Use of Blood Biomarkers in the Assessment of Sports-Related Concussion—A Systematic Review in the Context of Their Biological Significance

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Abstract

Objectives: To critically review current knowledge on the positive and negative predictive value of blood biomarkers for concussion; to illustrate the clinical and biological contexts that help evaluate the use of these markers in sport-related traumatic brain injuries (TBIs). **Methods:** This systematic review was performed in accordance with PRISMA guidelines. We reviewed the measurement, clinical utility, endpoint, and biological significance of blood biomarkers in concussion. **Results:** A total of 4352 publications were identified. Twenty-six articles relating to blood biomarkers were included in the review. Four common blood biomarkers, namely S100B, tau, neuron-specific enolase (NSE), and glial fibrillary acidic protein (GFAP), were examined. Overall, the studies showed S100B measurement and use, either acutely or at several time points, can distinguish injured from noninjured patients with an uncertain degree of utility in predicting mortality. At present, S100B has largely become an acceptable biomarker of TBI; however, studies have begun to highlight the need to incorporate clinical symptoms instead of S100B concentration in isolation on the basis of inconsistent results and lack of specificity across published studies. Further research is needed to evaluate and validate the use of tau, NSE, and GFAP as a diagnostic aid in the management of concussion and TBI. **Conclusions:** At present, blood biomarkers have only a limited role in the evaluation and management of concussion. Although several biomarkers of brain injury have been identified, continued research is required. S100B holds promise as the most clinically useful diagnostic biomarker. Blood biomarkers, in combination with other clinical data, such as head computed tomography, would maximize the diagnostic accuracy. The methodological limitations evident in blood biomarker research results in the need for the clinical utility of blood biomarker use in concussion to be further explored.

Key Words: concussion, brain injury, blood biomarkers, sports, S100B, tau, GFAP, NSE

(*Clin J Sport Med* 2017;00:1–11)

INTRODUCTION

Concussion is defined as a traumatically induced transient disturbance of brain function that involves a complex pathophysiological process.[1] It is considered a subset of mild traumatic brain injury (mTBI) characterized by a broad range and graded set of clinical symptoms that may or may not involve loss of consciousness. Concussion results from a complex biomechanical process resulting from direct or indirect hits,[2] leading to brain injury because of the ensuing rapid acceleration/deceleration of the brain that is encased in a rigid skull and surrounded by incompressible fluid.[3] In mTBI, the acute clinical symptoms largely reflect a functional disturbance rather than a structural injury with a rapid onset of short-lived impairment of neurological function that often resolves spontaneously. Specific risk factors for concussion exist. A history of concussion is associated with a higher risk of sustaining further concussion.[4] The likelihood of mTBI is almost 2 times higher among players who reported having sustained either one or more mTBIs.[5] There is an increased risk of long-term neurological deficits when athletes return to play prematurely because of undetected mTBI.[6] Fuller et al,[7] performed a large epidemiological study in rugby union and found that the overall incidence was 4.5 concussions/1000 player-match-hours with tackling (44.1%) accounting for the main causes of concussion.

To date, diagnosis of TBI depends on: neurological examination, clinical assessment scales, neuroimaging, patient history and patient's signs and symptoms. Although the body of research is growing, there is a paucity of research in sports-related concussion. For this reason, management principles reflect practice in managing TBI in all settings. This may be appropriate as the magnitude of reported head impacts from rugby and American Football are broadly similar to those observed in staged pedestrian impact studies at 40 km/h before head contact on a windscreen.[8] For this reason, it seems reasonable that assessment of TBI in the sports environment should reflect those in any other trauma setting. A multitude of assessment tools are available to evaluate a suspected concussion. Standardized assessments, such as the SCAT3 are used.[9]

Submitted for publication April 13, 2016; accepted March 23, 2017.

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D. Janigro has 3 patents on the use of peripheral markers of the blood–brain barrier. The remaining authors report no conflicts of interest.

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Supplemental digital content is available for this article. Direct URL citations appear in the printed text and are provided in the HTML and PDF versions of this article on the journal's Web site (www.cjsportmed.com).

http://dx.doi.org/10.1097/JSM.0000000000000478

Neuroimaging and computerized neuropsychological testing are growing in importance. An important concept in the detection of concussions in sports is the fact that while assessments are done typically at one time point, the biological consequences of traumatic head hits takes time to develop. It is essential to find approaches that not only diagnose concussions but also prognosticate their immediate or delayed consequences. A summary of common methods for assessing the utility of brain injury outcomes is presented in Table 1.

Clinical biomarkers of neuronal, axonal, and astroglial damage can be used to diagnose mTBI and predict clinical outcomes of patients with head trauma. Cerebrospinal fluid (CSF) and blood biomarkers of brain injury have been proposed as a means by which cellular damage may be detected if present. Biomarkers may be analyzed by examining CSF or blood. Potential biomarkers for brain damage include neuron-specific enolase (NSE), total tau (T-tau), glial fibrillary acidic protein (GFAP), and S100B.

Cerebrospinal fluid sampling is a high-risk and impractical method and thus blood biomarkers present the most appropriate option. A lumbar puncture is required to sample CSF. A lumbar puncture is an invasive technique that accesses the restricted compartment of the subarachnoid space. Various risks are associated with a lumbar puncture. These risks include local infection at the site of the lumbar puncture, uncorrected bleeding diathesis, local discomfort, radicular pain, spinal haematoma, meningitis, headache, persistent CSF leak, and an epidural blood patch.[10]

There is a need for reliable blood tests to assess concussion and TBI early and accurately, to prevent poor clinical outcomes, and to predict the most favorable point of time to return to physical activity.[11] However, there is a lack of consensus regarding which is the most appropriate biomarker for brain damage and what method of analysis is most applicable, especially in the sports setting.

AIMS AND OBJECTIVES

The aim of this review is to examine specific blood biomarkers that may be present as a measure of brain injury, including concussion, in both sport and in emergency department settings.

The objectives of this review are:

1. To review the prognostic value and validity of clinical-grade blood biomarkers used in sport and emergency department settings
2. To outline the differences between markers of brain damage and markers of cerebrovascular integrity
3. To outline when markers of brain damage versus markers of blood–brain barrier (BBB) function should be used in the management of TBI
4. To underscore differences between prognostic and diagnostic markers in the context of mTBI

METHODS

This systematic review was performed in accordance with PRISMA guidelines.[12] See **Appendix 2, Supplemental Digital Content 1**, http://links.lww.com/JSM/A150, for the PRISMA guidelines.

Eligibility Criteria

Inclusion criteria:

1. Participants aged 18 and older were diagnosed with "concussion" or "traumatic brain injury."
2. Studies examining blood biomarkers in both sports and emergency department settings. Plasma or serum proteins examined include total tau (T-tau), GFAP, S100B, and NSE.

Exclusion criteria:

1. Review articles, case reports (with 5 or less participants), and commentaries.
2. Studies examining participants with any comorbid conditions that may confound results, eg, seizures, migraines and, neurological disorders
3. Studies involving any medications that may interfere with results

Search Strategies

Studies were retrieved by searching electronic databases (MEDLINE/PubMed, EMBASE, and CINAHL) from their inception to November 2015. Search terms were adapted for use with each database (see **Appendix 3, Supplemental Digital Content 1**, http://links.lww.com/JSM/A150, for search strategy). No search restrictions (date or language) were imposed. The electronic database searches were supplemented by searching the abstracts of the annual meetings of the World Confederation for Concussion. When only abstracts were available in the published literature, authors were contacted to seek the full text of relevant studies. Finally, a hand search of the

TABLE 1. Assessing the Utility of Brain Injury Outcomes

Assessment	Pros	Cons
Side line assessment (SCAT3, Impac)	Easy, fast; combines cognitive and physical assessment	Requires baseline values to be reliable; highly subjective
Helmet accelerometers/telemetry (Riddell system)	Quantitative; objective	Does not correlate with true impact of head hits; expensive; impact threshold for concussion/mTBI shows great individual variability and no standard reference is available
Imaging	Different modalities encompass a broad range of parenchymal, functional, cerebrovascular or structural changes	Expensive; somewhat invasive (CT = radiation, MRI, CT = contrast agents) often unavailable
Serum or plasma biomarkers	Inexpensive; excellent NPV in mTBI against CTs	No point of contact (POC) testing available; many markers have different properties; may not be robust in the presence of peripheral trauma because of lack of specificity (positive predictive value)

reference lists of included studies was conducted. Two reviewers (B.O.C. and F.W.) independently screened titles and abstracts to identify studies that potentially met the eligibility criteria. Full texts of these reports were retrieved and independently assessed for eligibility by the same 2 reviewers. Any disagreements on inclusion were resolved by discussion to achieve consensus, and failing agreement, a third reviewer (A.K.) was consulted.

Data Collection and Analysis

A data extraction template based on Cochrane guidelines (www.cochrane.org) was adapted. One reviewer (B.O.C.) recorded (1) participant characteristics, (2) details and measurements of blood measures, and (3) relevant outcome data. In the event that the published data from included studies were insufficient to calculate pooled effects, study authors were contacted requesting additional data.

Potential Biases in the Overview Process

A risk of bias appraisal of included studies was performed independently by 2 reviewers (B.O.C. and F.W.). Disagreements between the reviewers were resolved through discussion to achieve consensus; failing agreement, a third reviewer arbitrated. The Quality in Prognostic Studies (QUIPS) risk of bias tool rated risk of bias across 3 domains as high, moderate, or low. The risk of bias for each study is illustrated in Table 2. See **Appendix 1, Supplemental Digital Content 1**, http://links.lww.com/JSM/A150, for the QUIPS tool.

RESULTS

Identification of Studies

The Prisma flowchart presented in Figure 1 provides an outline of the stratification process that was performed for this review of the literature. From the initial search strategy, 4352 studies were identified. After the removal of duplicates and extensive screening, 25 studies were examined as part of this systematic review.

Data Extraction

The following information was extracted from each study: study design, participant numbers, study objectives, primary outcome measures used, and study results.

Data Synthesis

After analysis, the 25 studies broadly fell under the following categories:

1. Blood biomarker analysis in athletic settings only

TABLE 2. Risk of Bias—QUIPS Tool

	QUIPS Tool Rating		
Study	**Study Participation**	**Study Attrition**	**Prognostic Factor Management**
Kiechle et al (2014)	Low	Low	Low
Shahim et al (2014)	Low	Low	Moderate
Marchi et al (2013)	Moderate	Low	Low
Dambinova et al (2013)	Low	Low	Moderate
Graham et al (2011)	Moderate	Moderate	Moderate
Stalnacke et al (2003)	Moderate	Moderate	Moderate
Neselius et al (2013)	Moderate	Low	High
Zetterberg et al (2009)	Moderate	Low	High
Straume-Naesheim et al (2008)	Low	Low	Moderate
Stalnacke et al (2006)	Moderate	Moderate	Moderate
Puvenna et al (2014)	Moderate	Low	Moderate
Arslan et al (2010)	Moderate	Moderate	Moderate
Stalnacke et al (2004)	Moderate	Low	Low
DeKruijk et al (2001)	Low	Moderate	Moderate
Calcagnile et al (2012)	Low	Low	Low
Savola et al (2004)	Low	Low	Low
Naeimi et al (2006)	Moderate	Moderate	Moderate
Egea-Guerrero et al (2012)	Low	Low	Low
Topolovec-Vranic et al (2011)	Low	Low	Low
Sojka et al (2006)	Moderate	Low	Moderate
De Boussard et al (2004)	Low	Low	High
Herrmann et al (2001)	Moderate	Moderate	Moderate
Honda et al (2010)	High	Moderate	Moderate
Korfias et al (2007)	Moderate	Low	Moderate
Bazarian et al (2006)	High	Low	Moderate
Biberthaler et al (2001)	Moderate	Moderate	Moderate

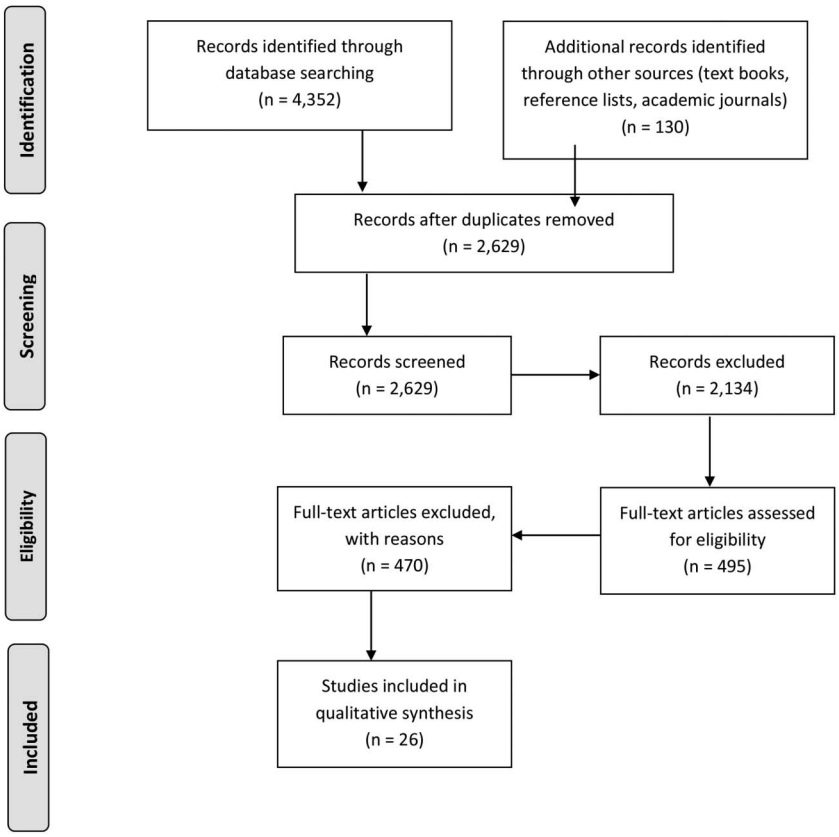

Figure 1. Breakdown of search results.

2. Blood biomarker analysis in TBI patients which were not specifically categorized as caused by sport or recreation (including emergency departments)

The studies are summarized and presented in Tables 3 and 4. Study designs varied between articles with most studies being prospective, cross-sectional (noncase controlled) in design. The number of participants ranged from 16 to 535. The primary outcome measures used most frequently are displayed in Table 5. The clinical utility of blood biomarkers is shown in Table 6.

DISCUSSION

The identification of clinically useful biomarkers for brain injury, especially for mTBI, poses several challenges. A biomarker is an indicator of a specific biological or disease state that can be measured using samples taken from either the affected tissue or peripheral body fluids. These markers can be altered by enzymatic activity, changes in protein expression or post-translational modification, altered gene expression, protein or lipid metabolites, or a combination of these changes. As a consequence, a variety of strategies have been used for biomarker discovery including transcriptional profiling and proteomic approaches. Five blood biomarkers were examined from the 25 papers included in this review. S100B was the most frequently examined blood biomarker and provided the most promising results. The other blood biomarkers included were NSE, tau, GFAP, and AMPAR (Alpha-amino-hydroxy-5-methyl-4-isoxazolepropionic acid receptor). However, the findings of the studies reviewed were compromised by limitations in study design. As a result, findings must be interpreted with caution.

S100B is mainly found in astroglia and Schwann cells and is one of the most well-known biomarkers of brain damage. The concentration of S100B is known to increase in the CSF and serum after injury making this protein a potential biomarker for TBI.[13] Neuron-specific enolase is highly expressed in neuronal cytoplasm. Neuron-specific enolase has been shown to have the sensitivity and specificity to detect neuronal cell death.[14] Neuron-specific enolase is localized mainly not only in neurons but also in smooth muscle fibers and adipose tissue. Glial fibrillary acidic protein is found only in astroglial cytoskeleton. Glial fibrillary acidic protein is an intermediate filament protein that forms networks that support the astroglial cells. Damage to the astroglial cells shows a subsequent upregulation of GFAP. Tau protein is widely distributed in the CNS (central nervous system) and systemic tissues. The distinct regional distribution of tau is likely to indicate the components of the brain being affected by injury with raised CSF T-tau protein representing axonal damage in grey matter neurons. Total tau protein levels in ventricular CSF correlate with lesion size and clinical outcome in patients with TBI.[15]

Blood Biomarkers in Sport

Significant increases in S100B, NSE, and tau levels were shown following a variety of different sports. Kiechle et al[16]

TABLE 3. Summary of Studies Included—Blood Biomarkers in Sport

Authors	Study Design	N	Control	Objectives	Outcome Measures	Imaging	Results
Kiechle et al (2014)	Longitudinal cohort study	46 (male = 41, female = 6) "contact sport" athletes from football, soccer, and basketball	Participant's own preseason baseline	Changes in S100B before and after sports-related concussion	S100B at preseason baseline, within 3 hours of injury and at days 2, 3, and 7 after sports-related concussion	None	The mean 3-hour post-SRC S100B was significantly higher than preseason baseline (0.099 ± 0.008 μg/L vs 0.058 ± 0.006 μg/L). "Post exertion" (nonconcussed) measure not significantly different than baseline.
Shahim et al (2014)	Prospective cohort study	288 professional ice hockey players all male	Used another group of players as control and for baseline levels (n = 47)[14]	Comparison of clinical preseason baseline testing and blood samples	Total tau, S100B and NSE in plasma and serum, SAC, Rivermead postconcussion symptoms questionnaire	None	Concussed players had increased levels T-tau and S100B levels compared with preseason values. Significant rise in T-tau (median 10 pg/mL) and S100B (median 0.075 μg/L).
Marchi et al (2013)	Prospective cohort study	57 American football players all male	Athletes are their own controls	Blood samples collected before and after 5 consecutive American football games. Only subconcussive hits were included.	S100B, Game film review, Head Hit Index, ImPACT testing	DTI	S100B increases were detected only in players experiencing the greatest number of subconcussive head hits. The average S100B was 0.04 ng/mL for postgame–pregame while the average S100B was 0.05 ng/mL for postgame–baseline.
Graham et al (2011)	Retrospective cohort study	16 Boxers	Pre bout compared with postbout, no baseline values	Blood serum biomarkers measured before and after boxing bouts	Punches to the head, punches to the body, S100B, NSE, creatine kinase and cortisol	None	Significant increases were found in prebout and postbout levels of S100B (0.35 ± 6.10 vs 0.54 ± 0.73 ngL), and NSE (19.7 ± 14 vs 31.3 ± 26.6 ngL).
Stalnacke et al (2003)	Descriptive clinical research	26 male ice hockey players and 18 basketball players	None	S100B blood samples were taken from players 1–2 h before and within 1 h after the game. Head accelerations/decelerations were measured.	S100B, NSE, Rivermead Post Concussion Symptoms Questionnaire, video analysis	None	S100B increases were statistically significant after either game (ice hockey, 0.072 ± 0.108 μg/L, $P = 0.00004$; basketball, 0.076 ± 0.091 μg/L, $P = 0.001$). In basketball, there was significant correlation between the change in S100B and jumps (postgame–pregame values) and jumps, which were the most frequent acceleration/deceleration (r = 0.706, $P = 0.002$).
Neselius et al (2013)	Prospective follow-up study	30 Olympic boxers	None	Olympic boxers competing in at least 47 bouts were compared with 25 controls.	Tau, S100B, Glial fibrillary acidic protein, brain-derived neurotrophic factor and amyloid beta	None	Plasma-tau was significantly increased in the boxers after a bout compared with controls (mean ± SD, 2.46 ± 5.10 vs 0.79 ± 0.961 ngL, $P = 0.038$).
Zetterberg et al (2009)	Observational case–control study	44 Amateur boxers	23 Controls	Comparison of a panel of serum biomarkers taken	S100B, BDNF, h-FABP, GFAP, and NSE	None	Boxers had higher serum levels of NSE (median range 11, 2.3-41 ng/mL) than controls (median range 4.8, 0.78-27 ng/mL, $P = 0.014$) but unchanged levels of the other brain damage biomarkers.

TABLE 3. Summary of Studies Included—Blood Biomarkers in Sport *(Continued)*

Authors	Study Design	N	Control	Objectives	Outcome Measures	Imaging	Results
Straume-Naesheim et al (2008)	Prospective cohort study	535 Norwegian professional soccer players	None	Comparison of baseline S100B levels after matches and different training intensities	Serum level of S100B, head impact	None	Serum S100B increased from baseline for all groups A total of 39 players (33.9%) had elevated S100B values (≥0.12 ng/mL) after a match.
Stalnacke et al (2006)		44 female soccer players	None	Venous blood samples obtained before and after a competitive game	S100B, NSE, video recordings of head impacts and trauma, scale assessing number of headers made (0-3)	None	Concentrations of S100B and NSE were significantly increased after the game. (S-100B, 0.18 (0.11) v 0.11 (0.05) mg/l ($P = 0.000$); NSE, 10.14 (1.74) v 9.05 (1.59) mg/L ($P = 0.001$). Significant correlation between changes in S-100B concentrations and both the number of headers ($P = 0.004$) and the number of other trauma events ($P < 0.001$).
Arslan et al (2010)	Repeated measures design	Greco-Roman (n = 15) and free style (n = 16) wrestling groups of >19 year old, healthy, male wrestlers	None	Serum samples analyzed after wrestling matches	Video analysis, S100B, H-FABP	None	Study results showed increments of 109% ($P = 0.007$) and 145% ($P = 0.001$) in serum S100B from prematch to postmatch in the free and Greco-Roman style groups.
Stalnacke et al (2004)		28 players from 4 elite soccer teams	None	Blood samples examined after a competitive game. The number of headers and trauma events were assessed.	S100B, NSE, video analysis of headers and trauma events	None	Both S100B (0.118 ± 0.04 vs 0.066 ± 0.025 ngL, $P <$ 0.001) and NSE (10.29 ± 2.16 vs 8.57 ± 2.31 ngL, $P < 0.001$) were significantly raised. S100B concentrations were statistically significantly and correlated to the number of headers and to the number of other trauma events.

demonstrated elevated S100B levels within 3 hours after sport-related concussion. Shahim et al,[17] showed that, within 20 to 60 minutes after game, concussed ice hockey players had increased tau and S100B levels. The highest concentrations of tau were measured immediately after the injury, and the levels declined during the first 12 hours followed by a second peak at between 12 and 36 hours. Marchi et al,[18] when examining American football players, found postgame BBB damage measured by serum S100B was detected only in players experiencing the greatest number of subconcussive head hits. Similarly, Stalnacke et al,[19] found statistically significant increases in S100B levels in male ice hockey and basketball players. Dambinova et al,[20] by contrast, measured the blood biomarker AMPAR in concussed athletes. AMPAR peptide values significantly increased in concussed athletes. The study endorses AMPAR as a brain-specific biomarker. Graham et al, Neselius et al, and Zetterberg et al focused on blood biomarker research in boxing.[21–23] Significant increases were found in prebout and postbout levels of S100B and NSE.[21] By contrast, Neselius et al,[22] discovered that only plasma-tau was significantly increased in the boxers after a bout compared with controls, whereas Zetterberg et al[23] postulated that boxers only had higher serum levels of NSE but unchanged levels of the other brain damage biomarkers. Total tau protein levels were elevated in lumbar CSF in boxers 4 to 10 days after a bout and in boxers who had not been knocked out; hence, TBI is a common exposure in the boxing ring, also in the absence of frank knockouts.[22,23] Total tau protein levels normalize within the 8 to 12 weeks, provided that boxers have not been subjected to further bouts.[22,23] S100B and GFAP represent astroglial injury and have been shown to increase after TBI but to a lesser degree than T-tau protein.[22,23] Soccer has also been a major focus of blood biomarker research. Heading in soccer has been postulated as a mechanism of blood biomarker release. Stalnacke et al, and Stalnacke et al showed that concentrations of S100B and NSE were increased after the game.[24,25] There was a significant correlation between changes in S100B concentrations and both the number of headers and the number of other trauma events. Similarly, Straume-Naesheim et al[26] revealed that S100B increases in professional soccer players with elevated concentrations correlating to an increased amount of headers.

TABLE 4. Summary of Studies Included—Blood Biomarkers in Emergency Departments

Authors	Study Design	N	Controls	Objectives	Outcome Measures	Imaging	Results
DeKruijk et al (2001)		91 MTBI patients	92 healthy controls	Blood samples from 104 MTBI patients were taken shortly after the trauma for measurement of S100B and NSE in serum.	NSE, S100B, Glasgow Coma Scale	None	Median NSE concentration was only slightly higher in patients than in controls (9.8 mg/L; 10-90 percentile range 6.9-14.3 mg/L) than in controls (9.4 mg/L; 6.3-13.3 mg/L). Median S100B concentration was significantly higher in patients (0.25 mg/L; 0.00-0.68 mg/L) than in controls (0.02 mg/L; 0.00-0.13 mg/L).
Calcagnile et al (2012)	Prospective cohort validation study	512 patients	None	S100B sampling of TBI patients.	S100B, GCS, CT scanning, symptom questionnaire	CT	73% showed elevated S100B. S100B had a sensitivity of 100% and a specificity of 28% for significant intracranial complications.
Savola et al (2004)		379 consecutive trauma patients	None	Serum protein S100B levels were determined after a trauma event.	Injury Severity Score, Rivermead Post Traumatic Amnesia Protocol, CT scanning, S100B	CT	The head trauma patients had a significantly higher median S100B than the patients with extra-cranial injuries ($P < 0.001$). Serum S100B levels also correlated with the severity of brain injury ($P < 0.001$), the highest values occurring in the patients with moderate-to-severe brain injury.
Naeimi et al (2006)	Prospective study	45 patients with TBI	None	Neurologic examination and CCT were performed. S-100B and NSE were analyzed.	GCS, S100B, NSE, CT scanning	CT	Significant difference between the S-100B levels of minor and severe head injuries. Statistically significant correlation between an increase of S-100B and NSE and a cerebral pathological finding in CTs ($P = 0.0497$).
Egea-Guerrero et al (2012)		143 mild TBI patients	None	Blood sample analysis at 6 hours post-TBI. A routine CT scan was obtained within 24 hours postinjury.	S100B, CT scanning	CT	Significant differences were found between S100B levels and CT findings ($P = 0.007$). S100B was found to be a useful tool for detecting the presence of brain injury in CTs ($P = 0.007$).
Topolovec-Vranic et al (2011)	Prospective observational study	141 patients	None	Blood samples for serum protein S100B and NSE were collected from 141 emergency department patients within 4 hours of a suspected mild TBI (mTBI).	The Rivermead Post-Concussion Symptoms Questionnaire (RPQ), Galveston Orientation and Amnesia test, S100B, NSE	None	Levels of S100B and NSE increased in 49% and 65% of patients with TBI, respectively. 68% and 38% of the patients were considered impaired at 1 wk and 6 weeks after injury, respectively.
Sojka et al (2006)	Research project	88 patients	None	S-100B and NSE assessed in acute phase in mild TBI patients. The occurrence of post-traumatic stress-related symptoms 1 yr after the trauma was analyzed.	GCS, S100B, NSE, Impact of Event Scale questionnaire	None	Only S100B in the second sample was statistically significantly ($P < 0.05$) associated to symptoms.

TABLE 4. Summary of Studies Included—Blood Biomarkers in Emergency Departments *(Continued)*

Authors	Study Design	N	Controls	Objectives	Outcome Measures	Imaging	Results
De Boussard et al (2004)	Prospective cohort study	122 patients	None	S100B, S100A1B and S100BB concentrations were examined in MTBI patients.	GCS, CT scan, S100, MRI, RPQ	CT and MRI	Mean values and proportions of subjects above cutoff limits for S100B and S100A1B were significantly higher in each trauma group than in noninjured controls, but only for S100A1B when patients with MTBI were compared with controls with orthopedic injuries.
Herrmann et al (2001)		69 patients	None	Blood samples were analyzed at day 1, day 2 and day 3 after TBI.	S100B, NSE, cranial CT, GCS, Neuropsychological assessment	CT	Patients with short- and long-term neuropsychological disorders had significantly higher NSE and S100B levels.
Honda et al (2010)	Retrospective analysis	34 trauma patients	None	Serum GFAP, S-100B, and NSE concentrations were collected from the patients for 3 days.	CT scanning, Serum GFAP, S100B, NSE	CT	Serum GFAP, S100B, and NSE were significantly higher in the TBI patients than in the non-TBI patients.
Korfias et al (2007)	Prospective observational study	102 adult patients with severe TBI	None	Serum S-100B levels were measured on admission and every 24 h thereafter for a maximum of 7 d.	GCS, CT scanning, S100B	CT	Initial S100B levels were significantly related to pupillary status, CT severity, and 1-mo survival.
Bazarian et al (2006)	Prospective study	35 mild TBI patients	None	Mild TBI patients presented to the emergency department. S100B and tau were compared to 3 month RPQ scores.	GCS, CT scanning, cleaved tau, S100B, RPQ	CT	The linear correlation between marker levels and RPCQ scores was weak. There was no statistically significant correlation between marker levels and 3-month PCS.
Biberthaler et al (2001)	Prospective study	52 patients with minor head trauma	Positive control group of 10 severe TBI patients and for a negative control group with 20 healthy volunteers	At admission the patients underwent a routine CCT to detect intracerebral lesions, and blood samples were drawn to investigate circulating levels of S-100b.	GCS, CT scanning, S100B	CT	The initial S-100B serum levels of MHT patients were 0.470 $\pm$ 0.099 ng/mL, those of the positive control group were 7.16 $\pm$ 3.77 ng/mL, and those of the negative control group were 0.05 $\pm$ 0.01 ng/mL. Relevant pathologic CCTs were detected in 28.8% of MHT patients.

Finally, Arslan et al[27] showed increases in both S100B and H-FABP levels after Greco-Roman and free style wrestling. The study indicates that the increase in S100B may be extracerebral in nature. In addition, it is postulated that the exercise associated with wrestling may influence the permeability of the BBB and that wrestling-related impact on the brain may then result in the demonstrable release of S100B in the blood.

Limitations are prevalent in the research examining blood biomarkers in sports. Some of the studies only examined isolated injuries to the head, without considering the potentially confounding effects of extracranial injuries on S100B elevations.[16] S100B and NSE are considered to be quantitative markers of the extent of damage of brain tissue. Yet, S100B and NSE may also occur in cells outside the central nervous system. Accordingly, contribution from these sources to the increases in serum concentration cannot be ruled out. Even if most of the increases of S100B originated from brain tissue, it is not known whether this increase is due to the destruction or the activation of astroglial cells or whether it is merely due to the opening of the BBB. Relatively small sample sizes were used, which restricts the possibilities of examining biomarker levels in relation to different forms and severities of concussion.[20,21,23,26] Some studies did not have access to preseason samples for all players, which would have made it easier and more accurate to evaluate the longitudinal change in biomarker levels after concussion.[20,26] Possible reasons for undetectable concentrations in some studies could be the time of sampling. Blood biomarkers, such as S100B, have a short time frame for detection. Neselius et al[22] examined S100B levels 1 to 6 days after a bout. Similarily, Zetterberg et al[23] measured biomarker levels after 2 months. These extended periods may not be optimal for accurate biomarker level

TABLE 5. List of Primary Outcome Measures

Outcome Measures	No. of Studies
AMPAR	1
CT Scanning	10
DTI	1
GCS	8
GFAP	3
ImPACT	2
MRI	1
NSE	12
RPQ	7
S100B	25
Tau	3

detection. In addition, another limitation was the grading of injury in some studies. Some subjects did not lose consciousness and only one reported symptoms, which is possibly why the mTBI was very mild.[22] Finally, a wide range of the studies included in this review did not use imaging to classify concussion or mTBI.[27] Computed tomography (CT) scanning is best used acutely for evaluating bone fracture and intracranial bleeding, contusion, mass effects, and/or brain stem herniation. MRI is more sensitive for evaluating persistent or worsening symptoms or concern for underlying pathology.[28] The use of imaging results in more accurate classifications of concussion. As a result, the absence of imaging in some studies means that the findings must be interpreted with caution.

Blood Biomarkers in Emergency Departments

Another major area of blood biomarker research involves examining TBI patients in an emergency department setting. Blood biomarkers have been proposed as a prognostic tool to help aid management. By contrast, DeKruijk et al[29] investigated serum NSE and S100B concentrations in a group of patients with clearly defined MTBI and a group of nontrauma controls. S100B was found to be a potentially useful marker for brain damage in mTBI. Honda et al[30] found serum GFAP was significantly higher in the TBI group than in the non-TBI group at all 3 sampling points. Serum S100B was significantly higher in the TBI group than in the non-TBI group on day 2, and NSE was significantly higher in the TBI group on day 2 and day 3. Sojka et al[31] singled out concentrations of S100B in blood samples obtained roughly 10 hours after trauma as being the only statistically significant variable associated with patients complaining about post-traumatic stress-related symptoms reported at follow-up 1 year after the trauma. Topolovec-Vranic et al[32] found that 65% and 49% of TBI patients had increased levels of

TABLE 6. Studies Indicating the Clinical Utility of Blood Biomarkers

	S100B			NSE			Tau			GFAP			AMPAR		
Study	Yes	No	NA	Yes	No	NA	Yes	No	NA	Yes	No	NA	Yes	No	NA
Arslan et al (2010)	X														
Bazarian et al (2006)		X						X							
Calcagnile et al (2012)	X														
Dambinova et al (2013)													X		
De Boussard et al (2004)	X														
DeKruijk et al (2001)	X			X											
Egea-Guerrero et al (2012)	X														
Graham et al (2011)	X			X											
Herrmann et al (2001)	X			X											
Honda et al (2010)	X			X											
Kiechle et al (2014)	X														
Korfias et al (2007)	X														
Marchi et al (2013)	X														
Naeimi et al (2006)	X														
Neselius et al (2013)	X						X								
Puvenna et al (2014)	X														
Savola et al (2004)	X														
Shahim et al (2014)	X						X								
Sojka et al (2006)	X														
Stalnacke et al (2003)	X														
Stalnacke et al (2004)	X			X											
Stalnacke et al (2006)	X			X											
Straume-Naesheim et al (2008)	X														
Topolovec-Vranic et al (2011)	x			X											
Zetterberg et al (2009)				X											
Biberthaler et al (2001)	X														

NSE and S100B, respectively. The findings indicate that a few factors readily available at, or shortly after, the time of injury can predict poor outcome at 1 week and 6 weeks after injury. Egea-Guerrero et al[33] indicated a strong correlation between the existence of intracranial lesions and high levels of S100B. Savola et al (2004)[34] indicated that both brain injuries and extracranial injuries independently increase serum S100B in trauma patients with multiple injuries. The severity of injury correlated positively with S100B values, the highest levels in head trauma patients being found in those with moderate-to-severe brain injuries. In the absence of head trauma, large extracranial injuries increase S100B levels considerably, whereas small extracranial injuries only rarely lead to elevated values. By stark contrast, Naeimi et al[35] could not find a clear correlation between S100B and the outcome of brain injury. The study concluded that the clear correlation between S100B and NSE values with the CT findings in this study did not validate both markers as independent predictors of diagnosis and prognosis. De Boussard et al[36] found that S100A1B seems to be more specific for brain injury than S100B in patients within the milder part of the mTBI spectrum. Herrmann et al[37] described the differences in S100B secretion in correlation with CT severity. Patients with short- and long-term neuropsychological disorders had significantly higher NSE and S100B serum concentrations and a significantly longer lasting release of both markers. Korfias et al[38] indicated that initial serum S100B protein is correlated with clinical admission and the severity displayed by neuroimaging and this has been supported by other studies.[37–39] In addition, Calcagnile et al[42] concluded that the clinical use of S100B for management of mild head injury (MHI) is safe and effective. Adult MHI patients, without additional risk factors and with normal S100B levels within 3 hours of injury, can safely be discharged from the hospital.

Similarities exist between research into blood biomarkers in an emergency setting and research into blood biomarkers in sports. Limited biomarker predictive power is a common issue. This could be due to the possibility that not all patients with clinically defined mild TBI have brain injury, resulting in a misclassification bias. Low-marker accuracy could be due to their expression in the serum in the absence of brain injury. S100B and other CNS proteins are known to reside in small amounts in normal CSF; however, a closed BBB prevents these proteins from entering the serum. Iatrogenic opening of the BBB using parenteral mannitol in subjects without brain injury was recently shown to result in increases in serum S100B levels. This possibility cannot be confirmed until a gold standard for the detection of brain injury after mild TBI has been developed. The use of small sample sizes and the lack of control groups indicate that further research is required to support findings of the studies.[30,35] Similar to the research in sport, the blood sampling points were not optimal for diagnosis and treatment selection in the clinical context in some of the studies. The severity of the brain injury was not assessed with CT, as management routines were followed in the hospital.[31] Some of the follow-up rates were low and the follow-up periods were too short.[32] The use of subjective self-reported outcome measures must be questioned as it calls into question the validity and reliability of the responses.[31] The interference from traumatic lesions associated with TBI may have caused limitations in some studies. The S100B protein has exogenous origins which could increase its levels in serum. This must be accounted for when proposing blood biomarker use as a diagnostic tool. Finally, some studies[30] only offered prehospital triage to patients with severe trauma. As a result, the assessment of TBI may only reflect a partial portion of the complete trauma population. It is important to note that some of the biomarker research in an emergency setting examined biomarkers as predictors of mortality as opposed to morbidity.

Although limitations exist, the overall findings of the studies reviewed suggest that S100B may be the most clinically useful blood biomarker. Brain injury is a complex process and blood biomarker analysis is only a small part of a comprehensive test battery. S100B was found to have a sensitivity of 100% for significant intracranial complications[42] and it also correlated well with brain injury detected by CT scanning.[35] S100B has been found to predict severity of brain injury (Savola et al, 2004) and is known as a good predictor of abnormal initial CT results.[43] Previous research supports this. The sensitivity for S100B has been found to be 98% for all types of intracranial pathology on CT scanning.[44] In addition, S100B correlated with CTs regarding TBI with a sensitivity of 96.8% and a specificity of 42.5%.[45] In sports, such as boxing and ice hockey, S100B increased significantly after activity.[17,21] However, S100B lacks specificity for identifying and isolating brain injury.[46] The specificity of S100B is further complicated by the fact that various types of physical activities affect S100B concentrations in apparently healthy athletes.[31] Schulte et al[47] suggest that variations in brain activity after intensive exercise may increase S100B levels without any head trauma. This lack of specificity diminishes the clinical utility of S100B as a blood biomarker for concussion and indicates that it should be used as part of a test battery along with other clinical markers. Further research is required to examine and validate the specificity of S100B as a biomarker for brain injury.

CONCLUSIONS

In summary, an overview of common TBI biomarkers, namely S100B, NSE, tau, and GFAP, provides contradictory results in regard to the overall utility in diagnostic and prognostic roles. Most reviewed studies indicated that S100B measurement and use, either acutely or at several time points, can distinguish injured from noninjured patients with an uncertain degree of utility in predicting mortality. At present, S100B has largely become an acceptable biomarker of TBI; however, studies have begun to highlight the need to incorporate clinical symptoms instead of S100B concentration in isolation on the basis of inconsistent results across published studies. Further research is needed to evaluate and validate the use of tau, NSE, and GFAP as a diagnostic aid in the management of concussion and TBI.

Any diagnostic or prognostic information that can be obtained is important when managing athletes with concussion. Unfortunately, at this time, biomarkers have only a limited role in the evaluation and management of concussion. Although several biomarkers of brain injury have been identified, continued research is required. S100B holds promise as the most clinically useful diagnostic biomarker. However, several biomarkers suffer from a lack of specificity, often being induced or released into the serum in response to other diseases or bodily injuries. This lack of specificity has hampered the effort to identify markers of mild TBI, especially in the context of trauma. A single biomarker may not have desired sensitivity and specificity for diagnosis nor for predicting outcome. Biomarkers, in combination with other clinical data, such as head CT, would maximize the diagnostic accuracy. The methodological limitations evident in blood

biomarker research results in the need for the clinical utility of blood biomarker use in concussion to be further explored.

References

1. McCrory P, Meeuwisse W, Aubry M, et al. Consensus statement on concussion in sport—the 4th International Conference on Concussion in sport held in Zurich, November 2012. *Clin J Sport Med*. 2013;23:89–117.
2. Meehan W, d'Hemecourt P, Comstock R. High school concussions in the 2008–2009 academic year: mechanism, symptoms, and management. *Am J Sports Med*. 2010;38:2405–2409.
3. Barkhoudarian G, Hovda D, Giza C. The Molecular pathophysiology of concussive brain injury. *Clin Sports Med*. 2011;30:33–48.
4. Abrahams S, Fie S, Patricios J, et al. Risk factors for sports concussion: an evidence-based systematic review. *Br J Sports Med*. 2014;2:91–97.
5. Hollis S, Stevenson M, McIntosh A, et al. Incidence, risk, and protective factors of mild traumatic brain injury in a cohort of Australian nonprofessional male rugby players. *Am J Sports Med*. 2009;37:2328–2333.
6. Bey T, Ostick B. Second impact syndrome. *West J Emerg Med*. 2009;10: 6–10.
7. Fuller CW, Taylor A, Raftery M. Epidemiology of concussion in men's elite Rugby-7s (Sevens World Series) and Rugby-15s (rugby World Cup, Junior World Championship and rugby Trophy, Pacific Nations Cup and English Premiership). *Br J Sports Med*. 2015;49:478–483.
8. Pellman E, Viano D, Tucker A, et al. Concussion in professional football: Reconstruction of Game impacts and injuries. *Neurosurgery*. 2003;53: 799–814.
9. Guskiewicz K, Register-Mihalik J, McCrory P, et al. Evidence-based approach to revising the SCAT2: introducing the SCAT3. *Br J Sports Med*. 2013;47:289–293.
10. Wright B, Lai J, Sinclair A. Cerebrospinal fluid and lumbar puncture: a practical review. *J Neurol*. 2012;259:1530–1545.
11. Len TK, Neary JP. Cerebrovascular pathophysiology following mild traumatic brain injury. *Clin Physiol Funct Imaging*. 2011;2:85–93.
12. Moher D, Liberati A, Tetzlaff J, et al; PRISMA group. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *PLoS Med*. 2009;6:e1000097.
13. Townend W, Ingebrigtsen T. Head injury outcome prediction: a role for protein S-100B? *Injury*. 2006;12:1098–1108.
14. Selakovic V, Raicevic R, Radenovic L. The increase of neuron-specific enolase in cerebrospinal fluid and plasma as a marker of neuronal damage in patients with acute brain infarction. *J Clin Neurosci*. 2005;12:542–547.
15. Franz G, Beer R, Kampfl A, et al. Amyloid beta 1-42 and tau in cerebrospinal fluid after severe traumatic brain injury. *Neurology*. 2003;60:1457–1461.
16. Kiechle K, Bazarian J, Merchant-Borna K, et al. Subject-specific increases in serum S-100B distinguish sports related concussion from sports-related exertion. *PLoS One*. 2014;9:e84977.
17. Shahim P, Tegner Y, Wilson D, et al. Blood biomarkers for brain injury in concussed professional ice hockey players. *JAMA Neurol*. 2014;71:367.
18. Marchi N, Bazarian J, Puvenna V, et al. Consequences of Repeated blood-brain barrier disruption in football players. *PLoS One*. 2013;8:e56805.
19. Stalnacke B, Tegner Y, Sojka P. Playing ice hockey and basketball increases serum levels of S-100B in elite players: a pilot study. *Clin J Sport Med*. 2003;13:292–302.
20. Dambinova S, Shikuev A, Weissman J, et al. AMPAR peptide values in blood of Nonathletes and Club sport athletes with concussions. *Mil Med*. 2013;178:285.
21. Graham M, Myers T, Evans P, et al. Direct hits to the head during amateur boxing is associated with a rise in serum biomarkers for brain injury. *Int J Immunopathol Pharmacol*. 2011;24:119–125.
22. Neselius S, Zetterberg H, Blennow K, et al. Olympic boxing is associated with elevated levels of the neuronal protein tau in plasma. *Brain Inj*. 2013; 27:425–433.
23. Zetterberg H, Tanriverdi F, Unluhizarci K, et al. Sustained release of neuron-specific enolase to serum in amateur boxers. *Brain Inj*. 2009;23:723–726.
24. Stalnacke B, Ohlsson A, Tegner Y, et al. Serum concentrations of two biochemical markers of brain tissue damage S 100B and neurone specific enolase are increased in elite female soccer players after a competitive game. *Br J Sports Med*. 2006;40:313–316.
25. Stalnacke B, Tegner Y, Sojka P. Playing soccer increases serum concentrations of the biochemical markers of brain damage S-100B and neuron-specific enolase in elite players: a pilot study. *Brain Inj*. 2004;18: 899–909.
26. Straume-Næsheim T, Andersen TE, Jochum M, et al. Minor head trauma in soccer and serum levels of S100B. *Neurosurgery*. 2008;62: 1297–1306.
27. Arslan F, Büyükyazi G, Ulman C, et al. Examining acute changes in some serum biochemical markers of brain tissue damage after free and Greco-Roman Style wrestling. *Turkish J Biochem*. 2010;35: 307–312.
28. Harmon KG, Drezner JA, Gammons M, et al. American medical Society for sports medicine position statement: concussion in sport. *Br J Sports Med*. 2013;47:15–26.
29. De Kruijk J, Leffers P, Menheere P, et al. S-100B and neuron-specific enolase in serum of mild traumatic brain injury patients. A comparison with healthy controls. *Acta Neurol Scand*. 2001;103:175–179.
30. Honda M, Tsuruta R, Kaneko T, et al. Serum glial fibrillary acidic protein is a highly specific biomarker for traumatic brain injury in humans compared with S 100B and neuron-specific enolase. *J Trauma*. 2010;69: 104–109.
31. Sojka P, Stalnacke B, Bjornstig U, et al. One year follow-up of patients with mild traumatic brain injury: occurrence of post-traumatic stress-related symptoms at follow-up and serum levels of cortisol, S-100B and neuron-specific enolase in acute phase. *Brain Inj*. 2006;20: 613–620.
32. Topolovec-Vranic J, Pollmann-Mudryj M, Ouchterlony D et al. The value of serum biomarkers in prediction Models of outcome after mild traumatic brain injury. *J Trauma*. 2011;71(5 suppl 1):S478–S486.
33. Egea-Guerrero J, Revuelto-Rey J, Murillo-Cabezas F, et al. Accuracy of the S100b protein as a marker of brain damage in traumatic brain injury. *Brain Inj*. 2012;26:76–82.
34. Savola O, Pyntinen J, Tuomo K, et al. Effects of head and extracranial injuries on serum protein S100B levels in trauma patients. *J Trauma Acute Care Surg*. 2004;56(6):1229–1234.
35. Naeimi Z, Weinhofer A, Sarahrudi K, et al. Predictive value of S-100B protein and neuron specific-enolase as markers of traumatic brain damage in clinical use. *Brain Inj*. 2006;20:463–468.
36. De Boussard C, Fredman P, Lundin A, et al. S100 in mild traumatic brain injury. *Brain Inj*. 2004;18:671–683.
37. Herrmann M, Curio N, Jost S, et al. Release of biochemical markers of damage to neuronal and glial brain tissue is associated with short and long term neuropsychological outcome after traumatic brain injury. *J Neurol Neurosurg Psychiatry*. 2001;70:95–100.
38. Korfias S, Stranjalis G, Boviatsis E, et al. Serum S-100B protein monitoring in patients with severe traumatic brain injury. *Intensive Care Med*. 2007; 33:255–260.
39. Biberthaler P, Linsenmeier U, Pfeifer KJ, et al. Serum S-100B concentration provides additional information for the indication of computed tomography in patients after minor head injury—a prospective multicenter study. *Shock*. 2006;25:446–453.
40. Biberthaler P, Mussack T, Wiedemann E, et al. Elevated serum levels of S-100B reflect the extent of brain injury in alcohol intoxicated patients after mild head trauma. *Shock*. 2001;16:97–101.
41. Biberthaler P, Mussack T, Wiedemann E, et al. Evaluation of S-100b as a specific marker for neuronal damage due to minor head trauma. *World J Surg*. 2001;25:93–97.
42. Calcagnile O, Undén L, Undén J. Clinical validation of S100B use in management of mild head injury. *BMC Emerg Med*. 2012;12:13.
43. Bazarian J, Zemlan F, Mookerjee S, et al. Serum S-100B and cleaved-tau are poor predictors of long-term outcome after mild traumatic brain injury. *Brain Inj*. 2006;20:759–765.
44. Undén J, Bellner J, Reinstrup P, et al. Serial S100B levels before, during and after cerebral herniation. *Br J Neurosurg*. 2004;18: 277–280.
45. Pandor A, Goodacre S, Harnan S, et al. Diagnostic management strategies for adults and children with minor head injury: a systematic review and an economic evaluation. *Health Technol Assess*. 2011;15: 1–202.
46. Di Battista A, Rhind S, Baker A. Application of blood-based biomarkers in human mild traumatic brain injury. *Front Neurol*. 2013;4:44.
47. Schulte S, Schiffer T, Sperlich B, et al. The impact of increased blood lactate on serum S100B and prolactin concentrations in male adult athletes. *Eur J Appl Physiol*. 2013;113:811–817.