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The use of blood biomarkers in the detection and management of traumatic brain injury

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Thesis

THE USE OF BLOOD BIOMARKERS IN THE DETECTION AND MANAGEMENT OF TRAUMATIC BRAIN INJURY

by

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ABSTRACT

This thesis explores the evolving landscape of blood and serum biomarkers in the detection and management of traumatic brain injury (TBI) and highlights the potential for these biomarkers to revolutionize current practices. TBI, a leading cause of morbidity and mortality globally, presents significant challenges in diagnosis and management owing to its complex pathophysiology and limitations in current imaging techniques. Recent advances in biomarker research have opened new avenues for improving TBI diagnostics and prognostics with the hope of leading to better therapeutic strategies. This review focuses on the diagnostic accuracy, prognostic value, and potential utility in guiding treatment decisions for five primary biomarkers: S100 Calcium-Binding Protein B (S100B), Glial fibrillary acidic protein (GFAP), ubiquitin C-terminal hydrolase L1 (UCH-L1), neurofilament light chain (NF-L), and Tau.

S100B, a glial protein, has been extensively studied for its role in the acute phase of TBI, offering valuable insights into injury severity and patient outcomes. GFAP and UCH-L1 similarly provide information on glial and neuronal damage, respectively, further enriching the biomarker toolkit for TBI assessment. Neurofilament proteins (NF) and Tau, both associated with axonal injury, have emerged as promising indicators of

neurodegeneration, offering prognostic information and a window into the chronic effects of TBI. Looking ahead, inflammatory markers, extracellular vesicles (EV), and microRNAs are identified as promising candidates for TBI biomarker research. These novel biomarkers hold the potential for even more precise diagnosis, understanding the complex biological responses to TBI, and tailoring individualized management plans.

While many of these biomarkers hold promise, GFAP stands above them. With well-validated diagnostic capabilities and defined cutoff values for TBI patients, it currently has the most potential for clinical use and has been approved for use by the U.S. Food and Drug Administration (FDA) in a biomarker panel alongside UCH-L1 to screen patients who may require a head computerized tomography (CT) scan based on biomarker levels. S100B has also been suggested for use as diagnostic criteria to determine a patient's eligibility for head CT through the establishment and validation of the Scandinavian Guidelines. Further experimentation is necessary to fully validate NF and Tau for diagnostic capabilities, but a lack of clinically relevant cutoff values holds most other biomarkers back in their usefulness for diagnostics. While there does seem to be a significant place for most biomarkers in outcome monitoring and prediction, blood biomarker sampling currently only has enough evidence to slightly impact treatment decisions. However, although they have not been widely implemented, the use of blood biomarkers as a criterion for head CT scans has already lowered the number of unnecessary head CT scans performed and will continue to improve with further research.

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LIST OF ABBREVIATIONS

AD .... Alzheimer's Disease

APP .... Acute Phase Proteins

BBB .... Blood-Brain Barrier

CDC .... Centers for Disease Control and Prevention

CNS .... Central Nervous System

CRP .... C-Reactive Protein

CSF .... Cerebrospinal Fluid

CT .... Computerized Tomography

CTE .... Chronic Traumatic Encephalopathy

DAI .... Diffuse Axonal Injury

DTI .... Diffusion Tensor Imaging

ED .... Emergency Department

EV .... Extracellular Vesicle

FDA .... U.S. Food and Drug Administration

GCS .... Glasgow Coma Scale

GFAP .... Glial Fibrillary Acidic Protein

GOS .... Glasgow Outcome Scale

GOSE .... Glasgow Outcome Scale Extended

HIC.. High-Income Countries

LMIC.. Lower Middle-Income Countries

miRNA.. Micro Ribonucleic Acid

MRI .... Magnetic Resonance Imaging

mTBI .... Mild Traumatic Brain Injury

MV .... Microvesicle

NF .... Neurofilament Protein

NF-H .... Neurofilament Heavy Chain

NF-L.. Neurofilament Light Chain

NF-M.. Neurofilament Medium Chain

NFT... Neurofibrillary Tangles

NIH .... National Institutes of Health

PCS .... Post Concussion Syndrome

PNS .... Peripheral Nervous System

p-tau .... Hyperphosphorylated Tau

PTA.. Post-Traumatic Amnesia

PTSD.. Post-Traumatic Stress Disorder

S100B.. S100 Calcium-Binding Protein B

TBI .... Traumatic Brain Injury

t-tau ....Total Tau

INTRODUCTION

Definition of Traumatic Brain Injury, Epidemiology, and Prevalence

TBI is defined as a disruption of brain function as a result of damage and pathology caused by an external physical force and is linked to severe alterations of mental health and neurodegenerative disorders such as depression, post-traumatic stress disorder (PTSD), and chronic traumatic encephaloapathy (CTE) (Khellaf et al., 2019; Taylor et al., 2017). TBI may cause neuronal cell death and lead to cellular and molecular molecular effects in the brain that result in sensory, motor, and behavioral issues (Ackermans et al., 2021). Understanding the epidemiology of TBI highlights the importance of developing effective acute management protocols and optimizing long-term care. In 2006, the Centers for Disease Control and Prevention (CDC) estimated that there were about 1.7 million TBI-related ED visits, hospitalizations, and deaths in the United States and a 2013 study estimated that there were 2.8 million TBI-related visits to the emergency department (ED) in the United States alone (Faul et al., 2010). This is a drastic increase in a short time. With the ever-rising popularity of contact sports such as American football and other contact sports in addition to the increasing use of explosive devices in modern conflicts, the prevalence of TBI will continue to rise (Pavlovic et al., 2019; Taylor et al., 2017). Recent data from the CDC shows that, during 2018 and 2019, there were approximately 120,000 TBI-related deaths in the United States (Centers for Disease Control and Prevention, 2022). Including direct and indirect medical costs, the lifetime economic cost of moderate to severe TBI was estimated to be approximately $76.5 billion in 2010 and is estimated to cost the world economy up to $400 billion annually (Finkelstein et al., 2009; Maas et al., 2022). With the potential for disruption of relationships and work productivity, the chronic effects of TBI may significantly quality of life and lifetime earnings (Rosenfeld et al., 2012).

Global modeling suggests that the incidence of TBI is significantly higher in Lower Middle-Income Countries (LMIC) than in High-Income Countries (HIC), with the main factor being driving incidents (Dewan et al., 2018; GBD 2016 Traumatic Brain Injury and Spinal Cord Injury Collaborators, 2019). The Global Neurotrauma Outcomes Study (Clark et al., 2020) confirmed this conclusion and reported that there is a clear relationship between the mechanism of injury and the United Nations Human Development Index, whereas the CENTER-TBI registry reports that the majority of TBIs in HICs are caused by falls (Clark et al., 2022; Lecky et al., 2021). As expected, the frequency of hospital admission for TBI is highest in elderly patients (≥ 65 years) followed by children and adolescents (≤ 19 years) (Majdan et al., 2016). Motor vehicle accidents are noted as a leading cause of TBI-related hospitalizations in young adults while falls account for the highest cause of TBI in both children and elderly patients (Faul et al., 2010). Children and adolescents with previous TBI have a four-fold increased risk of recurrent injury and increased risk of mental health issues compared to those with an orthopedic injury (Ledoux et al., 2022; van Ierssel et al., 2021).

A vast majority, around 90%, of patients presenting to the hospital with TBI fall into the mild TBI (mTBI) category and more than a third of patients admitted to the intensive care unit for TBI have mTBI (Maas et al., 2022). mTBI is a challenging diagnosis as some of the hallmark neurological symptoms of TBI may be absent, such as loss of consciousness (LOC) or post-traumatic amnesia (PTA), but it is generally defined as a head injury with LOC <30 minutes and PTA <24 hours (Posti & Tenovuo, 2022). About 55% of ICU cases with mTBI are admitted for additional extracranial injuries, indicating the co-occurrence of TBI with other traumatic injuries which further complicates the clinical picture (Huijben et al., 2020). This prevalence of mTBI is particularly concerning given that all severities of TBI have been implicated in the development of neurodegenerative diseases, sleep disturbances, neuroendocrine dysregulation, and behavioral disorders (Bramlett & Dietrich, 2015). Therefore, there is a critical need to adequately manage and provide ongoing treatment for TBI from the outset to mitigate the risk of long-term complications and improve overall outcomes for affected individuals.

Consequences and Heterogeneity of TBI

A consequence of TBI that has gained significant notoriety in the past decade is CTE which is important when considering the negative impact of TBI. Many concussion symptoms may resolve within days to weeks post-injury, but repeated mTBI has the potential to cause symptoms to become chronic. This, in turn, may develop into CTE which can manifest in a multitude of physical and mental effects. CTE is characterized as a progressive neurodegeneration with widespread deposition of hyperphosphorylated tau (p-tau) as neurofibrillary tangles (NFT) and is differentiated from other tauopathies like Alzheimer's Disease (AD) by the general atrophy of the cerebral cortex, medial temporal lobe, diencephalon, and mammillary bodies with enlarged ventricles (McKee et al., 2013). Headaches, loss of concentration, depression, and explosive behavior may be effects of stages I and II while executive dysfunction, cognitive impairment, and dementia characterize more severe and later stages III and IV (Singla et al., 2019). Considering CTE may only be diagnosed at autopsy, it is important to effectively manage even mTBI as repeated trauma may lead to potentially devastating effects in the future (Iverson, Gardner, et al., 2019). Increases in the development of other neurodegenerative disorders have also been noted in association with a history of TBI, although research suggests that not everyone with a TBI is at a heightened risk. AD is regarded as the leading cause of dementia in older adults and is of specific interest in studies investigating the link between TBI and age-related neurodegenerative diseases (P. Lei et al., 2021). While conflicting evidence does exist, there is mounting evidence supporting the idea that even a single instance of TBI can accelerate neurodegenerative diseases like AD and Parkinson's disease (Mckee & Daneshvar, 2015).

Increased neurobehavioral sequelae have also become a topic of research and concern for all severities of TBI. Following a moderate to severe TBI, it has been found that there is a significant increase in the prevalence of affective disorders, such as anxiety or depressive disorders, in the first year post-injury with a decrease in the following 5 years (Alway et al., 2016). In mTBI patients at level 1 trauma centers, it was found that there was a significantly higher prevalence of psychological distress than in orthopedic controls at 1 year post-injury (McMahon et al., 2014). Additionally, it was found that American servicemembers returning from Iraq who had suffered TBI had significantly higher rates of PTSD than those without a TBI, and those who had experienced an mTBI in combat had a higher rate of PTSD than those without a combat-related mTBI (Hoge et al., 2008; Schneiderman et al., 2008). While these statistics demonstrate the widespread impact that acute and chronic TBI has on ED and hospital resources, they underline the importance of having robust systems of detection and treatment to mitigate this dangerous condition safely and effectively.

TBI is a highly heterogenous disease (**Figure 1**). It may result from a light injury such as rapid acceleration or deceleration of the head, exposure to a blast, or something more severe such as blunt force trauma or a penetrating injury to the skull (Ghaith et al., 2022). While all result in TBI, mechanistic differences may result in TBI with distinct characteristics. For example, impact-related TBI may result from intertial, rotational, and shear direct damage to the brain tissue. However, in blast-related TBI, the injury results from the interaction between the pressure wave and brain tissue with the cerebral vasculature particularly implicated in injury (Elder et al., 2019). Symptoms of mTBI may include dizziness, headache, confusion, blurred vision, concentration issues, and loss of consciousness. Moderate or severe TBI may present with the same symptoms but can also include nausea and vomiting, seizures, dilation of one or both pupils, slurred speech, weakness or numbness in the extremities, loss of coordination, and in some cases death (Capizzi et al., 2020). Although symptoms of TBI are typically seen directly after the insult, symptoms like neuropsychiatric sequelae may be delayed in manifestation (Menon et al., 2010).

The result of trauma may include injury to axons or blood vessels through direct, rotational, or sheer forces which have a variety of consequences (Posti & Tenovuo, 2022). Focal injuries are insults to the axons in a directed manner, generally due to direct or indirect contact caused by a coup-countercoup mechanism of injury where the brain contusion is caused by the contact with both sides of the skull as it moves in the cerebrospinal fluid (CSF) enveloping it (Capizzi et al., 2020). Diffuse axonal injury (DAI) occurs as a result of a rotational injury and may lead to axonal damage and disconnection (Siedler et al., 2014). This axonal injury leads to local swelling and can slow the transmission of surrounding axons. Ischemia due to damaged blood vessels, compression due to mass lesions, and apoptosis are other consequences of TBI (Capizzi et al., 2020; Khellaf et al., 2019).

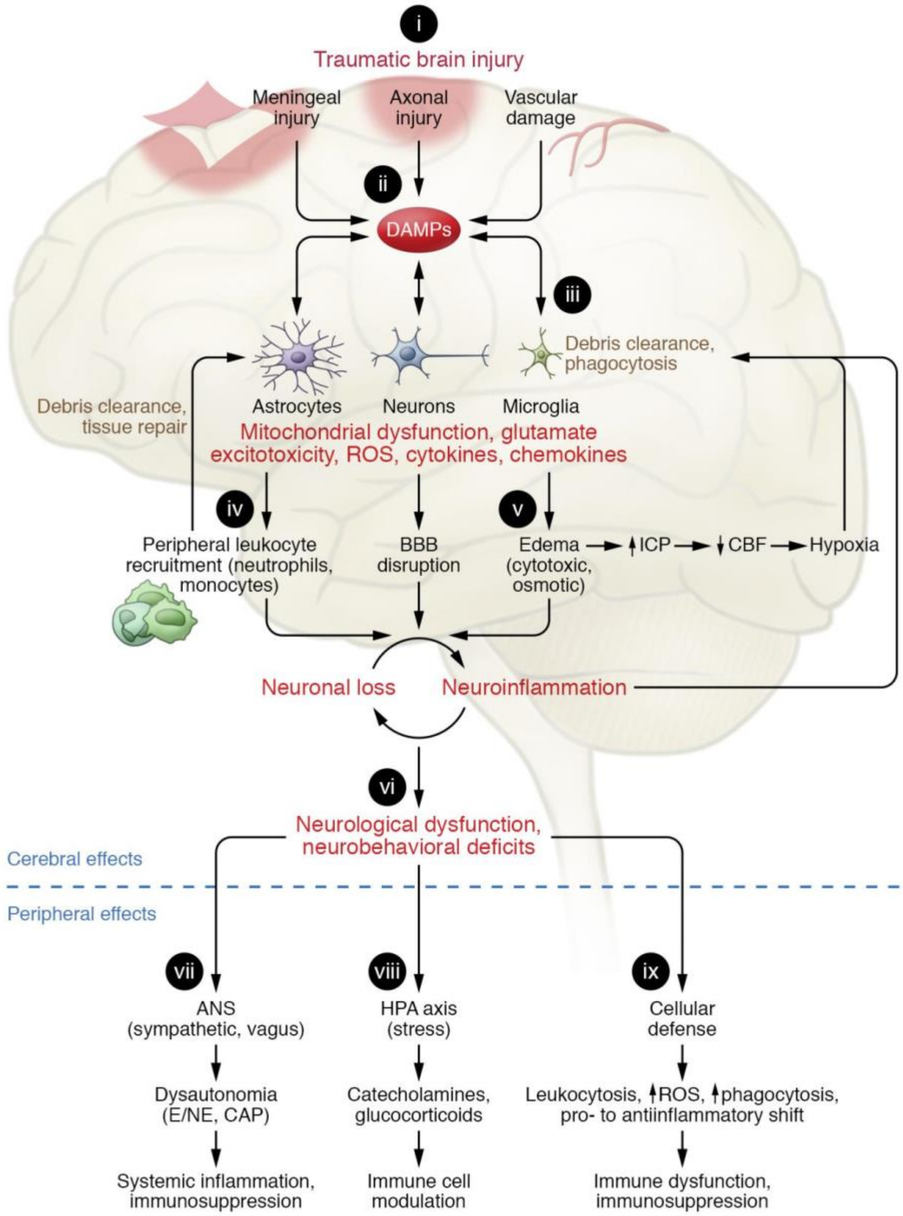

Figure 1. TBI Heterogeneity. Physical trauma to the head can cause a variety of injuries such as meningeal and axonal injury or vascular damage. Cell types implicated in these injuries are astrocytes, neurons, and microglia that may be injured or activated to result in different pathologies. The result is neuronal loss and neuroinflammation which may exacerbate the pathologies and lead to neurological dysfunction seen in both behavioral and physiological changes. Figure taken from (Hanscom et al., 2021).

These different mechanisms can be separated into two categories, primary and secondary injury. Simply, primary injury can be defined as the direct mechanical damage resulting from the insult and secondary injury can be defined as the molecular mechanisms that act post-injury (Hanscom et al., 2021). The focal and diffuse injury mechanisms are both primary as they happen at the time of injury and are examples of direct contusion. Primary injuries also involve direct vascular impacts such as subdural hematoma which can injure axons via mass effects due to the accumulating blood (Cernak, 2005). Secondary injury may begin minutes to hours after primary injury and can continue for days, months, or years, contributing to neuroinflammation, neurodegeneration, and neurological deficits (Hanscom et al., 2021). Oxidative stress from reactive oxygen and nitrogen species, prolonged inflammatory processes that result in neurotoxicity, and abnormal neurological excitation that results in post-traumatic epilepsy are major contributors to secondary injury associated with TBI (Bramlett & Dietrich, 2015). This wide variety of possible injury mechanisms underscores the heterogeneity of TBI and the importance of early management and detection. Indeed, early medical stabilization, acquisition and repetition of a Glasgow Coma Scale (GCS) score, imaging, and procedures like decompressive craniectomy minimize TBI-associated risks (Capizzi et al., 2020; Zweckberger et al., 2006).

Table 1. Primary versus secondary traumatic brain injury. Summary of differences found between primary and secondary TBI. Adapted from (Capizzi et al., 2020).

Primary Injury	**Secondary Injury**
• Direct hit can produce indirect damage thorugh acceleration-deceleration (coup contrecoup) mechanism • Penetrating (open) vs nonpenetrating (closed) • Subdural hematoma and mass effects	• Damage at the cellular/molecular level • Ischemia may cause cell death • Neuroinflammation and degeneration • Oxidative stress • Abnormal excitation and post-traumatic epilepsy

TBI Detection, Classification, and Treatment

CT and magnetic resonance imaging (MRI) scans are considered to be the gold standard of TBI detection and diagnosis. CT is the predominant imaging method in emergency settings and is used to determine the need for surgical interventions in cases of subdural or epidural hematomas due to its ability to efficiently identify acute intracranial hemorrhages (Smith et al., 2019). However, despite its widespread use, CT has limitations, particularly in cases of mTBI. Guidelines have been established to determine the necessity of head CT scans for patients with mTBI, but they tend to be inefficient as 90-95% of CT scans performed show no intracranial injury (Maas et al., 2022). This suggests that for lower-grade TBI, such as mTBI which has more subtle pathology not detected on CT, more sensitive methods such as MRI may be highly beneficial (Pavlovic et al., 2019).

Advanced MRI techniques, including susceptibility-weighted imaging, are effective in visualizing microhemorrhages caused by DAI that are not seen by CT.

Additionally, perfusion computerized tomography and dynamic susceptibility-weighted contrast-enhanced perfusion MRI are valuable tools in detecting areas of hypoperfusion, an outcome of mTBI that may lead to secondary ischemic injury and subsequent clinical deterioration (Smith et al., 2019). Despite the advantages offered by advanced imaging techniques, their implementation faces practical challenges. These services are expensive, require specialized equipment and expertise, and may not be readily available in all healthcare settings. Furthermore, their utilization may strain hospital resources, particularly in environments with limited access to imaging facilities. Therefore, while advanced imaging holds significant potential for improving TBI diagnosis and management, its benefit must be weighed against practical considerations such as cost, accessibility, and resource utilization.

The level and severity of TBI can also be classified without imaging. A widely accepted and quantitative tool is the GCS (**Table 2**), which assesses the patient's level of consciousness following TBI. This scale is based on three criteria of eye-opening, verbal, and motor response where the three scores are added and total between 3 and 15. Scores of 13-15 signify mTBI, 9-12 signify moderate TBI, and 3-8 signify severe TBI (Mehta et al., 2019). Importantly, the motor score is the only portion of GCS that has prognostic value (Capizzi et al., 2020). This score is important for a variety of reasons, mainly that it can be used outside of a hospital to decide which patients need to be transported to trauma centers, and may be used as an indicator of severe head injury. Being a quantifiable measure of TBI, it can be rapidly and repeatedly used and interpreted in emergencies and as monitoring for patients who have suffered TBI. A rapid decrease in

GCS may be indicative of a need for imaging, such as CT and MRI. It is limited, however, by the subjectivity of the administrator and other procedures or treatments administered to the patient in the hospital. The same patient may be scored differently by two different administrators due to the flexibility of the criteria, hence the importance of this test as a preliminary measure to determine if further assessments are needed (Mehta et al., 2019). Additionally, the use of prehospital sedation and tracheal intubation confounds the ability to assess a valid GCS (Maas et al., 2017).

Table 2. Glasgow coma scale. TBI patients are given a score for eye opening, verbal, and motor abilities. This is added together to give patients a GCS score. Taken from (Capizzi et al., 2020).

SCORE	EYE OPENING	VERBAL	MOTOR
1	None	None	None
2	To pain	Incomprehensible speech	Extension (decerebrate posturing) to pain
3	To speech	Inappropriate speech	Flexor (decorticate posturing) to pain
4	Spontaneous	Confused	Withdraws to pain
5	-	Oriented	Localizing response
6	-	-	Follows directions

Another measure of TBI severity is the assessment of PTA, which is defined as the period in which an individual is unable to form new memories following TBI, and greater PTA is generally a reliable indicator of TBI severity (Parker et al., 2022). Duration of loss of consciousness and posttraumatic confusion and disorientation are two additional measures used to measure the severity of TBI and when used in conjunction with PTA and GCS, these parameters provide a thorough assessment of TBI severity

(Carroll et al., 2004). These parameters of TBI classification can be found summarized in **Table 3**.

Table 3. Classification of traumatic brain injuries. TBI is classified into mild, moderate, or severe based on the absence, presence, duration, or level of GCS, loss of consciousness, alteration of consciousness, imaging findings, and PTA. Taken from (Capizzi et al., 2020).

	GCS (first 24 hours)	**Loss of Consciousness**	**Alteration of Consciousness**	**Imaging**	**PTA**
Mild	13-15	0-30 min	Up to 24 hours	Normal	0-1 days
Moderate	9-12	>30 min and <24 hours	>24 hours	Normal or abnormal	>1 day and <7 days
Severe	3-8	>24 hours	>24 hours	Normal or abnormal	>7 days

The Glasgow Outcome Scale (GOS) and Glasgow Outcome Scale Extended (GOSE) are further quantifiable measures of TBI used to evaluate severity, functional outcome, and disability; they measure the quality of survival and life post-injury rather than only focusing on whether the patient survived. Both GOS and GOSE are typically administered 3-12 months post-injury, but GOS has been determined to be superior. GOS assesses outcomes across the categories of death, persistent vegetative state, severe disability, moderate disability, and good recovery (McMillan et al., 2016).

Research in the past few decades has provided a deeper understanding and better detection of TBI, and great strides have been made in the prevention of TBI. Cars are safer and helmets are constantly improving for contact sports and combat (Khellaf et al., 2019). However, for most notably mTBI, there is a lack of objective tools to measure those who are at increased risk of developing further symptoms or conditions like Post

Concussion Syndrome (PCS) or CTE. The management of TBI is based entirely on the presentation of clinical symptoms with a lack of quantifiable measures (Kamins et al., 2017). An area that has been growing in recognition for its potential role in the detection and treatment of TBI is blood biomarkers. Biomarkers hold significant promise for revolutionizing TBI diagnosis and treatment by offering new prognostic indicators, and target identification for therapeutic intervention. The FDA has approved one blood test that assays biomarkers to determine whether patients with suspected mTBI qualify for a CT scan, and more private companies will continue to seek FDA approval for different tests (Papa et al., 2016). This field considers a diverse array of proteins, enzymes, cytokines, EVs, and microRNAs whose levels in the bloodstream can reflect various aspects of TBI pathology such as neuroinflammation, blood-brain barrier (BBB) disruption, axonal injury, and neuronal cell death (Ghaith et al., 2022).

Blood-based assays of TBI benefit the field in a multitude of ways. Collection of blood samples is a fairly non-invasive and easily trained procedure that is easily performed with a smaller pool of resources. A blood test would circumvent the need for more invasive procedures, like lumbar puncture, or technologically-intensive imaging procedures that may not be available in all clinical settings (Shahim et al., 2020). Greater accessibility allows for rapid information on the condition and can aid in early diagnosis of TBI allowing clinicians to address the injury earlier, and also may inform the decision to transfer a patient to a facility with the required advanced care depending on the results of the assay.

Blood biomarker assays also have the advantage of being highly specific to the molecule they are measuring and may provide a greater understanding of the heterogeneity of TBI. Using the unique molecular profile of a TBI in conjunction with other assessment methods may help determine an individualized approach to treatment. In this review, blood biomarkers identified in the literature until 2023 as promising targets will be assessed for their clinical viability based on ease of detection, sensitivity, and specificity for detecting and managing TBI.

SPECIFIC AIMS

1. Characterize the most robust blood biomarkers induced by traumatic brain injury.
2. Investigate the usefulness of these blood biomarkers in differentiating between severities of traumatic brain injury and patients with and without traumatic findings on commonly used imaging platforms.
3. Determine the ability of blood biomarkers to predict the functional outcomes of traumatic brain injury patients.

BIOMARKERS INDICATED AS CANDIDATES FOR BLOOD BIOMARKER ASSAYS IN THE CONTEXT OF TBI

Definition of Biomarkers

Biomarkers are defined by the National Institutes of Health (NIH) as a measurable indicator of the severity or presence of some disease state and can be used as an indicator of the physiological state of an organism. These can be, for example, the amount of a certain circulating protein in the blood of an organism. Biomarkers are distinct from measures of how a person feels, functions, or survives, measures known as a clinical outcome assessment, and can be used to link an objective measure of a substance to a likely clinical outcome measure (Califf, 2018). In 2016, the FDA-NIH Joint Leadership Council compiled definitive guidelines for researchers to use when considering what a biomarker is. The BEST (Biomarkers, EndpointS, and other Tools) Resource lists seven types of biomarkers: diagnostic, monitoring, response, predictive, prognostic, safety, and susceptibility/risk. The most important biomarkers in the context of TBI would be diagnostic, monitoring, and prognostic. A diagnostic biomarker would confirm the presence of TBI, a monitoring biomarker is repeatedly measured to assess the status or progression of TBI, and a prognostic biomarker is used to identify the likelihood of symptom development and progression in those suffering from a TBI (FDA-NIH Biomarker Working Group, 2016).

Of all possible definitions for what a biomarker may be, only biochemical biomarkers will be considered in this review. GCS, PTA, and duration of loss of consciousness could also be considered biomarkers and are used in clinical practice for assessing TBI severity and guiding treatment. This review selects promising biochemical biomarkers and focuses on the comparative efficacy of diagnosing and treating a TBI against these established measures. To qualify as a clinically useful biomarker, Wang et al. detailed the following characteristics a biomarker should possess: 1) The biomarker should exist in an accessible biofluid at low levels in non-injured populations and elevated levels during the acute phase while allowing for repeated detections within a 48-hour window post-TBI; 2) The biomarker should derive or originate from the injured brain; 3) The biomarker should be able to be quantified by sensitive assays such as sandwich ELISA or similar immunoassays and have one assay platform able to meet reliability and reproducibility standards set by the FDA while being translational in animal models; 4) The biomarker should correlate with severity of TBI as defined by GCS or CT abnormality and have acute levels that correlate with GOS or GOSE. An expanded list can be found in **Table 4** (K. K. Wang et al., 2018). In practice, protein biomarkers are most frequently quantified by sandwich ELISA, but a multiplex ELISA may be employed if a panel of biomarkers is being investigated. Mass spectrometry could also used to detect small molecules in an antibody-independent manner (Ghaith et al., 2022).

Table 4. Ideal serum-based biomarker attributes. Taken from (K. K. Wang et al., 2018).

1	The TBI biomarker levels can be readily measured in accessible biofluid. The biofluids will likely include whole blood, serum and/or plasma for moderate and mild TBI patients with the inclusion of CSF in cases of severe TBI patients.
2	The biomarkers are elevated in native and/or modified various forms which directly correlates to the degree of severity of human TBI in the acute phase (3 h to 24 h post-injury).
3	The biomarker has low background or basal biofluid levels in >95% of general non-injured healthy control population.
4	The biomarker detected in biofluid after TBI is the direct result of brain trauma.
5	The biomarker levels in the above-stated biofluids can be readily determined and quantified using at least two independent assay formats or platforms.
6	There are one or more available assays for such biomarker with test-retest reliability and reproducibility that meet analytical performance requirements acceptable to FDA.
7	The biomarker is translationally relevant relative to the model systems with demonstrated evidence that there are parallel biofluid-based biomarker profile patterns in at least two different animal models of TBI (e.g. rodent control cortical impact, fluid percussion injury, close head injury, penetrating brain injury or blast overpressure-wave brain injury).
8	The TBI protein biomarker should be qualitatively and quantitatively related to severity of TBI as defined by GCS, cranial CT and/or MRI abnormality.
9	The TBI protein biomarker should allow for repeated detections in one of the above mentioned biofluids within a 24–48 h window following brain injury.
10	The TBI protein biomarker should have initial acute levels (within first 24 h post-injury) that correlate with currently available and commonly accepted TBI patient outcome indices (such as GOS-E).
11	The post-TBI biofluid levels of the TBI biomarker is responsive to therapeutic treatments (i.e. Therapeutic treatment following TBI might reduce biomarker loads).

Blood biomarkers have the potential to be incredibly useful in a variety of clinical contexts. One example is in determining the need for acute head imaging such as CT scans. Some TBI patients, particularly those with mTBI, have been shown to not meet the clinical criteria for head CT, even though there is the possibility for significant traumatic intracranial findings during an mTBI, and thus may be discharged without receiving imaging. The opposite is also true. There have been different guidelines created to determine whether head CT is needed in the context of a TBI, however, many times head CT will show no significant findings and the patient will be exposed to unnecessary radiation (Posti et al., 2019). This information lends itself to the necessity of finding one or multiple adequate biomarkers to aid in the decision to perform a head CT and both minimize harm and maximize the benefits of imaging procedures. Another useful clinical context for biomarkers is the promise they hold in providing an objective measure for patients with sports or military-related mTBI to either avoid or return to play or duty. An ideal biomarker for TBI will provide a quantifiable measurement of pathophysiological processes occurring in the brain following injury. It could also elucidate some subtle cellular or molecular changes that may not be evident through clinical examination alone. As one of the most notable risks of mTBI is the damage further TBIs can cause, a biomarker reflective of these characteristics would be able to identify someone with an mTBI quickly and exclude them from a return to play or service due to the possibility of acquiring further injury (McDonald et al., 2021). Biomarkers also may aid in outcome prediction after TBI. While the IMPACT and CRASH models provide methods for predicting outcomes, biomarkers with high reliability and rapid results may be able to enhance the efficacy of these models (Collaborators, 2008; Maas et al., 2007). Clinicians will acquire another tool with which to stratify patients based on risk profile and tailor personalized treatment (Visser et al., 2022).

TYPES OF BLOOD BIOMARKERS IMPLICATED IN TBI DETECTION AND CLASSIFICATION

S100 Calcium-Binding Protein B

S100B is the 11 kDa ß-subunit of a calcium-binding protein, expressed in glial and Schwann cells in the central and peripheral nervous systems (PNS; Mercier et al., 2013). Typically, it is involved in cytoskeleton formation and cellular proliferation. After TBI, astroglial cells are activated and release S100B into the extracellular space where they diffuse into the CSF, and then into the bloodstream. While these proteins help to protect neurons against the inflammatory response following TBI, they also increase the phosphorylation of Tau proteins that contribute to neurodegenerative diseases (Ghaith et al., 2022). It is one of the most widely studied biomarkers concerning TBI. It has notably been approved for use in clinical practice with the establishment of the Scandinavian Guidelines for Initial Management of Minimal, Mild and Moderate Head Injuries in Adults (**Figure 2**; Minkkinen et al., 2019). S100B levels are significantly elevated in those over the age of 65 compared to those younger and vary significantly between different body fluids, specifically, its presence can be around 100 times greater in CSF than in blood due to S100B's diffusion from the CSF to the blood through the arachnoid villi (Calcagnile et al., 2013; E. P. Thelin, Nelson, et al., 2017).

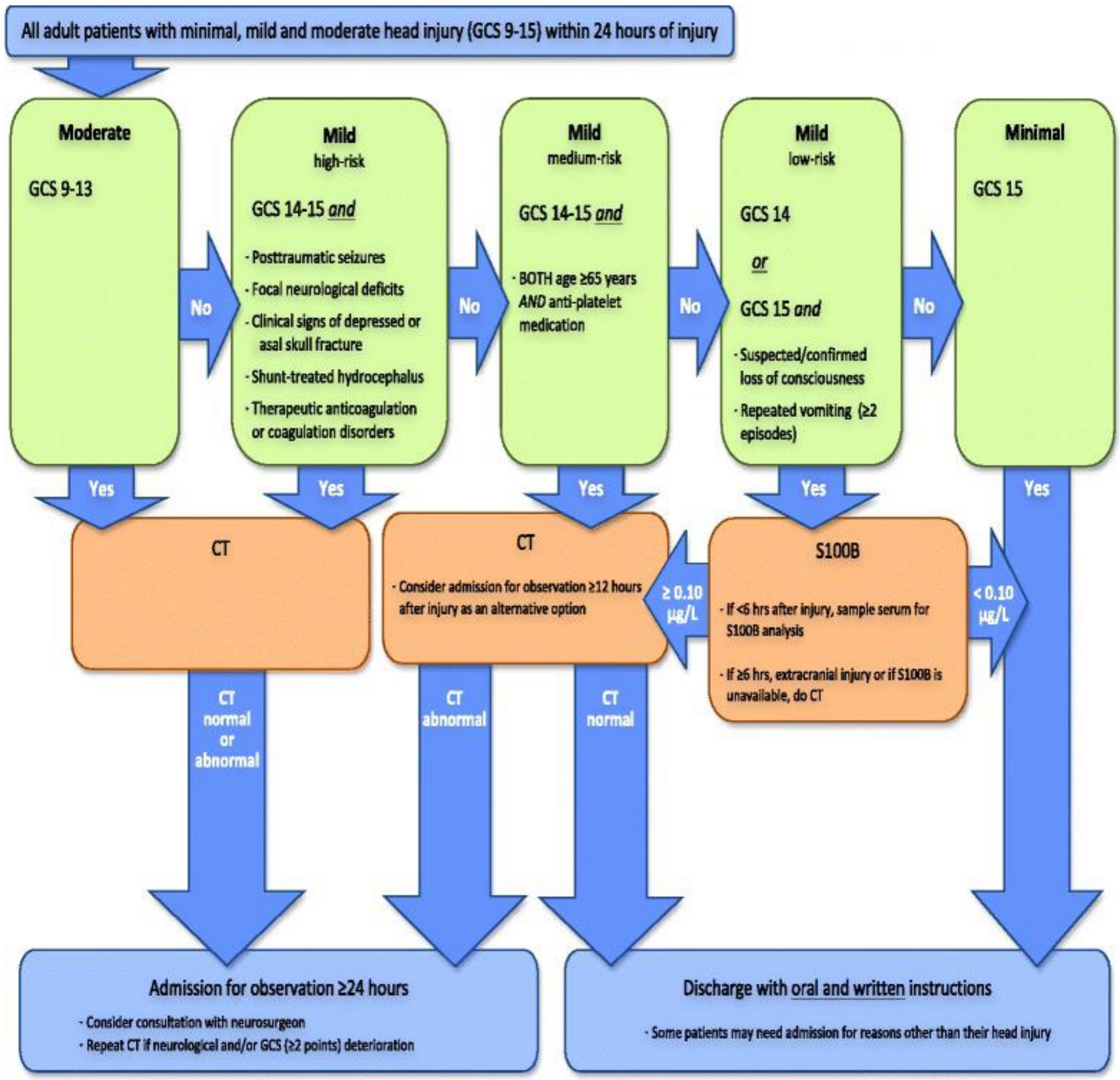

Figure 2. Scandinavian Neurotrauma Committee Guidelines. This flowchart is a graphical representation of the Scandinavian Guidelines for Initial Management of Minimal, Mild and Moderate Head Injuries in Adults. It outlines the decisions that are to be made based on GCS score and other associated symptoms of TBI at presentation, when to perform a head CT scan, and when to use S100B concentration to determine if a head CT scan is necessary. Taken from (L. Undén et al., 2015).

Multiple studies have reported a rapid appearance of S100B in the blood following TBI. S100B is detectable within an hour of injury, peak elevations over a baseline of 0.11 µg/L occur around 27 hours post-TBI, and it has a half-life of 1 to 2 hours (Ercole et al., 2016; Papa et al., 2014; E. P. Thelin, Nelson, et al., 2017). Interestingly, however, multiple studies also report a secondary spike in S100B level and the presence of further secondary injury in patients (Mercier et al., 2013; E. P. Thelin, Nelson, et al., 2017). There is some evidence that suggests an increase in BBB permeability is responsible for S100B elevation in the circulation of those with TBI, however, other research suggests that S100B may enter the circulation as a result of glymphatic drainage (Kanner et al., 2003; Kapural et al., 2002; Plog et al., 2015). This conflicting information suggests that further research is needed to elucidate the exact mechanism of S100B entry into the blood.

In clinical practice, S100B has become an effective tool to determine whether a patient in the ED requires a head CT. It is primarily used in the acute phases of moderate to severe TBI and its potential application in mTBI is an area that requires further investigation. The Scandinavian Guidelines suggest that patients presenting with TBI symptoms and are measured to have less than 0.1 µg/L of S100B in blood may be advised to not receive a head CT (Minkkinen et al., 2019). Studies done after the implementation of these guidelines have corroborated their usefulness but focused on the high negative predictive power of the S100B test. In a study by Minkkinen et al. in 2019, the guidelines-based sensitivity was 0.94 while specificity was 0.19, and the positive predictive value for positive head CT was 0.18 while the negative predictive value was 0.94. They report that 100% adherence to the Scandinavian Guidelines would have decreased the need for head CT in their population by 9% and a result of S100B <0.1

μg/L was able to exclude CT-positive findings in the mTBI group (Minkkinen et al., 2019). In a similar study done by Undén et al. in 2015, similar results were found but for the detection of intracranial hemorrhage using CT. The sensitivity was 0.97 while the specificity was 0.34. Following the Scandinavian guidelines in this context would result in a 32% decrease in head CT administration for patients who otherwise would be directed to receive one (L. Undén et al., 2015). Other studies report similar findings all seeming to centralize on the fact that S100B is an excellent negative predictor of CT-positive TBI and has the potential, with widespread application of the Scandinavian guidelines, to significantly reduce costs in EDs and reduce the amount of unnecessary radiation patients are exposed to (Calcagnile et al., 2016).

Another advantage of using S100B is the association that exists between increasing S100B levels and worsening clinical outcomes. It has been shown that S100B levels can be used as a marker for further development of PCS and CT abnormality in mTBI patients (Pfortmueller et al., 2016; Zongo et al., 2012). Specifically, it was found that serum levels of S100B collected within 12-36 hours following TBI correlate significantly with patient outcomes (K. K. Wang et al., 2018). S100B concentration in serum had a significant negative correlation with GOS score at 6 months post-TBI, and serum levels of >0.7 μg/L in serum correlated with 100% mortality. Additionally, patients presenting to the ED with initial serum levels >0.2 μg/L tended to have worse outcomes than those with <0.2 μg/L (Kellermann et al., 2016). Further research also corroborates the claim that higher concentrations of S100B correlate to worse outcomes (**Figure 3**; Goyal et al., 2013; Rainey et al., 2009). Other research has suggested that

S100B can be used as a reliable indicator for brain death or mortality after severe TBI. Although there seem to be significant variations concerning what the cutoff for serum concentration of S100B that correlates with unfavorable prognosis as defined by GOS is, the range seems to fall somewhere between 0.5 µg/L and 1.2 µg/L with some reported to be as low as 0.372 µg/L (Egea-Guerrero et al., 2013; Zongo et al., 2012). However, the values were determined by the sensitivity and specificity of each data set as determined by different groups of researchers. These data were also not gathered in uniform methods, with some research groups collecting serum based on time after admission while others were based on time after injury (Rainey et al., 2009). These cutoffs were also chosen for different reasons with some representing high negative predictive values while others targeted high positive predictive values, but are acknowledged not to be perfect prognostic indicators. The values proposed by the aforementioned studies begin to show that the administration of head CT imaging may be beneficial at >0.1 µg/L due to the increased chance of revealing CT abnormalities and the significantly increased chance of unfavorable outcomes at increasing values.

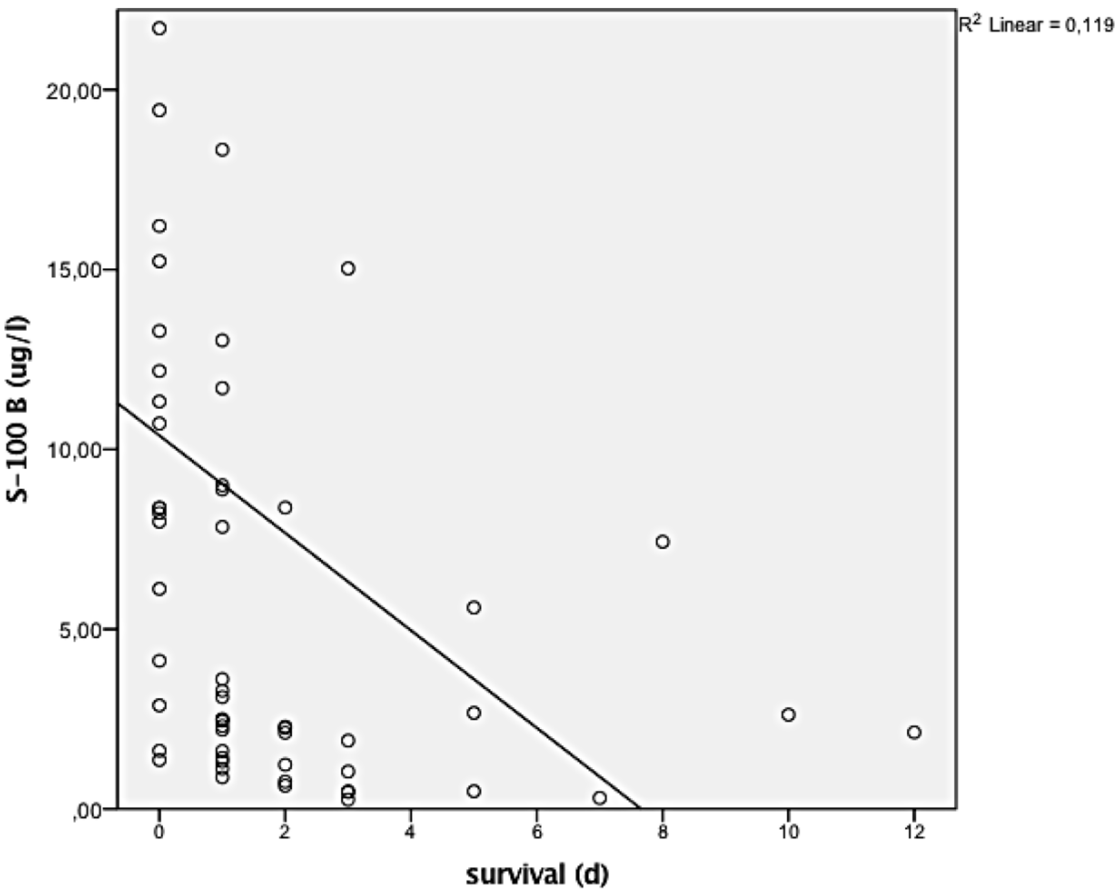

Figure 3. S100B concentration correlates negatively with survival. It was found that S100B serum concentration correlates negatively with days of survival post-TBI ($p < 0.0001$). Taken from (Pfortmueller et al., 2016).

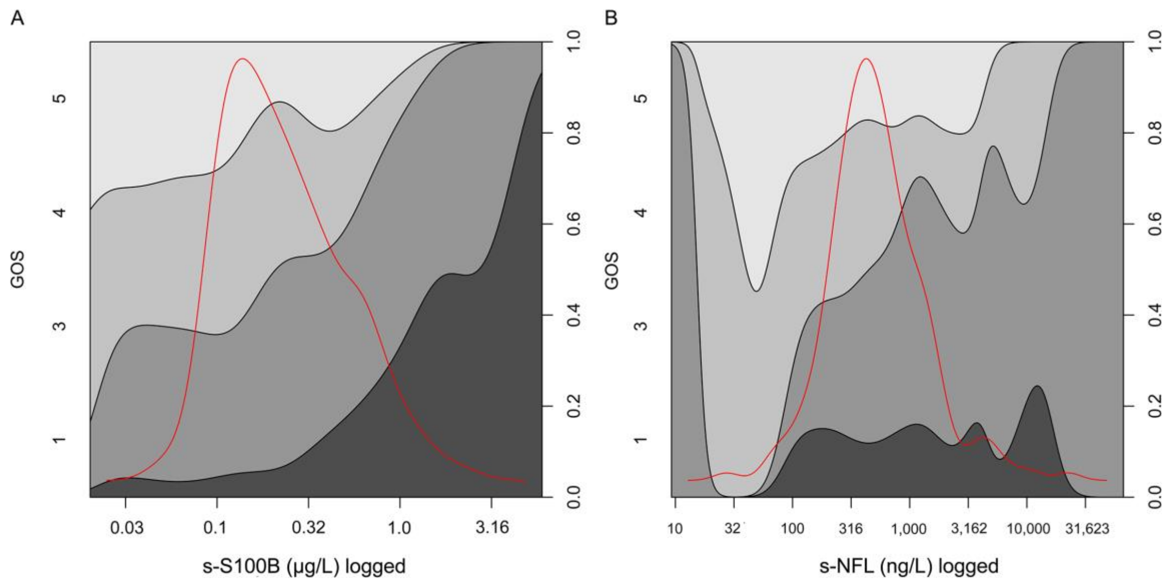

Figure 4. Both Serum S100B and NF-L Correlate to TBI Outcome. Conditional density plot of serum levels of S100B (*A*) and NF-L (*B*) sampled from 187 patients with TBI. Shown is the relationship between increasing S100B and NF-L concentration and GOS. Taken from (Al Nimer et al., 2015).

Considering the predictive power added by S100B serum sampling, it is unsurprising to find it included in blood panels being investigated for TBI detection. Elevation of a combination of S100B and GFAP is similarly implicated in unfavorable outcomes in severe TBI patients and another study concerning a panel of S100B, GFAP, and UCH-L1 in severe TBI found that elevation correlated significantly with injury severity and unfavorable clinical outcome (Lee et al., 2015; Vos et al., 2010).

Perhaps contrary to the evidence presented, S100B is also found by studies to have no relation to 1-month neuropsychological outcomes and performance, as well as being found to have no relation to imaging results (Abbasi et al., 2014; Metting et al., 2012). Additionally, there is evidence to suggest that S100B levels in serum may not accurately reflect their levels in the central nervous system (CNS). The passage of S100B from CSF to serum while there was an established “open” BBB was low. As such, it is difficult to definitively conclude that S100B levels in serum show the entire picture of what is going on and accurately reflect the extent of the injury (Kleindienst et al., 2010).

However, some issues may arise when considering the use of S100B as a biomarker for TBI as there seems to be conflicting evidence on the efficacy of S100B as a useful biomarker. As the field of TBI-related biomarkers is still young, an accumulation of studies that work to verify if guidelines, like the Scandinavian Guidelines, should elucidate this answer. S100B exists outside of the CNS and is significantly elevated in orthopedic injuries not involving the head (Papa et al., 2014). However, it has been shown that in patients with significant extracranial injury, suspected TBI, and elevated S100B levels there is a higher likelihood of a head CT showing some sort of abnormality (L. Undén et al., 2015). This broad distribution complicates the interpretation of elevated S100B levels as they may not be reflective of only CNS pathology. It is also true that while this biomarker may be a useful tool for a physician to decide about the necessity of a head CT, it is not 100% accurate at ruling patients in or out. The aforementioned reasons may partly or all be the cause for the slow adoption of S100B use in EDs worldwide.

Glial Fibrillary Acidic Protein

GFAP is a ~50 kDa monomeric filament protein expressed in the intermediate filaments that make up the cytoskeleton of astrocytes in the white and grey matter of the brain and helps to support the structure of these cells and additionally aids in the activation of glial cells. It is also present in non-myelinating Schwann cells in the PNS, enteric glial cells, chondrocytes, liver, kidney peritubular fibroblasts and glomeruli, and pancreas cells (Ghaith et al., 2022; Posti & Tenovuo, 2022). Following TBI, astroglial cells in injured brain tissue are activated and either are induced to undergo gliosis or aid in glial scar formation. As a result, GFAP expression increases and is released into the bloodstream where the biomarker form is detected either as a fully intact protein or as breakdown products ranging from 18-42 kDa in size (Ghaith et al., 2022; Posti & Tenovuo, 2022; K. K. Wang et al., 2018). With an accumulation of evidence supporting its efficacy and recent inclusion in an FDA-approved biomarker panel for identifying patients that require a head CT, GFAP has emerged as one of the most robust TBI biomarkers for astroglial injury.

GFAP has been determined to be detectable as early as 1 hour post-TBI. Its presence in serum may stay elevated for around 3-34 hours in all severities of TBI (K. K. Wang et al., 2018). The peak was found to be around 20-24 hours post-TBI with a subsequent decline in concentration. However, it tends to remain elevated for 7 days with a proposed half-life of around 24-48 hours (**Figure 5**; E. P. Thelin, Zeiler, et al., 2017; Welch et al., 2017). There is also evidence to suggest that there is a biphasic serum profile, where acute GFAP increases are followed by a decrease around 30 days, only for it to increase again around 90 days post-TBI (Shahim et al., 2020). GFAP has been found to stay persistently elevated for more than 5 years post-TBI, and it is found to continue increasing suggesting that TBI is an injury with continuous astrogliosis that can be detected. When taken into consideration with another protein, neurofilament light, the combined elevations are significantly related to microstructural injury detected via diffusion tensor imaging (DTI; Newcombe et al., 2022). Translational studies in rat models corroborate evidence suggesting that following severe, moderate, and mTBI, GFAP levels are significantly elevated over baseline with multiple types of TBI (penetrating, blast overpressure, neurotoxicity) and may even increase proportionally to injury severity (Glushakova et al., 2012; M.-D. Liu et al., 2014; Zoltewicz et al., 2013). Due to the highly specific mechanism of injury in these rodent models, one that does not involve extracranial injury, it can be inferred that the GFAP elevations are indeed due solely to brain injury. This evidence supports claims that GFAP elevation in humans is due to brain damage.

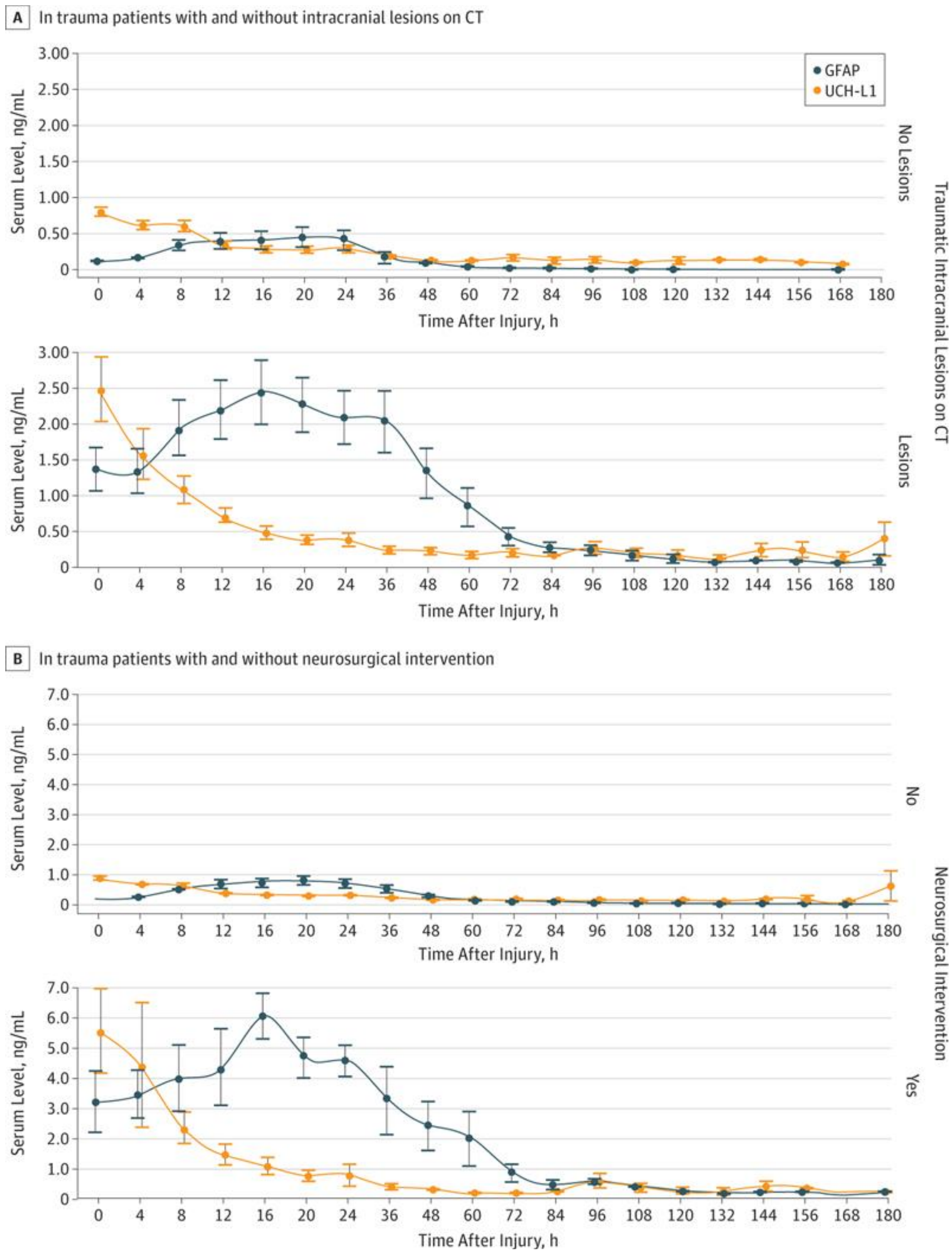

Figure 5. Serum Profiles of GFAP and UCH-L1. (*A*) Temporal profile of GFAP and UCH-L1 serum level in patients with and without intracranial lesions found on head CT scan following TBI. In those with lesions, UCH-L1 is elevated early and tapers as time passes, while GFAP does not peak until around 16 hours post-TBI. (*B*) Temporal profile of GFAP and UCH-L1 serum level in patients with and without neurosurgical intervention following TBI. UCH-L1 and GFAP patterns mimic patterns seen in patients with/without head CT lesions. Taken from (Papa et al., 2016).

Importantly, GFAP levels in TBI patients are significantly linked to pathological CT abnormalities and patient outcome measures (**Figure 6**). Compared to non-TBI trauma controls, GFAP was elevated in patients with mTBI and has been confirmed that serum GFAP can be used as a specific marker for brain damage following head trauma (Mondello et al., 2011). Patients who presented to a neurotrauma center in China with severe TBI, and were placed under hypothermia treatment, showed elevated serum GFAP at admission with a gradual decrease until discontinuation of hypothermia where GFAP levels once again rose (J. Lei et al., 2015). In TBI patients where head CT detected intracranial lesions, GFAP levels were found to be significantly elevated compared to those without detected lesions over 7 days of monitoring (Gill et al., 2018; Papa et al., 2016; Posti et al., 2019). The elevation of GFAP has also been shown to significantly relate to the level of grey matter atrophy after the first 6 months post-TBI (Graham et al., 2021). Additionally, GFAP has been implicated as a possible biomarker to predict pathological findings on MRI when there is nothing found on a CT scan. It has also been found to reliably discriminate patients with mTBI from healthy controls (Gill et al., 2018). Elevated serum GFAP has been found to significantly correlate to the detection of hemorrhages in TBI patients via MRI, and has also been demonstrated to distinguish between patients who needed neurosurgical intervention compared to those who did not (Kou et al., 2013; Papa et al., 2016).

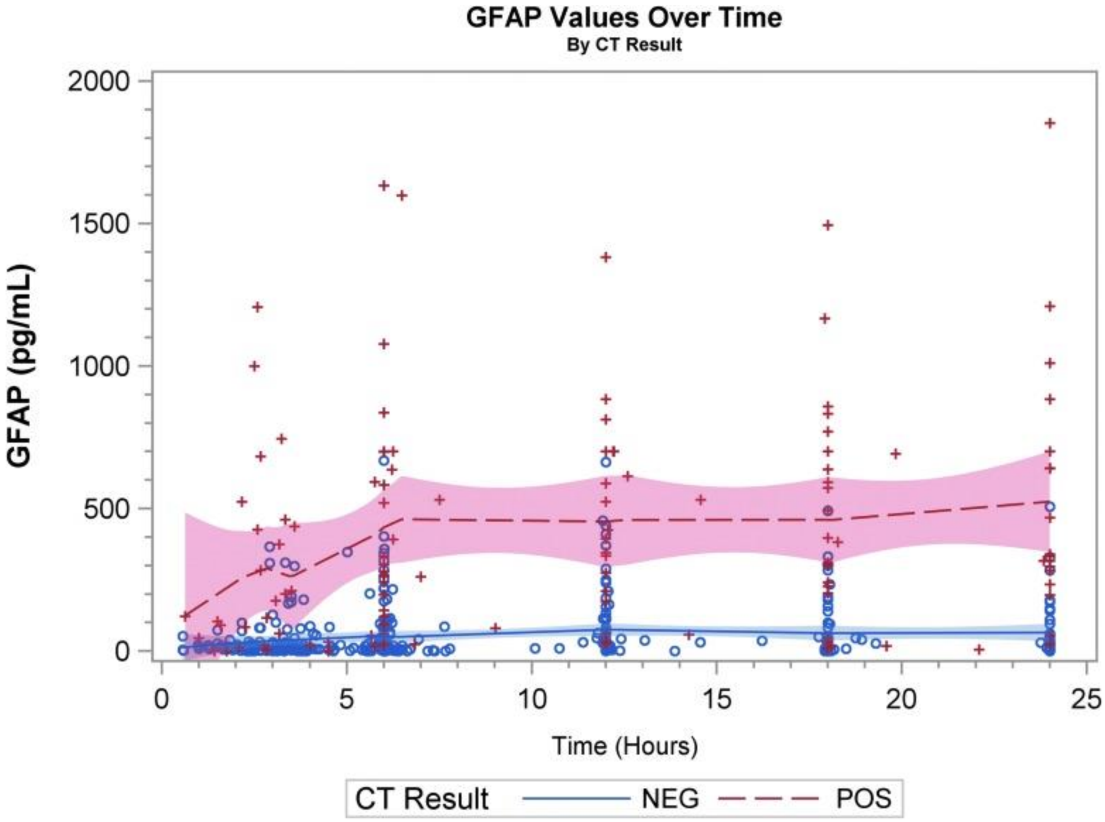

Figure 6. Elevated GFAP in CT+ Patients. Scatter plot of serum GFAP levels in patients undergoing head CT following TBI. Locally weighted scatter plot smoothing regression lines demonstrate the average concentration of serum GFAP found in CT-positive (red) and CT-negative (blue) patients. Red "+" markers signify CT-positive patients while blue "o" markers signify CT-negative patients. Taken from (Welch et al., 2017).

Plasma GFAP levels measured in the acute phase tend to have a loose correlation with predicting favorable versus unfavorable outcomes as determined by the GOSE level being either higher or lower than 4 (Diaz-Arrastia et al., 2014). There seems to be a strong correlation, however, between maximum levels of GFAP and unfavorable outcomes. Those with unfavorable outcomes had significantly higher maximal acute GFAP than those with favorable outcomes in early studies (Nylén et al., 2006; Pelinka et al., 2004). Additional research supports this claim and suggests that GFAP levels were significantly higher at early time points in those with unfavorable outcomes and may be able to modestly discriminate between patients with favorable and unfavorable outcomes (**Figure 7**; Hossain et al., 2019; J. Lei et al., 2015; Takala et al., 2016). In one study by Okonkwo et al., elevated GFAP levels were significantly associated with GOSE scores of under 7, signifying an unfavorable outcome and a lack of return to baseline function after 6 months. Additionally, with increasing serum GFAP there was an inverse relationship with favorable outcomes but no association with the ability of GFAP to predict a full recovery (Okonkwo et al., 2013). GFAP has also been found to be a significant predictor of mortality at 6 months post-injury (Czeiter et al., 2012). In children, increasing serum GFAP measured on day 1 of severe TBI correlates with decreasing functional outcome scores at 6 months as determined by the Pediatric Cerebral Performance Category scores (Fraser et al., 2011). It is important to note that there is conflicting evidence suggesting GFAP may not adequately predict inadequate recovery, even when combined with other biomarkers (Diaz-Arrastia et al., 2014). GFAP's ability to discriminate has been deemed not clinically useful (Takala et al., 2016). However, recovery is not necessarily standardized as when patients return home it is difficult to keep track of variables that may affect recovery. Injury severity is also able to be reliably predicted by GFAP elevation in the first 24 hours post-TBI where increasing GFAP levels are significantly correlated with decreasing GCS (Okonkwo et al., 2013).

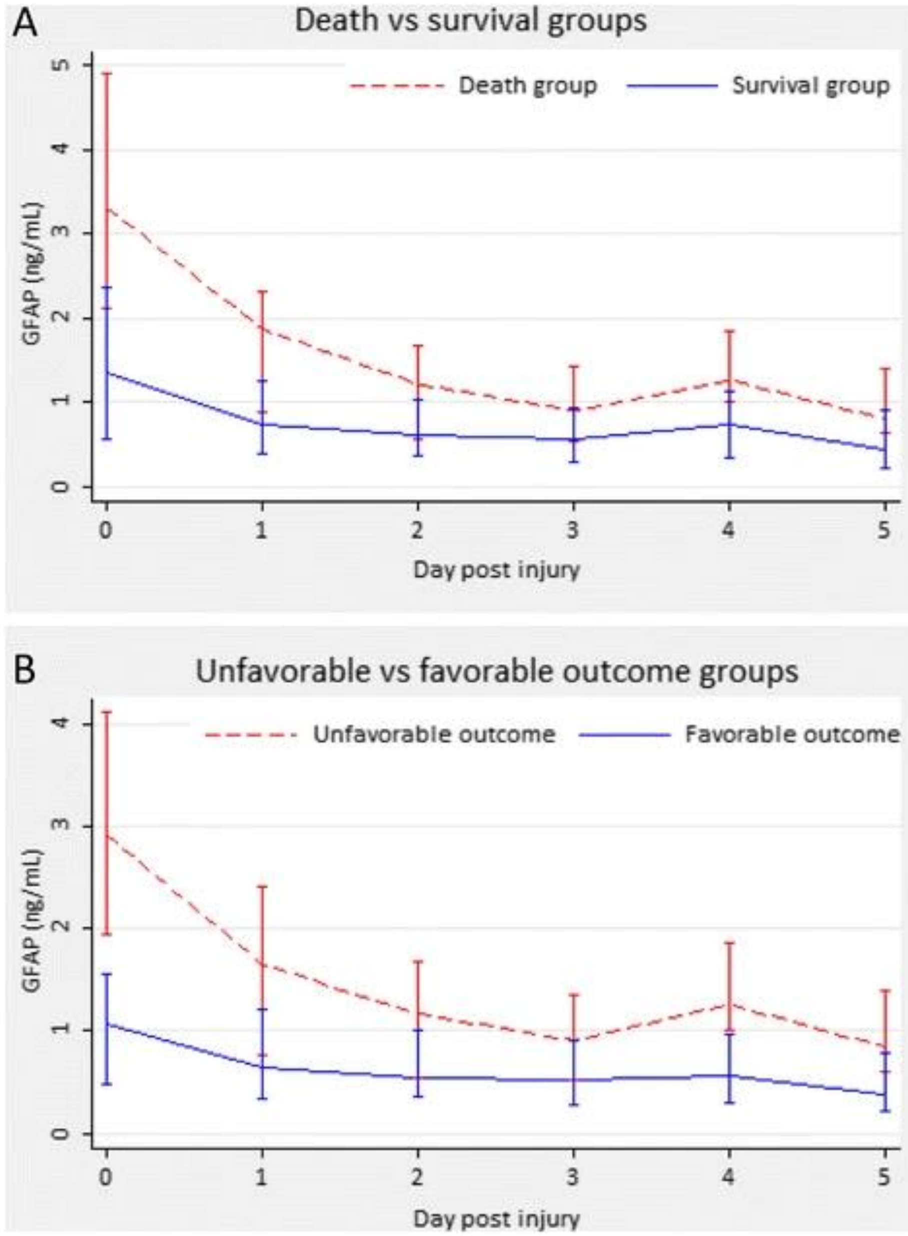

Figure 7. Median Serum GFAP Levels by Outcome Group. (_A_) The median GFAP level between those who survived or died following TBI with no significant difference. (_B_) The median GFAP level between those with favorable and unfavorable outcomes for 5 days post-TBI. Those with unfavorable outcomes had significantly higher serum GFAP levels than those with favorable outcomes. Error bars show interquartile ranges. Taken from (J. Lei et al., 2015).

Similar to S100B, with the evidence supporting the predictive power of GFAP in detecting TBI, its inclusion in panels of biomarkers has been a topic of research. Serum concentrations of GFAP and UCH-L1 when sampled together are strong predictors of poor outcome following TBI. These predictions may also outperform the outcome predictions made by S100B alone, lending credence to the idea that biomarker panels are highly beneficial (Mondello et al., 2016). It has also been used in panels with S100B as mentioned in the previous section to great effect in outcome prediction (Gradisek et al., 2012; Lee et al., 2015; Vos et al., 2010).

Most importantly, GFAP has recently been used in a combination panel with UCH-L1 that has gained FDA approval for the detection of TBI patients who may require a head CT. The ALERT-TBI study in 2018 validated the ability of a panel of GFAP and UCH-L1 to predict CT abnormalities within 12 hours of TBI in a prospective, multicenter observational study. It was found that patients positive for CT-detected lesions had significantly higher GFAP and UCH-L1 than CT-negative patients, and the test for GFAP and UCH-L1 had a sensitivity of 0.976, specificity of 0.364, and a negative predictive value of 0.996 for patients with acute CT-detected intracranial injury. The specificity, sensitivity, and negative predictive value were 0.973, 0.367, and 0.995 for patients with GCS of 14-15 (Bazarian et al., 2018). This research is the basis of the FDA-approved biomarker panel for the necessity of head CT following TBI. It is not without criticism, however, as the role of UCH-L1 in the panel has been questioned as well as the role of biomarkers as a determination for head CT in place of medical history taking (Maas & Lingsma, 2018). As a result, it has been suggested that GFAP alone may be sufficient as a biomarker test for CT necessity following TBI (Czeiter et al., 2020). Clinical covariates such as extracranial injury, injury mechanism, and use of anticoagulants are also not taken into account in this data set (Posti & Tenovuo, 2022). Extracranial injury has been shown to have a large effect on serum S100B detection and should be considered in the context of GFAP. Anticoagulants may predispose patients to intracranial hemorrhage and thus induce a CT-positive finding in a patient who otherwise may not have one.

The cutoff values for serum levels of GFAP are varied but results from the ALERT-TBI trial, and now the cutoff used in the FDA-approved panel to determine whether a patient is eligible for head CT following TBI, is 22 pg/mL for GFAP (Bazarian et al., 2018). Other reported cutoff values have shown that 0.68 ng/mL had a sensitivity of 0.73 and specificity of 0.89 with a positive predictive value of 0.87 for patients who will have pathological CT abnormalities (Okonkwo et al., 2013). Additionally, a cutoff value of 1.559 ng/mL has a sensitivity of 0.853 and specificity of 0.774 while having a positive predictive value of 0.806 and a negative predictive value of 0.828 (J. Lei et al., 2015).

Significant research has shown the potential power GFAP has as a diagnostic biomarker, but there are some negative considerations. Age may have a large effect on GFAP levels in the blood and its predictive power when identifying intracranial trauma via CT, and older people are the most at-risk group for TBI. Using the data set provided by the ALERT-TBI study, it was found that there is significantly reduced diagnostic accuracy for those over 60 years old as opposed to individuals less than 60 years old (Gardner et al., 2018). Similarly, it has previously been reported that individuals with unfavorable or lethal outcomes following TBI tended to be older (J. Lei et al., 2015). While this is likely a function of the vulnerability of the older population, it is important not to rule out the possibility of age-related differences in GFAP detection and conduct further research in this regard. GFAP serum levels alone as of now have also not been shown to accurately predict whether patients who have lower levels during the acute phase will show complete recovery and future investigations should consider the predictive power in this regard (Diaz-Arrastia et al., 2014; Okonkwo et al., 2013). It is also not currently included in any clinical guidelines akin to the Scandinavian guidelines, although it has been shown to outperform other similar biomarkers in its correlation with intracranial findings on CT scans (Posti et al., 2019).

Ubiquitin C-terminal Hydrolase-L1

UCH-L1 is another highly researched biomarker that has implications for TBI detection in clinical practice. It is a 25 kDa enzyme located in neuronal cell bodies that functions to add and remove ubiquitin from proteins as a method of homeostasis and metabolism. Given its location in the neuron cytoplasm, it is believed to be a functional biomarker that may reflect the extent of neuron cell body injury (Ghaith et al., 2022; Posti & Tenovuo, 2022; K. K. Wang et al., 2018). As stated before, it is important to consider that UCH-L1 exists outside of the CNS, mainly in those of the testis, ovaries, and kidneys (Posti & Tenovuo, 2022). Acting as the second half of the FDA-approved test for CT necessity in TBI patients, its elevation in the serum of patients presenting to the ED may direct treatment decisions in the future and may act as a powerful biomarker of neuronal cell injury (K. K. Wang et al., 2018).

Early studies of UCH-L1 generally observed the serum levels of patients with moderate to severe TBI, GCS of 3-8, and found that there was significant release of UCH-L1 into the serum in the first 24 hours following TBI compared to UCH-L1 levels of uninjured controls (Brophy et al., 2011; Mondello et al., 2012). Although the absolute levels of UCH-L1 were higher in CSF than in serum, there was a correlation between the two (Mondello et al., 2012). Increased levels in serum are associated with an increased albumin quotient, a value used to determine BBB function, after 12 hours post-severe TBI while these values have no relation in non-TBI models (Blyth et al., 2011). Thus, researchers suspect that BBB breakdown is related to the appearance of UCH-L1 following TBI (Li et al., 2015). Translational models for UCH-L1 elevation in serum were also found to work with similar kinetics in rats, lending credence to observational research in humans (M. C. Liu et al., 2010). Later research found that UCH-L1 is indeed released into the plasma following mTBI as well (Diaz-Arrastia et al., 2014). Serum UCH-L1 levels appear to be elevated in professional breacher trainees who are exposed to repeated blast overpressure injuries and in athletes post-concussion (Puvenna et al., 2014; Tate et al., 2013). Considering recent research in animal models has suggested that repeated blast overpressure mTBI disrupts cerebral vasculature, it may be feasible to hypothesize that UCH-L1 detection following mTBI injury may be a reflection of acute BBB disruption (Gama Sosa et al., 2021; Puvenna et al., 2014; Tate et al., 2013). Serum concentrations of UCH-L1 in humans are detectable within 1 hour from TBI and peak at around 8 hours with levels staying elevated from 6-24 hours post-TBI (**Figure 5**). Kinetic studies have found that levels are highest in the hours directly following injury and gradually decrease over time with no spikes at later time points. The proposed half-life in serum is around 7-8 hours (Brophy et al., 2011; Mondello et al., 2012; Papa et al., 2016). This short half-life has been the source of criticism regarding its inclusion in the FDA-authorized biomarker panel as in cases where sampling is done past 12 hours, the ability to detect UCH-L1 has been expected to decrease drastically (Czeiter et al., 2020). Importantly, serum elevation was significant when comparing patients with TBI to trauma controls suggesting brain injury is a significant cause for UCH-L1 detection (Papa et al., 2016).

The concentration of serum UCH-L1 was found to significantly correlate with both GCS score at the time of admission and the presence of lesions on head CT scans (**Figure 8**; Mondello et al., 2011; Papa et al., 2012). In the ALERT-TBI study, it was found that levels were significantly higher in patients who were CT-positive than CT-negative when using a cutoff value of 327 pg/mL (Bazarian et al., 2018). A meta-analysis corroborated this evidence. It found that, for the detection of intracranial lesions via CT at cutoffs ranging from 41 pg/mL to 327 pg/mL, the sensitivity was 0.97 and specificity was 0.40. When UCH-L1 was sampled within 6 hours post-mTBI, the sensitivity and specificity were 0.99 and 0.44 respectively. These cutoffs are widely varied, and it is acknowledged that further studies are needed to determine and potentially standardize this important value (Ramezani et al., 2018). Along with the ability to discriminate CT-positive from CT-negative patients, UCH-L1 was found by two studies to have significant power to discriminate between athletes with concussions in a contact sport from non-TBI contact sport injury controls in an acute post-TBI period (McCrea et al., 2020; Meier et al., 2020). Additionally, UCH-L1 levels were significantly elevated in patients requiring neurosurgical intervention following TBI than those who did not (Papa et al., 2012). There is, however, conflicting research in terms of the ability to UCH-L1 to differentiate between patients with normal and abnormal imaging findings. It has been suggested that UCH-L1 is not associated with abnormal findings through MRI or DTI (Kou et al., 2013). There is also a dearth of research to support the correlation of UCH-L1 levels to imaging findings when compared to the body of research supporting GFAP (Ramezani et al., 2018). GFAP has also been found to outperform UCH-L1 in its ability to discriminate between patients with and without mTBI as well as those with and without intracranial lesions on CT (Papa et al., 2016).

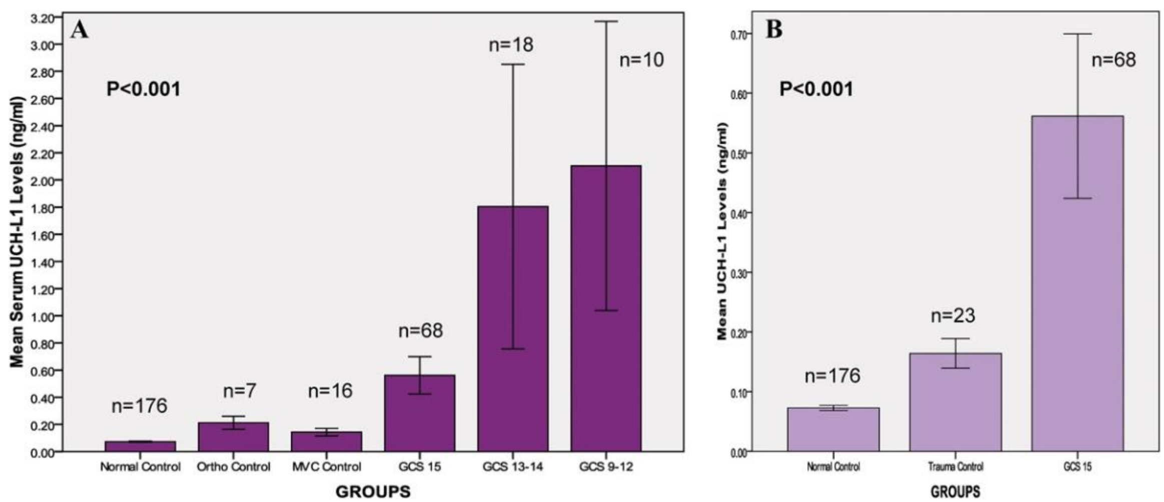

Figure 8. UCH-L1 Levels Correlate with GCS at Admission. A comparison of serum UCH-L1 levels in patients taken within 4 hours post-TBI. (*A*) Serum levels increased significantly with lower GCS scores, and compared to healthy, orthopedic, and motor vehicle collision groups. (*B*) Serum levels are significantly increased in patients with an ED GCS score of 15 as compared to healthy and trauma controls. Taken from (Papa et al., 2012).

There has been a significant amount of research concerning UCH-L1 and patient outcomes. After severe TBI, mortality within 3 months is associated with elevation of serum UCH-L1 in the first 6 hours after injury (Mondello et al., 2012). The use of CSF UCH-L1 has been found to enhance clinical outcome predictors of mortality following non-penetrating TBI and it was found that UCH-L1 may be useful in predicting the long-term prognosis of severe TBI (Zhang et al., 2014). In the first 24 hours following injury, it was found that there were significantly greater acute increases in serum UCH-L1 in patients who died than in those who did not, and there was a significant difference in UCH-L1 concentration between patients who died due to TBI and survived (Brophy et al., 2011). This is corroborated by evidence supporting the correlation between elevated UCH-L1 concentration and negative outcomes (Czeiter et al., 2012). UCH-L1 can modestly discriminate between patients with favorable and unfavorable outcomes, but the discriminatory power was not clinically useful for distinguishing between those with complete and incomplete recovery or for predicting mortality. It can distinguish between patients with GOS scores from 1-3 from those with scores from 4-5. However, UCH-L1 is unable to outperform commonly used tools to predict the outcome of TBI thus it may not be suitable to include in the current methods of outcome prediction (Takala et al., 2016). Similarly, in a pediatric TBI study, increasing UCH-L1 level was significantly correlated with decreasing GOS (Al Nimer et al., 2015). In sampling done in patients 24 hours post-TBI, UCH-L1 serum levels were not predictive of complete recovery in 3 months. The levels did, however, suggest that there was a modest correlation between favorable outcomes and serum UCH-L1 levels (Diaz-Arrastia et al., 2014). A recent observational cohort study using the data from the TRACK-TBI study moderately supported these claims and concluded that UCH-L1 sampled on the day of injury has a good prognostic value in predicting both death and unfavorable outcomes in patients following TBI, but cannot predict recovery at 6 months post-TBI (Korley et al., 2022).

If a biomarker is to be used in a clinical context, cutoff values are important. The FDA-approved biomarker panel set the cutoff of UCH-L1 to 327 ng/mL to determine if a patient requires a head CT (Bazarian et al., 2018). In the aforementioned meta-analysis of UCH-L1 literature, the cutoff values were wildly varied and changing these values may drastically change the conclusions of each study (Ramezani et al., 2018). In terms of UCH-L1 research, an accepted cutoff value should be thoroughly tested so further research can more reliably predict whether patients would benefit from UCH-L1 monitoring. Additionally, the clinical use of UCH-L1 has been called into question in various contexts. As previously mentioned, the use of UCH-L1 has been determined to be outperformed by GFAP, and while the ALERT-TBI data support its usefulness in a panel biomarker test, it may be unnecessary if GFAP can validate the test reliably by itself (Maas & Lingsma, 2018). While the use of one versus two biomarkers has no physical drawbacks to the patient, the inclusion of UCH-L1 in the panel has the potential to disqualify patients who require a head CT from receiving one.

Neurofilament Protein

NF has three detectable forms in body fluids, a 200 kDa heavy chain (NF-H), a 150 kDa medium chain (NF-M), and a 68 kDa light chain (NF-L). It is a major component of the axonal cytoskeleton as neurofibrils and is found exclusively in neurons (Khalil et al., 2018; K. K. Wang et al., 2018). Because NF proteins are only located in neurons, serum detection has been investigated as a fantastic marker for neuronal cell death. However, detectable levels of NF proteins are not yet used diagnostically for any disease (Yuan et al., 2017). Neurons exist outside of the brain and there is evidence that NF presence in blood may increase following extracranial injury (Posti et al., 2019). Despite this, NF-L has been shown to increase up to 10 times greater in TBI patients than in extracranial injury controls. Additionally, in an experimental TBI model, it was shown that serum NF-L becomes elevated proportionally to the extent of axonal injury as defined by histopathology (Graham et al., 2021). NF-H specifically is a target of phosphorylation, which increases neuron cytoskeleton stability (Ghaith et al., 2022). Because NF is localized in axons, it is seen as a valuable structural biomarker of axonal damage. Intracellular calcium increases following neuron damage and activates proteases that break down the proteins, destabilize the neuron, and lead to the release of NF into extracellular fluid (Ghaith et al., 2022; K. K. Wang et al., 2018). In support of this claim, NF-H is a specific biomarker for axonal injury, especially injury of white matter axons, and its presence in blood has been recognized as indicative of injury to these cell types (Anderson et al., 2008). NF protein release into the serum is a delayed process that occurs as an injury progresses and more neurons break down due to continued cell death. As a result, its presence in the blood may reflect ongoing pathogenic processes, like axonal degradation and cognitive decline, in those suffering from chronic TBI and indicate that neuron death is still occurring far past the initial injury (K. K. Wang et al., 2018). The half-life in the blood seems to be unknown but it is thought to be one of the longest of the TBI biomarkers (Posti & Tenovuo, 2022).

There is significant data to claim that following TBI, NF-L is elevated in the blood (Gao et al., 2020). NF-L and NF-H levels were observed to increase significantly during the first 2 weeks following severe TBI (Lambertsen et al., 2020). Importantly, there seems to be a strong correlation between NF-L levels in the blood and increasing age. In a study examining the NF-L levels following mTBI in older adults with pre-existing neurological disorders, NF-L increases with age in those with mTBI and no neurological disorders, as well as in those with pre-existing neurological disorders (Iverson, Reddi, et al., 2019). These are interesting findings, as they both suggest that NF-L levels will be different in older patients and that NF-L levels are generally higher in patients with neurological disorders. In the context of treating TBI, this factor is important to consider. Older individuals are at the highest risk for both neurological disorders and TBI. If NF-L is to be used as a diagnostic tool, it will be important to understand this interplay to make the most informed treatment decisions.

NF-L levels tend to increase in the first 2 weeks post-TBI, peak somewhere between 10 days and 6 weeks, and then decrease. However, levels have been reported to stay elevated for up to 1 year or in some cases up to 13 years later (Graham et al., 2021; Newcombe et al., 2022; Shahim et al., 2020). Additionally, it was found to increase early post-TBI and show a consistent decline with time (Shahim et al., 2020) Because of this long period of NF-L increase, it may be less useful as a biomarker in the first few hours following injury in the ED than it is as a method of monitoring TBI progression (Iverson, Reddi, et al., 2019). Many studies have found significant evidence of elevated serum NF-L in American football players throughout a season and in TBI patients, and it has been deemed to be a sensitive and reliable biomarker of sports-related concussion (Al Nimer et al., 2015; Khalil et al., 2018, p. 20; Neselius et al., 2013; Oliver et al., 2016)

The NF-L level in serum at 30 days was found to be associated with both later outcomes and greater grey and white matter loss at 90 days post-TBI. This association was not found with GFAP, perhaps owing to the elongated kinetics compared to GFAP (Shahim et al., 2020). The maximum NF-L peak was also correlated with the extent of white matter injury and was able to predict the extent of white matter injury between 6 and 12 months post-TBI. Additionally, the peak NF-L level predicted the extent of grey matter atrophy in the first 6 months post-TBI (Graham et al., 2021). Persistent elevation of NF-L is significantly related to microstructural injury as detected by DTI and was determined to correlate with diffusion MRI measures of axonal injury (Graham et al., 2021; Newcombe et al., 2022). These results suggest that serum NF-L measured in the weeks following TBI may be a valuable tool to monitor axonal injury. Additionally, initial serum levels at admission to the hospital were found to significantly correlate with the presence of intracranial lesion detection on CT (Shahim et al., 2016). Complicating these findings, a retrospective study that looked back at patients who had multiple serum samples taken over the first 15 days post-TBI did not find any correlation between DAI detection with MRI or intracranial damage detected on CT with the presence of NF-L in blood. This is interesting because, given that elevated NF-L correlates with axonal damage, retrospective studies with serial samples could be expected to show a more complete prediction of imaging findings than NF-L levels at admission. However, it is acknowledged that the data from this study does not necessarily preclude NF-L from having predictive power in terms of detecting DAI via MRI or CT abnormalities (Al Nimer et al., 2015). Significant increases in NF-L are correlated with patients who underwent neurosurgical intervention when compared to those who did not. As neurosurgical intervention generally indicates a more severe injury, this supports the evidence found in the previously mentioned experimental TBI model (Graham et al., 2021).

Due to the slow kinetics of NF-L when compared to other biomarkers, it has promise as a useful tool in outcome prediction. Its ability to discriminate between patients with favorable and unfavorable outcomes 6-12 months post-TBI was shown to outperform GFAP and low NF-L levels were significantly correlated with full recovery (Hossain et al., 2019). Blood NF-L levels were also able to distinguish players with PCS lasting over 1 year from patients who saw PCS resolve within 1 year. However, in this cohort, there was no association between NF-L and the functional outcome as measured by GOSE 30 days post-TBI (Shahim et al., 2016). In other studies, levels of serum NF-L have been shown to predict TBI outcomes assessed at 6 to 12 months post-TBI as evaluated by GOS (**Figure 4**; Al Nimer et al., 2015; Graham et al., 2021). This is corroborated by another study in which initial serum levels of NF-L significantly correlated with clinical outcomes as assessed by GOS at 12 months post-TBI (Shahim et al., 2016). When NF-L was tracked over 12 days in patients with severe TBI, the increase between day 0 and day 12 was significant when compared to controls. Levels were also predictive of patient survival when measured at baseline and when comparing cumulative 12-day levels to outcomes at 1-year post-TBI. There was a significant negative correlation between higher levels of NF-L and lower GOS scores (**Figure 4**; Shahim et al., 2016). Initial NF-L levels are significantly correlated with outcomes 12 months post-TBI (Shahim et al., 2016; Tomar et al., 2018) and levels measured early post-TBI have a significant predictive value in the outcome of mTBI where NF-L levels had a significant negative correlation with GOSE score (Hossain et al., 2019). Interestingly, NF-L has also been shown to be a significant predictor for complete recovery of CT-positive patients where complete recovery and better outcomes were correlated with significantly lower serum NF-L levels than those without complete recovery and worse outcomes (Hossain et al., 2019). It was found that serum NF-L levels do have predictive value in TBI outcomes, especially when measured in the first 2 weeks, but the predictive power is less than using the IMPACT model of outcome prediction. It is suggested that adding serum NF-L can greatly enhance prediction rather than only using one (Al Nimer et al., 2015; Lambertsen et al., 2020).

A combination panel of NF-L and GFAP provided the best improvement to the predictive performance of the IMPACT model of outcome prediction (E. Thelin et al., 2019). Similarly, a combination panel of NF-L and S100B has been shown to have high predictive value for TBI outcome and, when combined with the IMPACT model, may be able to produce even more valid outcome predictions (Al Nimer et al., 2015). Robust cutoff values are also vital to understanding when NF-L is considered elevated. In a study that showed the ability of NF-L to predict complete recovery and favorable outcomes, the cutoff values were 28.15 pg/mL with a sensitivity of 0.94 and specificity of 0.44 and 53.6 pg/mL with a sensitivity of 0.90 and specificity of 0.67 respectively (Hossain et al., 2019). To detect severe TBI with serum levels of NF-L, a cutoff of 24.0 pg/mL was used with a sensitivity of 0.97 and specificity of 0.96 (Shahim et al., 2016). More research must be done to determine more useful cutoff values. But, due to its kinetics, NF-L has the potential to add significant outcome prediction power when combined with biomarkers that support injury detection such as S100B or GFAP.

Tau

Tau is a microtubule-associated protein that acts as a structural component of the cytoskeleton of the axon and axon terminal in both the CNS and PNS (Posti & Tenovuo, 2022). Multiple isoforms of tau exist, but six are located in the CNS that range from 33 kDa to 46 kDa. Most research on tau as a biomarker has focused on using the total tau protein content in serum (Hossain et al., 2020). During normal functioning tau is phosphorylated, creating phosphorylated tau, to regulate the movement of microtubules. This phosphorylated version of Tau can be detected via immunolabeling assays separate from the unphosphorylated Tau protein. Phosphorylated tau is of particular interest as excessive accumulation can lead to NFTs which may impair neuronal functioning and lead to neurodegenerative diseases (Ghaith et al., 2022). Serum tau is thought to be an indicator of neurodegeneration. Considering that TBI damages axons which causes tau release, it is believed that tauopathies and hyperphosphorylated tau may be a result of the chronic effects of multiple TBIs or one sufficiently severe or repetitive TBI. Evidence has accumulated suggesting that TBI may be a risk factor for the development of neurodegenerative diseases, potentially due to the initiation of a protein aggregation that leads to protein aggregate accumulation, a hallmark of many neurodegenerative diseases (K. K. Wang et al., 2018). Total tau (t-tau), p-tau, and T-tau/P-tau ratio are currently used clinically as a biomarker for AD and is diagnostically relevant at even early stages of the disease (Blennow & Zetterberg, 2018). One specifically pertinent consequence of TBI and repeated mTBI is the development of CTE, a disease characterized by the accumulation of tau and TAR DNA binding protein-43 kDa in the cerebral cortex. These tau proteins can become phosphorylated and are detected by p-tau antibodies post-autopsy of patients with suspected CTE (McKee et al., 2009, 2013). Tau hyperphosphorylation following TBI, like that found in those who suffered from CTE, has been reproduced in animal models of TBI (Goldstein et al., 2012; Perez Garcia et al., 2021). TBI is known to cause hyperphosphorylation and cleavage of tau proteins, both of which are targets for biomarker investigation (Rubenstein et al., 2017; K. K. Wang et al., 2018). Tau is also linked to the S100B protein, as S100B has been shown to increase the phosphorylation of tau proteins and even exacerbate cerebral amyloidosis in an AD mouse model (Hearst et al., 2011). T-tau peaks around 12-48 hours post-TBI and levels decline slowly (Randall et al., 2013; Rubenstein et al., 2017; Shahim et al., 2014; J. Wang et al., 2016). It is reported to have a half-life of around 10 hours in the serum and, when comparing those from 45-64 years old and 65+, tau is found to be significantly elevated in older age groups (Chiu et al., 2017; E. Thelin et al., 2019).

T-tau measured a few hours post-TBI in ice hockey plays showed significant elevation compared to a preseason baseline suggesting that tau is elevated early following TBI (Shahim et al., 2014). Considering tau's localization to the axonal cytoskeleton, acute damage to the axons should elevate tau early in the serum. Tau is also observed, however, to be significantly elevated in the acute, subacute, and chronic stages following mTBI in both hockey players and military veterans (Olivera et al., 2015; Shahim et al., 2014). These findings are also supported in human cohorts of moderate to severe TBI where plasma t and p-tau elevations were detected in both the acute and chronic phases of injury (Rubenstein et al., 2017). This elongated kinetic profile of tau elevation supports earlier suggestions made about the progressive nature of TBI due to prolonged cell death. As vascular damage, remodeling, and inflammation continue to damage the brain, axonal proteins will continue to be elevated in the serum. Another interesting finding suggests that serum tau levels correlate significantly with injury severity as higher serum tau levels are correlated in more severe TBI (Rubenstein et al., 2017; J. Wang et al., 2016). Some issues with t-tau measurement following TBI have been raised. For instance, studies conducted in fairly similar clinical settings with similar assays report wide variation in the correlation of t-tau to TBI. This could suggest that some of the methods for t-tau measurement are different or that t-tau, as of yet, has not been studied enough to provide strong evidence of a reliable biomarker (Giza et al., 2021; Meier et al., 2020; Shahim et al., 2014). However, there is evidence to suggest that serum Tau does not correlate with CT lesions and cannot predict PCS in TBI patients (Papa et al., 2015). While serum tau may correlate with the presence of TBI and even injury severity, it may not be useful as a tool to predict whether a patient requires a CT scan.

Tau is a reliable outcome predictor for those suffering from TBI. P-tau and t-tau levels in patients with TBI, as well as the p-tau/t-tau ratio, were higher than in healthy controls. P-tau, t-tau, and p-tau/t-tau ratio levels were able to discriminate patients with mTBI from healthy controls based on GCS score. Acute p-tau levels and p-tau/t-tau ratio have also been found to weakly correlate with outcomes as defined by GOSE score as shown in **Figure 9**, but are not able to predict a full recovery in patients (Rubenstein et al., 2017). This has also been supported by another study that claims p-tau levels and p-tau/t-tau ratio outperformed t-tau alone in discriminating between mTBI patients with favorable outcomes from those with unfavorable outcomes, along with others that find a significant correlation between serum tau levels and clinical outcome (Hossain et al., 2020; J. Wang et al., 2016). When considering severe TBI, similar data is presented. It was found that following severe TBI, tau protein levels in the serum were found at significantly higher concentrations in those with poor outcomes than in those with good outcomes, and serum cleaved-tau levels were significantly lower in patients with good outcomes than those with bad outcomes (Liliang et al., 2010; Pandey et al., 2017). These data suggest that while Tau may not be sufficient to inform clinicians about which patients may require a head CT, it can be a powerful outcome prediction tool. When considering the role of tau in many neurodegenerative diseases, it becomes an even more attractive target to monitor over time. Additional research needs to be conducted regarding this relationship, but current tau serves a useful purpose as a TBI biomarker.

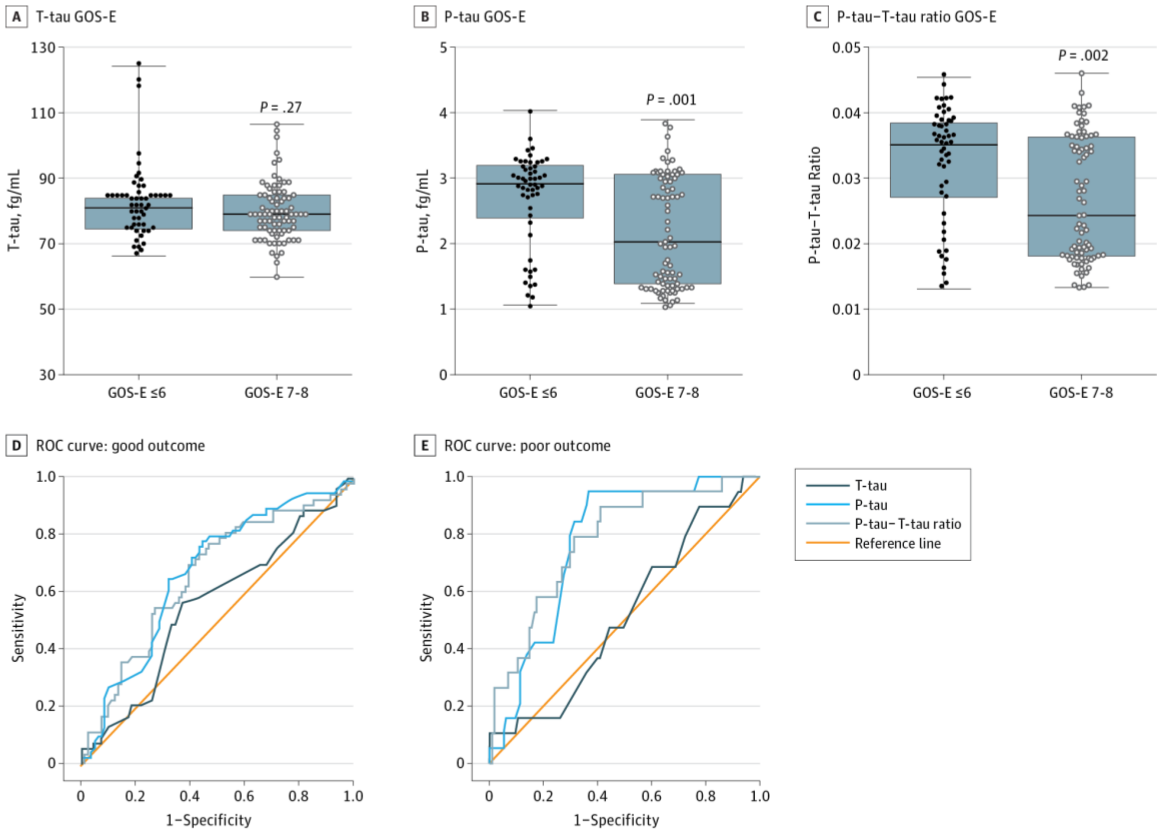

Figure 9. T-tau, P-tau, and P/T-tau Ratio Weakly Correlate with GOSE. Acute serum (*A*) T, (*B*) P, and (*C*) T/P-tau ratio of patients is shown in relation to the six-month GOSE score of TBI patients where <6 is considered a poor outcome and 7 or 8 is considered a good outcome. Receiver operating characteristic curves for (D) good and (E) poor outcomes also shown. P-tau and P/T-tau ratio have an inverse relationship with GOSE, but there is weak correlation between levels of P, T, and P/T-tau ratio and GOSE. Taken from (Rubenstein et al., 2017).

Seeing the value of tau, researchers have begun to look at the prediction power it may add to biomarker panels. It was found that, by using a combination of S100B, UCH-L1, GFAP, and tau, there is a possibility to make a biomarker panel with significant outcome prediction power as determined by GOS score (E. Thelin et al., 2019). Given the concomitant presence of tau and S100B during TBI, it is worth further investigating the link between these two proteins and the development of neurodegenerative diseases. A study concerned with using multiple potential biomarkers for neurodegenerative disease looked at combining T-tau, Aß40, and Aß42 to predict the outcome of mTBI when sampled during the first 24 hours post-admission. Only T-tau levels were significantly correlated with outcomes both for the entire study population and the CT-positive population. However, it was not found that Aß40 and Aß42 were able to enhance the predictive qualities of T-tau and instead, the panel of these three was unable to find any correlation between their levels and recovery. Aß40 and Aß42 are considered markers of AD and of plaques found in CTE patients and reflect amyloidogenic amyloid precursor protein metabolism, and as such may be better investigated at later timepoints following chronic TBI than in the acute setting (Hossain et al., 2020).

While cutoff values for the prediction of TBI outcome based on tau levels are not explicitly discussed in many studies, the average serum cleaved-tau found in severe TBI patients with good outcomes was 74.26 which is significantly lower than 127.32 pg/mL, the value found for poor outcome patients (Pandey et al., 2017). Additionally, another study identified a cutoff value of 12.84 pg/mL for a sensitivity of 0.90 for the prediction of a good or poor outcome following TBI (Hossain et al., 2020). These are drastically different values and necessitate more research.

FUTURE DIRECTIONS AND CONCLUSION

Future Directions

Inflammation biomarkers are a relatively new topic in the field of TBI biomarkers. Primary and secondary TBI activate the inflammatory process via the release of cytokines from activated microglia and leukocytes. These include IL-1, IL-6, IL-8, IL-10, and TNF-α and some research suggests they can be correlated with injury severity (Ghaith et al., 2022). IL-1 is involved in activating leukocytes and serum IL-1ß is a major component of the acute inflammatory response (Kaneko et al., 2019). Effects of the inflammatory response may lead to significant tissue damage and IL-1ß has been found to correlate with injury severity, potentially as a consequence of this inflammation (Taşçi et al., 2003). IL-6 is a key player in acute inflammation as levels increase quickly and it is known to induce production of acute phase proteins (APP) and C-reactive proteins (CRP) by the liver to mediate the inflammatory response. Importantly, it has been implicated in vasculogenesis in injured brain tissue and increasing BBB permeability following injury (Ciryam et al., 2023). IL-6 serum levels have been found to reliably increase around 100 times normal after TBI and are correlated with intracranial pressure measurements in TBI patients, but do not necessarily correlate to injury severity. IL8, IL-10, and TNF-α do not seem to be correlated with TBI severity, although more research is necessary. These have all been shown to have the ability to predict outcomes and when combined with other measures like GCS, do seem to improve outcome predictability (Woodcock & Morganti-Kossmann, 2013). Evidence suggests that there is a large systemic inflammatory response following mTBI with inflammatory markers being present as early as 6 hours post-TBI and up to 12 months post-TBI (Visser et al., 2022). In a review of studies of inflammatory biomarkers following mTBI, it was found that IL-6 was most often elevated. It is also found that patients with intracranial pressure (ICP) ≥ 25 mmHg had significantly higher levels of IL-6 than those under 25 mmHg. This is similar to systemic elevations found in moderate to severe TBI in other studies. A serum cutoff for IL-6 of >128 pg/mL was used to correctly identify TBI patients who developed high ICP with 85% efficacy. It was also found that IL-6 is elevated for a much shorter time in mTBI of athletes and military personnel when compared to other methods of TBI that are seen in the ED. Athletes and military personnel generally had elevated IL-6 levels for only the first 24 hours whereas ED populations may see elevations for as long as 6 months post-mTBI (Hergenroeder et al., 2010; Lustenberger et al., 2016; Rodney et al., 2018; Visser et al., 2022; Yan et al., 2014).

Some research claims that cytokine and APP production happens in response to extracranial injury too. This is a confounding variable that more studies need to investigate to confirm whether these inflammatory biomarkers are elevated uniquely in TBI compared to orthopedic controls (McDonald et al., 2016). APPs, like CRP, are found to be elevated relative to controls and orthopedic trauma controls in some studies, but in others, they are also significantly elevated in controls. In a cohort of patients from the TRACK-TBI study, it was found that TBI patients with worse outcomes as defined by GOSE < 5 had significantly elevated CRP compared to those with higher GOSE scores. Because the response of APPs is heterogeneous and extracranial injuries have the potential to cause elevation, more research is needed in the context of TBI to elucidate their potential as a biomarker for acute injury (Visser et al., 2022; Xu et al., 2021). This is the biggest concern when considering using inflammatory biomarkers for TBI, as displayed in **Figure 10**, as analyzing them may not be helpful unless the clinician knows for certain that only the head is implicated in the injury. Galectin-3 is another protein implicated in the inflammatory response following TBI. It is part of the lectin family and is involved in the activation of microglia, a process that is upregulated following TBI (Ghaith et al., 2022). Plasma concentrations of galectin-3 increase significantly following TBI as compared to controls. Levels of galectin-3 and GCS scores had a similar correlation to the prediction of in-hospital mortality and in-hospital major adverse events, suggesting that galectin-3 may be a suitable biomarker candidate for in-hospital monitoring if more research is done to validate these findings (Shen et al., 2016). These markers of inflammation show promise in the detection and outcome prediction of TBI. However, a lack of research, meaningful cutoff values for detection, and their overlap as markers for extracranial injury currently handicap their clinical potential.

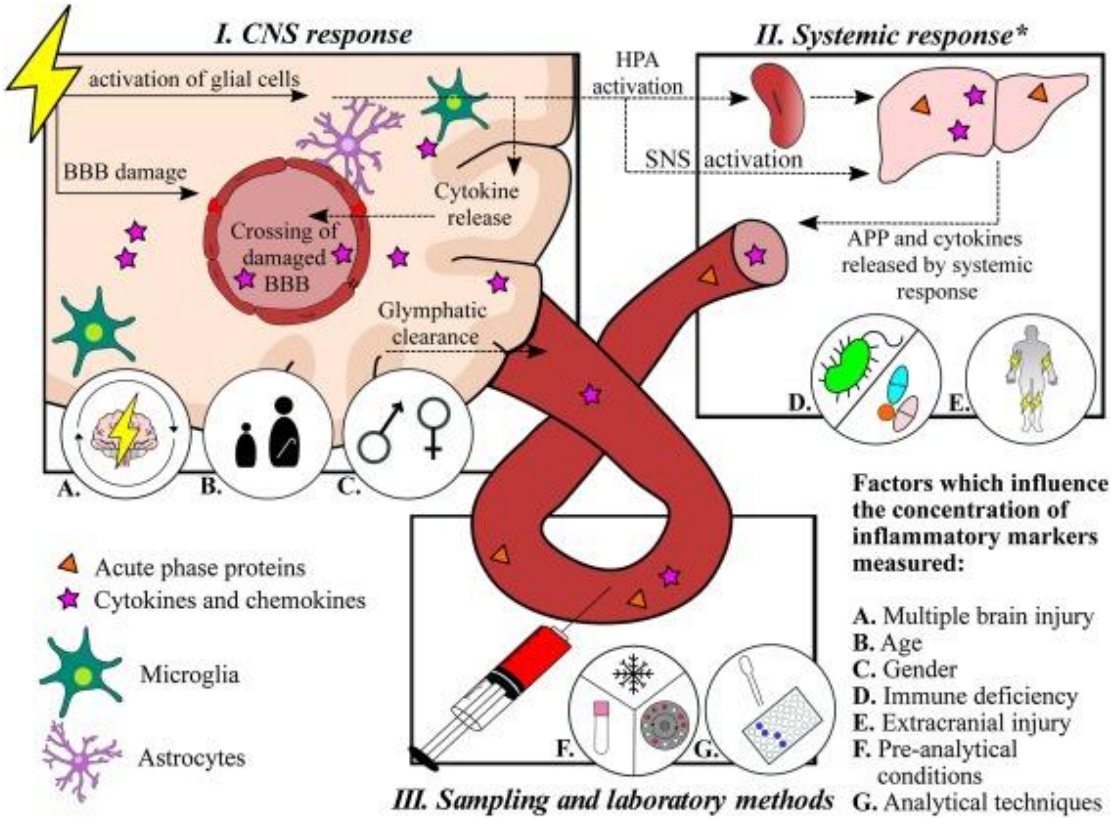

Figure 10. Heterogeneity of Inflammatory Response Following TBI. Diagram of the (I) CNS and (II) systemic response following TBI, and (III) sampling and laboratory methods used to detect inflammatory-related biomarkers. Multiple factors may lead to the production of cytokines and chemokines, posing a challenge in isolating inflammatory markers specifically from TBI. Taken from (Visser et al., 2022).

EVs are another serum-borne particle that has been investigated as a potential TBI biomarker. EVs are membranous particles secreted by different cells into body fluids. They are lined by a lipid bilayer and contain specific markers on their surface that act as indicators for the status of glial cells and neurons. EVs are split into three categories, apoptotic vesicles, microvesicles (MV), and exosomes (Karttunen et al., 2019). Apoptotic vesicles are secreted during apoptosis to remove cellular components to not cause an inflammatory response. If incorrectly executed, the failure of clearance is associated with chronic inflammatory diseases. They are large (50-5000 nm) and contain the dying cell's

DNA and other proteins (Wickman et al., 2012). MVs are between 100-1000 nm and are created through the extracellular budding of cell membranes. Exosomes are 40-100 nm, are contained in fluids such as serum and breast milk, and are formed from the inward budding of endosomal compartments of cells which then fuse with the plasma membrane. They contain non-coding RNA, proteins, and mRNA (Borges et al., 2013; Ghaith et al., 2022). EVs are important for healing processes following neuronal injury. Oligodendrocytes release EVs in response to oxidative stress in an attempt to control damage. In experimental models, exposure to oligodendroglial exosomes before oxidative stress leads to a significant increase in neuronal metabolic activity and an alleviation of oxidative stress (Frühbeis et al., 2013). It has also been shown that EVs from microglia containing the micro RNA miR-124 contribute to regeneration and help decrease inflammation (Beard et al., 2020). This suggests that EVs are found in the serum as a response to trauma in the brain and their levels may be indicative of the extent of the damage done. EVs from TBI patients have distinct sets of proteins correlated to GCS scores when investigated via mass spectrometry and MVs isolated from the plasma of TBI patients are significantly elevated in both GFAP and aquaporin-4 when compared to healthy controls (Moyron et al., 2017; Nekludov et al., 2017). Exosomes with embedded tau in TBI patients may be a diagnostic tool for chronic TBI and may help identify patients at risk of developing CTE. From a group of ex-National Football League players, exosomal tau was significantly elevated when compared to a control group. Interestingly, higher concentrations of exosomal tau were associated with worse memory test performance (Stern et al., 2016). While EVs show promise for the detection of TBI, they are currently very difficult, time-consuming, and expensive to isolate from plasma (Ghaith et al., 2022). Given the need for rapid and reliable sources of biomarker detection, there is significant progress that will need to be made to make EVs a more attractive option than the well-established proteins already in use.

MicroRNA are small endogenous RNA molecules ranging from 19-28 nucleotides long and play various roles in the regulation of gene expression. Detected by real-time quantitative reverse transcription PCR following serum isolation, they have recently become a topic of research as TBI biomarkers as micro ribonucleic acids (miRNA) have been found elevated following TBI in both rodent models and humans. In a model of blast overpressure injury in rats, miR-Let-7i was elevated in the serum immediately following TBI, and closed-head TBI rodent models have also found elevations in miRNAs (Balakathiresan et al., 2012; K. K. Wang et al., 2018). MiR-16, miR-92a, and miR-765 were found to be significantly elevated during the acute phase of severe TBI in humans, and by combining these miRNAs the diagnostic accuracy increased to 1.00 for both sensitivity and specificity compared to healthy and orthopedic injury controls. For patients with mTBI, miR-16 and miR-92a were significantly increased while miR-765 did not differ from controls (Redell et al., 2010). A different set of miRNA that includes miR-92a (miR-151–5p, miR-195, miR-20a, miR328, miR-362–3p, miR30d, miR-451, miR-486, miR-505, and miR-92a) was found to be elevated in the serum of humans with mild to severe TBI when compared to healthy and orthopedic injury controls (**Figure 11**). Apart from miR-328 and miR-151, all miRNAs displayed significant differences in groups with positive CT scans. Similarly, apart from miR-151, all miRNAs displayed the ability to diagnose TBI in patients due to the aforementioned significant changes and may be potential candidates for future research (Bhomia et al., 2016).

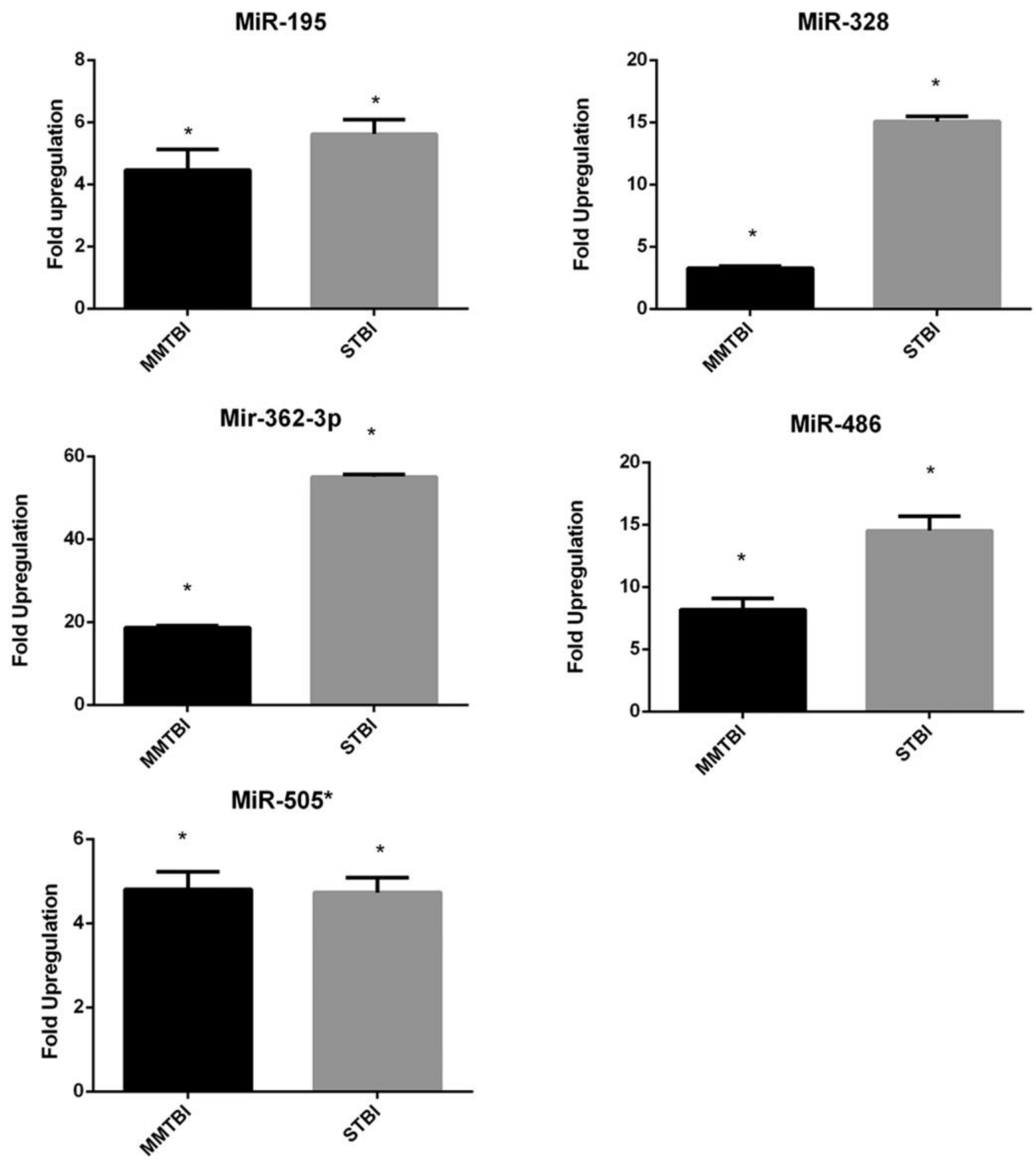

Figure 11. MiRNA Upregulation Following Mild, Moderate, and Severe TBI. Displayed is the fold upregulation of miRs 195, 328, 362-3p, 486, 505 as compared to healthy controls. There is significant upregulation in all miRNAs presented in mild/moderate and severe TBI, with specifically miRs 328, 362-3p, and 486 elevated further in severe TBI than mild/moderate TBI. Taken from (Bhomia et al., 2016).

MiR-92a is a promising candidate for biomarker detection of TBI. It has been found elevated in the serum of patients in the acute phase of mild to severe TBI and some research shows that, in a rodent model, systemic administration of miR-92a inhibitor led to enhanced blood vessel growth and increased recovery of damaged tissue. Ischemic events are known to cause secondary TBI and the elevation of miR-92a may be a culprit of this (Balakathiresan et al., 2012; Bonauer et al., 2009; Redell et al., 2010). Two different miRNAs, miR-142-3p and miR-423-3p, were demonstrated to distinguish between mTBI patients who were likely to develop PCS and those who were not. These two were also shown to decrease over time, which the researchers speculated was due to brain healing (Mitra et al., 2017). MiR-320c-1, miR-629, and let-7b-5p are three more promising biomarker candidates due to their association with specific symptoms when tested in the saliva of pediatric patients with mTBI. There was a significant correlation between changes in these three miRNAs and TBI, and their detection more accurately predicted the presence of injury than a test for the symptom burden (Johnson et al., 2018). MiR-320c-1 also has a role in neuroplasticity and is seen to change in the cerebral cortex of depressed individuals. It is viewed as a potential target due to the association between depression and PCS, so its detection may be able to tell clinicians about the mental state of their patients (Ghaith et al., 2022). Of the new biomarker candidates rapidly being discovered, miRNAs show the most promise. They seem to have the most correlation directly with TBI and there are already established methods of isolation and detection of these short nucleic acid chains. However, like the issues facing EVs, these detection methods are currently less accessible than the assays for proteins which currently makes them a less attractive candidate.

Conclusions

While there has been a growing effort to research biomarkers related to TBI, the implementation of research into clinical practice has been slow. Using biomarkers to determine the need for head CT and outcome prediction is what most of the research has concerned and there are a few notable examples in this regard: the Scandinavian guidelines and most recently the FDA-approved GFAP/UCH-L1 biomarker panel (Bazarian et al., 2018; J. Undén et al., 2013). There have been significant strides made in TBI biomarker research since the Scandinavian guidelines were implemented in 2000. These guidelines themselves have been updated and validated to reflect the advances made. Important updates include that anyone under a GCS of 15, with severe neurological response, or with altered coagulation should receive a head CT following TBI. If GCS is 15, they are either eligible for a blood test of S100B and released without a head CT if [S100B] is < 0.10 μg/L (J. Undén et al., 2013).

Currently, one of the largest issues with serum biomarker implementation in a clinical setting is the lack of consensus about relevant cutoff values. Large study populations are difficult to gather and studies on the scale of the ALERT-TBI trial require significant resources and research personnel to complete. Importantly, it is necessary to develop tests and panels with cutoff values in mind so the assays can be clinically useful.

These values differ between trials based on the population studied and are generally defined as the values with the highest sensitivity, specificity, and negative predictive value for finding lesions on a head CT scan. Further steps must be taken to validate promising cutoff values for serum biomarkers, especially for emerging biomarkers like miRNA and EVs.

To be clinically useful, guidelines have to be broadly generalizable to a large patient population. Researching biomarkers in varying populations is beneficial to understanding how the biomarker presents in a variety of different groups. However, current studies differ in many important variables. Cutoff values are one of these variables but injury severity, injury type, extracranial injury, age, biomarker levels in different ethnic populations, and medications at the time of injury are all variables that need to be considered. When designing future research, study populations must be controlled for these variables similar to how the Scandinavian guidelines set parameters for who is qualified or disqualified for head CT based on variables apart from S100B concentration (J. Undén et al., 2013).

This thesis has explored the potential of blood biomarkers for the detection and management of TBI. Currently, it is difficult to recommend the widespread use of biomarkers as a unique clinical tool for these purposes. While there has been significant research surrounding the topic, the literature is fraught with confounding variables such as age-related variation in biomarker levels and wide ranges of cutoff values for TBI detection. Additionally, many biomarkers seem to have potential as tools in outcome prediction rather than enhancing current, well-established methods of TBI detection.

When considering the several biomarkers investigated, GFAP emerges as the most promising biomarker in a clinical setting. GFAP has significant evidence to suggest that it is reliably increased in serum during the acute phase of TBI and has been validated for clinical use in this context by multiple studies. The specificity to the detection of astroglial injury is additionally important as neuroinflammation is known to be heavily implicated in secondary TBI. This is a process astroglial cells are known to be involved in, and the sensitivity of GFAP in detecting even subtle astroglial damage post-TBI makes it a valuable tool in identifying patients at risk of secondary TBI. The kinetics of GFAP in serum also makes it a more ideal candidate than other molecules. The long elevation and serum half-life compared to other biomarkers lends itself well to easier period of detection. This makes it a strong candidate for clinical use because, in reality, patients may not be able to make it to a hospital promptly or may not seek medical attention in the first few hours post-TBI. It is also a well-defined protein with established assay methods, setting it apart from newer biomarkers that are currently more difficult and costly to detect.

GFAP is not a perfect biomarker as age is a major confounding factor for its clinical use. It has specifically been shown to have decreasing accuracy in people over the age of 65, the population who is most affected by TBI. However other options that do not decrease significantly with age, such as p-tau, do not yet have evidence to suggest they are more adequate predictors of TBI than GFAP. Ultimately, the evidence presented in this thesis strongly supports the use of GFAP as a biomarker useful in the clinical management of TBI. Its continued inclusion promises to improve the precision of diagnostics, individualized treatment plans, and the quality of life for TBI patients. While there are still challenges regarding its widespread clinical adoption, further research to enhance the accuracy of previous investigations will expand its use cases over time.

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