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Biological Significance of Serum Biomarkers in Sports-Related Concussion Injury-A Systematic Review

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ABSTRACT

AIM: To evaluate all serum biomarkers in sports-related concussion injury (SRC) to determine diagnostic validity, changes with symptom severity, and return to play, as well as detect early changes in serum concentration.

MATERIAL and METHODS: Studies were searched in various electronic databases (MEDLINE/PubMed, EMBASE, CINAHL, Scopus and Cochrane databases) from their commencement to May 2021. Studies were included if athletes aged 12 years and older were diagnosed with a concussion injury and evaluated using serum biomarkers. Studies including athletes with injuries other than concussion injuries were excluded. Articles with fewer than 20 concussed athletes were excluded. There were 1782 articles identified.

RESULTS: After exclusion a total of 17 articles qualified for systematic review. S100 calcium binding protein β (S-100β) and ubiquitin carboxyl-terminal hydrolase L1 (UCH-L1) showed promising results in distinguishing concussed athletes from contact sports and non-athlete controls. Most of the serum biomarkers increased within 6 hours of SRC. Serum neurofilament light protein (NFL) positively correlated with the severity of post-concussion symptoms. NFL, tau and Interleukins (IL-1 Ra and IL-6) have the potential to determine return to play.

CONCLUSION: Serum biomarker measurement is an objective tool that aids in early diagnosis and predicts the severity and prognosis of injury.

KEYWORDS: Serum biomarkers, Sports-related concussion injury, Mild traumatic brain injury, Concussed athletes, Return to play

INTRODUCTION

Sports often result in musculoskeletal injuries. Concussion (mild traumatic brain injury often due to head impacts) injury is equally important and is often unintentionally ignored due to subtle symptoms, failure of diagnosis and athletes attempting to return to the field early. Athletes at all levels are pressured to be physically superior, which can cause many to overlook their mental health needs. We need to consider the importance of mental health and physical fitness for athletes. Concussion is defined by the Concussion in Sports Group as a “traumatic brain injury induced by biomechanical forces” (17). Concussion is often used synonymously with the term mild traumatic brain injury (mTBI). Concussion is an acute disturbance of neuronal function associated with damage to neuronal and glial cells, which may result in subacute and chronic consequences (5). Neurological impairments can occur which are generally short-lived, with symptoms, such as headache, dizziness and memory disturbances. Most concussions resolve within 7-10 days but in 10-15 % of individuals, the symptoms may persist for more than 10 days (16). Repeated episodes and long-term sequelae of concussion injury can lead to chronic traumatic encephalopathy (CTE), a term used to describe a constellation of symptoms such as changes in mood, behaviour, personality, cognition, and movement. In these situations mental fitness becomes the key concern after injury (10,11,26). Conventional imaging techniques often fail to detect these concussion injuries, allowing a number of

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athletes who might have subtle symptoms to evade clinical testing and continue to play. There is an increased risk of long-term neurological deficits when athletes return to play prematurely because of undetected sports-related concussion (SRC) injury (4). Due to the lack of definitive tools, both the diagnosis of concussion injury and the decision regarding return to play of the athletes are based on the resolution of self-reported symptoms. Thus, we require a reliable objective tool to diagnose sports-related concussion injury and predict the safe time to return to the field. Cerebrospinal fluid (CSF) is in direct contact with brain tissue and it can be suitable to detect pathological changes in the central nervous system. However, due to the invasive nature of lumbar puncture, it is unfeasible to use it in the routine course and follow-up of athletes. Thus, the assessment of concussion injury biomarkers in serum would be preferable (30) and may be a safe, less time-consuming and cost-effective tool. The aim of this study was to systematically review all serum biomarkers available in published literature that may present as a marker of concussion injury in all sports-related activities. The study was done to review the diagnostic validity of available serum markers in sports-related concussion injury, and help with the early detection of concussion so that clinicians should not depend entirely on self-reported symptoms by sportspersons, which sometimes can be biased. The study also examined changes in serum biomarkers with the severity of concussion symptoms and specified the biomarkers that could predict return to play.

MATERIAL and METHODS

The systematic review was performed in accordance with PRISMA guidelines (21).

Selection and Eligibility Criteria

Prespecified eligibility criteria using the PICO (P—Population, I—Index test, C—Comparison, O—Outcome) worksheet (Table I).

Studies evaluating serum biomarkers in sports-related concussion injury with participants aged 12 years and older were included. Articles including participants other than sports-related concussion injury as a primary focus (bouts/head impacts or any moderate/severe traumatic brain injury) and articles with fewer than 20 concussed participants were excluded. Review articles, opinion papers, case reports and articles published in languages other than English were also excluded.

Search Strategies

Studies were searched in various electronic databases (MEDLINE/PubMed, EMBASE, CINAHL, Scopus and Cochrane databases) from their commencement to May 2021. Searches were performed using the MESH terms biomarker OR serum marker AND Sports OR sport OR athlete AND concussion OR mild traumatic brain injury OR TBI. These terms were searched in the entire text of the article (e.g., title, abstract, and keywords). When only abstracts were available, authors were contacted to obtain the full text of the articles. Reference lists of all original and review articles were examined in search of other relevant articles. The abstracts were screened to identify articles that potentially met the inclusion criteria, and in case of dubiety regarding eligibility, the full text of the article was read.

Data Collection and Analysis

The entire text of the articles was gathered and reviewed by two independent authors to identify the articles that met the eligibility criteria. If the published data in studies was not sufficient for evaluation, then authors were contacted and requested to provide additional data. After including all eligible articles, they were reviewed using a systematized review process. A combined evidentiary table was framed including the study design, sample size, aim of the study, sample collection schedule, clinical symptoms assessed, outcome measures and results.

Assessment of Risk of Bias and Level of Evidence

Two authors independently assessed the risk of bias using the QUADAS-2 tool, a validated tool for evaluating the quality of diagnostic and prognostic studies (34). Four domains were assessed to rate the quality of the included studies: patient selection, index test, reference standard, plus flow and timing. Each study was assessed in terms of four domains of risk of bias and the first three of these domains were assessed in terms of concerns about applicability. Signalling questions as defined in the QUADAS-2 tool enable the reviewer to give a rating to each domain as high, low or unclear. Discrepancies were resolved by consensus after consulting with an independent third author. Figure 1 provides quality assessment of each study.

Table I: PICO Worksheet

Population	Athletes diagnosed to have concussion injury during sports related activity
Index test	Measuring the serum biomarkers concentration
Comparison	Athletes with concussion injury versus contact sports healthy athletes/noncontact healthy controls
Outcome	To review the diagnostic and prognostic validity of available serum markers. To examine the relation between serum biomarkers and the severity of symptoms. To determine return to play on field based on serum biomarkers

RESULTS

The PRISMA flowchart shown in Figure 2 gives an overview of the systematic process that was performed. From the initial search, 1745 studies were identified. Thirty-seven additional studies were identified after searching the reference list. After removing 839 duplicate articles, 943 remained for title and abstract screening. Studies that did not meet eligibility criteria were not considered for full-text review, implying review articles, cross-sectional studies and studies in which the number of concussed athletes was less than 20 or exposure was not concussion (head impacts/number of bouts). Forty full-text articles were closely examined for eligibility, leading to 17 eligible articles from which further data was retrieved, and the results were summarized.

All the included seventeen studies were prospective and were published in the literature either in or after 2013. All studies included control populations for comparison. Fifteen studies were cohort studies and 2 were case-control studies. Study participants were both high school/collegiate athletes and professional athletes. We included athlete participants aged more than 12 years as sports-related activities are also common in high school/college students. Although traumatic brain injuries in children and adults have different pathological mechanisms and recovery processes, serum biomarkers were reported to be increased and correlated with the recovery period in both age groups (2,12,18,20,22). The range of sample size was from 20 to 264. We excluded articles with low sample size (<20). Sample size was taken as the number of athletes diagnosed with concussion injury and not the number of participants included at the start of study. Sports in these studies included football (9 studies), hockey (9 studies), soccer (6 studies), rugby (4 studies), lacrosse (4 studies), basketball (4 studies), boxers (2 studies), mountain biking (1 study), volleyball (1 study) and water polo (1 study). Nineteen serum biomarkers were assessed that could potentially prove to be

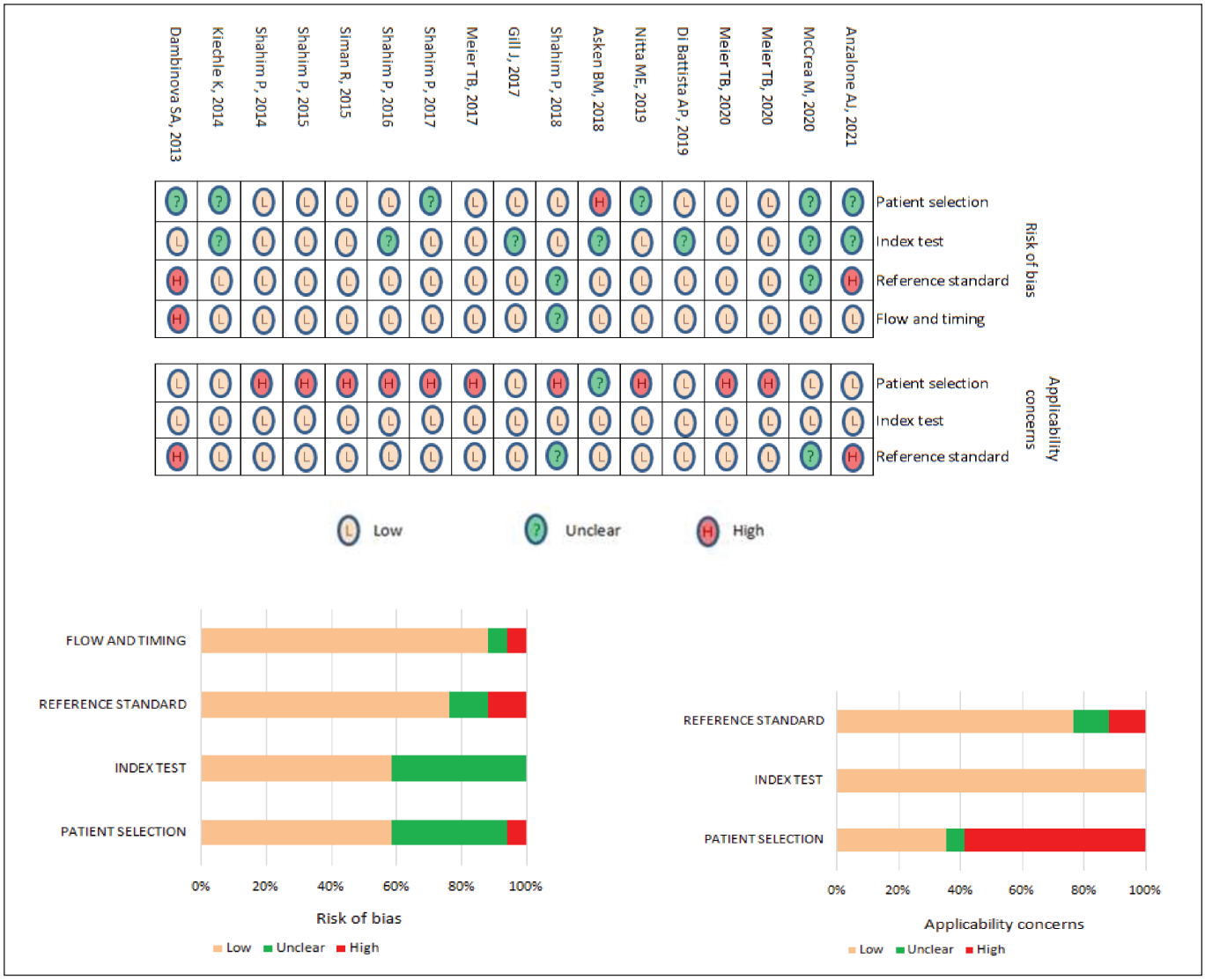

Figure 1: The quality assessment of included studies using QUADAS-2 tool.

an adjuvant in the diagnosis of SRC: Tau in 8 studies, S100 calcium binding protein β (S100β) in 7 studies, neurofilament light protein (NFL) in 4 studies, ubiquitin carboxyl-terminal hydrolase L1(UCH-L1) in 4 studies, glial fibrillary acidic protein (GFAP) in 4 studies, neuron-specific enolase (NSE) in 2 studies, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor peptide (AMPAR) in 1 study and others, such as inflammatory biomarkers. These serum biomarkers were measured prior to the start of sports-related activity (baseline), immediately after SRC injury, and then at various follow-up periods. Most of the studies used two control groups: one contact sport control group and another healthy non-athlete control group for comparison. In addition to comparing biomarker concentrations pre- and post-play, other outcome measures included changes in serum biomarker levels compared to the severity of symptoms and predicting safe return to play. The study results are summarized in Table II.

Diagnostic Validity of Serum Biomarkers

Athletes with SRC injury had significantly elevated S-100β and UCH-L1 in all included studies when compared to their preseason baseline value, contact sport control and healthy athlete control. In addition, most of the studies also showed that NFL and GFAP were significantly increased in concussed athletes compared to their preseason baseline values, contact sport athletes and healthy athletes.

Early Detection of Concussion Using Serum Markers

All studies assessing S-100β and UCH-L1 reported an increase in serum levels within 6 hours of SRC injury compared to baseline levels, contact sport controls and healthy controls. NFL levels could rise as early as 1 hour post-SRC when compared to controls.

Changes in Serum Biomarkers with Severity of Concussion Symptoms

Most of the studies suggested serum NFL as a biomarker that positively correlated with the severity of post-concussion symptoms after SRC.

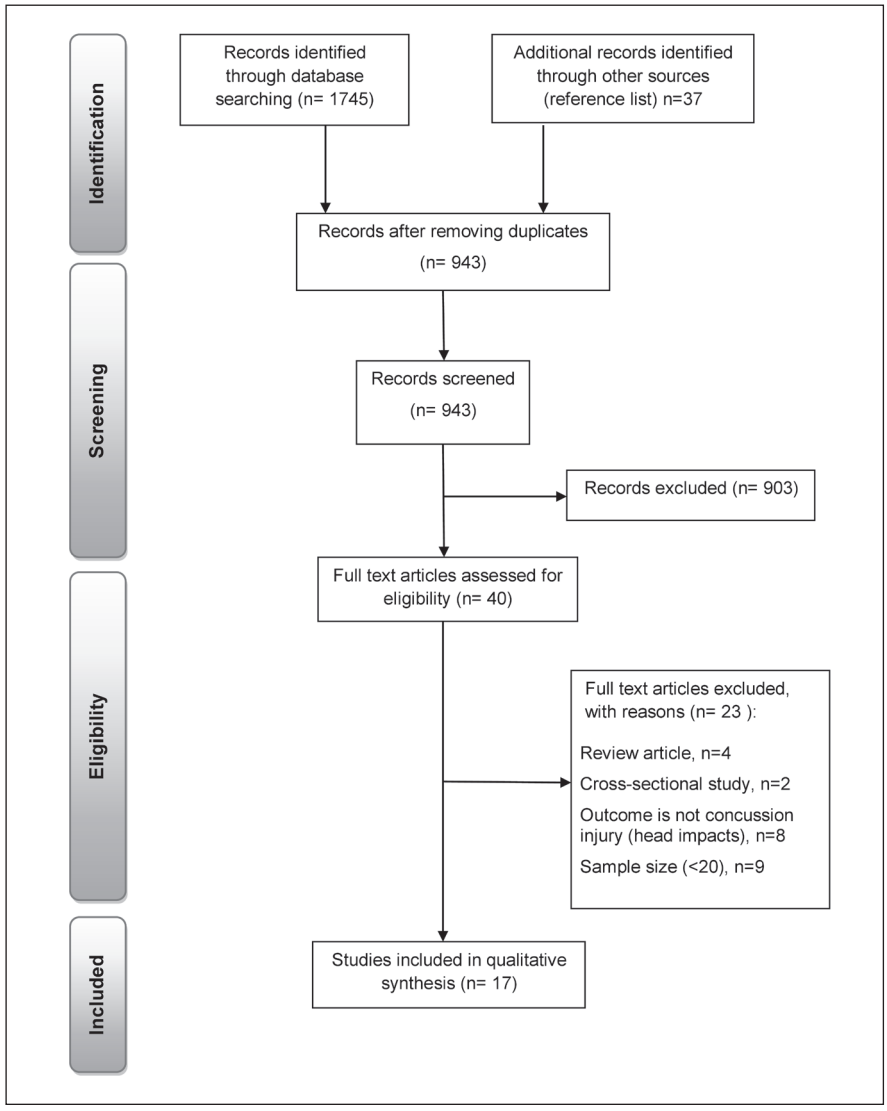

Figure 2: The flowchart of literature search and selection of articles.

Table II: Summary of Studies

Authors/ Year	Study design	Type of sports	Sample size	Control	Serum biomarkers	Outcome measures	Results
Anzalone AJ et al, 2021 (1)	Prospective cohort study	Football, Soccer, basketball and others	97 concussed athletes (62 males and 32 females)	30 non concussed athletes as contact controls.	tau and NFL	Blood biomarkers measured during the first visit (<7 days post injury) and again 6 months later.	**Diagnostic validity-** 1) NFL was higher for concussed athletes compared with non concussed control athlete. In contrast, tau was significantly lower for concussed athletes compared with non concussed control athletes. **Symptom severity and Return to play-** 1) No significant relations were observed between NFL or tau and the number of symptoms reported, total PCSS score (Postconcussion symptom scale) and, or recovery duration.
McCrea M et al, 2020 (15)	Prospective Case control study	Football, soccer, lacrosse, ice hockey, rugby and others	264 athletes (211 males and 53 females)	138 contact sport controls and 102 non–contact sport controls	UCH-L1, tau, NFL, and GFAP	blood sample collected at the acute postinjury period, 24-48 hours, the point of reporting being asymptomatic, and 7 days after return to play.	**Diagnostic validity-** 1) Athletes with concussion had a significant elevation in GFAP, UCH-L1 and tau levels compared with baseline, contact sport and non–contact sport controls. **Symptom severity-** 1) Significant increase in serum GFAP and NFL levels in concussed athletes group having severe symptoms.
Meier TB et al, 2020 (18)	Prospective cohort study	Football	106 High school and collegiate athletes (all males)	84 contact control athletes (football players) and 50 non-contact control athletes	UCH-L1, GFAP, SBDP150,S100B, IL-6, IL-1RA, and CRP	Blood biomarkers within 6 hours and at 24-48 hours, 8 days, 15 days, and 45 days post-injury	**Diagnostic validity-** 1) UCH-L1,S100β, SBDP150, IL-1RA and IL-6 levels were significantly elevated in SRC compared to baseline, contact control and non-contact control. 2) No conclusive evidence on CRP and GFAP drawn. **Return to play-** 1) Higher levels of IL-1RA were significantly associated with greater symptom duration following concussion.
Meier TB et al, 2020 (20)	Prospective cohort study	Football	59 High school and collegiate athletes	contact controls (CC group,n=54) and 30 noncontact control (NCC) athletes.	tryptophan (TRP), kynurenine (KYN), kynurenic acid (KynA), 3 hydroxy kynurenine (3HK), quinolinic acid (QuinA) and c-reactive protein (CRP)	Blood biomarkers measured within 6-hours (early-acute), at 24-48 hours(late acute) , at 8, 15, and 45 days post injury.	**Diagnostic validity-** 1) Significantly elevated KynA/3HK in SRC compared to the baseline. 2)Elevated CRP in SRC and CC athletes relative to NCC. **Symptom severity-** 1) Higher KynA/3HK at the early-acute (i.e., within 6 hours) visit was significantly associated with lower BSI-GSI scores.
Di Battista AP et al, 2019 (8)	Cohort study	Basketball, field hockey, football, ice hockey, lacrosse, rugby, soccer and others	43 athletes (21 males and 22 females)	102 healthy athletes	interferon (IFN)-γ, interleukin (IL)-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12p70, IL-13, and TNF-α, and Chemokine- macrophage inflammatory protein (MIP)-1α, MCP-1β.	Blood was sampled from injured athletes within 7 days. A second blood sample was taken within a median of 5 days of medical clearance to return to play.	**Diagnostic validity-** 1) Higher concentrations of MCP-4 and MIP-1β significantly contributed to class discrimination between athletes with SRC and healthy athletes. **Return to play-** 1) There was a significant positive correlation between days to recovery and both MCP-1 and MCP-4 in athletes with SRC.
Nitta ME et al, 2019 (22)	Cohort study	High school and collegiate football players	41 athletes with concussion (all males)	43 control athletes (football players)	(IL)–6, IL-1β, IL-10, tumor necrosis factor, C-reactive protein, interferon-γ, and IL-1 receptor antagonist	Blood biomarkers measured at preinjury baseline, 6 and 24–48 hours postinjury, and 8, 15, and 45 days following concussion.	**Diagnostic validity-** 1) Both IL-1RA and IL-6 levels were significantly higher in athletes with concussion compared to controls. **Return to play-** 1) IL-6 levels were significantly associated with symptom duration.

Table II: Cont.

Authors/ Year	Study design	Type of sports	Sample size	Control	Serum biomarkers	Outcome measures	Results
Asken BM et al, 2018 (2)	Case control study	Student athletes	36 concussed athletes (18 males and 18 females)	86 healthy controls (collegiate athletes)	β-amyloid peptide 42 (Aβ42), total tau, S100B, UCH-L1, GFAP, microtubule associated protein 2 (MAP2), and 2,3-cyclic-nucleotide 3phosphodiesterase (CNPase)	Blood biomarkers measured as baseline and immediately after concussion diagnosis was established (mean=10hrs)	**Diagnostic validity-** 1) Only S100β increased after SRC compared to baseline. 2) Concussed patients had higher Aβ42 , total tau, S100B, and GFAP than matched controls. **Return to play-** 1) No associations between acute serum biomarker concentrations and clinical recovery time after SRC.
Shahim P et al, 2018 (29)	Cohort study	Professional hockey players	87 players sustained SRC	28 contact sport controls, 12 noncontact athletes (gymnasts,GC) and 19 healthy nonathletic Controls(HC)	NFL, tau, S100B and NSE	Serum biomarkers measured at 1, 12, 36, and 144 hours after SRC and at the RTP time point.	**Diagnostic validity-** 1) Serum NFL and S100β after SRC were higher than baseline, healthy nonathletic control (HC) and noncontact sport athletes(GC) 2) Plasma tau after SRC was higher compared to preseason, HC but not GC. 3) Serum NSE increased after SRC (compared with HC, but not GC or preseason samples). **Symptom severity-** 1) Both NFL and tau level after SRC correlated with RPQscores. **Return to play-** 1) Serum NFL and tau were increased in players with RTP >10 days compared with players with RTP ≤10 days.
Gill J et al, 2017 (12)	Cohort study	Soccer, football, hockey, basketball and lacrosse	43 collegiate sport athletes (23 males and 20 females)	37 contact control athletes and 21 non athletes control	tau	Sampling at 6 hours, and at 24 hours, 72 hours, and 7 days after SRC.	**Diagnostic validity-** 1) SRC athletes had significantly lower total tau compared to control athletes. **Return to play-** 1) Compared to SRC athletes with short RTP (<10d), those with long RTP(≥10d) had higher tau concentrations.
Meier TB et al, 2017 (19)	Cohort study	Football	32 concussed athletes (all males)	29 Contact Sport control athletes	UCH-L1, S100B and GFAP	Sampling at 6 h, 24–48 h, and 8, 15, and 45 days post-concussion.	**Diagnostic validity-** 1) Concussed athletes had significantly elevated S100β and UCH-L1 levels relative to controls.
Shahim P et al, 2017 (31)	Cohort study	Boxing and Hockey	14 Swedish amateur boxers and 35 Swedish professional hockey players	14 contact sport controls and 12 gymnasts	NFL	Blood sampling at 1, 12, 36, and 144 hours after concussion, and on the day of RTP.	**Diagnostic validity-** 1) Serum NFL levels were higher after concussion as compared with controls. **Symptom severity and Return to play-** 1) The levels of serum NFL normalized (to the same levels as controls) at RTP in all the players except for one, who also had LOC and who returned to play after 3 months. 2) Serum NFL remained elevated in players with prolonged PCS (>6 days), as compared to controls.
Shahim P et al, 2016 (27)	Cohort study	Ice hockey	28 athletes	Contact athletes without concussion	Serum tau fragments (tau-A and tau-C)	Blood sampling at 1, 12, 36, and 144 hours after the trauma, or when the player returned to play	**Symptom severity-** 1) Serum tau-A levels post-concussion were significantly elevated in players with persistent post-concussive symptoms lasting longer than 10 days compared to players with symptoms resolving within 10 days. **Return to play-** 1) Serum tau-A concentrations after concussion correlated with the number of days it took for players to return to play.

Table II: Cont.

Authors/ Year	Study design	Type of sports	Sample size	Control	Serum biomarkers	Outcome measures	Results
Siman R et al, 2015 (32)	Cohort study	Ice hockey	28 athletes	17 concussion free athletes	Serum all-spectrin N-terminal fragment (SNTF), tau and S100B	Blood samples were obtained at 1h, 12h, 36h, and 144 h after concussion as well as on the day of return to play.	**Diagnostic validity-** 1)Serum SNTF and tau significantly increased compared to pre-season and controls. **Return to play** 1) Serum SNTF and tau levels were higher in the subset of concussions requiring more than 6 days for return to play, compared with cases with shorter-lasting PCS.
Shahim P et al, 2015 (28)	Cohort study	Ice hockey	28 athletes	24 athletes without concussion	Serum visinin-like protein-1	Blood sampling at 1, 12, 36 and 144 hours after the trauma or when the player returned to play	**Diagnostic validity-** 1)Serum VILIP-1 can't be used to differentiate SRC and without SRC. **Symptom severity and Return to play-** 1)levels of VILIP-1 did not correlate significantly with duration and severity of post-concussive symptoms.
Shahim P et al, 2014 (30)	Cohort study	Ice hockey	28 athletes	47 athletes without concussion injury	Total tau, S100B and NSE	Blood sampling at 1, 12, 36, and 144 hours and when the players returned to play	**Diagnostic validity-** 1)Athletes with concussions had an increased T-tau levels compared to controls. **Symptom severity and Return to play-** 1) The concentration of T-tau after concussion correlated with the number of days it took for concussion symptoms to resolve. 2)The levels of S-100β (1 hour) after concussion were significantly higher in players with loss of consciousness and those with RTP more than 10 days after concussion.
Kiechle K et al, 2014 (13)	Cohort study	Football, Soccer and Basketball	22 athletes	30 non contact sport athletes post exertion	Serum S100β	Blood sampling at pre-season baseline, within 3 hours of injury, and at days 2, 3 and 7.	**Diagnostic validity-** 1) S100β was significantly higher post-SRC than pre-season baseline and was accurate discriminator of SRC from noncontact exertion without SRC.
Dambinova SA et al, 2013 (7)	Cohort study	Rugby, soccer, lacrosse	33 athletes (24 males and 9 females)	51 athletes without a history of recent Concussions and 40 non-athletes	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR) peptide	Selected subjects had two additional blood samples drawn during a follow up-visit.	**Diagnostic validity-** 1) Athletes with concussions had an increased AMPAR peptide level compared to controls.

BSI-GSI (BSI Global Severity Index) comprising 3 subscales (depression, anxiety, and somatization) used to assess overall psychological symptoms. RPQ score-Rivermead Post-Concussion Symptoms Questionnaire.

Biomarkers Predicting Return to Play

Although many biomarkers have been studied, NFL, tau, IL-1 RA and IL-6 have shown better potential to predict return to play than the rest of the others.

DISCUSSION

There is emerging evidence of a positive correlation between exposure to concussion and mental symptoms (24). The consensus statement on concussion in sports given in 2017, also emphasized the importance of post-concussion mental health and suggested that the occurrence of depressive symptoms tends to cause persistent or chronic post-concussion symptoms (17). Cognitive disturbances often accompany concussion injury, which may exacerbate the psychological response of the athlete due to removal from play and its career impact. The lack of knowledge about the repercussion of sport-related concussion injury and the general inclination of giving preferences to physical fitness tends to play a crucial role in the mismanagement of recovery from injury. Moreover, it is important to pay attention to all consequences of concussion injury because symptoms such as headache and fatigue are common immediately after the injury, while emotional and mood disturbances tend to develop later (often after 7 days) during recovery. Therefore, the resolution of physical symptoms does not necessarily reflect recovery from injury (14). Return to play should be gradual and under medical surveillance. Another major hurdle is the diagnosis of sports related concussion injury (SRC) because of its subjective symptoms and underreporting. It is a diagnosis of exclusion and lacks any specific signs on radiology and clinical symptoms, making it more complex and leading investigators to search for a surrogate marker that should be more objective than subjective. Hence, the importance of serum biomarkers has emerged that can act as a potential adjuvant to conventional imaging techniques (CT/MRI) in the diagnosis of mTBI or concussion injury. The study of biomarkers in SRC is in the precursory stage but over the last decade, a number of studies have been performed to identify serum biomarkers to assist in the diagnosis of concussion. There are several studies related to serum biomarkers, but despite this, there is no single approved serum biomarker to diagnose SRC and predict prognosis. Previous studies, including reviews performed to date, have included some serum biomarkers while examining only a few of their aspects. However, in this review, we tried to unravel every aspect of serum biomarkers.

S-100β is the most widely studied in the literature. S-100 β is a protein found abundantly in astrocytes and in lower concentrations in non-neuronal tissue outside the brain such as bone and soft tissue. Thus, S-100β can be expected to rise in various physical activities without head injury (23). The C-terminal hydrolase of ubiquitin-L1 (ubiquitin C-terminal hydrolase- L1, UCH-L1) is an E2 conjugation enzyme present in the cytoplasm of neurons. It is also found in neurons of the peripheral nervous system, especially in the neuromuscular junction, and the neuroendocrine system. Three isoenzymes of UCH (UCH-L1, UCH-L2, and UCH-L3) have been found, UCH-L1, is present in high concentrations in the CNS (25). Glial fibrillary acidic protein (GFAP) is a protein predominantly found in glial cells, and is a part of the intermediate filament of the cytoskeleton of astrocytes. It is believed to be a specific marker of CNS diseases, and is also released in several events that affect the integrity of the blood-brain barrier (25). NFL is a cytoskeletal component that provides structural support to neurons and is predominantly present in long myelinated subcortical axons. Tau, which is also abundantly present in axons, is critical for the stability of microtubules and their assembly (23). Neuron-specific enolase (NSE) is a glycolytic protein, that is present only in neurons of the brain and other CNS structures. Damage to neurons causes the release of NSE into the circulation (3). APP (amyloid precursor protein), a well-known protein can accumulate after traumatic brain injury due to disruption of axonal transport. Furthermore, enzymatic breakdown of APP can release amyloid-β (Aβ) peptides, which are neurotoxic and linked to long-term cognitive dysfunction and progression to Alzheimer's disease (33). Traumatic brain injury also causes oncotic necrosis of neurons which further leads to upregulation of calpains and caspases. α-II-spectrin is a cytoskeletal protein that acts as a substrate for calpains and caspases and is cleaved to produce α-II-spectrin breakdown products (SBDPs) (6). In response to traumatic brain injury, an inflammatory response may occur which includes immune-mediated activation of a key kynurenine (KYN) pathway. KYN is metabolized and eventually converted to quinolinic acid (QuinA) which is neurotoxic. KYN can also be metabolized into kynurenic acid (KynA), which is often considered to be neuroprotective (20). Another biomarker AMPAR (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor) is a type of glutamate receptor primarily present in the forebrain and subcortical fibres. Its presence signifies an ongoing degenerative process in neurons (7). Other inflammatory markers are also activated in response to tissue damage and hypoxia such as IL-1β, TNF-α, and IFN-γ. Interleukin-6 (IL-6) is involved in the neuroinflammatory cascade. It also acts as a VEGF-agonist, which modifies tight junction proteins to damage the integrity of the blood-brain barrier and can enhance the release of another marker IL-1Ra (9).

Diagnostic Validity of Serum Biomarkers

Studies included in this review measured serum biomarkers of athletes preseason (baseline) and then at various intervals to follow the longitudinal change in levels. Along with examining the longitudinal change in serum biomarker levels in athletes, most of the studies took two control groups (contact sport control and healthy athlete control) for comparison. S-100β and UCH-L1 were consistently reported to be increased in athletes with SRC injury when compared to their preseason baseline value, contact sport control and healthy athlete control, recognizing their strong diagnostic potential (2,13,15,18,19,29). One study quoted S-100β as an accurate discriminator of SRC from athletes without SRC (13). In addition, most of the studies also revealed that NFL and GFAP could be significantly increased in concussed athletes compared to controls (1,2,15,29,31). Studies measuring serum tau as a biomarker immediately after concussion injury showed an increase in its concentration compared to the preseason

baseline value and control athletes (2,15,30,32). In contrast, few studies have shown a decrease in tau after concussion compared to controls (1,12). Similarly, inconsistent findings have also been observed in relation to other biomarkers. One study in 2018, showed increased serum NSE levels in concussed athletes compared to healthy controls whereas another study showed that serum NSE was not significantly increased after concussion injury (29,30). One study examined calpain-derived aII-spectrin N-terminal fragment (SNTF) which accumulates in axons and increases in human blood after mild traumatic brain injury (mTBI). They suggested that serum SNTF is significantly increased after concussion injury compared to baseline and control values. No other study was done on this biomarker (32). A prospective cohort study identified new serum biomarkers, kynurenic acid (KynA), and 3-hydroxykynurenine (3HK). They showed that the KynA/3HK ratio was significantly elevated in SRCs in the early-acute visit (within 6 hours) compared to its baseline value, but no significant increase was observed when compared to controls (20). One study was performed on the novel biomarker serum visinin-like protein-1 but did not show any changes after concussion injury (28). Thereafter, few studies were performed to observe the inflammatory response in concussed athletes following SRC. One of these studies showed increase in SBDP150, IL-1 RA and IL-6 levels after SRC in early-acute visits relative to both contact control and non-contact control athletes as well as relative to their own baseline (18). One prospective cohort study demonstrated a significant increase in both IL-1 RA and IL-6 levels in athletes with concussion compared to contact control athletes (22). Another cohort study demonstrated higher concentrations of MCP-4 and MIP-1β which significantly contributed to discriminating athletes with concussion from healthy athletes (8). One study introduced a novel serum biomarker a-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR) peptide which was significantly increased in concussed athletes compared to healthy athletes and non-athlete controls (7). These studies examining inflammatory biomarkers indeed showed no consistent evidence on serum CRP levels to distinguish SRC from controls (18,20,22). Overall, we recommend S-100β and UCH-L1 as serum biomarkers to diagnose concussion injury in athletes, which can be reliably distinguished from non-concussed athletes. NFL, GFAP and tau have also shown potential, either in isolation or combination.

Early Detection of Concussion Using Serum Markers

We performed a comprehensive study to evaluate every aspect of serum biomarkers that could be used for further clinical studies. Apart from evaluating the increase in the levels of serum biomarkers for diagnosis, it was also important to determine the optimal time to measure their levels, which could increase from within a few minutes of concussion to a few days. Studies have invariably shown that S-100β and UCH-L1 levels increase in concussed athletes within 6 hours of SRC compared to baseline and controls (2,13,15,18,19,29). Few studies have also reported NFL levels to rise as early as 1 hour post-SRC when compared to controls (29,31). A case-control study in 2018 showed an increase in GFAP levels at approximately 10 hours post-SRC compared to controls but not to their baseline value (2). Another case-control study in 2020 demonstrated an increase in GFAP levels in SRCs immediately after concussion injury and 24-48 hrs post-SRC compared to their baseline levels and controls (15). However, one study concluded significantly higher GFAP at the 24–48 h visit relative to 6 h post-SRC (19). Studies examining serum tau levels revealed a common pattern of progression of its level in relation to time. Serum tau increased at approximately 10 hours post-SRC and then decreased at 24-72 hours post-SRC compared to controls (2,12,15). This reason could be why few studies reported an increase in serum tau, while others reported a decrease in its level post-SRC compared to controls, because they were done at different time intervals. Various other biomarkers, such as SBDP150, IL-1 RA, IL-6 levels and the KynA/3HK ratio, also increased within 6 hours of SRC compared to control athletes (18,20,22). Thus, most of the serum biomarkers increased within 6 hours of SRC compared to the baseline as well as controls.

Changes in Serum Biomarkers with Severity of Concussion Symptoms

There are various studies showing the level of serum biomarkers and their correlation with the severity of concussion symptoms, such as headache, dizziness, loss of consciousness, fatigue, restlessness and post-traumatic amnesia. There were different scoring systems that quantified the severity of concussion symptoms based on questionnaires. Therefore, we reviewed these studies to determine whether these increase in the levels of serum biomarkers were related to the severity of concussion symptoms. Most of the studies evaluating serum NFL revealed that its level was comparatively higher in concussed athletes with severe symptoms than in those with mild symptoms (15,29,31). Studies have also shown that serum tau and S100β levels were positively correlated with post-concussion symptom severity (27,29,30) whereas only a few studies have found no correlation with severity (1,29). One study evaluated GFAP with symptom severity and showed that it was increased in concussed athletes with loss of consciousness and post traumatic amnesia (LOC-PTA) compared to the no LOC-PTA group (15). A recent study dealing with a novel biomarker reported a negative correlation and stated that higher KynA/3HK was significantly associated with lower BSI-GSI scores (used to assess overall psychological symptoms) (20).

Biomarkers Predicting Return to Play

The toughest and most challenging part of using serum biomarkers is in predicting return to play. The duration of physiological recovery often exceeds that of clinical recovery. There is no definitive time course of recovery of these physiological parameters (serum biomarkers). One may wonder what is the need to study the time course of physiological recovery if someone has recovered clinically? There is limited but substantial evidence that repeated concussion can prolong recovery. Various studies suggest that repeated concussion prior to recovery can result in more severe physiological insult and clinical sequelae. Therefore, there should be a physiological parameter whose return to normal value would indicate physiological recovery. Various

studies performed to date have measured serum biomarker levels at follow-up visits after SRC and correlated their levels with clinical recovery. Studies have shown that athletes with higher concentrations of serum NFL and tau took more time to return to play (often >10 days) than athletes with low levels of these biomarkers (<10 days) (12,27,29-31). Few studies have shown that inflammatory markers (IL-1 RA, IL-6, MCP-1 and MCP-4) are positively correlated with the recovery period of concussed athletes(8,18,22). One study showed S100β to be correlated with the time period of return to play (30). On the other hand, few studies have shown no association between acute serum biomarker (NFL, tau, S100β, UCH-L1, GFAP, Aβ42, MAP2, CNPase) concentrations and clinical recovery time after SRC (1,2,15).

LIMITATIONS

This is a review that has presented comprehensive insight into all available serum biomarkers in sports-related concussion injury to date but it has shortcomings that need to be acknowledged. We did not include studies with sub-concussion/head-impact injuries that may affect serum biomarkers. The included studies had small sample sizes but all were prospective studies with fairly adequate follow-up until clinical recovery. These biomarkers can also be elevated in various extra cranial injuries, which is a major confounding factor and poses a challenge to establish their clinical utility. Apart from this, the criteria to diagnose or define concussed athletes are different in a few studies, which has led to a risk of bias in the domain of the reference standard, as shown in Figure 2. However, overall, the studies included in our systematic review had a low risk of bias. Of course, applicability concerns remain an issue because most of the studies have taken athletes from a particular kind of sport. Hence, studies with large sample sizes including at least 3-4 types of sports and adequate follow-up are required to characterize the natural course of serum biomarkers after concussion because there are large fluctuation in their levels over time.

CONCLUSION

Serum biomarker measurement is an objective tool, that aids in early diagnosis and predicts the severity and prognosis of injury. Of all serum biomarkers, S-100β and UCH-L1 can be reliably used in distinguishing concussed athletes from athletes without sports-related concussion injury. NFL, GFAP and tau levels could also be potential candidates in the future as surrogate markers for concussion injury. Serum biomarkers increase within 6 hours of SRC. Serum NFL correlated positively with the severity of post-concussion symptoms. A fair number of studies suggest the potential for NFL, tau, IL-1 RA and IL-6 to predict return to play but require further studies with large sample sizes and adequate follow-up. Overall, serum biomarkers can have different implications pertaining to the welfare of athletes not only to diagnose subclinical cases early but also to predict the severity of symptoms. Hence, higher levels of serum biomarkers could be associated with longer periods of rest. Prior to returning to the field, the clinician could obtain the level of serum biomarkers along with physical and cognitive tests to decide the timing for safe return to play.

DISCLOSURE and CONFLICT of INTEREST

The authors certify that there are no conflicts of interest with any financial organizations regarding the material discussed in the manuscript.

AUTHORSHIP CONTRIBUTION

Study conception and design: MA

Data collection: AKD, RB

Analysis and interpretation of results: AKD, MA, RB, DKP

Draft manuscript preparation: AKD, RB

Critical revision of the article: MA, DKP

All authors (AKD, MA, RB, DKP) reviewed the results and approved the final version of the manuscript.

REFERENCES

1. Anzalone AJ, Turner SM, Baleztena AC, McGuffin T, Creed K, Jeromin A, Wilson D, Hanlon D, Garrison JC, Blueitt D, Oliver JM: Blood biomarkers of sports-related concussion in pediatric athletes. Clin J Sport Med 31:250-256, 2021
2. Asken BM, Bauer RM, DeKosky ST, Svingos AM, Hromas G, Boone JK, DuBose DN, Hayes RL, Clugston JR: Concussion BASICS III: Serum biomarker changes following sport-related concussion. Neurology 91:e2133-2143, 2018
3. Bandyopadhyay S, Hennes H, Gorelick MH, Wells RG, Walsh-Kelly CM: Serum neuron-specific enolase as a predictor of short-term outcome in children with closed traumatic brain injury. Acad Emerg Med 12:732-738, 2005
4. Bey T, Ostick B: Second impact syndrome. Western J Emerg Med 10:6, 2009
5. Blennow K, Hardy J, Zetterberg H: The neuropathology and neurobiology of traumatic brain injury. Neuron 76:886-899, 2012
6. Chen S, Shi Q, Zheng S, Luo L, Yuan S, Wang X: Role of α-II-spectrin breakdown products in the prediction of the severity and clinical outcome of acute traumatic brain injury. Exp Ther Med 11:2049-2053, 2016
7. Dambinova SA, Shikuev AV, Weissman JD, Mullins JD: AMPAR peptide values in blood of nonathletes and club sport athletes with concussions. Mil Med 178:285-290, 2013
8. Di Battista AP, Churchill N, Rhind SG, Richards D, Hutchison MG: Evidence of a distinct peripheral inflammatory profile in sport-related concussion. J Neuroinflammation 16:1-12, 2019
9. Dugue R, Nath M, Dugue A, Barone F: Roles of pro-and anti-inflammatory cytokines in traumatic brain injury and acute ischemic stroke. In: Abreu GEA (ed). Mechanisms of Neuroinflammation. London: IntechOpen, 2017
10. Gavett BE, Cantu RC, Shenton M, Lin AP, Nowinski CJ, McKee AC, Stern RA: Clinical appraisal of chronic traumatic encephalopathy: Current perspectives and future directions. Curr Opin Neurol 24:525-531, 2011

11. Gavett BE, Stern RA, McKee AC: Chronic traumatic encephalopathy: A potential late effect of sport-related concussive and subconcussive head trauma. Clin Sports Med 30:179-188, 2011
12. Gill J, Merchant-Borna K, Jeromin A, Livingston W, Bazarian J: Acute plasma tau relates to prolonged return to play after concussion. Neurology 88: 595-602, 2017
13. Kiechle K, Bazarian JJ, Merchant-Borna K, Stoecklein V, Rozen E, Blyth B, Huang JH, Dayawansa S, Kanz K, Biberthaler P: Subject-specific increases in serum S-100B distinguish sports-related concussion from sports-related exertion. PloS one 9: e84977, 2014
14. Kontos AP, Elbin RJ, Schatz P, Covassin T, Henry L, Pardini J, Collins MW: A revised factor structure for the post-concussion symptom scale: Baseline and postconcussion factors. Am J Sports Med 40:2375-2384, 2012
15. McCrea M, Broglio SP, McAllister TW, Gill J, Giza CC, Huber DL, Harezlak J, Cameron KL, Houston MN, McGinty G, Jackson JC: Association of blood biomarkers with acute sport-related concussion in collegiate athletes: Findings from the NCAA and Department of Defense CARE Consortium. JAMA Network Open 3:e1919771, 2020
16. McCrory P, Meeuwisse WH, Aubry M, Cantu RC, Dvorak J, Echemendia RJ, Engebretsen L, Johnston KM, Kutcher JS, Raftery M, Sills A: Consensus statement on concussion in sport—the 4th International Conference on Concussion in Sport held in Zurich, November 2012. Br J Sports Med 5:255-279, 2013
17. McCrory P, Meeuwisse W, Dvorak J, Aubry M, Bailes J, Broglio S, Cantu RC, Cassidy D, Echemendia RJ, Castellani RJ, Davis GA: Consensus statement on concussion in sport—the 5th international conference on concussion in sport held in Berlin, October 2016. Br J Sports Med 51:838-847, 2017
18. Meier TB, Huber DL, Bohorquez-Montoya L, Nitta ME, Savitz J, Teague TK, Bazarian JJ, Hayes RL, Nelson LD, McCrea MA: A prospective study of acute blood-based biomarkers for sport-related concussion. Ann Neurol 87:907-920, 2020
19. Meier TB, Nelson LD, Huber DL, Bazarian JJ, Hayes RL, McCrea MA: Prospective assessment of acute blood markers of brain injury in sport-related concussion. J Neurotrauma 34: 3134-3142, 2017
20. Meier TB, Nitta ME, Teague TK, Nelson LD, McCrea MA, Savitz J: Prospective study of the effects of sport-related concussion on serum kynurenine pathway metabolites. Brain Behav Immun 87:715-724, 2020
21. Moher D, Liberati A, Tetzlaff J, Altman DG: Preferred reporting items for systematic reviews and meta-analyses: The PRISMA statement. Int J Surg 8:336-341, 2010
22. Nitta ME, Savitz J, Nelson LD, Teague TK, Hoelzle JB, McCrea MA, Meier TB: Acute elevation of serum inflammatory markers predicts symptom recovery after concussion. Neurology 93: e497-507, 2019
23. Papa L: Potential blood-based biomarkers for concussion. Sports Med Arthrosc Rev 24:108-115, 2016
24. Rice SM, Parker AG, Rosenbaum S, Bailey A, Mawren D, Purcell R: Sport-related concussion and mental health outcomes in elite athletes: A systematic review. Sports Med 48:447-465, 2018
25. Rodríguez A, Cervera E, Villalba P: Neuronal and glial biomarkers research for traumatic brain injury. In: Zhou Y (ed), Traumatic Brain Injury-Neurobiology, Diagnosis and Treatment. London: IntechOpen, 2019
26. Sandel N, Reynolds E, Cohen PE, Gillie BL, Kontos AP: Anxiety and mood clinical profile following sport-related concussion: From risk factors to treatment. Sport Exerc Perform Psychol 6:304-323, 2017
27. Shahim P, Linemann T, Inekci D, Karsdal MA, Blennow K, Tegner Y, Zetterberg H, Henriksen K: Serum tau fragments predict return to play in concussed professional ice hockey players. J Neurotrauma 33:1995-1999, 2016
28. Shahim P, Mattsson N, Macy EM, Crimmins DL, Ladenson JH, Zetterberg H, Blennow K, Tegner Y: Serum visinin-like protein-1 in concussed professional ice hockey players. Brain Injury 29:872-876, 2015
29. Shahim P, Tegner Y, Marklund N, Blennow K, Zetterberg H: Neurofilament light and tau as blood biomarkers for sports-related concussion. Neurology 90:e1780-e1788, 2018
30. Shahim P, Tegner Y, Wilson DH, Randall J, Skillbäck T, Pazooki D, Kallberg B, Blennow K, Zetterberg H: Blood biomarkers for brain injury in concussed professional ice hockey players. JAMA neurology 71:684-692, 2014
31. Shahim P, Zetterberg H, Tegner Y, Blennow K: Serum neurofilament light as a biomarker for mild traumatic brain injury in contact sports. Neurology 88:1788-1794, 2017
32. Siman R, Shahim P, Tegner Y, Blennow K, Zetterberg H, Smith DH: Serum SNTF increases in concussed professional ice hockey players and relates to the severity of postconcussion symptoms. J Neurotrauma 32:1294-1300, 2015
33. Tsitsopoulos PP, Marklund N: Amyloid-β peptides and tau protein as biomarkers in cerebrospinal and interstitial fluid following traumatic brain injury: A review of experimental and clinical studies. Front Neurol 4:79, 2013
34. Whiting PF, Rutjes AW, Westwood ME: QUADAS-2: A revised tool for the quality assessment of diagnostic accuracy studies. Ann Intern Med 155:529-536, 2011