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A Meta-Analysis: Significance of Biofluid Biomarkers in Sports-Related Traumatic Brain Injury

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A META-ANALYSIS: SIGNIFICANCE OF BIOFLUID BIOMARKERS IN SPORTS-RELATED TRAUMATIC BRAIN INJURY

by

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ABSTRACT

Background: To reduce the reliance on clinical judgment for the regulation of sports-related traumatic brain injury, identifying and measuring objective to biofluid biomarkers can provide important insight into the diagnosis (Determining the type and origin of a disorder) and prognosis (Determining the chance of survival of a disorder) of SR-TBIs. A biomarker is a qualitative or quantitative measurement that provides a measure of a subject's physiological or pathological condition at a specific time or during a disease state. Recent literature has suggested that biomarkers can help in the screening of patients exhibiting symptoms of mild traumatic brain injury (mTBI). Despite insights from recent research, it is not clear whether biomarkers and assessments of sports-related TBI are well-aligned. The objective of this study sought to review the current literature on predictive values of biomarkers: glial fibrillary acidic protein (GFAP), calcium channel binding protein S100 subunit beta (S100β), total-tau and neuron-specific enolase (NSE) for sports-related Traumatic Brain Injuries (SR-TBIs) to improve comprehension of biological and clinical contexts that can help evaluate the use of these biomarkers in sports-related TBIs and their potential function.

Methods: The study was reported based on guidelines recommended by the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA: 2020 Edition) of 8 studies related to the assessment of biomarkers concerning SR-TBI. Literature searches were carried out on PubMed, Google Scholar, ScienceDirect, and ResearchGate. With an evidentiary table, the characteristics of the studies included in the meta-analysis (n = 14 studies) were presented. A significant role for biomarkers in the management of mild traumatic brain injury is suggested by the results of this analysis. From the literature, the significance of biomarkers in SR-TBI was identified along with the biomarkers that can facilitate more accurate clinical decision-making.

Results: The initial search resulted in 73 articles, and the application of exclusion criteria and removal of duplicates resulted in the inclusion of 14 articles. Eight of the included studies were ([26], [27], [28], [30], [34], [39], [40], [41]), three were cohort studies ([25], [37], [45]) one was a pilot study [32], one interview, and an observational study [44]. The review was carried out to determine the efficacy of Biomarkers GFAP, S100β, Total-tau, and NSE to help in the screening of mild traumatic brain injury (mTBI) in patients showing symptoms. The focus is on athletes presenting at an emergency department with possible mTBI requiring a CT scan based on the application of a clinical algorithm. A forest plot was utilized, and the studies had low heterogeneity or variability ($P<0.23$) and I2 statistic is equal to 26%. A random-effects model was used. The plot shows the odds ratio favoring the control group thus depicting the negative predictive value of the biomarkers such that in the control group no mTBI case was reported. The biomarkers were only present in the experimental group which had cases of mTBI. A funnel plot was also used to determine negative predictive values and to provide data screening for the distribution of 14 studies, resulting in a lower quadrant precision overall.

Conclusions: It was established that the utility of biofluid biomarkers in the prediction of mild traumatic brain injury due to SRC is significant when the markers are used in large combinations. The four biofluid biomarkers (S100β, total-tau, GFAP, NSE) under study have strong predictive ability for mTBI, and their use can reduce the number of CT scans among TBI patients participating in athletic activities. Although preliminary evidence shows that other diagnostic treatments may help to mitigate traumatic brain injury sequelae, clinical trials are needed to further test their efficacy, specifically with diverse and high-risk populations. Luckily, the research on mTBI biomarkers is rapidly advancing, and should these biomarkers be better established clinically, they could easily hold many important roles.

DEDICATION

For my thesis chair, Dr. Tina Dow, and committee members, Dr. Camilla Ambivero and Dr. Jonathan Caranto, who encouraged, guided, and advised me throughout the process of making this thesis feasible.

For my family, who are always in my corner pushing me to be a better version of myself every day. Thank you, Mom, for always guiding me on my journey to becoming the woman I am today.

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ABBREVIATION

TBI: Traumatic Brain Injury

mTBI: Mild Traumatic Brain Injury

SR-TBI: Sports-Related Traumatic Brain Injury

SRC: Sports-Related Concussion

CTE: Chronic traumatic encephalopathy

PRISMA: Preferred Reporting Items for Systematic Reviews and Meta-Analyses

GFAP: Glial Fibrillary Acidic Protein

S100β: S100 Subunit Beta Protein

T-tau or tau: Total-Tau

NSE: Neuron-Specific Enolase

UCH-L1: Ubiquitin C-Terminal Hydrolase-L1

NF-L: Neurofilament Light Chain

CSF: Cerebrospinal Fluid

CT: Computerized Tomography

MRI: Magnetic resonance imaging

GSC: Glasgow Coma Scale

PRDX-6: Peroxiredoxin-6 protein

POI: Point-Of-Injury

BM: Biomarker

OR: Odds Ratio

FIGURES

TABLES

Keywords: concussion, sports-related concussion (SRC), clinical studies, predictive value, biofluid biomarkers, or NSE or GFAP or S100β; mild traumatic brain injury; mTBI; TBI pathophysiology; meta-analysis review

INTRODUCTION

When engaging in sporting activities, most athletes tend to engage in physical touch, which may sometimes expose them to the risk of getting an internal brain injury commonly known as Traumatic Brain Injury (TBI). A traumatic brain injury, or concussion, results from a drastic external blow to the head that can be diagnosed through evidence of alterations in brain function and/or behavioral patterns. [1] Concussions will result from exposing the head to significant impacts that may result in the change of position of the brain in the skull, causing a rupture of blood vessels and injury of several cells present in the brain. [2] A TBI will pathologically develop from primary and secondary injuries which will lead to brain injury. The primary injury is related to the first onset of external impact on the skull resulting in the shaking of the skull. The secondary injury will occur minutes or days following the primary injury and can result in both physical and/or chemical changes inside the brain. [3] This secondary injury in the pathophysiology of a TBI is what brings about the concern for possible behavioral change post-injury. Traumatic brain injury (TBI) continues to progressively become more of a public health concern worldwide. According to the CDC, between the years 2001 and 2010, deaths and visits to the hospital drastically increased in cases related to brain trauma injuries. [3] Specifically, males in contact sports, such as football, soccer, and ice hockey, are at a higher risk of being diagnosed with a TBI, and adolescents and young adults had the highest incidence of a sports-related TBI. [4]

Current literature indicates that the impacts of a TBI may go beyond physical, affecting mental health. [5] Recent studies have identified that athletes who suffer a concussion will exhibit significant behavioral changes. [5] According to a study, high school and college athletes who suffered and were diagnosed with concussions developed depressive symptoms, confusion, and mood changes within three weeks following the diagnosis. [6] A study found that, in an older population, social behavior and etiquette were worse after a TBI diagnosis than before. [7] One study notes that the negative social impacts of a TBI perhaps are exacerbated within children, also affecting family dynamics, academic performance, and athletics. [8] Collectively, these studies indicate that TBIs can alter behavior in athletes in general. Athletes who participate in high-contact sports such as football, soccer, and ice hockey are more likely to suffer a traumatic brain injury. [9] Damaging consequences that follow a TBI within these sports can be short-term or long-term depending on some important factors such as the severity of the TBI. [10] Therefore, in anticipation of addressing these concerns in the literature surrounding this consequential topic, this study will examine biomarkers in sports-related TBIs to determine if these biological measures are significant enough to possibly prevent such consequences.

Short-term Consequences

Short-term complications in a sports-related traumatic brain injury (SR-TBI) can occur immediately after the injury. The signs and symptoms of short-term complications can depend on factors, such as TBI severity and/or physical characteristics of the injury. [11] These factors can help categorize the degree of the following types of TBIs: Mild traumatic brain injury (mTBI) and

Moderate to severe traumatic injury. Temporarily, traumatic brain injuries may damage your brain cells, but a more serious traumatic brain injury can result in damage to the brain such as bleeding and bruising. Normally, patients who suffer from mild traumatic brain injuries (mTBI) can show physical, sensory, and/or cognitive symptoms. [10] Physical symptoms of a mTBI include but are not limited to headaches, nausea, dizziness, and more. [10] Sensory symptoms that can be observed in a person after a mTBI are light-sensitive, while cognitive/behavior symptoms can be observed as abrupt changes in mood or the possible feeling of anxiety or depression. [10] Patients dealing with untreated moderate to severe TBI can show the following physical symptoms: loss of consciousness or coordination, or even possible seizures. Cognitive or mental symptoms in moderate to severe TBIs can cause agitation or other unusual behavior in the patient. [11]

Long-term Consequences

Untreated brain injuries can lead to several complications immediately after the incident. The severity of an injury following a traumatic brain injury can also increase the risk of more serious complications. [12] A prolonged injury to the brain that is moderate to severe can cause a persistent change in a person's consciousness, awareness, and responsiveness. There are dangerous levels of consciousness, including but not limited to comas and/or a vegetative state. A coma results in complete unawareness and the person does not respond to a stimulus. Vegetative states indicate extensive brain damage resulting in the unawareness of surroundings, but the person may respond to partial stimulation. [13]

Chronic traumatic encephalopathy (CTE) is a progressive neuropsychological condition caused by repeated force to the head. Athletes who play high collision sports are at a higher risk of developing

CTE compared to nonphysical sports. [10] Cognitive or behavioral changes that occur years after a concussion are known as CTE. [14] CTE has been linked with the following high collision sports: football, hockey, soccer, and others. [14] Particularly in sports, early diagnosis is critical to prevent CTE and long-term consequences. [14] Signs and symptoms can delay coming forward after a person has sustained a brain injury. To provide optimal care to athletes suffering from CTE, which is thought to be primarily caused by repetitive head trauma, early detection methods are fundamental. [14] In addition to headaches, dizziness, unsteadiness in walking, and fatigue, patients with CTE may suffer from cognitive and psychosocial symptoms.

Biomarkers in traumatic brain injury

A biomarker can be detected in bodily fluids or tissues and is referred to as a biological molecule or substance that can be examined to predict the incidence or outcome of a disease. Understanding the relationship between quantifiable biological processes and clinical consequences is critical for expanding mTBI therapy options. On the contrary, many of the more well-studied biomarkers are susceptible to deterioration. Biomarkers are currently only used in clinical practice to rule out the need for a CT scan. However, an exciting and growing area of research focuses on blood tests for TBI diagnosis. A blood test approved by the FDA in 2018 detects two proteins released into the bloodstream by the brain following a mild concussion, UCH-L1, and GFAP. [23] After brain injury, proteins would be synthesized and secreted from neurological cells into the CSF, where they can possibly cross the blood-brain barrier (BBB) and enter the peripheral circulation. [15] The neuron-specific enolase (NSE) and the ubiquitin C-terminal hydrolase (UCH-L1) are both neuron-derived proteins, while protein S100 Beta (S100β) and glial fibrillary acidic protein (GFAP) are both glial-derived proteins. Over the past decade, a growing body of research has

supported the use of biomarkers to detect concussions in children and adults. [16] Over a dozen studies have measured different biomarkers including Protein S100 Beta (S100β), glial fibrillary acidic protein (GFAP), and neuron-specific enolase (NSE). [12] Protein S100 Beta (S100β) and glial fibrillary acidic protein (GFAP) have been the most helpful thus far in the growing body of research supporting biomarkers in patients with TBI. [12] Biomarkers may be the best way for athletes to prevent further injuries if they receive early and personalized treatment after suffering a concussion. Those who are trained in sports medicine, such as athletic trainers, coaches, and clinicians, could detect concussions with a point-of-care test, for instance. Clinically, it can be used to determine whether a patient has suffered a concussion and to screen them for neuroimaging such as CT or MRI. The incorporation of biomarkers into guidelines for returning to sports could be crucial to monitoring injury development and improvement in athletes at high risk of repeated injuries. In the evaluation of new treatments and therapies for concussion, biomarkers could even serve as surrogate measures of efficacy. [16] In sports such as football, ice hockey, and soccer, where concussions are common, early diagnosis and predictive tools are becoming increasingly important to prevent CTE and other long-term effects of concussions.

It is possible that diagnostic testing and a comprehensive medical evaluation may reveal the issues following a TBI injury, but the entirety of the injury may not be completely understood immediately after. Diagnosis of a TBI is individualized, depending on many factors such as age, health, medical history, the extent of the condition, and the presence of other injuries. [22] Currently, healthcare providers utilize different tests and measures to diagnose TBI such as the Glasgow coma scale (GSC), blood tests, and others. Although imaging can be found to be accurate, it is possible that false positives can occur and that flaws can be evident. Also, patients are exposed

to more radiation utilizing these current imaging techniques. In some cases, a biomarker blood test can possibly eliminate the need for a CT scan on individuals whose injury is unlikely to show up on the scan.

Computed tomography (CT) and Magnetic resonance imaging (MRI) are imaging brain tests, which have been identified to be the most efficient way currently of observing the condition of internal body parts. [22] The functions of these tests specifically include imaging techniques to diagnose brain injuries such as a TBI in the brain. [23] Specific to traumatic brain injuries, the most common technology used to determine patients with a more severe TBI is a CT. [22] As a diagnostic tool, it uses X-rays, which are electromagnetic waves that create an image of soft tissues and bones to help determine where brain damage occurred. [24] An MRI can be used to identify changes in the performance and structure of the brain after an injury, as well as assess the structure of the brain at a more detailed level. [22] After initial treatment and assessment, an MRI may be used. Since the test is more sensitive, it can detect the slightest of changes in the brain that can be missed on a CT scan. [22,24] Much of what occurs to the brain following a TBI occurs at the cellular level, so brain scans can be utilized to determine the magnitude of the brain injury and whether surgery is required to repair any damage. [22] The Glasgow Coma Scale is one of the most widely used tools for assessing the level of consciousness after TBI. [23] This method is commonly a 15-point, standardized test that assesses verbal abilities, eye functions, and physical awareness of body movements. There are also numerous in-depth tests such as cognitive or speech/language tests that can identify levels of injury or damage in TBI patients, in addition to neurological exams, which are a series of quick questions and exercises that assess how well the brain and body are functioning after a TBI. [22] As part of a neurological exam, you can assess

abilities such as but not limited to the patient's motor skills, senses, coordination, emotions, and changes in behavior. For medical evaluation, screening tools can identify the most concerning concussions. [24]

Currently, diagnosis of TBIs relies on neurological examination, use of Computerized tomography (CT), X-rays, patient history, and presenting symptoms. [35] The difficulty arises in the fact that symptoms of mTBIs can present themselves despite a negative neuroimaging result and that diagnosis can be subjective when based on self-reported neurological symptoms. [33] The diagnosis of mTBIs is also made difficult due to the quick resolution of symptoms and the lack of objective evidence for a positive diagnosis using neuroimaging techniques. [33] Accurate diagnosis can also be impeded by the desire to conceal signs or the inability of the patient to identify subtle changes in consciousness and other dysfunctions. [33] The tendency to conceal symptoms is often motivated by an athlete and/or coach's desire for a return to play. [42] A premature return to play, however, can be unsafe for the athlete due to their increased risk of developing pronounced neurological injuries if not fully recovered. [42] Thus, there is a necessity for objective indicators of traumatic brain injury.

Neuroimaging techniques can potentially be unavailable because of patient radiation exposure and their high-cost healthcare expenditures compared to the sparse distribution of subjects across regions. The need to attend to patients with possible mTBI in an efficient and economically effective manner has led to significant research and development in accurate biofluid biomarkers. Designations of biomarkers connected to TBI pathophysiology are of clinical value due to their

potential for increased accuracy in the characterization and risk analysis of TBI. Personalized and targeted therapeutic interventions can be made possible as a result.

This meta-analysis aims to review the available literature on the predictive value of biofluid biomarkers for concussions and to emphasize the biological and clinical contexts that can help evaluate the use of these biofluid biomarkers in efficiently diagnosing and treating SR-TBIs. This meta-analysis concerned itself with biomarkers that hold promising value in diagnosing TBIs within the clinical setting. These biomarkers include S100 calcium-binding protein B (S100β) and glial fibrillary acidic protein (GFAP).

METHODOLOGY

Overview

A comprehensive review was conducted to evaluate the predictive value of biofluid biomarkers for concussions to improve comprehension of biological and clinical contexts that can help evaluate the use of these biofluid biomarkers in sports related TBIs. This review was conducted concurrently from January 2022 to March 2022. The methodology for this meta-analysis literature review is in accordance with the PRISMA 2020 statement guidelines [47].

Eligibility Criteria

This research paper was reported based on the 2020 Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) edition. Literature searches were carried out on PubMed, Google Scholar, ScienceDirect, and ResearchGate databases. A second search was performed on the reference lists of the studies obtained in the initial search. Similar articles recommended by the digital libraries mentioned above were included in the results of the second literature search. The search included studies on the utility of S100 calcium-binding protein B (S100β), glial fibrillary acidic protein (GFAP), neuron-specific enolase (NSE), and tau in the prediction of mild traumatic brain injury. Only the results of the first five pages were included to ensure the relevance of the articles to this meta-analysis was assured to some degree. This was also done to ensure the most recently published and most cited articles were obtained in the initial search. Search results were limited to articles published from 2000 to 2021.

Information Sources

A meta-analysis literature search began in January 2021 using the following electronic databases: PubMed (2000-2020), Google Scholar (2000-2020), ScienceDirect (2000-2020), and ResearchGate databases. All databases were last searched in March 2022.

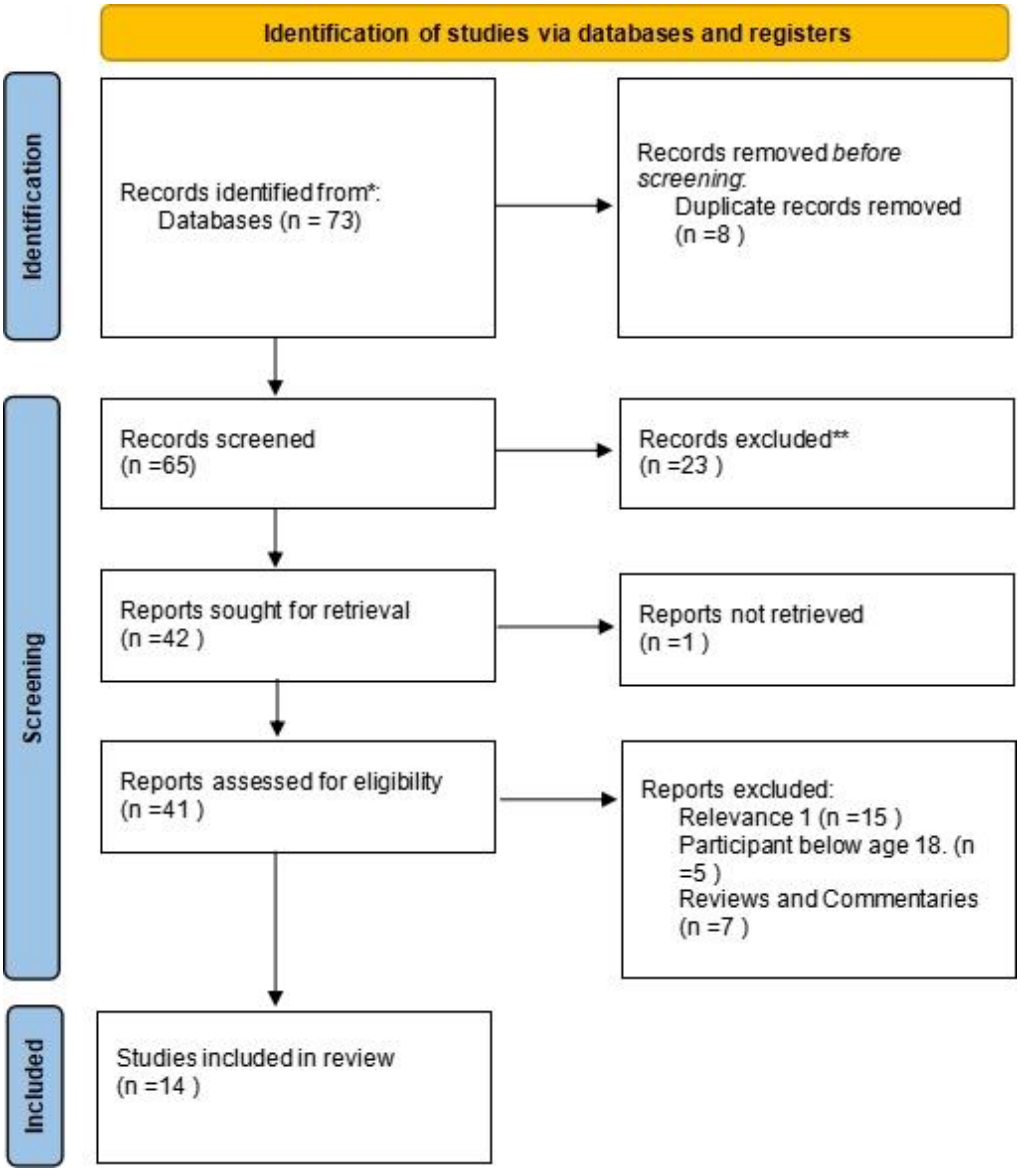
Search Strategy

Keywords used in the search included "concussion," "sport-related concussion (SRC) ", "clinical studies", "predictive value", " biofluid biomarkers", or "NSE " or "GFAP " or " S100β." After the initial search and assessment of the results, new keywords were added, such as “mild traumatic brain injury," " mTBI," and “TBI pathophysiology." The keywords were used in different combinations to guide the literature search and ensure the relevance of the articles in the search results.

Selection Process

An initial screening of articles to remove duplicates and assess inclusion/exclusion criteria by title and abstract was conducted independently. Full-text analysis was then conducted to determine the eligibility of the remaining studies. Reference lists of all eligible articles were then reviewed and screened for additional articles. All eligible studies were included in the meta-analysis review (see **Figure 1**).

Figure 1: Flow chart of the Meta-Analysis review search selection process



Guidelines and Selection Criteria

In this meta-analysis, studies that focused on biofluid biomarkers for concussion and that described the biological and clinical contexts that could help evaluate the use of these biofluid biomarkers in

SR-TBI were included. Studies that were only done on adults, that is, participants who are 18 years and older, diagnosed with a concussion were included. Studies that examine biofluid biomarkers S100 calcium-binding protein B (S100β), glial fibrillary acidic protein (GFAP), neuron-specific enolase (NSE), and tau in the prediction of mild traumatic brain injury in sports were included.

The titles of the articles obtained in the initial search were analyzed to determine that they covered the subject of study of this research paper. The abstracts of the remaining articles were then studied to establish the relevance of the given articles to the current study, such that they tried to answer the questions posed herein. At this point, the articles that passed the described eligibility criteria were then fully read to determine their utility and significance in achieving the goal of meta-analysis. The exclusion criteria included the similarity of studies obtained and the evidence of a clearly defined study objective mentioning the predictive value of biofluid biomarkers for concussion. Studies that focused on health and social care staff were not included. Review articles, case reports of fewer than 5 participants, commentaries, studies that examine participants with conditions that can be confounders, and studies involving the use of medication were excluded. Studies that were not reported in English were not included. Articles with individual keywords were excluded as most of them did not contain information relevant to this research paper.

Data Collection

The data was collected from the selected studies to establish a suitable answer to the questions of this research paper. The data from the studies that passed the exclusion and inclusion criteria were extracted into a predefined extraction table. The author's name, publication date, type of study, sample size, demographics, and the studies' results were recorded. The extraction table provided a

means of assessing the reliability of the included papers according to the parameters described below. Review Manager 5.4 (RevMan) is Cochrane's software that facilitates the preparation of study data. Within this meta-analysis, a forest plot and funnel plot were designed and created using RevMan version 5.4.

RESULTS

Study Selection

For the literature review, the initial search of electronic databases resulted in 73 studies, and the application of exclusion criteria by title and abstract and removal of duplicates resulted in the inclusion of 14 studies. A 2020 PRISMA diagram detailing the process of eligible article selection was included in this meta-analysis and can be found in **Figure 1.**

Study Characteristics

Table 3 illustrates the characteristics of the included studies. Information of characteristics including author, type of study/research design, biomarker protein analyzed, number of participants in the study, sampling type-assay analyzer, results/conclusions/recommendations of the studies, the mean age of participants, biomarker levels in TBI patients, and biomarker levels in controls. Eight of the included studies were ([26], [27], [28], [30], [34], [39], [40], [41]), three were cohort studies ([25], [37], [45]) one was a pilot study [32], one interview, and an observational study [44]. The studies essentially represented the current body of knowledge concerning the prediction of mild traumatic brain injury using biomarkers. Regarding the assays used among the 14 studies evaluated, 4 used electrochemiluminescence immunoassay (ECLIA) on an Elecsys analyzer or the Cobas 6000 analyzer (Roche Diagnostics), 1 used venipuncture (Fujirebio Diagnostics), 1 used Human Neurology 4-Plex A assay (N4PA) on an HD-1 Single molecule array, 2 used immunoluminometric assays, 3 used immunosorbent assays(ELISA), and 1 used serum digital array technology, 1 used sandwich chemiluminescence immunoassay (LIAISON®), 1 used immunoradiometric assay kit (Sangtec®).

Table 1: Reference table for peak levels and characteristics of investigated biomarkers

Biomarker	Peak Levels after mTBI	Cell	Mechanism
S100β	1-3 hours	astrocyte	astrogliosis
GFAP	About 24 hours	astrocyte	glial damage
NSE	About 12 hours	neuron	neuronal damage
tau-T	About 1 hour	neuronal axon	axonal injury

Table 1 represents the characteristics of the 4 investigated biomarkers: S100β, GFAP, NSE, and tau-T. Properties of these biomarkers included in the reference table are peak levels after a mTBI, the type of cell that expresses that biomarker, and the mechanism of causation.

Table 2: Studies Indicating Clinical Utility of Investigated Biomarkers

	s100B		NSE		GFAP		tau	
Author	Yes	No	Yes	No	Yes	No	Yes	No
(Blais-L'Écuyer et al., 2020)	☐	☐	☐	☑	☑	☑	☐	☑
(Schulte et al., 2015)	☑	☐	☑	☐	☐	☑	☐	☑
(Bogoslovsky et al., 2017)	☐	☑	☐	☑	☑	☐	☑	☐
(HERRMANN et al., 2000)	☑	☐	☑	☐	☐	☐	☐	☐
(Papa et al., 2014)	☑	☐	☐	☑	☑	☐	☐	☑
(Pelinka et al., 2004)	☑	☐	☐	☑	☑	☐	☑	☑
(Thelin et al., 2019)	☐	☑	☐	☑	☑	☐	☑	☐
(Janigro et al., 2020)	☑	☐	☐	☑	☐	☑	☐	☑
(Papa et al., 2016)	☐	☑	☐	☑	☑	☐	☐	☑
(Wolf et al., 2013)	☑	☐	☑	☐	☐	☑	☐	☑
(Romner et al., 2000)	☑	☐	☐	☑	☐	☑	☐	☑
(Lewis et al., 2017)	☑	☐	☐	☑	☑	☐	☐	☑
(Bouvier et al., 2016)	☑	☐	☐	☑	☐	☑	☐	☑
(Di Battista et al., 2018)	☐	☑	☐	☑	☐	☑	☑	☐

Table 2 shows which studies indicate the clinical utility of the investigated biomarkers. Demonstrating a correlation between the 14 studies included in this review and the four investigated biomarkers: s100β, NSE, GFAP, and T-tau.

Table 3: Evidence table summarizing studies assessing biomarkers in athletes

Author	Type of study/ Research Design	BM Protein analyzed	# Of Participants- Experiment/Control	Sampling type- Assay analyzer	Results, Conclusion And Recommendations	Mean age of participants	BM levels in TBI patients	BM levels in controls
(Blais-L'Écuyer et al., 2020)	Cohort study- Quantitative analysis	S100β serum protein	476/N/A	Serum (Venous) -enzyme-linked immunosorbent assay (ELISA)	S100β median value was for patients with clinically important brain injury versus the median for patients without clinically important brain injury. Twenty-four (5.0%) patients had a clinically significant intracranial hemorrhage while 37 (7.8%) patients had any type of intracranial bleeding. Sensitivity and specificity of the S100β protein level, if used alone to detect clinically important brain injury, were 16.7% (95% CI 4.7-37.4) 88.5% (95% CI 85.2-91.3), respectively. S100β serum protein level was not associated with clinically significant intracranial hemorrhage in mTBI patients. This protein did not appear to be useful to reduce the number of CTs prescribed in the ED and would have missed many clinically important brain injuries. Future research should focus on different ways to assess mTBI patients and ultimately reduce unnecessary head CT.	41 ± 18	S100β Median: 0.043 µg/L Range: 0.008-0.080	S100β Median: 0.039 µg/L Range: 0.023-0.059

Author	Type of study/ Research Design	BM Protein analyzed	# Of Participants- Experiment/Control	Sampling type- Assay analyzer	Results, Conclusion And Recommendations	Mean age of participants	BM levels in TBI patients	BM levels in controls
(Schulte et al., 2015)	Clinical study- Quantitative analysis	NSE and S100β enzyme	127/N/A	Venipunctures- (Fujirebio Diagnostics, Inc., Göteborg, Sweden	For S100β, 50 % had follow-up testing results lower than the post-injury result. For NSE, 92 % of NSE follow-up results were lower than post-injury. 100% of the results for S100β and NSE were within the athlete-derived reference intervals upon return-to-play and season end. The reported workflow provides a framework for the eventual implementation of biomarkers for concussion assessment into existing assessment protocols and strengthens the need for reliance on clinical laboratory testing. Athlete-specific reference intervals will be required to adequately interpret results.	21	NSE Range: 6.7 to 23.9 µg/L S100β Range: 32 to 250 ng/L	NSE Range: 3.7 to 8.9 µg/L S100β Range: 0 to 96 ng/L
(Bogoslovsky et al., 2017)	Clinical trial- Quantitative analysis	tau, GFAP, and Ab42	34/69	Serum (Venous) - Digital array technology (Quanterix Corporation, Lexington, MA)	Plasma levels of GFAP, tau, and Ab42 are all increased up to 90 days after TBI compared with controls. The levels are maximal on Day 0 for GFAP and tau and on Day 30 for Ab42. Total tau levels: Day 30 correlated with clinical and radiological variables of TBI severity. Ab42 plasma levels:	39	Total panel levels: 1.350 pg/mL Range: 0.8870–2.280	0.80 pg/mL Range: 0.8–1.070

Author	Type of study/ Research Design	BM Protein analyzed	# Of Participants- Experiment/Control	Sampling type- Assay analyzer	Results, Conclusion And Recommendations	Mean age of participants	BM levels in TBI patients	BM levels in controls
					Day 30 correlated with clinical outcome. A combination of all three biomarkers on Days 0 and 30 can be used to differentiate controls from mTBI populations and may be useful as biomarkers of TBI in both acute and subacute phases.			
(HERRMANN et al., 2000)	Clinical study- Quantitative analysis	NSE and S100β	66	Venous blood samples- immunoluminometric assays (Sangtec®) and a fully automated LIA-mat® system	The significant positive correlation between S100β and NSE concentrations and volume of contusions as demonstrated in the present indicates that increased levels of both proteins must be attributed to brain injury. Data indicates that early release patterns of NSE and S100β may reflect the pathophysiological consequences of traumatic brain injury and may mirror complex neuronal-glial interactions	31.5 ± 13.3	S100β Range: 13.2 ± 22.2 NSE Range: 328.0 ± 261.1	N/A
(Papa et al., 2014)	Prospective Cohort Study- Quantitative analysis	GFAP and S100β	208/109	Blood samples- sandwich enzyme-linked immunosorbent assays (ELISAs)	In a general trauma population with and without mTBI, GFAP out-performed S100β in detecting traumatic intracranial CT lesions, particularly in the setting of extracranial lesions on head CT and fractures to the torso and extremities. Both GFAP and S100β demonstrated a rapid appearance in serum post-injury with levels detectable within an hour of injury. The	40	GFAP cutoff level of 0.067 ng/mL S100β cutoff level of 0.020 ng/mL	N/A

Author	Type of study/ Research Design	BM Protein analyzed	# Of Participants- Experiment/Control	Sampling type- Assay analyzer	Results, Conclusion And Recommendations	Mean age of participants	BM levels in TBI patients	BM levels in controls
					temporal profile of GFAP and S100β differed slightly. S100β rose quicker and peaked within the first 2 h, whereas GFAP rose more steadily over 4 h and tapered slightly at 4 h.			
(Pelinka et al., 2004)	Clinical study- Quantitative analysis	GFAP/ S100β	92	Blood samples- a monoclonal immunoluminometric assay (LIAISON® GFAP and S100β assay, AB Sangtec Medical, Bromma, Sweden)	Both GFAP and S100β were related to brain damage, but some (but not all) patterns of TBI appeared to be associated with differences between GFAP and S100β release. Both GFAP and S100β are good predictors of mortality, and the correlation between GFAP and S100β release improves with survival after TBI and is strong beyond 36 h after TBI.	35	GFAP and S100β were lower in ICP of 25 than ICP of 25 mm H2O (p 0.0005) CPP of 60 than CPP of 60 mm Hg (p 0.0005) MAP of 70 than MAP of 70 mm Hg (p 0.0005). GFAP and S100β were lower in GOS 4–5 than in GOS	N/A

Author	Type of study/ Research Design	BM Protein analyzed	# Of Participants- Experiment/Control	Sampling type- Assay analyzer	Results, Conclusion And Recommendations	Mean age of participants	BM levels in TBI patients	BM levels in controls
(Thelin et al., 2019)	Observational study- Quantitative analysis	(S100β), (NSE), (GFAP), (UCH-L1), tau, and (NF-L	172	Blood samples- Human Neurology 4-Plex A assay (N4PA) on an HD-1 Single molecule array (Simoa)	In summary, a panel of several different protein biomarkers, all associated with injury severity, with the different cellular origin and temporal trajectories, improve outcome prediction models.	Median-55	NF-L: 8.8% at 103 pg/mL 8.4% at 7.4 pg/mL tau: 11.9% at 1.2 pg/mL 9.7% at 22.5 pg/mL GFAP: 9.0% at 72 pg/mL 7.3% at 88 pg/mL UCH-L1: 33.3% at 11.4 pg/mL 34.0% at 10.8 pg/mL	N/A
(Janigro et al., 2020)	Pilot Study- Quantitative analysis	S100β	15/15	Saliva, Serum (Venous) - chemiluminescence and automated sandwich ELISA (LIAISON; Diasorin,	The results have shown that: (1) salivary levels of the astrocytic protein S100β are higher than those measured in serum (2) the diagnostic properties of S100β in the blood are like those in saliva (3) a correlation exists between salivary and blood levels of S100β in control and post-TBI conditions.	47	N/A	Below 0.02 ng/mL

Author	Type of study/ Research Design	BM Protein analyzed	# Of Participants- Experiment/Control	Sampling type- Assay analyzer	Results, Conclusion And Recommendations	Mean age of participants	BM levels in TBI patients	BM levels in controls
				Stillwater, MI)	A crucial aspect of TBI diagnostics is the fact that although serum markers such as GFAP, UCH-L1, or S100β already rule out clinically important concussion sequelae with an excellent negative predictive value and a low limit of detection in laboratory-based approaches, there is currently no translation of this technology to a saliva-based POI or POC device.			
(Papa et al., 2016)	Prospective cohort study- Quantitative analysis	GFAP and UCH-L1	584	Blood samples- sandwich enzyme-linked immunosorbent assays	GFAP performed consistently in detecting mTBI, CT lesions, and neurosurgical intervention across 7 days. UCH-L1 performed best in the early post-injury period.	40	GFAP levels Median: 0.112 ng/mL IQR: 0.030-0.462 ng/mL Range: 0.008-8.078 ng/mL UCH-L1 were significantly higher Median: 0.258 ng/mL IQR: 0.109-0.627 ng/mL Range: 0.045-	GFAP levels Median, 0.008 ng/mL IQR, 0.008-0.030 ng/mL Range, 0.008-0.773 ng/mL) ($P < .001$). UCH-L1 Median, 0.171 ng/mL IQR, 0.100-0.417 ng/mL Range, 0.045-4.241 ng/mL

Author	Type of study/ Research Design	BM Protein analyzed	# Of Participants- Experiment/Control	Sampling type- Assay analyzer	Results, Conclusion And Recommendations	Mean age of participants	BM levels in TBI patients	BM levels in controls
							9.000 ng/mL	(P < .001)
(Wolf et al., 2013)	Cohort study- Quantitative analysis	S100β and (NSE)	114	Peripheral venous blood samples- sandwich technique with NSE kits and the Elecsys 2010 analyzer (Roche Diagnostics)	The integration of the biomarker panel as part of a diagnostic rule including high-risk factors symptoms is safe and reliable in determining a diagnosis, pending the availability of more brain-specific biomarkers.	59 ± 23	In patients with a subarachnoid hemorrhage, S100β Median: 0.98 μg/L NSE Median: 18.14 μg/L.	N/A
(Romner et al., 2000)	Clinical study- Quantitative analysis	S100β protein	278/110	Blood samples- immunoradiometric assay kit (detection limit, 0.2 m g/L. Sangtec Medical, Bromma, Sweden)	S100β protein is established as a serum marker of traumatic brain injury. An undetectable serum level of S100β protein predicts normal intracranial findings on a CT scan. Determination of S100β protein in serum may be used to select patients for scanning in situations where CT is in short supply or where patients need to be sent long distances to obtain a CT.	32	Patients suffering severe head injury had detectable S100β levels within 24 h Post-injury. Mean: 3.6 m g/L Range: 1.2–12.5 m g/L S100β was detected in 75% of moderate	N/A

Author	Type of study/ Research Design	BM Protein analyzed	# Of Participants- Experiment/Control	Sampling type- Assay analyzer	Results, Conclusion And Recommendations	Mean age of participants	BM levels in TBI patients	BM levels in controls
							head injuries and 35% of mild. Mean: 0.7 m g/L Range: 0.2–2.2 m g/L	
(Lewis et al., 2017)	Clinical study - Quantitative analysis	(GFAP) S100β, and (UCH-L1)	247/247	Blood samples- electrochemiluminescence immunoassay designed for in vitro diagnostic testing (Cobas 6000, Roche), enzyme-linked immunosorbent assay	A single serum concentration of GFAP, UCH-L1, or S100β within 6 hours of a head injury may be useful in identifying and stratifying the severity of brain injury in emergency department patients with head trauma but cannot reliably exclude a diagnosis of a concussion. A positive GFAP was associated with the presence of concussion.	48.4	GFAP Median: 20.0 pg/mL Range: 20.0–36.7 UCH-L1 Median: 64.0 pg/mL Range: 30.0–110.4 S100β Median: 120.0 pg/mL Range: 75.0–240.0	S100β are given in μg/L Standard normal reference intervals: 0.00 to 0.09 μg/L.
(Bouvier et al., 2016)	Clinical study - Quantitative analysis	S100β	39/27	Venous blood samples- serum S100β concentrations by electro-chemilu	S100β measured 36 h after a match is thus a discriminating test to identify concussion in a male rugby player, with a 100 % negative predictive value.	28.6	The median basal concentration over the whole season was 0.05 lg/L	N/A

Author	Type of study/ Research Design	BM Protein analyzed	# Of Participants- Experiment/Control	Sampling type- Assay analyzer	Results, Conclusion And Recommendations	Mean age of participants	BM levels in TBI patients	BM levels in controls
				minescence immunoassay on a Roche Diagnostics Cobas e411 instrument (Meylan, France)			Minimum: 0.02, Maximum: 0.15 IQR: 0.04–0.06	
(Di Battista et al., 2018)	Clinical study - Quantitative analysis	GFAP, S100β, NSE, Total-tau (NRGN), (CKBB), (VILIP)-1, (vWF), (BDNF), (PRDX)-6, and (MCP)-1	19/19	Venous blood samples: electrochemiluminescent SULFO-TagTM, sensitive electrochemiluminescence detection via sandwich immunoassay	Findings support the continued application of blood biomarkers to help elucidate the pathophysiology of secondary injury after SRC. Higher circulating levels of PRDX-6 in athletes with SRC compared to control athletes in the subacute phase after injury and identified a relationship between SRC and higher PRDX-6 and T-tau levels at medical clearance.	19	PRDX-6 24.6 vs. 20.7 ng/mL T-tau 20.4 vs. 14.4 pg/mL	N/A

Quality Score and Level of Evidence

The overall quality score did not vary for the articles, with 14 of the studies having a quality range of between 8. Based on the Research and Quality Scoring Method, all the included studies were considered high quality. The studies were rated according to their characteristics, and the score

determines the reliability of their published findings. However, none of the studies performed sample justification. (See quality **Table 4**)

Table 4: Quality Assessment Scoring

Quality Assessment

element of assessment	scoring criteria	(Blais-L'Écuyer et al., 2020)	(Schulte et al., 2015)	(Bogoslovsky et al., 2017)	(HERRMANN et al., 2000)	(Papa et al., 2014)	(Pelinka et al., 2004)	(Thelin et al., 2019)	(Janigro et al., 2020)	(Papa et al., 2016)	(Wolf et al., 2013)	(Romner et al., 2000)	(Lewis et al., 2017)	(Bouvier et al., 2016)	(Di Battista et al., 2018)
Study design	0 = Non-randomized, systematic review, observational, cohort study 1 = Randomized experiment, quasi-experiment	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Outcome measure	0 = measure of the outcome is self report 1 =measure of the outcome been mentioned using validated measure	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Clarity of outcome	0 = no definition of study outcome 1 = Clearly defined the study outcome	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Information on withdrawal or dropout rate	0 = not stated or discussed 1 = Clearly stated [number and reasons	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Research question	0 = not clear 1 = Clearly stated	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Participants in sample	0 = not clear 1 = Clearly identified and described	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Participant inclusion/ exclusion criteria	0 = not clear 1 = specified	1	1	1	1	1	1	1	1	1	1	1	0	1	1
Type of location where study conducted and type of data collection performed	0 = not clear 1 = Clearly stated	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Sample size justification and power analysis	0 = Unclear/not provided 1 = Sufficiently described and justified before the study	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total		8	8	8	8	8	8	8	8	8	8	8	7	8	8

the score will be divided into 3 groups:

0 to 5	low quality
6 to 9	high quality

Biomarker Utility in Sport Setting

The review was carried out to determine the efficacy of biomarkers GFAP, S100β, Total-tau, and NSE to aid the screening patients presenting with symptoms of mild traumatic brain injury (mTBI). The focus is on athletes presenting at an emergency department with possible mTBI requiring a CT scan based on the application of a clinical algorithm. The mentioned biofluid biomarkers have a strong predictive ability for mTBI, and their use could reduce the number of CT scans among athletic TBI patients. Athletes who play in higher divisions such as professional or collegiate often sustain one or multiple concussions. The important things in this setting are to determine whether a concussion has happened, and the level of injury sustained. It is possible to make real-time

decisions regarding "return to play" decisions for athletes using a biomarker that is detected in circulation within 30 minutes post-impact. [46] If they are tested again within days of a concussion, combined with other measures such as the Glasgow coma scale (GCS), a scoring system used to determine the level of consciousness post mTBI [23], they could be useful in "return to play" decisions. A key characteristic of sports-related mTBI is repeated concussions over time, both in a recreational and professional sports setting. The forest plot below shows the negative predictive value of the four biomarkers.

Figure 2: Forrest Plot on predictive utility

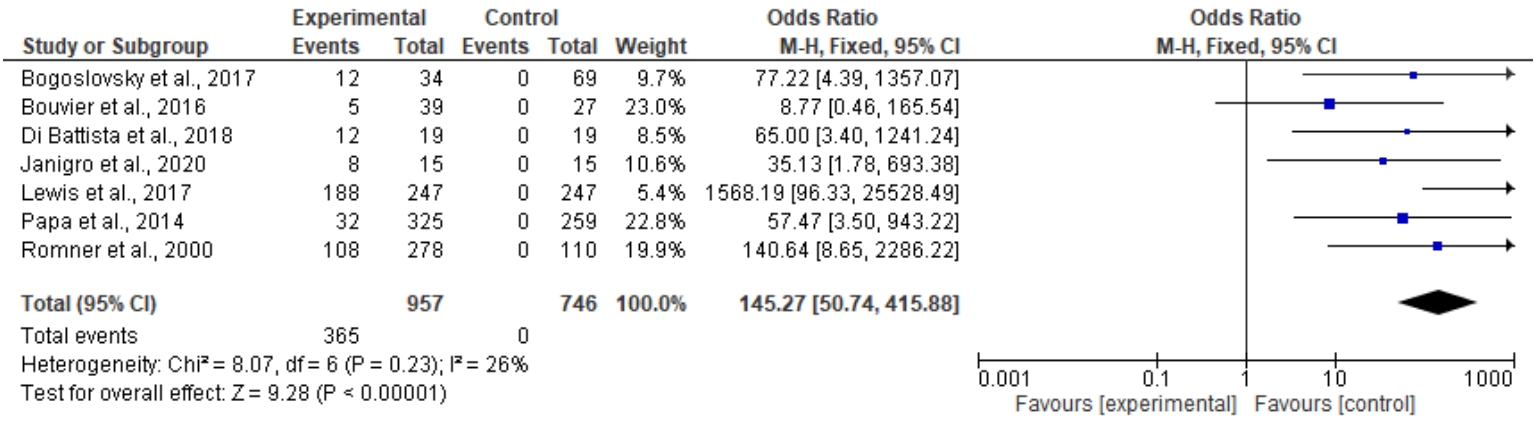


Figure 2 categorizes selected quantitative articles. This allowed me to directly compare the chosen studies and determine quality all in one platform. The studies had low heterogeneity or variability (P<0.23) and the I^2 statistic equaled 26%. A random-effects model was used to estimate the mean of a distribution of effects. The weight (%) reflects how much of an impact a study or subgroup had on the overall findings. The vertical line represents the null effect. The horizontal lines on the plot represent the scale for statistics of Odds Ratio and the 95% confidence intervals. The blue boxes located on the horizontal lines represent the estimate of the study result. The smaller the blue box, the fewer participants in the study and vice versa. The diamond located towards the bottom of the plot illustrates the point estimate and confidence

intervals after the studies or subgroups were averaged. A test of heterogeneity is the I^2 statistic. The formula for calculating I^2 from Cochran's Q is I^2 = 100 percent X (Cochran's Q – degrees of freedom). [50] The plot shows the odd ratios favor the control group thus depicting the negative predictive value of the biomarkers such that in the control group no mTBI case was reported. The biomarkers were only present in the experimental group which had cases of mTBI. Biomarkers may be of considerable use in the management of SRC mild traumatic brain injury. This figure was created using a 5.4 version of the Revman software.

Figure 3: Funnel Plot on the efficacy of biomarkers

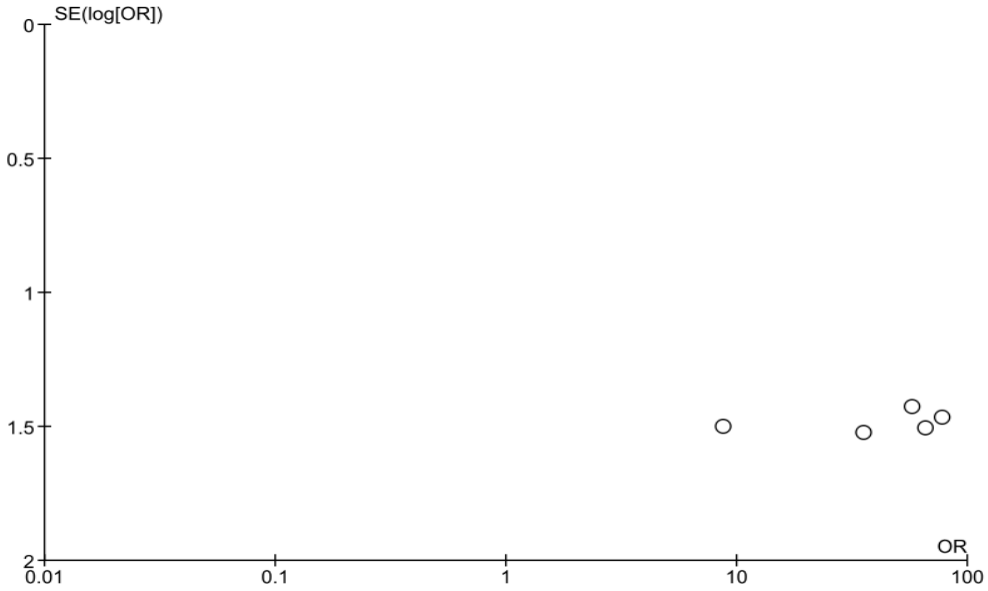


Figure 3 also categorizes selected quantitative articles and assesses the potential role of publication bias. This figure shows lower quadrant precision. The standard error of the effect estimate was chosen as the measure of study size and plotted on the y-axis. This funnel plot screened the distribution of 14 studies for negative predictive value according to the average shown on the y-axis SE (log [OR]) and the mean result on the x-axis (OR). This plot depicted the probability of biomarkers and assessment of publication bias. This figure was also created using a 5.4 version of the Revman software.

DISCUSSION

From the review findings, it was established that the use of biofluid biomarkers in the prediction of mild traumatic brain injury due to SRC is significant when the markers are used in large combinations. Notably, Schulte et al. (2015) determined that NSE biomarkers, the reference intervals that are being used, should not be applied to athletes. This discovery supports clinical medicine and raises the bar for the development and implementation of population-specific reference intervals. They establish that in the context of physical activity, provision of established reference intervals can be difficult; therefore, strict control should be maintained during experimental design determination. This tight control should also be carried over during sample collection for studies and in the establishment of protocols for the implementation of biomarkers in the diagnosis of mTBI, especially among athletes. Schulte et al. (2015), however, report that at the time an athlete returns to play and at the end of the season the results for the given biomarkers were 100% and within athlete reference intervals. According to Herrmann et al. (2000) the considerable positive correlation between S100β and NSE concentrations and the number of impacts in their study suggests there is a direct correlation between increased concentrations of the proteins and traumatic brain injury. As such these proteins can be useful in the diagnosis of SRC mild traumatic brain injury. Herrmann et al. (2000) also determine that the way NSE and S100β are released can describe the pathophysiological impacts of brain injury and illustrate complex neuronal-glial interactions.

S100β protein, according to Romner et al. (2000) has been use as a serum marker for traumatic brain injury. Minute serum concentrations of S100β protein suggest normal intracranial results from neurological imaging techniques such as CT and X-ray scans. Significant concentrations of

the protein in serum can be used to inform the decision of which patients qualify for CT scans in scenarios where the CT is not a viable option such as when patients must travel long distances to obtain one. From their study, Bouvier et al. (2016) conclude that the S100β biomarker can be used as a discriminating test to predict traumatic brain injury after thirty-six hours of impact. This test provides a 100% negative predictive value. Blais-L'Écuyer et al. (2020) determine that S100β serum protein concentration in blood serum samples is unrelated to clinically significant intracranial hemorrhage in patients with mild traumatic brain injury. The protein biomarker was also not effective and productive in reducing the number of patients prescribed with CT scans in the emergency department. Significant traumatic brain injuries would have gone unaddressed if this protein was solely used in the diagnosis of mTBI.

GFAP out-performed S100β in the prediction of traumatic CT lesions, especially in the context of extracranial lesions on head scans and the extremities Papa et al., (2014). The two proteins were detectable in blood serum, within the general trauma population after about an hour after impact to the head. The temporal patterns of the two biomarkers, however, differed somewhat. S100β protein concentration in the serum increased significantly and peaked within the preceding 2 hours of injury while GFAP increased gradually and climaxed after about four hours. Pelinka et al. (2004) findings support the conclusions arrived at by Papa et al. (2014), although some forms of TBI were not associated with differences between the two biomarkers released. According to Pelinka et al. (2004), GFAP and S100β are reliable predictors of mortality, and their correlation in their release amends and strengthens after thirty-six hours. Papa et al. (2016) compared the predictive utility of GFAP in diagnosing mild and moderate traumatic brain and determined that the former effectively performed injury within seven days of injury while the utility of the latter

became apparent in the very early post-injury period. According to Lewis et al. (2017), a single blood test for the concentration of GFAP or S100β within 6 hours of a head impact may be useful in predicting the severity of brain injury in patients with a head injury, but it cannot effectively rule out a diagnosis of concussion.

The use of biomarkers in the prediction of mild traumatic brain injury is substantiated by the use of multiple biomarkers such as in a panel. According to Thelin et al. (2019), a panel of several different protein biomarkers, all related to the degree of injury, improve the results of prediction models. These biomarkers usually have different cellular origins and temporal trajectories. The implementations of such a panel, concurrently with observable high-risk factors symptoms, provide a reliable protocol for diagnosing mTBI. Wolf et al. (2013). This is, however, a temporary solution as research continues to identify more effective and conclusive biomarkers for diagnosis.

The pathophysiology of sports-related concussion can be clarified using biofluid biomarkers, according to the findings of Di Battista et al. (2018). In the subacute phase after injury, significant circulating levels of PRDX-6 are found in athletes with SRC. Furthermore, the high levels identified a relationship between SRC and higher PRDX-6 and T-tau levels at medical clearance. While there is not enough evidence to support the importance of tau biomarkers alone in the management of patients with SR-TBI, understanding the relationship between PRDX-6 and tau, suggests great potential for future research. Bogoslovsky et al. (2017) establish that plasma biomarkers GFAP, tau, and Ab42, can be used in combination within the first thirty days of injury to differentiate controls of complicated traumatic brain injury populations and can be reliable predictors of TBI in both acute and subacute phases of injury. Janigro et al. (2020) determine that

salivary concentration of astrocytic protein S100β are higher than those measured in serum after head injury. S100β levels in saliva are similarly predictive to those in the blood, and there is a correlation between the two. [49] However, currently, there exists no implementation of this technology for saliva-based POI. [49] GFAP and S100β, biofluid biomarkers with an excellent negative predictive value and a low limit of detection in laboratory-based approaches, tend to miss clinically significant concussion sequelae.

Study Strengths and Limitations

Meanwhile, some limitations noted throughout this meta-analysis are to be discussed. The focus of this meta-analysis was on the four most investigated biofluid biomarkers after mTBI, but other biomarkers including neurofilament light protein (NF-L) were not included in this meta-analysis. [16] In this meta-analysis, I looked specifically at biomarkers in SR-TBI. I omitted studies that were not SR-TBI-specific and some of these studies showed biomarker levels to be significantly correlated with TBI. The S100β biomarker levels in the blood are equivalent to those in the saliva, and there is a link between salivary and blood S100β levels in the control and post-TBI conditions. [49] However, no deployment of this technology to saliva-based POI currently exists for biofluid protein biomarkers. [49] Furthermore, this meta-analysis did not conduct or implement any detailed research into the evaluation of a mTBI using POI salivary tests for biofluid protein biomarkers. The lack of description or the lack thereof of control groups in most of the studies, questions the reliability of the findings of this meta-analysis. The following studies failed to describe or did not include control groups in their research meaning that they are less effective, and their findings require more careful considerations: [25], [41], [30], [38], [39], [44], [45]. Despite these limitations, the evaluated biofluid biomarkers confirmed some success in predicting

positive intracranial lesions in CT scans after mTBI. Hence, these biomarkers may be reflective of a brain injury. The forest plot showed the odds ratio favoring the control group thus depicting the negative predictive value of the biomarkers such that in the control group no mTBI case was reported. This analysis also confirmed that the utility of biomarkers in the management of SRC mild traumatic brain injury is considerable.

Conclusions and Future Directions

When engaging in sports-related activities, most athletes tend to engage in physical touch, which may sometimes expose them to the risk of getting internal brain injuries, commonly known as Traumatic Brain Injury. It has become increasingly important to diagnose concussions as early as possible, especially in athletes who participate in sports and who suffer concussions regularly. Luckily, the research on TBI biomarkers is rapidly advancing, with data suggesting several promising candidates. Should these biomarkers be better established clinically, they could easily hold many important roles. It was established that the utility of biomarkers in the prediction of mTBI is significant when the markers are used in large combinations. These biomarkers can potentially aid in more accurate clinical decision-making by clarifying injury severity and monitoring injury progression and recovery. Utilizing biomarkers can reduce the cost of diagnosing mTBI, and athletes will be exposed to less radiation than with current imaging techniques. Developing biomarkers and incorporating them into guidelines could help athletes who participate in collision sports, which are commonly known for being at high-risk of repeated head injuries, return to sports safely. Biomarkers can potentially be the solution for accurate identification and treatment of Traumatic Brain Injuries. Regarding future reference in data collection, an alternative measure to the funnel plot is recommended to be employed to improve

future data collection, such as a forest plot, in which more significant conclusions were found within this review. Although several biomarkers of brain injury have been identified, continued research is required. Biofluid biomarkers, in combination with other clinical data, would maximize the diagnostic accuracy for athletes who suffer a mTBI, but the methodological limitations are evident in biomarker research. This results in the need for the clinical utility of biomarker use in mTBI to be further explored.

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