The Potential of Human Serum S100 Calcium-binding Protein B, Glial Fibrillary Acidic Protein, Neuron-Specific Enolase, and Serum Amyloid A as biomarkers for Traumatic Brain Injury in Moderate and Severe Cohort

By

Seke Nzau Mafuika
(Student No.: 215001367)

Submitted in fulfillment of the requirements for the degree of Doctor of Philosophy in Medical Science (Anatomy), In the Discipline of Clinical Anatomy, School of Medicine, College of Health Sciences, University of KwaZulu-Natal

Supervisor: Prof. Lelika Lazarus

Co-supervisors: Dr. Rohen Harrichandparsad

2026

PREFACE

This study represents original work by the author conducted from 2020 to 2026 and has not been submitted in any other form to any other University. Where use was made of the work of others, it has been duly acknowledged in the text.

The research described in this dissertation was carried out in the Optics and Imaging Centre, Doris Duke Medical Research Institute, Department of Clinical Medicine, and Department of Clinical Anatomy, College of Health Sciences, University of KwaZulu-Natal, Durban, South Africa, under the supervision of Professor Lelika Lazarus and under the co-supervision of Dr. Rohen Harrichandparsad.

Seke Nzau Mafuika
(Student number: 215001367)

Professor Lelika Lazarus
(Supervisor)

Dr. Rohen Harrichandparsad
(Co-Supervisor)

DECLARATION

I, **Seke Nzau Mafuika,** declare that:

- The research reported in this dissertation, except where otherwise indicated, is my original work.
- This dissertation has not been submitted for any degree or examination at any other university.
- This dissertation does not contain other persons' data, pictures, graphs, or other information, unless specifically acknowledged as being sourced from other persons.
- This dissertation does not contain other persons' writing, unless specifically acknowledged as being sourced from other researchers. Where other sources have been quoted, then:
- Their words have been rewritten, but the general information attributed to them has been referenced.
- Where their exact words have been used, their writing has been placed inside quotation marks and referenced.
- Where I have reproduced a publication of which I am the author or co-author, I have indicated in detail which part of the publication was written by me alone and have fully referenced such publications.
- This dissertation does not contain text, graphics, or tables copied and pasted from the internet, unless specifically acknowledged and the source is detailed in the dissertation and the reference sections.

Signed:  Date: 31/03/2026

DEDICATION

- This work is dedicated, first and foremost, to my Lord and Savior, Jesus Christ. In all things, I believe in Him.
- I dedicate this work to my biological father, Gregoire Mafuika Tsingulu Seke, in gratitude for the many sacrifices he made to ensure that I received an education.
- To my wife, Chika Amelia Obumnaeme and my baby Jason Mafuika, thank you for walking this journey with me and for your unwavering love, patience, and support.

ACKNOWLEDGEMENT

The successful completion of this research would not have been possible without the invaluable support and contributions of many individuals and institutions.

- First and foremost, I give thanks to the Almighty God, Jesus Christ, for the gift of life, strength, and guidance throughout this journey.
- I extend my deepest gratitude to my biological father, Mr. Gregoire Mafuika, and my entire Mafuika family for believing in me and continually supporting me through your prayers and encouragement.
- My heartfelt appreciation goes to my spiritual parents, Apostle Jonas and Pastor Prisca Mpoyi, for their unwavering moral and spiritual support.
- I am sincerely grateful to my supervisor, Professor L Lazarus, and my co-supervisor, Dr. R. Harrichandparsad, for their guidance, academic insight, and mentorship throughout this research.
- I would also like to thank Professor T Naicker for her assistance and guidance throughout this project.
- Special thanks to Mr. Denis Makhubela for his technical assistance in the Physiology Laboratory.
- I also wish to acknowledge all the staff members, doctors, and nurses from the Department of Neurosurgery at Inkosi Albert Luthuli Central Hospital for their support and cooperation during the data collection process.
- My sincere appreciation is extended to all the patients who generously consented to participate in this study.
- Lastly, I gratefully acknowledge the College of Health Sciences for granting me tuition remission and awarding me the CHS scholarship, which provided both operational funding and a stipend to support this research.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS AND ACRONYMS

BBB:	Blood-brain barrier
BDNF:	Brain-Derived Neurotrophic Factor
Ca 2+:	Calcium
CPP:	Cerebral perfusion pressure
CSF:	Cerebrospinal fluid
CT:	Computed Tomography
ERK:	Extracellular signal-regulated kinase
GCS:	Glasgow coma score
GFAP:	Glial fibrillary acidic protein
IF:	Intermediate filament
JNK:	c-Jun N-terminal kinase
KZN:	KwaZulu-Natal
MAPK/ERK:	Mitogen-activated protein kinase/extracellular signal-regulated kinase.
MAPK:	Mitogen-activated protein kinase
MRI:	Magnetic Resonance Imaging
mTBI:	mild TBI
mTOR:	Mechanistic target of rapamycin
NBL1:	Neuroblastoma suppressor of tumorigenicity 1
Nrf2:	Nuclear factor erythroid 2-related factor 2
NSE:	Neuron-specific enolase
PI3K:	Phosphoinositide 3-kinase
PI3K/AKT:	Phosphatidylinositol-4,5-bisphosphate 3-kinase/protein kinase B
RAGE:	Receptor for advanced glycation end-products

ROS:	Reactive oxygen species
S100B:	S100 calcium-binding protein B
SA:	South Africa
SAA:	Serum Amyloid A
SCLC:	Small-cell lung cancer
TBI:	Traumatic brain injury
TLR-4:	Toll-like receptor 4
TNFR:	Tumor necrosis factor receptor
TRAF-6:	Tumor necrosis factor receptor-associated factor 6
TrkB:	Tyrosine kinase
Zn 2+:	Zinc

Publication outcomes

Table 1: Research output

S/N	Titles	Journals	Author contributions	Q-index and Impact factor	DOI	Status
1	The potential of serum S100 calcium-binding protein B and glial fibrillary acidic protein as biomarkers for traumatic brain injury	**Translational Research in Anatomy (Elsevier)**	Seke Mafuika: Material preparation, data collection, conducted all experimental assays, performed data analysis and interpretation, and wrote all manuscripts. Co-supervisors: Dr. R. Harrichandparsad contributed to the study conception and design and read and approved the final manuscripts. Supervisor: Prof L. Lazarus contributed to the study conception and design, assisted with the methodology, read and approved the final manuscripts.	**Q2, IF= 3.2**	https://doi.org/10.1016/j.tria.2022.100228	Published
2	Evaluating Traumatic Brain Injury Severity Through GFAP and S100B Biomarkers: An Immunoassay-Based Approach	**Journal of Neurocritical Care**		**Q3, IF= 3.6**	Under review Manuscript number: JNC-25-031	
3	Evaluation of Serum Neuron-Specific Enolase and Serum Amyloid A in Moderate-to-Severe Traumatic Brain Injury in Association with Glasgow Coma Scale and CT Findings for Improved Classification in a South African Cohort	**The South African Medical Journal**		**Q3, IF= 2.2**		

ABSTRACT

Background: Traumatic brain injury (TBI) occurs due to a severe head injury caused by an external force during a vehicle accident, fall, domestic violence, or explosion. In sub-Saharan Africa, the prevalence of TBI is 150–170 per 100,000 individuals. It is estimated that by 2050, the prevalence of TBI in Africa will increase to 14.25±0.75 million. TBI is clinically evaluated and classified using the Glasgow Coma Score (GCS), with CT and MRI. However, GCS has many limitations. Therefore, the aim of this study was to investigate the role of serum GFAP, S100B, NSE and SAA as potential biomarkers for the detection and diagnosis of TBI.

Methodology: The first phase of the experiment was the scan analysis (n=50) as recorded in the hybrid electronic medical registry, MediTech, at a large urban tertiary referral hospital in eThekwini, South Africa. The second phase utilized a multiplex immunoassay (ProcartaPlex, Thermo Fisher Scientific, 2024) to detect human serum GFAP, S100B, NSE and SAA in n=50 patients with moderate to severe TBI and n=25 control patients. Ethics approval was obtained from the Biomedical Research Ethics Committee (Ref. BREC/00002147/2020) of the University of KwaZulu-Natal. Statistical analysis was achieved using Statistical Package for the Social Sciences version 30.0.0.0 (SPSS; IBM Corporation 2025).

Results: Serum GFAP and S100B concentrations were significantly elevated in patients with moderate and severe TBI compared with the control group ($P < 0.001$). No significant differences between the moderate and severe groups were detected for GFAP or S100B ($P=0.2623$, $P>0.999$). The serum concentrations of NSE and SAA proteins were also significantly upregulated in the moderate and severe TBI compared to the control group ($p<0.0001$) and ($p= 0.0002$), respectively.

Conclusion: These findings suggest that elevated serum concentrations of GFAP, S100B, NSE and SAA are indicative of increasing severity of TBI. These findings suggest that combining GFAP, S100B, NSE, and SAA biomarkers with GCS and CT findings enhances the accuracy of TBI classification.

CHAPTER ONE

Introduction

Traumatic brain injury (TBI) is the most frequent cause of paralysis, disability and death of many individuals, especially in urban regions (Ghajar, 2000). Globally, the burden of TBI is increasing significantly. The reported global prevalence of TBI is 37.93 million [95% uncertainty interval (UI) = 36.33-39.77] (Zhong et al., 2025).

Studies report that in Africa, there are approximately two million cases of TBI per annum, which require neurosurgical care (Embolo *et al.*, 2021). The prevalence of TBI in Sub-Saharan Africa remains unclear due to several factors, including unreported injuries and inadequate technology for documenting reported cases (Staton *et al.*, 2017). Available cases are primarily hospital-reported and may offer an incomplete picture of actual prevalence (Eaton et al., 2017).

Developing countries experience a disproportionate burden of TBIs, possibly due to rapid urban growth, expanding transport systems lacking proper risk assessment, and weak enforcement of road safety regulations (Roozenbeek et al., 2013). The National Institute for Occupational Health of South Africa reported approximately 89,000 new traumatic brain injury (TBI) cases annually (Kong et al., 2017), with 39.4% attributed to interpersonal violence, 16.7% to motor vehicle collisions, 14.1% to vehicle–pedestrian incidents, 10.3% to falls and 19.5% to other causes (Jerome et al., 2017).

Bradshaw et al. (2005) found higher TBI incidence rates in more developed provinces, including Gauteng, Mpumalanga, and the Western Cape. Within KwaZulu-Natal, research has highlighted a substantial burden of TBI among children in Pietermaritzburg (Buitendag et al., 2017).

Additionally, an earlier study reported that in 2000, 40% of patients admitted to a public acute neurological unit in the province were diagnosed with TBI (Kalyan, 2007).

Traumatic brain injury is categorized as mild (GCS 13-15), moderate (GCS 9-12), or severe (GCS <9) based on the Glasgow Coma Scale (GCS), which evaluates the level of consciousness through verbal, motor, and ocular responses (Bodien *et al.*, 2021). The Glasgow Coma Scale (GCS) is widely used to assess cerebral function; however, it has notable limitations in reliability and in accurately classifying TBI severity, particularly among intubated patients and those with severe trauma (Andraos et al., 2025; Niessen et al., 2025). It demonstrates only a mild to moderate correlation with anatomical injury severity, while underestimating the injury's underlying severe physiological effects (Bodien *et al.*, 2021). Moreover, the GCS score fails to account for factors such as brainstem reflexes, anesthesia, aphasia, timing of assessment, pre-hospital interventions, medication and associated injuries, thereby compromising its reliability (Savitsky *et al.*, 2016). Recognizing the limitations of the GCS is essential for physicians in decision-making in certain contexts, while also fostering exploration of potential enhancements to the GCS by considering biomarkers as an emerging tool for TBI diagnosis. More recently, the NINDS-CBI-M has been used to classify injuries based on clinical tests, biomarkers, brain scans and individual factors such as the cause of injury and medical history. These factors may offer a more precise characterization and classification of brain injuries (Peralta et al., 2026).

Biomarkers are quantitatively measurable proteins that serve as indicators of standard biological processes, pathogenic processes, or responses to therapeutic interventions (Wagner and Atkinson Jr., 2015). These biomarkers are important across various fields of biology and medicine, including oncology, psychiatry, cardiovascular disease, reproductive medicine and neuroscience (Figg and Newell, 2014). In precision medicine, biomarkers are crucial for identifying patient subgroups that most benefit from specific treatments, predicting individual drug responses, and identifying patients at risk of unfavorable outcomes (Leung *et al.*, 2019). They are useful for prediction, diagnosis and prognosis, supporting treatment decisions, patient classification and monitoring therapeutic responses (Huss, 2015). Identification and validation of appropriate and accurate biomarkers in clinical research and drug development are required to address challenges in disease treatment and drug approval (Amur *et al.*, 2015). There is a paucity of TBI biomarker data from South African and broader African populations. This is not simply a geographic omission. However, biomarker concentrations and diagnostic thresholds may be influenced by demographic characteristics, comorbidities, renal function, assay characteristics, timing of sampling and biological differences between populations. Consequently, evidence generated predominantly in North American and European cohorts cannot automatically be assumed to have identical diagnostic characteristics in South African population (Wroblewski et al., 2025). Therefore, the aim of this study is to investigate potential biomarkers, including S100 calcium-binding protein B (S100B), Glial Fibrillary Acidic Protein (GFAP), Neuron-Specific Enolase (NSE), and Serum Amyloid A, for the diagnosis and prognosis of TBI in a South African cohort.

Overview of Anatomy of the Brain

The brain is composed of the cerebrum, cerebellum and brainstem, of which the cerebrum is the largest part (Bui and Das, 2020). The cerebrum is made up of two hemispheres joined together by the corpus callosum. It is further divided into five lobes: the frontal, parietal, temporal, occipital and insula lobes (Bui and Das, 2023). The cerebrum is responsible for various functions, including motor control, sensory perception, cognitive functions, emotions, speech and language and spatial reasoning. Executive functions include creativity, consciousness, and learning and memory (Catani, 2017).

The cerebellum is the part of the hindbrain located in the posterior cranial fossa. The extension of the dura mater called the tentorium cerebelli separates the cerebellum from the cerebrum. It is comprised of two hemispheres joined together by the vermis and is subsequently segregated into three lobes, namely, the anterior, posterior, and flocculonodular lobes (Van Essen *et al.*, 2018).

Damage to any part of the brain and its components can cause impairment and a range of neurological conditions and malfunctions, ranging from mild to severe. However, sometimes these conditions may not be obvious immediately after surgery in the recovery room (Baker *et al.*, 2018).

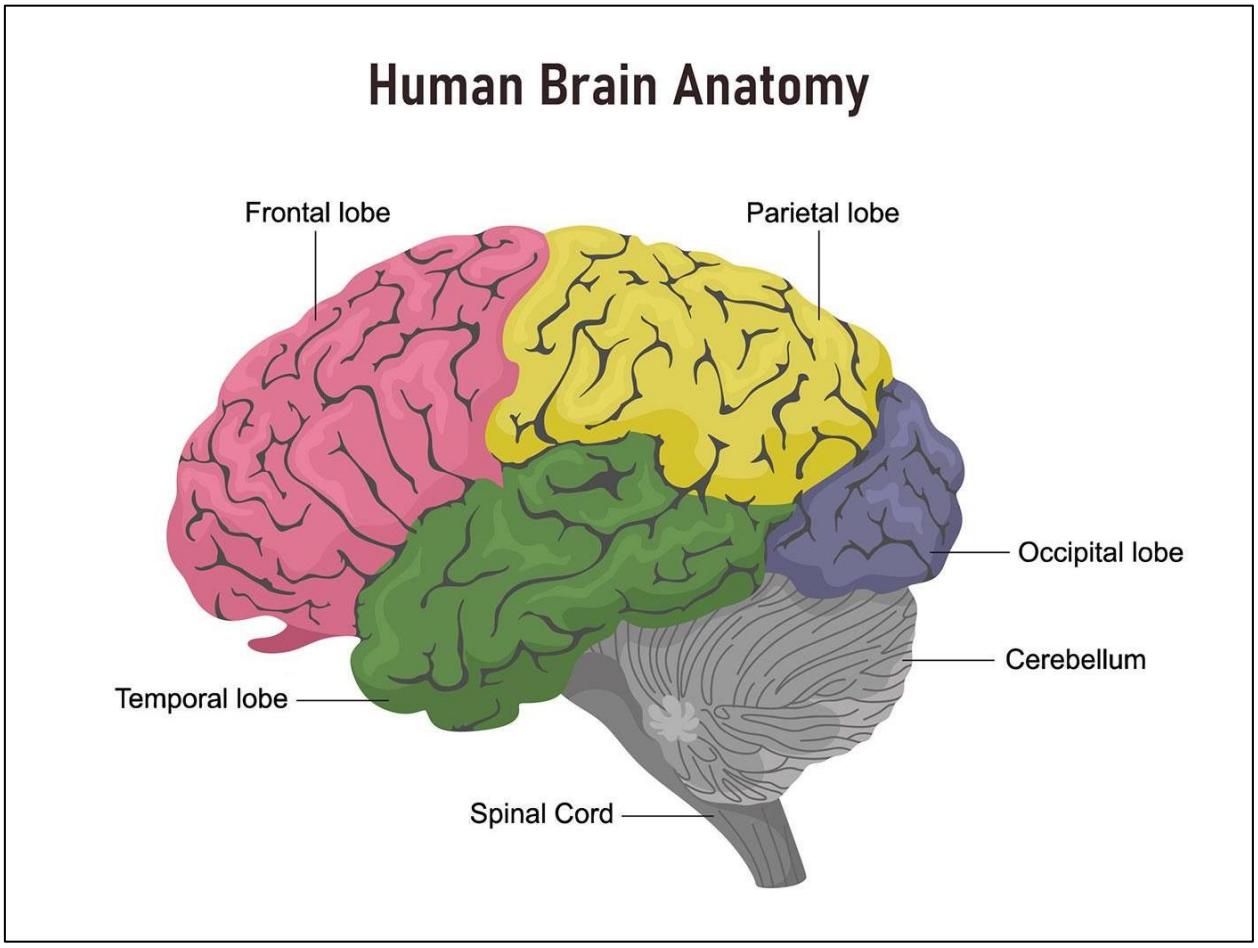


Figure 1: Illustration of the human brain and its lobes (*Adapted from:* Fahim et al., 2024)

Pathophysiological process of TBI and phases of brain injury

TBI is divided into two phases, namely, the primary and secondary phases. The primary phase is a direct result of mechanical force exerted on the head, leading to hematoma, lacerations, contusions and shearing of neurons, glial cells and axons (Nazwar *et al.*, 2023). The primary phase triggers systemic inflammation, increasing blood-brain barrier (BBB) permeability and triggering the secondary phase of the injury (Kinoshita, 2016). During the secondary phase, neurotransmitters such as glutamate, glycine, and aspartate are released, leading to the production of proinflammatory cytokines, reactive oxygen species (ROS), ischemia, and apoptosis (Greve and

Zink, 2009). The injury can be focal or widespread, depending on the impact or force applied to the brain (Dolenec *et al.*, 2020).

It is inevitable for the primary injury to happen; however, the secondary injury can be managed and controlled through suitable and proper techniques, such as oxygen pressure monitoring for severe traumatic brain injury (OXY-TC) and brain oxygen optimization in severe TBI, Phase 3 (BOOST3) (Diaz-Arrastia et al., 2024), to avert subsequent events such as hypotension, hypoxia and excessive cerebral perfusion pressure (CPP) (Vella *et al.*, 2017).

During the second phase of TBI, a complex cascade of cellular and molecular events occurs, mainly through the RhoA/Rho kinase (ROCK) and NLRP3 pathways, influencing the neuropathology and neurological dysfunction of the brain (Mulherkar and Tolias, 2020). These pathways play key roles in cell survival, oxidative stress, inflammation, neuronal plasticity, and cognitive function (Freire *et al.*, 2023). The known signaling pathways include nuclear factor-kappa B (NF-κB), a modulator of cellular activity that binds to a promoter region, inducing the transcription of various protein genes (Singh and Singh, 2020). Stimulation of NF-kB in neuronal and glial cells has been associated with neuroprotective activity and neurodegenerative conditions, including TBI (Singh and Singh, 2020). These genes induce inflammation and cell survival by acting as downstream mediators of receptor signaling, such as Toll-like receptor 4 (TLR-4) and tumor necrosis factor receptor-associated factor 6 (TRAF-6), in individuals with TBI. Therefore, inhibiting these receptors will consequently reduce apoptosis and inflammatory response after injury (Freire *et al.*, 2023).

The mitogen-activated protein kinase (MAPK) pathway is another crucial pathway in TBI. However, it has mostly been associated with neuronal apoptosis, neuroinflammation and cognitive impairments following TBI. The MAPK pathway comprises numerous protein kinases, including

c-Jun N-terminal kinase (JNK), extracellular signal-regulated kinase (ERK), and p38 MAPK (Kalra *et al.*, 2022; Karve *et al.*, 2016).

The phosphoinositide 3-kinase (PI3K)/Akt pathway is another pathway implicated in TBI pathology. It is responsible for regulating cell survival, neurogenesis, and synaptic plasticity. Studies have shown that initiation of the PI3K/Akt pathway promotes neuronal survival and improves recovery post-TBI (Kalra *et al.*, 2022). Thus, activating Brain-Derived Neurotrophic Factor (BDNF)/receptor tyrosine kinase (TrkB) signaling via PI3K/Akt/MAPK appears to be a promising neuroprotective treatment option for TBI (Cente *et al.*, 2023). BDNF has recently been the subject of several studies on neuroprotection following a TBI incident (Di Battista et al., 2015). This molecule binds to two types of receptors: TrkB, which has the highest affinity and the pan-neurotrophin receptor p75NTR, which belongs to the tumor necrosis factor receptor (TNFR) family and has the lowest affinity (Gustafsson *et al.*, 2021). The TrkB receptor activates MAPK, PI3K/mTOR, and phospholipase C-γ (PLC-γ) pathways. These pathways work together to promote the expression of genes involved in important brain functions such as neuronal survival, axonal sprouting, dendritic growth and synaptogenesis (Wang *et al.*, 2022). In an experiment by Wang et al. (2014), BDNF levels in CSF increased between 1 and 6 hours after brain trauma in rats. Augmented BDNF levels in CSF following TBI may be detrimental due to injury and the subsequent increase in pro-apoptotic BDNF target receptors (Failla *et al.*, 2016).

The mechanistic target of the mammalian target of rapamycin (mTOR) signaling pathway has also piqued the interest of TBI researchers. In non-pathological conditions, this pathway is vital for brain development, synaptic plasticity, metabolic regulation and memory (Movahedpour *et al.*, 2022). However, deviations in the mTOR signaling pathway can contribute to the development of a wide range of brain diseases, including TBI (Maiese, 2016).

Another main pathway is the Janus kinase/signal transducer and activator of transcription (JAK/STAT) pathway. This pathway is activated by cytokine and growth factor receptors and oversees signal transduction from the cellular surface to the nucleus (Kumar *et al.*, 2023). An experiment conducted by Zhao et al. (2011) reported that, 3 hours after cortical compression-induced TBI, the JAK2/STAT pathway was activated in neurons and astrocytes. This subsequently increased the secretion of inflammatory cytokines by T cells and macrophages (Zhao *et al.*, 2011).

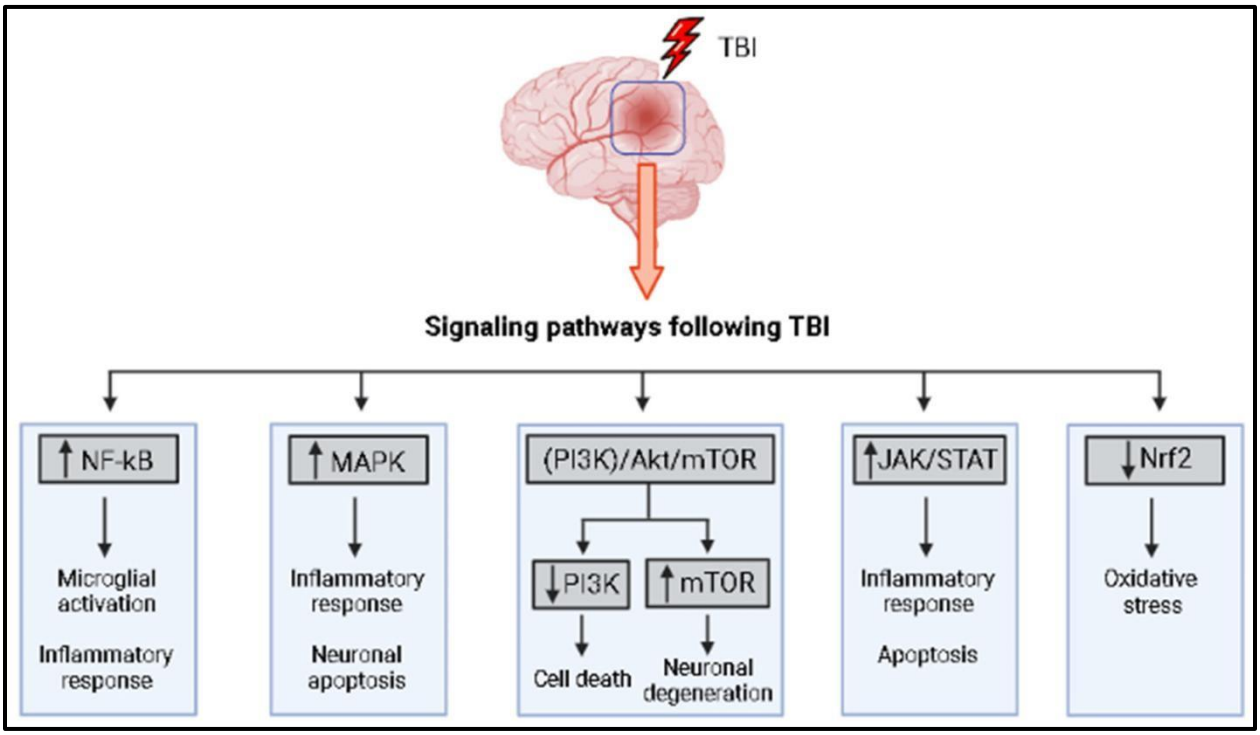


Figure 2: Summarized signaling pathways after TBI. Nuclear factor-kappa B (NF-κB) signaling pathway, mitogen-activated protein kinase (MAPK) pathway, PI3K/Akt/mTOR signaling pathway, Janus kinase/signal transducer and activator of transcription (JAK/STAT) pathway, nuclear factor erythroid 2-related factor 2 (Nrf2) pathway (Adapted from: Freire et al., 2023).

Biomarkers and their roles

S100 calcium-binding protein B

S100 calcium-binding protein B (S100B) derives from the S100 family of proteins, categorized by two EF-hand-type calcium-binding proteins, and plays a vital role in cell morphology. It has been found in high concentrations in the cytoplasm of astrocytes and other glial cells, including oligodendrocytes, ependymal cells, Schwann cells, retinal Muller cells and enteric glial cells (Du *et al.*, 2021; Mbele *et al.*, 2002). It plays a crucial role in several cellular processes, both intracellular and extracellular, due to its affinity for calcium (Ca^{2+}) and Zinc (Zn^{2+}) (Tsoporis *et al.*, 2010). S100B is a small, dimeric protein associated with diseases such as cancer, making it a potential target for therapeutic inhibitors (Wilder *et al.*, 2019). Furthermore, S100B interacts with a variety of cellular targets in a calcium-dependent manner, leading to conformational changes that enable its involvement in cell signaling (Malik *et al.*, 2008). The protein's function includes regulating trace metal homeostasis and excitotoxicity in the brain. Furthermore, S100B has been linked to neuropathological conditions and is regarded as a potential biomarker for brain-related conditions (Sen and Belli, 2007; Hagmeyer *et al.*, 2018). Changes in serum S100B concentrations can indicate the severity of neuroglial injury, making it a possible marker for neuronal injury (Hamadi *et al.*, 2023). S100B has been widely investigated and clinically used to rule out mild TBI (mTBI) (Oris *et al.*, 2023). However, the sensitivity of S100B alone in detecting TBI severity has been found to be relatively low in mTBI (Blais Lécuyer *et al.*, 2021). S100B is a reliable biomarker unaffected by hemolysis or storage conditions, making it suitable for clinical use. Serum levels are stable for up to 8 hours at room temperature and 48 hours at 2-8°C (Oris *et al.*, 2019).

The S100 calcium-binding protein B is involved in a range of physiological and pathological functions. It has been associated with calcium homeostasis, glial proliferation and axon growth (Alatas *et al.*, 2015). Following injury or ischemia, astrocytes release S100B, which can serve as a marker of BBB disruption (Lopez *et al.*, 2020). However, other studies of TBI patients have found no correlation between BBB disruption and peak S100B levels (Thelin *et al.*, 2017). The mechanisms that drive this diffusion across the BBB are not entirely clear. After diffusion into the bloodstream, S100B is eliminated through the kidneys and has been associated with renal failure in some reported cases (Li *et al.*, 2011). In addition to being a biomarker, S100B plays a dual role in molecular pathophysiological processes. At low concentrations, S100B exhibits neuroprotective and neurotrophic effects, preventing neuronal degeneration and promoting neuronal survival. At elevated concentrations, it can trigger the brain's inflammatory response by binding to the receptor for advanced glycation end products (RAGE) and activating the NF-κB pathway, thereby increasing the expression of pro-inflammatory cytokines (Oris *et al.*, 2023), Figure 3. Therefore, inhibiting the S100B/RAGE signaling pathway has emerged as a promising treatment strategy for TBI (Oris *et al.*, 2023).

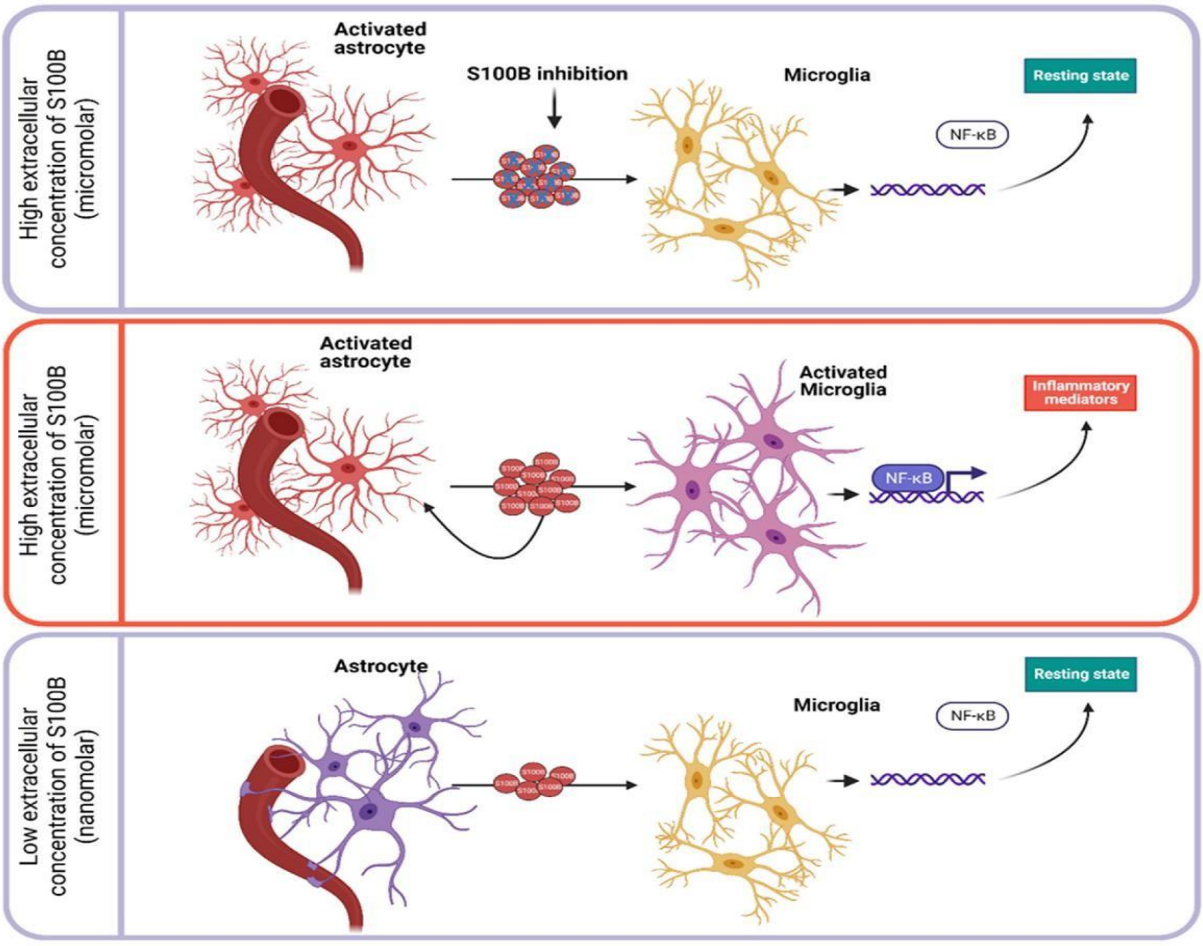


Figure 3: A schematic representation of extracellular S100B astrocytes regulation through the Nuclear factor-kappa B (NF-κB) signaling pathway.
(Adapted from: Michetti et al., 2023).
Pathological elevation of S100B to high extracellular concentrations (micromolar) by activated astrocytes induces microglia activation and triggers the NF-κB signaling pathway, leading to the production of inflammatory mediators and an astroglial reactivation feedback loop.
At low extracellular concentrations (nanomolar), astrocytes and microglia maintain a baseline resting state. Targeted S100B inhibition blocks this pathological cascade, suppressing downstream NF-κB activation and restoring microglia to a resting state.

At nanomolar concentrations, S100B has no effect on microglial homeostasis. Microglia exhibit pro-inflammatory phenotypes when exposed to micromolar concentrations of S100B. Negative regulation of extracellular S100B can shift microglia from an inflammatory to a homeostatic state by suppressing NF-κB signaling (Michetti *et al.*, 2023).

Glial Fibrillary Acidic Protein

Glial fibrillary acidic protein (GFAP) is an intermediate filament (IF) III protein that is encoded by the GFAP gene in humans chromosome 17q21 and is distinctively found in astrocytes in the central nervous system, enteric glial cells and non-myelinating Schwann cells in the peripheral nervous system (PNS) (Isaacs *et al.*, 1998; Yang and Wang, 2015). GFAP is the primary structural protein responsible for the IF cytoskeleton in astrocytes. GFAP, along with other cytoskeletal components such as microtubules and microfilaments, mechanically supports, stabilizes and reorganizes the astrocyte cell membrane and processes, which is significant for astrocyte morphological changes and reactivity in response to brain and spinal cord injury or disease (Hol and Pekny, 2015; Yang and Wang, 2015). Astrocyte cells respond to injuries, including TBI and other destructive neurological conditions, by undergoing a process known as "reactive astrogliosis," which involves cellular hypertrophy, proliferation and inflammation, which subsequently lead to increased GFAP levels (Eng *et al.*, 2000; Tzeng *et al.*, 2005), Figure 4. This increase in GFAP levels is associated with injury severity: reactive astrocytes near the injury site express the most GFAP, while levels decrease farther away (Burda *et al.*, 2016).

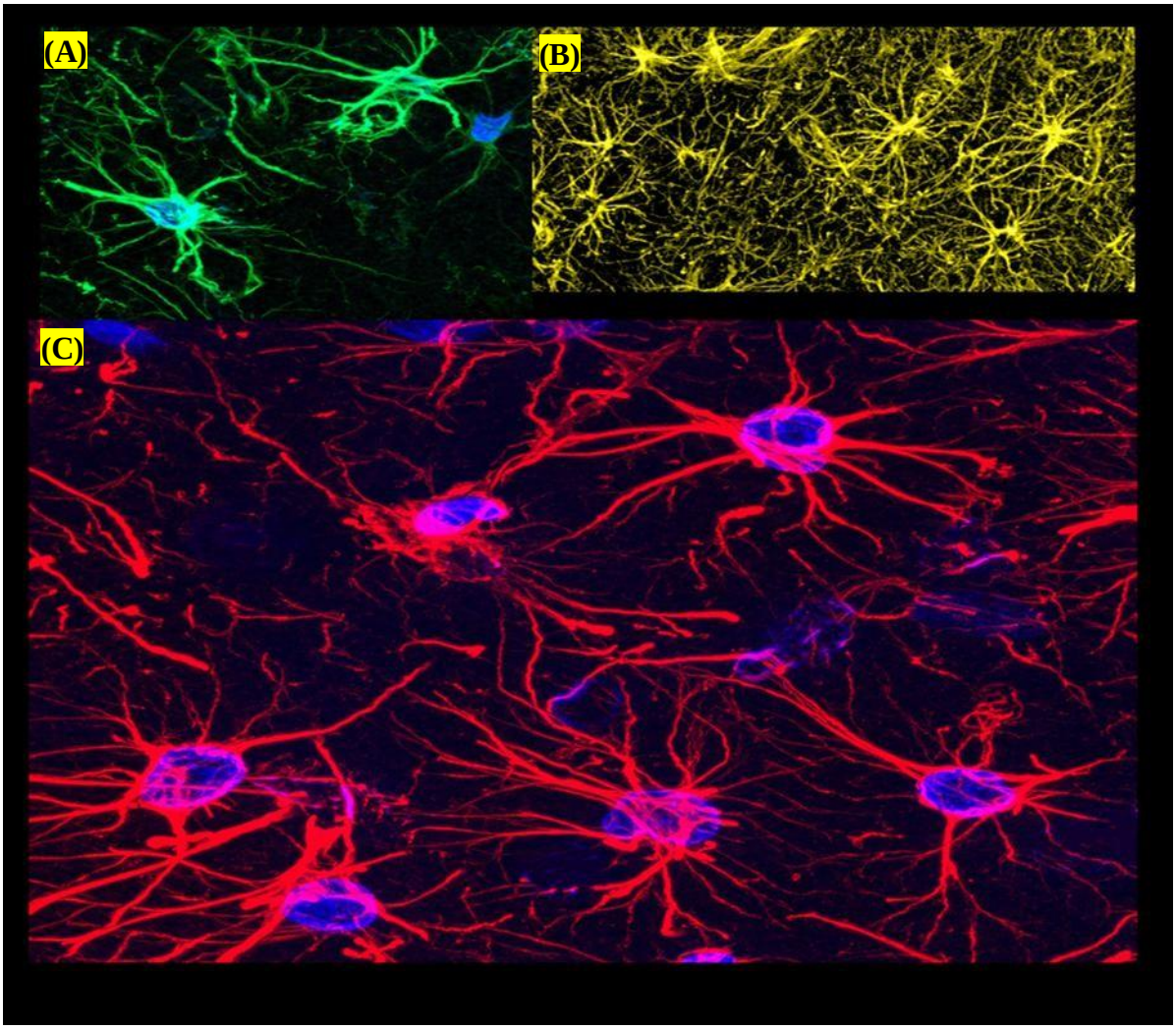


Figure 4: (A) and (B) Reactive astrocytes stained with vimentin showing structural intermediate filaments and nuclei (blue) of astrocytes GFAP (C) undergoing a cellular transformation triggered by TBI. Scale bar, 50 µm, (Adapted from: Le Douce et al., 2020).

Studies have shown that elevated serum GFAP levels can aid as a blood biomarker and a prognostic and diagnostic tool to detect the early development of a variety of neurological diseases and injuries, including TBI, stroke and other neurodegenerative disorders (Wang et al., 2021; Heimfarth *et al.*, 2022).

GFAP expression is regulated by several nuclear receptor hormones, growth factors and lipopolysaccharides, including ciliary neurotrophic factor, which activates the JAK/STAT signaling cascade, thereby inducing GFAP transcription (Gomes, Paulin et al. 1999). Post-translational modifications to GFAP include phosphorylation by kinases such as PKC and PKA, which are important for cytoplasmic signal transduction. One of the key phosphorylation pathways involves the G-protein-coupled mGluR receptor, which causes calcium influx and CaMKII activation (Yang and Wang, 2015).

In summary, GFAP is a critical structural and signaling protein in astrocytes that is dynamically regulated at the transcriptional and post-translational levels to mediate astrocyte activation and response to brain injuries. Understanding GFAP signaling pathways provides insight into astrocyte biology in both health and disease. GFAP has been recognized as a biomarker of astrocyte activation and as a marker of brain injury severity. Higher GFAP levels in blood or CSF after injury can help determine the extent of astrocytic damage and activation, allowing for a better assessment of injury severity and recovery outcomes (Abdelhak *et al.*, 2022).

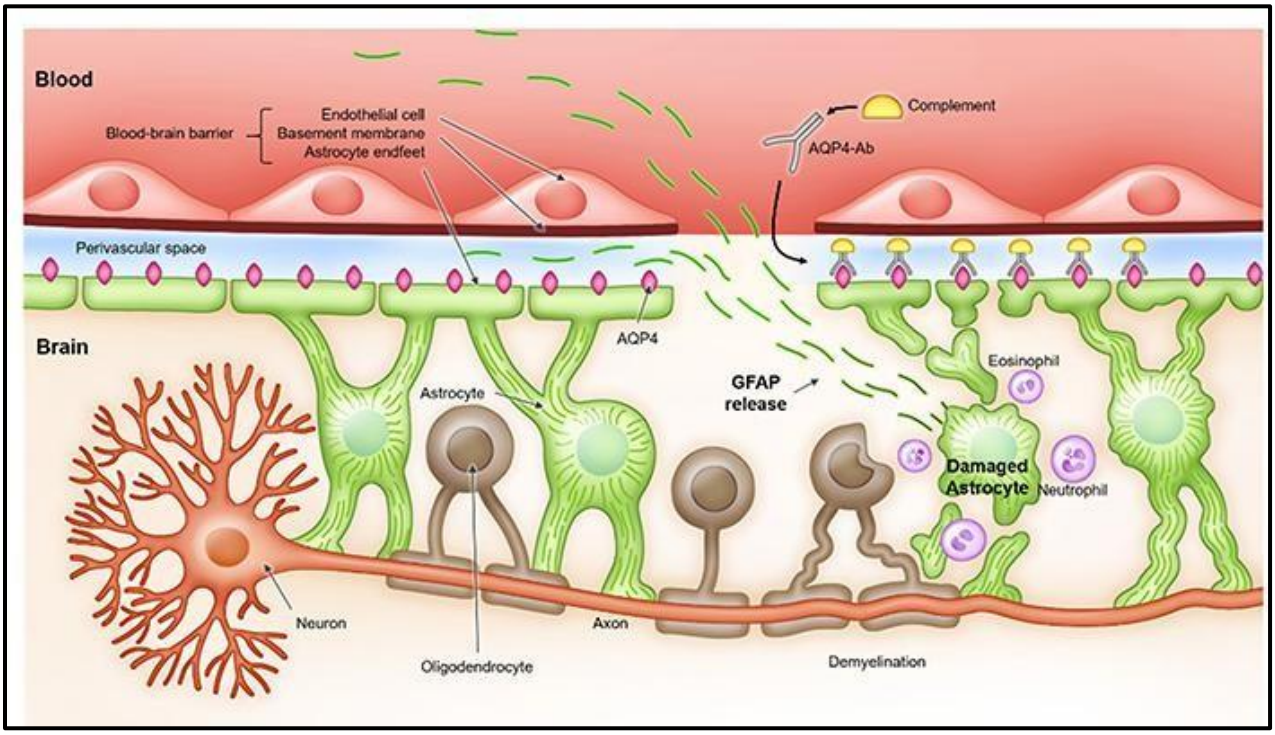


Figure 5: Dispensation of GFAP after astrocyte injury.
Anti-aquaporin-4 autoantibodies (AQP4-Ab) pass through a damaged blood-brain barrier (BBB) consisting of endothelial cells, basement membrane, and astrocytic endfeet. In the perivascular region, AQP4 antibodies attach to AQP4 water channels located on astrocytic endfeet, triggering the complement cascade and resulting in astrocyte destruction. Astrocytic damage results in the secretion of glial fibrillary acidic protein (GFAP) into the extracellular environment and circulation. Adapted from: (Kim et al., 2022).

Antibody and complement-dependent cellular cytotoxicity leads to the recruitment of inflammatory cells, demyelination, astrocyte damage and neuronal apoptosis. Following astrocyte damage, GFAP, an astrocytic scaffold protein, is released into the interstitial and cerebrospinal fluids, eventually reaching the bloodstream via an impaired BBB and/or glymphatic efflux (Kim et al., 2022).

Neuron-specific enolase

Neuron-specific enolase (NSE) is characterized as a cell-specific isoenzyme of the glycolytic enzyme enolase, specifically in the gamma form (γ). It serves as a biomarker for neurons and neuroendocrine cells and is associated with various physiological and pathological conditions, as well as TBI (Isgrò et al., 2015).

Studies have shown that elevated levels of NSE in serum are associated with neuronal damage, making it a potential biomarker in conditions like traumatic brain injury and stroke. It has also been found that the level of concentration of NSE correlates with the extent of brain insult, providing vital information for prognosis, diagnosis, and treatment planning (Lee *et al.*, 2021).

The signaling pathways of NSE known so far are the phosphatidylinositol 3-kinase/protein kinase B (PI3K/AKT) and mitogen-activated protein kinase/extracellular signal-regulated kinase (MAPK/ERK) pathways. These pathways control pro-inflammatory mechanisms, neurotrophic activity and neuronal growth regulation (McCoy *et al.*, 2023).

NSE measurement in clinical settings has the potential to identify patients at risk of poor outcomes, guide treatment decisions and monitor therapy response. However, further research is needed to fully understand its clinical utility and limitations.

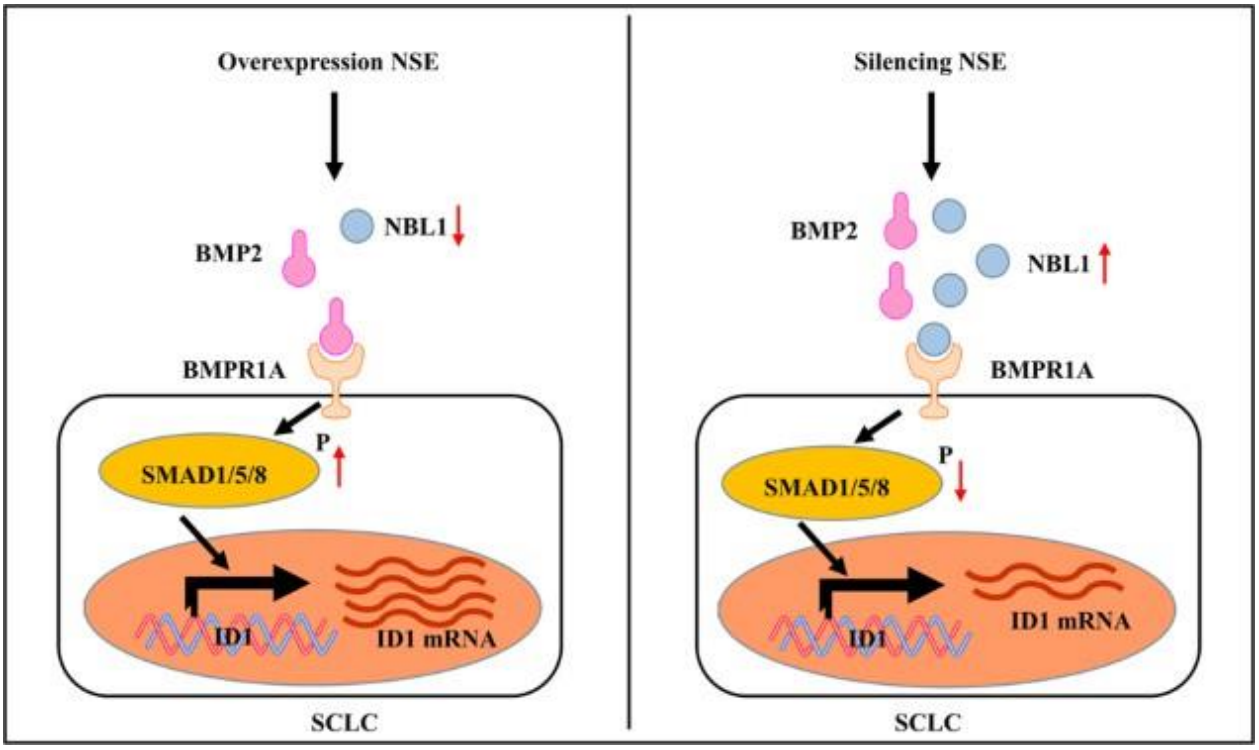


Figure 6: Signaling pathway of NSE regulating stem cell-like traits in Small-Cell Lung Cancer (SCLC) cells by modulating the BMP2/Smad/ID1 pathway via the antagonist NBL1 when silenced and overexpressed (Adapted from (Lu et al., 2022).

Serum Amyloid A

Serum Amyloid A (SAA) is a protein family that consists of SAA1, SAA2, and SAA4. SAA1 and SAA2 are the primary hepatic acute-phase proteins (APP) produced by hepatocytes; however, extrahepatic production has been reported (Yamada, 2006).

Serum amyloid A (SAA) is an acute-phase protein that is upregulated in response to TBI. SAA levels reach their peak in the bloodstream shortly after the insult and are associated with neuroinflammatory responses. Studies have shown that SAA is expressed in the brain following TBI, predominantly in microglia and astrocytes, emphasizing its role in the acute inflammatory response. Remarkably, SAA expression appears to be sex-dependent, with higher levels observed in males (Wicker *et al.*, 2019; Soriano *et al.*, 2020). In a clinical study, SAA concentrations in

serum on the first day of hypoxic-ischemic encephalopathy were found to be significantly correlated with the severity of damage in neonates (Aly *et al.*, 2011). Additionally, SAA levels in adult TBI patients with severe polytrauma have been documented (Rael *et al.*, 2009). However, it remains unclear whether circulating SAA levels are directly correlated with TBI severity. Specifically, there is currently a lack of experimental models of TBI that investigate SAA as a potential biomarker of injury severity.

Problem statement

Currently, the prognostic and diagnostic methods for TBI assessment during the patient admission and characteristic clinical outcomes for brain surgery include Glasgow coma score (GCS), which is the most commonly used, along with other assessments such as the absence of pupillary reactivity, activated partial thromboplastin time, lymphocyte ratio, international normalized ratio and platelet count (Kim *et al.*, 2020). TBI is classified as mild (score: 13–15), moderate (score: 9–12), and severe (score: <9) based on the level of consciousness, also known as the Glasgow coma scale (GCS) score after resuscitation (Maas *et al.*, 2012). Mild TBI (GCS 13–15) is also referred to as a concussion and is usually followed by a full neurological recovery. Although the majority of patients may develop short-term memory loss and concentration difficulties (Jerome *et al.*, 2017). In moderate TBI (GCS 9–13), the patient is usually in a confused and slow to react and in severe injury (GCS <9), the patient is in a coma state, unresponsive to light and other motor stimuli (Fernández-Salgueró *et al.*, 2002; Rauchman *et al.*, 2023). Computed Tomography (CT) and Magnetic Resonance Imaging (MRI) are additional tools to GCS, used to assess the severity of the injury (Fadalla *et al.*, 2022). However, recent investigations have reported several limitations and the unreliability of the GCS scoring system in assessing injury severity in TBI patients (Sherer *et*

al., 2008). Studies have reported several factors, such as administration of sedatives, early intubation, facial injuries and administration of paralyzing agents or intoxicants, that may hinder the reliability and accuracy of the GCS scoring system (Murray *et al.*, 1999). GCS consists of three components: eye, verbal and motor responses. However, GCS does not include evaluation of brainstem reflexes, which determine brainstem stimulation activity (Matis and Birbilis, 2008). Other limitations have been associated with the low sensitivity of CT and MRI scanning for diffuse axonal injury and diffuse vascular injury (Amyot *et al.*, 2015), failure to predict the risk of cerebral vasospasm or hypoperfusion (Fatima *et al.*, 2019) and different scores assessed on the same patient by different physicians (Baratloo *et al.*, 2016). These limitations have led to the search for more reliable approaches to assess TBI by incorporating imaging and biomarkers (Diaz-Arrastia & Kochanek, 2023). Therefore, given the high morbidity and mortality associated with TBI, this study was set out to examine different biomarkers and compare their predictive value as risk indicators for TBI.

Justification for the study

Among common neurological disorders, TBI has the highest incidence and carries a significant public health burden (Bruns Jr and Hauser, 2003; Owolabi *et al.*, 2023). There is growing evidence that TBI is not just an acute problem but also a chronic illness with long-term effects, such as a higher chance of late-onset neurodegeneration (Maas *et al.*, 2022). The current GCS diagnostic tools do not detect additional neurological signs, such as focal lesions, brainstem reflexes, corneal reflexes, pupil response and oculocephalic reflexes, which are often indicative of significant midbrain or pontine injury and are frequently observed in severe TBI (Mena *et al.*, 2011). These gaps often lead to underdiagnosis of transtentorial herniation or locked-in syndrome, in which motor responses appear intact despite significant impairment (Bodien *et al.*, 2021). Additionally, it has been reported that symptoms of TBI can overlap with those of other conditions, like obsessive-compulsive disorder (OCD), post-traumatic stress disorder (PTSD), impaired memory and cognitive function. Hence, misdiagnosis of TBI through the GCS system can be a significant setback, emphasizing the importance of proper management (Sumpter and McMillan, 2005). However, biomarkers such as GFAP, S100B, NSE and SAA, among others, provide objective biological indicators of brain injury, pathological conditions, including stroke, hypoxic-ischemic injury, seizures, neuroinflammation, and systemic trauma or inflammation. Therefore, addressing significant limitations of the GCS, which relies subjectively on behavioral evaluations (Hossain *et al.*, 2024). In contrast to GCS, biomarkers directly quantify subtle lesions and neuronal injury, axonal damage and gliosis, facilitating accurate categorization of TBI severity beyond the limited correlation of GCS with morphological damage (National Academies of Sciences *et al.*, 2023). The study addresses whether the relationships between the aforementioned biomarkers, clinical TBI severity and GCS (which have predominantly been characterized in international cohorts), are reproducible and clinically informative in a South African population.

Expected Contribution to Knowledge

The Glasgow Coma Scale (GCS) has certain limitations in TBI, particularly in its short- and long-term use, because it relies on observable behaviours rather than underlying physiological and pathological factors. GCS inadequately predicts outcomes, frequently results in suboptimal recovery due to unrecognized axonal injury and the subscale combinations obscure prognostic details such as motor abnormalities. Several factors, such as intubation, anesthesia, intoxication, or facial trauma, obscure initial evaluations, whereas interobserver reliability among novice clinicians diminishes accuracy and dependability. Therefore, interest in developing a more accurate or complementary tool, such as GFAP, S100B, NSE, and SAA biomarkers, to enhance the accuracy and reliability of TBI diagnosis is vital to improve patient outcomes and deepen understanding of other neurological phases associated with TBI. Biomarkers substantially improve TBI diagnosis by providing objective evidence of cerebral injury, inflammation, neuronal apoptosis and neuroimmunological changes.

Research questions

- Can biomarkers predict the severity of brain injury?
- Are biomarkers better diagnostic tools than the GCS scoring system?

Aim of the study

The aim of this study is to investigate potential biomarkers, including S100 calcium-binding protein B (S100B), Glial Fibrillary Acidic Protein (GFAP), Neuron-Specific Enolase (NSE), and Serum Amyloid A, for the diagnosis and prognosis of traumatic brain injury.

Objectives

The specific objectives of this study are as follows:

1. To determine the concentration of serum S100B protein versus the control, and its effect in TBI.
2. To determine the concentration of serum GFAP versus control, and its effect in TBI.
3. To determine the concentration of serum NSE protein versus control, and its effect in TBI.
4. To determine the concentration of serum Amyloid A protein versus control, and its effect in TBI.
5. To compare the concentration of serum S100B protein, GFAP, NSE, and amyloid A in TBI.

Overview of Methodology

The following design was adopted for the collection, analysis and interpretation of this study. Ethical approval for this study was obtained from the Biomedical Research Ethics Committee (BREC) of the University of KwaZulu-Natal (Ethics number: BREC/00002147/2020).

Study population

The study was conducted on male and female patients diagnosed with severe to moderate TBI from the Inkosi Albert Luthuli Central Hospital (IALCH), Durban, KwaZulu-Natal, South Africa. The control group consisted of normal individuals with no underlying neurological disease or history of TBI.

Sample size

This is a prospective study that used purposive sampling of serum samples from TBI patients.

The sample size was determined by using the following parameters:

N=75, where n1=n2=n3=25

Power calculation

For comparing three independent groups using a one-way ANOVA, assume: Number of groups k=3, total sample N=75, n=25 per group (control, moderate and severe TBI), significance level α=0.05, two-sided hypothesis, and a desired power = 80%. Using the **Cohen's formula,** $f=\sqrt{\sum^{3}_{i}=1 p_i(\mu_i-\mu)^2/\sigma^2}$ with 75 participants (25 per group) provide approximately 80% power to detect a large effect (f=0.40).

Table 2: Demographic profile of samples

TBI group	Age range	Male	Female
Control	18-45^{+}	10	15
Moderate		24	1
Severe		23	2

Inclusion/ exclusion criteria

Inclusion criteria: Male and female patients diagnosed with moderate (9-12) to severe < 9 TBI based on the Glasgow Coma Scale (GCS), above the age of 18 years old. Age, gender and informed consent were obtained from participants and recorded in an Excel file. All patients were managed according to the Brain Trauma Foundation guidelines, including surgery and ICP monitoring for patients with severe TBI.

Exclusion criteria: Patients had the right to choose whether to participate; therefore, any patient who declined to participate was excluded. In addition, any patient who fell under the age of 18 years old, had mild TBI (13-15) based on (GCS) and other neurological conditions based on patient's hospital record was excluded. Patients that had a prior history of TBI were also excluded. The control group consisted of patients that had no prior history of TBI or other neurological or systemic conditions. Lifestyle choices were not recorded. Temporal changes of biomarkers over time and follow-up data were not collected.

Blood collection

After obtaining informed consent from eligible patients, blood samples were collected by Inkosi Albert Luthuli physicians within 24 hours of traumatic head injury using serum separator tubes. The sample tubes were appropriately labeled and immediately sent to a local laboratory at the Westville campus. Samples were allowed to clot overnight at room temperature (due to logistical limitations in infrastructural support in sample processing), then centrifuged at 22°C or lower for 20 minutes at 1000xg. Undiluted serum samples were then aliquoted and stored at -80 °C. The samples underwent a total of two freeze-thaw cycles.

Scan details collection

All CT scans were performed in accordance with the Neuroradiology Department's protocol. Investigators who read the scans were blinded to clinical information. CT classification was done by a radiologist and categorized as traumatic subarachnoid hemorrhage, extradural hematoma, subdural hematoma or diffuse axonal injury. The classification was based on GCS: moderate (9-12) and severe (< 9). Axial CT images of specific patients were obtained from the hospital's Picture Archiving and Communication System and stored in Digital Imaging and Communication in Medicine format. These CT scans were obtained during standard clinical

procedures using either a 128-slice SOMATOM Definition AS scanner or a SOMATOM Definition Flash CT Scanner (Siemens Definition Edge Plus and Siemens Definition Flash).

Reconstruction: 1.0mm x 0.7mm (Thin slice/source images for reporting and planning of 3D reconstruction).

Brain reconstruction: Kernel –Hr38, Window - Cerebrum, 5.0mm x5.0 mm Axial

Bone reconstruction: Kernel Hr60- , Window- Inner ear, 5.0mm x5.0 mm Axial

Exposure: Dose and exposure factors are modulated automatically by the scanner.

Multiplex immunoassay procedures

ProcartaPlex kits: EPX010-12339-901 HU S100B Simplex, EPX010-12336-901 HU GFAP, EPX010-12335-901 HU NSE Simplex and EPX01A-12136-901 HU SAA Simplex were purchased from Life Sciences Solutions Group, Thermo Fisher Scientific, Waltham, MA, USA. The immunoassay procedures were each performed according to the manufacturer's instructions for the analyte. Samples were run once in singlicate.

Statistical Analysis

Analysis was achieved using the Statistical Package for the Social Sciences, version 30.0.0.0 (SPSS, IBM Corporation). Kruskal-Wallis and Pearson Chi-Square tests were used to assess the association between variables, with 95% confidence intervals. Results with two-tailed $p < 0.05$ were considered significant, p-values were not corrected for multiple comparisons and the results obtained were expressed as median and interquartile range.

Overview of thesis

This thesis is written and submitted in manuscript form. As recommended by the University of KwaZulu-Natal College of Health Sciences guidelines, this thesis comprises 5 chapters and appendices as follows:

Chapter 1: This chapter provides background information on the study, the literature review, the problem statement and research gap, the study justification, the research questions, the research objectives, the general overview of the research methodology, the overview of the thesis, and the references.

Chapter 2: This chapter contains a review article titled "The potential of serum S100 calcium-binding protein B and glial fibrillary acidic protein as biomarkers for traumatic brain injury," published in the Translational Research in Anatomy Journal. It summarized the most suitable potential biomarkers for TBI diagnosis. DOI: https://doi.org/10.1016/j.tria.2022.100228.

Chapter 3: This chapter contains the first manuscript from the study on S100B and GFAP expressed in astrocytes and reports results for objectives 1 and 2. In this chapter, we determined the concentration of S100B and GFAP in TBI patients. The findings were presented in a manuscript titled "Evaluating Traumatic Brain Injury Severity Through GFAP and S100B Biomarkers: An Immunoassay-Based Approach." The manuscript was submitted to and is under review by the Journal of Neurocritical Care.

Chapter 4: This chapter contains the second manuscript from the study on NSE and SAA biomarkers, expressed in neurons, neuroendocrine cells, and microglia/macrophages. The chapter reports results for objectives 3 and 4. In this chapter, we determined the concentration of NSE and SAA in TBI patients. The findings were presented in a manuscript titled Evaluation of Serum Neuron-Specific Enolase and Serum Amyloid A in Moderate-to-Severe Traumatic Brain Injury in

Association with Glasgow Coma Scale and CT Findings for Improved Classification in a South African Cohort.

Chapter 5: This chapter contains the synthesis, conclusion, recommendations, suggestions for future research, limitations, a summary of findings and contributions to knowledge, and references. The chapter reports results for objective 5.

Appendices: This contains the ethical and hospital approvals, data collection sheet, standard curves and the informed consent form.

References

1) Abdelhak, A., Foschi, M., Abu-Rumeileh, S., Yue, J. K., D'Anna, L., Huss, A., Oeckl, P., Ludolph, A. C., Kuhle, J., Petzold, A., Manley, G. T., Green, A. J., Otto, M., & Tumani, H. (2022). Blood GFAP as an emerging biomarker in brain and spinal cord disorders. *Nature Reviews Neurology*, *18*(3), 158-172.
2) Alatas, Ö. D., Gürger, M., Atesçelik, M., Yildiz, M., Demir, C. F., Ekingen, E., Kalayci, M., Ilhan, N., & Acar, E. (2015). Neuron-Specific Enolase, S100 Calcium-Binding Protein B, and Heat Shock Protein 70 Levels in Patients with Intracranial Hemorrhage. *Medicine*, *94*(45), e2007.
3) Aly, H., Hamed, Z., Mohsen, L., Ramy, N., Arnaoot, H., & Lotfy, A. (2011). Serum amyloid A protein and hypoxic ischemic encephalopathy in the newborn. *Journal of Perinatology*, *31*(4), 263-268.
4) Al-Rawi NH, Atiyah KM. 2009. Salivary neuron-specific enolase: an indicator for neuronal damage in patients with ischemic stroke and stroke-prone patients. Clin Chem Lab Med, 47(12):1519-1524.
5) Amur, S., LaVange, L., Zineh, I., Buckman-Garner, S., & Woodcock, J. (2015). Biomarker Qualification: Toward a Multiple Stakeholder Framework for Biomarker Development, Regulatory Acceptance and Utilization. *Clinical Pharmacology & Therapeutics*, *98*(1), 34-46.
6) Amyot, F., Arciniegas, D. B., Brazaitis, M. P., Curley, K. C., Diaz-Arrastia, R., Gandjbakhche, A., Herscovitch, P., Hinds, S. R., 2nd, Manley, G. T., Pacifico, A., Razumovsky, A., Riley, J., Salzer, W., Shih, R., Smirniotopoulos, J. G., & Stocker, D. (2015). A Review of the Effectiveness of Neuroimaging Modalities for the Detection of Traumatic Brain Injury. *J Neurotrauma*, *32*(22), 1693-1721.

7) Andraos, C., Siddiqi, A., Brazdzionis, J. & Siddiqi, J. 2025. Limitations of The Glasgow Coma Scale: Challenges And Considerations. *Cureus,* 17**,** E78900.

8) Baker, C. M., Burks, J. D., Briggs, R. G., Conner, A. K., Glenn, C. A., Sali, G., McCoy, T. M., Battiste, J. D., O'Donoghue, D. L., & Sughrue, M. E. (2018). A Connectomic Atlas of the Human Cerebrum—Chapter 1: Introduction, Methods, and Significance. *Operative Neurosurgery*, *15*(suppl_1), S1-S9.

9) Baratloo, A., Shokravi, M., Safari, S., & Kamal Aziz, A. (2016). Predictive value of Glasgow Coma Score and Full Outline of Unresponsiveness Score on the Outcome of Multiple Trauma Patients [Article]. *Archives of Iranian Medicine*, *19*(3), 215-220.

10) Blais Lécuyer, J., Mercier, É., Tardif, P.-A., Archambault, P. M., Chauny, J.-M., Berthelot, S., Frenette, J., Perry, J., Stiell, I., Émond, M., Lee, J., Lang, E., McRae, A., Boucher, V., & Le Sage, N. (2021). S100B protein level for the detection of clinically significant intracranial haemorrhage in patients with mild traumatic brain injury: a subanalysis of a prospective cohort study. *Emergency Medicine Journal*, *38*(4), 285.

11) Bodien, Y. G., Barra, A., Temkin, N. R., Barber, J., Foreman, B., Vassar, M., Robertson, C., Taylor, S. R., Markowitz, A. J., Manley, G. T., Giacino, J. T. & Edlow, B. L. 2021. Diagnosing Level Of Consciousness: The Limits Of The Glasgow Coma Scale Total Score. *J Neurotrauma,* 38**,** 3295-3305.

12) Bradshaw, D., Nannan, N., Groenewald, P., Joubert, J., Laubscher, R., Nojilana, B., Norman, R., Pieterse, D., & Schneider, M. (2005). Provincial mortality in South Africa, 2000: priority-setting for now and a benchmark for the future. *South African Medical Journal*, *95*(7), 496-503.

13) Bruns Jr, J. & Hauser, W. A. 2003. The Epidemiology Of Traumatic Brain Injury: A Review. *Epilepsia,* 44**,** 2-10.

14) Bui, T. T., & Das, J. M. (2020). Neuroanatomy, Cerebral Hemisphere.

15) Bui, T. & Das, J. M. 2023. Neuroanatomy, Cerebral Hemisphere. StatPearls. Treasure Island (FL) companies. Disclosure: Joe Das declares no relevant financial relationships with ineligible companies.: StatPearls Publishing.

16) Buitendag, J., Kong, V., Bruce, J., Laing, G., Clarke, D., & Brysiewicz, P. (2017). The spectrum and outcome of paediatric traumatic brain injury in KwaZulu-Natal Province, South Africa, have not changed over the last two decades. *South African Medical Journal*, *107*(9), 777-780.

17) Burda, J. E., Bernstein, A. M., & Sofroniew, M. V. (2016). Astrocyte roles in traumatic brain injury. *Exp Neurol*, *275 Pt 3*(0 3), 305-315.

18) Catani, M. (2017). The clinical anatomy of the temporal and parietal lobes. *Cortex*, *97*, 160-163.

19) Cente, M., Matyasova, K., Csicsatkova, N., Tomikova, A., Porubska, S., Niu, Y., Majdan, M., Filipcik, P., & Jurisica, I. (2023). Traumatic MicroRNAs: Deconvolving the Signal After Severe Traumatic Brain Injury. *Cellular and Molecular Neurobiology*, *43*(3), 1061-1075.

20) Dewan, M. C., Rattani, A., Gupta, S., Baticulon, R. E., Hung, Y.-C., Punchak, M., Agrawal, A., Adeleye, A. O., Shrime, M. G., Rubiano, A. M., Rosenfeld, J. V., & Park, K. B. (2019). Estimating the global incidence of traumatic brain injury. *Journal of Neurosurgery*, 1-18.

21) Di Battista, A. P., Buonora, J. E., et al. 2015. Blood biomarkers in moderate-to-severe traumatic brain injury: potential utility of a multi-marker approach in characterizing outcome. Frontiers in Neurology, 6, 110.

22) Dolenec, P., Pilipović, K., Janković, T. V., & Župan, G. (2020). Pattern of Neuronal and Axonal Damage, Glial Response, and Synaptic Changes in Rat Cerebellum within the First

Week following Traumatic Brain Injury. *Journal of neuropathology and experimental neurology*.

23) Diaz-Arrastia, R., Bernard, F., et al. 2024. Monitoring patients with severe traumatic brain injury. The Lancet Neurology, 23, 230-231.

24) Diaz-Arrastia, R. & Kochanek, P. M. 2023. Pharmacotherapy for Traumatic Brain Injury: The Next Generation of Clinical Trials. Neurotherapeutics, 20, 1428-1432.

25) Du, J., Yi, M., Zhou, F., He, W., Yang, A., Qiu, M., & Huang, H. (2021). S100B is selectively expressed by gray matter protoplasmic astrocytes and myelinating oligodendrocytes in the developing CNS. *Molecular Brain*, *14*(1), 154.

26) Eaton, J., Hanif, A. B., Grudziak, J., & Charles, A. (2017). Epidemiology, Management, and Functional Outcomes of Traumatic Brain Injury in Sub-Saharan Africa. *World Neurosurgery*, *108*, 650-655.

27) Embolo, F. N., Bellamkonda, N., Dickson, D., Motwani, G., Mbeboh, S. N., Mbiarikai, M., Oben, E., Oke, R. A., Dicker, R. A., Juillard, C., Christie, S. A., & Chichom-Mefire, A. (2021). Epidemiology of traumatic brain injury based on clinical symptoms amongst inhabitants of the Southwest Region of Cameroon: a community-based study. *Brain Injury*, *35*, 1184 - 1191.

28) Eng, L. F., Ghirnikar, R. S., & Lee, Y. L. (2000). Glial fibrillary acidic protein: GFAP-thirty-one years (1969-2000). *Neurochem Res*, *25*(9-10), 1439-1451.

29) Fadalla, T., Jalaleldean, B., Suliman, M., Elsayed, M., Elmahdi, M., & Elsalawi, W. (2022). Post-traumatic bilateral synchronous acute extradural hematomas: A case report and review of the literature. *Annals of Medicine and Surgery*, *75*, 103377.

30) Failla, M. D., Conley, Y. P., & Wagner, A. K. (2016). Brain-Derived Neurotrophic Factor (BDNF) in traumatic brain injury–related mortality: interrelationships between genetics and

acute systemic and central nervous system BDNF profiles. *Neurorehabilitation and neural repair*, *30*(1), 83-93.

31) Fahim, David & Grewal, Joshveer & Ellendula, Ritvik. (2024). A Novel Approach to Image EEG Sleep Data for Improving Quality of Life in Patients Suffering From Brain Injuries Using DreamDiffusion. 10.48550/arXiv.2407.02673.

32) Fatima, N., Shuaib, A., Chughtai, T. S., Ayyad, A., & Saqqur, M. (2019). The Role of Transcranial Doppler in Traumatic Brain Injury: A Systemic Review and Meta-Analysis. *Asian J Neurosurg*, *14*(3), 626-633.

33) Fernández-Salgueiro, P., Vargas, C., & Chacón, J. M. (2002). Traumatismos craneoencefálicos. Escalas de valoración para la medida de resultados en rehabilitación. *Rehabilitación*, *36*, 408–417.

34) Figg, W. D., & Newell, D. R. (2014). Pharmacologic Biomarkers in the Development of Stratified Cancer Medicine. *Clinical Cancer Research*, *20*(10), 2525-2529.

35) Freire, M. A. M., Rocha, G. S., Bittencourt, L. O., Falcão, D., Lima, R. R., & Cavalcanti, J. R. L. P. (2023). Cellular and Molecular Pathophysiology of Traumatic Brain Injury: What Have We Learned So Far? *Biology*, *12*(8), 1139.

36) Ghajar, J. (2000). Traumatic brain injury. *Lancet*, *356*(9233), 923-929.

37) Gomes, F. C. A., Paulin, D., & Moura Neto, V. (1999). Glial fibrillary acidic protein (GFAP): modulation by growth factors and its implication in astrocyte differentiation. *Brazilian Journal of Medical and Biological Research*, *32*.

38) Greve, M. W., & Zink, B. J. (2009). Pathophysiology of traumatic brain injury. *Mount Sinai Journal of Medicine: A Journal of Translational and Personalized Medicine*, *76*(2), 97-104.

39) Gustafsson, D., Klang, A., Thams, S., & Rostami, E. (2021). The role of BDNF in experimental and clinical traumatic brain injury. *International Journal of Molecular Sciences*, *22*(7), 3582.

40) Hagmeyer, S., Cristóvão, J. S., Mulvihill, J. J. E., Boeckers, T. M., Gomes, C. M., & Grabrucker, A. M. (2018). Zinc Binding to S100B Affords Regulation of Trace Metal Homeostasis and Excitotoxicity in the Brain [Original Research]. *Frontiers in Molecular Neuroscience, 10*.

41) Hamadi, N., Beegam, S., Zaaba, N. E., Elzaki, O., Altamimi, M. A., & Nemmar, A. (2023). Neuroinflammation, Oxidative Stress, Apoptosis, Microgliosis and Astrogliosis in the Cerebellum of Mice Chronically Exposed to Waterpipe Smoke. *Biomedicines*, *11*(4), 1104.

42) Heimfarth, L., Passos, F. R. S., Monteiro, B. S., Araújo, A. A. S., Quintans Júnior, L. J., & Quintans, J. S. S. (2022). Serum glial fibrillary acidic protein is a body fluid biomarker: A valuable prognostic for neurological disease - A systematic review. *Int Immunopharmacol*, *107*, 108624.

43) Hol, E. M., & Pekny, M. (2015). Glial fibrillary acidic protein (GFAP) and the astrocyte intermediate filament system in diseases of the central nervous system. *Curr Opin Cell Biol*, *32*, 121-130.

44) Hossain, I., Marklund, N., Czeiter, E., Hutchinson, P. & Buki, A. 2024. Blood Biomarkers For Traumatic Brain Injury: A Narrative Review Of Current Evidence. *Brain Spine,* 4**,** 102735.

45) Huss, R. (2015). Chapter 19 - Biomarkers. In A. Atala & J. G. Allickson (Eds.), *Translational Regenerative Medicine* (pp. 235-241). Academic Press.

46) Isaacs, A., Baker, M., Wavrant-De Vrièze, F., & Hutton, M. (1998). Determination of the gene structure of human GFAP and absence of coding region mutations associated with frontotemporal dementia with Parkinsonism linked to chromosome 17. *Genomics*, *51*(1), 152-154.

47) Isgrò, M. A., Bottoni, P., & Scatena, R. (2015). Neuron-Specific Enolase as a Biomarker: Biochemical and Clinical Aspects. *Adv Exp Med Biol*, *867*, 125-143.

48) Jerome, E., Laing, G. L., Bruce, J. L., Sartorius, B., Brysiewicz, P., & Clarke, D. L. (2017). *An audit of traumatic brain injury (TBI) in a busy developing-world trauma service exposes a significant deficit in resources available to manage severe TBI* (Vol. 107) [Traumatic brain injury; Trauma; Audit; Developing world].

49) Kalra, S., Malik, R., Singh, G., Bhatia, S., Al-Harrasi, A., Mohan, S., Albratty, M., Albarrati, A., & Tambuwala, M. M. (2022). Pathogenesis and management of traumatic brain injury (TBI): role of neuroinflammation and anti-inflammatory drugs. *Inflammopharmacology*, *30*(4), 1153-1166.

50) Kalyan, S., Nadasan, T. and Puckree, T. (2007). The Epidemiology of Traumatic Brain Injuries (TBI): A Literature Review. *South African Journal of Physiotherapy*, *63*(3), 32.

51) Karve, I. P., Taylor, J. M., & Crack, P. J. (2016). The contribution of astrocytes and microglia to traumatic brain injury. *British Journal of Pharmacology*, *173*(4), 692-702.

52) Kim, H., Lee, E.-J., Lim, Y.-M., & Kim, K.-K. (2022). Glial Fibrillary Acidic Protein in Blood as a Disease Biomarker of Neuromyelitis Optica Spectrum Disorders [Mini Review]. *Frontiers in Neurology*, *13*.

53) Kim, N. Y., Lim, J., Lee, S., Kim, K., Hong, J. H., & Chun, D. H. (2020). Hematological factors predicting mortality in patients with traumatic epidural or subdural hematoma undergoing emergency surgical evacuation: A retrospective cohort study. *Medicine (Baltimore)*, *99*(37), e22074.

54) Kinoshita, K. (2016). Traumatic brain injury: pathophysiology for neurocritical care. *Journal of Intensive Care*, *4*(1), 29.

55) Kong, V. Y., Odendaal, J. J., Bruce, J. L., Laing, G. L., Jerome, E., Sartorius, B., Brysiewicz, P., & Clarke, D. L. (2017). Quantifying the funding gap for management of traumatic brain

injury at a major trauma centre in South Africa [Article]. *South African Journal of Surgery*, *55*(4), 26-30.

56) Kumar, S., Mehan, S., & Narula, A. S. (2023). Therapeutic modulation of JAK-STAT, mTOR, and PPAR-γ signaling in neurological dysfunctions. *Journal of Molecular Medicine*, *101*(1), 9-49.

57) Lee, D., Cho, Y., Ko, Y., Heo, N. H., Kang, H. G., & Han, S. (2021). Neuron-specific enolase level as a predictor of neurological outcome in near-hanging patients: A retrospective multicenter study. *PLoS One*, *16*(2), e0246898.

58) Leung, J. M., Obeidat, M. e., Sadatsafavi, M., & Sin, D. D. (2019). Introduction to precision medicine in COPD. *European Respiratory Journal*, *53*(4), 1802460.

59) Li, J. P., Lu, L., Wang, L. J., Zhang, F. R., & Shen, W. F. (2011). Increased serum levels of S100B are related to the severity of cardiac dysfunction, renal insufficiency and major cardiac events in patients with chronic heart failure. *Clin Biochem*, *44*(12), 984-988.

60) Lopez, M. G., Hughes, C. G., DeMatteo, A., O'Neal, J. B., McNeil, J. B., Shotwell, M. S., Morse, J., Petracek, M. R., Shah, A. S., Brown, N. J., & Billings, F. T., IV. (2020). Intraoperative Oxidative Damage and Delirium after Cardiac Surgery. *Anesthesiology*, *132*(3), 551-561.

61) Lu, L., Zha, Z., Zhang, P., Wang, P., Liu, X., Fang, X., Weng, C., Li, B., Mao, H., Wang, L., Guan, M., Wu, Y., Xu, Z., Liu, Z., & Liu, G. (2022). Neuron-specific enolase promotes stem cell-like characteristics of small-cell lung cancer by downregulating NBL1 and activating the BMP2/Smad/ID1 pathway. *Oncogenesis*, *11*(1), 21.

62) Maas, A. I., Menon, D. K., Lingsma, H. F., Pineda, J. A., Sandel, M. E., & Manley, G. T. (2012). Re-orientation of clinical research in traumatic brain injury: report of an international workshop on comparative effectiveness research. *J Neurotrauma*, *29*(1), 32-46.

63) Maas, A. I. R., Menon, D. K., Manley, G. T., Abrams, M., Åkerlund, C., Andelic, N., Aries, M., Bashford, T., Bell, M. J., Bodien, Y. G., Brett, B. L., Büki, A., Chesnut, R. M., Citerio, G., Clark, D., Clasby, B., Cooper, D. J., Czeiter, E., Czosnyka, M., . . . Zemek, R. (2022). Traumatic brain injury: progress and challenges in prevention, clinical care, and research. *Lancet Neurol*, *21*(11), 1004-1060.

64) Maiese, K. (2016). Targeting molecules to medicine with mTOR, autophagy and neurodegenerative disorders. *British Journal of Clinical Pharmacology*, *82*(5), 1245-1266.

65) Malik, S., Revington, M., Smith, S. P., & Shaw, G. S. (2008). Analysis of the structure of human apo-S100B at low temperature indicates a unimodal conformational distribution is adopted by calcium-free S100 proteins. *Proteins: Structure, Function, and Bioinformatics*, *73*(1), 28-42.

66) Matis, G., & Birbilis, T. (2008). The Glasgow Coma Scale--a brief review. Past, present, future. *Acta Neurol Belg*, *108*(3), 75-89.

67) Mbele, G. O., Deloulme, J. C., Gentil, B. T. J., Delphin, C., Ferro, M., Garin, J., Takahashi, M., & Baudier, J. (2002). The Zinc- and Calcium-binding S100B Interacts with and Co-localizes with IQGAP1 during Dynamic Rearrangement of Cell Membranes. *Journal of Biological Chemistry*, *277*(51), 49998-50007.

68) McCoy, H. M., Polcyn, R., Banik, N. L., & Haque, A. (2023). Regulation of enolase activation to promote neural protection and regeneration in spinal cord injury. *Neural Regen Res*, *18*(7), 1457-1462.

69) Michetti, F., Clementi, M. E., Di Liddo, R., Valeriani, F., Ria, F., Rende, M., Di Sante, G., & Romano Spica, V. (2023). The S100B Protein: A Multifaceted Pathogenic Factor More Than a Biomarker. *International Journal of Molecular Sciences*, *24*(11), 9605.

70) Movahedpour, A., Vakili, O., Khalifeh, M., Mousavi, P., Mahmoodzadeh, A., Taheri-Anganeh, M., Razmeh, S., Shabaninejad, Z., Yousefi, F., & Behrouj, H. (2022). Mammalian target of rapamycin (mTOR) signaling pathway and traumatic brain injury: A novel insight into targeted therapy. *Cell biochemistry and function*, *40*(3), 232-247.

71) Mulherkar S, Tolias KF. RhoA-ROCK. 2020. Signaling as a Therapeutic Target in Traumatic Brain Injury. Cells. Jan 18;9(1):245.

72) Murray, G. D., Teasdale, G. M., Braakman, R., Cohadon, F., Dearden, M., Iannotti, F., Karimi, A., Lapierre, F., Maas, A., Ohman, J., Persson, L., Servadei, F., Stocchetti, N., Trojanowski, T., & Unterberg, A. (1999). The European Brain Injury Consortium survey of head injuries. *Acta Neurochir (Wien)*, *141*(3), 223-236.

73) National Academies Of Sciences, E., Medicine, Health, Medicine, D., Board On Health Sciences, P., & Forum On Traumatic Brain Injury. 2023. The National Academies Collection: Reports Funded By National Institutes Of Health. *In:* Bowman, K., Matney, C. & Snair, M. (Eds.) *Biomarkers For Traumatic Brain Injury: Proceedings Of A Workshop.* Washington (Dc): National Academies Press (Us).

74) Nazwar, T. A., Triangto, I., Pringga, G. A., Bal'afif, F., & Wardana, D. W. (2023). Mobilization phases in traumatic brain injury. *Acute Crit Care*, *38*(3), 261-270.

75) Niessen, P. D. M., Krijnen, P., Leijdesdorff, H. A., Peul, W. C. & Schipper, I. B. 2025. Interpreting Traumatic Brain Injury Severity: Analysis of The Correlation Between Glasgow Coma Scale And Abbreviated Injury Scale. *Eur J Trauma Emerg Surg,* 51**,** 239.

76) Owolabi, E. O., Nyamathe, S., Joseph, C., Khuabi, L.-A. J.-N., English, R. G., Vlok, A., Erasmus, E., Geduld, H. I., Lategan, H. J. & Chu, K. M. 2023. Mapping Access To Care And

Identification Of Barriers For Traumatic Brain Injury In A South African Township. *Journal Of Evaluation In Clinical Practice,* 29**,** 380-391.

77) Oris, C., Chabanne, R., Durif, J., Kahouadji, S., Brailova, M., Sapin, V., & Bouvier, D. (2019). Measurement of S100B protein: evaluation of a new prototype on a bioMérieux Vidas® 3 analyzer. *Clin Chem Lab Med, 57*(8), 1177-1184.

78) Oris, C., Kahouadji, S., Durif, J., Bouvier, D., & Sapin, V. (2023). S100B, Actor and Biomarker of Mild Traumatic Brain Injury. *Int J Mol Sci, 24*(7).

79) Peralta R, Cardona-Collazos S, Msheik A, Gonzalez WD, Restrepo AS, Loaiza-Cardona LM, Rubiano AM. Updating Traumatic Brain Injury Classification for Surgeons: Integrating the CBI-M Framework Into Trauma and Acute Care Practice. World J Surg. 2026 Jul;50(7):2042-2049. doi: 10.1002/wjs.70440. Epub 2026 Jun 2. PMID: 42227605; PMCID: PMC13356529.

80) Rael, L. T., Bar-Or, R., Salottolo, K., Mains, C. W., Slone, D. S., Offner, P. J., & Bar-Or, D. (2009). Injury severity and serum amyloid A correlate with plasma oxidation-reduction potential in multi-trauma patients: a retrospective analysis. *Scandinavian Journal of trauma, resuscitation and emergency medicine, 17*, 1-7.

81) Rauchman, S. H., Pinkhasov, A., Gulkarov, S., Placantonakis, D. G., De Leon, J., & Reiss, A. B. (2023). Maximizing the Clinical Value of Blood-Based Biomarkers for Mild Traumatic Brain Injury. *Diagnostics, 13*.

82) Roozenbeek, B., Maas, A. I. R., & Menon, D. K. (2013). Changing patterns in the epidemiology of traumatic brain injury. *Nature Reviews Neurology, 9*(4), 231-236.

83) Savitsky, B., Givon, A., Rozenfeld, M., Radomislensky, I. & Peleg, K. 2016. Traumatic Brain Injury: It Is All About Definition. *Brain Inj,* 30**,** 1194-200.

84) Sen, J., & Belli, A. (2007). S100B in neuropathologic states: The CRP of the brain? *Journal*

of Neuroscience Research, 85(7), 1373-1380.

85) Sherer, M., Struchen, M. A., Yablon, S. A., Wang, Y., & Nick, T. G. (2008). Comparison of indices of traumatic brain injury severity: Glasgow Coma Scale, length of coma and post-traumatic amnesia. *J Neurol Neurosurg Psychiatry, 79*(6), 678-685.

86) Singh, S., & Singh, T. G. (2020). Role of Nuclear Factor Kappa B (NF-Κ B) Signalling in Neurodegenerative Diseases: A Mechanistic Approach. *Current Neuropharmacology, 18*(10), 918-935.

87) Soriano, S., Moffet, B., Wicker, E., & Villapol, S. (2020). Serum Amyloid A is Expressed in the Brain After Traumatic Brain Injury in a Sex-Dependent Manner. *Cell Mol Neurobiol, 40*(7), 1199-1211.

88) Staton, C. A., Msilanga, D., Kiwango, G., Vissoci, J. R., de Andrade, L., Lester, R., Hocker, M., Gerardo, C. J., & Mvungi, M. (2017). A prospective registry evaluating the epidemiology and clinical care of traumatic brain injury patients presenting to a regional referral hospital in Moshi, Tanzania: challenges and the way forward. *International Journal of Injury Control and Safety Promotion, 24*(1), 69-77.

89) Sumpter, R. E., & McMillan, T. M. (2005). Misdiagnosis of post-traumatic stress disorder following severe traumatic brain injury. *British Journal of Psychiatry, 186*(5), 423-426.

90) Thelin, E. P., Nelson, D. W., & Bellander, B. M. (2017). A review of the clinical utility of serum S100B protein levels in the assessment of traumatic brain injury. *Acta Neurochir (Wien), 159*(2), 209-225.

91) Tsoporis, J. N., Mohammadzadeh, F., & Parker, T. G. (2010). Intracellular and Extracellular Effects of S100B in the Cardiovascular Response to Disease. *Cardiovascular Psychiatry and Neurology, 2010*(1), 206073.

92) Tzeng, S. F., Hsiao, H. Y., & Mak, O. T. (2005). Prostaglandins and cyclooxygenases in glial

cells during brain inflammation. *Curr Drug Targets Inflamm Allergy*, *4*(3), 335-340.

93) Van Essen, D. C., Donahue, C. J., & Glasser, M. F. (2018). Development and Evolution of Cerebral and Cerebellar Cortex. *Brain Behav Evol*, *91*(3), 158-169.

94) Vella, M. A., Crandall, M. L., & Patel, M. B. (2017). Acute Management of Traumatic Brain Injury. *Surg Clin North Am*, *97*(5), 1015-1030.

95) Wagner, J., & Atkinson Jr., A. (2015). Measuring Biomarker Progress. *Clinical Pharmacology & Therapeutics*, *98*(1), 2-5.

96) Wang, C. S., Kavalali, E. T., & Monteggia, L. M. (2022). BDNF signaling in context: From synaptic regulation to psychiatric disorders. *Cell*, *185*(1), 62-76.

97) Wang, K. K., Munoz Pareja, J. C., et al. 2021. Blood-based traumatic brain injury biomarkers – Clinical utilities and regulatory pathways in the United States, Europe and Canada. Expert Review of Molecular Diagnostics, 21, 1303-1321.

98) Wicker, E., Benton, L., George, K., Furlow, W., & Villapol, S. (2019). Serum Amyloid A Protein as a Potential Biomarker for Severity and Acute Outcome in Traumatic Brain Injury. *BioMed Research International*, *2019*(1), 5967816.

99) Wilder, P. T., Varney, K. M., & Weber, D. J. (2019). Targeting S100 Calcium-Binding Proteins with Small-Molecule Inhibitors. In C. W. Heizmann (Ed.), *Calcium-Binding Proteins of the EF-Hand Superfamily: From Basics to Medical Applications* (pp. 291-310). Springer New York.

100) Wroblewski, T. H., Ajmal, E., Ononogbu-Uche, F., Lerner, D. P., Bigdeli, T. B., Divers, J., & Barthélemy, E. J. (2025). Molecular Biomarkers Associated with Traumatic Brain Injury Outcome in Individuals of Black Racial Identity or African Ancestry: A Narrative Review. World neurosurgery, 194, 123620.

101) Yamada, T. (2006). Serum amyloid A (SAA)--pathogenicity and implication of appearance in plasma. *Rinsho byori. The Japanese Journal of Clinical Pathology*, *54*(5), 509-512.

102) Yang, Z., & Wang, K. K. (2015). Glial fibrillary acidic protein: from intermediate filament assembly and gliosis to neurobiomarker. *Trends Neurosci*, *38*(6), 364-374.

103) Zhao, J.-b., Zhang, Y., Li, G.-z., Su, X.-f., & Hang, C.-h. (2011). Activation of the JAK2/STAT pathway in the cerebral cortex after experimental traumatic brain injury in rats. *Neuroscience letters*, *498*(2), 147-152.

104) Zhong, H., Feng, Y., et al. 2025. Global Burden of Traumatic Brain Injury in 204 Countries and Territories From 1990 to 2021. Am J Prev Med, 68, 754-763.

BRIDGING TEXT

FROM CHAPTER ONE TO TWO

Chapter one presents an introductory section and a review of the literature. It was evident from chapter one how TBI is a socioeconomic problem and how it adds a burden to the healthcare system. Furthermore, chapter one demonstrates that the current diagnostic tool, GCS, is limited and requires a new or complementary tool capable of detecting underlying neurological dysfunctions following TBI. Given this, investigating biomarkers as new or complementary tools for TBI diagnosis may be a tempting option to enhance diagnostic accuracy. Thus, the next chapter (Chapter Two) focuses on analyzing the most common and suitable biomarkers currently under investigation or already introduced into clinical settings. The results obtained on S100B and GFAP were presented as a manuscript and published in the Journal of Translational Research in Anatomy. DOI: https://doi.org/10.1016/j.tria.2022.100228

CHAPTER TWO

PUBLISHED REVIEW ARTICLE

The potential of serum S100 calcium-binding protein B and glial fibrillary acidic protein as biomarkers for traumatic brain injury

Seke Nzau Mafuika[1], Thajasvarie Naicker[2], Rohen Harrichandparsad[3], Lelika Lazarus[1]

[1]Discipline of Clinical Anatomy, School of Laboratory Medicine and Medical Science, College of Health Sciences, University of KwaZulu-Natal, Westville campus, F block, Private Bag X54001, Durban, 4000, South Africa

[2]Discipline of Optics and Imaging, Doris Duke Medical Research Institute, College of Health Sciences, School of Laboratory Medicine & Medical Sciences, Nelson R Mandela School of Medicine, University of KwaZulu-Natal, 719 Umbilo Road, Durban, South Africa.

[3]Department of Neurosurgery, Inkosi Albert Luthuli Central Hospital, Ethekwini, KwaZulu-Natal, 4000, South Africa.

Corresponding Author: Seke Nzau Mafuika, Discipline of Clinical Anatomy, School of Laboratory Medicine & Medical Sciences, Westville Campus, University of KwaZulu-Natal, Durban, South Africa

Sekemafuika2@gmail.com, 215001367@stu.ukzn.ac.za

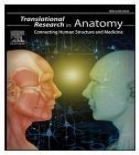
Published: Translational Research in Anatomy

Translational Research in Anatomy 29 (2022) 100228

Contents lists available at ScienceDirect

Translational Research in Anatomy

journal homepage: www.elsevier.com/locate/tria



The potential of serum S100 calcium-binding protein B and glial fibrillary acidic protein as biomarkers for traumatic brain injury

Seke Nzau Mafuika [a], Thajasvarie Naicker [b], Rohen Harrichandparsad [c], Lelika Lazarus [a,*]

[a] Discipline of Clinical Anatomy, School of Laboratory Medicine and Medical Science, College of Health Sciences, University of KwaZulu-Natal, Westville Campus, Private Bag X54001, Durban, 4000, South Africa
[b] Discipline of Optics & Imaging, Doris Duke Medical Research Institute, College of Health Sciences, University of KwaZulu-Natal, Durban, 4000, South Africa
[c] Department of Neurosurgery, Inkosi Albert Luthuli Central Hospital, Ethekwini, KwaZulu-Natal, 4000, South Africa

ARTICLE INFO

Keywords:
TBI
Serum S100B
Serum GFAP

ABSTRACT

Background: Traumatic brain injury (TBI) occurs as a result of a severe head injury caused by an external force during a vehicle accident, fall, domestic violence, or explosion. Clinically, TBI is evaluated and classified clinically according to the Glasgow Coma Scale (GCS), associated with Computed Tomography (CT) and Magnetic Resonance Imaging (MRI). However, there are many limitations associated with GCS; therefore, the aim of this review is to perform a systematic meta-synthesis that evaluates the various characteristics of TBI markers. Most especially, S100 calcium-binding protein B (S100B) and Glial Fibrillary Acidic Protein (GFAP).
Methods: To achieve this revised review of S100B and GFAP roles in human TBI, an electronic literature search was carried out among articles published in English from previous years up to December 2021. The review included 103 studies and was retrieved from PubMed, Google Scholar, PMC, BMC and Scopus, using the keywords "traumatic brain injury" and "neurobiomarkers". And "S100B and GFAP proteins".
Results: & Conclusion: This study demonstrates that S100B and GFAP have a high sensitivity for intracranial injuries and remain unchanged by external factors such as temperature, freeze-thaw cycles, and hemolysis. Moreover, both S100B and GFAP are specific to the brain. Hence a combination of the two proteins could improve the outcome of TBI diagnosis. However, the clinical test value and specific mechanisms of S100B and GFAP to predict TBI requires further investigation.

1. Introduction

Traumatic brain injury (TBI) is a change in brain function, or an indication of brain pathology caused by an external force [1,2]. Globally, TBI is considered a silent epidemic that contributes to major morbidity and mortality compared to other traumatic insults. It is a major health and socioeconomic problem affecting individuals irrespective of their race, gender, and age [3,4].

Worldwide, 69 million (95% CI: 64–74 million) individuals suffer from TBI [5]. TBI occurs in both developed and developing countries [6]. The prevalence of TBI is three-fold greater in low- and middle-income compared to high-income countries [5].In sub-Saharan Africa, the prevalence of TBI is 150–170 per 100 000 individuals [7]. It is estimated that by the year 2050, the TBI prevalence in Africa will increase to 14.25 ± 0.75 million due to increased urbanization [8]. Notably, the National Institute for Occupational Health of South Africa (SA) report an estimated 89 000 new cases of TBI annually [9], of which 39.4% are caused by interpersonal violence, 16.7% by a motor vehicle collision, 14.1% by vehicle-pedestrian accidents, 10.3% from falls and 19.5% from other causes [10].

Traumatic brain injury varies in gravity from minor to fatal, as well as by type, location, age and gender [11]. Acute trauma may result in hemorrhage within several intracranial segments arising from brain contusion, diffuse axonal shearing, and brain oedema [12]. Frequent TBI

Abbreviations: (TBI), Traumatic brain injury; (SA), South Africa; (ROS), Reactive oxygen species; (GCS), Glasgow Coma Scale; (CT), Computed Tomography; (NSE), Neuron-Specific Enolase; (GFAP), Glial Fibrillary Acidic Protein; (S100B), S100 calcium-binding protein B; (CNS), Central nervous; (TLR4), Toll-like receptor 4; (MAPK), Mitogen-activated protein kinases; (NF-kB), Nuclear factor kappa light chain enhancer of activated B cells; (iNOS), Inducible nitric oxide synthase; (RAGE), receptor for advanced glycation end products; (NADPH), Nicotinamide adenine dinucleotide phosphate.

* Corresponding author.
E-mail addresses: 215001367@stu.ukzn.ac.za (S.N. Mafuika), naickera@ukzn.ac.za (T. Naicker), Harrichandparsad@ukzn.ac.za (R. Harrichandparsad), Ramsaroopl@ukzn.ac.za (L. Lazarus).

https://doi.org/10.1016/j.tria.2022.100228
Received 16 August 2022; Received in revised form 22 October 2022; Accepted 28 October 2022
Available online 11 November 2022

may cause chronic traumatic encephalopathy, identified clinically by amnesia, executive dysfunction, aggression, depression, and Parkinsonism [13].

TBI is evaluated and classified clinically according to the GCS by imaging using axial Computed Tomography (CT) and Magnetic Resonance Imaging (MRI) [14,15]. Based on the GCS, TBI is categorized as mild (score: 13–15), moderate (score: 9–12), and severe (score: <9) [11]. However, using GCS as a diagnostic tool is associated with significant limitations [16]. These include its insensitivity to detect underlying neurological and non-neurological diseases [17], failure to evaluate verbal responses in intubated patients, an absence of brainstem reflex, and different scores assessed on the same patient [18]. Most physicians in emergency rooms often encounter the challenge of how to evaluate patients who are intoxicated with alcohol that have poor recall of events [19].

Moreover, detecting early symptoms in sport-related TBI is difficult as CTs cannot differentiate subtle changes in brain tissue. Also, it is costly to transport these scanners to sports sites. Hence, a considerable percentage of sport-related TBIs remain undiagnosed or undetected [20]. These limitations have driven the need for alternative, more reliable methods to assess TBI. Thus, in light of the high morbidity and mortality associated with TBI. This review examines different biomarkers and compares their values as predictor risk indicators for brain injury.

1.1. Pathophysiological process of TBI and phases of brain injury

TBI may comprise two separate phases rather than a single pathophysiological incident. The early insult stimulates the primary injury process, which causes tissue distortion, necrosis, and shearing of neurons, glial cells and axons [21]. The nervous tissue injury resulting from this primary phase causes the release of neurotransmitters such as glutamate, glycine and aspartate [22]. Thus, the latter phase activates a secondary phase associated with an elevation of proinflammatory cytokines with consequent production of reactive oxygen species (ROS) that cause mitochondrial dysfunction and subsequent ischemia and injury to the cell and apoptosis, as shown in Fig. 1 [23]. This inflammatory phase may persist for weeks to months, often culminating in death [24]. Moreover, disrupted blood-brain barrier (BBB) consequently causes invasion of peripheral inflammatory cells in the brain and activation of voltage-dependent calcium (Ca^{2+}) and sodium (Na^{+}) channels. Following Ca^{2+} and Na^{+} activation of phospholipases, proteases, and lipid peroxidases occur, followed by induction of apoptosis, membrane degradation and cell death [25].

1.2. Prognosis of TBI and TBI biomarkers

Currently, the prognostic approach for the TBI assessment during the patient admission and characteristic clinical outcomes for brain surgery include Glasgow coma score (GCS), which is the most commonly used, along with other assessments such as the absence of pupillary reactivity, activated partial thromboplastin time, lymphocyte ratio, international normalized ratio, and platelet count [26].

Biomarkers are quantifiable biological substances that serve as markers of physiological injury or inflammation. Currently, studies have highlighted many TBI biomarkers that are promising and clinically relevant [27]. Early studies investigated the presence of serum fructose 1,6-diphosphate aldolase, glutamic pyruvic transaminase, GOT, LDH, alpha-hydroxybutyric acid dehydrogenase, and malate transaminase in patients with head injury [28]. However, these studies had several methodology errors that were performed prior to the introduction of both CT scans and GCS [29]. Biomarkers that have received recent attention include Creatine Kinase (CK), Lactate Dehydrogenase (LDH), Myelin Basic Protein (MBP), Neuron-Specific Enolase (NSE), Creatine Kinase Isoenzyme (BB), Glial Fibrillary Acidic Protein (GFAP) and S100 calcium-binding protein B (S100B). Both GFAP and S100B have generated extensive scientific interest [29]; plausibly because both are parallel regarding detection of brain damage, higher level of accuracy for prediction mortality, as well as neuromonitoring parameters such as cerebral perfusion pressure, intracranial pressure and mean arterial

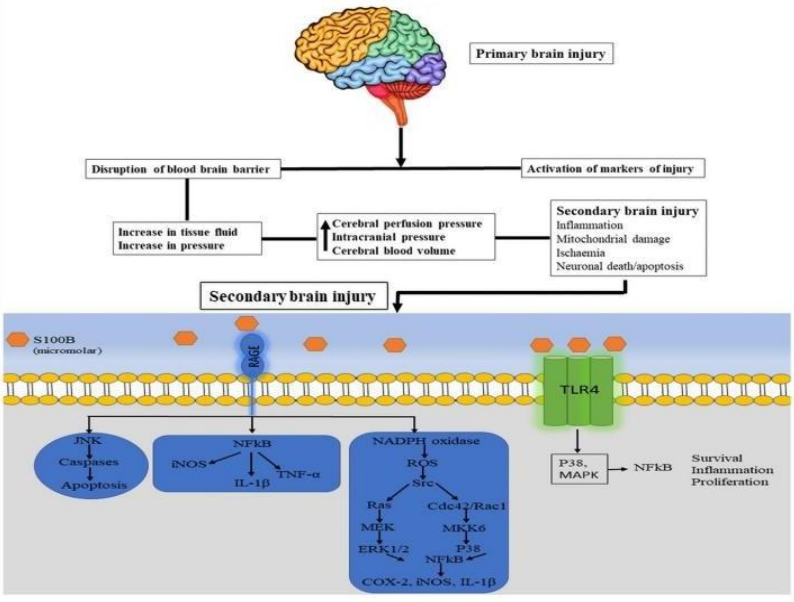


Fig. 1. The pathophysiology of TBI is initiated by an external force exerted on the head (i.e primary injury). The mechanical damage, resulting from the primary injury causes blood-brain barrier (BBB) disruption, brain tissue damage and altered vasculature. The emanating damaged neurons and glia release their intracellular contents into the extracellular space and stimulate adjacent neurons and glia generating the second injury. S100B proteins pathways during inflammation, proliferation, lipid peroxidation and apoptosis. S100B acts in a paracrine, autocrine and endocrine manner. It binds to toll-like receptor 4 (TLR4) and an immunoglobulin receptor for advanced glycation end products (RAGE) on the plasmalemma of astrocytes, microglia, Schwann cells and neurons. ROS is produced via activation of NADPH oxidase complex. This stimulates Src tyrosine kinase and recruits the Rac1-Cdc42-MKK6-p38-MAPK-NF-κB and Ras-MEK-ERK1/2-NF-κB signaling pathways. Consequently, there is an up-regulation of IL-1β, TNF-α, COX-2 and iNOS expression. Also, elevated ROS leads to lipid peroxidation and elevation of Bcl-2, and a Rac1-Cdc42 pathway leading to mitochondrial cytochrome-C release causing apoptosis.
Abbreviations: P38 MAPK: p38 mitogen-activated protein kinases, NF-kB: nuclear factor kappa light chain enhancer of activated B cells, JNK: c-Jun N-terminal kinase, TLR4: toll-like receptor 4, iNOS: inducible nitric oxide synthase, IL-β: interleukin 1 beta, and TNF-α: tumor necrosis factor-α. ROS: Reactive oxygen species.

pressure [30]. Hence, our review focuses on S100 calcium-binding protein B (S100B) and Glial fibrillary acidic protein (GFAP) as predictor markers for the prognosis of TBI.

1.2.1. Other TBI biomarkers their applications in TBI prognosis

TBI has been reported to induce tau aggregation and spreading, suggesting that TBI is a risk factor for tauopathies [31]. The primary function of tau in the brain is to stabilize the microtubules which support the long axon; other reported functions include regulation of the cell cycle via tyrosine kinase and promote the synaptic function [32,33]. Studies have reported that tau phosphorylation in the brain occurs during the early hours of TBI and induce progressive pathology for years after the initial injury [34,35]. However, several studies reported the failure to identify tau pathology within the short observation period and after TBI [36–38].

Similarly, accumulation of alpha-synuclein (α-Syn) in microglial cells and astrocytes has been reported in TBI, which promotes neuronal damage leading to neurodegeneration ([39]. Alpha-synuclein (α-Syn) is the most abundant protein in presynaptic terminals. Its normal expression is vital for neuronal survival [40]. The presence of exocytosis α-Syn during the neuronal mitochondrial dysfunction and the detention in the cerebrospinal fluid (CSF) with severe TBI makes a potential marker for TBI prognosis [41]. However, a study reported that there was no statistically significant when comparing the α-Syn level with initial GCS and GOS scores in CSF of 47 infants and children with severe TBI [42]. Other biomarkers such as BDNF, ICAM-5 and MCP-1, were evaluated in the blood sample of 85 isolated moderate-to-severe TBI patients to reveal the complex cellular and molecular mechanisms [43]. Using the biomarkers, Battista and colleagues reported the complex cellular and molecular mechanism underlying TBI. The results showed that S100B alone compared with other biomarkers, outperformed in discriminating unfavourable and favorable outcomes in the acute period from moderate to severe TBI [43].

1.3. The biological role of S100 and GFAP during the physiological brain process

S100B is a calcium-binding protein usually found in the glial cells and detected in definite extra-neural cell types. Its various biological roles in the normal physiological process are still debatable [44], but it is believed to regulate protein phosphorylation, cell growth, cell cycle, cell differentiation, and cell survival and act in intracellular and extracellular functions [45,46]. S100B has an autocrine or paracrine effect at physiological concentration when secreted. However, its toxic effects are manifested at a higher concentration which can be detected in biological fluids such as CSF, blood, urine, amniotic fluid, and saliva [47]. Therefore, it has been used for brain biomarkers during neuroinflammation, tumour, neurodegeneration, and traumatic brain injury [48]. More so, S100B has been reported to express in Schwann cells and in the cell that forms myelin. This suggests that it may be involved in the degree of myelination and response to injury [49].

GFAP is an intermediate filament structural protein that is highly expressed during the activation of glial cells, predominantly in astrocytes [50]. The intermediate filament in this protein forms a network that gives structural support and strength to cells. The functions of GFAP are not well understood, but it has been reported that it involves controlling the astrocytes' shape, movement and function [50]. Studies have also reported that GFAP plays several biological roles in CNS, such as suppression of neuronal proliferation, neurite extension in the mature brain, cerebellar motor learning process, regulation of blood flow after ischemia, providing support to the blood-brain barrier, and mechanical strength to the cell [46,51]. However, GFAP is not routinely found in the blood or biological fluid, and it is only released during neuroinflammation, neuronal injury or cell death. Thus, this attribute has made GFAP a potential marker for TBI [51]. For instance, GFAP has been used as a marker for the diagnosis of brain injury and its potential adverse effect related to death outcomes [43]. Another study reported that the higher GFAP levels were found to be associated with worse GCSs in TBI patients [52]. More recently, the expression of GFAP in astrocytes has been used as a major marker in neurological, neuroinflammation and neurogenesis to predetermine and monitor neurological severity in TBI patients [53].

1.3.1. Pros and cons of the commonly used biomarkers in TBI; advantages and disadvantages of S100B and GFAP as biomarkers

Neuron-specific enolase (NSE) is uniquely known for its specificity to detect direct functional damage to neurons [54]. Unlike normal physiological conditions, during cellular injury or death, NSE leaks into the extracellular space and is upregulated. One of the major problems related to the use of NSE as a biomarker for TBI, is hemolysis. Red blood cells contain a substantial amount of NSE, hemolysis during TBI may cause an increase of NSE in the blood, making it difficult to interpret and may reflect inaccurate values [55]. Nonetheless, despite NSE having a longer half-life than S100B yet equivalent to GFAP, its slow release from plasma makes it difficult to differentiate between primary and secondary injury to the brain [56]. Some studies have reported no difference in NSE levels between control and TBI patients [57]. Generally, NSE is mostly associated with severe injury rather than mild and moderate injury [58]. Its poor sensitivity and specificity limit its use in TBI as a good biomarker since it also does not correlate with mild and moderate TBI compared to S100B and GFAP.

Contrary to S100B and NSE effect, *ubiquitin C-terminal hydrolase -L1* (UCH-L1) is enhanced and very specific for neurons and is absent-low in other tissues [59]. Studies have reported that serum UCH-L1 measured within 48 h following injury distinguishes patients with intracranial hemorrhage from controls [60]. In contrast, shahim et al. [61], noted an increase in UCH-L1 concentration in patients with TBI seven months after the first measurement, and there was no correlation between the level of UCH-L1 and the severity of the injury. These finding questions the specificity and accuracy of UCH-L1 levels post-injury as similar to NSE, it has a very low sensitivity for mTBI compared to S100B and GFAP [62].

Neurofilament light (NF-L) is the core protein of CNS neurofilaments expressed within long myelinated subcortical axons [63]. It is made up of a heteromeric protein, divided into three groups with different molecular weights, that is, light (NF-L)-68 kDa, moderate-sized (NF-M)-150 kDa, and heavy (NF–H)- 200 kDa NF proteins respectively, with NF-L acting as the core protein [64]. Although S100B and GFAP have a shorter half-life compared to NF-L, they are considered a better biomarker than NF-L due to their dynamic release. With NF-L, the initial increase is highly correlated to primary brain injury and weakly correlated to secondary brain injury, while S100B and GFAP is more effective for detection of secondary brain injury, thus are considered better biomarkers [65]. Whilst the predictive value of NF-L is considered independent of association with other biomarkers, contradictory reports show weak predictive values compared to S100B and GFAP [66].

1.3.1.1. S100 calcium-binding protein B (S100B). Twenty-one S100 proteins occur exclusively in vertebrates and are encoded in the human genome [67]. They share sequence and structural homology, but are not functionally interchangeable with their functional roles in proliferation, migration and/or invasion, inflammation and differentiation [68].

S100B has been immunohistochemically identified in astrocytes of brain tissues as well as within other organs such as bladder, colon, liver, lungs, kidney, pancreas, tonsil and stomach [69]]. Therefore, S100B measurements taken at the time of trauma, may not be derived primarily from the CNS but may also emanate from other organs [70].

S100B is a small dimeric cytosolic and calcium-binding protein with a molecular size that ranges between 9 and 21 kDa. It is soluble in 100% ammonium sulfate liquid [71]. Its normal level in serum is 0.14 μg/l [72], and 1.8 μg/l in CSF [73]. Notably the S100B increase measured at

the time of trauma correlates poorly with TBI outcome [74]. However, 12 h after the trauma, there is a considerable association with TBI outcome [75]. Following brain injury, S100B is released as a structural factor in high concentration and as a reconstructive protein in low concentration [76]. In CNS development and TBI recovery, S100B may be correlated to its neurotrophic and gliotrophic actions [77]. A low S100B secretion at nanomolar concentrations strengthens neural survival, however, at micromolar levels it may have a toxic effect [76].

Physiologically, S100B is implicated in the development and maintenance of CNS homeostasis. In astrocytes, S100B is located within the cytoplasm and nucleus, where it regulates various cellular activities [78]. It binds to crucial synaptic proteins such as toll-like receptor 4 (TLR4) (Fig. 1), thereby inhibiting their phosphorylation [79], and signal transduction is affected via the mitogen-activated protein kinases (MAPK) and nuclear factor kappa light chain enhancer of activated B cells (NF-kB) pathways [80]. The mechanism of secretion and action of S100B remains unclear [81] however, it may be associated to different factors such as interleukin β, tumor necrosis factor alpha α-1, the proinflammatory cytokines and metabolic stress [82]]. In contrast, several studies have demonstrated a positive correlation between S100B release after brain trauma and the degree of severity of injury [83]. Furthermore, S100B protects neurons against excitotoxic damage, demonstrating a positive role for S100B in decreasing brain injury and in stimulating recovery [84]. However, toxic levels of glutamate in neurons down-regulates serum deprivation induced S100B secretion by a process reliant upon the glutamate transporter [85]. Glutamate is the dominant free amino acid with excitatory lethal effects on nerve cells in the brain [86]. In an experiment conducted by *Guerra* et al. [87], it was observed that S100B was upregulated by phospholipid 1-palmitoyl-2-arachidonoyl-sn-glycerol-3-phosphatidylcholine (PAPC), which has anti-inflammatory properties [88]. *Ke* et al. [89] showed that endothelin-1 (Et-1) also upregulates S100B secretion in astrocyte cultures, however this was time dependent, after an hour Et-1 decreased S100B secretion.

It is worth noting that individuals with darker skin have higher serum levels of S100B compared to individuals with lighter skin [90], due to higher levels of melanocytes in darker-skinned individuals [91]. This should be taken into consideration as it implies the concentration of S100B may be affected by race and that careful observations are required during measurements of serum S100B and interpretation of results in correlation with skin color [92].

1.3.1.2. S100B and Ca^{2+} pathway. Several S100 proteins are dependent on Ca^{2+} activation for relocation from one specific cellular compartment to another [93]. Ca^{2+} ions are vital secondary messengers in all active cells [94]. During intracellular signaling, S100B regulates cytosolic Ca^{2+} level thereby transducing Ca^{2+} signaling at the plasmalemma [95]]. In response to Ca^{2+} increase, S100B translocates as vesicle-like structures from the perinuclear zone towards the periphery of the cell where it induces astrocyte proliferation [93]. In contrast, Ca^{2+} ions were reported to be ineffective on S100B release, in a study on rat cortical slices [96]. The omission of Ca^{2+} ions from incubation medium containing oxygen and glucose did not affect S100B release. However, when extracellular Ca^{2+} ions are chelated with bis (alpha-aminophenoxy) ethane-N, N, N′, N′-tetraacetic acid) (BAPTA) there is significant elevation in S100B release [97].

Furthermore, S100B stimulates glial inducible nitric oxide synthase (iNOS) through a signal transduction pathway that involves the transcription factor nuclear factor kappa-light-chain-enhancer of activated B cells (NFkB). It is plausible that this signal transduction pathway regulates S100B activation of glia which ultimately contributes to oxidative and neurotoxic responses [98]. Additionally, extracellular S100B is encapsulated by vesicles and reabsorbed by astrocytes in a receptor for advanced glycation end products (RAGE)-dependent manner (Fig. 1) [99]. Reabsorption of S100B via RAGE activates and further endorses the shedding of endothelial glycocalyx, exacerbates the dysfunction of BBB, and increases vascular permeability [100]. Recent studies have shown the role of S100B in neurodegenerative diseases and neurodegenerative brain injury via mitochondria-derived Reactive Oxygen Species (mROS) [101,102]. ROS is produced via activation of Nicotinamide adenine dinucleotide phosphate (NADPH) oxidase complex [103]. This triggers Src tyrosine kinase and recruits the Rac1-Cdc42-MKK6-p38-MAPK-NF-κB and Ras-MEK-ERK1/2-NF-κB signaling pathways, at low and high concentration of ROS respectively [104]. Resulting in anti-apoptotic expression and neurite extension respectively (Fig. 1).

1.3.1.3. S100B and apoptosis. Notably, at high concentrations, S100B induces apoptosis of an *in vitro* cell line (N18) in a RAGE-dependent manner (Fig. 1) [105]. In malignant melanomas, S100B downregulates apoptosis by binding directly to the p53 tumour suppressor protein, thereby decreasing and inhibiting the p53 protein levels [106]. Overexpression of S100B prevents apoptosis due to lower levels of p53 and phosphorylated p53 protein [106]. To decrease the survival ability of malignant melanoma, S100B levels must be 2-fold lower in order to activate apoptosis [107].

Moreover, based on its concentration, secreted glial S100B has both trophic and toxic effects [108]. At high levels, S100B stimulates neurite outgrowth and boosts the survival of neurons during development. In contrast, at low levels, S100B induces the expression of proinflammatory cytokines and apoptosis [109].

1.3.1.4. Brain immune response during TBI. During TBI, the BBB is disrupted, resulting in immune cell infiltration into the CNS, leading to neuroinflammation and activating proinflammatory markers. The prolonged chronic inflammatory process often leads to secondary cell death [110]. More so, the disruption of BBB plays a significant role in Osmotic demyelination syndrome (ODS) ODS pathology, which could lead to microglia activation and astrocyte death [111].

1.3.2. Glial fibrillary acidic protein (GFAP)

Glial fibrillary acidic protein (GFAP) is a protein derived from glial cells of the central nervous system (CNS). It forms a portion of the intermediate filament of the cytoskeleton of astrocytes [112]. Astrocytes play a critical function in cellular restoration and healing following TBI [113]. Following TBI, spinal cord injury and stroke, GFAP and its contents are rapidly released into biofluids, making them potential candidate biomarkers for neurological disorders [114,115].

GFAP is related to agents that compromise the integrity of the blood-brain barrier (BBB) [50]. The level of GFAP increases in many neurodegenerative illnesses such as Alzheimer's disease (AD), dementia with Lewy bodies (DLB), and frontotemporal lobar degeneration (FTLD) [112]. The normal level of GFAP in serum is < 0.03 ng/mL [29]. However, in TBI, the more severe the damage, the higher the serum GFAP expression [116]. In CSF, the normal concentration of GFAP is 2.27 ng/mL [112]. Studies have highlighted that GFAP in CSF has greater diagnostic accuracy compared to S100B in differentiating mild-TBI patients from controls [117]. GFAP mRNA expression is regulated by numerous nuclear-receptor hormones, growth factors, and lipopolysaccharides (LPSs) [50]. GFAP is also subject to several post-translational modifications. GFAP protein induction seems to play a vital role in astrocyte activation subsequent to CNS injuries and neurodegeneration [114]. Nonetheless, the mechanism of action of GFAP is poorly understood. However, it is thought to give structural support and tensile strength to the cytoskeleton of astrocytes [118].

1.3.2.1. GFAP mechanism. Studies suggest that GFAP plays a vital defensive role in nervous system injury (NSI), where the local inflammatory response is directly proportional to GFAP inhibition [119]. Toll-like receptor 4 (TLR4) plays a vital role in pathogen recognition and

activation of the innate immune system; it is believed to decrease GFAP levels post-TBI [120]. Elevated GFAP release has also been associated with elevated inflammatory responses in patients with neurodegenerative diseases, including neuromyelitis Optica (NMO) [121]. However, it has been demonstrated that GFAP may be detrimental depending on the NFkB signalling pathways [122]. GFAP is considered a strong predictor of mortality; that is, a high concentration of this protein can lead to death [123]. GFAP expression is also upregulated upon TBI by the JAK/STAT pathway (Fig. 2), which in turn is activated by Interleukin 6 (IL-6), suggesting that reactive astrogliosis depends on IL-6 [124]. IL-6 is inhibited by allyl isothiocyanate (AITC), and inhibition of IL-6 downregulates GFAP, which in turn prevents astroglial activation in TBI patients [124]. Other studies have reported that IL-6 upregulates GFAP via the MAPK pathway (Fig. 2) [125]. In contrast, no association between IL-6 and GFAP have been demonstrated, raising uncertainties about its involvement in the JAK/STAT pathway [126].

Unlike S100B, several studies have demonstrated a strong association and correlation between GFAP and MRI [127], making it an important biomarker that may be associated with current diagnostic tools. A limiting factor of GFAP as a reliable biomarker in clinical practice is the varying sensitivity and specificity of the protein, different cut-off values have been reported [128]. Nonetheless, it is important to note that S100B and GFAP have separate regulatory mechanisms of expression [129]. However, studies report that a combination of S100B and GFAP exhibits better prediction of TBI and outperforms other biomarker combinations [130]. Therefore, S100B and GFAP are potential candidates for diagnoses and prognoses of mild to severe TBI.

TBI biomarkers properties, as suggested by *Thelin* et al. [72] include:

- It must show a considerable sensitivity and particularity for brain injury
- Must demonstrate a compliant release from the CNS without any accelerated dynamic release
- It must be convenient to differentiate patients by the gravity of the injury and provide information about injury mechanisms
- Must have a rapid occurrence of inaccessible biological fluids and a limitless passage from the brain
- Have unambiguous bio-kinetic properties and control the progress of disease and reaction to treatment, and predict functional outcome

S100B and GFAP fulfil most of these properties; however, their mechanisms are not yet completely understood. S100B is a stable protein, comparatively unaltered by storing, fluctuating temperatures, freeze-thaw cycles and hemolysis in the sample, which greatly simplifies the processing of the protein and reliability of the analyses, therefore, making it a suitable biomarker in the acute setting [131]. Moreover, both S100B and GFAP have high sensitivity for intracranial injuries despite them differing considerably in their specificity (5% and 55%, respectively) [132].

Limitations of S100B are mostly associated with its inability to exclude moderate to severe TBI; neither has it been specifically quantified according to the TBI category. Most probably due to its fluctuating values in brain injury and other extracranial sources of S100B, which may affect the overall concentration of the protein. In contrast, GFAP is brain-specific, and a combination of the two proteins could improve the outcome of TBI diagnosis.

2. Correlation between radiological examination and blood plasma levels of S100B and GFAP

Studies have reported the correlation between Computed tomography images and the two biomarkers (S100B and GFAP) in TBI [51,132]. S100B, of all the biomarkers, is the most studied biomarker in mild TBI patients for possible head computed tomography [132]. The use of S100B for CT screening in TBI is debatable, and Studies have reported significant correlations between CT image abnormalities and elevated serums levels of S100B [133,134]. Conversely, other studies failed to detect any association between the two [135,136]. Recent studies showed that S100B alone could be used as a predictor for TBI, and it will reduce the risk of exposing patients to radiation, especially in pediatric patients [137,138].

Similarly, GFAP outperformed S100B when detecting intracranial CT lesions. This means GFAP could be a valuable biomarker in TBI [132]. In addition, a study has reported that GFAP levels remain elevated for several months and even years after TBI, and this elevation was

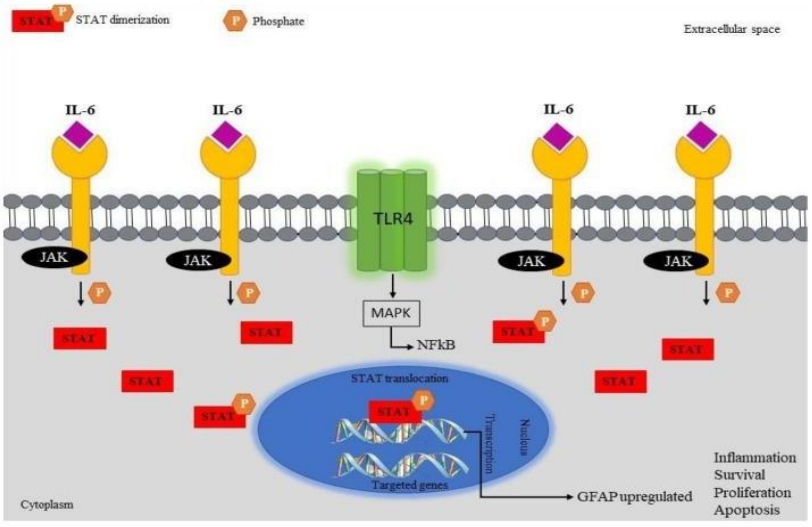


Fig. 2. GFAP pathophysiology via the MAPK and STAT pathways. MAPK: mitogen-activated protein kinases, NF-kB: nuclear factor kappa light chain enhancer of activated B cells, TLR4: toll-like receptor 4, IL-6: interleukin 6. The MAPK pathway usually leads to the increase of GFAP level, while the STAT pathway leads to decrease of GFAP level, which eventually induces multiple cellular processes such as cell differentiation, migration, apoptosis, proliferation, and inflammation.

correlated with changes in brain microscopic structure using quantitative diffusion tensor imaging. This suggests that GFAP may be a useful tool to identify TBI survivors overt time who are at high risk of progressive neurological damage [139].

3. Summary of findings for the commonly used biomarkers

As shown in Table 1, an extensive literature review comparing the serum concentration of NSE with mortality and neurological outcome in TBI patients showed that the higher NSE concentration was significant with increased mortality and unfavourable outcomes in TBI patients [58]. Similarly, a study reported the correlation of S100B with intra-and-extracranial injury using the Stockholm CT score. The reports showed that there were higher S100B levels in both patients with intracranial and extracranial compared with patients without injury. This study revealed that S100B levels were independently correlated with TBI, which could be a potential marker for monitoring aggregated brain injury [70]. Furthermore, the study reported that the serum levels of S100B and GFAP in 49 patients with TBI, both S100B and GFAP showed a significant negative correlation with GOS score in the 6th month. Interesting, there was an increase in medium serum levels of S100B and GFAP in patients with unfavourable conditions and those who later dead compared with those alive with improving health [140].

Ubiquitin C-terminal hydrolase L1 (UCH-L1) is another biomarker used to determine the severity of TBI due to its abundance in the brain, particularly neurons. The expression of UCH-L1 has been reported in patients with moderate and severe TBI after 10 h of injury [141]. Nimer and colleagues investigated the NF-L as a marker of neuroaxonal injury using 182 patients admitted to the neurointensive care unit who were suffering from TBI. Their reports revealed that serum NF-L levels, S100B and NSE are highly expressed in TBI patients. Their results showed that serum NF-L is well-correlated to TBI outcome and neuroaxonal injury [66].

4. Conclusion

Given the high prevalence of TBI globally, there is an urgent requirement for a reliable predictor risk indicator that would enable rapid patient management. This review demonstrates that S100B and GFAP have good potential as predictors for TBI compared to NSE, UCH-L1 and NF-L biomarkers. They show high specificity for neural networks related to mortality and are associated with good prognoses. They are useful and are potential screening means for physicians who may not consider performing head CT cases, such as in younger patients and pregnant women, to prevent exposure to radiation. Both S100B and GFAP have high sensitivity for intracranial injuries and are unchanged by external factors such as temperature, freeze-thaw cycles, and hemolysis. In addition, S100B and GFAP testing is cost-effective; the blood sample is not limited by the duration of testing time, making analysis simple for suitable health professionals using commercially available kits. More studies should be done on the quantification of both S100B and GFAP levels according to TBI severity, that is, mild, moderate, and severe. Reflecting the concentration of TBI severity will ease medical management. However, at this introductory stage, S100B and GFAP measurements should be combined with correct imaging for anatomical visualization of the injury as the levels of S100B and GFAP can be affected by the amount of tissue damaged. Long term studies in a large cohort are required to better elucidate S100B and GFAP in TBI patients as well as investigate their mechanisms of action in TBI.

Ethical statement

This study has been reviewed and approved by the institutional ethics committee (Biomedical Research Ethics Committee, Reference number: BREC/00002721/2021) of the University of KwaZulu-Natal. Ethical approval was dependant on review by the medical institution as well as the Department of Health of South Africa. Individual patient consent was required since the present study involved the use of human blood samples.

Table 1
Five mostly investigated biomarkers for TBI.

Authors	Biomarkers	Molecular weight	Primary origin	TBI type	Effective half-life
[134]	S100B	9–21 kDa	Astrocytes	Mild, moderate & severe	24 h
[72,74]	GFAP	52 kDa	Astrocytes	Mild, Moderate & Severe	48 h
[58,92]	NSE	47 kDa	Neurons	Severe	48 h
[141]	UCH-L1	24 kDa	Neurons	Moderate & Severe	10 h
[66]	NF-L	Light 68 kDa Mid-sized 150 kDa Heavy 200 kDa	Neurons	Mild & Severe	(>1 weeks)

Funding disclosure

Financial assistance was received from the University of KwaZulu-Natal (UKZN), postgraduate scholarship, College of Health Sciences [Student number: 215001367]. The opinions expressed and conclusions arrived at are those of the authors and are not necessarily attributed to the UKZN.

Declaration of competing interest

The authors declare that they have no conflicts of interest to report.

Acknowledgements

The author declares no conflicts of interest in this study. The author wishes to acknowledge the family, friends, and colleagues for moral and spiritual support towards this research.

References

[1] D.K. Menon, et al., Position statement: definition of traumatic brain injury, Arch. Phys. Med. Rehabil. 91 (11) (2010) 1637–1640.
[2] M. Anto-Ocrah, et al., The elephant in the room: intimate partner violence, women, and traumatic brain injury in sub-Saharan Africa, Front. Neurol. 13 (2022), 917967.
[3] T.B. Cole, Global road safety crisis remedy sought: 1.2 million killed, 50 million injured annually, JAMA 291 (21) (2004) 2531–2532.
[4] O. Ogunfolaji, et al., Misconceptions about traumatic brain injuries in five sub-saharan African countries, Cureus 13 (9) (2021), e18369.
[5] M.C. Dewan, et al., Estimating the global incidence of traumatic brain injury, J. Neurosurg. (2018) 1–18.
[6] S. Koskinen, H. Alaranta, Traumatic brain injury in Finland 1991-2005: a nationwide register study of hospitalized and fatal TBI, Brain Inj. 22 (3) (2008) 205–214.
[7] J.J.P. Buitendag, et al., Validation of the Simplified Motor Score in patients with traumatic brain injury at a major trauma centre in South Africa, S. Afr. Med. J. 108 (2) (2018).
[8] J.C. Wong, et al., Traumatic brain injury in Africa in 2050, a modeling study 23 (2) (2016) 382–386.
[9] V.Y. Kong, et al., Quantifying the funding gap for management of traumatic brain injury at a major trauma centre in South Africa, S. Afr. J. Surg. 55 (4) (2017) 26–30.
[10] E. Jerome, et al., An audit of traumatic brain injury (TBI) in a busy developing-world trauma service exposes a significant deficit in resources available to manage severe TBI %, J SAMJ: S. Afr. Med. J. 107 (2017) 621–625.
[11] M. Prins, et al., The pathophysiology of traumatic brain injury at a glance, Disease models & mechanisms 6 (6) (2013) 1307–1315.
[12] R.A. Stern, et al., Clinical presentation of chronic traumatic encephalopathy, Neurology 81 (13) (2013) 1122–1129.

[13] S.A. Acosta, et al., Alpha-synuclein as a pathological link between chronic traumatic brain injury and Parkinson's disease, J. Cell. Physiol. 230 (5) (2015) 1024–1032.
[14] G. Matis, T. Birbilis, The Glasgow Coma Scale–a brief review. Past, present, future, Acta Neurol. Belg. 108 (3) (2008) 75–89.
[15] T. Fadalla, et al., Post-traumatic bilateral synchronous acute extradural hematomas: a case report and review of literature, Ann Med Surg (Lond) 75 (2022), 103377.
[16] P. Forastero Fernández-Salgueró, C. Echevarría Ruiz de Vargas, J.M. Barrera Chacón, Traumatismos craneoencefálicos. Escalas de valoración para la medida de resultados en rehabilitación, Rehabilitacion 36 (6) (2002) 408–417.
[17] P.V. Dong, O.L. Cremer, Limitations of the use of the Glasgow Coma Scale in intensive care patients with non-neurological primary disease: a search for alternatives, Crit. Care 15 (Suppl 1) (2011) P506. P506.
[18] A. Baratloo, et al., Predictive value of glasgow coma score and full outline of unresponsiveness score on the outcome of multiple trauma patients, Arch. Iran. Med. 19 (3) (2016) 215–220.
[19] J.H. Olson-Madden, et al., Substance use and mild traumatic brain injury risk reduction and prevention: a novel model for treatment, Rehabilitation Research and Practice (2012), 174579, 2012.
[20] S. Schulte, et al., A systematic review of the biomarker S100B: implications for sport-related concussion management, J. Athl. Train. 49 (6) (2014) 830–850.
[21] Y. Xiong, A. Mahmood, M. Chopp, Animal models of traumatic brain injury, Nat. Rev. Neurosci. 14 (2) (2013) 128–142.
[22] K. Kinoshita, Traumatic brain injury: pathophysiology for neurocritical care, Journal of Intensive Care 4 (2016).
[23] M.W. Greve, B.J. Zink, Pathophysiology of traumatic brain injury, Mt. Sinai J. Med. 76 (2) (2009) 97–104.
[24] Y. Xiong, A. Mahmood, M. Chopp, Emerging treatments for traumatic brain injury, Expet Opin. Emerg. Drugs 14 (1) (2009) 67–84.
[25] H.H. Dash, S. Chavali, Management of traumatic brain injury patients, Korean journal of anesthesiology 71 (1) (2018) 12–21.
[26] N.Y. Kim, et al., Hematological factors predicting mortality in patients with traumatic epidural or subdural hematoma undergoing emergency surgical evacuation: a retrospective cohort study, Medicine (Baltim.) 99 (37) (2020), e22074.
[27] R. Ved, M. Zaben, Biomarkers for traumatic brain injury, J. Neurol. 265 (5) (2018) 1241–1243.
[28] H. Takahashi, H. Sato, Y. Tsuji, Biochemical markers in the acute stage of head injury—Aldosterone and CK-BB&mdash, Neurol. Med.-Chir. 29 (3) (1989) 192–195.
[29] T. Ingebrigtsen, B. Romner, Biochemical serum markers of traumatic brain injury, J. Trauma 52 (4) (2002) 798–808.
[30] L.E. Pelinka, et al., Glial fibrillary acidic protein in serum after traumatic brain injury and multiple trauma, J. Trauma 57 (5) (2004) 1006–1012.
[31] G. Edwards 3rd, et al., Traumatic brain injury induces tau aggregation and spreading, J. Neurotrauma 37 (1) (2020) 80–92.
[32] N. El Mammeri, et al., Microtubule-binding core of the tau protein, Sci. Adv. 8 (29) (2022) eabo4459.
[33] R. Dixit, et al., Differential regulation of dynein and kinesin motor proteins by tau, Science 319 (5866) (2008) 1086–1089.
[34] A. Katsumoto, H. Takeuchi, F. Tanaka, Tau pathology in chronic traumatic encephalopathy and Alzheimer's disease: similarities and differences, Front. Neurol. 10 (2019) 980.
[35] S.M. Lippa, et al., APOE is associated with serum tau following uncomplicated mild traumatic brain injury, Front. Neurol. 13 (2022), 816625.
[36] C. Smith, et al., Tau immunohistochemistry in acute brain injury, Neuropathol. Appl. Neurobiol. 29 (5) (2003) 496–502.
[37] K. Uryu, et al., Multiple proteins implicated in neurodegenerative diseases accumulate in axons after brain trauma in humans, Exp. Neurol. 208 (2) (2007) 185–192.
[38] J.J. Bazarian, et al., Serum S-100B and cleaved-tau are poor predictors of long-term outcome after mild traumatic brain injury, Brain Inj. 20 (7) (2006) 759–765.
[39] D. Impellizzeri, et al., Traumatic brain injury leads to development of Parkinson's disease related pathology in mice, Front. Neurosci. 10 (2016) 458.
[40] L.D. Bernal-Conde, et al., Alpha-synuclein physiology and pathology: a perspective on cellular structures and organelles, Front. Neurosci. 13 (2019) 1399.
[41] L. Agnello, et al., Evaluation of alpha-synuclein cerebrospinal fluid levels in several neurological disorders, J. Clin. Med. 11 (11) (2022).
[42] E. Su, et al., alpha-Synuclein levels are elevated in cerebrospinal fluid following traumatic brain injury in infants and children: the effect of therapeutic hypothermia, Dev. Neurosci. 32 (5–6) (2010) 385–395.
[43] A.P. Di Battista, et al., Blood biomarkers in moderate-to-severe traumatic brain injury: potential utility of a multi-marker approach in characterizing outcome, Front. Neurol. 6 (2015) 110.
[44] A. Abdelhak, et al., Blood GFAP as an emerging biomarker in brain and spinal cord disorders, Nat. Rev. Neurol. 18 (3) (2022) 158–172.
[45] M. Brenner, Role of GFAP in CNS injuries, Neurosci. Lett. 565 (2014) 7–13.
[46] A. Ganne, et al., Glial fibrillary acidic protein: a biomarker and drug target for Alzheimer's disease, Pharmaceutics 14 (7) (2022).
[47] F. Michetti, et al., The S100B protein in biological fluids: more than a lifelong biomarker of brain distress, J. Neurochem. 120 (5) (2012) 644–659.
[48] G. Sreejit, et al., S100 family proteins in inflammation and beyond, Adv. Clin. Chem. 98 (2020) 173–231.
[49] M. Mata, D. Alessi, D.J. Fink, S100 is preferentially distributed in myelin-forming Schwann cells, J. Neurocytol. 19 (3) (1990) 432–442.
[50] Z. Yang, K.K. Wang, Glial fibrillary acidic protein: from intermediate filament assembly and gliosis to neurobiomarker, Trends Neurosci. 38 (6) (2015) 364–374.
[51] E. Czeiter, et al., Blood biomarkers on admission in acute traumatic brain injury: relations to severity, CT findings and care path in the CENTER-TBI study, EBioMedicine 56 (2020), 102785.
[52] N.A. Huebschmann, et al., Comparing glial fibrillary acidic protein (GFAP) in serum and plasma following mild traumatic brain injury in older adults, Front. Neurol. 11 (2020) 1054.
[53] M. Abedi, et al., Exosome derived from human neural stem cells improves motor activity and neurogenesis in a traumatic brain injury model, BioMed Res. Int. 2022 (2022), 6409346.
[54] S. Giacoppo, et al., Predictive biomarkers of recovery in traumatic brain injury, Neurocritical Care 16 (3) (2012) 470–477.
[55] E. Toman, S. Harrisson, T. Belli, Biomarkers in traumatic brain injury: a review, J. Roy. Army Med. Corps 162 (2) (2016) 103.
[56] R. Ergün, et al., Prognostic value of serum neuron-specific enolase levels after head injury, Neurol. Res. 20 (5) (1998) 418–420.
[57] S.A. Ross, et al., Neuron-specific enolase as an aid to outcome prediction in head injury, Br. J. Neurosurg. 10 (5) (1996) 471–476.
[58] F. Cheng, et al., The prognostic value of serum neuron-specific enolase in traumatic brain injury: systematic review and meta-analysis, PLoS One 9 (9) (2014) e106680.
[59] K.D. Wilkinson, et al., The neuron-specific protein PGP 9.5 is a ubiquitin carboxyl-terminal hydrolase, Science 246 (4930) (1989) 670–673.
[60] J.J. Bazarian, et al., Serum GFAP and UCH-L1 for prediction of absence of intracranial injuries on head CT (ALERT-TBI): a multicentre observational study, Lancet Neurol. 17 (9) (2018) 782–789.
[61] P. Shahim, et al., Time course and diagnostic utility of NfL, tau, GFAP, and UCH-L1 in subacute and chronic TBI, Neurology 95 (6) (2020) e623.
[62] R. Diaz-Arrastia, et al., Acute biomarkers of traumatic brain injury: relationship between plasma levels of ubiquitin C-terminal hydrolase-L1 and glial fibrillary acidic protein, J. Neurotrauma 31 (1) (2014) 19–25.
[63] H. Zetterberg, D.H. Smith, K. Blennow, Biomarkers of mild traumatic brain injury in cerebrospinal fluid and blood, Nat. Rev. Neurol. 9 (4) (2013) 201–210.
[64] N. Norgren, et al., Monoclonal antibodies selective for low molecular weight neurofilaments, Hybrid. Hybridomics 21 (1) (2002) 53–59.
[65] E.P. Thelin, D.W. Nelson, B.-M. Bellander, Secondary peaks of S100B in serum relate to subsequent radiological pathology in traumatic brain injury, Neurocritical Care 20 (2) (2014) 217–229.
[66] F. Al Nimer, et al., Comparative assessment of the prognostic value of biomarkers in traumatic brain injury reveals an independent role for serum levels of neurofilament light, PLoS One 10 (7) (2015) e0132177.
[67] D.B. Zimmer, et al., Evolution of the S100 family of calcium sensor proteins, Cell Calcium 53 (3) (2013) 170–179.
[68] A. Hermann, et al., S100 calcium binding proteins and ion channels, Front. Pharmacol. 3 (2012) 67.
[69] N. Pham, et al., Extracranial sources of S100B do not affect serum levels, PLoS One 5 (9) (2010), e12691.
[70] E.P. Thelin, et al., Assessing bicycle-related trauma using the biomarker S100B reveals a correlation with total injury severity, Eur. J. Trauma Emerg. Surg. 42 (5) (2016) 617–625.
[71] A. Raabe, et al., Serum S-100B protein in severe head injury, Neurosurgery 45 (3) (1999) 477–483.
[72] E.P. Thelin, D.W. Nelson, B.-M. Bellander, A review of the clinical utility of serum S100B protein levels in the assessment of traumatic brain injury, Acta Neurochir. 159 (2) (2017) 209–225.
[73] M. Huang, et al., High S100B levels in cerebrospinal fluid and peripheral blood of patients with acute basal ganglial hemorrhage are associated with poor outcome, World journal of emergency medicine 1 (1) (2010) 22–31.
[74] E.P. Thelin, et al., S100B is an important outcome predictor in traumatic brain injury, J. Neurotrauma 30 (7) (2013) 519–528.
[75] E.P. Thelin, et al., Utility of Neuron-specific Enolase in Traumatic Brain Injury; Relations to S100B Levels, Outcome, and Extracranial Injury Severity, vol. 20, Critical care, London, England), 2016, p. 285, 285.
[76] R. Donato, S100: a multigenic family of calcium-modulated proteins of the EF-hand type with intracellular and extracellular functional roles, Int. J. Biochem. Cell Biol. 33 (7) (2001) 637–668.
[77] J. Sen, A. Belli, S100B in neuropathologic states: the CRP of the brain? J. Neurosci. Res. 85 (7) (2007) 1373–1380.
[78] F. Brozzi, et al., S100B protein regulates astrocyte shape and migration via interaction with Src kinase: implications for astrocyte development, activation, and tumor growth, J. Biol. Chem. 284 (13) (2009) 8797–8811.
[79] W. Liu, et al., S100β in heavy metal-related child attention-deficit hyperactivity disorder in an informal e-waste recycling area, Neurotoxicology 45 (2014) 185–191.
[80] B. Qin, K.S. Panickar, R.A. Anderson, Cinnamon polyphenols regulate S100β, sirtuins, and neuroactive proteins in rat C6 glioma cells, Nutrition 30 (2) (2014) 210–217.
[81] R. Donato, et al., S100B's double life: intracellular regulator and extracellular signal, Biochim. Biophys. Acta 1793 (6) (2009) 1008–1022.
[82] M.C. Guerra, et al., Lipopolysaccharide modulates astrocytic S100B secretion: a study in cerebrospinal fluid and astrocyte cultures from rats, J. Neuroinflammation 8 (2011) 128.

[83] R. Donato, et al., S100B's double life: intracellular regulator and extracellular signal, Biochim. Biophys. Acta Mol. Cell Res. 1793 (6) (2009) 1008–1022.
[84] K. Willoughby, et al., S100B protein is released by in vitro trauma and reduces delayed neuronal injury, J. Neurochem. 91 (2005) 1284–1291.
[85] F. Tramontina, et al., High glutamate decreases S100B secretion by a mechanism dependent on the glutamate transporter, Neurochem. Res. 31 (6) (2006) 815–820.
[86] Y. Zhou, N.C. Danbolt, Glutamate as a neurotransmitter in the healthy brain, J. Neural. Transm. 121 (8) (2014) 799–817. Vienna, Austria : 1996.
[87] M.C. Guerra, et al., Lipopolysaccharide modulates astrocytic S100B secretion: a study in cerebrospinal fluid and astrocyte cultures from rats, J. Neuroinflammation 8 (1) (2011) 128.
[88] Y. Ke, et al., Anti-inflammatory effects of OxPAPC involve endothelial cell-mediated generation of LXA4, Circ. Res. 121 (3) (2017) 244–257.
[89] M.C. Leite, et al., Gap junction inhibitors modulate S100B secretion in astrocyte cultures and acute hippocampal slices, J. Neurosci. Res. 87 (11) (2009) 2439–2446.
[90] B. Jean-Louis, L. Said, La protéine S100B, marqueur biologique de tri pour le diagnostic du traumatisme crânien léger, Ann. Biol. Clin. 71 (1) (2013) 71–78.
[91] K.E. Andersen, H.I. Maibach, Black and white human skin differences, J. Am. Acad. Dermatol. 1 (3) (1979) 276–282.
[92] E.P. Thelin, D.W. Nelson, B.M. Bellander, A review of the clinical utility of serum S100B protein levels in the assessment of traumatic brain injury, Acta Neurochir. 159 (2) (2017) 209–225.
[93] G.E. Davey, et al., Calcium-dependent translocation of S100A11 requires tubulin filaments, Biochim. Biophys. Acta 1498 (2–3) (2000) 220–232.
[94] M.J. Berridge, M.D. Bootman, H.L. Roderick, Calcium signalling: dynamics, homeostasis and remodelling, Nat. Rev. Mol. Cell Biol. 4 (7) (2003) 517–529.
[95] G. Sorci, et al., S100B protein, A damage-associated molecular pattern protein in the brain and heart, and beyond, Cardiovasc Psychiatry Neurol 2010 (2010).
[96] P. Nardin, et al., S100B secretion in acute brain slices: modulation by extracellular levels of Ca2+ and K+, Neurochem. Res. 34 (9) (2009) 1603–1611.
[97] M. Gürsoy, R.L. Büyükuysal, Mechanism of S100b release from rat cortical slices determined under basal and stimulated conditions, Neurochem. Res. 35 (3) (2010) 429–436.
[98] A.G.M. Lam, et al., Mechanism of glial activation by S100B: involvement of the transcription factor NFκB, Neurobiol. Aging 22 (5) (2001) 765–772.
[99] E. Lasič, et al., Time-dependent uptake and trafficking of vesicles capturing extracellular S100B in cultured rat astrocytes, J. Neurochem. 139 (2) (2016) 309–323.
[100] Z. Zou, et al., The role of S100B/RAGE-enhanced ADAM17 activation in endothelial glycocalyx shedding after traumatic brain injury, J. Neuroinflammation 19 (1) (2022) 46.
[101] L. Rodrigues, et al., Longterm increased S100B enhances hippocampal progenitor cell proliferation in a transgenic mouse model, Int. J. Mol. Sci. 23 (17) (2022).
[102] X. Xiao, et al., S100 proteins in atherosclerosis, Clin. Chim. Acta 502 (2020) 293–304.
[103] M.Y. Zeng, et al., The roles of NADPH oxidase in modulating neutrophil effector responses, Mol Oral Microbiol 34 (2) (2019) 27–38.
[104] J. Checa, J.M. Aran, Reactive oxygen species: drivers of physiological and pathological processes, J. Inflamm. Res. 13 (2020) 1057–1073.
[105] H.J. Huttunen, et al., Coregulation of neurite outgrowth and cell survival by amphoterin and S100 proteins through receptor for advanced glycation end products (RAGE) activation, J. Biol. Chem. 275 (51) (2000) 40096–40105.
[106] J. Lin, et al., The calcium-binding protein S100B down-regulates p53 and apoptosis in malignant melanoma, J. Biol. Chem. 285 (35) (2010) 27487–27498.
[107] M.R. Fernandez-Fernandez, D.B. Veprintsev, A.R. Fersht, Proteins of the S100 family regulate the oligomerization of p53 tumor suppressor, Proc. Natl. Acad. Sci. U. S. A 102 (13) (2005) 4735.
[108] R. Astrand, J. Undén, Clinical use of the calcium-binding S100B protein, a biomarker for head injury, in: C.W. Heizmann (Ed.), Calcium-Binding Proteins of the EF-Hand Superfamily: from Basics to Medical Applications, Springer New York, New York, NY, 2019, pp. 679–690.
[109] M. Rothermundt, et al., S100B in brain damage and neurodegeneration, Microsc. Res. Tech. 60 (2003) 614–632.
[110] C.A. McKee, J.R. Lukens, Emerging roles for the immune system in traumatic brain injury, Front. Immunol. 7 (2016) 556.
[111] C. Nicaise, et al., Osmotic demyelination: from an oligodendrocyte to an astrocyte perspective, Int. J. Mol. Sci. 20 (5) (2019).
[112] A. Ishiki, et al., Glial fibrillar acidic protein in the cerebrospinal fluid of Alzheimer's disease, dementia with Lewy bodies, and frontotemporal lobar degeneration, J. Neurochem. 136 (2) (2016) 258–261.
[113] C. Farina, F. Aloisi, E. Meinl, Astrocytes are active players in cerebral innate immunity, Trends Immunol. 28 (3) (2007) 138–145.
[114] Z. Yang, K.K.W. Wang, Glial fibrillary acidic protein: from intermediate filament assembly and gliosis to neurobiomarker, Trends Neurosci. 38 (6) (2015) 364–374.
[115] D.V. Agoston, A. Shutes-David, E.R. Peskind, Biofluid biomarkers of traumatic brain injury, Brain Inj. 31 (9) (2017) 1195–1203.
[116] Y. Surjawan, et al., The different pattern of blood S100B protein and GFAP concentrations in ischemic stroke, Medical Journal of Indonesia 22 (4) (2013) 215–220, https://doi.org/10.13181/mji.v22i4.602. In this issue.
[117] T. Bogoslovsky, et al., Increases of plasma levels of glial fibrillary acidic protein, tau, and amyloid beta up to 90 Days after traumatic brain injury, J. Neurotrauma 34 (1) (2017) 66–73.
[118] A. Peraud, et al., Expression of fascin, an actin-bundling protein, in astrocytomas of varying grades, Brain Tumor Pathol. 20 (2) (2003) 53–58.
[119] T. Lefrançois, et al., Neuritic outgrowth associated with astroglial phenotypic changes induced by antisense glial fibrillary acidic protein (GFAP) mRNA in injured neuron-astrocyte cocultures, J. Neurosci. : off J Soc Neurosci 17 (11) (1997) 4121–4128.
[120] A. Ahmad, et al., Absence of TLR4 reduces neurovascular unit and secondary inflammatory process after traumatic brain injury in mice, PLoS One 8 (3) (2013), e57208.
[121] R. Kassubek, et al., GFAP in early multiple sclerosis: a biomarker for inflammation, Neurosci. Lett. 657 (2017) 166–170.
[122] S. Liddelow, et al., Neurotoxic reactive astrocytes are induced by activated microglia, Nature (2017) 541.
[123] L.E. Pelinka, et al., GFAP versus S100B in serum after traumatic brain injury: relationship to brain damage and outcome, J. Neurotrauma 21 (11) (2004) 1553–1561.
[124] T. Woodcock, M.C. Morganti-Kossmann, The role of markers of inflammation in traumatic brain injury, Front. Neurol. 4 (2013) 18.
[125] D.F. de Souza, et al., Interleukin-6-induced S100B secretion is inhibited by haloperidol and risperidone, Prog. Neuro-Psychopharmacol. Biol. Psychiatry 43 (2013) 14–22.
[126] A.D. Lafrenaye, et al., Circulating GFAP and Iba-1 levels are associated with pathophysiological sequelae in the thalamus in a pig model of mild TBI, Sci. Rep. 10 (1) (2020), 13369.
[127] S. Mondello, et al., Insight into pre-clinical models of traumatic brain injury using circulating brain damage biomarkers: operation brain trauma therapy, J. Neurotrauma 33 (2015).
[128] J. Lei, et al., Glial fibrillary acidic protein as a biomarker in severe traumatic brain injury patients: a prospective cohort study, Crit. Care 19 (2015) 362, 362.
[129] A. Buffo, et al., Origin and progeny of reactive gliosis: a source of multipotent cells in the injured brain, Proc. Natl. Acad. Sci. USA 105 (9) (2008) 3581.
[130] P.E. Vos, et al., Glial and neuronal proteins in serum predict outcome after severe traumatic brain injury, Neurology 62 (8) (2004) 1303–1310.
[131] A. Raabe, et al., Measurements of serum S-100B protein: effects of storage time and temperature on pre-analytical stability, Clin. Chem. Lab. Med. 41 (5) (2003) 700–703.
[132] L. Papa, et al., GFAP out-performs S100β in detecting traumatic intracranial lesions on computed tomography in trauma patients with mild traumatic brain injury and those with extracranial lesions, J. Neurotrauma 31 (22) (2014) 1815–1822.
[133] J. John, et al., Predicting functional outcomes in severe traumatic brain injury: role of S100B along with other clinical and imaging parameters, Current Medical Issues 20 (2) (2022).
[134] K. Muller, et al., S100B serum level predicts computed tomography findings after minor head injury, J. Trauma 62 (6) (2007) 1452–1456.
[135] K. Bechtel, et al., Relationship of serum S100B levels and intracranial injury in children with closed head trauma, Pediatrics 124 (4) (2009) e697–e704.
[136] A. David, et al., Evaluation of S100B blood level as a biomarker to avoid computed tomography in patients with mild head trauma under antithrombotic medication, Diagn Interv Imaging 98 (7–8) (2017) 551–556.
[137] F.M. Kelmendi, et al., Serum S100B levels can predict computed tomography findings in paediatric patients with mild head injury, BioMed Res. Int. 2018 (2018), 6954045.
[138] S. Kahouadji, et al., S100B blood level determination for early management of ski-related mild traumatic brain injury: a pilot study, Front. Neurol. 11 (2020) 856.
[139] V.F.J. Newcombe, et al., Post-acute blood biomarkers and disease progression in traumatic brain injury, Brain 145 (6) (2022) 2064–2076.
[140] R. H, et al., Can serum biomarkers prognosticate out come of traumatic brain injury (TBI)? J Med Sci Res 7 (2) (2019) 43–50.
[141] P. Bishop, D. Rocca, J.M. Henley, Ubiquitin C-terminal hydrolase L1 (UCH-L1): structure, distribution and roles in brain function and dysfunction, Biochem. J. 473 (16) (2016) 2453–2462.

BRIDGING TEXT

FROM CHAPTER TWO TO THREE

In the manuscript from Chapter Two, we noted that GFAP and S100B originate primarily from astrocytes, while NSE is mainly associated with neuronal damage and SAA is a hepatocyte and is associated with inflammatory cytokines like IL-1, IL-6, and TNF-α. GFAP, S100B, NSE and SAA were identified as the most suitable biomarkers for TBI diagnosis and have been implemented in some clinical settings in developed countries. Therefore, Chapter Three was designed to investigate GFAP and S100B separately from NSE and SAA based on their origins. A novel multiplex immunoassay was used to detect GFAP and S100B levels and assess their ability to predict the severity and progression of TBI. The results of this Chapter have been presented as a manuscript and are under review in the Journal of Neurocritical Care **(Manuscript number: JNC-25-031).**

CHAPTER THREE

MANUSCRIPT TWO

Evaluating Traumatic Brain Injury Severity Through GFAP and S100B Biomarkers: An Immunoassay-Based Approach

[1]Seke Nzau Mafuika, [2]Rohen Harrichandparsad, and [1]Lelika Lazarus

[1]Discipline of Clinical Anatomy, School of Laboratory Medicine & Medical Sciences, University of KwaZulu-Natal, Durban, South Africa

[2] Department of Neurosurgery, Inkosi Albert Luthuli Central Hospital, School of Clinical Medicine, College of Health Sciences, Nelson R Mandela School of Medicine, University of KwaZulu-Natal, Durban, South Africa

Corresponding Author: Seke Nzau Mafuika, Discipline of Clinical Anatomy, School of Laboratory Medicine & Medical Sciences, Nelson R Mandela School of Medicine, University of KwaZulu-Natal, 719 Umbilo Road, Durban, South Africa. Sekemafuika2@gmail.com

AUTHOR ORCID NUMBERS:

SN Mafuika: 0000-0003-1002-6565
R Harrichandparsad: 0000-0002-0391-8496
L Lazarus: 0000-0002-3113-3143

Abstract

Diagnosis and detection of low levels of serum GFAP and S100B biomarkers in traumatic brain injury (TBI) using highly sensitive blood and fluid assays, such as fluorescence-based techniques, enzyme-linked immunosorbent assays (ELISA), and electrochemiluminescence (ECL), remain major challenges despite improved predictive accuracy.

This prospective study utilizes a multiplex immunoassay (ProcartaPlex, Thermo Fisher Scientific, 2024) to detect human GFAP and S100B proteins in serum from 75 patients with moderate to severe TBI. Statistical analysis was achieved using Statistical Package for the Social Sciences version 30.0.0.0 (SPSS; IBM Corporation). Kruskal-Wallis, Dunn's multiple comparisons, and Pearson's chi-squared test were used to assess the association between variables. The results obtained were expressed as median and interquartile range, at $P< 0.05$.

Serum concentrations of GFAP and S100B were significantly elevated in the moderate and severe TBI groups compared with the control group ($P < 0.001$). No significant differences between the moderate and severe groups were detected for GFAP or S100B (P=0.2623, P>0.999).

These findings suggest that elevated serum concentrations of GFAP and S100B are indicative of increasing severity and progression of TBI, supporting the utility of multiplex immunoassay as a predictive tool. These findings suggest that the combined use of GFAP and S100B biomarkers enhances the accuracy of distinguishing between moderate and severe TBI as predictor test indicators of the condition.

Keywords: Traumatic brain injury, GFAP, S100B, neurology, Immunoassay, GCS.

TEXT

Introduction

Globally, traumatic brain injury (TBI) is the principal cause of ill health, disability, mental instability, and death (Kalyan, 2007; Williamson and Rajajee, 2021). TBI has a wide range of aetiology, which includes mechanical assault, firearm-related accidents, falls, or motor vehicle accidents (CDC, 2018; Miller et al., 2020).

Its complex pathophysiology is caused by primary and secondary trauma, resulting in either short-term or long-term neurological impairment (Xiong et al., 2013; Galgano et al., 2017). The primary injury is characterized by short-term impairment and primarily affects gross anatomical structures such as the skull, brain, blood vessels, and surrounding tissues of the head. In contrast, the secondary trauma occurs immediately following the primary impact, triggering pathophysiological pathways that lead to various cellular dysfunctions, including inflammation, altered cell differentiation and proliferation, as well as apoptosis (Galgano et al., 2017). This preempts a secondary injury that involves chemical, molecular, and inflammatory responses, thereby eliciting further cerebral damage. This cascade of events involves neuronal neutralization followed by the release of excitatory neurotransmitters such as aspartate and glutamate that cause a surge in intracellular calcium (Galgano et al., 2017; Abdelmalik et al., 2019). Consequently, this triggers the activation of apoptotic enzymes such as caspases and free radicals that degrade neuronal cells and impair the blood-brain barrier (BBB), causing cerebral damage (Annunziato et al., 2003; Galgano et al., 2017).

It is estimated that approximately 50 million individuals sustain TBI worldwide each year, with the highest incidence reported in Southeast Asia and Europe (Dewan et al., 2018; James et al., 2019). Africa and Southeast Asia are notably regarded as the epicentre of road traffic collision-

related TBIs (Dewan et al., 2018). Empirical evidence suggests that TBI affects every individual regardless of age and sex, but some specific groups are more vulnerable and at greater risk of prolonged disability and mortality (Rutland-Brown et al., 2005).

The severity of TBI is determined by a simple blow to the head, compared to a penetrating injury to the brain. It is classified into mild, moderate, and severe subtypes (CDC, 2015). The mild form is viewed as an injury without any obvious morphological damage secondary to a non-penetrating TBI with loss of consciousness not more than 30 minutes and with post-traumatic amnesia (PTA) not greater than 24 hours (CDC, 2015). Moderate TBI is characterized by the loss of consciousness and PTA lasting for 1-24 hours, whilst severe TBI is the loss of consciousness for more than 24 hours and PTA of more than 7 days (CDC, 2015).

Several clinical indices have been developed and utilized to grade the severity of TBI (Hill *et al*., 2016). These tools include the Galveston Orientation and Amnesia Test, Glasgow Coma Scale (GCS), and the Marshall Computed Tomography classification. Of these clinical tools, GCS is the most accurate, easy to use, and widely used to classify the severity of TBI into three subtypes (Syed *et al*., 2007). On the GCS scale, grades 13-15 indicate mild TBI, which accounts for approximately 80% of cases. A grading of 9-12 represents moderate TBI, which accounts for 10% of cases, whilst a grading of 3-8 represents severe TBI, which accounts for 10% of cases (Syed et al., 2007). Despite its acceptability and the widespread utilization of GCS in evaluating the severity of TBI, its limitations include subjectivity and the loss of verbal elements following patient intubation (Gardner and Zafonte, 2016).

Until recently, the evaluation of acute TBI was restricted to radiographic imaging and physical examination (Mahan et al., 2019). Biomarkers such as ubiquitin C-terminal hydrolase L1 (UCH-L1), S100 calcium-binding protein B (S100B) and glial fibrillary acidic protein (GFAP) have been

put forward as potential clinical predictive tools for the determination of TBI severity (Welch et al., 2017; Mahan et al., 2019).

The S100B protein is a class of calcium-binding proteins produced by neuroglial cells within the brain. This protein acts as a neurotrophic factor for neuroprotection and has been detected in serum after the opening of the BBB following brain injury (Xiong et al., 2009). The latter study proposed that a high concentration of S100B increases neuroinflammation and worsens neural survival. Numerous experimental studies have also interrogated the practicality of measuring blood S100B for the exclusion of mild TBI patients (Bouvier et al., 2009). More recently, GFAP has been suggested as an important brain fluid biomarker (Benedet et al., 2021). It is expressed on mature astrocytes within both white and grey matter, cerebellum, sub-granular and subventricular zones (Middeldorp and Hol, 2011). Several immunoassay methods can be used to detect S100B and GFAP in body fluids. Hence, this study aimed to employ a novel multiplex immunoassay to evaluate S100B and GFAP levels and predict TBI severity, with the aim of better understanding the progression of the injury and developing appropriate therapeutic modalities.

Materials and Methods

Study population

This is a prospective study that used purposive sampling of serum from TBI (n=50; determined by GCS) and control (n=25) patients. This TBI group was further stratified by severity into moderate (n=25) and severe (n=25) sub-groups. The control group consisted of healthy individuals with no underlying neurological disease or history of TBI.

Inclusion criteria

Male and female adult patients, irrespective of race and ethnicity (African, Indian, Coloured, and White), aged >18 years, were diagnosed with TBI based on the Glasgow Coma Scale (GCS), [moderate (9-12) to severe < 9]. Informed consent was obtained from all participants or family members.

Exclusion criteria

Exclusion criteria were participants who did not agree to participate in the study, participants younger than 18 years old, patients with mild TBI (13-15), and patients with other neurological conditions.

Reagents and Kits

ProcartaPlex kits: EPX010-12339-901 HU S100B Simplex and EPX010-12336-901 HU GFAP Simplex were purchased from Life Sciences Solutions Group, Thermo Fisher Scientific, Waltham, MA, USA.

GFAP and S100B concentrations in serum samples were measured using digital array technology (ProcartaPlex), which employs Luminex xMAP technology with magnetic beads for multiplexing and fluorescent detection via spectrally unique beads, enabling high sensitivity and specificity

(Kroll et al., 2023). ProcartaPlex Immunoassay is a novel technology that utilizes highly sensitive immunoassays with the lowest limit of quantification (LLOQ) of 36.72 pg/mL and the lowest limit of detection (LLOD) of 1.79 pg/mL for GFAP, and (LLOQ) of 1.84 pg/mL and (LLOD) of 0.19 pg/mL for S100B.

Blood collection and multiplex immunoassay

Blood samples were collected from patients within 3 hours of TBI from the time of presentation to the hospital to blood sampling using serum separator blood sample tubes. The sample tubes were appropriately labelled; samples were centrifuged for 10 minutes at 1000xg at 20-25°C. Undiluted serum samples were stored at -80°C. The S100B and GFAP concentrations were determined using ProcartaPlex Simplex Basic kits following the manufacturer's instructions. Additionally, CT scan results from each TBI group were analysed, and brain injuries were classified as either extradural, subdural, or subarachnoid.

Immunoassay procedure- Samples were brought to room temperature as per the manufacturer's instructions. The beads were vortexed for 30 seconds, and 50 µL of the beads was added to each well. The plate was subsequently washed with wash buffer. 25 µL of serum samples and standards were added to their designated wells and incubated overnight. Thereafter, the plate was washed, and 25 µL of biotinylated detection antibody mix was added to each well. The plate was incubated on a shaker at room temperature for 30 minutes before subsequent washing. 50 µL of Streptavidin-phycoerythrin was added to each well. The plate was incubated on a shaker for an additional 30 minutes at room temperature. Thereafter, the plate was washed. Lastly, 120 µL of reading Buffer was added to each well, and concentration was detected on the MAGPX18251721 system.

Data analysis

Statistical analysis was achieved using the Statistical Package for the Social Sciences, version 30.0.0.0 (SPSS, IBM Corporation). Kruskal-Wallis and Pearson Chi-Square tests were used to test the association of variables. Results with two-tailed $P < 0.05$ were considered significant, p-values were not corrected for multiple comparisons, and the results obtained were expressed as median and interquartile range.

Results

We report that interpersonal violence and assault occurred in 33.3% of TBI patients. Other causes emanated from sports (13.3 %), motor vehicle accidents (12.0%), and falls (8.0%) (Fig. 1).

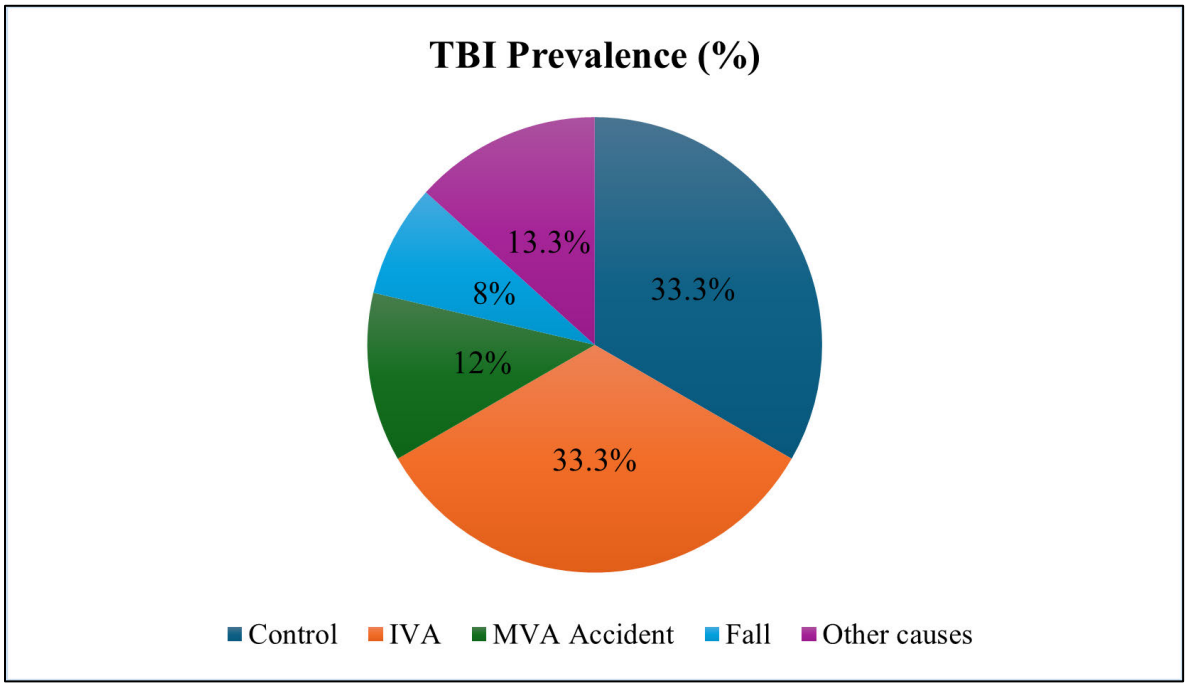


Figure 1: Total study population – Control (33%) and TBI (67%) prevalence.
Key: IVA: Interpersonal violence/assault; MVA: Motor Vehicle Accident

There was a significant difference in the cause of TBI by gender (P < 0.001). Males were more likely to sustain TBI compared to females, as shown in Table 1. Statistical significance was determined using the Pearson Chi-Square test.

Table 1: Causes of TBI compared to sex

Cause of TBI		**Sex (%)**		**Total (%)**	***P-value***
		Male	**Female**		
Control (no TBI)	Count	10	15	25	*P<0.001*
	% within Causes/History	40.0	60.0	100.0	
IVA	Count	25	0	25	
	% within Causes/History	100.0	0.0	100.0	
MVA	Count	7	2	9	
	% within Causes/History	77.8	22.2	100.0	
Fall	Count	6	0	6	
	% within Causes/History	100.0	0.0	100.0	
Other causes	Count	9	1	10	
	% within Causes/History	90.0	10.0	100.0	
Total	Count	57	18	75	
	% within Causes/History	76.0	24.0	100.0	

Key: IVA: Interpersonal violence/assault; MVA: Motor Vehicle Accident, P-value for comparisons between Control and TBI

There was a significant difference (*P<.001)* between the cause of TBI *vs.* Age. Patients aged 18-29 years had the highest IVA score (56.0%) compared with other age groups. Patients aged 45+ had the highest falls score (66.7%). Patients aged 30-44 years and 45+ years had the same prevalence (40.0%) of other causes as the 18-29 years age group (20.0%). However, all age groups had an equal prevalence of 33.3% for MVA as shown in Table 2. Statistical significance was determined using the Pearson Chi-Square test.

Table 2: Causes of TBI compared to age

Causes of TBI		Age group			Total	*P-value*
		18-29	30-44	45+		
Control	Count	23	2	0	25	
	% within Causes/History	92.0	8.0	0.0	100.0	
Interpersonal violence (IVA) / Assault	Count	14	11	0	25	
	% within Causes/History	56.0	44.0	0.0	100.0	
Motor Vehicle Accident (MVA)	Count	3	3	3	9	
	% within Causes/History	33.3	33.3	33.3	100.0	*p<0.001*
Fall	Count	2	0	4	6	
	% within Causes/History	33.3	0.0	66.7	100.0	
Other causes	Count	2	4	4	10	
	% within Causes/History	20.0	40.0	40.0	100.0	
Total	Count	44	20	11	75	
	% within Causes/History	58.7	26.7	14.7	100.0	

CT Scan results showed that the moderate group had a higher percentage of extradural and subarachnoid hematoma injuries (61.9% and 66.7%, respectively) compared to the severe group (38.1% and 33.3%, respectively). However, the severe group had a higher percentage of subdural hematoma injuries (61.5 %) compared to moderate (38.5 %), as shown in Figure 2. Statistical significance was observed between injury type and GCS score (p<0.001).

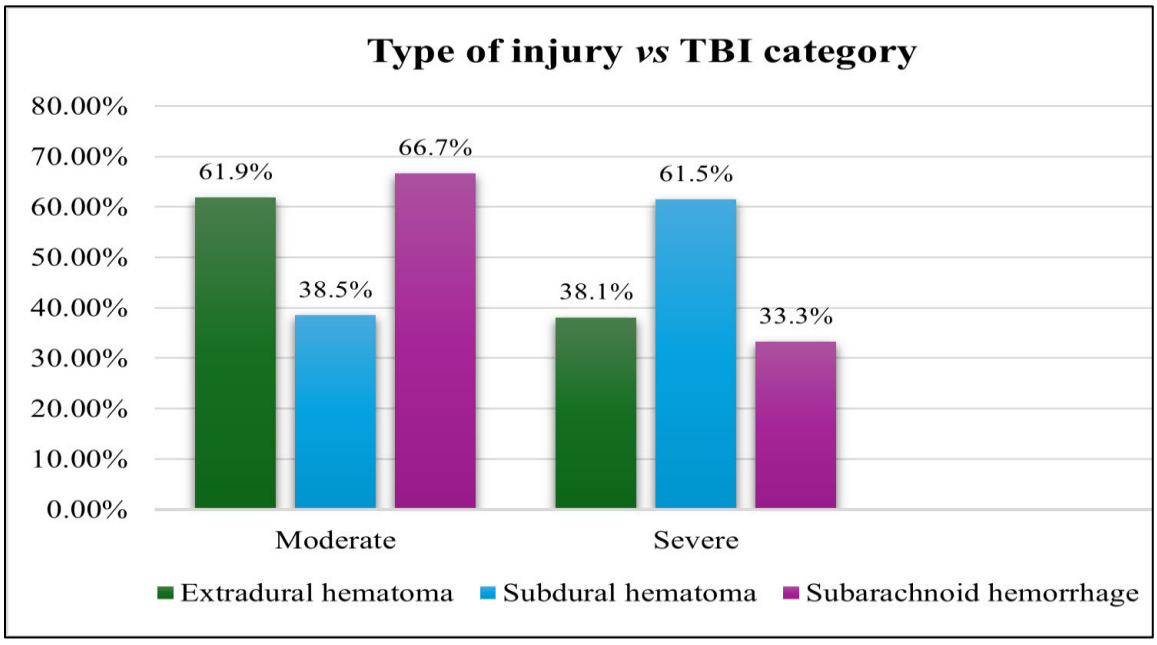


Figure 2: Comparison between the types of injury based on GCS

There was a significant increase between the control (median = 1.79 pg/ml, IQR =1.79-2.19 pg/ml) and moderate (median = 155.40 pg/ml, IQR = 36.72-1819 pg/ml) groups for GFAP ($p<0.0001$), Figure 3(a). The serum concentration of GFAP was significantly higher in the severe group (median = 3240 pg/ml, IQR = 111.70-8839 pg/ml) than in the control group ($p<0.001$). There was no significant difference in serum concentration of GFAP between moderate and severe groups [$p=0.2623$, Figure 3(a)]. Statistical significance was determined using Dunn's multiple-comparison test with a 95% confidence interval.

Similarly, S100B was significantly upregulated in moderate (median = 0.40 pg/ml, IQR = 0.23-1.16 pg/ml) and severe (median = 0.59 pg/ml, IQR = 0.23-1.84 pg/ml) groups compared to the control group (median = 0.19 pg/ml, IQR = 0.19-0.23 pg/ml) ($P<0.0001$). However, there was no significant difference in serum concentration of S100B between the moderate and severe groups [($P>0.999$); Figure 3(b)]. Statistical significance was determined using Dunn's multiple-comparison test with a 95% confidence interval.

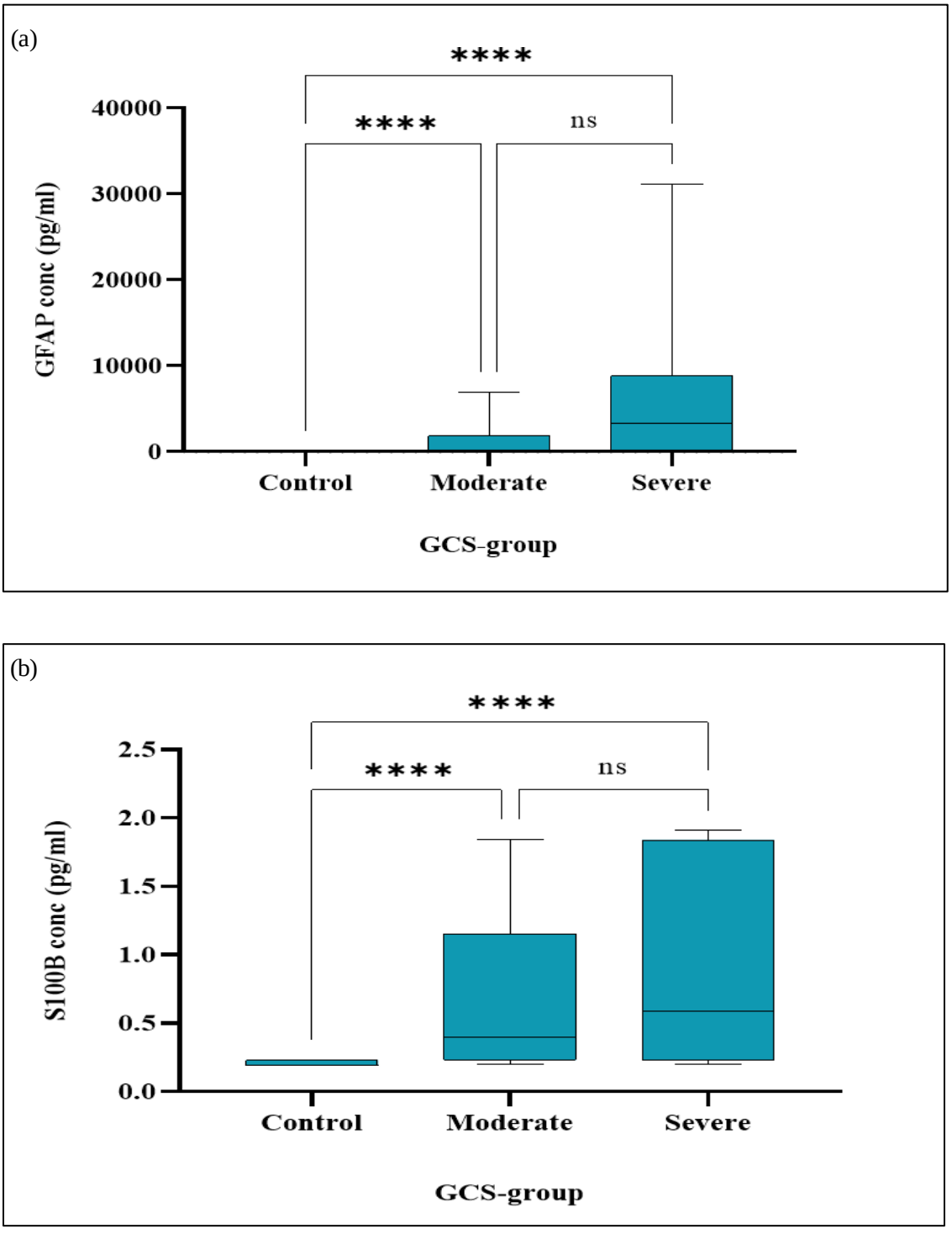


Figure 3: (a) GFAP, (b) S100B concentrations *vs*. GCS category, n=25 for each group.

****** statistical significance, ns: not significant. Data is represented as median and interquartile range**

A further analysis was made to test the correlation of GFAP and S100B. A strong positive correlation between GFAP and S100B concentrations was demonstrated [Kruskal-Wallis Test - *p<0,001*, r = 0,731], as shown in Table 3.

Table 3: Correlation analysis between concentrations of GFAP and S100B protein

Correlations				
			GFAP conc (pg/ml)	**S100B conc (pg/ml)**
Spearman's rho	GFAP conc (pg/ml)	Correlation Coefficient	1.000	0.731**
		Sig. (2-tailed)	.	*P<0.001*
		n	75	75
	S100B conc (pg/ml)	Correlation Coefficient	0.731**	1.000
		Sig. (2-tailed)	*P<0.001*	.
		n	75	75
**. Correlation is significant at the 0.01 level (2-tailed)				

Serum GFAP concentrations did not differ significantly by gender, age group, injury type, or patient outcome. However, S100B showed statistical significance for injury type and patient outcome (p<0.001), as shown in Tables 4 and 5 using the Mann-Whitney U and Kruskal-Wallis Tests, respectively.

Table 4: Cross analysis of analytes across sex (n= 75)

Gender	**GFAP conc (pg/ml)** Median & Interquartile Range (IQR) (pg/mL)	**S100B conc (pg/ml)** Median & Interquartile Range (IQR) (pg/mL)
Male (n=57)	155.43 (29.32-3386.69)	0.40 (0.23-0.95)
Female (n=18)	1.79 (1.79-8.15)	0.23 (0.19-0.23)
P-value	*<0.001*	0.008

Table 5: Cross analysis of analytes across participants' demographics (n= 75)

		Analyte concentration Median & Interquartile Range (IQR) (pg/mL)		***P-value***	
		GFAP	**S100B**	**GFAP**	**S100B**
Age group	18-29 (n=44)	8.15 (1.79-1608.83)	0.23 (0.19-0.59)	0.515	0.030
	30-44 (n=20)	157.28 (25.61-1980.98)	0.25 (0.23-0.54)		
	45+ (n=11)	166.76 (36.72-4380.54)	0.92 (0.23-1.84)		
Type of injury	Control (n=25)	2.00 (1.79-2.20)	1.00 (0.19-0.23)	0.049	<0.001
	Extradural (n=21)	211.89 (36.72-4020.77)	0.40 (0.23-1.73)		
	Subdural (n=26)	1324.79 (36.72-4729.25)	0.40 (0.23-1.84)		
	Subarachnoid (n=3)	1895.98 (155.43-5450.58)	0.98 (0.23-1.61)		
Patient outcome	Control group (n=25)	1.79 (2.00-2.20)	1.00 (0.19-0.23)	0.021	<0.001
	Discharged (n=42)	479.63 (36.72-4403.38)	0.59 (0.23-1.84)		
	Passed away (n=8)	992.96 (141.23-5412.31)	0.32 (0.23-0.40)		

Discussion

The National Institute of Occupational Health (NIOH) in South Africa (SA) has reported TBI as a significant public health issue. Of note, SA has an incidence of TBI of 89,000 per annum, with the main cause being motor vehicle, bicycle, or pedestrian–vehicle accidents accounting for 50% (Dewan et al., 2018). In a 2007 audit of TBI care in KwaZulu-Natal, a concerning lack of understanding and prioritization of secondary brain injury prevention and classification of TBI was identified (Alexander et al., 2009). This study reports that interpersonal violence/assault (IVA) is the main cause of TBI, accounting for 33.3%, followed by other (non-intentional trauma) causes (13.3%), motor-vehicle accident (MVA) (12%) and fall (8%), Fig. 1. Furthermore, we report the effect of gender and age, as males between the ages of 18- 29 years (56%) and 30-44 years (44%)

were most susceptible to sustaining TBI compared to females of the same age. The TBI group analysis demonstrated that males (100%) were from IVA, 77.8% from MVA, 100% from a fall, and 90% from other causes (Tables 1 and 2). These results are corroborated by Ramdheen and Naicker in 2021, where 78.7% of males and 59.7% of males emanated from IVA as the main contributing factor to the aetiology of TBI (Ramdheen and Naicker, 2021). However, it is worth noting that the incidence reported in this study was based on patients who reported and were admitted to a tertiary hospital. Many patients with unreported TBIs who died at the sites of the accident or during transfer to a hospital, or due to lack of accessibility to a hospital, are not accounted for in this report.

Our study quantified the concentration of the most promising GFAP and S100B biomarkers for the diagnosis of TBI. Due to several limitations of conventional tools for diagnosing TBI, such as GCS, CT, MRI and functional MRI, biomarkers have the potential to improve the accuracy of TBI diagnosis. The United States of America's National Institutes of Health and the National Institute of Neurological Disorders and Stroke have recently proposed a new approach for diagnosing and categorizing TBI. This approach consists of four components: a clinical component (comprehensive GCS and pupillary reactivity); a biomarker component (blood-based metrics); an imaging component (pathoanatomical assessments); and a modifier component (factors affecting clinical presentation and outcomes) (Manley et al., 2025). Several biomarkers have shown suitability; however, many have not been applicable in clinical settings. To be appealing and considered for use in the clinic, such markers must be readily accessible, easy to interpret and quick and inexpensive to process (Okonkwo et al., 2013). Therefore, this study investigated the concentrations of relevant biomarkers, such as GFAP and S100B, which have been shown to be suitable, using a novel multiplex technique. Hence, this experimental investigation reports increased GFAP levels in moderate and severe compared to control patients ($p<0.0001$). However,

there was no significant difference in serum GFAP levels between the moderate and severe groups [p=0.262; Fig. 3(a)]. It is worth noting that GFAP is released during astrocytic activity, and its increased levels reflect the brain's response to injury (Okonkwo et al., 2013). Also, GFAP functions in providing strength and support for the neurons and astrocytes as well as safeguarding the BBB (McKeon and Benarroch, 2018). Many studies have shown that GFAP is released into the interstitial fluid and then crosses the BBB into the bloodstream following brain injury (Mondello et al., 2011; Huebschmann et al., 2020). Hence, GFAP upregulation may be directly proportional to injury severity. Despite a lack of upregulation of serum GFAP in patients with severe TBI compared with those with moderate TBI, we observed a higher serum GFAP concentration in patients with severe TBI. The precise mechanisms that trigger changes in GFAP levels are still unclear, but they are known to be associated with astrocyte and glial cell damage or activation (Okonkwo et al., 2013). GFAP has both neurotrophic and neurotoxic effects (Gust et al., 2023). It is proposed that following the secondary phase of the injury, cytokines such as Oncostatin M (OSM), Ciliary neurotrophic factor (CNTF) and Leukaemia Inhibitory Factor (LIF) activate the Janus kinase (JAK) pathway. The JAK then activates the Signal Transducer and Activator of Transcription 3 (STAT3) pathway through phosphorylation, which subsequently leads to GFAP expression (Brenner and Messing, 2021). Additionally, Smad1 aids STAT3 in stimulating GFAP, following the binding of Bone Morphogenetic Protein 2 (BMP2) to its plasma membrane receptor (Abidin et al., 2017). Neurogenin (Ngn), an abundant transcription factor expressed during neuronal differentiation, inhibits JAK-mediated STAT3 phosphorylation, thereby suppressing GFAP expression and neurotrophic effects (Agarwal et al., 2015).

This study also showed a significant upregulation of S100B in the moderate and severe groups compared with the control group (*P<0.0001*). Similar to GFAP, there was no significant difference in serum S100B concentration between the moderate and severe groups (P>0.999), as shown in

Fig. 3(b). In an experiment conducted by Anderson et al., (2001), the S100B level in normal individuals was 10 pg/mL. It is unexpected, but we report a lower level of 0.19 pg/mL in normal patients, suggesting that the immunoassay used in this experiment can detect lower concentrations of biomarkers.

S100B proteins are mainly expressed in the supporting cells of the brain, such as astrocytes and Schwann cells, and are usually found in the extracellular space and blood following brain injury (Gerlach et al., 2006). This protein is essential for brain development and recovery from injury, which substantiates its role as a marker of neuronal death, glial activation, and the severity and progression of TBI (Yardan et al., 2011). In our study, elevated serum S100B protein levels were observed in the moderate and severe TBI groups compared with the control group. Our results support S100B's ability to predict the progression and severity of TBI, consistent with a previous study by Kövesdi et al. (2010). Similarly, a study found that high concentrations of S100B increase neuroinflammation and worsen neural survival (Xiong et al., 2009). This result confirms that a significantly increased concentration of S100B protein exacerbates the severity and progression of TBI. However, studies have demonstrated that S100B is also present in extracerebral tissues and fluids, including bone marrow, heart, and muscle (Anderson et al., 2001). Hence, the concentration of S100B detected in our study may not be specific to the brain but may also emanate from other extracerebral organs. The mechanism of action of S100B remains unclear, but it is generally thought to interact with the receptor for advanced glycation end products (RAGE), which can exacerbate the inflammatory response and progression of injury (Zou et al., 2022). S100B has been reported to stimulate glial iNOS via a signal transduction pathway involving the transcription factor NF-κB. Consequently, NF-κB triggers activities in neuronal and glial cells that ultimately lead to cell proliferation and apoptosis, as well as neural and immune development and

inflammation (Lam et al., 2001). Therefore, the elevated S100B levels in our study may reflect an inflammatory response following brain injury.

In our study, increases in GFAP and S100B were significant ($P < 0.001$). A correlation ($r = 1$) between GFAP and S100B was also observed, as shown in Table 3. Thus, we conclude that GFAP and S100B concentrations increase simultaneously following TBI, making them a good pair of complementary biomarkers for TBI diagnosis. However, Janigro et al. (2022) reported conflicting results, stating that GFAP release may be delayed by a simultaneous, passive release of serum S100B from damaged astrocytes. Nevertheless, elevated levels of GFAP and S100B may be attributed to several factors, including bone fractures, cerebral injury, endothelial cell dysfunction, and systemic inflammation (Anderson et al., 2001). The proteins may trigger or reflect endothelial dysfunction and elevated inflammatory cytokines (IL-6, IL-8), both of which are common in severe trauma and the systemic inflammatory response (Dang et al., 2014). The non-significant increase of GFAP and S100B between the moderate and severe groups may be attributed to several factors, such as cerebral ischemia, increased intracranial pressure, reduced cerebral perfusion pressure, complete vascular collapse, blood or fluid transfusions during resuscitation, which may cause rapid clearance of biomarkers and massive tissue necrosis, which can cause complete astrocytes destruction in severe injury (Pelinka et al., 2004; Janigro et al., 2022).

We also observed that both serum GFAP and S100B levels were higher in males than in females, and they increased proportionally with age, as shown in Table 4 and 5. Contrary to our study, Castellani et al. (2009) reported a significant increase in S100B in younger patients. We observed significant differences in serum levels of the two proteins between extradural, subdural, and subarachnoid injuries ($P=0.021$ and $P<0.001$, respectively; Table 5). Serum GFAP and S100B levels were higher in patients with subarachnoid injury. This is true, as it reflects the extent of the

injury up to the brain. Thus, GFAP and S100B may be used in conjunction with CT or MRI to aid in diagnosing TBI and assessing its severity. Previous studies found GFAP and S100B to be sensitive enough to detect and assess a variety of traumatic intracranial lesions, including cerebral contusions, subdural and extradural hematomas, as well as subarachnoid haemorrhages (Thelin et al., 2017).

Elevated GFAP and S100B on intensive care unit admission have been correlated to mortality in severe TBI patients with greater accuracy when measured 24 hours after admission (Wu et al., 2020; Gyldenholm et al., 2022). Persistent high levels of serum GFAP and S100B have been previously linked to fatal neuronal damage, resulting in death, regardless of GCS scores (Wu et al., 2020; Trung Nguyen et al., 2024). In contrast to previous studies, we observed a significant increase in serum GFAP and S100B levels in discharged patients (Table 5).

The significantly elevated serum concentrations of GFAP and S100B in the moderate and severe TBI groups in our study indicate the suitability of the multiplex technique for detecting GFAP and S100B levels, confirming the ability of these two biomarkers to distinguish between patients with moderate and severe brain injury. A similar investigation documented the usefulness of serum GFAP concentration in predicting TBI severity (Abdelhak et al., 2022). However, emerging evidence suggests that serum concentrations of GFAP and S100B have not yet been widely explored as predictors of TBI severity and progression (Vos et al., 2004). This finding revealed that the serum levels of GFAP and S100B increase as the TBI progresses from moderate to severe. Moreover, GFAP is a constituent of the cytoskeleton that anchors neurons and neuroglial cells, especially astrocytes. Nevertheless, careful interpretation of serum S100B should be considered, as this protein can be detected outside the nervous system and is elevated in polytrauma patients, unlike GFAP, which is specific to the brain (Anderson et al., 2001).

Conclusion

The study reports that progression from moderate to severe TBI increases serum concentrations of GFAP and S100B. However, for more accurate prediction of TBI severity and progression across the moderate-to-severe categories, combining these two biomarkers may yield better results. GFAP and S100B may be useful adjuncts for characterising TBI severity, but should not be interpreted as discriminatory tools between moderate and severe TBI without further investigation. This study also emphasises that the findings support the promise of multiplex immunoassays in detecting low concentrations of GFAP and S100B, rather than asserting that these biomarkers alone can classify severity grades. Our dataset was limited to single-time-point sampling and a modest sample size, which restricted the ability to perform secondary analyses, such as examining temporal changes in biomarker levels. This is a limitation of the study, and future studies should incorporate longitudinal sampling and expanded statistical modeling to better characterize biomarker dynamics. Furthermore, the study did not permit parallel testing with established methods such as ELISA. Future work should include comparisons with established platforms.

Limitations of the study

Limitations of this study include a small sample size. The inability to obtain consent from relatives of TBI patients affected the sample size. Also, it must be noted that some of the patients may be HIV positive and have received ART, which may have influenced apoptosis, endothelial cell injury, as well as inflammation. Additionally, biological sex should ideally be evaluated while controlling for major confounding factors, including injury severity, mechanism of injury, age, time from injury to sampling, and other clinical characteristics known to influence biomarker release. The candidate acknowledges that differences in the distribution of TBI severity, injury mechanisms, and sample sizes between male and female participants may have contributed to the

observed variation in GFAP concentrations. Therefore, the reported sex differences should not be interpreted as being solely attributed to biological sex. We recommend conducting further large-scale experiments to compare GFAP and S100B with other biomarkers as tools for diagnosing TBI at different time points.

Declaration of competing interests

The authors have no conflicts of interest to declare.

Funding disclosure

This work was supported by the University of KwaZulu-Natal (UKZN), Postgraduate Fund, College of Health Sciences [Student number: 215001367]. The opinions expressed and conclusions arrived at are those of the authors and are not necessarily attributed to the UKZN.

Acknowledgments

The author declares no conflicts of interest in this study. The author wishes to acknowledge the Discipline of Clinical Medicine, University of KwaZulu-Natal, Medical School Campus; family and friends; neurosurgeons at Inkosi Albert Luthuli Central Hospital; and colleagues for their support in completing this research.

References

1) Abdelhak A, Foschi M, Abu-Rumeileh S, Yue JK, D'anna L, Huss A, Oeckl P, Ludolph AC, Kuhle J, Petzold A, Manley GT, Green AJ, Otto M & Tumani H. 2022. Blood GFAP as an Emerging Biomarker in Brain and Spinal Cord Disorders. *Nature Reviews Neurology,* 18**,** 158-172.
2) Abdelmalik PA, Draghic N & Ling GSF. 2019. Management of Moderate and Severe Traumatic Brain Injury. *Transfusion,* 59**,** 1529-1538.
3) Abidin SZ, Leong JW, Mahmoudi M, Nordin, Abdullah S, Cheah PS & Ling KH. 2017. In Silico Prediction and Validation of GFAP as an miR-3099 Target In Mouse Brain. *Neuroscience Bulletin,* 33**,** 373-382.
4) Agarwal V, Bell, GW, Nam JW & Bartel DP. 2015. Predicting Effective MicroRNA Target Sites in Mammalian MRNAs. *Elife,* 4**,** E05005.
5) Alexander T, Fuller G, Hargovan P, Clarke DL, Muckart DJ & Thomson SR. 2009. An Audit of The Quality of Care of Traumatic Brain Injury at a Busy Regional Hospital In South Africa. *S Afr J Surg,* 47**,** 120-2, 124-6.
6) Anderson RE, Hansson LO, Nilsson O, Dijlai-Merzoug R & Settergren G. 2001a. High Serum S100B Levels for Trauma Patients Without Head Injuries. *Neurosurgery,* 48**,** 1255-1260.
7) Annunziato L, Amoroso S, Pannaccione A, Cataldi M, Pignataro G, D'alessio A, Sirabella R, Secondo A, Sibaud L & Di Renzo GF. 2003. Apoptosis Induced In Neuronal Cells By Oxidative Stress: Role Played By Caspases And Intracellular Calcium Ions. *Toxicol Lett,* 139**,** 125-33.
8) Bouvier D, Oddoze C, Ben Haim D, Moustafa F, Legrand A, Alazia M, Jehle E, Schmidt J & Sapin V. 2009. [Interest of S100B Protein Blood Level Determination For The Management of Patients with Minor Head Trauma]. *Ann Biol Clin (Paris),* 67**,** 425-31.

9) Brenner M., & Messing, A. 2021. Regulation Of GFAP Expression. *Asn Neuro,* 13**,** 1759091420981206.

10) Castellani C, Bimbashi P, Ruttenstock E, Sacherer P, Stojakovic T & Weinberg AM. 2009. Neuroprotein S100b -- A Useful Parameter in Paediatric Patients with Mild Traumatic Brain Injury? *Acta Paediatr,* 98**,** 1607-12.

11) CDC 2015. Report to Congress on Traumatic Brain Injury in the United States: Epidemiology and Rehabilitation. *National Center For Injury Prevention And Control,* 2**,** 1-72.

12) CDC 2018. Report to Congress: The Management of Traumatic Brain Injury in Children, National Center For Injury Prevention and Control. *Division of Unintentional Injury Prevention, Atlanta, Ga.*

13) Dang X, Guan L, Hu W, Du G & Li J. 2014. S100b Ranks as a New Marker of Multiple Traumas in Patients and May Accelerate Its Development by Regulating Endothelial Cell Dysfunction. *Int J Clin Exp Pathol,* 7**,** 3818-26.

14) Dewan MC, Rattani A, Gupta S, Baticulon RE, Hung YC, Punchak M, Agrawal A, Adeley, AO, Shrime MG & Rubiano AM. 2018. Estimating The Global Incidence of Traumatic Brain Injury. *Journal Of Neurosurgery,* 130**,** 1080-1097.

15) Galgano M, Toshkezi G, Qiu X, Russell T, Chin L & Zhao LR. 2017. Traumatic Brain Injury: Current Treatment Strategies And Future Endeavors. *Cell Transplant,* 26**,** 1118-1130.

16) Gardner AJ & Zafonte R. 2016. Neuroepidemiology of Traumatic Brain Injury. *Handb Clin Neurol,* 138**,** 207-23.

17) Gerlach R, Demel G, König HG, Gross U, Prehn J, Raabe A, Seifert V & Kögel D. 2006. Active Secretion of S100B from Astrocytes during Metabolic Stress. *Neuroscience,* 141**,** 1697-1701.

18) Gust J, Rawlings-Rhea SD, Wilson AL, Tulberg NM, Sherman AL, Seidel KD, Wu QV, Park JR, Gardner RA, & Annesley CE. 2023. GFAP and NFL Increase During Neurotoxicity From High Baseline Levels In Pediatric CD19-Car T-Cell Patients. *Blood Adv,* 7**,** 1001-1010.

19) Gyldenholm T, Hvas CL, Hvas AM & Hviid CVB. 2022. Serum Glial Fibrillary Acidic Protein (GFAP) Predicts Outcome After Intracerebral And Subarachnoid Hemorrhage. *Neurol Sci,* 43**,** 6011-6019.

20) Huebschmann NA, Luoto TM, Karr JE, Berghem K, Blennow K, Zetterberg H, Ashton NJ, Simrén J, Posti JP, Gill JM & Iverson GL. 2020. Comparing Glial Fibrillary Acidic Protein (GFAP) in Serum and Plasma Following Mild Traumatic Brain Injury in Older Adults. *Front Neurol,* 11**,** 1054.

21) James SL, Theadom A, Ellenbogen RG, Bannick MS, Montjoy-Venning W, Lucchesi LR, Abbasi N, Abdulkader R, Abraha HN & Adsuar JC. 2019. Global, Regional, and National Burden of Traumatic Brain Injury and Spinal Cord Injury, 1990–2016: A Systematic Analysis For The Global Burden of Disease Study 2016. *The Lancet Neurology,* 18**,** 56-87.

22) Janigro D, Mondello S, Posti JP & Unden J. 2022. GFAP and S100B: What You Always Wanted to Know and Never Dared to Ask. *Frontiers In Neurology,* Volume 13 - 2022.

23) Kalyan S, Nadasan T, and Puckree T. 2007. The Epidemiology of Traumatic Brain Injuries (TBI): A Literature Review. *South African Journal Of Physiotherapy,* 63**,** 32.

24) Kövesdi E, Lückl J, Bukovics P, Farkas O, Pál J, Czeiter E, Szellár D, Dóczi T, Komoly S & Büki A. 2010. Update On Protein Biomarkers In Traumatic Brain Injury With Emphasis On Clinical Use In Adults And Pediatrics. *Acta Neurochirurgica,* 152**,** 1-17.

25) Kroll KW, Woolley G, Terry K, Premeaux TA, Shikuma CM, Corley MJ, Bowler S, Ndhlovu, LC & Reeves RK. 2023. Multiplex Analysis of Cytokines and Chemokines in Persons Aging With or without HIV. *Aids Research and Human Retroviruses,* 39**,** 367-380.

26) Lam AGM, Koppal T, Akama KT, Guo L, Craft JM, Samy B, Schavocky JP, Martin Watterson D & Van Eldik LJ. 2001. Mechanism of Glial Activation by S100b: Involvement of the Transcription Factor Nfκb. *Neurobiology Of Aging,* 22**,** 765-772.

27) Mahan MY, Thorpe M, Ahmadi A, Abdallah T, Casey H, Sturtevant D, Judge-Yoakam S, Hoover C, Rafter D, Miner J, Richardson C & Samadani U. 2019. Glial Fibrillary Acidic Protein (GFAP) Outperforms S100 Calcium-Binding Protein B (S100b) and Ubiquitin C-Terminal Hydrolase L1 (UCH-L1) as a Predictor for Positive Computed Tomography of the Head in Trauma Subjects. *World Neurosurg,* 128**,** E434-E444.

28) Manley GT, Dams-O'Connor K, Alosco et al. 2025. A New Characterisation of Acute Traumatic Brain Injury: The NIH-NINDS TBI Classification and Nomenclature Initiative. *The Lancet Neurology,* 24**,** 512-523.

29) McKeon A & Benarroch EE. 2018. GLIAL Fibrillary Acid Protein: Functions and Involvement in Disease. *Neurology,* 90**,** 925-930.

30) Middeldorp J & Hol EM. 2011. GFAP In Health And Disease. *Prog Neurobiol,* 93**,** 421-43.

31) Miller GF, Kegler SR & Stone DM. 2020. Traumatic Brain Injury–Related Deaths From Firearm Suicide: United States, 2008–2017. *American Journal Of Public Health,* 110**,** 897-899.

32) Mondello S, Muller U, Jeromin A, Streeter J, Hayes RL & Wang KK. 2011. Blood-Based Diagnostics of Traumatic Brain Injuries. *Expert Review Of Molecular Diagnostics,* 11**,** 65-78.

33) Okonkwo DO, Yue JK, Puccio AM, Panczykowski DM, Inoue T, McMahon PJ, Sorani MD, Yuh EL, Lingsma HF, Maas AI, Valadka AB, & Manley GT. 2013. GFAP-BDP as an Acute Diagnostic Marker In Traumatic Brain Injury: Results From The Prospective Transforming Research And Clinical Knowledge In Traumatic Brain Injury Study. *J Neurotrauma,* 30**,** 1490-7.

34) Pelinka, L. E., Kroepfl, A., et al. 2004. GFAP Versus S100B in Serum after Traumatic Brain Injury: Relationship to Brain Damage and Outcome. Journal of Neurotrauma, 21, 1553-1561.

35) Ramdheen S & Naicker B. 2021. Evaluating the Burden of Head Injuries on a Rural Emergency Department in South Africa. *S Afr Fam Pract (2004),* 63**,** E1-E6.

36) Syed AT, Lone NA, Wani MA & Bhat AS. 2007. Clinical Management of Patients with Minor Head Injuries. *Int J Health Sci (Qassim),* 1**,** 131-40.

37) Thelin EP, Nelson DW & Bellander BM. 2017. A Review of the Clinical Utility of Serum S100b Protein Levels in the Assessment of Traumatic Brain Injury. *Acta Neurochir (Wien),* 159**,** 209-225.

38) Trung Nguyen K, Dinh Le T, Dang Le M, Viet Tran T, Van Pham C, Van Dang B, Tien Nguyen S, Chi Nguyen T, Quang Nguyen H, The Vu A, Luong Cong T, Duong Huy H & Anh Vu H. 2024. Changes in the Levels of Serum Glial Fibrillary Acidic Protein and the Correlation with Outcomes in Severe Traumatic Brain Injury Patients. *Sage Open Medicine,* 12**,** 20503121241260006.

39) Vos PE, Lamers K, Hendriks J, Van Haaren M, Beems T, Zimmerman C, Van Geel W, De Reus H, Biert J & Verbeek M. 2004. Glial and Neuronal Proteins in Serum Predict Outcome after Severe Traumatic Brain Injury. *Neurology*, 62**,** 1303-1310.

40) Welch RD, Ellis M, Lewis LM, Ayaz SI, Mika VH, Millis S & Papa L. 2017. Modeling the Kinetics of Serum Glial Fibrillary Acidic Protein, Ubiquitin Carboxyl-Terminal Hydrolase-L1, and S100b Concentrations in Patients With Traumatic Brain Injury. *J Neurotrauma*, 34**,** 1957-1971.

41) Williamson C & Rajajee V. 2021. Traumatic Brain Injury: Epidemiology, Classification, And Pathophysiology. Uptodate.

42) Wu L, Feng Q, Ai ML, Deng SY, Liu ZY, Huang L, Ai YH & Zhang L. 2020. The Dynamic Change of Serum S100B Levels From Day 1 To Day 3 Is More Associated With Sepsis-Associated Encephalopathy. *Scientific Reports,* 10**,** 7718.

43) Xiong Y, Mahmood A & Chopp M. 2009. Emerging Treatments For Traumatic Brain Injury. *Expert Opin Emerg Drugs,* 14**,** 67-84.

44) Xiong Y, Mahmood A & Chopp M. 2013. Animal Models of Traumatic Brain Injury. *Nat Rev Neurosci,* 14**,** 128-42.

45) Yardan T, Erenler AK, Baydin A, Aydin K & Cokluk C. 2011. Usefulness of S100b Protein in Neurological Disorders. *Jpma-Journal of the Pakistan Medical Association,* 61**,** 276.

46) Zou Z, Li L, Li Q, Zhao P, Zhang K, Liu C, Cai D, Maegele M, Gu Z & Huang Q. 2022. The Role of S100b/Rage-Enhanced Adam17 Activation in Endothelial Glycocalyx Shedding After Traumatic Brain Injury. *Journal Of Neuroinflammation,* 19**,** 46.

BRIDGING TEXT

FROM CHAPTER THREE TO FOUR

In Chapter Three, the detection and quantification of GFAP and S100B in astrocytes showed substantial differences in GFAP and S100B levels between healthy individuals and patients with moderate to severe TBI, and can be useful for TBI prognosis and diagnosis. However, no significant difference in GFAP and S100B levels was found between the moderate and severe groups. These findings required further investigation, especially to establish the difference in severity between moderate and severe TBI. On this basis, Chapter four will explore additional biomarkers, NSE and SAA, their roles and usefulness in TBI classification based on their neuronal and hepatocytic origins, respectively. The results obtained from this investigation were prepared as a manuscript to be submitted for publication.

CHAPTER FOUR

MANUSCRIPT THREE

TITLE: Evaluation of Serum Neuron-Specific Enolase and Serum Amyloid A in Moderate-to-Severe Traumatic Brain Injury in Association with Glasgow Coma Scale and CT Findings for Improved Classification in a South African Cohort

AUTHORS: SN Mafuika[1], R Harrichandparsad[2], L Lazarus[1]

AFFILIATIONS:
[1]Discipline of Clinical Anatomy
School of Medicine
College of Health Sciences
University of KwaZulu-Natal
Durban, South Africa

[2]Department of Neurosurgery
Inkosi Albert Luthuli Central Hospital
School of Medicine
College of Health Sciences
Nelson R Mandela School of Medicine
University of KwaZulu-Natal
Durban, South Africa

RUNNING HEADLINE: Redefining TBI classification with Biomarkers

CORRESPONDING AUTHOR: Mr. SN Mafuika
Department of Clinical Anatomy
School of Medicine
College of Health Sciences
University of KwaZulu-Natal
Private Bag X54001
Durban
4000
South Africa

EMAIL: sekemafuika2@gmail.com

AUTHOR ORCID NUMBERS: SN Mafuika: 0000-0003-1002-6565
R Harrichandparsad: 0000-0002-0391-8496
L Lazarus: 0000-0002-3113-3143

Abstract

The current classification and prognostic system for TBI assessment includes the GCS and CT scans. However, recent research has highlighted the limitations and unreliability of the GCS scoring system in assessing injury severity in TBI patients. Biomarkers, CT and MRI scans, as well as pre-existing medical conditions, environmental factors and the manner in which the injury occurred, are now included in the new assessment criteria.

This prospective study used a multiplex immunoassay (ProcartaPlex, Thermo Fisher Scientific, 2024) to compare the concentrations of human NSE and SAA biomarkers in serum from 75 patients with moderate to severe TBI and from normal individuals. Statistical analysis was achieved using Statistical Package for the Social Sciences version 30.0.0.0 (SPSS; IBM Corporation). The results obtained were expressed as medians and interquartile ranges, with $p < 0.05$.

These findings suggest that elevated serum concentrations of NSE and SAA are indicative of increasing severity of TBI. These findings suggest that combining NSE and SAA biomarkers with GCS and CT findings enhances the accuracy of TBI classification.

Keywords: Traumatic brain injury, NSE, SAA, serum biomarkers, new classification

TEXT

Introduction

Traumatic brain injury (TBI) is one of the most common causes of disability and death across all age groups (Yan et al., 2025). TBI occurs when there is a sudden or physical assault that damages the brain, resulting in physical disability, long-term cognitive and social defects (Richmond and Rogol, 2014). Over 50 million individuals are reported to suffer from TBI each year, which accounts for 30–40% of all injury-related fatalities globally (Zhong et al., 2025). Over 80% of neurosurgical admissions for TBI in South Africa (SA) consist of young adult males between the ages of 17-40 years (Maasdorp et al., 2020). In SA, reports show that TBI undoubtedly constitutes a public health emergency, adding more weight to the already overwhelmed South African healthcare system (Jerome et al., 2017). In KwaZulu-Natal Province (KZN, SA), a study reported a significant burden of TBI amongst children in Pietermaritzburg (Buitendag et al., 2017). Kalyan (2007) also reported that 40% of the patients admitted to the public acute neurological unit in KZN were diagnosed with TBI. The SA statistic reports that the high crime rate in SA is a major contributor to the high TBI prevalence, with assault and Road Traffic Incidents (RTIs) accounting for 26.5% of all non-natural deaths (Gettleman, 2009; Ramdheen and Naicker, 2021). In SA, crime, RTIs and violence are the main contributors to the high TBI incidence compared to high-income countries (HICs)(Seedat et al., 2009). In HICs, falls are the major cause of TBI, particularly among older individuals (Maas et al., 2022).

Currently, the classification and prognostic system for TBI assessment during patient admission includes the Glasgow Coma Score (GCS), international normalized ratio, absence of pupillary reactivity, platelet count, activated partial thromboplastin time and neutrophil-to-lymphocyte ratio

(Kim et al., 2020). Investigating the above-mentioned clinical characteristics is essential before entering the operating room.

Conventional Classification of TBI

Traumatic Brain Injury may be classified based on several factors, including type, severity, location, injury mechanism and physiological response (Carney et al., 2017). *Classification by clinical severity* is based on the GCS system, which has been widely and extensively used to classify TBI into sub-categories of mild (GCS 13-15), moderate (GCS 9-12), or severe (GCS <9) (Stiell et al., 2001). *The Mayo TBI classification system* divides patients into three categories, namely, definite, probable and possible, based on clinical and CT findings (Malec et al., 2007). *Classification by etiology* distinguishes between blunt, penetrating, and blast injuries (Ling et al., 2009). *Classification by injury progression* differentiates between primary and secondary injuries. The primary injury results from the immediate mechanical force applied to the head, whether blunt, penetrating, or blast, leading to structural damage such as concussion, skull fracture, contusion, hematoma (subdural, intracerebral, or subarachnoid), axonal shear, or laceration (Silver et al., 2012). In contrast, the secondary injury encompasses the complex cascade of pathophysiological processes that unfold following the initial insult. These include cerebral oedema, increased intracranial pressure, ischemia, seizures, inflammation and traumatic venous sinus thrombosis (Maas et al., 2008). *Classification by area of involvement* is based on the distribution of the injury within the brain, categorized as focal, diffuse, or a combination of both (Maas et al., 2008).

In South Africa, the GCS is the most commonly used marker for assessing the level of consciousness in patients following TBI (Salottolo et al., 2014). However, recent research has reported limitations and unreliability in the GCS scoring system for determining injury severity in TBI patients (Sherer et al., 2008). Studies have reported several factors, such as administration of

sedatives, early intubation, facial injuries and administration of paralyzing agents or intoxicants, that may hinder the reliability and accuracy of the GCS scoring system (Zafonte et al., 1996; Murray et al., 1999).

Therefore, in recent times, the use of additional and accurate laboratory prognostic markers to determine the severity of TBI has been highly sought (Wang et al., 2021). In 2022, a new framework was proposed by the USA National Institutes of Health-National Institute of Neurological Disorders and Stroke. Biomarker evaluation, CT and MRI scans, as well as preexisting medical conditions, environmental factors and the manner in which the injury occurred, are now included in the new assessment criteria (Manley et al., 2025).

Neuron-specific enolase (NSE) is a key cytoplasmic enzyme present in neurons that plays a central role in the glycolytic pathway. Elevated extracellular NSE reflects neuronal injury (Mollayeva et al., 2018). NSE has been investigated as an acute-phase biomarker in stroke, potentially signaling early neuronal damage (Nguyen et al., 2018). Interestingly, NSE has also been detected in the saliva of stroke patients and individuals at risk, suggesting disruption of the blood-brain barrier (BBB) and leakage of the enzyme from the central nervous system (CNS) (Al-Rawi and Atiyah, 2009).

Serum Amyloid A is an acute-phase protein that is primarily released by hepatocytes in response to infection, tissue damage and cancer (Villapol et al., 2015). Several previous studies have reported elevated SAA concentrations in TBI patients and shown that SAA is highly sensitive to disease severity (Rael et al., 2009; Aly et al., 2011). Its release may aid in the accurate detection and direct measurement of the severity of brain injury and may be a useful tool for TBI diagnosis when pathological symptoms or neuroimaging analysis fail to provide this information (Wicker et al., 2019).

Hence, this study aimed to evaluate the role of NSE and SAA biomarkers in moderate-to-severe TBI, in association with GCS and CT findings, to improve classification in a South African Cohort. We hypothesize that NSE and SAA levels correlate positively with TBI severity, as indicated by GCS and CT findings.

Materials and methods

The study population consisted of 25 controls and 50 patients diagnosed with TBI, as recorded in the hybrid electronic medical registry, MediTech, at a large urban tertiary referral hospital in eThekwini, South Africa. This included 25 patients with severe TBI and 25 with moderate TBI. Standard demographic data were analyzed and patients were classified according to the severity of their head injury, as well as their gender and age. The severity of TBI was determined based on the GCS scoring system, in line with international standards, as follows: severe (GCS <9), moderate (GCS 9-12) and mild (GCS 13-15). The control (n = 25) group consisted of normal individuals with no underlying neurological disease or history of TBI.

All CT scans were performed in accordance with the Neuroradiology Department's protocol. Investigators who read the scans were blinded to clinical information. CT classification was done by a radiologist and categorized as traumatic subarachnoid hemorrhage, subdural hematoma, extradural hematoma, or diffuse axonal injury. Ethics approval was obtained from the Biomedical Research Ethics Committee (Ref. BREC/00002147/2020) of the University of KwaZulu-Natal.

For biomarkers to be used as tools for TBI diagnosis, we first need to determine whether their concentrations differ significantly by injury severity and other demographic factors. Therefore, we measured and determined the concentrations of NSE and SAA in each TBI group according to GCS score. Subsequently, the concentrations of NSE and SAA were further analyzed and compared against the types and extent of the injury based on the analyzed scan results.

Reagents and multiplex immunoassay Kits

ProcartaPlex kits: EPX010-12335-901 HU NSE Simplex and EPX01A-12136-901 HU SAA Simplex were purchased from Life Sciences Solutions Group, Thermo Fisher Scientific, Waltham, MA, USA.

NSE and SAA concentrations in serum samples were measured using digital array technology (ProcartaPlex), which employs Luminex xMAP technology with magnetic beads for multiplexing and fluorescent detection, utilizing spectrally unique beads to achieve high sensitivity and specificity (Kroll et al., 2023). ProcartaPlex Immunoassay is a novel technology that utilizes highly sensitive immunoassays with the lowest limit of quantification (LLOQ) 42564.06 pg/mL for NSE and (LLOQ) 169.73 pg/mL for SAA.

Immunoassay procedure- Samples were brought to room temperature as per the manufacturer's instructions. The beads were vortexed for 30 seconds and 50 µL of beads were added to each well. The plate was subsequently washed with wash buffer. A volume of 25 µL of serum samples and standards were added to their designated well and left to incubate overnight. Thereafter, the plate was washed and 25 µL of biotinylated detection antibody mix was added to each well. The plate was incubated on the shaker for 30 minutes at room temperature before being washed subsequently. A total of 50 µL of Streptavidin-phycoerythrin was added to each well. The plate was then incubated on a shaker for an additional 30 minutes at room temperature. Thereafter, the plate was washed. Lastly, 120 µL of reading Buffer was added to each well and concentrations were detected on the MAGPX18251721 system. The recommended sample dilution factor for SAA was 100; NSE did not require any sample dilution.

Inclusion criteria encompassed male and female patients over 18 years of age, of any race or ethnicity, who were admitted to the hospital with isolated head trauma resulting in TBI.

Exclusion criteria included individuals under 18 years of age, patients with mild TBI (GCS 13–15) and those admitted for medical conditions other than TBI.

Analysis was achieved using the Statistical Package for the Social Sciences, version 30.0.0.0 (SPSS, IBM Corporation). Kruskal-Wallis and Pearson Chi-Square tests were used to assess the association between variables, with 95% confidence intervals. Results with two-tailed $p < 0.05$ were considered significant, p-values were not corrected for multiple comparisons and the results obtained were expressed as median and interquartile range.

Result

The following results were obtained from patients with moderate and severe TBI. There was a substantial increase of serum NSE in the moderate (median = 863.7 ng/mL, IQR = 15.44-2917 ng/mL) and severe (median = 626.8 ng/mL, IQR = 334.1-626.8 ng/mL) TBI groups compared to the control group (median = 115.5 ng/mL, IQR = 68.67-518.0 ng/mL), Figure 1(a).

A significant difference in serum NSE concentration was observed among the control, moderate, and severe TBI groups ($p < 0.001$). However, no significant difference was observed between the moderate and severe groups ($p > 0.99$), as shown in Figure 1(a).

Similarly, SAA was significantly up-regulated in moderate (median = 2308 ng/mL, IQR = 1283-2828 ng/mL) and severe (median = 1678 ng/mL, IQR = 1162-2319 ng/mL) groups compared to the control group (median = 418.7 ng/mL, IQR = 291.2-987.5 ng/mL) ($p<0.001$) and ($p= 0.002$), respectively. However, there was no significant difference in serum concentration of SAA between the moderate and severe groups [($p= 0.59$); Figure 1(b)].

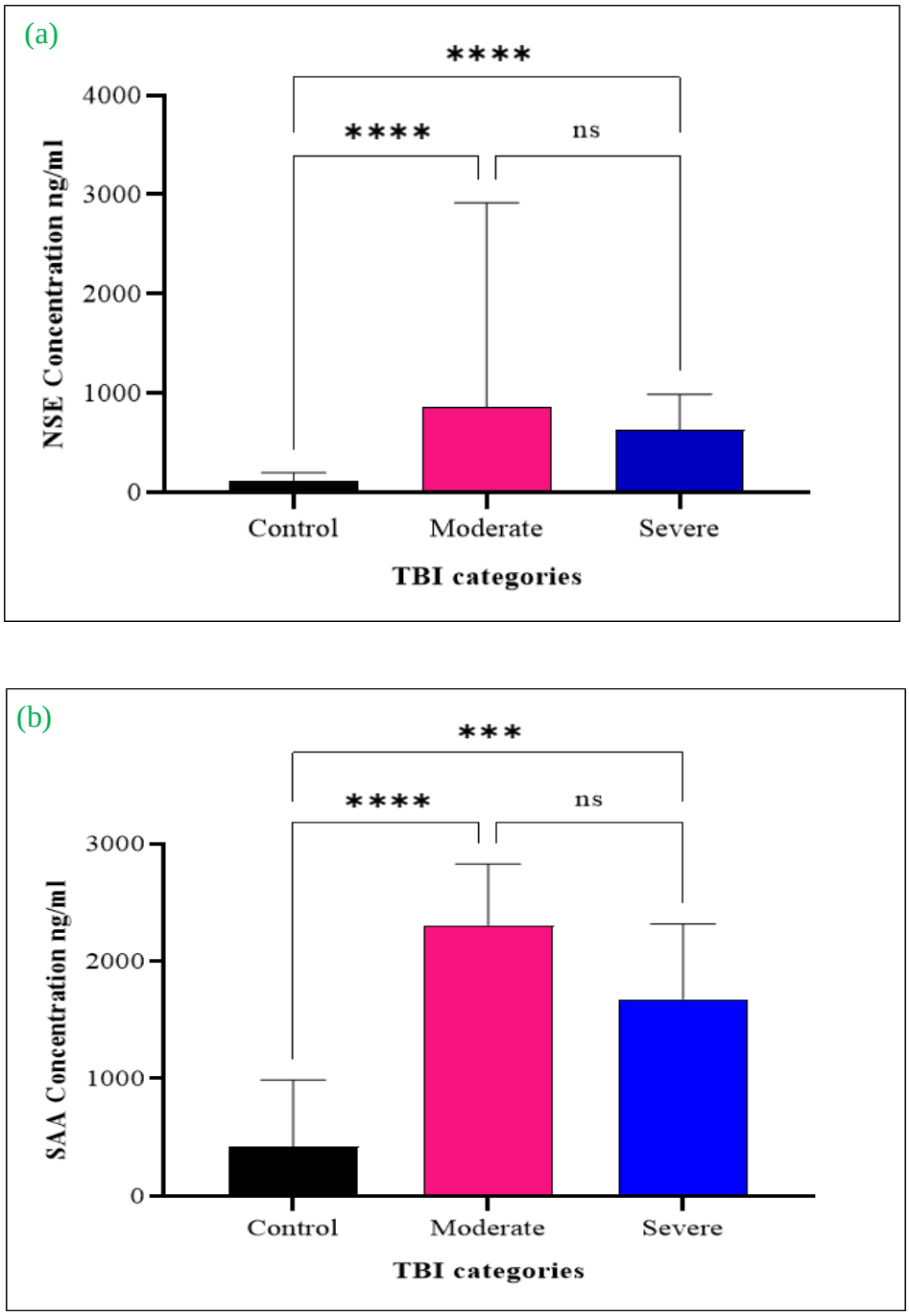


Figure 1: (a) NSE, (b) SAA concentrations *vs.* GCS category, n=25 for each group

Results show a relatively positive correlation between NSE and SAA (Spearman's rho Test), as shown in Table 1.

Table 1: Correlation between NSE and SAA biomarkers

Correlation			
		NSE (ng/mL)	**SAA (ng/mL)**
NSE (ng/mL)	Correlation Coefficient	1	0.374**
	Sig. (2-tailed)	0	<0.001
	n	75	75
SAA (ng/mL)	Correlation Coefficient	0.374**	1
	Sig. (2-tailed)	<0.001	0
	n	75	75
**. Correlation is significant at the 0.01 level (2-tailed).			

There was no substantial difference in the concentration of NSE and SAA between focal and diffuse brain injury, *p-value 0.314* and *p-value 0.472*, respectively, as shown in Table 2. The statistical significance was determined using the Mann-Whitney U test. However, a substantial difference has been noted between injury types, with a p-value < *0.001* (Table 3). Statistical significance was determined using the Kruskal-Wallis multiple-comparisons test with a 95% confidence interval. Therefore, the null hypothesis that the distributions of NSE and SAA (ng/mL) are the same across the different types of injury is rejected.

Table 2: NSE and SAA concentrations *vs.* extent of injury

	Extent of injury	n	Median (ng/mL)	Interquartile Range (IQR) (ng/mL)	*p-value*
NSE	Control	25	115.533	57.46-350.04	0.314
	Focal	37	626.780	233.31-2675.19	
	Diffuse	13	1007.778	113.41-4606.36	
	Total	75			
SAA	Control	25	418.712	192.27-1288.61	0.472
	Focal	37	1861.340	1161.512-2881.406	
	Diffuse	13	1870.465	691.26-3390.67	
	Total	75			

Table 3: NSE and SAA concentrations across types of injury

		NSE (ng/mL) n=75		SAA (ng/mL) n=75		*p-value across haemorrhage*	*Overall p-value*
		Median	**Interquartile Range (IQR) (ng/mL)**	**Median**	**Interquartile Range (IQR) (ng/mL)**		
Type of injury	**Control**	115.533	350.043-57.464	418.712	192.27-1288.61		*p<0.001*
	Extradural hematoma	809.472	3092.343-295.606	2317.084	1161.69-3390.669	*p= 0.37*	
	Subdural hematoma	628.246	3420.943-160.422	1740.819	943.06-3294.671	*p= 0.18*	
	Subarachnoid haemorrhage	2675.188	3159.257-0	1161.512	0-2275.596	*p= 0.33*	

A relatively positive correlation between the serum concentration of NSE, SAA and age was observed (Spearman's rho Test), as shown in Table 4.

Table 4: Correlation between age and concentration of NSE and SAA

Correlations				
		NSE (ng/mL)	**SAA (ng/mL)**	**Age**
NSE (ng/mL)	Correlation Coefficient	1	0.374**	0.286*
	Sig. (2-tailed)	.	<0.001	0.013
	n	75	75	75
SAA (ng/mL)	Correlation Coefficient	0.374**	1	0.293*
	Sig. (2-tailed)	<0.001	.	0.011
	n	75	75	75
Age	Correlation Coefficient	0.286*	0.293*	1
	Sig. (2-tailed)	0.013	0.011	.
	n	75	75	75
** Correlation is significant at the 0.01 level (2-tailed).				
* Correlation is significant at the 0.05 level (2-tailed).				

The serum concentrations of both NSE and SAA varied by gender, with p-values of *<0.001* and *0.002*, respectively. The concentration of NSE and SAA was higher in males compared to females, as shown in Table 5.

Table 5: Comparison of NSE and SAA concentrations across gender

		NSE (ng/mL)		*p-value*	SAA (ng/mL)		*p-value*
		Median	**Interquartile range**		**Median**	**Interquartile range**	
Sex	**Male**	542.631	149.67-2482.16	**0.001**	1678.160	677.87-2775.39	0.002
	Female	127.719	75.87-3092.34		873.350	192.27-2016.74	
	Total	350.043	113.41-2415.24		1288.612	411.98-2634.796	

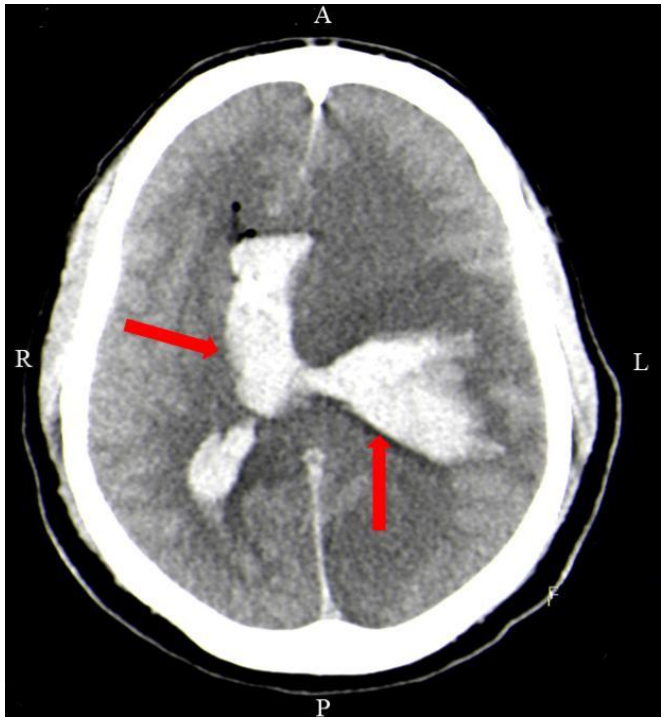
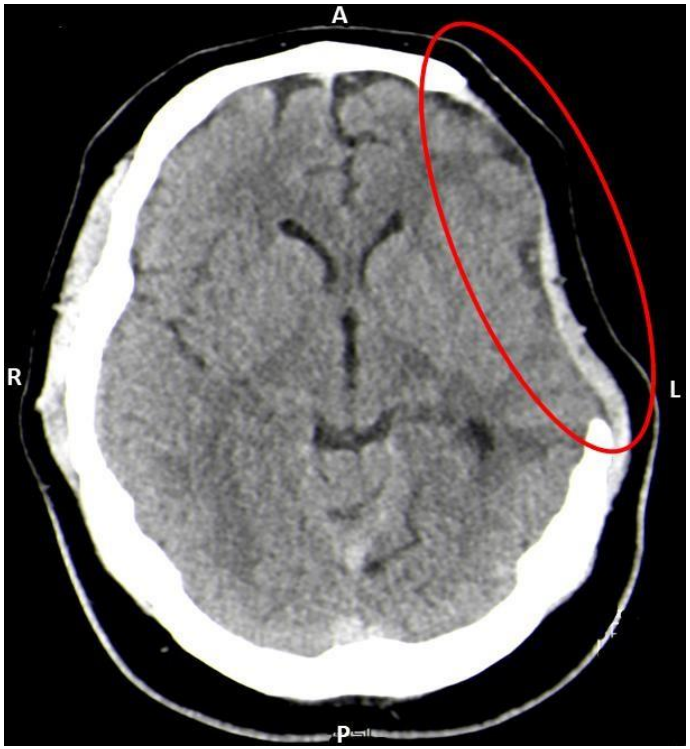
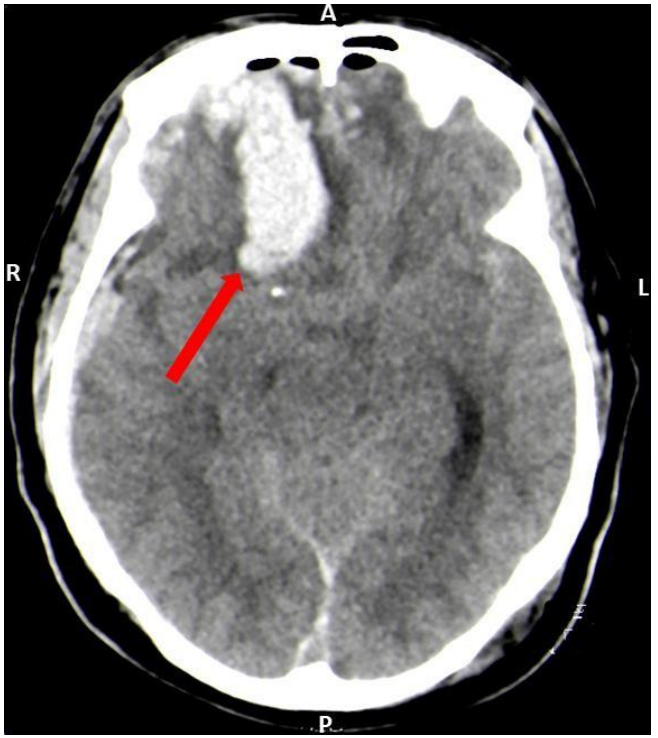
A	B	C
		
Clinical: GCS score: 6	**Clinical: GCS score**: 7	**Clinical: GCS score**: 7
Biomarker: NSE concentration: 542.631 ng/mL **SAA concentration**: 1161.689 ng/mL	**Biomarker: NSE concentration**: 115.269 ng/mL **SAA concentration**: 1678.16 ng/mL	**Biomarker: NSE concentration**: 149.669 ng/mL **SAA concentration**: 3525.845 ng/mL
Scan results: soft tissue swelling, no skull fracture, left parieto-occipital intracranial hematoma, right intraventricular hematoma and entrapment hydrocephalus; there is a midline shift to the right. **Extent of injury**: diffuse **Type of injury**: extradural hematoma	**Scan results**: left parietal skull fracture, large left parietal acute subdural hematoma 8mm, coup and contre-coup contusion. There is a midline shift to the right of 4.5 mm, subfalcine herniation and soft tissue swelling. **Extent of injury**: focal **Type of injury**: subdural hematoma	**Scan results**: left occipital skull fracture extending to basal skull, transverse petrous temporal bone fracture, fracture of right temporal bone, frontal pneumocephalus, large frontal intraparenchymal hemorrhage, and right frontal acute subdural hematoma. **Extent of injury**: focal **Type of injury**: subdural hematoma
Modifiers: assaulted with an unknown object	**Modifiers**: fell off a moving vehicle	**Modifiers**: fell from the stairs

Figure 2: TBI Classification System with biomarkers incorporated (severe TBI case)

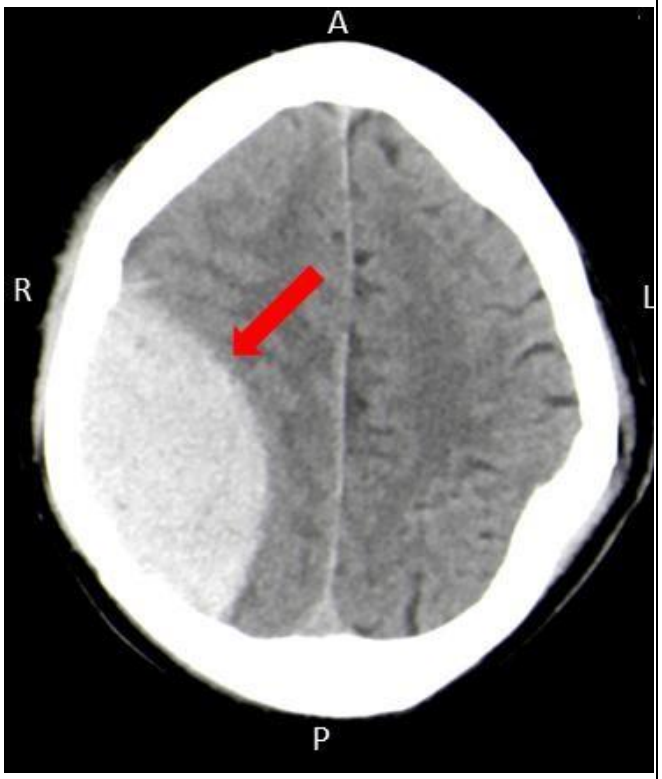
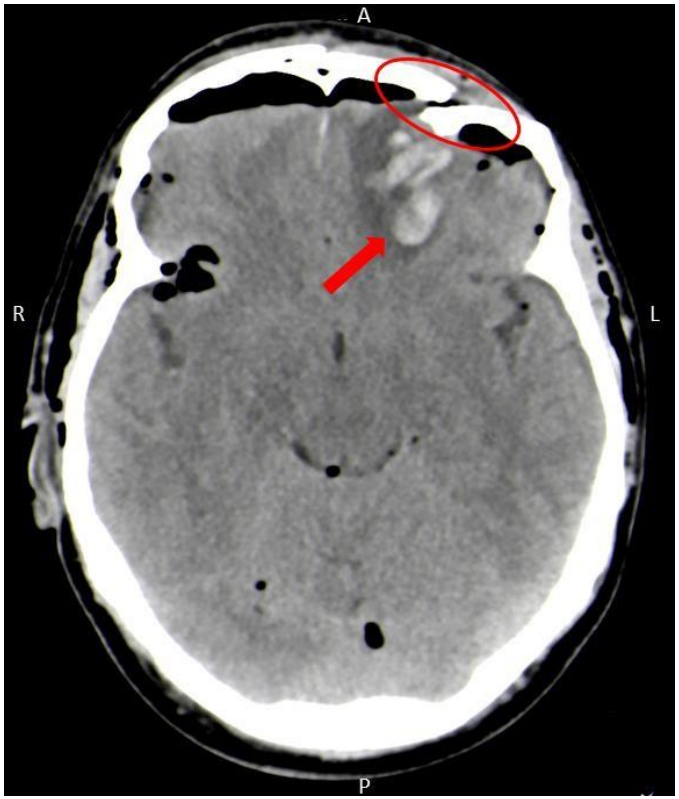
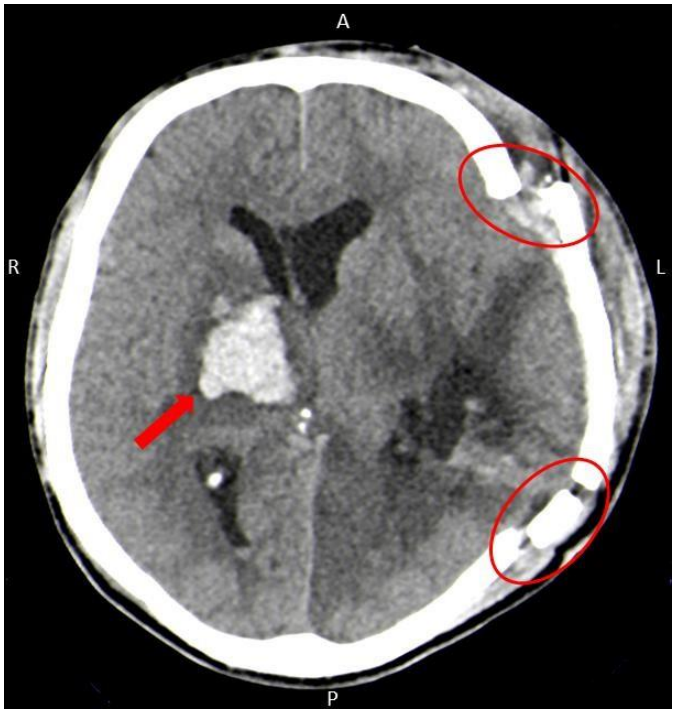
A	B	C
		
Clinical: GCS score: 11	**Clinical: GCS score**: 13	**Clinical: GCS score**: 12
Biomarker: NSE concentration: 2415.244 ng/mL **SAA concentration**: 2547.604 ng/mL	**Biomarker: NSE concentration**: 295.606 ng/mL **SAA concentration**: 3306.983 ng/mL	**Biomarker: NSE concentration**: 342.335 ng/mL **SAA concentration**: 869.515 ng/mL
Scan results: right-sided acute extradural hematoma, right parietal skull fracture with underlying parieto-occipital acute extradural hematoma, temporal contusion and basal cisterns are patent. **Extent of injury**: Focal **Type of injury**: Extradural hematoma	**Scan results**: multiple small skull lacerations sutured from base, periorbital ecchymosis and oedema, left supraorbital instability and crepitus on palpation, epistaxis and left otorrhea. **Extent of injury**: diffuse **Type of injury**: Extradural hematoma	**Scan results**: fronto-parietal and posterior-parietal skull fractures, right post-traumatic intracranial hemorrhage with extension to the left, midline shift of 10mm to the left with cytotoxic, partial cisternal effacement. **Extent of injury**: diffuse **Type of injury**: subdural hematoma
Modifiers: Pedestrian motor vehicle accident	**Modifiers**: Patient fell from the second floor of a building while doing maintenance work	**Modifiers**: Fell off the bike and landed on the head

Figure 3: TBI Classification System with biomarkers incorporated (moderate TBI case)

Discussion

The development of diagnostic tools and recovery predictors for TBI is hindered by the heterogeneity of injuries, the inaccuracy of the GCS system and existing limitations in imaging sensitivity (Eierud et al., 2014). The newly proposed TBI classification comprises four main pillars and includes biomarkers as one of the pillars: a *modifier pillar* made up of features influencing clinical presentation and outcome; a *biomarker pillar* (blood-based measures); an *imaging pillar* reporting pathoanatomical measures; and a *clinical pillar* reporting full GCS and pupillary reactivity (Manley et al., 2025). However, several biomarkers have not yet been clinically investigated or approved for use. For a more accurate diagnosis and a more accurate predictive value of functional outcomes, the combination of multiple biomarkers is vital, as each biomarker may reflect different pathological processes associated with injury, such as neuronal, glial, or axonal injury (Al Nimer et al., 2015; Sahu et al., 2025). Figures 2 and 3 illustrate an example of the potential new classification system, incorporating biomarkers investigated in this study.

While some biomarkers, like S100B and GFAP, have been approved for clinical use, others, including NSE and SAA, are still under scientific investigation (Puccio and Brody, 2024). Hence, this study investigated the concentration of NSE and SAA using a novel immunoassay technique.

In this study, we report a substantial increase in the concentration of serum NSE in moderate (median = 863.7 ng/mL, IQR = 15.44-2917 ng/mL) and severe (median = 626.8 ng/mL, IQR = 334.10-626.80 ng/mL) TBI groups compared to the control (median = 115.5 ng/mL, IQR = 68.67-518.0 ng/mL), as shown in Figure 1(a). Notably, the glycolytic enzyme NSE is predominantly found in neurons and is activated when neurons are damaged, subsequently being released into the bloodstream (Kawata et al., 2016). Hence, damaged neurons release NSE when their membrane integrity is compromised, thereby indicating the severity of

neuronal injury (Bezek et al., 2020). In addition to its enzymatic activity, NSE also triggers intracellular signaling pathways that promote neuronal survival, regeneration, and neurotrophic functions, such as the phosphoinositide 3-kinase/protein kinase B (PI3K/Akt) and mitogen-activated protein kinase/extracellular signal-regulated kinase (MAPK/ERK) pathways. Moreover, NSE interacts with the RhoA/Rho kinase pathway, thereby inducing inflammation that results in neuronal death and subsequent regeneration (Zhang et al., 2021). Therefore, the increase observed in our study suggests that NSE attempts to induce neuronal survival and regeneration. However, Smith et al. (2019) stated that approximately 10 ng/mL of serum NSE levels at baseline can be released from red blood cells and hemolysis may elevate circulating NSE concentrations, making interpretation more challenging in clinical settings. Higher mortality and worse neurological outcomes are frequently associated with elevated serum NSE levels in brain injury cases, such as TBI and sepsis-associated encephalopathy (SAE) (El-Maraghi et al., 2013). In our study, an elevated serum NSE was observed in patients with subarachnoid hemorrhage injury (median = 2675.188 ng/mL, IQR = 0-3159.257 ng/mL) (Table 3). Hence, the NSE concentration detected in our study reflects the severity of the injury. Contrary to our findings, Zhang et al. (2025) observed that NSE levels decreased with increasing disease severity. Nonetheless, several studies corroborate our finding that serum NSE levels increase in proportion to injury severity (El-Maraghi et al., 2013; Thelin et al., 2016; Park et al., 2019).

Similarly, SAA was significantly upregulated in moderate (median = 2308 ng/mL, IQR = 1283-2828 ng/mL) and severe (median = 1678 ng/mL, IQR = 1162-2319 ng/mL) groups compared to the control group (median = 418.7 ng/mL, IQR = 291.2-987.5 ng/mL) ($p<0.0001$) and ($p= 0.0002$), respectively. Astrocytes and microglia release SAA in the brain in response to injury and inflammation (Yu et al., 2014). Following brain injury, SAA levels increase due to a significant release of inflammatory mediators, including IL-1β, IL-6 and

TNF-α (Soriano et al., 2020). When the BBB is disrupted, the systemic SAA can enter the brain and activate microglial cells through pathways such as the Nucleotide-binding domain, Leucine-rich–containing family, Pyrin domain–containing-3 (NLRP3) inflammasome, cathepsin B release and reactive oxygen species (ROS) formation (Yu et al., 2019; Erickson and Mahankali, 2024). It also binds to high-density lipoprotein (HDL) in plasma. Post-injury neuroinflammation is sustained by this activation, which triggers the brain to generate more inflammatory cytokines such as IL-1β (Erickson and Mahankali, 2024).

Studies have reported a positive correlation between NSE and SAA levels with the severity of injury since both represent exacerbated inflammatory and neurodegenerative processes (El-Maraghi et al., 2013; Wicker et al., 2019). Similarly, our study shows a significant positive correlation between NSE and SAA ($r = 0.374$, p-value < 0.005; see Table 1). Although other studies have reported a better correlation between NSE and S100B, our findings also reveal a positive correlation between NSE and SAA (Al Nimer et al., 2015; Sahu et al., 2025). This finding highlights the correlation between NSE and SAA in determining the extent of neurological damage and the possible prognosis after TBI or encephalopathy. As complementary prognostic biomarkers, higher levels of SAA and NSE generally indicate worse outcomes and more extensive damage and they also tend to increase in tandem (Wicker et al., 2019).

Further analysis in our study revealed that serum NSE and SAA levels increased in patients with different types of TBI. An increase in serum NSE (median = 1007.778 ng/mL, IQR = 113.411-4606.364 ng/mL) and SAA (median = 1870.465 ng/mL, IQR = 691.256-3390.669 ng/mL) levels were observed in patients with diffuse TBI compared to those with focal TBI, as shown in Table 2. However, the difference was not statistically significant. Similarly, another study found that patients with diffuse axonal injuries had significantly higher NSE levels within 6 hours of injury than those with focal injuries (Chen et al., 2022). In our study,

the lack of significance may reflect the smaller sample size, as the p-values (p=0.314 and p=0.472) are close to 0.05. However, as the injury progresses from extradural to subdural or subarachnoid hemorrhage, levels of NSE and SAA increase significantly ($p < 0.001$), as shown in Table 3. Hence, deducing that the more neuronal damage and axonal shearing occur, the higher the concentrations of NSE and SAA are released. This can be interpreted as the deeper the penetration in the brain and the more damaged the cortical regions are, the higher the concentrations of serum NSE and SAA (Polcyn et al., 2017). In our study, we report a positive correlation between age and serum NSE and SAA levels, with r values of 0.286 and 0.293, respectively. These correlations were statistically significant, with p-values of *0.013 and 0.011,* respectively (Table 4). Our findings, along with other studies, clearly reveal that injury severity and SAA levels are highly correlated. However, no clear investigations have reported any correlation between SAA levels and patient age (Wicker et al., 2019; Soriano et al., 2020). According to a thorough analysis of biomarker dynamics following TBI, NSE levels in healthy individuals did not correlate with age, unlike other biomarkers. NSE levels in TBI patients were less predictable and did not exhibit a distinct age-related pattern (Chen et al., 2022; Gardner et al., 2022). Our study also reports substantial differences in NSE and SAA levels across genders, with p-values of <0.001 and 0.002, respectively. The concentrations of NSE and SAA were higher in males than in females, as shown in Table 5. Similarly, Soriano et al. (2020) found elevated SAA levels in males compared to females. According to research, specifically in animal models, males had higher levels of SAA than females following TBI, particularly during the acute phase following the brain damage. This discrepancy in sexual dimorphism is strongly associated with a more robust neuroinflammatory response in males (Soriano et al., 2020). Unlike for NSE, no current investigations have highlighted this discrepancy in sexual dimorphism.

Limitations of the study

Limitations of this study include a small sample size. The inability to obtain consent from relatives of TBI patients affected the sample size. Exclusion of mTBI patients, as this group was not referred to the hospital site where the study was conducted. Also, it must be noted that some of the patients may be HIV positive and have received ART, which may have influenced apoptosis, endothelial cell injury, as well as inflammation. Additionally, SAA in polytrauma may reflect systemic rather than neuroinflammation. We recommend conducting additional large-scale experiments to compare NSE and SAA with other biomarkers for diagnosing TBI.

Conclusion

In conclusion, we report a significant upregulation of NSE and SAA in TBI patients compared to controls, which was associated with injury severity. We also noted that serum NSE and SAA concentrations are higher in patients with diffuse TBI than in those with focal TBI. Furthermore, we report a positive proportional increase of both biomarkers following TBI, and they were further correlated with age. No substantial difference in the concentration of NSE and SAA between focal and diffuse brain injury was observed. These findings suggest that combining NSE and SAA biomarkers with GCS and CT findings enhances the accuracy of TBI classification. Higher levels of NSE and SAA indicate worse outcomes and more extensive damage to the brain. The incorporation of NSE and SAA alongside other biomarkers into the new classification system provides a multidimensional characterization of TBI, advancing our understanding of the initial injury among TBI experts, scientists, and patients.

In summary, serum NSE and SAA are useful for diagnosing TBI and can be incorporated into the new classification system. However, for more accurate and robust deduction, further studies on a larger population are required. Further studies should also examine the positive predictive value of NSE and SAA in mild TBI and incorporate these findings into the new classification.

Funding disclosure

This work was supported by the University of KwaZulu-Natal (UKZN), Postgraduate Fund, College of Health Sciences.

Conflict of interest

The author declares no conflicts of interest in this study. The opinions expressed and conclusions arrived at are those of the authors and are not necessarily attributed to the UKZN.

Acknowledgment

The author wishes to acknowledge the Discipline of Clinical Medicine at the University of KwaZulu-Natal, Medical School Campus, and neurosurgeons from Inkosi Albert Luthuli Central Hospital.

References

1) Al Nimer, F., Thelin, E., Nyström, H., Dring, A. M., Svenningsson, A., Piehl, F., Nelson, D. W. & Bellander, B. M. 2015. Comparative Assessment of The Prognostic Value of Biomarkers in Traumatic Brain Injury Reveals An Independent Role For Serum Levels of Neurofilament Light. *Plos One,* 10**,** E0132177.
2) Aly, H., Hamed, Z., Mohsen, L., Ramy, N., Arnaoot, H. & Lotfy, A. 2011. Serum Amyloid A Protein and Hypoxic Ischemic Encephalopathy in the Newborn. *Journal of Perinatology,* 31**,** 263-268.
3) Bezek, S., Biberthaler, P., Martinez-Espina, I. & Bogner-Flatz, V. 2020. Chapter 11 - Pathophysiology and Clinical Implementation of Traumatic Brain Injury Biomarkers: Neuron-Specific Enolase. *In:* Wu, A. H. B. & Peacock, W. F. (Eds.) *Biomarkers For Traumatic Brain Injury.* Academic Press.
4) Buitendag, J. J. P., Kong, V. Y., Bruce, J. L., Laing, G. L., Clarke, D. L. & Brysiewicz, P. 2017. The Spectrum and Outcome of Paediatric Traumatic Brain Injury in KwaZulu-Natal Province, South Africa, has not Changed over the last two decades. *S Afr Med J,* 107**,** 777-780.
5) Carney, N., Totten, A. M., O'reilly, C., Ullman, J. S., Hawryluk, G. W., Bell, M. J., Bratton, S. L., Chesnut, R., Harris, O. A., Kissoon, N., Rubiano, A. M., Shutter, L., Tasker, R. C., Vavilala, M. S., Wilberger, J., Wright, D. W. & Ghajar, J. 2017. Guidelines For The Management of Severe Traumatic Brain Injury, Fourth Edition. *Neurosurgery,* 80**,** 6-15.
6) Chen, W., Wang, G., Yao, C., Zhu, Z., Chen, R., Su, W., & Jiang, R. 2022. The Ratio of Serum Neuron-Specific Enolase Level to Admission Glasgow Coma Scale Score is Associated with Diffuse Axonal Injury in Patients with Moderate to Severe Traumatic Brain Injury. *Front Neurol,* 13**,** 887818.

7) Eierud, C., Craddock, R. C., Fletcher, S., Aulakh, M., King-Casas, B., Kuehl, D., & Laconte, S. M. 2014. Neuroimaging After Mild Traumatic Brain Injury: Review and Meta-Analysis. *Neuroimage Clin,* 4**,** 283-94.

8) El-Maraghi, S., Yehia, H., Hossam, H., Yehia, A. & Mowafy, H. 2013. The Prognostic Value of Neuron-Specific Enolase in Head Injury. *The Egyptian Journal of Critical Care Medicine,* 1**,** 25-32.

9) Erickson, M. A. & Mahankali, A. P. 2024. Interactions of Serum Amyloid A Proteins With The Blood-Brain Barrier: Implications For Central Nervous System Disease. *Int J Mol Sci,* 25.

10) Gardner, R. C., Puccio, A. M., Korley, F. K., Wang, K. K. W., Diaz-Arrastia, R., Okonkwo, D. O., Puffer, R. C., Yuh, E. L., Yue, J. K., Sun, X., Taylor, S. R., Mukherjee, P., Jain, S., Manley, G. T. & Investigators, T. T.-T. 2022. Effects of Age and Time Since Injury on Traumatic Brain Injury Blood Biomarkers: A Track-TBI Study. *Brain Communications,* 5.

11) Gettleman, J. 2009. The Most Dangerous Place In The World. *Foreign Policy***,** 61.

12) Kalyan, S., Nadasan, T., And Puckree, T. 2007. The Epidemiology of Traumatic Brain Injuries (TBI): A Literature Review. *South African Journal of Physiotherapy,* 63**,** 32.

13) Kawata, K., Liu, C. Y., Merkel, S. F., Ramirez, S. H., Tierney, R. T., & Langford, D. 2016. Blood Biomarkers For Brain Injury: What Are We Measuring? *Neuroscience & Biobehavioral Reviews,* 68**,** 460-473.

14) Kim, N. Y., Lim, J., Lee, S., Kim, K., Hong, J. H. & Chun, D. H. 2020. Hematological Factors Predicting Mortality In Patients With Traumatic Epidural or Subdural Hematoma Undergoing Emergency Surgical Evacuation: A Retrospective Cohort Study. *Medicine (Baltimore),* 99**,** E22074.

15) Kroll, K. W., Woolley, G., Terry, K., Premeaux, T. A., Shikuma, C. M., Corley, M. J., Bowler, S., Ndhlovu, L. C. & Reeves, R. K. 2023. Multiplex Analysis of Cytokines And

Chemokines In Persons Aging With or Without Hiv. *Aids Research And Human Retroviruses,* 39**,** 367-380.

16) Maas, A. I., Stocchetti, N. & Bullock, R. 2008. Moderate And Severe Traumatic Brain Injury In Adults. *Lancet Neurol,* 7**,** 728-41.

17) Maas, A. I. R., Menon, D. K., Manley, G. T., Abrams, Et al. 2022. Traumatic Brain Injury: Progress And Challenges In Prevention, Clinical Care, And Research. *The Lancet Neurology,* 21**,** 1004-1060.

18) Maasdorp, S. D., Swanepoel, C. & Gunter, L. 2020. Outcomes Of Severe Traumatic Brain Injury At Time Of Discharge From Tertiary Academic Hospitals In Bloemfontein. *Afr J Thorac Crit Care Med,* 26.

19) Malec, J. F., Brown, A. W., Leibson, C. L., Flaada, J. T., Mandrekar, J. N., Diehl, N. N. & Perkins, P. K. 2007. The Mayo Classification System For Traumatic Brain Injury Severity. *J Neurotrauma,* 24**,** 1417-24.

20) Manley, G. T., Dams-O'connor, K., Alosco, M. Et Al. 2025. A New Characterisation Of Acute Traumatic Brain Injury: The Nih-Ninds Tbi Classification And Nomenclature Initiative. *The Lancet Neurology,* 24**,** 512-523.

21) Mollayeva, T., Mollayeva, S. & Colantonio, A. 2018. Traumatic Brain Injury: Sex, Gender And Intersecting Vulnerabilities. *Nat Rev Neurol,* 14**,** 711-722.

22) Murray, G. D., Teasdale, G. M., Braakman, R., Cohadon, F., Dearden, M., Iannotti, F., Karimi, A., Lapierre, F., Maas, A., Ohman, J., Persson, L., Servadei, F., Stocchetti, N., Trojanowski, T. & Unterberg, A. 1999. The European Brain Injury Consortium Survey Of Head Injuries. *Acta Neurochir (Wien),* 141**,** 223-36.

23) Nguyen, K. P. L., Pai, V., Rashid, S., Treece, J., Moulton, M. & Baumrucker, S. J. 2018. Prognostication In Anoxic Brain Injury. *American Journal Of Hospice And Palliative Medicine®,* 35**,** 1446-1455.

24) Park, D.-W., Park, S.-H. & Hwang, S.-K. 2019. Serial Measurement Of S100b And Nse In Pediatric Traumatic Brain Injury. *Child's Nervous System,* 35**,** 343-348.

25) Polcyn, R., Capone, M., Hossain, A., Matzelle, D., Banik, N. & Haque, A. 2017. Enolase And Acute Spinal Cord Injury. *Journal Of Clinical & Cellular Immunology,* 08.

26) Puccio, A. & Brody, D. L. 2024. Biomarkers, Biomarkers, Biomarkers, And More Biomarkers. *Journal Of Neurotrauma*.

27) Rael, L. T., Bar-Or, R., Salottolo, K., Mains, C. W., Slone, D. S., Offner, P. J. & Bar-Or, D. 2009. Injury Severity And Serum Amyloid A Correlate With Plasma Oxidation-Reduction Potential In Multi-Trauma Patients: A Retrospective Analysis. *Scandinavian Journal Of Trauma, Resuscitation And Emergency Medicine,* 17**,** 57.

28) Ramdheen, S. & Naicker, B. 2021. Evaluating The Burden Of Head Injuries On A Rural Emergency Department In South Africa. *South African Family Practice; Vol 63, No 1 (2021): Part 4do - 10.4102/Safp.V63i1.5327*.

29) Richmond, E. & Rogol, A. D. 2014. Traumatic Brain Injury: Endocrine Consequences In Children And Adults. *Endocrine,* 45**,** 3-8.

30) Sahu, S., Marlecha, P., Patel, T., Gadad, B. & Anand, N. 2025. Integrating Biomarkers For Real-Time Risk Assessment And Outcome Prediction In Traumatic Brain Injury Management (P6-7.010). *Neurology,* 104**,** 4942.

31) Salottolo, K., Levy, A. S., Slone, D. S., Mains, C. W. & Bar-Or, D. 2014. The Effect Of Age On Glasgow Coma Scale Score In Patients With Traumatic Brain Injury. *Jama Surg,* 149**,** 727-34.

32) Seedat, M., Van Niekerk, A., Jewkes, R., Suffla, S. & Ratele, K. 2009. Violence And Injuries In South Africa: Prioritising An Agenda For Prevention. *The Lancet,* 374**,** 1011-1022.

33) Sherer, M., Struchen, M. A., Yablon, S. A., Wang, Y. & Nick, T. G. 2008. Comparison Of Indices Of Traumatic Brain Injury Severity: Glasgow Coma Scale, Length Of Coma and Post-Traumatic Amnesia. *J Neurol Neurosurg Psychiatry,* 79**,** 678-85.

34) Smith, R., Chepisheva, M., Cronin, T. & Seemungal, B. M. 2019. Chapter 16 - Diagnostic Approaches Techniques In Concussion/Mild Traumatic Brain Injury: Where Are We? *In:* Hoffer, M. E. & Balaban, C. D. (Eds.) *Neurosensory Disorders In Mild Traumatic Brain Injury.* Academic Press.

35) Soriano, S., Moffet, B., Wicker, E. & Villapol, S. 2020. Serum Amyloid A Is Expressed In The Brain After Traumatic Brain Injury In A Sex-Dependent Manner. *Cell Mol Neurobiol,* 40**,** 1199-1211.

36) Stiell, I. G., Wells, G. A., Vandemheen, K., Clement, C., Lesiuk, H., Laupacis, A., Mcknight, R. D., Verbeek, R., Brison, R., Cass, D., Eisenhauer, M. E., Greenberg, G. & Worthington, J. 2001. The Canadian CT Head Rule For Patients With Minor Head Injury. *Lancet,* 357**,** 1391-6.

37) Thelin, E. P., Jeppsson, E., Frostell, A., Svensson, M., Mondello, S., Bellander, B.-M. & Nelson, D. W. 2016. Utility Of Neuron-Specific Enolase In Traumatic Brain Injury; Relations To S100b Levels, Outcome, And Extracranial Injury Severity. *Critical Care,* 20**,** 285.

38) Villapol, S., Kryndushkin, D., Balarezo, M. G., Campbell, A. M., Saavedra, J. M., Shewmaker, F. P. & Symes, A. J. 2015. Hepatic Expression Of Serum Amyloid A1 Is Induced By Traumatic Brain Injury And Modulated By Telmisartan. *The American Journal Of Pathology,* 185**,** 2641-2652.

39) Wang, K. K., Munoz Pareja, J. C., et al. 2021. Blood-based traumatic brain injury biomarkers – Clinical utilities and regulatory pathways in the United States, Europe and Canada. Expert Review of Molecular Diagnostics, 21, 1303-1321.

40) Wicker, E., Benton, L., George, K., Furlow, W. & Villapol, S. 2019. Serum Amyloid A Protein As A Potential Biomarker For Severity And Acute Outcome In Traumatic Brain Injury. *Biomed Res Int,* 2019**,** 5967816.

41) Yan, J., Wang, C. & Sun, B. 2025. Global, Regional, And National Burdens Of Traumatic Brain Injury From 1990 To 2021. *Frontiers In Public Health,* Volume 13 - 2025.

42) Yu, J., Zhu, H., Taheri, S., Mondy, W., Bonilha, L., Magwood, G. S., Lackland, D., Adams, R. J. & Kindy, M. S. 2019. Serum Amyloid A-Mediated Inflammasome Activation Of Microglial Cells In Cerebral Ischemia. *J Neurosci,* 39**,** 9465-9476.

43) Yu, Y., Liu, J., Li, S. Q., Peng, L. & Ye, R. D. 2014. Serum Amyloid A Differentially Activates Microglia And Astrocytes Via The PI3K Pathway. *J Alzheimers Dis,* 38**,** 133-44.

44) Zafonte, R. D., Hammond, F. M., Mann, N. R., Wood, D. L., Black, K. L. & Millis, S. R. 1996. Relationship Between Glasgow Coma Scale And Functional Outcome 1. *American Journal Of Physical Medicine & Rehabilitation,* 75**,** 364-369.

45) Zhang, B., Wu, Q. & Wu, J. 2025. Association Of Serum Netrin-1, Nse, And S100β With Brain Injury Severity And Prognosis In Patients With Sepsis-Associated Encephalopathy. *Biomol Biomed,* 25**,** 2755-2765.

46) Zhang, S., Lachance, B. B., Mattson, M. P. & Jia, X. 2021. Glucose Metabolic Crosstalk And Regulation In Brain Function And Diseases. *Progress In Neurobiology,* 204**,** 102089.

47) Zhong, H., Feng, Y., Shen, J., Rao, T., Dai, H., Zhong, W. & Zhao, G. 2025. Global Burden Of Traumatic Brain Injury In 204 Countries And Territories From 1990 To 2021. *Am J Prev Med,* 68**,** 754-763.

CHAPTER FIVE

SYNTHESIS, CONCLUSION AND RECOMMENDATION

Synthesis

The causes of TBI vary from one country to another. In the US, for instance, the major causes of TBI are falls and firearm-related injury (Daugherty *et al.*, 2019). While in SA, it is primarily caused by car accidents, violence and falls and affects men nearly twice as much as women under the age of 45 (Johnson and Griswold, 2017). TBI occurs at various stages, with interconnected pathophysiological pathways that begin with mechanical disruption and progress through biochemical cascades (Algattas and Huang, 2013). The primary or immediate phase is caused by direct mechanical pressure, which induces focal contusions, diffuse axonal injury and vascular ruptures, resulting in prompt cellular necrosis, bleeding, and ischemia (Maas et al., 2008). The second phase is triggered 24 hours following the injury. Energy failure diminishes ATP levels, leading to excitotoxicity from glutamate excess, calcium influx, lactic acidosis and mitochondrial impairment (Freire *et al.*, 2023). Neuroinflammation ensues when the BBB is compromised, facilitating cytokine production (e.g., TNF-α and IL-1), oxidative stress, edema and leukocyte infiltration via reactive oxygen species, thereby exacerbating damage (Ng and Lee, 2019). Additionally, the apoptotic demise of neurons and oligodendrocytes is a characteristic of the secondary phase (Grady *et al.*, 2003). Certain neurochemical, cellular, and molecular pathways, including extracellular signal-regulated kinase (ERK), p38 MAPK, and JAK/Signal transducer and activator of transcription, can interact to trigger apoptotic events that involve the activation of cysteine proteases such as caspases and calpains (Zhao *et al.*, 2011). Since the primary phase of TBI typically involves immediate physical injury and irreversible necrotic cell death, treatment primarily aims to stabilize the injury site and prevent progression to the secondary phase (Ng and Lee, 2019).

To diagnose and classify TBI, physicians rely on several parameters, including severity, mechanism, location and type. Based on the GCS score, TBI is classified as mild (GCS 13-15), moderate (GCS 9-12) and severe (GCS<9) (Carney *et al.*, 2017). In most clinical settings, the initial severity of a TBI is quantified using the GCS score (Bodien *et al.*, 2021). However, several investigations have identified flaws and limitations of GCS, such as inaccurate scores in intubated, intoxicated, or sedated patients (Bodien *et al.*, 2021). Additionally, there is ongoing debate concerning the inclusion of a GCS of 13 in the category of mild TBI, due to the increased risk of intracranial injury in these patients compared to those with a GCS of 14 or 15 (Türedi *et al.*, 2008; Pearson *et al.*, 2012).

These limitations have led physicians and researchers to investigate better or complementary tools, such as biomarkers, to improve TBI prognosis and diagnosis ((Diaz-Arrastia and Kochanek, 2023; Wilde *et al.*, 2022). Several studies have proven that biomarkers such as GFAP, S100B, NSE and SAA have gained popularity and approval to help detect brain lesions, abnormalities and reduce the need for head CT scan procedures (Wicker *et al.*, 2019; Chen *et al.*, 2022; Hossain *et al.*, 2024). Similarly, our study reports elevated serum GFAP, S100B, NSE and SAA levels in patients with moderate and severe TBI compared with healthy individuals. Hence, the upregulation of serum GFAP, S100B, NSE and SAA observed in our study reflects the severity of the injury and supports the ability of these biomarkers to predict its progression. Furthermore, our study has shown a positive correlation between the four biomarkers GFAP, S100B, NSE and SAA, suggesting that they can be used in combination to improve the accuracy of TBI diagnosis (Table 1).

The exact mechanisms of action of GFAP, S100B, NSE and SAA are not yet fully understood, but they have been reported to interact with signaling pathways such as RAGE, the transcription factor NF-kB, P13K/Akt, MAPK/ERK, RhoA/Rho kinase and NLRP3 (Yu *et al.*, 2019; Zhang *et al.*, 2021; Zou *et al.*, 2022; Erickson and Mahankali, 2024).

Nevertheless, the significant upregulation of serum GFAP, S100B, NSE and SAA in moderate and severe TBI in this study may contribute to TBI prognosis and diagnosis. It also implies that these biomarkers can detect underlying neurological changes following brain injury. Based on the biological sources of the biomarkers, a comparison has been made to predict injury severity (Table 1).

Table 1: Receiver Operating Characteristic (ROC) curve analysis of GFAP, S100B, NSE, and SAA

Biomarker	Diagnostic Accuracy	Calculated Area Under the Curve (AUC) Value
GFAP	Excellent	0.990
S100B	Excellent	0.938
NSE	Very Good	0.904
SAA	Very Good	0.890

The ROC curve analysis was performed to evaluate the diagnostic performance of S100B, GFAP, NSE, and SAA to distinguish moderate-severe TBI patients from the control group. It was observed that GFAP achieved the highest overall diagnostic performance (AUC= 0.990), representing a near-perfect separation between the controls and TBI patients. S100B also exhibited remarkable discriminative ability (AUC= 0.938), revealing sharp baseline concentrations in both TBI severity categories compared to controls. Both NSE (AUC= 0.904) and SAA (AUC= 0.890) demonstrated reliable clinical classification, however they showed a slightly higher level of background crossover noise in the control population when compared to GFAP.

Table 2: Comparison of concentration thresholds in the literature review

Biomarker & Type of cell	Literature Threshold	Study threshold	Pathophysiologic Cascade	Reference
GFAP (Astrocyte)	67 pg/mL	14.77 pg/mL	Astrocyte cytoskeletal breakdown	Papa et al., 2014; Welch et al., 2016; (Receiver Operating Characteristic (ROC) Amoo et al., 2021; Janigro et al., 2022 (Meta-analysis) Ahmadi et al., 2023 (Receiver Operating Characteristic (ROC),
S100B (Astrocyte)	100 pg/mL	0.27 pg/mL	Reversible glial activation & BBB disruption and permeability	Papa et al., 2014 (Receiver Operating Characteristic (ROC) Amoo et al., 2021; Janigro et al., 2022 (Meta-analysis)
NSE (Neuron)	20–35ng/mL	233.31 ng/mL	Neuronal cell membrane disruption/necrosis	Vos et al., 2006 Amoo et al., 2021 (Receiver Operating Characteristic (ROC), multivariate logistic regression)
SAA (Glioneuronal)	10 µg/mL	1032.06 ng/mL	Neuroinflammatory magnification	Papa et al., 2014; Janigro et al., 2022 Salgado et al., 2026 (Meta-analysis, meta-analysis, Receiver Operating Characteristic (ROC)

The clinical cutoffs were calculated using Youden's J. The moderate and severe TBI cohorts were combined and compared with the control group. It is worth noting that GFAP emerged as the strongest standalone biomarker, with a cutoff of 14.77 pg/mL, although the threshold was found to be lower than that reported in the literature (67 pg/mL). The optimal S100B concentration found was 0.27 pg/mL, which is < 100 pg/mL reported in the literature. The NSE threshold found in this study was 233.31 ng/mL, which is >35 ng/mL. Finally, the SAA threshold was 1032.06 ng/mL, which is < 10 µg/mL.

Practical limitations: the threshold can be limited by the type of immunoassay employed; local validation and certification are important before implementing into hospital protocols (Papa et al., 2014; Gnyliukh et al., 2025). Collection timing, all GFAP, S100B, NSE and SAA must be collected within 3–6 hours for accurate thresholds; delayed collection reduces negative predictive value (NPV) and specificity (Welch et al., 2016). Another limitation is that, in pediatric settings, thresholds for moderate-to-severe TBI differ from those in adults (Salgado et al., 2026).

Conclusion

The introduction of biomarkers has tremendously impacted the use of the GCS score in diagnosing TBI. With the new proposed framework, biomarkers have the potential to improve patients' experience and outcome after sustaining a head injury. TBI biomarkers such as GFAP, S100B, NSE and SAA have the potential to improve prehospital transport decisions and reduce unnecessary head CT scans. This improvement may change patients' status from misdiagnosis to accurate diagnosis. However, prolonged exposure to higher concentrations of these biomarkers may lead to more severe conditions, such as neural inflammation and apoptosis. Moreover, inadequate biomarker measurements may obscure the precise condition. Additionally, we report positive correlations between GFAP, S100B, NSE and SAA and age, patient outcome and injury severity. This study has demonstrated that GFAP, S100B, NSE and SAA could be potential tools for TBI prognosis and diagnosis. However, further studies should also examine the positive predictive values of GFAP, S100B, NSE and SAA in mild TBI and incorporate these findings into the new classification, as the mild group was not included in this study. To successfully adopt blood-based biomarkers for TBI diagnosis and classification in routine clinical practice, the healthcare sector, clinical laboratory specialists

and the in vitro diagnostics sector will need to work together. This next phase is at least as challenging as biomarker discovery and characterization.

Recommendation and further study

The observed sex-related difference in GFAP concentrations warrants interpretation. We recommend that biological sex should ideally be evaluated while controlling for major confounding factors, including injury severity, mechanism of injury, age, time from injury to sampling, and other clinical characteristics known to influence biomarker release. The differences in the distribution of TBI severity, injury mechanisms, and sample sizes between male and female participants may have contributed to the observed variation in GFAP concentrations. Therefore, the reported sex differences should not be interpreted as being solely attributed to biological sex. Regarding the markedly larger difference observed for GFAP compared with S100B, several biological and methodological explanations may account for this finding. Although both are considered astrocytic markers, they differ substantially in their cellular distribution, release kinetics, and clearance profiles. GFAP is a structural intermediate filament protein that is relatively specific to astrocytes and is released predominantly following astrocytic injury and cellular disruption. In contrast, S100B is more widely distributed, can be released during astrocyte activation without overt cell damage, and may also originate from extracranial tissues such as adipose tissue, skeletal muscle, and melanocytes. Consequently, S100B concentrations may be less sensitive to sex-specific differences in central nervous system injury and more susceptible to background biological variability. Furthermore, GFAP has been shown to correlate strongly with structural brain injury and injury severity, whereas S100B frequently demonstrates earlier and more transient elevations. Differences in sampling times between studies may therefore disproportionately affect S100B measurements while preserving larger differences in GFAP concentrations.

Although some biomarkers have already been used in clinical settings, further research is needed across numerous domains. Existing research on biomarkers for TBI is frequently restricted by small sample sizes and short follow-up periods. Additional research is also needed on the use of biomarkers in prehospital and chronic care settings. In this study, the influence of comorbidities was not considered as it fell outside the scope of this study. The cohort of patients was treated as homogenous as the study was based solely on the diagnosis of moderate-severe TBI. Therefore, future studies should evaluate the influence of comorbidities in TBI. In South African healthcare, integrating biomarkers into TBI diagnosis can be achieved by promoting local, large-scale research that aligns with the National Health Laboratory Service (NHI) guidelines, advocate for South African National Accreditation System (SANAS)-accredited TBI biomarker testing platforms in KZN provincial hospitals, and conduct local health technology assessments, prioritising point-of-care biomarker testing, and implementing national training programmes for healthcare professionals.

References

1) Ahmadi, S., Roshdi Dizaji, S., et al. 2023. Serum Glial Fibrillary Acidic Protein in Detecting Intracranial Injuries Following Minor Head Trauma: A Systematic Review and Meta-Analysis. Arch Acad Emerg Med, 11, e9.
2) Algattas, H. & Huang, J. H. 2013. Traumatic Brain Injury pathophysiology and treatments: early, intermediate, and late phases post-injury. *Int J Mol Sci,* 15**,** 309-41.
3) Amoo, M., Henry, J., et al. 2022. S100B, GFAP, UCH-L1 and NSE as predictors of abnormalities on CT imaging following mild traumatic brain injury: a systematic review and meta-analysis of diagnostic test accuracy. Neurosurg Rev, 45, 1171-1193.
4) Bodien, Y. G., Barra, A., et al. 2021. Diagnosing Level of Consciousness: The Limits of the Glasgow Coma Scale Total Score. *J Neurotrauma,* 38**,** 3295-3305.
5) Carney, N., Totten, A. M., et al. 2017. Guidelines for the Management of Severe Traumatic Brain Injury, Fourth Edition. *Neurosurgery,* 80**,** 6-15.
6) Chen, W., Wang, G., et al. 2022. The ratio of serum neuron-specific enolase level to admission glasgow coma scale score is associated with diffuse axonal injury in patients with moderate to severe traumatic brain injury. *Frontiers in Neurology,* Volume 13 - 2022.
7) Daugherty, J., Waltzman, D., et al. 2019. Traumatic Brain Injury-Related Deaths by Race/Ethnicity, Sex, Intent, and Mechanism of Injury - United States, 2000-2017. *MMWR. Morbidity and mortality weekly report,* 68**,** 1050-1056.
8) Erickson, M. A. & Mahankali, A. P. 2024. Interactions of Serum Amyloid A Proteins with the Blood-Brain Barrier: Implications for Central Nervous System Disease. *Int J Mol Sci,* 25.
9) Freire, M. A. M., Rocha, G. S., et al. 2023. Cellular and Molecular Pathophysiology of Traumatic Brain Injury: What Have We Learned So Far? *Biology (Basel),* 12.
10) Gnyliukh, N., Wei, J., et al. 2025. The role of S100B protein as a diagnostic biomarker for brain injury. Sensing and Bio-Sensing Research, 50, 100888.

11) Grady, M. S., Charleston, J. S., et al. 2003. Neuronal and glial cell number in the hippocampus after experimental traumatic brain injury: analysis by stereological estimation. *Journal of neurotrauma,* 20**,** 929-941.

12) Hossain, I., Marklund, N., et al. 2024. Blood biomarkers for traumatic brain injury: A narrative review of current evidence. *Brain Spine,* 4**,** 102735.

13) Institute of Medicine Committee on Nutrition, T. & the B. 2011. *In:* Erdman, J., Oria, M. & Pillsbury, L. (eds.) *Nutrition and Traumatic Brain Injury: Improving Acute and Subacute Health Outcomes in Military Personnel.* Washington (DC): National Academies Press (US). Copyright 2011 by the National Academy of Sciences. All rights reserved.

14) Janigro, D., Mondello, S., et al. 2022. GFAP and S100B: What You Always Wanted to Know and Never Dared to Ask. Front Neurol, 13, 835597.

15) Johnson, W. D. & Griswold, D. P. 2017. Traumatic brain injury: a global challenge. *Lancet Neurol,* 16**,** 949-950.

16) Ng, S. Y. & Lee, A. Y. W. 2019. Traumatic Brain Injuries: Pathophysiology and Potential Therapeutic Targets. *Front Cell Neurosci,* 13**,** 528.

17) Papa, L., Silvestri, S., et al. 2014. GFAP outperforms S100β in detecting traumatic intracranial lesions on computed tomography in trauma patients with mild traumatic brain injury and those with extracranial lesions. J Neurotrauma, 31, 1815-22.

18) Pearson, W. S., Ovalle, F., Jr., et al. 2012. A review of traumatic brain injury trauma center visits meeting physiologic criteria from The American College of Surgeons Committee on Trauma/Centers for Disease Control and Prevention Field Triage Guidelines. *Prehosp Emerg Care,* 16**,** 323-8.

19) Pelinka, L. E., Kroepfl, A., et al. 2004. GFAP Versus S100B in Serum after Traumatic Brain Injury: Relationship to Brain Damage and Outcome. Journal of Neurotrauma, 21, 1553-1561.

20) Türedi, S., Hasanbasoglu, A., et al. 2008. Clinical decision instruments for CT scan in minor head trauma. *J Emerg Med,* 34**,** 253-9.

21) Wicker, E., Benton, L., et al. 2019. Serum Amyloid A Protein as a Potential Biomarker for Severity and Acute Outcome in Traumatic Brain Injury. *BioMed Research International,* 2019**,** 5967816.

22) Wilde EA, Wanner IB, Kenney K, et al. 2022. A Framework to Advance Biomarker Development in the Diagnosis, Outcome Prediction, and Treatment of Traumatic Brain Injury. J Neurotrauma;39(7-8):436-457.

23) Yu, J., Zhu, H., et al. 2019. Serum Amyloid A-Mediated Inflammasome Activation of Microglial Cells in Cerebral Ischemia. *J Neurosci,* 39**,** 9465-9476.

24) Zhang, S., Lachance, B. B., et al. 2021. Glucose metabolic crosstalk and regulation in brain function and diseases. *Progress in Neurobiology,* 204**,** 102089.

25) Zhao, J.-b., Zhang, Y., et al. 2011. Activation of the JAK2/STAT pathway in the cerebral cortex after experimental traumatic brain injury in rats. *Neuroscience letters,* 498**,** 147-152.

26) Zou, Z., Li, L., et al. 2022. The role of S100B/RAGE-enhanced ADAM17 activation in endothelial glycocalyx shedding after traumatic brain injury. *Journal of Neuroinflammation,* 19**,** 46.

APPENDICES

APPENDIX A: ETHICAL APPROVAL

UNIVERSITY OF KWAZULU-NATAL
INYUVESI YAKWAZULU-NATALI

10 April 2025

Mr Seke Nzau Mafuika (215001367)
School of Laboratory Medicine & Medical Science
Westville

Dear Mr Mafuika,

Protocol reference number: BREC/00002147/2020
Project title: The potential of serum S100B and Glial Fibrillary Acidic Protein as biomarkers for traumatic brain Injury
Degree: PhD
New Title: The potential of serum S100 calcium-binding protein B, Glial fibrillary acidic protein, Neuron-Specific Enolase and Serum Amyloid A as potential biomarkers for traumatic brain injury.

We wish to advise you that your application for amendments listed below received on 27 March 2025 and your response to BREC queries dated 03 April 2025 for the above study has been **noted and approved** by a subcommittee of the Biomedical Research Ethics Committee.

Amendments noted and approved:

1. Change of study title to new title above
2. New sample size 75
3. additional biomarkers added

The committee will be advised of the above at its next meeting to be held on 13 May 2025.

Yours sincerely

[redacted]

Ms. A Marimuthu
(for) Prof S Singh
Chair: Biomedical Research Ethics Committee

Biomedical Research Ethics Committee
Chair: Professor S Singh
UKZN Research Ethics Office Westville Campus, Govan Mbeki Building
Postal Address: Private Bag X54001, Durban 4000
Email: BREC@ukzn.ac.za
Website: http://research.ukzn.ac.za/Research-Ethics/Biomedical-Research-Ethics.aspx

UNIVERSITY OF KWAZULU-NATAL
INYUVESI YAKWAZULU-NATALI

05 October 2023

Mr Seke Nzau Mafuika (215001367)
School of Laboratory Medicine & Medical Science
Westville

Dear Mr Mafuika,

Protocol reference number: BREC/00002147/2020
Project title: The potential of serum S100B and Glial Fibrillary Acidic Protein as biomarkers for traumatic brain Injury **Degree: PhD**

RECERTIFICATION APPLICATION APPROVAL NOTICE

Approved:	**17 June 2023**
Expiration of Ethical Approval:	**16 June 2024**

I wish to advise you that your application for Recertification for the above protocol has been **noted and approved** by a sub-committee of the Biomedical Research Ethics Committee (BREC) for another approval period. The start and end dates of this period are indicated above.

Your response to BREC letter dated 24 August 2023 has been noted by a sub-committee of the Biomedical Research Ethics Committee.

Notes to PI:

1. RIG entry suggests that data collection is complete/closed and that only data analysis and reporting are taking place.
2. However, pending amendment request suggests that this is not the case.
   Please clarify.

If any modifications or adverse events occur in the project before your next scheduled review, you must submit them to BREC for review. Except in emergency situations, no change to the protocol may be implemented until you have received written BREC approval for the change.

The committee will be notified of the above approval at its next meeting to be held on 14 November 2023.

Yours sincerely

[redacted]

.....

Ms A Marimuthu
(for) Prof D Wassenaar
Chair: Biomedical Research Ethics Committee

Biomedical Research Ethics Committee
Chair: Professor D R Wassenaar
UKZN Research Ethics Office Westville Campus, Govan Mbeki Building
Postal Address: Private Bag X54001, Durban 4000
Email: BREC@ukzn.ac.za
Website: http://research.ukzn.ac.za/Research-Ethics/Biomedical-Research-Ethics.aspx
Founding Campuses: Edgewood Howard College Medical School Pietermaritzburg Westville

UNIVERSITY OF KWAZULU-NATAL
INYUVESI YAKWAZULU-NATALI

17 June 2021

Mr Seke Nzau Mafuika (215001367)
School of Lab Med & Medical Sc
Westville

Dear Mr Mafuika,

Protocol reference number: BREC/00002147/2020
Project title: The potential of serum S100B and Glial Fibrillary Acidic Protein as biomarkers for traumatic brain injury
Degree: PhD

EXPEDITED APPLICATION: APPROVAL LETTER

A sub-committee of the Biomedical Research Ethics Committee has considered and noted your application.

The conditions have been met and the study is given full ethics approval and may begin as from 17 June 2021. Please ensure that outstanding site permissions are obtained and forwarded to BREC for approval before commencing research at a site.

This approval is subject to national and UKZN lockdown regulations, see (http://research.ukzn.ac.za/Libraries/BREC/BREC_Lockdown_Level_3_Guidelines.sflb.ashx). Based on feedback from some sites, we urge PIs to show sensitivity and exercise appropriate consideration at sites where personnel and service users appear stressed or overloaded.

This approval is valid for one year from 17 June 2021. To ensure uninterrupted approval of this study beyond the approval expiry date, an application for recertification must be submitted to BREC on the appropriate BREC form 2-3 months before the expiry date.

Any amendments to this study, unless urgently required to ensure safety of participants, must be approved by BREC prior to implementation.

Your acceptance of this approval denotes your compliance with South African National Research Ethics Guidelines (2015), South African National Good Clinical Practice Guidelines (2020) (if applicable) and with UKZN BREC ethics requirements as contained in the UKZN BREC Terms of Reference and Standard Operating Procedures, all available at http://research.ukzn.ac.za/Research-Ethics/Biomedical-Research-Ethics.aspx.

BREC is registered with the South African National Health Research Ethics Council (REC-290408-009). BREC has US Office for Human Research Protections (OHRP) Federal-wide Assurance (FWA 678).

The sub-committee's decision will be noted by a full Committee at its next meeting taking place on 13 July 2021.

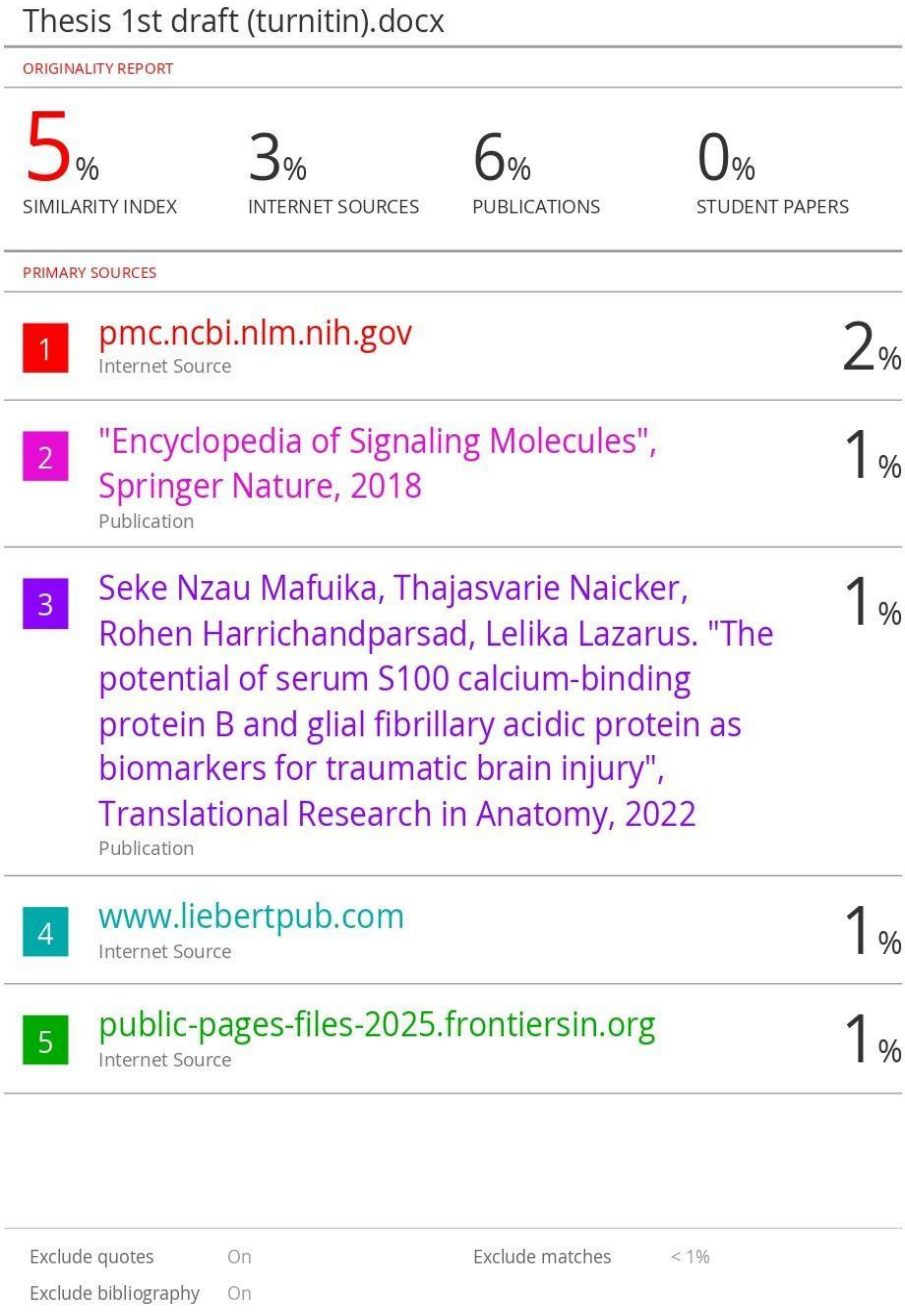
Yours sincerely,

[redacted]

Prof D Wassenaar
Chair: Biomedical Research Ethics Committee

Biomedical Research Ethics Committee
Chair: Professor D R Wassenaar
UKZN Research Ethics Office Westville Campus, Govan Mbeki Building
Postal Address: Private Bag X54001, Durban 4000
Email: BREC@ukzn.ac.za
Website: http://research.ukzn.ac.za/Research-Ethics/Biomedical-Research-Ethics.aspx

APPENDIX B: TURNITIN REPORT



Thesis 1st draft (turnitin).docx

ORIGINALITY REPORT

5%	3%	6%	0%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	pmc.ncbi.nlm.nih.gov Internet Source	2%
2	"Encyclopedia of Signaling Molecules", Springer Nature, 2018 Publication	1%
3	Seke Nzau Mafuika, Thajasvarie Naicker, Rohen Harrichandparsad, Lelika Lazarus. "The potential of serum S100 calcium-binding protein B and glial fibrillary acidic protein as biomarkers for traumatic brain injury", Translational Research in Anatomy, 2022 Publication	1%
4	www.liebertpub.com Internet Source	1%
5	public-pages-files-2025.frontiersin.org Internet Source	1%

Exclude quotes	On	Exclude matches	< 1%
Exclude bibliography	On		

APPENDIX C: STANDARD CURVES

GFAP

Belysa® Software Experiment Report

Test Info

File Name: C:/Users/dessaif/Desktop/Seke Magpix Runs/Run 1 GFAP/Run 1 Seke GFAP STD change.belysa
Source File Name: C:/Users/dessaif/Desktop/Seke Magpix Runs/Run 1 GFAP/Run 1 GFAP Seke.csv
Executed By: admin
Executed On: Tue Oct 1 15:40:00 2024
Instrument: MAGPX18251721
File Last Modified: Wed Feb 12 13:42:06 2025 GMT+0200
File Last Modified By: Dessaif **Instrument SW Version:** 4.2.1705.0 **Analysis SW Version:** Belysa 1.2.2
Printed By SW Version: 1.2.2
LLoQ Recovery Range [80% 120%]
LLoQ CV Limit 20%
LoD Coefficient 2.5
MDD Coefficient 2.5
Experiment Name: Experiment 1
Experiment Description:
Experiment Created By: Dessaif
Experiment Created On: Wed Feb 12 13:22:03 2025 GMT+0200
Messages: ND = Response is below limit of detection (LoD); EXT = Using extrapolated values; BLOQ = Response is below the lower limit of quantification (LLoQ); Defects = Well(s) have defects

GFAP

Original Name	Current Name	ID	Units	Precision
GFAP	GFAP	22	pg/mL	2

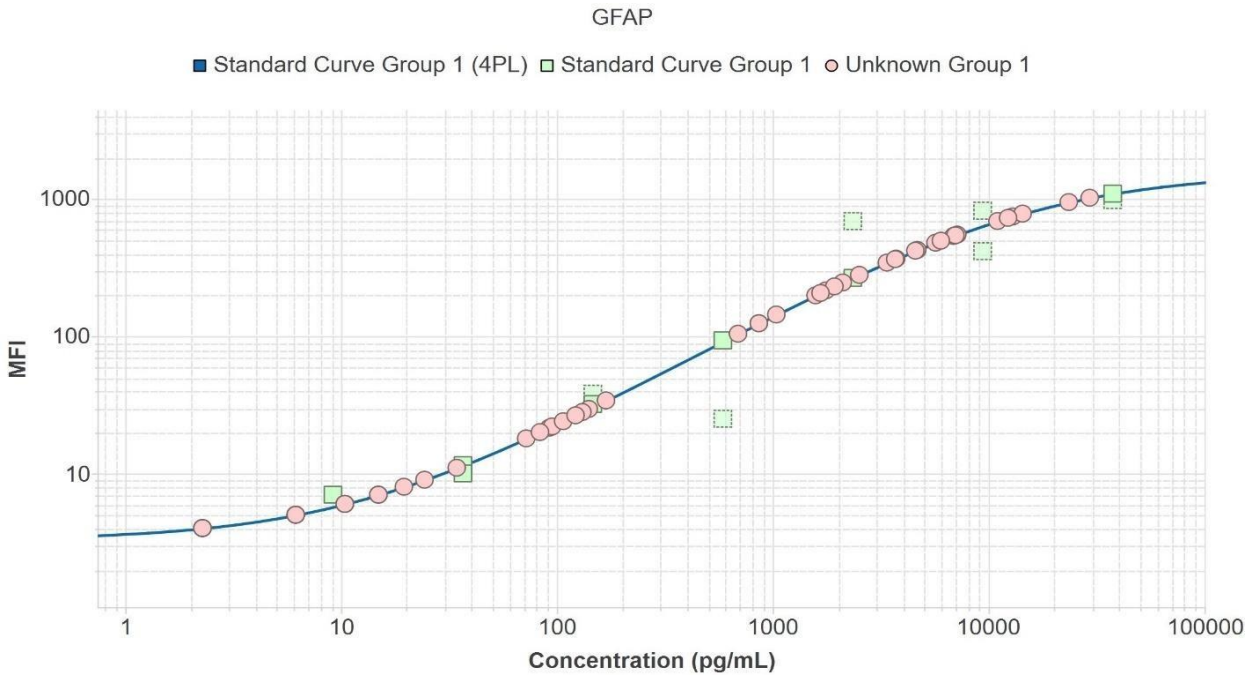


Figure 1: GFAP Curve Standard Curve Group 1 (4PL)

Key:

- **Bloq**: Below the Limit of Quantification
- **NaN**: Not a Number
- **LLD**: Lower Limit of Detection
- **LLOQ**: Lower Limit of Quantification
- **ND:** Not Detected

Table 1: GFAP Luminex results

Fit	LLoQ	MDD	LoD	R Squared	Slope	Weighting	Equation
4PL	36.72	2.77	1.79	1.00	0.03	1/y^2	y = 3.28 + (1564.12 - 3.28)/(1 + (14584.84/x)^0.876658)

Standards								
Location	**Sample ID**	**Expected (pg/mL)**	**MFI**	**Result (pg/mL)**	**Result SD**	**Result CV**	**Recovery**	**Message**
A1 A2	Standard1	37600.00	1089.00	37477.12	0.00	0.0%	99.7%	EXT
~~*A1*~~	~~*Standard1*~~	~~*37600.00*~~	~~*978.00*~~	~~*33092.38*~~			~~*88.0%*~~	~~*EXT*~~
A2	*Standard1*	*37600.00*	*1089.00*	*37477.12*			*99.7%*	*EXT*
~~B1 B2~~	~~Standard2~~	~~9400.00~~	~~0.00~~	~~0.00~~	~~0.00~~	~~0.0%~~	~~0.0%~~	
~~*B1*~~	~~*Standard2*~~	~~*9400.00*~~	~~*415.00*~~	~~*4522.95*~~			~~*48.1%*~~	
~~*B2*~~	~~*Standard2*~~	~~*9400.00*~~	~~*817.50*~~	~~*16100.57*~~			~~*171.3%*~~	
C1 C2	Standard3	2350.00	265.00	2345.26	0.00	0.0%	99.8%	
C1	*Standard3*	*2350.00*	*265.00*	*2345.26*			*99.8%*	
~~*C2*~~	~~*Standard3*~~	~~*2350.00*~~	~~*687.00*~~	~~*10977.44*~~			~~*467.1%*~~	
D1 D2	Standard4	587.50	93.00	600.11	0.00	0.0%	102.1%	
D1	*Standard4*	*587.50*	*93.00*	*600.11*			*102.1%*	
~~*D2*~~	~~*Standard4*~~	~~*587.50*~~	~~*25.00*~~	~~*113.01*~~			~~*19.2%*~~	
E1 E2	Standard5	146.88	32.00	156.24	0.00	0.0%	106.4%	
E1	*Standard5*	*146.88*	*32.00*	*156.24*			*106.4%*	
~~*E2*~~	~~*Standard5*~~	~~*146.88*~~	~~*38.00*~~	~~*194.86*~~			~~*132.7%*~~	
F1 F2	Standard6	36.72	10.75	33.12	5.39	16.3%	90.2%	
F1	*Standard6*	*36.72*	*11.50*	*36.93*			*100.6%*	
F2	*Standard6*	*36.72*	*10.00*	*29.32*			*79.8%*	
G1 G2	Standard7	9.18	7.00	14.90	0.00	0.0%	162.3%	
~~*G1*~~	~~*Standard7*~~	~~*9.18*~~	~~*NaN*~~	~~*NaN*~~			~~*nan%*~~	
G2	*Standard7*	*9.18*	*7.00*	*14.90*			*162.3%*	
H1 H2	Background0	0.00	3.25	-	-	-	-	EXT, ND
H1	*Background0*	*0.00*	*3.00*	-			-	*EXT*
H2	*Background0*	*0.00*	*3.50*	-			-	*ND*

Unknowns							
Location	**Sample ID**	**MFI**	**Result (pg/mL)**	**Result SD**	**Result CV**	**Unk. Dilution**	**Message**
A3	Unknown1	4.00	2.28	0.00	0.0%	1.00	BLOQ
A3	*Unknown1*	*4.00*	*2.28*			*1.00*	*BLOQ*
B3	Unknown2	6.00	10.41	0.00	0.0%	1.00	BLOQ
B3	*Unknown2*	*6.00*	*10.41*			*1.00*	*BLOQ*
C3	Unknown3	3.00	-	-	-	1.00	EXT
C3	*Unknown3*	*3.00*	-			*1.00*	*EXT*
D3	Unknown4	2.00	-	-	-	1.00	EXT
D3	*Unknown4*	*2.00*	-			*1.00*	*EXT*
E3	Unknown5	5.00	6.17	0.00	0.0%	1.00	BLOQ
E3	*Unknown5*	*5.00*	*6.17*			*1.00*	*BLOQ*
F3	Unknown6	NaN	NaN	0.00	0.0%	1.00	
F3	*Unknown6*	*NaN*	*NaN*			*1.00*	
G3	Unknown7	3.00	-	-	-	1.00	EXT
G3	*Unknown7*	*3.00*	-			*1.00*	*EXT*
H3	Unknown8	4.00	2.28	0.00	0.0%	1.00	BLOQ
H3	*Unknown8*	*4.00*	*2.28*			*1.00*	*BLOQ*
A4	Unknown9	5.00	6.17	0.00	0.0%	1.00	BLOQ

A4	*Unknown9*	*5.00*	*6.17*			*1.00*	*BLOQ*
B4	Unknown10	7.00	14.90	0.00	0.0%	1.00	BLOQ
B4	*Unknown10*	*7.00*	*14.90*			*1.00*	*BLOQ*
C4	Unknown11	6.00	10.41	0.00	0.0%	1.00	BLOQ
C4	*Unknown11*	*6.00*	*10.41*			*1.00*	*BLOQ*
D4	Unknown12	4.00	2.28	0.00	0.0%	1.00	BLOQ
D4	*Unknown12*	*4.00*	*2.28*			*1.00*	*BLOQ*
E4	Unknown13	4.00	2.28	0.00	0.0%	1.00	BLOQ
E4	*Unknown13*	*4.00*	*2.28*			*1.00*	*BLOQ*
F4	Unknown14	2.50	-	-	-	1.00	EXT
F4	*Unknown14*	*2.50*	-			*1.00*	*EXT*
G4	Unknown15	3.00	-	-	-	1.00	EXT
G4	*Unknown15*	*3.00*	-			*1.00*	*EXT*
H4	Unknown16	0.00	-	-	-	1.00	EXT
H4	*Unknown16*	*0.00*	-			*1.00*	*EXT*
A5	Unknown17	4.00	2.28	0.00	0.0%	1.00	BLOQ
A5	*Unknown17*	*4.00*	*2.28*			*1.00*	*BLOQ*
B5	Unknown18	4.00	2.28	0.00	0.0%	1.00	BLOQ
B5	*Unknown18*	*4.00*	*2.28*			*1.00*	*BLOQ*
C5	Unknown19	6.00	10.41	0.00	0.0%	1.00	BLOQ
C5	*Unknown19*	*6.00*	*10.41*			*1.00*	*BLOQ*
D5	Unknown20	7.00	14.90	0.00	0.0%	1.00	BLOQ
D5	*Unknown20*	*7.00*	*14.90*			*1.00*	*BLOQ*
E5	Unknown21	5.00	6.17	0.00	0.0%	1.00	BLOQ
E5	*Unknown21*	*5.00*	*6.17*			*1.00*	*BLOQ*
F5	Unknown22	7.00	14.90	0.00	0.0%	1.00	BLOQ
F5	*Unknown22*	*7.00*	*14.90*			*1.00*	*BLOQ*
G5	Unknown23	5.00	6.17	0.00	0.0%	1.00	BLOQ
G5	*Unknown23*	*5.00*	*6.17*			*1.00*	*BLOQ*
H5	Unknown24	0.50	-	-	-	1.00	EXT
H5	*Unknown24*	*0.50*	-			*1.00*	*EXT*
A6	Unknown25	4.00	2.28	0.00	0.0%	1.00	BLOQ
A6	*Unknown25*	*4.00*	*2.28*			*1.00*	*BLOQ*
B6	Unknown26	535.50	6878.22	0.00	0.0%	1.00	
B6	*Unknown26*	*535.50*	*6878.22*			*1.00*	
C6	Unknown27	28.00	131.28	0.00	0.0%	1.00	
C6	*Unknown27*	*28.00*	*131.28*			*1.00*	
D6	Unknown28	5.00	6.17	0.00	0.0%	1.00	BLOQ
D6	*Unknown28*	*5.00*	*6.17*			*1.00*	*BLOQ*
E6	Unknown29	2.00	-	-	-	1.00	EXT
E6	*Unknown29*	*2.00*	-			*1.00*	*EXT*
F6	Unknown30	NaN	NaN	0.00	0.0%	1.00	
F6	*Unknown30*	*NaN*	*NaN*			*1.00*	
G6	Unknown31	NaN	NaN	0.00	0.0%	1.00	
G6	*Unknown31*	*NaN*	*NaN*			*1.00*	
H6	Unknown32	34.00	168.96	0.00	0.0%	1.00	
H6	*Unknown32*	*34.00*	*168.96*			*1.00*	
A7	Unknown33	548.00	7162.00	0.00	0.0%	1.00	
A7	*Unknown33*	*548.00*	*7162.00*			*1.00*	
B7	Unknown34	215.00	1763.81	0.00	0.0%	1.00	
B7	*Unknown34*	*215.00*	*1763.81*			*1.00*	
C7	Unknown35	8.00	19.56	0.00	0.0%	1.00	BLOQ
C7	*Unknown35*	*8.00*	*19.56*			*1.00*	*BLOQ*
D7	Unknown36	124.00	862.59	0.00	0.0%	1.00	
D7	*Unknown36*	*124.00*	*862.59*			*1.00*	
E7	Unknown37	22.00	95.17	0.00	0.0%	1.00	
E7	*Unknown37*	*22.00*	*95.17*			*1.00*	
F7	Unknown38	104.00	690.62	0.00	0.0%	1.00	
F7	*Unknown38*	*104.00*	*690.62*			*1.00*	

G7	Unknown39	6.00	10.41	0.00	0.0%	1.00	BLOQ
G7	*Unknown39*	*6.00*	*10.41*			*1.00*	*BLOQ*
H7	Unknown40	279.00	2519.85	0.00	0.0%	1.00	
H7	*Unknown40*	*279.00*	*2519.85*			*1.00*	

Location	Sample ID	MFI	Result (pg/mL)	Result SD	Result CV	Unk. Dilution	Message
A8	Unknown41	2.00	-	-	-	1.00	EXT
A8	*Unknown41*	*2.00*	-			*1.00*	*EXT*
B8	Unknown42	4.00	2.28	0.00	0.0%	1.00	BLOQ
B8	*Unknown42*	*4.00*	*2.28*			*1.00*	*BLOQ*
C8	Unknown43	6.00	10.41	0.00	0.0%	1.00	BLOQ
C8	*Unknown43*	*6.00*	*10.41*			*1.00*	*BLOQ*
D8	Unknown44	18.00	72.13	0.00	0.0%	1.00	
D8	*Unknown44*	*18.00*	*72.13*			*1.00*	
E8	Unknown45	230.00	1931.53	0.00	0.0%	1.00	
E8	*Unknown45*	*230.00*	*1931.53*			*1.00*	
F8	Unknown46	366.00	3732.17	0.00	0.0%	1.00	
F8	*Unknown46*	*366.00*	*3732.17*			*1.00*	
G8	Unknown47	26.50	122.09	0.00	0.0%	1.00	
G8	*Unknown47*	*26.50*	*122.09*			*1.00*	
H8	Unknown48	11.00	34.37	0.00	0.0%	1.00	BLOQ
H8	*Unknown48*	*11.00*	*34.37*			*1.00*	*BLOQ*
A9	Unknown49	29.50	140.56	0.00	0.0%	1.00	
A9	*Unknown49*	*29.50*	*140.56*			*1.00*	
B9	Unknown50	245.50	2110.82	0.00	0.0%	1.00	
B9	*Unknown50*	*245.50*	*2110.82*			*1.00*	
C9	Unknown51	143.50	1039.29	0.00	0.0%	1.00	
C9	*Unknown51*	*143.50*	*1039.29*			*1.00*	
D9	Unknown52	NaN	NaN	0.00	0.0%	1.00	
D9	*Unknown52*	*NaN*	*NaN*			*1.00*	
E9	Unknown53	20.00	83.54	0.00	0.0%	1.00	
E9	*Unknown53*	*20.00*	*83.54*			*1.00*	
F9	Unknown54	198.00	1580.46	0.00	0.0%	1.00	
F9	*Unknown54*	*198.00*	*1580.46*			*1.00*	
G9	Unknown55	363.50	3694.05	0.00	0.0%	1.00	
G9	*Unknown55*	*363.50*	*3694.05*			*1.00*	
H9	Unknown56	542.00	7024.74	0.00	0.0%	1.00	
H9	*Unknown56*	*542.00*	*7024.74*			*1.00*	
A10	Unknown57	424.00	4677.67	0.00	0.0%	1.00	
A10	*Unknown57*	*424.00*	*4677.67*			*1.00*	
B10	Unknown58	478.50	5683.81	0.00	0.0%	1.00	
B10	*Unknown58*	*478.50*	*5683.81*			*1.00*	
C10	Unknown59	342.50	3381.86	0.00	0.0%	1.00	
C10	*Unknown59*	*342.50*	*3381.86*			*1.00*	
D10	Unknown60	124.00	862.59	0.00	0.0%	1.00	
D10	*Unknown60*	*124.00*	*862.59*			*1.00*	
E10	Unknown61	9.00	24.38	0.00	0.0%	1.00	BLOQ
E10	*Unknown61*	*9.00*	*24.38*			*1.00*	*BLOQ*
F10	Unknown62	779.00	14385.86	0.00	0.0%	1.00	
F10	*Unknown62*	*779.00*	*14385.86*			*1.00*	
G10	Unknown63	206.00	1665.86	0.00	0.0%	1.00	
G10	*Unknown63*	*206.00*	*1665.86*			*1.00*	
H10	Unknown64	945.00	23533.01	0.00	0.0%	1.00	
H10	*Unknown64*	*945.00*	*23533.01*			*1.00*	
A11	Unknown65	688.00	11010.08	0.00	0.0%	1.00	
A11	*Unknown65*	*688.00*	*11010.08*			*1.00*	
B11	Unknown66	418.50	4582.75	0.00	0.0%	1.00	
B11	*Unknown66*	*418.50*	*4582.75*			*1.00*	
C11	Unknown67	21.50	92.25	0.00	0.0%	1.00	
C11	*Unknown67*	*21.50*	*92.25*			*1.00*	
D11	Unknown68	1016.50	29425.60	0.00	0.0%	1.00	
D11	*Unknown68*	*1016.50*	*29425.60*			*1.00*	
E11	Unknown69	24.00	107.02	0.00	0.0%	1.00	
E11	*Unknown69*	*24.00*	*107.02*			*1.00*	

F11	Unknown70	726.00	12317.28	0.00	0.0%	1.00	
F11	*Unknown70*	*726.00*	*12317.28*			*1.00*	
G11	Unknown71	NaN	NaN	0.00	0.0%	1.00	
G11	*Unknown71*	*NaN*	*NaN*			*1.00*	
H11	Unknown72	495.00	6013.61	0.00	0.0%	1.00	
H11	*Unknown72*	*495.00*	*6013.61*			*1.00*	
A12	Unknown73	5.00	6.17	0.00	0.0%	1.00	BLOQ
A12	*Unknown73*	*5.00*	*6.17*			*1.00*	*BLOQ*
B12	Unknown74	743.00	12947.45	0.00	0.0%	1.00	
B12	*Unknown74*	*743.00*	*12947.45*			*1.00*	
C12	Unknown75	7.00	14.90	0.00	0.0%	1.00	BLOQ
C12	*Unknown75*	*7.00*	*14.90*			*1.00*	*BLOQ*

The following records were excluded from the analyzed results

Plate	Location	Sample ID Reason
Plate 1	A1	Standard1
Plate 1	B1	Standard2
Plate 1	B2	Standard2
Plate 1	C2	Standard3
Plate 1	D2	Standard4
Plate 1	E2	Standard5
Plate 1	G1	Standard7

S100B

Belysa® Software Experiment Report

Test Info

File Name: Untitled Test Results
Source File Name: C:/Users/dessaif/Desktop/Seke Magpix Runs/Run 1 S100B/Run1 Seke S100B_lxb/Run1 Seke S100B.csv
Executed By: admin
Executed On: Tue Oct 1 14:06:00 2024
Instrument: MAGPX18251721
File Last Modified: Tue Feb 11 16:33:07 2025 GMT+0200
File Last Modified By: Dessaif **Instrument SW Version:** 4.2.1705.0 **Analysis SW Version:** Belysa 1.2.2
Printed By SW Version: 1.2.2
LLoQ Recovery Range [80% 120%]
LLoQ CV Limit 20%
LoD Coefficient 2.5
MDD Coefficient 2.5
Experiment Name: Experiment 1
Experiment Description:
Experiment Created By: Dessaif
Experiment Created On: Tue Feb 11 16:23:11 2025 GMT+0200
Messages: ND = Response is below limit of detection (LoD); EXT = Using extrapolated values; BLOQ = Response is below the lower limit of quantification (LLoQ); Defects = Well(s) have defects

S100B

Original Name	Current Name	ID	Units	Precision
S100B	S100B	18	pg/mL	2

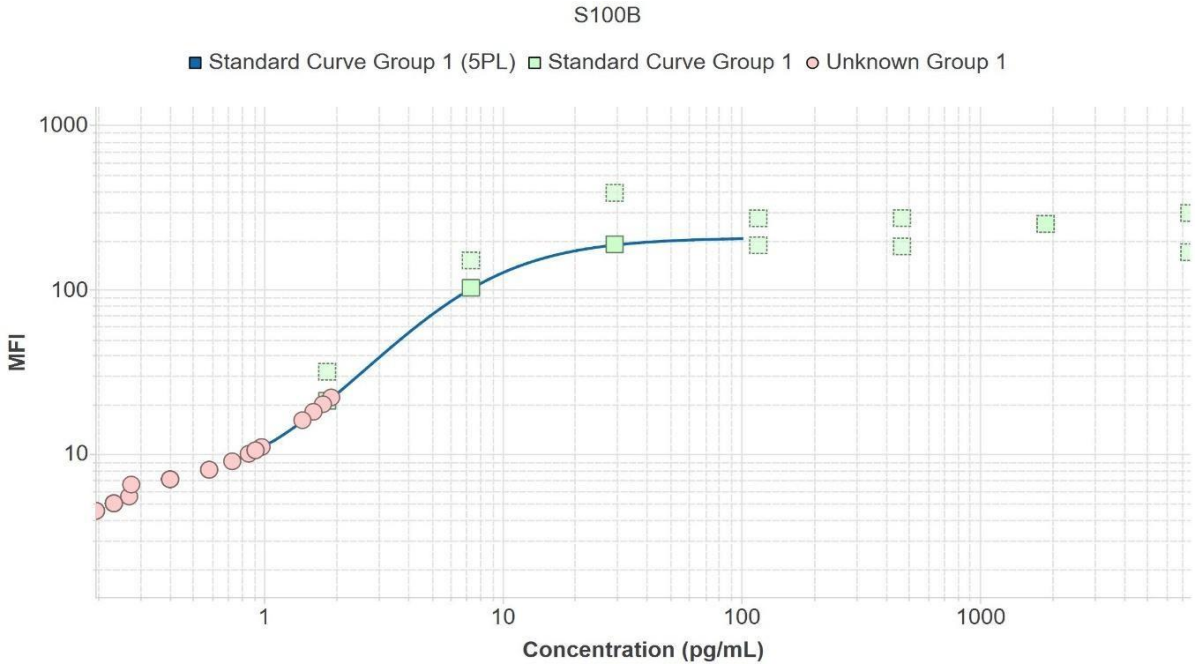


Figure 2: S100B Curve Standard Curve Group 1 (5PL)

Table 2: S100B Luminex results

Fit	LLoQ	MDD	LoD	R Squared	Slope	Weighting	Equation
5PL	1.84	0.00	0.19	1.00	5.41	1/y^2	y = 6.00 + (207.73 - 6.00)/((1 + (6.93/x)^1.617822))^1.151909

Standards								
Location	**Sample ID**	**Expected (pg/mL)**	**MFI**	**Result (pg/mL)**	**Result SD**	**Result CV**	**Recovery**	**Message**
~~A1 A2~~	~~Standard1~~	~~7550.00~~	~~0.00~~	~~0.00~~	~~0.00~~	~~0.0%~~	~~0.0%~~	
~~*A1*~~	~~*Standard1*~~	~~*7550.00*~~	~~*290.50*~~	~~*47.49*~~			~~*0.6%*~~	~~*EXT*~~
~~*A2*~~	~~*Standard1*~~	~~*7550.00*~~	~~*168.00*~~	~~*18.20*~~			~~*0.2%*~~	
~~B1 B2~~	~~Standard2~~	~~1887.50~~	~~0.00~~	~~0.00~~	~~0.00~~	~~0.0%~~	~~0.0%~~	
~~*B1*~~	~~*Standard2*~~	~~*1887.50*~~	~~*250.00*~~	~~*40.00*~~			~~*2.1%*~~	~~*EXT*~~
~~*B2*~~	~~*Standard2*~~	~~*1887.50*~~	~~*249.00*~~	~~*39.82*~~			~~*2.1%*~~	~~*EXT*~~
~~C1 C2~~	~~Standard3~~	~~471.88~~	~~0.00~~	~~0.00~~	~~0.00~~	~~0.0%~~	~~0.0%~~	
~~*C1*~~	~~*Standard3*~~	~~*471.88*~~	~~*270.50*~~	~~*43.79*~~			~~*9.3%*~~	~~*EXT*~~
~~*C2*~~	~~*Standard3*~~	~~*471.88*~~	~~*182.00*~~	~~*24.97*~~			~~*5.3%*~~	
~~D1 D2~~	~~Standard4~~	~~117.97~~	~~0.00~~	~~0.00~~	~~0.00~~	~~0.0%~~	~~0.0%~~	
~~*D1*~~	~~*Standard4*~~	~~*117.97*~~	~~*270.00*~~	~~*43.70*~~			~~*37.0%*~~	~~*EXT*~~
~~*D2*~~	~~*Standard4*~~	~~*117.97*~~	~~*185.00*~~	~~*27.22*~~			~~*23.1%*~~	
E1 E2	Standard5	29.49	187.50	29.49	0.00	0.0%	100.0%	
~~*E1*~~	~~*Standard5*~~	~~*29.49*~~	~~*385.00*~~	~~*64.98*~~			~~*220.3%*~~	~~*EXT*~~
E2	*Standard5*	*29.49*	*187.50*	*29.49*			*100.0%*	

F1 F2	Standard6	7.37	102.00	7.37	0.00	0.0%	100.0%	
~~*F1*~~	~~*Standard6*~~	~~*7.37*~~	~~*149.50*~~	~~*13.40*~~			~~*181.9%*~~	
F2	*Standard6*	*7.37*	*102.00*	*7.37*			*100.0%*	
G1 G2	Standard7	1.84	21.00	1.84	0.00	0.0%	100.0%	
~~*G1*~~	~~*Standard7*~~	~~*1.84*~~	~~*31.50*~~	~~*2.56*~~			~~*138.9%*~~	
G2	*Standard7*	*1.84*	*21.00*	*1.84*			*100.0%*	
H1 H2	Background0	0.00	6.00	-	-	-	-	ND
H1	*Background0*	*0.00*	*6.00*	-			-	*ND*
H2	*Background0*	*0.00*	*6.00*	-			-	*ND*
Unknowns								

Location	Sample ID	MFI	Result (pg/mL)	Result SD	Result CV	Unk. Dilution	Message
A3	Unknown1	3.50	-	-	-	1.00	EXT
A3	*Unknown1*	*3.50*	-			*1.00*	*EXT*
B3	Unknown2	5.00	0.23	0.00	0.0%	1.00	EXT
B3	*Unknown2*	*5.00*	*0.23*			*1.00*	*EXT*
C3	Unknown3	5.00	0.23	0.00	0.0%	1.00	EXT
C3	*Unknown3*	*5.00*	*0.23*			*1.00*	*EXT*

Location	Sample ID	MFI	Result (pg/mL)	Result SD	Result CV	Unk. Dilution	Message
D3	Unknown4	4.00	-	-	-	1.00	EXT
D3	*Unknown4*	*4.00*	-			*1.00*	*EXT*
E3	Unknown5	5.00	0.23	0.00	0.0%	1.00	EXT
E3	*Unknown5*	*5.00*	*0.23*			*1.00*	*EXT*
F3	Unknown6	4.00	-	-	-	1.00	EXT
F3	*Unknown6*	*4.00*	-			*1.00*	*EXT*
G3	Unknown7	5.00	0.23	0.00	0.0%	1.00	EXT
G3	*Unknown7*	*5.00*	*0.23*			*1.00*	*EXT*
H3	Unknown8	4.00	-	-	-	1.00	EXT
H3	*Unknown8*	*4.00*	-			*1.00*	*EXT*
A4	Unknown9	4.50	0.20	0.00	0.0%	1.00	EXT
A4	*Unknown9*	*4.50*	*0.20*			*1.00*	*EXT*
B4	Unknown10	4.00	-	-	-	1.00	EXT
B4	*Unknown10*	*4.00*	-			*1.00*	*EXT*
C4	Unknown11	4.00	-	-	-	1.00	EXT
C4	*Unknown11*	*4.00*	-			*1.00*	*EXT*
D4	Unknown12	4.00	-	-	-	1.00	EXT
D4	*Unknown12*	*4.00*	-			*1.00*	*EXT*
E4	Unknown13	4.00	-	-	-	1.00	EXT
E4	*Unknown13*	*4.00*	-			*1.00*	*EXT*
F4	Unknown14	4.00	-	-	-	1.00	EXT
F4	*Unknown14*	*4.00*	-			*1.00*	*EXT*
G4	Unknown15	4.00	-	-	-	1.00	EXT
G4	*Unknown15*	*4.00*	-			*1.00*	*EXT*
H4	Unknown16	4.00	-	-	-	1.00	EXT
H4	*Unknown16*	*4.00*	-			*1.00*	*EXT*
A5	Unknown17	4.00	-	-	-	1.00	EXT
A5	*Unknown17*	*4.00*	-			*1.00*	*EXT*
B5	Unknown18	4.00	-	-	-	1.00	EXT
B5	*Unknown18*	*4.00*	-			*1.00*	*EXT*
C5	Unknown19	5.00	0.23	0.00	0.0%	1.00	EXT
C5	*Unknown19*	*5.00*	*0.23*			*1.00*	*EXT*

D5	Unknown20	5.00	0.23	0.00	0.0%	1.00	EXT
D5	*Unknown20*	*5.00*	*0.23*			*1.00*	*EXT*
E5	Unknown21	5.00	0.23	0.00	0.0%	1.00	EXT
E5	*Unknown21*	*5.00*	*0.23*			*1.00*	*EXT*
F5	Unknown22	4.00	-	-	-	1.00	EXT
F5	*Unknown22*	*4.00*	-			*1.00*	*EXT*
G5	Unknown23	2.00	-	-	-	1.00	EXT
G5	*Unknown23*	*2.00*	-			*1.00*	*EXT*
H5	Unknown24	5.00	0.23	0.00	0.0%	1.00	EXT
H5	*Unknown24*	*5.00*	*0.23*			*1.00*	*EXT*
A6	Unknown25	4.50	0.20	0.00	0.0%	1.00	EXT
A6	*Unknown25*	*4.50*	*0.20*			*1.00*	*EXT*
B6	Unknown26	8.00	0.59	0.00	0.0%	1.00	BLOQ
B6	*Unknown26*	*8.00*	*0.59*			*1.00*	*BLOQ*
C6	Unknown27	6.00	-	-	-	1.00	ND
C6	*Unknown27*	*6.00*	-			*1.00*	*ND*
D6	Unknown28	5.00	0.23	0.00	0.0%	1.00	EXT
D6	*Unknown28*	*5.00*	*0.23*			*1.00*	*EXT*
E6	Unknown29	5.00	0.23	0.00	0.0%	1.00	EXT
E6	*Unknown29*	*5.00*	*0.23*			*1.00*	*EXT*
F6	Unknown30	5.00	0.23	0.00	0.0%	1.00	EXT
F6	*Unknown30*	*5.00*	*0.23*			*1.00*	*EXT*
G6	Unknown31	5.00	0.23	0.00	0.0%	1.00	EXT
G6	*Unknown31*	*5.00*	*0.23*			*1.00*	*EXT*
H6	Unknown32	5.00	0.23	0.00	0.0%	1.00	EXT
H6	*Unknown32*	*5.00*	*0.23*			*1.00*	*EXT*
A7	Unknown33	7.00	0.40	0.00	0.0%	1.00	BLOQ
A7	*Unknown33*	*7.00*	*0.40*			*1.00*	*BLOQ*
B7	Unknown34	7.00	0.40	0.00	0.0%	1.00	BLOQ
B7	*Unknown34*	*7.00*	*0.40*			*1.00*	*BLOQ*
C7	Unknown35	8.00	0.59	0.00	0.0%	1.00	BLOQ
C7	*Unknown35*	*8.00*	*0.59*			*1.00*	*BLOQ*
D7	Unknown36	4.00	-	-	-	1.00	EXT
D7	*Unknown36*	*4.00*	-			*1.00*	*EXT*
E7	Unknown37	10.00	0.86	0.00	0.0%	1.00	BLOQ
E7	*Unknown37*	*10.00*	*0.86*			*1.00*	*BLOQ*
F7	Unknown38	0.00	-	-	-	1.00	EXT
F7	*Unknown38*	*0.00*	-			*1.00*	*EXT*
G7	Unknown39	5.00	0.23	0.00	0.0%	1.00	EXT
G7	*Unknown39*	*5.00*	*0.23*			*1.00*	*EXT*
H7	Unknown40	4.00	-	-	-	1.00	EXT
H7	*Unknown40*	*4.00*	-			*1.00*	*EXT*

Location	Sample ID	MFI	Result (pg/mL)	Result SD	Result CV	Unk. Dilution	Message
A8	Unknown41	5.00	0.23	0.00	0.0%	1.00	EXT
A8	*Unknown41*	*5.00*	*0.23*			*1.00*	*EXT*
B8	Unknown42	7.00	0.40	0.00	0.0%	1.00	BLOQ
B8	*Unknown42*	*7.00*	*0.40*			*1.00*	*BLOQ*
C8	Unknown43	5.00	0.23	0.00	0.0%	1.00	EXT
C8	*Unknown43*	*5.00*	*0.23*			*1.00*	*EXT*
D8	Unknown44	4.50	0.20	0.00	0.0%	1.00	EXT
D8	*Unknown44*	*4.50*	*0.20*			*1.00*	*EXT*
E8	Unknown45	20.00	1.77	0.00	0.0%	1.00	BLOQ
E8	*Unknown45*	*20.00*	*1.77*			*1.00*	*BLOQ*
F8	Unknown46	9.00	0.74	0.00	0.0%	1.00	BLOQ
F8	*Unknown46*	*9.00*	*0.74*			*1.00*	*BLOQ*
G8	Unknown47	5.00	0.23	0.00	0.0%	1.00	EXT
G8	*Unknown47*	*5.00*	*0.23*			*1.00*	*EXT*
H8	Unknown48	16.00	1.45	0.00	0.0%	1.00	BLOQ
H8	*Unknown48*	*16.00*	*1.45*			*1.00*	*BLOQ*
A9	Unknown49	7.00	0.40	0.00	0.0%	1.00	BLOQ
A9	*Unknown49*	*7.00*	*0.40*			*1.00*	*BLOQ*
B9	Unknown50	5.50	0.27	0.00	0.0%	1.00	EXT
B9	*Unknown50*	*5.50*	*0.27*			*1.00*	*EXT*
C9	Unknown51	5.00	0.23	0.00	0.0%	1.00	EXT
C9	*Unknown51*	*5.00*	*0.23*			*1.00*	*EXT*
D9	Unknown52	7.00	0.40	0.00	0.0%	1.00	BLOQ
D9	*Unknown52*	*7.00*	*0.40*			*1.00*	*BLOQ*
E9	Unknown53	7.00	0.40	0.00	0.0%	1.00	BLOQ
E9	*Unknown53*	*7.00*	*0.40*			*1.00*	*BLOQ*
F9	Unknown54	4.00	-	-	-	1.00	EXT
F9	*Unknown54*	*4.00*	-			*1.00*	*EXT*
G9	Unknown55	5.00	0.23	0.00	0.0%	1.00	EXT
G9	*Unknown55*	*5.00*	*0.23*			*1.00*	*EXT*
H9	Unknown56	7.00	0.40	0.00	0.0%	1.00	BLOQ
H9	*Unknown56*	*7.00*	*0.40*			*1.00*	*BLOQ*
A10	Unknown57	8.00	0.59	0.00	0.0%	1.00	BLOQ
A10	*Unknown57*	*8.00*	*0.59*			*1.00*	*BLOQ*
B10	Unknown58	11.00	0.98	0.00	0.0%	1.00	BLOQ
B10	*Unknown58*	*11.00*	*0.98*			*1.00*	*BLOQ*
C10	Unknown59	6.50	0.28	0.00	0.0%	1.00	BLOQ
C10	*Unknown59*	*6.50*	*0.28*			*1.00*	*BLOQ*
D10	Unknown60	5.00	0.23	0.00	0.0%	1.00	EXT
D10	*Unknown60*	*5.00*	*0.23*			*1.00*	*EXT*
E10	Unknown61	5.00	0.23	0.00	0.0%	1.00	EXT
E10	*Unknown61*	*5.00*	*0.23*			*1.00*	*EXT*
F10	Unknown62	18.00	1.61	0.00	0.0%	1.00	BLOQ
F10	*Unknown62*	*18.00*	*1.61*			*1.00*	*BLOQ*
G10	Unknown63	1.00	-	-	-	1.00	EXT
G10	*Unknown63*	*1.00*	-			*1.00*	*EXT*
H10	Unknown64	10.50	0.92	0.00	0.0%	1.00	BLOQ
H10	*Unknown64*	*10.50*	*0.92*			*1.00*	*BLOQ*
A11	Unknown65	2.00	-	-	-	1.00	EXT
A11	*Unknown65*	*2.00*	-			*1.00*	*EXT*
B11	Unknown66	5.00	0.23	0.00	0.0%	1.00	EXT
B11	*Unknown66*	*5.00*	*0.23*			*1.00*	*EXT*
C11	Unknown67	4.50	0.20	0.00	0.0%	1.00	EXT
C11	*Unknown67*	*4.50*	*0.20*			*1.00*	*EXT*
D11	Unknown68	2.00	-	-	-	1.00	EXT
D11	*Unknown68*	*2.00*	-			*1.00*	*EXT*

E11	Unknown69	5.00	0.23	0.00	0.0%	1.00	EXT
E11	*Unknown69*	*5.00*	*0.23*			*1.00*	*EXT*
F11	Unknown70	10.50	0.92	0.00	0.0%	1.00	BLOQ
F11	*Unknown70*	*10.50*	*0.92*			*1.00*	*BLOQ*
G11	Unknown71	6.00	-	-	-	1.00	ND
G11	*Unknown71*	*6.00*	-			*1.00*	*ND*
H11	Unknown72	8.00	0.59	0.00	0.0%	1.00	BLOQ
H11	*Unknown72*	*8.00*	*0.59*			*1.00*	*BLOQ*
A12	Unknown73	1.00	-	-	-	1.00	EXT
A12	*Unknown73*	*1.00*	-			*1.00*	*EXT*
B12	Unknown74	22.00	1.91	0.00	0.0%	1.00	
B12	*Unknown74*	*22.00*	*1.91*			*1.00*	
C12	Unknown75	0.00	-	-	-	1.00	EXT
C12	*Unknown75*	*0.00*	-			*1.00*	*EXT*

The following records were excluded from the analyzed results

Plate	Location	Sample ID Reason
Plate 1	A1	Standard1
Plate 1	A2	Standard1
Plate 1	B1	Standard2
Plate 1	B2	Standard2
Plate 1	C1	Standard3
Plate 1	C2	Standard3
Plate 1	D1	Standard4
Plate 1	D2	Standard4
Plate 1	E1	Standard5
Plate 1	F1	Standard6
Plate 1	G1	Standard7

NSE

Belysa® Software Experiment Report

Test Info

File Name: Untitled Test Results
Source File Name: C:/Users/dessaif/Desktop/2 plex Run 1 18Mar2025/2 plex Run2 Seke 18Mar25.csv
Executed By: admin
Executed On: Tue Mar 18 10:13:00 2025
Instrument:
File Last Modified: Tue Mar 18 13:28:18 2025 GMT+0200
File Last Modified By: Dessaif **Instrument SW Version:** 4.2.1705.0 **Analysis SW Version:** Belysa 1.2.2
Printed By SW Version: 1.2.2
LLoQ Recovery Range [80% 120%]
LLoQ CV Limit 20%
LoD Coefficient 2.5
MDD Coefficient 2.5
Experiment Name: Experiment 1
Experiment Description:
Experiment Created By: Dessaif
Experiment Created On: Tue Mar 18 12:59:38 2025 GMT+0200
Messages: ND = Response is below limit of detection (LoD); EXT = Using extrapolated values; BLOQ = Response is below the lower limit of quantification (LLoQ); Defects = Well(s) have defects

NSE

Original Name	Current Name	ID	Units	Precision
NSE	NSE	12	pg/mL	2

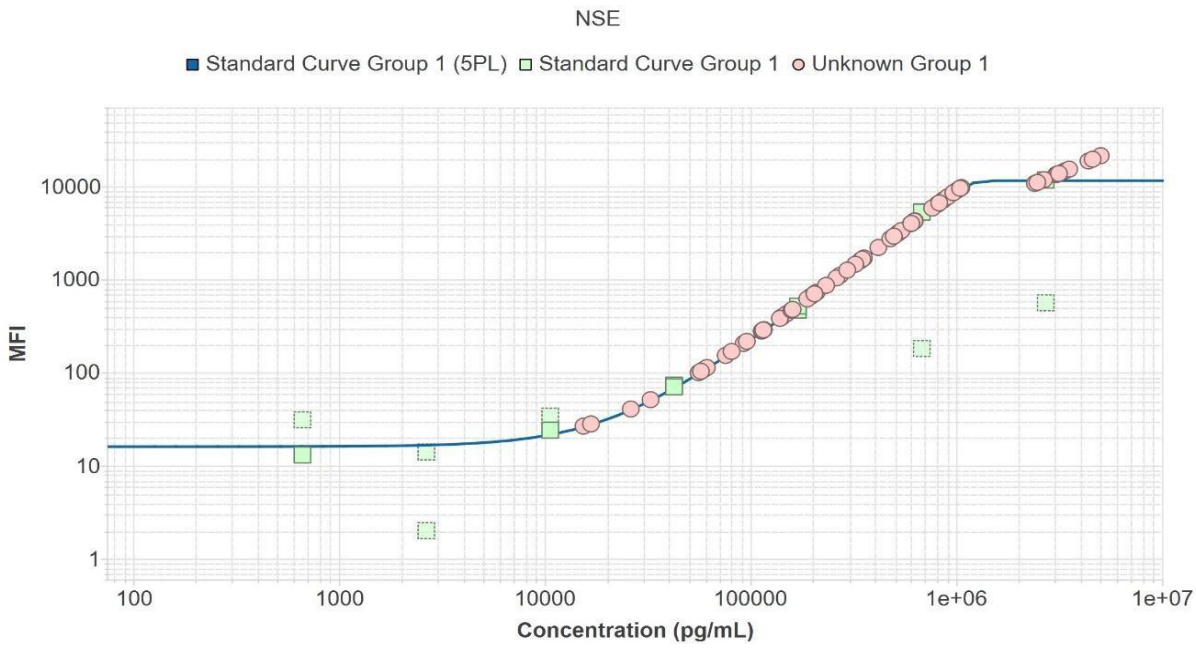


Figure 3: Curve Standard Curve Group 1 (5PL)

Fit	LLoQ	MDD	LoD	R^2 Squared	Slope	Weighting	Equation
5PL	42564.06	18496.59	N/A	1.00	0.00	1/y^2	y = 16.24 + (11669.48 - 16.24)/((1 + (1173841.51/ x)^15.283679))^0.106274

Standards

Location	Sample ID	Expected (pg/mL)		MFI	Result (pg/mL)	Result SD
Result CV	Recovery	Message				
A1 A2	Standard1	2724100.00	11669.00	2648571.97	0.00	0.0%	97.2%	EXT
A1	*Standard1*	*2724100.00*	*11669.00*	*2648571.97*			*97.2%*	*EXT*
~~*A2*~~	~~*Standard1*~~	~~*2724100.00*~~	~~*560.00*~~	~~*177881.86*~~			~~*6.5%*~~	
B1 B2	Standard2	681025.00	5276.00	719322.89	0.00	0.0%	105.6%	
B1	*Standard2*	*681025.00*	*5276.00*	*719322.89*			*105.6%*	
~~*B2*~~	~~*Standard2*~~	~~*681025.00*~~	~~*181.00*~~	~~*85285.76*~~			~~*12.5%*~~	
C1 C2	Standard3	170256.25	492.50	163891.32	7344.72	4.5%	96.3%	
C1	*Standard3*	*170256.25*	*468.00*	*158697.82*			*93.2%*	
C2	*Standard3*	*170256.25*	*517.00*	*169084.82*			*99.3%*	
D1 D2	Standard4	42564.06	71.50	43526.05	1028.81	2.4%	102.3%	
D1	*Standard4*	*42564.06*	*73.00*	*44253.53*			*104.0%*	
D2	*Standard4*	*42564.06*	*70.00*	*42798.57*			*100.6%*	
E1 E2	Standard5	10641.02	24.00	13001.15	0.00	0.0%	122.2%	
E1	*Standard5*	*10641.02*	*24.00*	*13001.15*			*122.2%*	
~~*E2*~~	~~*Standard5*~~	~~*10641.02*~~	~~*34.00*~~	~~*21642.66*~~			~~*203.4%*~~	
~~F1 F2~~	~~Standard6~~	~~2660.25~~	~~0.00~~	~~0.00~~	~~0.00~~	~~0.0%~~	~~0.0%~~	
~~*F1*~~	~~*Standard6*~~	~~*2660.25*~~	~~*2.00*~~	~~-~~			~~-~~	~~*EXT*~~
~~*F2*~~	~~*Standard6*~~	~~*2660.25*~~	~~*14.00*~~	~~-~~			~~-~~	~~*EXT*~~

G1 G2	Standard7	665.06	13.00----------					EXT
~~*G1*~~	~~*Standard7*~~	~~*665.06*~~	~~*31.00*~~	~~*19312.84*~~			~~*2903.9%*~~	
G2	*Standard7*	*665.06*	*13.00*	-			-	*EXT*
H1 H2	Background0	0.00	30.00	-	-	-	-	
~~*H1*~~	~~*Background0*~~	~~*0.00*~~	~~*0.00*~~	~~-~~			~~-~~	~~*EXT*~~
H2	*Background0*	*0.00*	*30.00*	-			-	

Table 3: NSE Luminex results

Unknowns							
Location	**Sample ID**	**MFI**	**Result (pg/mL)**	**Result SD**	**Result CV**	**Unk. Dilution**	**Message**
A3	Unknown1	1697.00	356343.59	0.00	0.0%	1.00	
A3	*Unknown1*	*1697.00*	*356343.59*			*1.00*	
B3	Unknown2	40.50	26223.48	0.00	0.0%	1.00	BLOQ
B3	*Unknown2*	*40.50*	*26223.48*			*1.00*	*BLOQ*
C3	Unknown3	397.00	142841.33	0.00	0.0%	1.00	
C3	*Unknown3*	*397.00*	*142841.33*			*1.00*	
D3	Unknown4	9.00	-	-	-	1.00	EXT
D3	*Unknown4*	*9.00*	-			*1.00*	*EXT*
E3	Unknown5	391.00	141451.31	0.00	0.0%	1.00	
E3	*Unknown5*	*391.00*	*141451.31*			*1.00*	
F3	Unknown6	381.00	139115.46	0.00	0.0%	1.00	
F3	*Unknown6*	*381.00*	*139115.46*			*1.00*	
G3	Unknown7	701.00	205011.86	0.00	0.0%	1.00	
G3	*Unknown7*	*701.00*	*205011.86*			*1.00*	
H3	Unknown8	286.00	115532.79	0.00	0.0%	1.00	
H3	*Unknown8*	*286.00*	*115532.79*			*1.00*	
A4	Unknown9	278.00	113411.20	0.00	0.0%	1.00	
A4	*Unknown9*	*278.00*	*113411.20*			*1.00*	
B4	Unknown10	205.00	92733.41	0.00	0.0%	1.00	
B4	*Unknown10*	*205.00*	*92733.41*			*1.00*	
C4	Unknown11	216.00	96024.17	0.00	0.0%	1.00	
C4	*Unknown11*	*216.00*	*96024.17*			*1.00*	
D4	Unknown12	1649.00	350043.32	0.00	0.0%	1.00	
D4	*Unknown12*	*1649.00*	*350043.32*			*1.00*	
E4	Unknown13	727.50	209860.79	0.00	0.0%	1.00	
E4	*Unknown13*	*727.50*	*209860.79*			*1.00*	
F4	Unknown14	466.00	158264.90	0.00	0.0%	1.00	
F4	*Unknown14*	*466.00*	*158264.90*			*1.00*	
G4	Unknown15	700.00	204827.48	0.00	0.0%	1.00	

G4	*Unknown15*	*700.00*	*204827.48*			*1.00*	
H4	Unknown16	103.00	57464.16	0.00	0.0%	1.00	
H4	*Unknown16*	*103.00*	*57464.16*			*1.00*	
A5	Unknown17	51.00	32722.15	0.00	0.0%	1.00	BLOQ
A5	*Unknown17*	*51.00*	*32722.15*			*1.00*	*BLOQ*
B5	Unknown18	3102.00	517984.74	0.00	0.0%	1.00	
B5	*Unknown18*	*3102.00*	*517984.74*			*1.00*	
C5	Unknown19	616.50	189044.27	0.00	0.0%	1.00	
C5	*Unknown19*	*616.50*	*189044.27*			*1.00*	
D5	Unknown20	99.50	56025.70	0.00	0.0%	1.00	
D5	*Unknown20*	*99.50*	*56025.70*			*1.00*	
E5	Unknown21	280.50	114076.84	0.00	0.0%	1.00	
E5	*Unknown21*	*280.50*	*114076.84*			*1.00*	
F5	Unknown22	107.50	59281.24	0.00	0.0%	1.00	
F5	*Unknown22*	*107.50*	*59281.24*			*1.00*	
G5	Unknown23	113.00	61455.99	0.00	0.0%	1.00	
G5	*Unknown23*	*113.00*	*61455.99*			*1.00*	
H5	Unknown24	168.00	81077.61	0.00	0.0%	1.00	
H5	*Unknown24*	*168.00*	*81077.61*			*1.00*	
A6	Unknown25	289.00	116322.14	0.00	0.0%	1.00	
A6	*Unknown25*	*289.00*	*116322.14*			*1.00*	
B6	Unknown26	152.50	75874.36	0.00	0.0%	1.00	
B6	*Unknown26*	*152.50*	*75874.36*			*1.00*	
C6	Unknown27	7130.50	866834.35	0.00	0.0%	1.00	
C6	*Unknown27*	*7130.50*	*866834.35*			*1.00*	
D6	Unknown28	14464.00	3343829.88	0.00	0.0%	1.00	EXT
D6	*Unknown28*	*14464.00*	*3343829.88*			*1.00*	*EXT*
E6	Unknown29	2731.00	478703.77	0.00	0.0%	1.00	
E6	*Unknown29*	*2731.00*	*478703.77*			*1.00*	
F6	Unknown30	10731.00	2415243.91	0.00	0.0%	1.00	EXT
F6	*Unknown30*	*10731.00*	*2415243.91*			*1.00*	*EXT*
G6	Unknown31	11000.00	2482157.82	0.00	0.0%	1.00	EXT
G6	*Unknown31*	*11000.00*	*2482157.82*			*1.00*	*EXT*
H6	Unknown32	28.00	16792.11	0.00	0.0%	1.00	BLOQ
H6	*Unknown32*	*28.00*	*16792.11*			*1.00*	*BLOQ*
A7	Unknown33	7089.50	863726.22	0.00	0.0%	1.00	
A7	*Unknown33*	*7089.50*	*863726.22*			*1.00*	
B7	Unknown34	3188.00	526825.70	0.00	0.0%	1.00	
B7	*Unknown34*	*3188.00*	*526825.70*			*1.00*	
C7	Unknown35	1105.50	272830.65	0.00	0.0%	1.00	
C7	*Unknown35*	*1105.50*	*272830.65*			*1.00*	

D7	Unknown36	14774.00	3420942.57	0.00	0.0%	1.00	EXT
D7	*Unknown36*	*14774.00*	*3420942.57*			*1.00*	*EXT*
E7	Unknown37	1257.00	295605.81	0.00	0.0%	1.00	
E7	*Unknown37*	*1257.00*	*295605.81*			*1.00*	
F7	Unknown38	861.00	233305.32	0.00	0.0%	1.00	
F7	*Unknown38*	*861.00*	*233305.32*			*1.00*	
G7	Unknown39	19539.50	4606363.53	0.00	0.0%	1.00	EXT
G7	*Unknown39*	*19539.50*	*4606363.53*			*1.00*	*EXT*
H7	Unknown40	15276.50	3545939.74	0.00	0.0%	1.00	EXT

Location	Sample ID	MFI	Result (pg/mL)	Result SD	Result CV	Unk. Dilution	Message
H7	*Unknown40*	*15276.50*	*3545939.74*			*1.00*	*EXT*
A8	Unknown41	390.00	141218.82	0.00	0.0%	1.00	
A8	*Unknown41*	*390.00*	*141218.82*			*1.00*	
B8	Unknown42	9696.00	1060342.42	0.00	0.0%	1.00	
B8	*Unknown42*	*9696.00*	*1060342.42*			*1.00*	
C8	Unknown43	26.50	15439.39	0.00	0.0%	1.00	BLOQ
C8	*Unknown43*	*26.50*	*15439.39*			*1.00*	*BLOQ*
D8	Unknown44	6470.00	816051.69	0.00	0.0%	1.00	
D8	*Unknown44*	*6470.00*	*816051.69*			*1.00*	
E8	Unknown45	18703.00	4398283.66	0.00	0.0%	1.00	EXT
E8	*Unknown45*	*18703.00*	*4398283.66*			*1.00*	*EXT*
F8	Unknown46	11776.00	2675188.28	0.00	0.0%	1.00	EXT
F8	*Unknown46*	*11776.00*	*2675188.28*			*1.00*	*EXT*
G8	Unknown47	5825.00	764706.23	0.00	0.0%	1.00	
G8	*Unknown47*	*5825.00*	*764706.23*			*1.00*	
H8	Unknown48	13722.00	3159256.94	0.00	0.0%	1.00	EXT
H8	*Unknown48*	*13722.00*	*3159256.94*			*1.00*	*EXT*
A9	Unknown49	8505.00	969105.79	0.00	0.0%	1.00	
A9	*Unknown49*	*8505.00*	*969105.79*			*1.00*	
B9	Unknown50	1581.50	341061.73	0.00	0.0%	1.00	
B9	*Unknown50*	*1581.50*	*341061.73*			*1.00*	
C9	Unknown51	1591.00	342334.68	0.00	0.0%	1.00	
C9	*Unknown51*	*1591.00*	*342334.68*			*1.00*	
D9	Unknown52	10980.00	2477182.81	0.00	0.0%	1.00	EXT
D9	*Unknown52*	*10980.00*	*2477182.81*			*1.00*	*EXT*
E9	Unknown53	427.00	149669.02	0.00	0.0%	1.00	
E9	*Unknown53*	*427.00*	*149669.02*			*1.00*	
F9	Unknown54	4222.00	626780.23	0.00	0.0%	1.00	
F9	*Unknown54*	*4222.00*	*626780.23*			*1.00*	
G9	Unknown55	3991.50	605401.63	0.00	0.0%	1.00	
G9	*Unknown55*	*3991.50*	*605401.63*			*1.00*	
H9	Unknown56	476.00	160422.18	0.00	0.0%	1.00	
H9	*Unknown56*	*476.00*	*160422.18*			*1.00*	
A10	Unknown57	656.00	196609.15	0.00	0.0%	1.00	
A10	*Unknown57*	*656.00*	*196609.15*			*1.00*	

B10	Unknown58	4256.00	629895.21	0.00	0.0%	1.00	
B10	*Unknown58*	*4256.00*	*629895.21*			*1.00*	
C10	Unknown59	6386.00	809472.22	0.00	0.0%	1.00	
C10	*Unknown59*	*6386.00*	*809472.22*			*1.00*	
D10	Unknown60	1036.50	262056.82	0.00	0.0%	1.00	
D10	*Unknown60*	*1036.50*	*262056.82*			*1.00*	
E10	Unknown61	6511.00	819252.32	0.00	0.0%	1.00	
E10	*Unknown61*	*6511.00*	*819252.32*			*1.00*	
F10	Unknown62	3344.00	542631.17	0.00	0.0%	1.00	
F10	*Unknown62*	*3344.00*	*542631.17*			*1.00*	
G10	Unknown63	8509.00	969401.97	0.00	0.0%	1.00	
G10	*Unknown63*	*8509.00*	*969401.97*			*1.00*	
H10	Unknown64	6621.00	827805.44	0.00	0.0%	1.00	
H10	*Unknown64*	*6621.00*	*827805.44*			*1.00*	
A11	Unknown65	21300.00	5044288.96	0.00	0.0%	1.00	EXT
A11	*Unknown65*	*21300.00*	*5044288.96*			*1.00*	*EXT*
B11	Unknown66	4220.00	626596.70	0.00	0.0%	1.00	
B11	*Unknown66*	*4220.00*	*626596.70*			*1.00*	
C11	Unknown67	13308.00	3056274.19	0.00	0.0%	1.00	EXT
C11	*Unknown67*	*13308.00*	*3056274.19*			*1.00*	*EXT*
D11	Unknown68	1453.00	323540.59	0.00	0.0%	1.00	
D11	*Unknown68*	*1453.00*	*323540.59*			*1.00*	
E11	Unknown69	9023.00	1007777.85	0.00	0.0%	1.00	
E11	*Unknown69*	*9023.00*	*1007777.85*			*1.00*	
F11	Unknown70	7713.50	910543.76	0.00	0.0%	1.00	
F11	*Unknown70*	*7713.50*	*910543.76*			*1.00*	
G11	Unknown71	2911.00	498003.50	0.00	0.0%	1.00	
G11	*Unknown71*	*2911.00*	*498003.50*			*1.00*	
H11	Unknown72	9505.50	1045021.13	0.00	0.0%	1.00	
H11	*Unknown72*	*9505.50*	*1045021.13*			*1.00*	
A12	Unknown73	2200.00	418668.10	0.00	0.0%	1.00	
A12	*Unknown73*	*2200.00*	*418668.10*			*1.00*	
B12	Unknown74	285.00	115268.93	0.00	0.0%	1.00	
B12	*Unknown74*	*285.00*	*115268.93*			*1.00*	
C12	Unknown75	1609.00	344738.52	0.00	0.0%	1.00	
C12	*Unknown75*	*1609.00*	*344738.52*			*1.00*	
D12	Unknown76	13453.00	3092343.03	0.00	0.0%	1.00	EXT

D12	*Unknown76*	*13453.00*	*3092343.03*			*1.00*	*EXT*
E12	Unknown77	3963.00	602725.62	0.00	0.0%	1.00	

Location	Sample ID	MFI	Result (pg/mL)	Result SD	Result CV	Unk. Dilution Message
E12	*Unknown77*	*3963.00*	*602725.62*			*1.00*
F12	Unknown78	9448.50	1040519.82	0.00	0.0%	1.00
F12	*Unknown78*	*9448.50*	*1040519.82*			*1.00*

The following records were excluded from the analyzed results

Plate	Location	Sample ID Reason
Plate 1	A2	Standard1
Plate 1	B2	Standard2
Plate 1	E2	Standard5
Plate 1	F1	Standard6
Plate 1	F2	Standard6
Plate 1	G1	Standard7
Plate 1	H1	Background 0

SAA

Belysa® Software Experiment Report

Test Info

File Name: Untitled Test Results
Source File Name: C:/Users/dessaif/Desktop/Run1 SAA Seke 11Apr2025/Run1 SAA Seke.csv
Executed By: admin
Executed On: Fri Apr 11 09:57:00 2025
Instrument: MAGPX18251721
File Last Modified: Fri Apr 11 13:58:22 2025 GMT+0200
File Last Modified By: Dessaif **Instrument SW Version:** 4.2.1705.0 **Analysis SW Version:** Belysa 1.2.2
Printed By SW Version: 1.2.2
LLoQ Recovery Range [80% 120%]
LLoQ CV Limit 20%
LoD Coefficient 2.5
MDD Coefficient 2.5
Experiment Name: Experiment 1
Experiment Description:
Experiment Created By: Dessaif
Experiment Created On: Fri Apr 11 13:48:34 2025 GMT+0200
Messages: ND = Response is below limit of detection (LoD); EXT = Using extrapolated values; BLOQ = Response is below the lower limit of quantification (LLoQ); Defects = Well(s) have defects

SAA

Original Name	Current Name	ID	Units	Precision
SAA	SAA	26	pg/mL	2

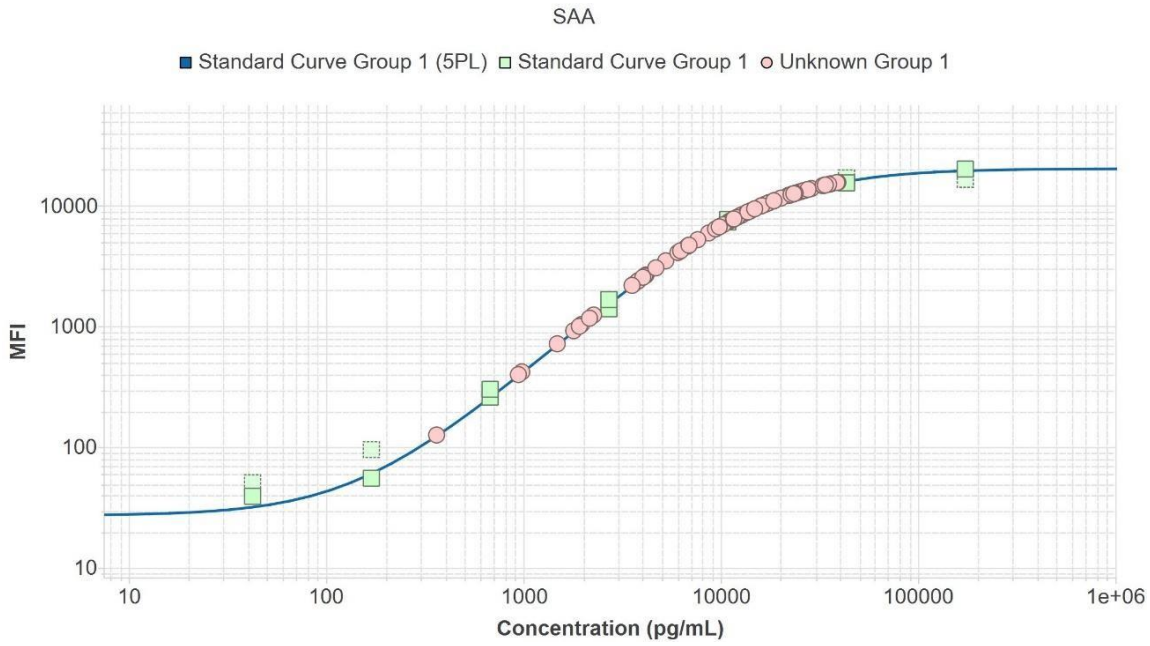


Figure 4: SAA Curve Standard Curve Group 1 (5PL)

Table 4: SAA Luminex results

Fit	LLoQ	MDD	LoD	R Squared	Slope	Weighting	Equation
5PL	169.73	40.36	23.05	1.00	0.11	1/y^2	y = 27.32 + (20400.95 - 27.32)/((1 + (16046.68/x)^1.343423))^1.047263

Standards								
Location	**Sample ID**	**Expected (pg/mL)**	**MFI**	**Result (pg/mL)**	**Result SD**	**Result CV**	**Recovery**	**Message**
A1 A2	Standard1	173800.00	19999.00	163963.00	0.00	0.0%	94.3%	EXT
A1	*Standard1*	*173800.00*	*19999.00*	*163963.00*			*94.3%*	*EXT*
~~*A2*~~	~~*Standard1*~~	~~*173800.00*~~	~~*16431.00*~~	~~*109201.66*~~			~~*62.8%*~~	~~*EXT*~~
B1 B2	Standard2	43450.00	15351.50	38139.25	0.00	0.0%	87.8%	
~~*B1*~~	~~*Standard2*~~	~~*43450.00*~~	~~*16855.50*~~	~~*53114.95*~~			~~*122.2%*~~	
B2	*Standard2*	*43450.00*	*15351.50*	*38139.25*			*87.8%*	
C1 C2	Standard3	10862.50	7460.00	11212.41	421.85	3.8%	103.2%	
C1	*Standard3*	*10862.50*	*7288.00*	*10914.12*			*100.5%*	
C2	*Standard3*	*10862.50*	*7632.00*	*11510.70*			*106.0%*	
D1 D2	Standard4	2715.63	1524.50	2674.39	261.53	9.8%	98.5%	
D1	*Standard4*	*2715.63*	*1391.00*	*2489.46*			*91.7%*	
D2	*Standard4*	*2715.63*	*1658.00*	*2859.32*			*105.3%*	
E1 E2	Standard5	678.91	279.25	714.52	60.12	8.4%	105.2%	
E1	*Standard5*	*678.91*	*258.50*	*672.00*			*99.0%*	
E2	*Standard5*	*678.91*	*300.00*	*757.03*			*111.5%*	
F1 F2	Standard6	169.73	55.00	147.33	0.00	0.0%	86.8%	
F1	*Standard6*	*169.73*	*55.00*	*147.33*			*86.8%*	
~~*F2*~~	~~*Standard6*~~	~~*169.73*~~	~~*95.00*~~	~~*278.65*~~			~~*164.2%*~~	
G1 G2	Standard7	42.43	39.00	79.73	0.00	0.0%	187.9%	
G1	*Standard7*	*42.43*	*39.00*	*79.73*			*187.9%*	
~~*G2*~~	~~*Standard7*~~	~~*42.43*~~	~~*50.50*~~	~~*129.84*~~			~~*306.0%*~~	
H1 H2	Background0	0.00	26.50	-	-	-	-	EXT, ND
H1	*Background0*	*0.00*	*28.00*	-			-	*ND*
H2	*Background0*	*0.00*	*25.00*	-			-	*EXT*

Unknowns							
Location	**Sample ID**	**MFI**	**Result (pg/mL)**	**Result SD**	**Result CV**	**Unk. Dilution**	**Message**
A3	Unknown1	1236.00	226983.46	0.00	0.0%	100.00	
A3	*Unknown1*	*1236.00*	*226983.46*			*100.00*	
B3	Unknown2	1032.00	197320.74	0.00	0.0%	100.00	
B3	*Unknown2*	*1032.00*	*197320.74*			*100.00*	
C3	Unknown3	2211.00	360423.40	0.00	0.0%	100.00	
C3	*Unknown3*	*2211.00*	*360423.40*			*100.00*	

Location	**Sample ID**	**MFI**	**Result (pg/mL)**	**Result SD**	**Result CV**	**Unk. Dilution**	**Message**
D3	Unknown4	7999.00	1217074.88	0.00	0.0%	100.00	
D3	*Unknown4*	*7999.00*	*1217074.88*			*100.00*	
E3	Unknown5	998.00	192273.85	0.00	0.0%	100.00	
E3	*Unknown5*	*998.00*	*192273.85*			*100.00*	
F3	Unknown6	125.50	36348.36	0.00	0.0%	100.00	
F3	*Unknown6*	*125.50*	*36348.36*			*100.00*	
G3	Unknown7	5199.00	765459.78	0.00	0.0%	100.00	

G3	*Unknown7*	*5199.00*	*765459.78*			*100.00*	
H3	Unknown8	6640.50	984289.72	0.00	0.0%	100.00	
H3	*Unknown8*	*6640.50*	*984289.72*			*100.00*	
A4	Unknown9	2651.00	418712.38	0.00	0.0%	100.00	
A4	*Unknown9*	*2651.00*	*418712.38*			*100.00*	
B4	Unknown10	2342.50	377893.99	0.00	0.0%	100.00	
B4	*Unknown10*	*2342.50*	*377893.99*			*100.00*	
C4	Unknown11	2600.00	411974.60	0.00	0.0%	100.00	
C4	*Unknown11*	*2600.00*	*411974.60*			*100.00*	
D4	Unknown12	2380.00	382866.13	0.00	0.0%	100.00	
D4	*Unknown12*	*2380.00*	*382866.13*			*100.00*	
E4	Unknown13	10900.00	1857625.81	0.00	0.0%	100.00	
E4	*Unknown13*	*10900.00*	*1857625.81*			*100.00*	
F4	Unknown14	8381.00	1288611.89	0.00	0.0%	100.00	
F4	*Unknown14*	*8381.00*	*1288611.89*			*100.00*	
G4	Unknown15	4210.00	626871.21	0.00	0.0%	100.00	
G4	*Unknown15*	*4210.00*	*626871.21*			*100.00*	
H4	Unknown16	2173.00	355362.90	0.00	0.0%	100.00	
H4	*Unknown16*	*2173.00*	*355362.90*			*100.00*	
A5	Unknown17	4678.00	691535.38	0.00	0.0%	100.00	
A5	*Unknown17*	*4678.00*	*691535.38*			*100.00*	
B5	Unknown18	915.50	179881.18	0.00	0.0%	100.00	
B5	*Unknown18*	*915.50*	*179881.18*			*100.00*	
C5	Unknown19	8298.00	1272808.56	0.00	0.0%	100.00	
C5	*Unknown19*	*8298.00*	*1272808.56*			*100.00*	
D5	Unknown20	14701.00	3376943.26	0.00	0.0%	100.00	
D5	*Unknown20*	*14701.00*	*3376943.26*			*100.00*	
E5	Unknown21	6621.50	981239.82	0.00	0.0%	100.00	
E5	*Unknown21*	*6621.50*	*981239.82*			*100.00*	
F5	Unknown22	3036.00	469565.57	0.00	0.0%	100.00	
F5	*Unknown22*	*3036.00*	*469565.57*			*100.00*	
G5	Unknown23	2535.00	403383.02	0.00	0.0%	100.00	
G5	*Unknown23*	*2535.00*	*403383.02*			*100.00*	
H5	Unknown24	9942.00	1617719.37	0.00	0.0%	100.00	
H5	*Unknown24*	*9942.00*	*1617719.37*			*100.00*	
A6	Unknown25	6681.00	990807.68	0.00	0.0%	100.00	
A6	*Unknown25*	*6681.00*	*990807.68*			*100.00*	
B6	Unknown26	714.00	148544.81	0.00	0.0%	100.00	
B6	*Unknown26*	*714.00*	*148544.81*			*100.00*	
C6	Unknown27	15087.00	3625581.00	0.00	0.0%	100.00	
C6	*Unknown27*	*15087.00*	*3625581.00*			*100.00*	
D6	Unknown28	10524.00	1759316.42	0.00	0.0%	100.00	
D6	*Unknown28*	*10524.00*	*1759316.42*			*100.00*	
E6	Unknown29	12372.00	2308498.41	0.00	0.0%	100.00	
E6	*Unknown29*	*12372.00*	*2308498.41*			*100.00*	
F6	Unknown30	13012.50	2547603.54	0.00	0.0%	100.00	
F6	*Unknown30*	*13012.50*	*2547603.54*			*100.00*	
G6	Unknown31	7939.50	1206199.31	0.00	0.0%	100.00	
G6	*Unknown31*	*7939.50*	*1206199.31*			*100.00*	
H6	Unknown32	12492.50	2351127.44	0.00	0.0%	100.00	
H6	*Unknown32*	*12492.50*	*2351127.44*			*100.00*	
A7	Unknown33	7.00	-	-	-	100.00	EXT
A7	*Unknown33*	*7.00*	-			*100.00*	*EXT*
B7	Unknown34	7707.00	1164354.08	0.00	0.0%	100.00	
B7	*Unknown34*	*7707.00*	*1164354.08*			*100.00*	
C7	Unknown35	13455.50	2733291.98	0.00	0.0%	100.00	
C7	*Unknown35*	*13455.50*	*2733291.98*			*100.00*	
D7	Unknown36	9849.00	1596171.11	0.00	0.0%	100.00	

D7	*Unknown36*	*9849.00*	*1596171.11*			*100.00*	
E7	Unknown37	12842.00	2480815.19	0.00	0.0%	100.00	
E7	*Unknown37*	*12842.00*	*2480815.19*			*100.00*	
F7	Unknown38	14584.00	3306983.32	0.00	0.0%	100.00	
F7	*Unknown38*	*14584.00*	*3306983.32*			*100.00*	
G7	Unknown39	13550.00	2775388.31	0.00	0.0%	100.00	
G7	*Unknown39*	*13550.00*	*2775388.31*			*100.00*	
H7	Unknown40	14723.50	3390668.53	0.00	0.0%	100.00	
H7	*Unknown40*	*14723.50*	*3390668.53*			*100.00*	

Location	Sample ID	MFI	Result (pg/mL)	Result SD	Result CV	Unk. Dilution	Message
A8	Unknown41	12080.00	2209257.42	0.00	0.0%	100.00	
A8	*Unknown41*	*12080.00*	*2209257.42*			*100.00*	
B8	Unknown42	6934.00	1032058.57	0.00	0.0%	100.00	
B8	*Unknown42*	*6934.00*	*1032058.57*			*100.00*	
C8	Unknown43	13779.00	2881406.15	0.00	0.0%	100.00	
C8	*Unknown43*	*13779.00*	*2881406.15*			*100.00*	
D8	Unknown44	14563.00	3294670.74	0.00	0.0%	100.00	
D8	*Unknown44*	*14563.00*	*3294670.74*			*100.00*	
E8	Unknown45	15443.00	3882942.79	0.00	0.0%	100.00	
E8	*Unknown45*	*15443.00*	*3882942.79*			*100.00*	
F8	Unknown46	8744.00	1359534.08	0.00	0.0%	100.00	
F8	*Unknown46*	*8744.00*	*1359534.08*			*100.00*	
G8	Unknown47	12277.00	2275595.78	0.00	0.0%	100.00	
G8	*Unknown47*	*12277.00*	*2275595.78*			*100.00*	
H8	Unknown48	4676.00	691255.80	0.00	0.0%	100.00	
H8	*Unknown48*	*4676.00*	*691255.80*			*100.00*	
A9	Unknown49	4580.00	677870.22	0.00	0.0%	100.00	
A9	*Unknown49*	*4580.00*	*677870.22*			*100.00*	
B9	Unknown50	12661.00	2412491.22	0.00	0.0%	100.00	
B9	*Unknown50*	*12661.00*	*2412491.22*			*100.00*	
C9	Unknown51	10451.00	1740881.30	0.00	0.0%	100.00	
C9	*Unknown51*	*10451.00*	*1740881.30*			*100.00*	
D9	Unknown52	5904.00	869514.98	0.00	0.0%	100.00	
D9	*Unknown52*	*5904.00*	*869514.98*			*100.00*	
E9	Unknown53	14937.50	3525844.52	0.00	0.0%	100.00	
E9	*Unknown53*	*14937.50*	*3525844.52*			*100.00*	
F9	Unknown54	12410.00	2321831.94	0.00	0.0%	100.00	
F9	*Unknown54*	*12410.00*	*2321831.94*			*100.00*	
G9	Unknown55	12595.00	2388205.52	0.00	0.0%	100.00	
G9	*Unknown55*	*12595.00*	*2388205.52*			*100.00*	
H9	Unknown56	7718.00	1166311.01	0.00	0.0%	100.00	
H9	*Unknown56*	*7718.00*	*1166311.01*			*100.00*	
A10	Unknown57	7346.00	1101328.73	0.00	0.0%	100.00	
A10	*Unknown57*	*7346.00*	*1101328.73*			*100.00*	
B10	Unknown58	10947.50	1870464.74	0.00	0.0%	100.00	
B10	*Unknown58*	*10947.50*	*1870464.74*			*100.00*	
C10	Unknown59	15503.50	3929752.00	0.00	0.0%	100.00	
C10	*Unknown59*	*15503.50*	*3929752.00*			*100.00*	
D10	Unknown60	7691.00	1161511.59	0.00	0.0%	100.00	
D10	*Unknown60*	*7691.00*	*1161511.59*			*100.00*	
E10	Unknown61	10450.50	1740755.73	0.00	0.0%	100.00	
E10	*Unknown61*	*10450.50*	*1740755.73*			*100.00*	
F10	Unknown62	7692.00	1161689.11	0.00	0.0%	100.00	
F10	*Unknown62*	*7692.00*	*1161689.11*			*100.00*	
G10	Unknown63	8815.00	1373765.20	0.00	0.0%	100.00	
G10	*Unknown63*	*8815.00*	*1373765.20*			*100.00*	
H10	Unknown64	9362.00	1487694.70	0.00	0.0%	100.00	
H10	*Unknown64*	*9362.00*	*1487694.70*			*100.00*	
A11	Unknown65	12036.00	2194776.47	0.00	0.0%	100.00	
A11	*Unknown65*	*12036.00*	*2194776.47*			*100.00*	
B11	Unknown66	3470.50	527202.78	0.00	0.0%	100.00	
B11	*Unknown66*	*3470.50*	*527202.78*			*100.00*	
C11	Unknown67	8239.50	1261758.54	0.00	0.0%	100.00	
C11	*Unknown67*	*8239.50*	*1261758.54*			*100.00*	
D11	Unknown68	12440.00	2332429.32	0.00	0.0%	100.00	
D11	*Unknown68*	*12440.00*	*2332429.32*			*100.00*	
E11	Unknown69	6381.00	943059.45	0.00	0.0%	100.00	
E11	*Unknown69*	*6381.00*	*943059.45*			*100.00*	

F11	Unknown70	13226.00	2634795.75	0.00	0.0%	100.00	
F11	*Unknown70*	*13226.00*	*2634795.75*			*100.00*	
G11	Unknown71	1163.50	216552.31	0.00	0.0%	100.00	
G11	*Unknown71*	*1163.50*	*216552.31*			*100.00*	
H11	Unknown72	10914.00	1861399.76	0.00	0.0%	100.00	
H11	*Unknown72*	*10914.00*	*1861399.76*			*100.00*	
A12	Unknown73	7558.00	1138062.94	0.00	0.0%	100.00	
A12	*Unknown73*	*7558.00*	*1138062.94*			*100.00*	
B12	Unknown74	10196.50	1678160.00	0.00	0.0%	100.00	
B12	*Unknown74*	*10196.50*	*1678160.00*			*100.00*	
C12	Unknown75	12396.50	2317083.59	0.00	0.0%	100.00	
C12	*Unknown75*	*12396.50*	*2317083.59*			*100.00*	
D12	Unknown76	11464.00	2016740.77	0.00	0.0%	100.00	
D12	*Unknown76*	*11464.00*	*2016740.77*			*100.00*	
E12	Unknown77	4068.00	607523.82	0.00	0.0%	100.00	
E12	*Unknown77*	*4068.00*	*607523.82*			*100.00*	

Location	Sample ID	MFI	Result (pg/mL)	Result SD	Result CV	Unk. Dilution Message
F12	Unknown78	9024.00	1416377.64	0.00	0.0%	100.00
F12	*Unknown78*	*9024.00*	*1416377.64*			*100.00*
G12	Unknown79	418.00	98244.74	0.00	0.0%	100.00
G12	*Unknown79*	*418.00*	*98244.74*			*100.00*
H12	Unknown80	397.00	94376.68	0.00	0.0%	100.00
H12	*Unknown80*	*397.00*	*94376.68*			*100.00*

The following records were excluded from the analyzed results

Plate	Location	Sample ID Reason
Plate 1	A2	Standard1
Plate 1	B1	Standard2
Plate 1	F2	Standard6
Plate 1	G2	Standard7

Quality assurance procedure for run acceptance

The following steps were observed to formally validate and accept data using the ProcartaPlex Analysis App and Luminex software:

- Instrument system suitability was verified: the instrument (Luminex 200, FLEXMAP 3D, INTELLIFLEX) passed its daily calibration and fluidic verification checks.
- Ensured the DD (Doublet Discriminator) gate was correctly set to 7,500 – 25,000 for standard magnetic bead assays.
- Inspected the 7-Point Standard Curve: fit the standard data using a 4PL (4-Parameter Logistic) or 5PL curve algorithm. The curve must present a clear monotonic sigmoidal shape with an R^2

value close to 1.0.

- Assess Standard Recovery: ensured individual standard concentrations back-calculate precisely to their nominal values within the 80–120% tolerance window.
- Checked Regional Bead Populations: Confirm that the instrument successfully counted ≥ 50 beads per region inside every target well.
- Confirmed Replicate Reproducibility: ensured the %CV across duplicate wells is strictly <15%.

APPENDIX D: RAW DATA

Table 1: Raw data (control group)

Control							
Demographics				**Assay results Concentration**			
Study No	Date	Age	Gender	**GFAP conc (pg/ml)**	**S100B (pg/ml)**	**NSE (ng/ml)**	**SAA (ng/ml)**
1	5-May-24	23	1	1.79	0.19	356.344	226.983
2	5-May-24	22	2	2.6	0.23	26.223	197.321
3	5-May-24	22	2	1.79	0.23	142.841	360.423
4	5-May-24	21	2	1.79	0.19	75.874	1217.075
5	5-May-24	21	2	1.79	0.23	141.451	192.274
6	5-May-24	21	2	1.79	0.19	139.115	36.348
7	5-May-24	24	2	1.79	0.23	205.012	765.46
8	5-May-24	23	2	1.79	0.19	115.533	984.29
9	5-May-24	23	1	1.79	0.2	113.411	418.712
10	5-May-24	39	2	8.15	0.19	92.733	377.894
11	5-May-24	20	2	2.6	0.19	96.024	411.975
12	5-May-24	21	1	1.79	0.19	350.043	382.866
13	5-May-24	32	2	1.79	0.19	209.861	148.545
14	5-May-24	25	1	1.79	0.19	158.265	1288.612
15	5-May-24	18	1	1.79	0.19	204.827	626.871
16	5-May-24	22	1	1.79	0.19	57.464	355.363
17	5-May-24	23	1	1.79	0.19	32.722	691.535
18	5-May-24	21	1	1.79	0.19	517.984	179.881
19	5-May-24	22	2	2.6	0.23	189.044	1272.809
20	5-May-24	21	2	8.15	0.23	56.026	3376.943
21	5-May-24	22	2	1.79	0.23	114.077	981.24
22	5-May-24	20	1	8.15	0.19	59.281	469.566
23	5-May-24	24	1	1.79	0.19	61.456	403.383
24	5-May-24	22	2	1.79	0.23	81.078	1617.719
25	5-May-24	21	2	1.79	0.2	116.322	990.808

Table 2: Raw data (moderate group)

TBI Moderate												
			Clinical Data			Demographics		Patients' outcome	Assay results Concentration			
Study No	Admission Date	Causes/History	GCS	Focal or diffuse	Type of injury	Age	Sex		GFAP (pg/ml)	S100B (pg/ml)	NSE (ng/ml)	SAA (ng/ml)
1	25-09-2023	4	12	Focal	2	41	1	2	6635.69	0.59	866.834	3625.581
2	19-10-2023	2	12	Focal	2	57	1	1	166.76	1.84	3343.83	1759.316
3	29-10-2023	1	13	Focal	1	33	1	1	36.72	0.23	478.704	2308.498
4	4-Nov-23	2	11	Focal	1	56	1	1	36.72	0.23	2415.244	2547.604
5	4-Nov-23	1	12	Diffuse	1	29	1	1	36.72	0.23	2482.158	1206.199
6	5-Nov-23	1	13	Diffuse	1	26	1	1	36.72	0.23	16.792	2351.127
7	5-Nov-23	1	13	Focal	1	25	1	1	211.89	0.23	863.726	1164.354
8	7-Nov-23	4	10	Focal	1	20	1	2	6920.71	0.4	526.825	2733.292
9	21-Nov-23	4	13	Focal	2	32	1	2	1742.18	0.4	272.831	1596.171
10	12-Feb-24	4	10	Focal	2	47	1	1	14.77	0.59	3420.943	2480.815
11	10-Feb-24	3	13	Diffuse	1	63	1	1	910.42	1.84	295.606	3306.983
12	3-Dec-23	4	12	Focal	2	29	1	1	121.28	0.86	233.305	2775.388
13	3-Dec-23	1	12	Diffuse	1	18	1	1	747.37	1.84	4606.364	3390.669
14	6-Dec-23	2	13	Diffuse	2	36	1	1	2.6	0.23	3545.94	2209.257
15	7-Dec-23	1	12	Focal	1	23	1	1	2437.33	1.84	141.219	1032.059
16	7-Dec-23	1	13	Focal	2	24	1	1	36.72	0.23	1060.342	2881.406
17	7-Dec-23	1	13	Diffuse	2	43	1	1	36.72	0.4	15.439	3294.671
18	10-Dec-23	1	13	Focal	1	42	1	1	2.59	0.23	816.052	3882.943
19	28-Dec-23	2	13	Focal	1	41	2	1	90.68	0.2	4398.284	1359.534
20	5-Feb-24	1	12	Focal	3	22	1	1	1895.98	1.77	2675.188	2275.596
21	5-Feb-24	1	13	Diffuse	1	18	1	1	3569.65	0.74	764.706	691.256
22	15-Feb-24	3	12	Diffuse	3	57	1	2	155.43	0.23	3159.257	677.87
23	15-Feb-24	1	12	Focal	1	16	1	1	36.87	1.45	969.106	2412.491
24	11-Feb-24	1	13	Focal	2	33	1	1	178.07	0.4	341.062	1740.881
25	11-Feb-24	1	12	Diffuse	2	40	1	1	2060.58	0.27	342.335	869.515

Table 3: Raw data (severe group)

TBI severe												
		Clinical Data				Demographics		Patient outcome	Assay results concentration			
Study No	Admission Date	Causes/History	GCS	Focal or Diffuse	Type of injury	Age	Sex		GFAP (pg/ml)	S100B (pg/ml)	NSE (ng/ml)	SAA (ng/ml)
1	24-Oct-2023	3	7	Focal	2	52	1	2	1075.5	0.23	149.669	3525.845
2	5-Nov-23	1	8	Focal	1	33	1	2	36.72	0.4	626.78	2321.832
3	3-Dec-23	3	7	Focal	1	19	1	1	106.01	0.4	605.402	2388.206
4	10-Dec-23	4	7	Focal	2	30	1	1	1574.07	1.84	160.422	1166.311
5	3-Mar-24	1	5	Focal	2	30	1	1	3533.64	0.23	196.609	1101.329
6	16-Mar-24	2	7	Diffuse	2	25	1	1	6782.7	0.4	629.895	1870.465
7	3-Apr-24	1	8	Focal	1	26	1	1	4471.89	0.59	809.472	3929.752
8	4-Apr-24	1	7	Focal	3	29	1	1	5450.58	0.98	262.057	1161.512
9	29-Dec-23	2	7	Focal	2	23	1	1	3239.73	0.28	819.252	1740.756
10	27-Dec-23	1	6	Diffuse	1	40	1	2	910.42	0.23	542.631	1161.689
11	1-Jan-24	1	8	Focal	2	32	1	1	21.91	0.23	969.402	1373.765
12	2-Apr-24	1	4	Focal	1	34	1	1	14509.23	1.61	827.805	1487.695
13	7-Apr-24	2	4	Focal	2	30	1	1	1652.38	1.84	5044.289	2194.776
14	11-Jul-24	4	8	Focal	2	51	1	1	24592.57	0.92	626.597	1416.378
15	11-Jul-24	4	8	Diffuse	2	43	1	1	10895.38	1.84	3056.274	1261.759
16	11-Jul-24	4	3	Focal	2	53	1	1	4380.54	0.23	323.541	2332.429
17	29-Jul-24	1	3	Diffuse	2	19	1	1	117.46	0.2	1007.778	943.059
18	29-Jul-24	1	8	Focal	2	27	1	1	31144.48	1.84	910.544	2634.796
19	29-Jul-24	1	7	Focal	2	35	1	2	136.49	0.23	498.004	216.552
20	31-Jul-24	1	8	Focal	1	21	1	1	12283.51	0.92	1045.021	1861.34
21	13-Aug-24	2	3	Focal	2	21	1	1	36.72	1.84	1040.52	1138.063
22	8-Sep-24	3	7	Focal	2	21	1	1	5775.38	0.59	115.269	1678.16
23	8-Nov-2024	3	8	Focal	1	59	1	1	36.72	1.84	344.739	2317.084
24	27-Jul-24	2	8	Diffuse	1	66	2	1	12958.09	1.91	3092.343	2016.741
25	16-May-24	4	8	Focal	2	82	2	1	8.15	1.84	602.726	607.524

Keys:

Interpersonal violence Assault (1)	Focal (1)	Extradural hematoma (1)	Male (1)	Discharged (1)
MVA Accident (2)	Diffuse (2)	Subdural hematoma (2)	Female (2)	Passed away (2)
Fall (3)		Subarachnoid hemorrhage (3)		
Other causes (4)				

APPENDIX E: SCANS

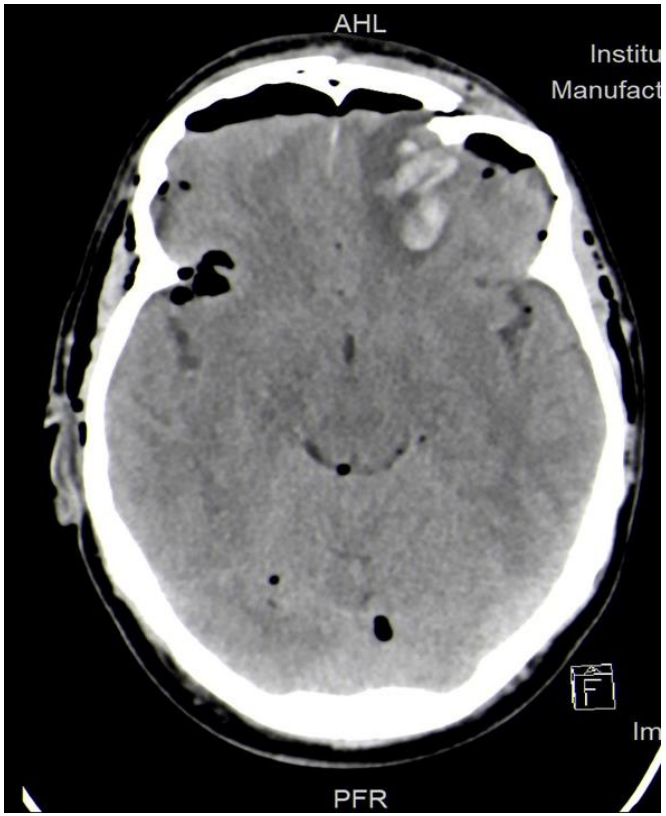
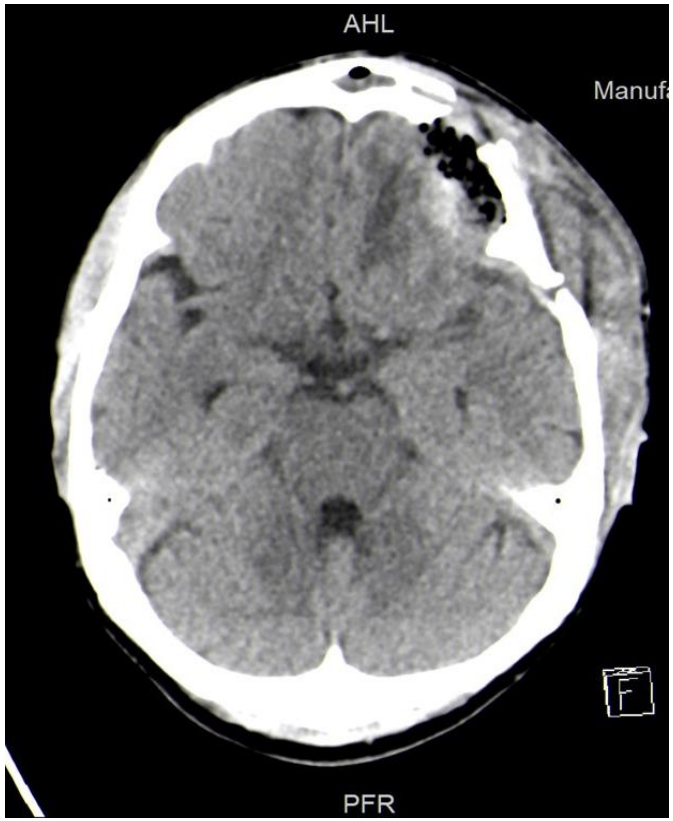
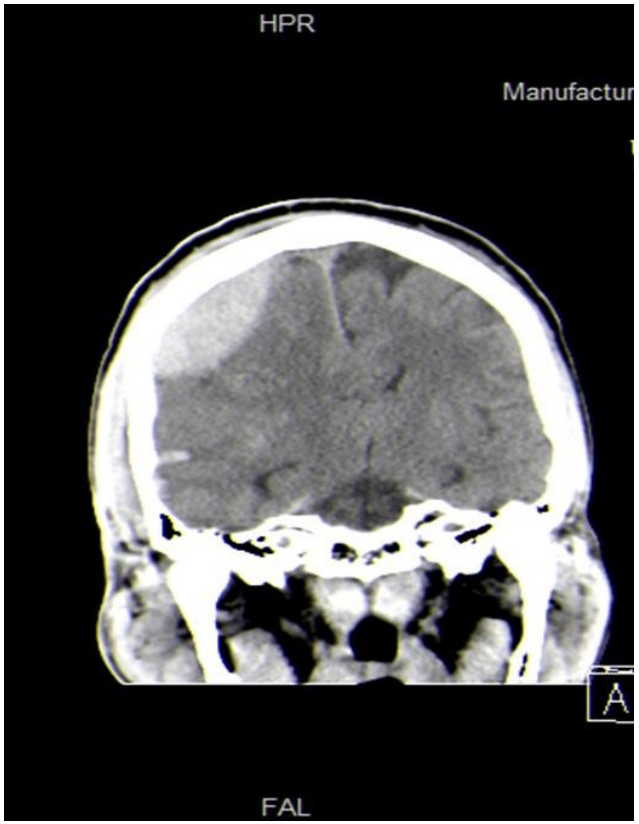
A	B	C
		
Clinical: GCS score: 11	**Clinical: GCS score**: 10	**Clinical: GCS score**: 12
Biomarker: S100B concentration: 1.84 pg/mL **GFAP concentration:** 166.76 pg/mL **NSE concentration**: 478.7 ng/mL **SAA concentration**: 1759.32 ng/mL	**Biomarker: S100B concentration:** 0.23 pg/mL **GFAP concentration:** 211.89 pg/mL **NSE concentration**: 863.72 ng/mL **SAA concentration**: 1164.35 ng/mL	**Biomarker: S100B concentration:** 0.86 pg/mL **GFAP concentration:** 121.28 pg/mL **NSE concentration**: 233.30 ng/mL **SAA concentration**: 2775.38 ng/mL

Figure 1: Additional TBI Classification System with biomarkers incorporated (moderate TBI case)

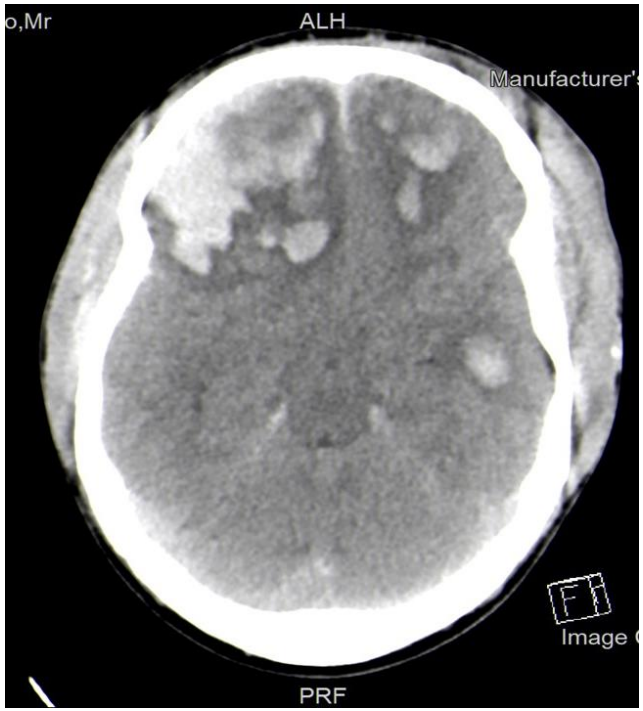
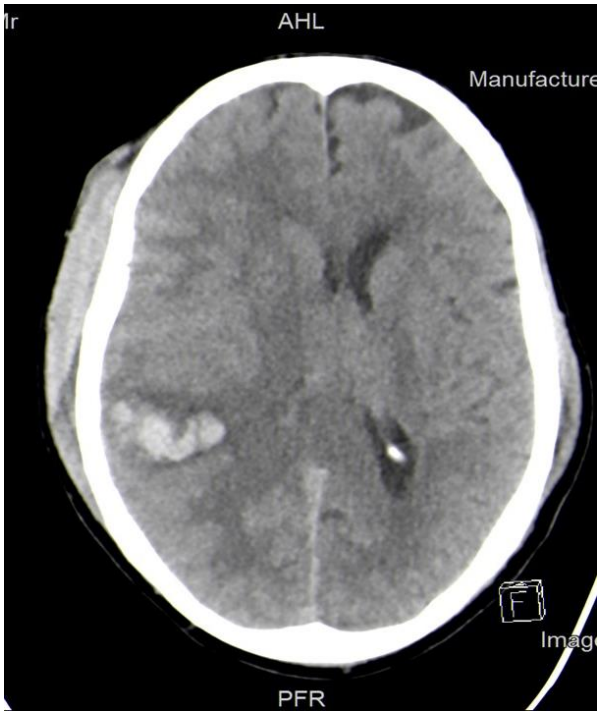
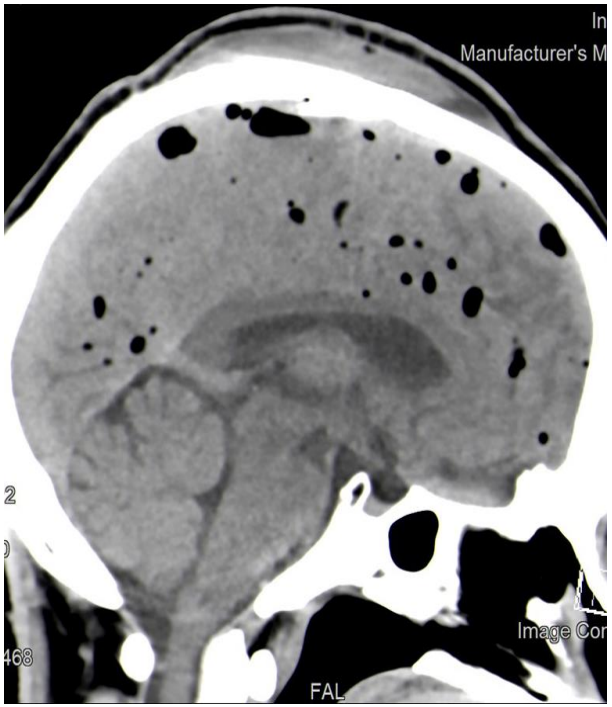
D	E	F
		
Clinical: GCS score: 10	**Clinical: GCS score**: 11	**Clinical: GCS score**: 11
Biomarker: S100B concentration: 0.23 pg/mL **GFAP concentration:** 36.72 pg/mL **NSE concentration**: 1060.34 ng/mL **SAA concentration**: 2881.40 ng/mL	**Biomarker: S100B concentration:** 0.2 pg/mL **GFAP concentration:** 90.68 pg/mL **NSE concentration**: 4398.28 ng/mL **SAA concentration**: 1359.53 ng/mL	**Biomarker: S100B concentration:** 1.45 pg/mL **GFAP concentration:** 36.87 pg/mL **NSE concentration**: 969.1 ng/mL **SAA concentration**: 2412.49 ng/mL

Figure 2: Additional TBI Classification System with biomarkers incorporated (moderate TBI case)

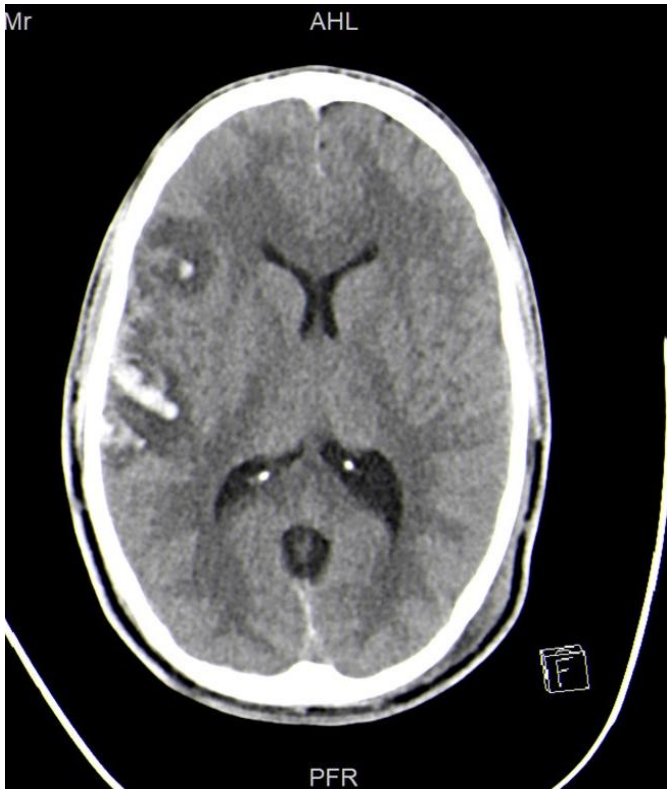
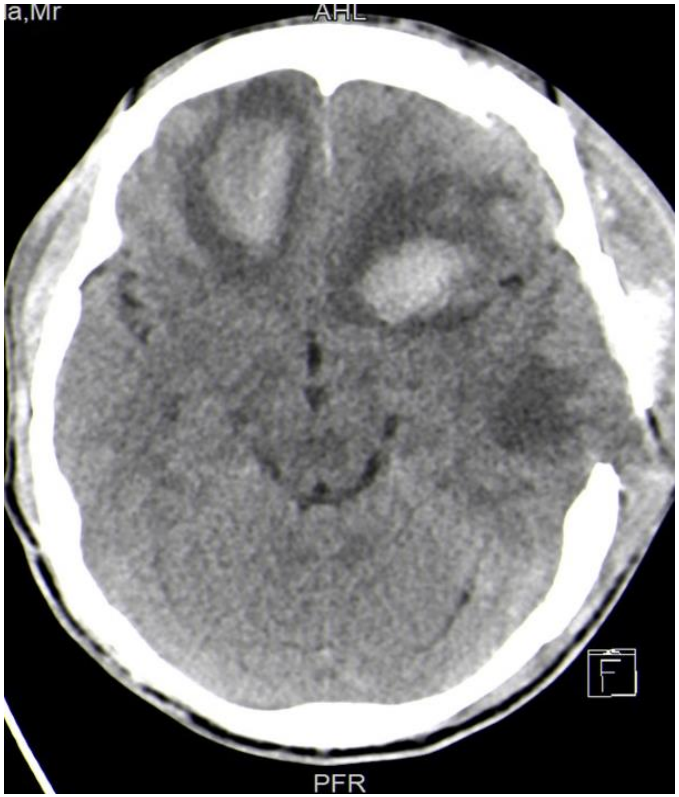
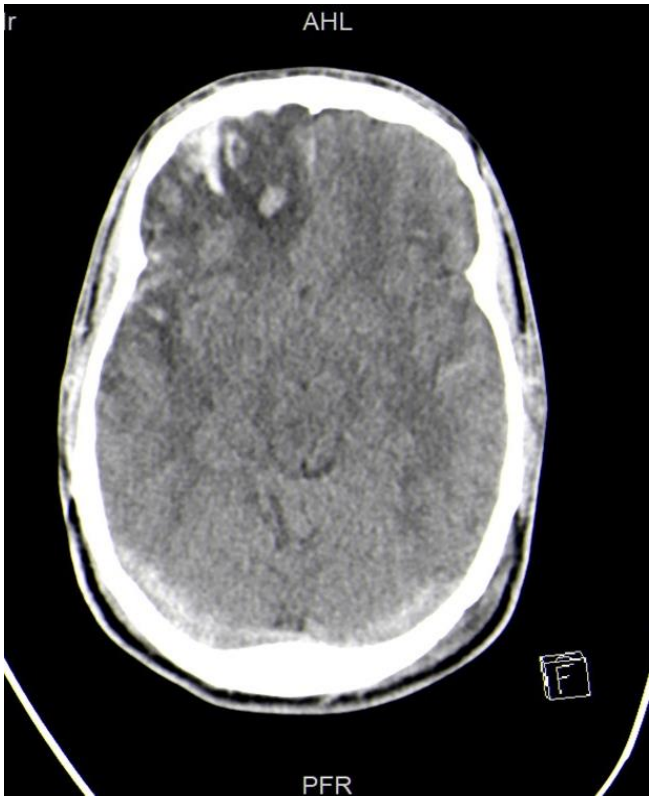
A	B	C
		
Clinical: GCS score: 4	**Clinical: GCS score**: 8	**Clinical: GCS score**: 5
Biomarker: S100B concentration: 0.59 pg/mL **GFAP concentration:** 4471.89 pg/mL **NSE concentration**: 809.47 ng/mL **SAA concentration**: 3929.75 ng/mL	**Biomarker: S100B concentration:** 1.61 pg/mL **GFAP concentration:** 14509.23 pg/mL **NSE concentration**: 827.80 ng/mL **SAA concentration**: 1487.69 ng/mL	**Biomarker: S100B concentration:** 1.84 pg/mL **GFAP concentration:** 1652.38 pg/mL **NSE concentration**: 5044.28 ng/mL **SAA concentration**: 2194 ng/mL

Figure 3: Additional TBI Classification System with biomarkers incorporated (severe TBI case)

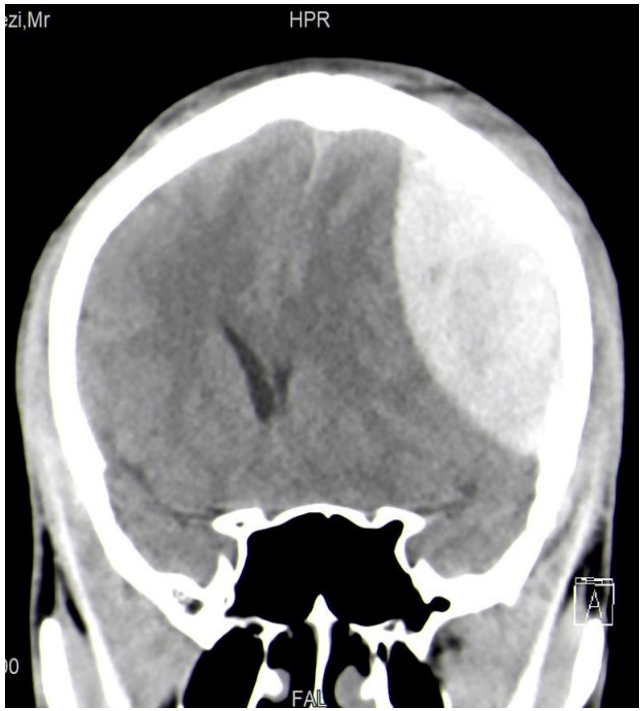
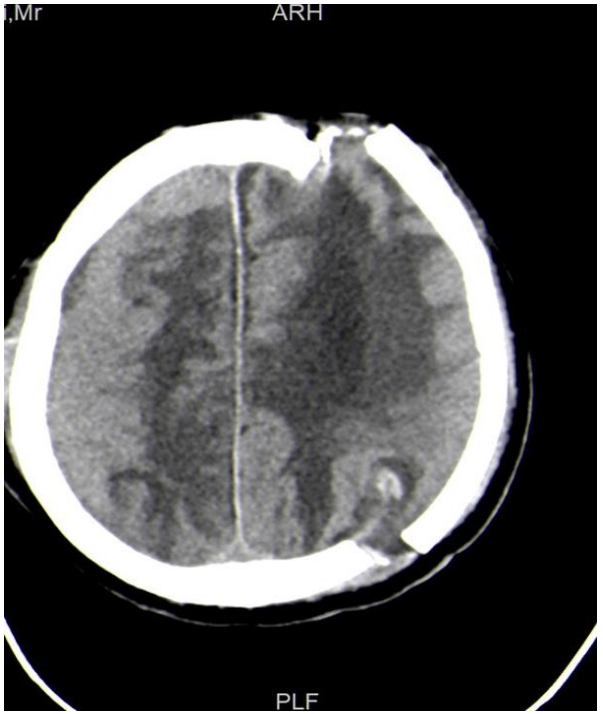
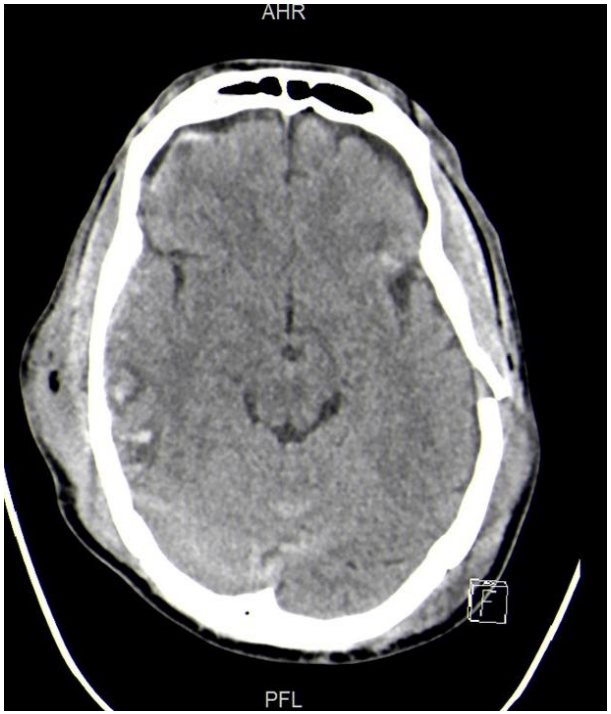
D	E	F
		
Clinical: GCS score: 8	**Clinical: GCS score**: 7	**Clinical: GCS score**: 8
Biomarker: S100B concentration: 1.91 pg/mL **GFAP concentration:** 12958.09 pg/mL **NSE concentration**: 3092.34 ng/mL **SAA concentration**: 2016.74 ng/mL	**Biomarker: S100B concentration:** 0.59 pg/mL **GFAP concentration:** 4471.89 pg/mL **NSE concentration**: 115.269 ng/mL **SAA concentration**: 1678.16 ng/mL	**Biomarker: S100B concentration:** 0.92 pg/mL **GFAP concentration:** 12283.51 pg/mL **NSE concentration**: 1045.02 ng/mL **SAA concentration**: 1861.34 ng/mL

Figure 4: Additional TBI Classification System with biomarkers incorporated (severe TBI case)