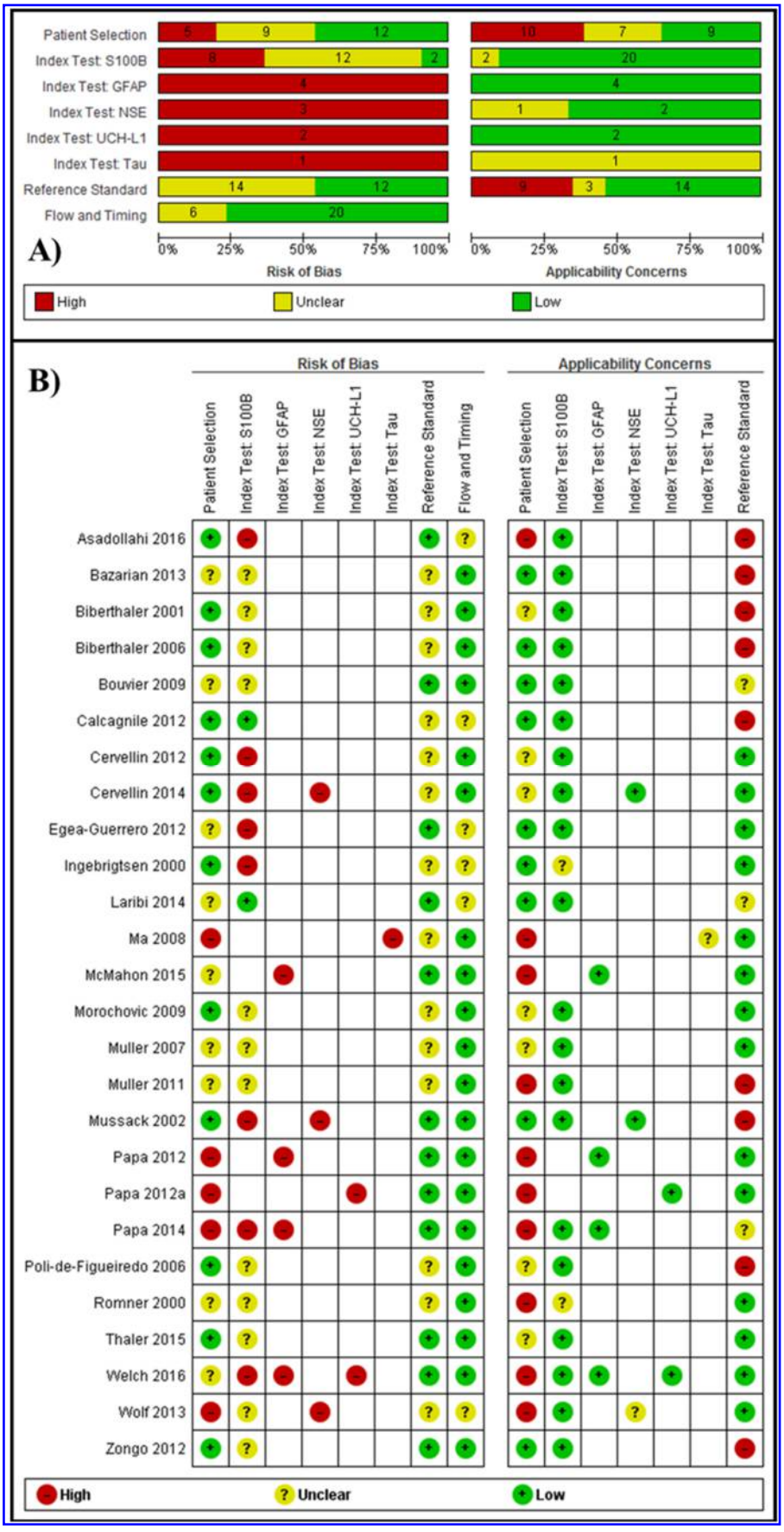

Figure 2S. (A) Risk of bias and applicability concerns graph by marker. Review authors' judgments about each domain presented as percentages across included studies. **(B) Risk of bias and applicability concerns summary by marker.** Review authors' judgments about each domain for each included study.

Study	TP	FP	FN	TN	Sampling Time	Polytrauma	CT abnormalities	GCS	Population Age	Sensitivity (95% CI)	Specificity (95% CI)
Asadollahi 2016	75	51	4	28	Within 3 h	No	Skull fracture included	13-15	Adult	0.95 [0.88, 0.99]	0.35 [0.25, 0.47]
Bazarian 2013	39	473	6	264	Within 6 h	Yes	Skull fracture included	13-15	Adult&Pediatric	0.87 [0.73, 0.95]	0.36 [0.32, 0.39]
Biberthaler 2001	15	22	0	15	Within 3 h	No	Skull fracture included	13-15	Unclear	1.00 [0.78, 1.00]	0.41 [0.25, 0.58]
Biberthaler 2006	92	855	1	361	Within 3 h	No	Skull fracture included	13-15	Adult	0.99 [0.94, 1.00]	0.30 [0.27, 0.32]
Bouvier 2009	16	60	0	29	Within 3 h	No	Unclear	13-15	Adult	1.00 [0.79, 1.00]	0.33 [0.23, 0.43]
Calcagnile 2012	24	350	0	138	Within 3 h	Unclear	Skull fracture included	14-15	Adult	1.00 [0.86, 1.00]	0.28 [0.24, 0.33]
Egea-Guerrero 2012	15	94	0	34	Within 6 h	Yes	Skull fracture excluded	15	Adult&Pediatric	1.00 [0.78, 1.00]	0.27 [0.19, 0.35]
Laribi 2014	26	231	0	143	Within 3 h	No	Unclear	13-15	Adult	1.00 [0.87, 1.00]	0.38 [0.33, 0.43]
Morochovic 2009	15	59	3	25	Within 3 h	Yes	Skull fracture excluded	13-15	Adult	0.83 [0.59, 0.96]	0.30 [0.20, 0.41]
Muller 2007	20	141	1	64	Within 12 h	No	Skull fracture excluded	13-15	Adult	0.95 [0.76, 1.00]	0.31 [0.25, 0.38]
Muller 2011	19	144	3	67	On admission	No	Skull fracture included	13-15	Adult&Pediatric	0.86 [0.65, 0.97]	0.32 [0.26, 0.38]
Poli-de-Figueiredo 2006	6	35	0	9	Within 3 h	No	Skull fracture included	13-15	Unclear	1.00 [0.54, 1.00]	0.20 [0.10, 0.35]
Thaler 2015	49	474	1	258	Within 3 h	No	Skull fracture excluded	13-15	Adult	0.98 [0.89, 1.00]	0.35 [0.32, 0.39]
Welch 2016	33	120	3	95	Within 6 h	Unclear	Skull fracture excluded	9-15	Adult	0.92 [0.78, 0.98]	0.44 [0.37, 0.51]
Wolf 2013	18	52	7	30	Within 3 h	No	Skull fracture excluded	13-15	Adult	0.72 [0.51, 0.88]	0.37 [0.26, 0.48]
Zongo 2012	110	1272	1	177	Within 6 h	No	Skull fracture included	13-15	Adult&Pediatric	0.99 [0.95, 1.00]	0.12 [0.11, 0.14]

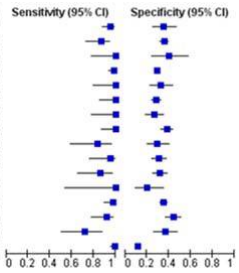

Figure 3S. Supplemental Figure 2. Forest plot of S100B 0.1-0.11µg/L cut-off value studies for detection of CT abnormalities. Information related to population, sampling time and reference test - potential sources of heterogeneity - are shown.

Journal of Neurotrauma
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 (DOI: 10.1089/neu.2017.5182)
This paper has been peer-reviewed and accepted for publication, but has yet to undergo copyediting and proof correction. The final published version may differ from this proof.

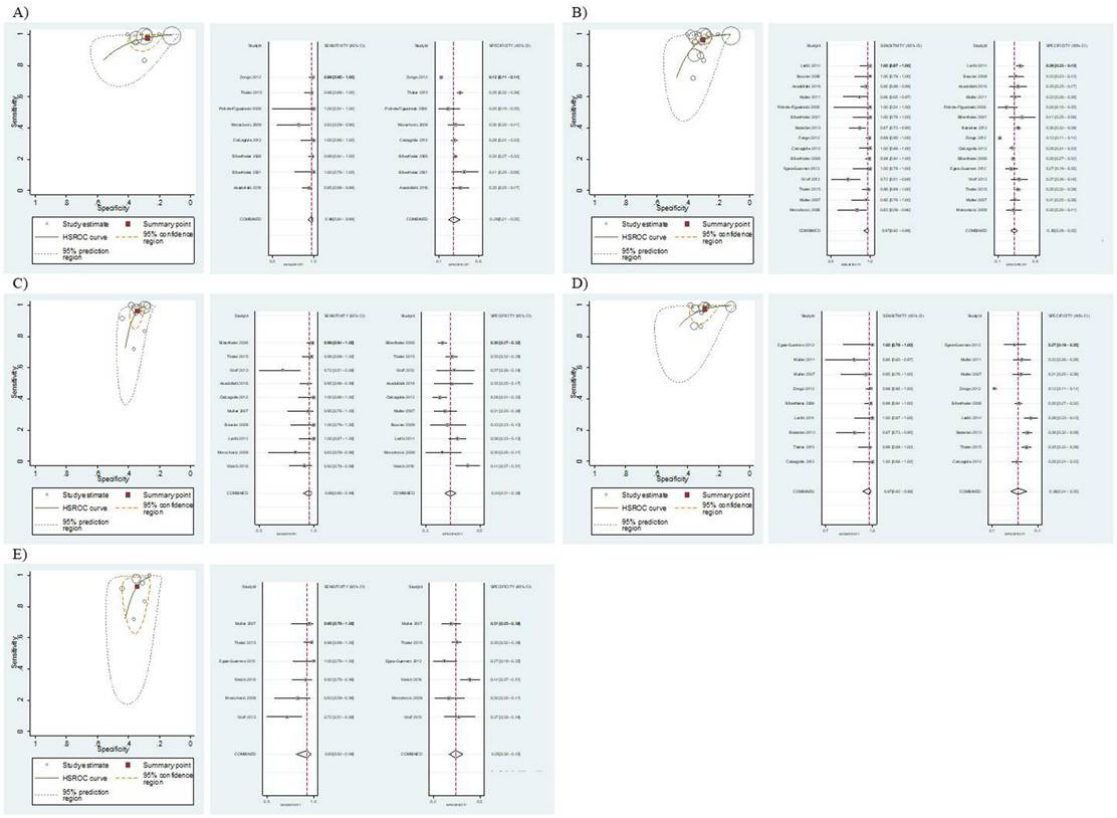

Figure 4S. Detection of abnormal CT sensitivity analyses. (A) Detection of abnormal CT sensitivity analyses excluding studies at high or unclear risk of bias for patient selection domain. **(B)** Detection of abnormal CT sensitivity analyses excluding Welch 2016. **(C)** Detection of abnormal CT sensitivity analyses excluding studies containing mixed pediatric and adult populations. **(D)** Detection of abnormal CT sensitivity analyses excluding studies having a high prevalence of CT findings (over 11%). **(E)** Detection of abnormal CT sensitivity analyses excluding studies at high or unclear risk to not properly classify the target condition.

Appendix 1. 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)