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by

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Abstract

Upon head trauma, neurons within the brain can become stretched and injured. Continuous mild traumatic brain injuries (mTBIs) without proper healing can lead to chronic neurological symptoms. This is especially problematic in contact sports and military personnel. The purpose of this literature review is to take an in depth look at fluid biomarkers that could aid in the diagnosis of a concussion at a point of injury. Databases were used including PubMed, Clinical Key, Embase and Access Medicine. The studies chosen were published within the last 7 years, applicable to the topic and absent of pronounced bias. The 19 works include clinical trials, randomized control trials, and systematic reviews. There proved to be more research in blood biomarkers than salivary biomarkers, but the evidence is still lacking with both in relation to mTBIs. GFAP and UCHL1 show to be sufficient serum markers for individuals who have a significant concussion history or recurrent head trauma. Additionally, continued research on saliva miRNAs could prove them to be a diagnostic tool for quick, point of injury tests. The data shows that serum proteins respond in a much more delayed response, whereas saliva miRNA biomarkers respond more acutely. Additional studies will need to be done to properly examine both genders, other contact activities, and the polytrauma associated with military personnel.

Key words: *microRNAs, polymerase chain reaction, biomarkers, brain concussion, saliva, traumatic brain injury, fluid biomarkers, TBI, mTBI, concussion, head injury, mRNA*

Diagnosing Mild TBIs with Fluid Biomarkers

The brain is the most vital aspect of the nervous system. It receives signals from external sources and responds by controlling movement, thought process, speech, memory, and vital organ function. The outer portion of the brain, the cerebral cortex, contains numerous neurons and is protected within the cranium. According to the CDC, in 2019, there were nearly 61,000 traumatic brain injury (TBI)-related deaths, with the majority of these being classified as a mild TBI. They most often occur from head trauma including direct or indirect contact through an abrupt acceleration-deceleration movement. The most common of these are motor vehicle accidents, falls, sports injuries, blast injuries in combat, and assault. The word “concussion” can also be used interchangeably with mild TBI, but not for more severe TBI classifications. At the cellular level, the axonal portion of the neurons can stretch and tear when the brain bounces against the intracranial surface. This results in inflammation and can alter brain function, leading to acute or chronic complications. The diagnosis of a mild TBI is most commonly a clinical diagnosis, in which the provider determines the injury based on physical appearance and subjective symptoms. The purpose of the following literature review is to take a closer look at other diagnostic tools, including blood and saliva tests, to determine their efficacy when compared to a standard clinical diagnosis.

Statement of the Problem

Mild TBIs are typically not visualized on routine imaging, leaving the clinical diagnosis to be both subjective and biased. This can lead to underreported symptoms and patients returning to activity too soon. Concerns for this arise especially in athletes and those who are not experiencing symptoms post-incident. These symptoms can include headache, dizziness, loss of consciousness (LOC), or significant neurogenic symptoms. Without a known cognitive baseline

for each patient, a clinical diagnosis can miss the microscopic damage within the brain's neurons. Newly discovered blood and saliva laboratory tests are promising diagnostic tools that could aid in this process. With these biomarker tests being both non-invasive and relatively time sensitive, it would be a game changer in solidifying a diagnosis of TBI. They could prevent future impaired cognitive outcomes for patients and also provide medical professionals with a tool to better manage and treat these patients appropriately following a head injury.

Research Question

In adult patients with a TBI, can biomarker testing compared to standardized clinical exams provide earlier diagnosis and improved cognitive outcomes?

Research Methods

An electronic literature review was completed by comprehensively compiling information through the following databases: PubMed, Clinical Key, Embase, and Access Medicine. The search was done using the mesh terms microRNAs, polymerase chain reaction, biomarkers, and brain concussion. This yielded a total of 230 articles, which was then filtered down to 92 articles by limiting the years from 2014 – 2021 and setting an age filter to 18 and older. Saliva and traumatic brain injuries were then added as mesh terms and used in combination with the others listed. Other key words that were used include fluid biomarkers, TBI, mTBI, concussion, head injury, and mRNA. These key words were also added in and used in several combinations with all of the mesh terms used. The literature used consisted of clinical trials, randomized control trials, and systematic reviews. The articles were then meticulously sifted through by choosing those that were applicable to the topic and also absent of a pronounced bias.

Literature Review

An Overview of Mild TBIs

Pathophysiology and Associated Complications with Mild TBI

Within the category of TBIs, concussions are discussed in a way that they are a mild form of a TBI. According to Zetterberg et al. (2019), studies have made it apparent that concussions are a separate injury state when compared to moderate-severe TBIs. This is due to the wide range of both acute and chronic symptoms that can occur and present so differently among individuals. The terms mild TBI and concussion can be used synonymously due to the fact that both have similar criteria involving acute loss of consciousness (LOC) and post-injury amnesia. With the understanding that most mild TBIs are unreported, there is estimated to be an annual incidence of around 3.8 million (Zetterberg et al., 2019). It is known that the pathophysiology of mild TBIs consist almost entirely of damage to the brain's axons, at the cellular level, which can then lead to alterations at the behavioral level, most commonly working memory deficits. Because of these microscopic injuries and the long-term effects that are not always made aware to external sources, concussions are said to be a "silent epidemic" according Laskowski et al. (2019). Even with the Glasgow Coma Scale (GCS) being used as a tool to measure the level of consciousness and severity of the brain injury, it cannot accurately correspond with the hidden cerebral pathology due to microscopic disturbances and their ability to externally display clinical similarities regardless of the extent of damage (Laskowski, Creed, & Raghupathi, 2015; Zetterberg et al., 2019).

The microscopic injuries that occur involve the axonal aspect of the brain's neurons. During a traumatic blow to the head, the axon is stretched and manipulated to the point of structural damage. Laskowski, Creed, & Raghupathi (2015) state that as a result of this, the axonal cell

membrane can have microscopic holes causing influx of certain molecules such as calcium. This results in continued structural alterations to the axonal cytoskeleton. This traumatic cascade can lead to transport disruption between neurons, swelling and inflammation of the axons, and possibly programmed cell death. The extent of this process depends solely on the amount of trauma that was applied, and therefore is not able to be easily predicted by providers. Neurons also contain memory fields within the prefrontal cortex part of the brain. In a post-concussive state, the excitatory and inhibitory signals through these memory fields can be faulty, causing an imbalance of dopamine. The altered dopaminergic signaling can cause the working memory deficits that present with concussions (Laskowski et al., 2015 & Zetterberg et al., 2019).

The microscopic damage is the concern. Because it is unseen and underdiagnosed, repetitive brain damage can occur. This is especially problematic in contact sports or blast injuries in combat. Although most concussions can heal on their own within a few weeks, Zetterberg et al. (2019) states that 10-15% of these patients can progress to developing post concussive syndrome (PCS), possibly leaving them with chronic neurological symptoms. PCS is difficult to establish and poorly defined due to the variability with symptoms and there being a blurred line between symptoms from a mild TBI and symptoms arising from PCS. The most common symptoms seen in PCS include headache, sleep disturbances, fatigue, and anxiety, which are also difficult symptoms to distinguish from individuals who deal with these even outside of a concussion (Laskowski, Creed, & Raghupathi, 2015; Zetterberg et al., 2019).

Glasgow Coma Scale

In 1974, two professors of neurosurgery at the University of Glasgow in Scotland created a scale that could be used to evaluate and assess the level of consciousness of an individual. The Glasgow coma scale (GSC) was initially created by Graham Teasdale and Bryan J. Jennett in

order to assess LOC in those who had suffered a head injury but is now a tool that is widely used for many acute situations including patients in intensive care units, elderly patients, and trauma patients (Mehta & Chinthapalli, 2019). The scale is composed of three main components that are given a score by the provider based on patient presentation: eye response, verbal response, and motor response. According to Mehta & Chinthapalli (2019), this triple criterion scoring system provides a total score between 3 and 15, showing to be a good prognostic indicator for the severity of the head injury. The CDC (2021) classifies a mild head injury with a GCS score between 13 and 15, a moderate head injury between 9 and 12, and a severe head injury between 3 and 8. Unfortunately, a GCS score between 13 and 15 is either normal or very close to normal, making it difficult to assess the microscopic severity of a concussion.

Current Clinical Diagnosis of Mild TBI

The current guidelines for a mild traumatic brain injury (mTBI) include first being thoroughly evaluated by a trained and licensed healthcare professional in whichever environment that may be: an office visit, the emergency room, or on the sideline during an athletic event. Evan & Whitlow (2021) explain that imaging is required after an acute evaluation only if the patient was unconscious for longer than one minute, if there are obvious findings when evaluating mental status, cranial nerves, and limb strength, if the patient is older than 64, if there is repetitive vomiting, or if there are any signs of skull trauma. Once these have been ruled out, there are a few standardized examinations that can be used; the Standardized Assessment of Concussion (SAC), the Sport Concussion Assessment Tool (SCAT5), Post-Concussion Symptom Scale and Graded Symptom Checklist, and Westmead posttraumatic amnesia scale (WPTAS). These aid in the diagnosis of a concussion only if appropriately used as a preinjury assessment in order to compare to the patient's baseline but are not able to rule out a mTBI.

Other measures used but not well validated include the Immediate Post-Concussion Assessment and Cognitive Testing (ImPACT), the Galveston Orientation and Amnesia Test (GOAT), the Military Acute Concussion Evaluation (MACE), and the Balance Error Scoring System (BESS) (Evan & Whitlow, 2021). Although there are two blood biomarkers that are FDA approved, they are not routinely used in clinical practice to diagnose and manage concussions.

Current FDA Approved Blood Biomarkers

The current FDA approved biomarker blood test was developed at Banyan Biomarkers Inc. in San Diego. Essentially, when a TBI occurs and it is still within 12 hours, the protocol is that the blood test is performed in order to guide the decision of ordering a CT scan (Voelker, 2018). Voelker explains that the test is measuring two biomarkers, glial fibrillary acidic protein (GFAP) and ubiquitin C-terminal hydrolase L1 (UCHL1), which are aimed to be measured within 1 hour after a head injury in order to measure the risk of the patient (2018). A common misconception is that these peripheral blood markers diagnose concussion, but in reality, they are really used to decide if a CT should be performed or not (Voelker, 2918.

According to Voelker (2018), the push for the development of this blood test came from the US Department of defense, due to the high number of battlefield blast injuries. Initially, this article points out that the military was actually interested in a point-of-care (POC) test that could be rapid in immediate situations, but Voelker (2018) explains that the science is just not there yet. There is a downside to this process; if the blood test is positive and the CT scan is performed, sometimes the result of the CT scan won't entirely change the course of action for the patient's treatment plan, therefore they may have just been exposed to unnecessary radiation (Voelker, 2018). On the other hand, the laboratory turn-around-time for this lab test alone takes 3 – 4 hours, whereas a CT scan can take less than 30 minutes. In conclusion, Voelker states that

the blood tests for GFAP and UCHL1 have an accuracy rate of 97.5% in showing intracranial lesions on a CT scan and a 99.6% accuracy in prediction of the patients that would not have lesions on a CT scan. In all reality, this screening tool is very useful in the way that unnecessary radiation to a patient can be avoided in certain instances when they are not clinically presenting as very high-risk or low-risk, and a decision needs to be made in next steps (Voelker, 2018).

Long-term Effects of TBIs

Traumatic brain injuries are most common in military environments and contact sports. Furthermore, these are both intensive settings that don't always allow for a solidified diagnosis or extended time to heal. With limited attention to mTBIs in combat and sports, repetitive concussions over and over can lead to long-term effects including memory loss, headaches and dizziness, personality and mood alterations, or concentration difficulties. Overall, not allowing the brain to heal between minor blows to the head can lead to cumulative effects down the road for these individuals.

Military Induced

A blast induced traumatic brain injury (bTBI) is a type of mild TBI that is among the most common head injuries affecting military personnel. Since 2001, the military conflicts in Afghanistan and Iraq deployed nearly 1.5 million service personnel. Of the 1.5 million individuals, Agoston and Kamnaksh (2015) state it is estimated that 15 – 30% of them suffered from TBIs and approximately 80% of those were in the bTBI category from an explosive. The complexity of these cases differs significantly due to the common polytrauma outcome of a blast injury, but even of those that have minimal physical damage, there are still neurobehavioral symptoms that can occur (Agoston & Kamnaksh, 2015).

Biologically, the extreme damage to the brain is most commonly from the rapid increase and decrease in pressure. Not only does the damage come from a direct blast injury to the head, but the explosive also affects the entire body. The large blood vessels in other parts of the body can take up the blast force causing the energy to travel to the brain (Agoston & Kamnaksh, 2015). As stated previously, around 80% of TBIs fall into the mild category: GCS score of 13 – 15, LOC or an altered state for less than 30 minutes, and amnesia lasting less than 24 hours. Agoston & Kamnaksh (2015) state that the recovery time for these individuals is usually within days to weeks from the injury; however, some lingering symptoms can include increased anxiety and depression, forgetfulness, slowed thinking, and overall impaired cognitive function. Within a war environment and with those symptoms seeming somewhat "minor", soldiers will return to duty while they are still in the post-concussive stage where they experience repeated blast injuries. The brain damage then becomes more severe, potentially leading to long-term adverse outcomes (Agoston & Kamnaksh, 2015).

Of note, Agoston and Kamnaksh (2015) explain that the methodology of recreating the physical components of a blast environment for research purposes is both challenging and restrictive due to safety reasons and difficulty in replicating the human brain and skull. Additionally, species contain different brains. This makes it difficult when selecting test subjects that contain a lissencephalic brain and comparing to the human gyrencephalic brain. Ethical and economic issues surrounding using animals as test subjects also makes the research testing challenging (Agoston & Kamnaksh, 2015).

An experimental study was done to compare military personnel and rats in relation to an exposed blast injury and the lagging symptoms that occurred. Within the clinical mild bTBI study, through survey, soldiers were found to be frequently dazed, confused and disoriented

within the acute timeline of <72 hours. Chronically, from 1.2 – 7.1 years, they were also found to frequently experience headaches, sleep disturbances, anxiety and depression, memory loss, impaired executive function, and altered sensory sensitivities and functions. Agoston & Kamnaksh (2015) make it a point that these symptoms may be difficult to distinguish from an underlying cause of brain injury or PTSD. Within the rat models, they were given a blast exposure and in <72 hours they were rarely observed to have anxiety and depression or memory loss. However, in 72 days which is relative to the chronic timeline of a human, they were frequently observed to have memory loss based on their tracked activity in a Barnes maze (Agoston & Kamnaksh, 2015).

In the military realm, overpressure (OP) is the pressure caused by a shock wave that is more than the typical atmospheric pressure. Soldiers who tactically enter unknown situations or closed spaces, also known as breachers, are regularly subject to repeated exposures. Boutté et al. (2019) explains that this slowly causes a decrease in their reaction time (RT), headaches, dizziness, fatigue, and tinnitus. This is referred to as “breacher’s brain”, which is clinically equivalent to individuals who are diagnosed with a mTBI (Boutté et al., 2019). This is of serious concern due to symptoms being subjective and severely underreported or managed.

A cross-sectional cohort study paired with observations of 29 males during the duration of their service aimed to objectively look at blood-based biomarkers as well as other parameters to measure the effects of their brain trauma. The biomarkers are central nervous system proteins and include neurofilament light chain (NFL), tau, glial fibrillary acidic protein (GFAP), and ubiquitin carboxy-terminal hydrolase L1 (UCH-L) (Boutté et al., 2019). These Active-duty United States Army personnel were among the trainees completing a two-week breacher training course (Boutté et al., 2019). Within the two weeks, one day included a heavy wall breaching

exercise, where blood samples, neurocognitive testing and symptoms were assessed. The overpressure events included back-to-back heavy wall breaches to destruct concrete walls and the psi exposed to each soldier was measured using a B3-H pressure sensor that was placed on their left shoulder. To assess neurocognitive performance, the Defense Automatized Neurocognitive Assessment (DANA) tool was completed by each participant 8 hours before the breach and 1 hour after the breach, as well as blood draws at these timeframes. According to Boutté et al. (2019), the DANA tool is a computerized test to measure the participant's simple reaction time (SRT). The men also completed a paper-and-pencil symptoms assessment 8 hours before exposure and 1 hour after exposure (Boutté et al., 2019).

The 29 participants in the study by Boutté et al. (2019) were males with a mean age of 29.5 years old, ranging from 21 – 43 years of age. Their duration of service ranged from 2 – 20 years with a mean duration of about 8.5 years. When comparing the pre-exposure and post-exposure symptoms assessment, the results showed that 52% reported an increase in headache, 41% reported slowed thinking, 31% reported an increase in dizziness, and 28% reported poorer concentration (Boutté et al., 2019). When looking at the DANA tool, assessing the men's SRT showed that after the exposure the median SRT increased by 30.5 mms with the pre-exposure median being 269.0 ms and post-exposure median being 299.9 ms. This revealed a significant 11% change ($p = 0.048$). The serum biomarkers that were tested revealed a marginal decrease in the concentration of GFAP with a pre-exposure median value of 59.2 and a post-exposure median value of 52.1. The results from Boutté et al. (2019) showed that NFL ($p = 0.035$) and tau ($p = 0.090$) almost doubled in concentration after exposure, although Nf-L was the only biomarker of the two that met the statistical threshold.

The top three symptoms of significance (headache, dizziness, and slowed thinking) were correlated with both the results of the neurocognitive testing with DANA and the biomarker levels (Boutté et al., 2019). The significant data showed that the SRT ($p = 0.04$) was slower in the individuals that reported an increase in dizziness post-exposure (n = 20). Other significant data showed that those with an increase in dizziness also showed a decrease in the biomarker GFAP ($p = 0.02$), and correlation with the increases in UCH-L1($p = 0.05$) and Nf-L ($p = <.001$). Symptoms of headache and slowed thinking did not have any statistically significant correlations (Boutté et al., 2019).

When the test subjects were exposed to OP, their NFL levels increased with significant p-values. Changes in GFAP and tau proteins also occurred but were not significant. The decrease in GFAP within this study is physiologically unknown and requires further evaluation given that the FDA approved test expects an increased level. The protein UCH-L1 did not show a significant increase, although they did prove to be significant when correlated with those who had concussion history. Participants also had a significant increase in their SRT and symptom assessment post-exposure, proving there was in fact a head injury (Boutté et al., 2019).

Another cohort study focused on serum biomarkers using 102 United States service members or veterans. The sample consisted of 84 participants who had sustained an mTBI and 18 as injured controls (IC) without a TBI. Pattinson et al. (2019) then separated these individuals into three groups based on the presence of PTSD: IC without PTSD (n = 18), mTBI without PTSD (n = 63), and mTBI with PTSD (n = 21). The mean age of these individuals was 34.17 years with 92.5% of the sample size being male. The 84 individuals that were included in the mTBI group had to have a previous brain injury that fell within the mTBI category. The IC group

were those that experienced a soft tissue injury during combat, had trauma to the brain but did not fit into the mTBI category, or had no history of a TBI (Pattinson et al., 2019).

The participants all provided a non-fasting blood sample to test tau levels. At this time, they also completed a 2.5-hour survey of self-reported symptoms. When looking at the results, Pattinson et al. (2019) noted it was obvious that the mTBI with PTSD group reported significantly higher scores on the assessments when compared to the mTBI without PTSD and IC without PTSD groups. The mTBI with PTSD group also exhibited a higher level of functional impairment due to having the lowest scores on cognitive scales compared to the other groups (Pattinson et al., 2019).

Pattinson et al. (2019) found that tau was significantly elevated in the mTBI with PTSD group. The mean tau protein levels were the following: mTBI with PTSD was 3.52, mTBI without PTSD was 2.84, and the IC group without PTSD was 2.57. With this trend, Pattinson et al. (2019) found there is a significant difference in these groups with the mTBI with and without PTSD (p = .43) and the mTBI with PTSD and IC without PTSD (p = .22). With that, there was not a significant difference between the IC group and mTBI without PTSD.

This study discovered that tau protein levels were significantly increased in soldiers and veterans that had a diagnosis of PTSD and those who have had a mTBI. Additionally, those with both mTBI and PTSD had the highest levels of tau, so this shows that PTSD had a large part in increased tau levels. This helps support previous studies in thoughts that relate high tau concentrations to neurological deficits, such as a mTBI (Pattinson et al., 2019).

Sport Induced

Along with military induced brain injuries, contact sports also play a large role in the silent epidemic of concussions and repeated exposures. The Journal of Head Trauma Rehabilitation

shared a study that looked at 52 athletes from university and sports medicine clinics based in Canada. The objective of the study was to examine and evaluate both psychological and physical results in concussed athletes over a period of time. Fourteen teams from the University of Toronto were used in a study by Hutchinson et al. (2017), which consisted of 7 male teams and 7 female teams. The sports that were used in this sample included the following: football (n = 7), soccer (n = 6), rugby (n = 14), hockey (n = 9), basketball (n = 4), volleyball (n = 5), lacrosse (n = 6), and baseball (n = 2). Data was gathered from a total of 26 concussed athletes (16 male, 10 female), with a matching control group of uninjured athletes. The mean age was 21 years and of the 52 athletes, 40.4% of them had no history of a concussion (Hutchinson et al., 2017).

When an athlete experienced a concussion, the report prompted a diagnosis by a sports medicine provider using clinically diagnostic approaches as well as the athlete taking the Sport Concussion Assessment Tool (SCAT). The athletes then followed the 6-stage Return to Play (RTP) protocol which includes stages that are not advanceable until the participant completes the exercise level without any post-concussion symptoms (Hutchinson et al., 2017). During the study, Hutchinson et al. (2017) stated that athletes who were concussed were assessed at 3 recovery milestones: (a) during the symptomatic phase (within the first week post-injury), (b) after the symptoms had cleared and the participant started the RTP program, and (c) during the post-RTP phase (one week after RTP clearance). Non-concussed, healthy athletes were tested in linearity to the concussed subjects (Hutchinson et al., 2017).

During all 3 phases, the concussed athletes, as well as the control group, took self-assessments that displayed scores they reported for themselves on mood, perceived stress, sleep quality, post-concussion symptoms, depression, anger, vigor, confusion, fatigue, and tension. The results showed that at the symptomatic phase, the concussed athletes had a significant

worsening in mood, sleep, post-concussion symptoms, depression, anger, vigor, confusion, and tension. At the second phase, the concussed athletes and control groups all revealed similar results despite the concussed group showing a high self-rank for post-concussion symptoms. Interestingly enough, when progressing to the third phase, Hutchinson et al. (2017) found that the concussed athletes had significantly better rankings than the control group when looking at overall mood, depression, and fatigue (Hutchinson et al., 2017).

The overall conclusion of Hutchinson et al. (2017) study showed that within the first week post-injury, concussed athletes will have significantly lower sleep quality and elevations in emotional disturbances compared to the controls. Over time this improves and during the post-RTP phase, the concussed athletes showed better scores than the controls, which is thought to be due to their "return to normalcy" scores being heightened compared to those who did not just recover from a concussion (Hutchinson et al., 2017). Unfortunately, an individual's eagerness to continue to play or return to play may be so enthusiastic that symptoms are masked and subjectively reported as normal or even better than normal. This can falsely allow athletes to return to play too soon if the healthcare professional has no physical findings.

Because symptomatic monitoring may not be completely reliable, protein biomarkers are of high interest following concussions involved in athletics. Three of them were closely looked at in a cohort study that included 18 Division I college football players in order to evaluate the changes in blood levels while monitoring head impacts with accelerometer-embedded mouthguards (Rubin et al., 2019). Tau, neurofilament light chain (NFL), and S100 calcium-binding protein B (S100B) were three proteins that were monitored, but Rubin et al. (2019) states that NFL is one that has recently been of more interest involving mTBIs. The median age of the male participants was 20.5 years old. Demographics for previous concussions showed 50% of

them had never had one, 39% of them have had only one and 11% of them had a total of three concussions. The study was completed during pre-season for a total of one month where the participants were training for their upcoming season. Baseline levels were received via blood draws two months before preseason began, when the athletes did not have any contact practices or training. Blood draws were then also taken from the athletes one hour before practices and one hour after practices during pre-season (Rubin et al., 2019).

The first result that Rubin et al. (2019) looked at after 1 month of collecting data from these participants was the number of hits each player endured and compared that with NFL level changes from pre-post practice. This trend showed that as the number of hits increased, there was a significant correlation with the increasing NFL levels. The mouthguards that Rubin et al. (2019) used in this study measured the peak accelerations of the head during practices. The head impact magnitudes were also shown to be associated with significant changes in NFL levels pre-post practice. To ensure that these results were not due to years of football experience or a past history of numerous concussions, football playing history as well as concussion history were added into the models which showed to not change the correlation between head impacts kinetics and the increase in NFL levels (Rubin et al., 2019).

In order to further confirm that the changes in NFL concentrations were not due to other factors, the participants were then divided up into two groups: a lower (n = 6) and a higher (n = 12) impact group. These separations were based on the head impact kinetics received and how extreme the number of hits and head accelerations were for each player. Rubin et al. (2019) found that the lower and higher groups differed in all results of the head kinetics. The median number of hits was 32 versus the lower group which only had 4, as well as all other head impact kinetics showing significant difference between the lower and higher groups. This proves that the

NFL concentration changes are not correlated with each athlete's football history, concussion history, age, or BMI, but strictly by the data that was picked up by the mouthguards. There also showed to be a significant correlation between increases in NFL and increases in S100B (p = <.001) (Rubin et al., 2019).

This study was the first one that looked at the actual head impact kinetics with mouthguards and correlated this with biomarker levels in the blood, showing that these levels increase as the number and severity of head impacts increase. According to Rubin et al. (2019), the protein NFL is more associated with axonal injury and S100B is more associated with damage to the blood brain barrier. With this, it is difficult to pinpoint exactly what is being altered within the brain from this study due to two mechanisms. Furthermore, NFL is known to be within larger and longer axons whereas tau is more numerous in thin and unmyelinated axons (Rubin et al., 2019). Because the tau concentrations were not significant in this study, this could tell us something about which axons experienced damage and at which locations in the brain. This study revealed that NFL and S100B could be potential peripheral biomarkers for an acute finding in mTBIs; however, Rubin et al. (2019) did not evaluate females and the sample size was relatively small. Although football is well known as a high impact sport and highly associated with head injuries, other sports need to be investigated at as well.

Fluid Biomarker Use in Mild TBI Diagnosis

Fluid biomarkers are proteins made by our body in response to internal or external stimuli that can clue us in on information regarding the pathophysiological state of a patient. Because TBIs are very difficult to diagnose through subjective source, the heterogeneity of it makes it unclear what each clinical outcome will be. Biomarkers, in response to a TBI, can be released from the brain cells, then having the ability to travel across the blood brain barrier (BBB) into the

peripheral blood or into the mouth via salivary transport (Dadas et al., 2018). Dadas et al. (2018) discusses and compares the biokinetics of blood and saliva biomarkers.

The majority of TBI biomarker testing has been accomplished through blood and CSF, but as of late there has been some evidence that biomarker S100B may be identified in saliva samples as well, according to Dadas et al. (2018). S100B is a specific biomarker that has been found to reveal itself within the peripheral blood after being released from brain cells, as discussed previously in the 2019 study by Rubin et al. The presence of S100B is a sign that there has been some BBB disruption, giving its ability to surpass the BBB and be detected in the blood, which is what Dadas et al. wanted to evaluate further in this 2018 study. S100B and GFAP are both biomarkers that are released following brain damage, but based on their kinetics, it is known that they will show in the peripheral blood at different timeframes according to Dadas et al. (2018). They found that S100B has a sharp increase in concentration just minutes after injury and will return to normal in just 1 – 2 hours, whereas GFAP does not raise as suddenly and stays elevated for days. This is most likely due to S100B having a much smaller molecular weight than GFAP (Dadas et al., 2018).

Compared to a blood draw, saliva samples for detection of a concussion are a lot simpler and also noninvasive for the individual. They don't require a need for specialty personnel to draw the blood or a centrifugation machine to separate a blood sample. Although this concept is very straight forward, Dadas et al. (2018) also discusses that the passage of these biomarkers into saliva is extremely complex. However, when using laboratory technology to measure the weights of the proteins in whole saliva versus blood, it was found that the protein fractions in saliva are much smaller in molecular weight compared to protein sizes in the blood (Dadas et al., 2018). With this information, it was clear to them that the consistency is apparent with these small

salivary proteins and the selective permeability that must take place when there is movement from the blood to the saliva for low-molecular weight proteins.

Dadas et al. (2018) explain that the salivary barrier is much more selective than the BBB and in contrast to a damaged disruption of the BBB, the salivary barrier occurs without disruption in healthy tissue. It is true that saliva biomarker tests are beginning to gain momentum, but because of their selective permeability even without disruption, further studies will need to be analyzed (Dadas et al., 2018). The complexity in figuring out the best biomarkers to use for the diagnosis and management of mTBIs not only stems from their mode of action, but also their varying molecular weights and timelines.

Blood Biomarker Use in Mild TBI Diagnosis

Correlation with CT scans

One way to accurately diagnose a mTBI is through CT imaging. Çevik et al. (2019) states that the downfall to this is the patient is exposed to unnecessary radiation and the cost is high. This is a big reason why blood biomarkers are a potential candidate to a quicker, more cost-effective method to detect if there was axonal damage that cannot be seen otherwise. Çevik et al. (2019) completed a cross-sectional study on patients who presented to the emergency room with a TBI between February and September of 2016. They aimed to measure the levels of S100B, GFAP, and a small neuronal protein neurogranin (NRGN). When brain trauma occurs, the proteins arise from brain and spinal cords cells, eventually making their way to the peripheral blood. The aim of this particular study was to compare the levels of these biomarkers with the results of CT scans to correlate the patterns in participants with mTBIs (Çevik et al., 2019).

The guidelines to be in the study with a confirmed TBI included a GCS score between 14 – 15 and presenting with one or more symptoms of either post-injury amnesia, nausea, vomiting,

or seizures. There were 48 patients total in this study; 24 of them had a positive CT scan showing intracranial traumatic pathology, and 24 of them had a negative CT scan. The venous blood draws for the biomarker testing were collected within the first 4 hours from the trauma, as well as a 16-slice CT scan without contrast. Positive traumatic pathology included findings of any type of hemorrhage or cerebral contusion (Çevik et al., 2019).

The average age of these participants was 24, ranging from 5 – 65. Demographically, 79% of them were male and 21% of them were female. The causes of the mTBIs in these individuals included traffic accidents (20.8%), assault (16.7%), falls (47.9%), and others (14.6%). Of the symptoms, headache and vomiting were the most common. NRGN concentration levels were significantly higher in the 24 patients who were CT positive than the patients who had negative findings on the CT scan (p = .001). Çevik et al. (2019) also found that S100B (p = <.001) and GFAP (p = 0.026) were significantly elevated in patients who were CT positive. According to the data and results, all three of these biomarkers were found to be statistically significant in correlation to CT scans and the presence of a mTBI, which provides some evidence that GFAP, S100B, and NRGN are reliable biomarkers for detecting a mTBI without obtaining a CT scan as the primary diagnostic tool (Çevik et al., 2019). However, these biomarkers were not evaluated in parallel to those with mTBIs from a contact sport or individuals who experienced head trauma and did not report to the emergency room. It is also difficult to know the accuracy of elevated biomarker levels without knowledge of the patient's baseline levels.

Correlation with Baseline Levels in Sports

Another recent 2020 cohort study by Meier et al. specifically looked at sport-related concussions (SRC) in high school and collegiate football players. Because most studies are unable to receive a baseline from participants, Meier et al. (2020) focused on obtaining

biomarker levels in the athletes prior to any injuries. Blood was also drawn two separate times post-injury. A control group was also administered into the study including participants who played football and were uninjured (n = 83) and athletes who were involved in non-contact sports (n = 50) (Meier et al., 2020). GFAP, UCH-L1, and S100B were measured in the athletes.

The prospective study was completed during the timeframe of 2015 – 2018 after having gained approval from the Medical College of Wisconsin. A total number of 1,136 football athletes were enrolled into the study before the season had started, which is when they did the baseline testing (Meier et al., 2020). The control group of football players who were uninjured had to be concussion free for at least 6 months prior to the study and the control group in non-contact sports had to have no experience playing football or no history of concussion in their lifetime. Of the 1,136 football players, a total of 106 of them obtained a concussion during the study timeframe and met the criteria in which they were diagnosed by a certified athletic trainer or team physician based off of the CDC's concussion definition. The 106 participants completed a blood draw within 6 hours of the injury and again within 24 – 48 hours (early-acute), 8 days, 15 days, and 45 days after the injury (late-acute) (Meier et al., 2020).

Demographically, Meier et al. (2020) found there to be very little differences between the sports-related concussion (SRC) group and the contact controls (CC). The non-contact controls (NCC) were slightly older in age, had lower BMIs, and had more years of participation in their non-contact sport. At baseline, Meier et al. (2020) found that the SRC group's symptom assessment scores were a little higher than the CC and NCC groups. Again, the GFAP biomarker did show significant elevation in the SRC group, but even at baseline, this concentration was higher than the other groups. Meier et al. (2020) state that this is most likely due to a high unlikelihood of any contact. In the early-acute phase, the SRC group concentrations were

significantly elevated compared to the others, and then subsided back down to baseline in the late-acute phase.

This study proves that it is difficult to know if GFAP is a reliable blood biomarker for a concussion due to the football players with mTBIs having higher baselines than the other groups, however, the spike in the early acute phase may be significant. The baseline symptom assessment scores of the SRC group were also significant when compared to other groups. This begins to raise the question of how accurate this study can be with a biased group of concussed football players already experiencing symptoms and having high biomarker levels at baseline. Additionally, continued studies and data points of early-acute concentrations would need to be obtained in order to establish what increased concentration of each biomarker would be diagnostic of a true brain injury (Meier et al., 2020).

Neurofilament Light Chain

In addition to the biomarkers discussed, the axonal protein neurofilament light (NFL) found in the peripheral blood has been of high interest in evaluating concussions, especially in athletes. Shahim et al. (2017) looked into this protein and how sensitive it can be for detecting mTBIs. This is important, especially for those athletes or nonathletes who experience post-concussion symptoms (PCS) that continue even months to years after the injury. Because it is known that NFL is released from axons and entered into the peripheral blood during injury when the cytoskeleton is disrupted, Shahim et al. (2017) discusses that it can be a promising test for detection. This protein is largely found in the CNS and most commonly in the long, myelinated axons (Shahim et al., 2017).

To research this further, a longitudal cohort study in 2017 enrolled numerous participants from different backgrounds (Shahim et al.). This included 14 amateur boxers who were tested 7

– 10 days after boxing and again 3 months after resting. Another 14 healthy, non-boxers were enrolled in order to compare to the athletes experiencing head impacts. In addition to the boxers and healthy nonathletes, levels were also measured in 12 gymnasts and 35 concussed professional hockey players from the Swedish Hockey League. The hockey players were tested at 1, 12, 36, and 144 hours after experiencing their concussion. Those who were experiencing PCS in length for more than 6 days were also tested on initiation of their return to play (RTP).

The 14 boxers (median age 21.5 years) were tested longitudinally with the 14 non-athlete controls (median age 23.5 years). The gymnasts (median age 19) were enrolled in order to also analyze athletes without direct impact to the head. Of note, Shahim et al. (2017) found there to be no difference in the NFL levels between the non-athletes and the gymnasts ($p = .9$). Overall, the results showed that the NFL levels in boxers were very significant. The serum levels were elevated significantly in boxers when compared to the controls ($p = <.001$) and the 7 – 10 days post-boxing levels were also significantly higher than the 3-month rest levels ($p = <.001$). Although the NFL serum levels did decrease as the 3-month mark, Shahim et al. (2017) found that they were still significantly higher than the control groups ($p = <.001$).

Furthermore, Shahim et al. (2017) then evaluated the data to distinguish between boxers who had experienced severe head impacts (more than 15 punches to the head and altered level of consciousness) and those who just had mild impacts (less than 15 punches to the head). These results were also significant, showing that the boxers who had severe head impacts had much higher NFL concentrations compared to the boxers with mild impacts ($p = 0.002$). The 3-month rest levels were also higher in the boxers with severe head impacts, although not as significant ($p = .54$). The mild impact boxers still had higher NFL concentrations at the 3-month testing compared to the control groups (Shahim et al., 2017).

In the evaluation of the 35 hockey players, serum NFL levels (1 – 144 hours) were significantly higher than the control groups ($p = 0.035$). Initially, the NFL levels increased rapidly in the hockey players after experiencing their concussion, with the highest result being at the 144-hour mark. Over time, these levels normalized and came to be to the same levels as the control groups (Shahim et al., 2017). To further assess prolonged PCS and how that is associated with NFL levels, the athletes with RTP < 6 days and > 6 days were evaluated (Shahim et al., 2017). It was found that the players who had PCS resolve quickly and returned to play in < 6 days had essentially unchanged serum levels between hours 1 and 144 after the brain injury when compared to the controls. For those who had PCS lasting longer than 6 days, Shahim et al. (2017) found that their serum levels remained elevated from 1 – 144 hours when compared to the controls. There was also a significant difference in serum levels between hours 1 and 36 for the groups of PCS > 6 days and < 6 days ($p = 0.02$ and $p = 0.006$) (Shahim et al., 2017).

When looking at the results of these two independent cohort studies, NFL does show to be a sensitive and specific biomarker for axon cytoskeleton disruption resulting in brain injury (Shahim et al., 2017). This is especially apparent in athletes, such as boxers who experience repeated hits to the head, as well as ice hockey players with concussions. These studies also showed that there may be a correlation between athletes who will have prolonged PCS and their serum levels of NFL in samples taken between the hours of 1 and 144 after concussion (Shahim et al., 2017).

Saliva Biomarker Use in Mild TBI Diagnosis

Saliva Biomarkers Timeline

One of the main goals of an advanced saliva test in diagnosing a concussion is for it to be a point of injury (POI) test. This means at the time of injury, the method of testing that is being

used needs to be able to pick up biomarkers that are peaking immediately after the head injury. In 2019, LaRocca et al. (2019) used 50 MMA fighters as participants in order to analyze both serum and saliva. This allowed for a comparison in timelines of peripheral blood and salivary collection in diagnosing mTBI. The samples were collected before a and again after the fight at different timeframes; immediately post-fight, 2 – 3 days, 1 week, and 3 or more weeks. These MMA fighter participants included mostly males with an average age of 26.5 years. Of note, 29% of the fighters reported a history of concussions with no complications that followed. In addition to collection of the samples, all fighters were analyzed by an FDA approved device that assessed balance and cognitive function. The fighters were also split into groups based on the number of hits to the head (HTH) they received during their fights. Based off of the number of HTH, the groups were compared by sectioning into low, moderate, and very likely risk of a mTBI (LaRocca et al., 2019).

In LaRocca et al. (2019) study, all of the balance/cognitive tests were completed, and the amount of swaying was measured for each participant. The results showed that the fighters who were in the low-risk group had less sway in some tests when compared to the moderate and very likely groups, although not all of the balance/cognitive tests showed this trend. Additionally, there were 10 peripheral blood biomarker proteins that were analyzed in the fighters before their fight and after their fight that showed potential changes. Four of these proteins showed significant increases in the serum samples when comparing pre-fight and post-fight samples. These peripheral proteins included S100B ($p = 0.006$), GFAP ($p = <.001$), NSE2 ($p = 0.037$), and MBP ($p = 0.003$). Because the average increases were found in low, moderate, and very high-risk groups, further analysis was completed in order to see if there was a correlation between the protein increases and the specific number of HTH. The only biomarker of the 10 analyzed that

showed significant correlation to HTH was UCHL1, but UCHL1 was not one of the 4 biomarkers that showed a significant change in pre- and post-fight (p = .934) (LaRocca et al., 2019).

To analyze miRNA biomarkers from the fighters' saliva, 925 miRNAs were found by an RNA quantification technique. These biomarkers were then analyzed by their sphericity and normality. After the biomarkers were investigated and sifted through, 21 miRNAs showed significant changes when correlated with the HTH and mTBI risk classification (LaRocca et al., 2019). At the end of the study, LaRocca et al. (2019) found that UCHL1 was the only biomarker that correlated quantitatively with the number of HTH compared to the other biomarkers in this study, although it did not show a significant difference in pre- and post-fight results. With all of the data retrieved, the serum proteins responded later in more of a delayed response and the saliva miRNA responded more acutely, picking up level changes closer to the head injury.

Study of Concussion in Rugby Union Through MicroRNAs

Testing for and diagnosing a concussion through a saliva test has been a popular topic lately as researchers in the United Kingdom (UK) have identified and tested biomarkers successfully. The study was completed using UK rugby players and is paving the way for the first non-invasive clinical tool to be used in sports or other settings (Yakoub et al., 2018). It has been called "ground-breaking" research as the use of miRNAs has been of large interest lately, according to Yakoub et al. (2018), especially with new discoveries in their possibility as potential biomarkers for cancers and neurogenerative diseases. This study was conducted in hopes of being able to use these biomarkers for brain trauma, specifically in athletics, so that quick, non-invasive saliva samples can be provided in the diagnosis of a concussion. A panel of

biomarkers would be a quick and objective test, inhibiting athletes to return to play too early (Yakoub et al., 2018).

Currently, the protocol for athletes who experience a head injury in the UK is managed through the Head Injury Assessment (HIA) in an effort to try and reduce the common occurrence of sports allowing athletes to continue to play following a traumatic event, according to Yakoub et al. (2018). If a player exhibits any 'criteria 1' symptoms, they are removed from the game that day. If none of the symptoms are met, the player must still step out of the game in order to go through an off-field assessment lasting a minimum of 10 minutes, known as HIA1. With that being said, athletes who experience a head injury are then split into 3 different groups: (1) Players have met the criteria 1 symptoms and are removed from the game, (2) players did not meet criteria 1 symptoms and enter HIA1 assessment off the field, leading to a diagnosed concussion, and (3) players did not meet criteria 1 symptoms and enter HIA1 assessment off the field, but are not diagnosed with a concussion. Although this clinical diagnostic approach seems thorough, the symptoms are subjective and easily avoided by the athlete in order to continue to play (Yakoub et al., 2018).

The prospective observational cohort study was performed on around 1,100 professional male rugby players in England in 2018. The participants were all asked to provide baseline samples of their saliva at the beginning of the study, as well as baseline questionnaires with respect to concussion history and symptoms. As discussed previously, there is the HIA1 off-field assessment phase right after the head injury, then leading to HIA2 which identifies an early concussion and HIA 3 which identifies a late concussion. Throughout the first season, Yakoub et al. (2018) had the players provide saliva samples at all three phases. Alongside an athlete that had a concussion, a healthy, non-concussed player supplied samples as a control. The control

was a player that had played a game or practiced in the same environment as the concussed athlete and for a similar amount of time. Lastly, there were orthopedic controls who were players that had experienced musculoskeletal injuries. This was part of the study in order to rule out changes in biomarkers due to other injuries and traumas that occur during rugby. Within the first season, there were 106 confirmed concussions. (Yakoub et al., 2018).

To deduce all of the data from season one, next generation gene sequencing and RNA PCR data analysis was performed. Once all of the proteins were found in the samples, they were compared using a heat map amongst the different groups of the study including HIA (+) at phases 1 – 3, HIA (-) at phases 1 – 3, uninjured controls and those with musculoskeletal injuries (Yakoub et al., 2018). The heat map displayed the average concentrations of each type of miRNA across all of the participant groups. The data showed that a significant number of miRNAs were either significantly increased or decreased in the concussed athletes when compared to all of the control groups at the "post-injury" phase. (Yakoub et al., 2018).

There were 32 miRNAs that were found to be expressed differently at all of the different time phases (T1, T2, and T3). When these 32 biomarkers were looked into deeper in correspondence with not only their baseline values, but also specific time points, Yakoub et al. (2018) found that 14 of them were highly accurate in correspondence with the concussed players in comparison to the other groups. Overall, the HIA (-) group's biomarkers all kept fairly close to their baseline during all of the HIA time phases when compared to the HIA (+) group (Yakoub et al., 2018).

In season two, Yakoub et al. (2018) explains that the sample size was much smaller, but the panel of 14 miRNAs that were discovered from season one was used for testing. Again, the panel showed a significant difference between HIA (+) and HIA (-) ($p = 0.007$), especially at the 36 –

48-hour mark. This reveals that this panel is a potential biomarker to accurately diagnose a mTBI due to its very acute changes (Yakoub et al., 2018). Furthermore, the 14 salivary biomarkers that were identified in this study also showed high accuracy in being over or under expressed both immediately after a head injury and 36 – 48 hours after. This is promising in being able to separate those with a true concussion from those who are uninjured or have musculoskeletal injuries.

In summary, the panel of 14 small noncoding RNAs that were identified throughout the two rugby seasons were highly accurate (96%) in differentiating those players with an actual concussion versus those with a suspected concussion that was ruled out during the HIA. The biomarker levels also showed significant differentiation both immediately after the head injury as well as 36 – 48 hours after (Yakoub et al., 2018).

Other Salivary Biomarker Methods

MicroRNAs are the noncoding molecules that play a large role in how proteins are made in the body, and they can arrive in saliva through transport micro vesicles and exosomes; this occurs in the area of space around the cells in our body (Hicks et al., 2020). Along with the detectable miRNAs, there are also some additional mechanisms within this transport process that could be candidates for other modes of detection within the saliva.

Upon a brain injury, extracellular vesicles (EVs) are released by our cells into the extracellular environment where they transport RNAs and proteins, including mRNA and miRNA, from the brain to the oral cavity. Because of this, there is the ability to be able to detect these indicators within an individual's saliva. With that, it is predicted that the saliva of someone who has just experienced a brain injury will be different than that of normal saliva without trauma (Cheng et al., 2019). 54 participants were enrolled into a study: 23 without history of any

brain trauma, 16 with history of an outpatient concussion, and 15 with an emergency room brain injury within 24 hours. The participants provided saliva samples by spitting into the test tubes every 60 seconds until there was a minimum of at least 5 mLs (Cheng et al., 2019).

The EVs were then isolated and analyzed from each participant's saliva specimen using several different methods including transmission electron microscopy (TEM). Cheng et al. (2019) explains that TEM was performed in order to compare the morphology and the size of the EVs between each participant population. Cheng et al. (2019) found that both the size and concentration of the EVs were increased by 2-fold in the ER brain injury patients when compared with the healthy controls. With this information, an Alzheimer's disease array analysis was used to compare with the participant's EVs. These results showed that 57 of the 93 genes in this analysis were increased. In comparison, the outpatient participants showed 56 gene increases in the analysis when compared with the controls. Out of all the genes upregulated in the ER participants and outpatient participants, there were 3 genes that were similar between these two.; CDC2, CSNK1A1, and CTSD (Cheng et al., 2019).

CTSD was found in 12 of the 15 ER patients and 15 out of the 16 outpatient concussion participants. According to Cheng et al. (2019), this gene is known to be associated with neural plaque formation and was significantly higher when compared to the controls ($p = <.001$). The CNSK1A1 gene was found in 13 of the ER patients and all of the outpatient concussion patients. With these results, this study has shown that significant EVs could be detected in minimally invasive saliva samples for an objective diagnosis of mTBIs (Cheng et al., 2019).

Another study by Olczak et al. (2019) looked at urine, saliva, and vitreous body fluids in post-mortem individuals in order to analyze the biomarkers that could be present after death. Specifically, the microtubule associated protein tau (MAPT) was analyzed. This is a coding gene

for making the tau protein and is highly expressed in the axonal aspect of the CNS. Because it has been identified as being in large association with axons, axonal damage will show increased levels of MAPT, possibly leading to an ability to diagnose and differentiate TBIs (Olczak et al., 2019).

The participants included 14 individuals that died of a severe head injury and 13 controls that died of other sudden death causes, such as cardiopulmonary failure. The mean age of the deceased individuals was 48 years old in the severe head injury group and 52 years old in the control group. The fluids tested were collected from each corpse during their routine autopsy and within 24 hours after death. Olczak et al. (2019) explain that specific cases that were excluded from this study were individuals that received any rescue measure or therapeutic procedures, those with head injuries that had healing features, ocular damage, or corneal donation. After the participants passed away, the bodies were kept in a cold temperature until they were able to be autopsied (Olczak et al., 2019).

In addition to the body fluids that were collected using aseptic techniques, tissue samples of the frontal lobe and kidney were also extracted (Olczak et al., 2019). The body fluids were tested for MAPT using an ELISA technique. The brain tissue specimen was stained using a hematoxylin and eosin, as well as a trichrome stain, and the kidney tissue was stained using an anti-MAPT antibody. Olczak et al. (2019) found that the results of the body fluids showed that the study group had a significantly higher level of MAPT in both urine and saliva compared to the control group ($p = <.001$). The study group averaged a urine MAPT of 5.86 and saliva MAPT of 8.76, whereas the control group averaged a urine MAPT of 0.9 and saliva MAPT of 1.71. Olczak et al. (2019) found there was no significant difference in vitreous fluid between the study and control group. The immunohistochemical and histological staining of the brain and

kidney tissues were viewed under the microscope. The study group's tissues revealed ruptures of several blood vessels and hemorrhages compared to the control group. The severity of these damages varied depending on how bad the head trauma was that the deceased had endured. No neurogenerative changes were found in either the study or control group. Lastly, there weren't any differences found in the kidney specimens between the two groups (Olczak et al., 2019).

Essentially, when blood-brain barrier damage occurs, the MAPT concentration is leaked out from the axons in the CNS, causing it to be detectable upon concerns of a concussion (Olczak et al., 2019). MAPT levels could also be considered as a non-invasive diagnostic tool from body fluids such as saliva and urine for mTBI diagnosis.

Discussion

As a result of this literature review, overall, the data revealed that although there is more research in blood biomarkers than saliva biomarkers, the evidence is still lacking with both in relation to mTBIs. With the blood tests being newly FDA approved and saliva biomarker testing also being fairly new as a possible diagnostic choice, the literature selection was fairly minimal. Because concussions are typically not visualized on routine imaging, the current FDA approved GFAP and UCHL1 blood test protocol is used as a screening tool to aid in the decision of ordering a CT scan (Evan & Whitlow, 2021). Although this protocol has high sensitivity and specificity, routine clinical practices do not utilize it often due to the fact that the result of the CT scan won't entirely change the course of action for the patient's treatment plan anyway. If a CT scan is ruled out, the current concussion protocol guidelines consist of subjective symptoms, a physical exam, and standardized exams; however, the standardized exams are only useful if there is a pre-injury assessment available to compare to their baseline (Evan & Whitlow, 2021). Furthermore, they do not rule out a mTBI.

The hope for the future is to have some sort of point of injury (POI) test that can be used rapidly in immediate situations, but according to Voelker (2018) and the research in this literature review, the science is just not quite there yet. This is due to the complexity of the fluid biomarkers and the difficulty in recreating the physical components within studies. Humans and other species contain different brains, making it difficult to replicate a human head trauma incident; ethical and economic issues are also of high concern (Agoston & Kamnaksh, 2015). With that, it is challenging to obtain accurate and realistic research, and this is perhaps why the studies are very limited. Additionally, PCS involves chronic neurological symptoms, which makes it difficult to differentiate between acute concussion symptoms and PCS symptoms in a patient.

Cohort studies were performed within military research by using soldiers as test subjects over time. Throughout the studies, they completed assessments and wore devices to measure their blast exposures. Boutté et al. (2019) discovered there were significant increases in the blood biomarker NFL; however, GFAP levels were found to be decreased and UCHL1 levels were only significant when they were correlated to the individual's concussion history. This decrease in GFAP is physiologically unknown and requires further evaluation as it opposes the expected result for the FDA approved test. The UCHL1 results align with the study by LaRocca et al. (2019); UCHL1 was the only biomarker in this study to correlate quantitively with the number of hits to the head in the MMA fighters but did not show significance in pre- and post-fight differences.

In contrast to the study by Boutté et al. (2019), GFAP levels were found to be elevated in individuals who showed a positive CT scan (Cevik et al., 2019). Furthermore, GFAP levels were also higher at baseline in football players who reported already experiencing concussion related

symptoms before the study by Meier et al. (2020) even began. Its concentration in the blood also raises slowly over time and stays elevated for days. The blood biomarker S100B on the other hand, was found to have a sharp increase in concentration just minutes after an injury, as well as in individuals who were CT positive (Cevik et al., 2019 & Dadas et al., 2018). The most likely reason for the rapid appearance of S100B is due to its smaller molecular weight in comparison to the others (Dadas et al., 2018). The different modes of transportation and molecular weights of each biomarker is where the complexity of this research comes into play.

Both GFAP and UCHL1 show to be a great biomarker for individuals who have a significant history of concussions or head trauma incidents recurrently, therefore being able to identify those who are at greatest risk for prolonged or chronic neurological symptoms. Due to the smaller molecular weight of S100B, continued research on this biomarker could prove it to be a diagnostic tool for a quick, point of injury test.

The biomarker Tau did not show significant concentration increases in footballs players; however, it was increased in military personnel, especially in individuals who were also diagnosed with PTSD. PTSD is another aspect of the research that can make things challenging, especially in soldiers. This reveals that Tau may have a correlation to blast injuries specifically, polytrauma in the military, or PTSD. Further studies will need to be performed. NFL was another biomarker studied extensively throughout this research; overall, the increased levels were found to be sensitive and specific in relation to mTBIs. Based on the study by Shahim et al. (2017), the levels may even be correlated with the ability to predict if an individual will advance to PCS.

Although there was limited research on salivary biomarkers, the data retrieved showed that serum proteins respond in a much more delayed response, whereas the saliva miRNA biomarkers respond more acutely. They can pick up level changes closer to the head injury. This

is because protein fractions in saliva are much smaller and more selective in the salivary barrier. The 14 small non-coding RNAs that were identified through the UK Rugby team research were highly accurate in both sensitivity and specificity (Yakoub et al., 2018), and showed differentiation both immediately after the head injury as well as 36 – 48 hours after.

Currently, there are a limited number of studies that include females as well as other contact sports. These limitations could alter the results. Most of the military personnel that participated in the studies also had PTSD as well as other brain injuries from combat. This is hard to distinguish between military polytrauma and mTBIs. Additional studies will need to be completed in the future in order to properly assess these biomarkers.

Applicability to Clinical Practice

Head trauma can occur from direct blows to the head or even indirect contact through abrupt acceleration-deceleration movements. There are many ways these commonly occur including sports injuries, MVAs, assault, falls, and blast injuries in combat, all of which a primary care provider will encounter. The challenging part about head injuries are mild TBIs, specifically the ones that provide no clinical evidence and only contain microscopic damage that cannot be seen on routine imaging. Most patients with concussions commonly do not recognize it and are not treated; this can lead to chronic neurological outcomes. Fluid biomarkers at the time of injury, such as blood and saliva, are diagnostic tools that could guide post-injury care of a patient in hopes that either a CT scan can be ordered if necessary or the return to activity timeframe will be extended. With having the ability to diagnostically provide care for an individual, medical providers can treat objectively and not have to base their care strictly from a history and physical. This will especially be of use in athletes and military personnel who may withhold serious symptoms or underreport the extent of the injury in order to avoid healing time.

The purpose of this literature review was to provide information on newer blood and saliva biomarkers in the diagnosis of a mild TBI in hopes that providers will have more knowledge and insight with this diagnostic approach.

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