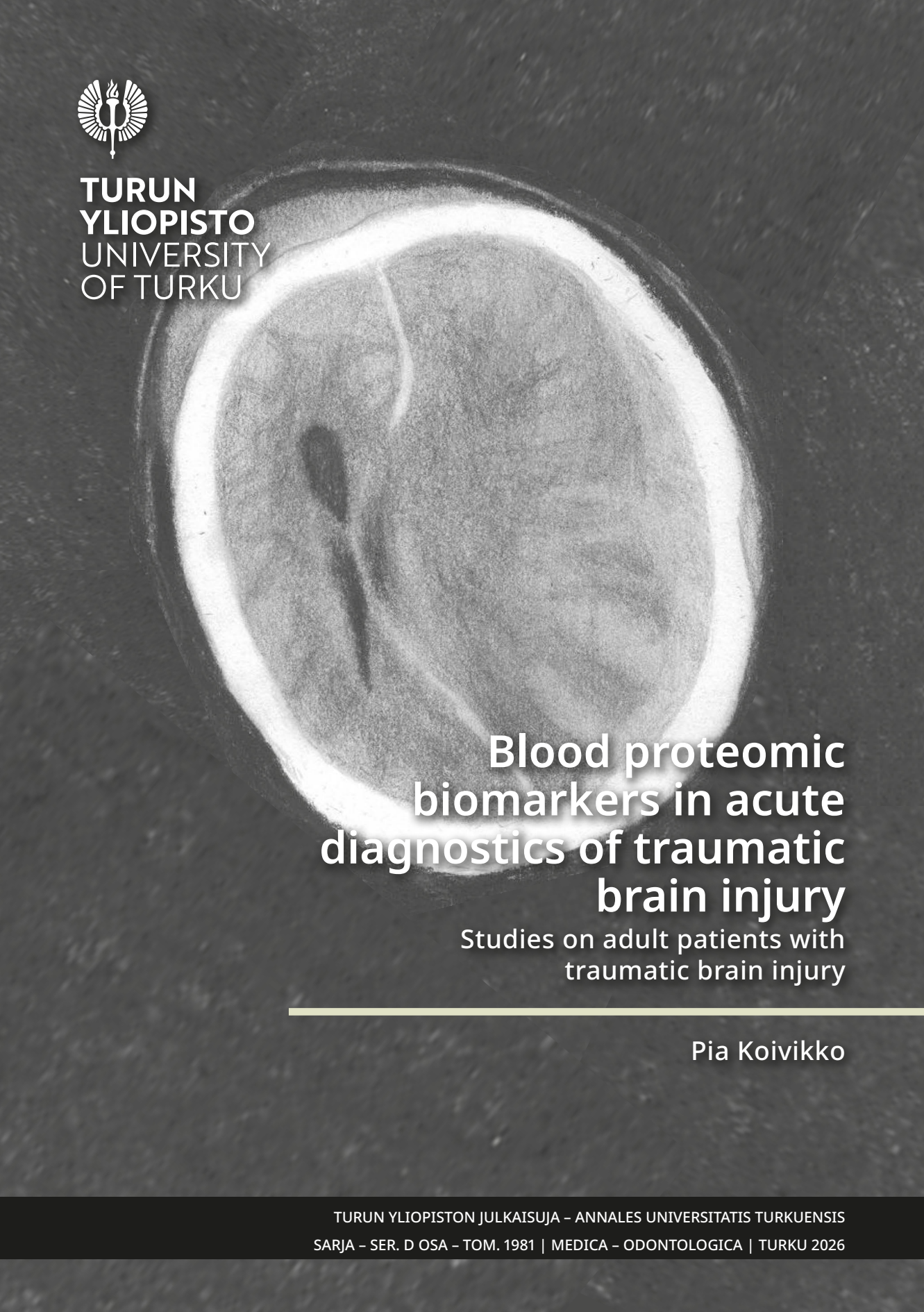

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UNIVERSITY OF TURKU

Blood proteomic biomarkers in acute diagnostics of traumatic brain injury

Studies on adult patients with traumatic brain injury

Pia Koivikko

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To my family,
who were convinced this project
would never be finished.
Now it is.

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ABSTRACT

Traumatic brain injury (TBI) is a leading cause of death and disability worldwide; in more severe cases, it may require long-term intensive care treatment and rehabilitation for survivors. Diagnosis has long been based on clinical signs, the Glasgow Coma Scale (GCS), and head computed tomography (CT) scanning. TBI is a highly complex condition affecting several structures; however, the GCS score does not account for the underlying pathophysiological injuries in TBI. In addition, CT findings may be absent in mild TBI (mTBI), the most common form of TBI. This study examined the use of blood biomarkers [β-amyloids 1–40 (Aβ40) and 1–42 (Aβ42), glial fibrillary acidic protein (GFAP), heart fatty acid-binding protein (H-FABP), interleukin-10 (IL-10), neurofilament light (NfL), S100 calcium-binding protein B (S100B), and total tau (t-tau)] in TBI research. In the first study, the ability of single biomarkers and their combinations to discriminate between different severities of TBI was examined, along with their capacity to distinguish between mTBI and orthopaedic controls. The ability of the biomarkers to distinguish between different lesion combinations observed on head CT scans was examined in the second study. In the third study, the association between biomarker outliers and clinically significant events and outcomes in ICU-treated patients was evaluated. In the first study, the results indicated that the biomarkers could distinguish mTBI from more severe forms but could not differentiate between mTBI and the orthopaedic controls. In the second study, the biomarkers identified the most severely injured patients, but the sample size was too small to draw conclusions about their ability to differentiate between lesion combinations. In the third study, biomarker outliers appeared to be associated with epileptic seizures/status epilepticus and decompressive hemicraniectomy; however, this finding may be attributable to more severe TBI.

KEYWORDS: Acute diagnostics, blood biomarkers, computed tomography, severity assessment, traumatic brain injury

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TIIVISTELMÄ

Tapaturmainen aivovamma on maailmanlaajuisesti merkittävä kuolinsyy, joka saattaa aiheuttaa eloon jääville pysyviä vaurioita sekä vaikeampien vammojen jälkeen pitkiä tehohoito- ja kuntoutusjaksoja. Diagnostiikan kulmakivinä ovat pitkään olleet kliiniset oireet, Glasgow Coma Scale (GCS) ja pään tietokonekuvantaminen. Tapaturmainen aivovamma on monimutkainen vamma ja vaikuttaa useisiin aivojen rakenteisiin, mutta GCS ei huomioi vammojen patofysiologista taustaa. Suurin osa aivovammoista on lieviä, jolloin myöskään pään tietokonekuvantamisessa ei välttämättä ole löydöksiä. Väitöstutkimuksessa tutkittiin veren biomarkkereiden [β-amyloidit 1-40 (Aβ40) ja 1-42 (Aβ42), GFAP, H-FABP, interleukiini-10 (IL-10), NfL, S100B, t-tau] käyttöä aivovammatutkimuksessa. Ensimmäisessä osatyössä tutkittiin sekä yksittäisten biomarkkereiden että niiden yhdistelmien kykyä erottaa vammojen eri vakavuusasteet sekä kykyä erottaa lievät aivovammapotilaat ortopedisista kontrollipotilaista. Toisessa osatyössä analysoitiin biomarkkereiden kykyä erottaa erilaisia vammakombinaatioita vertaamalla biomarkkeritasoja pään tietokonekuviin. Kolmannessa osatyössä tutkittiin teho-osastolla hoidettujen aivovammapotilaiden biomarkkeritason poikkeamia ja niiden yhteyttä kliinisesti merkittäviin tapahtumiin sekä toipumiseen. Ensimmäisen osatyön tuloksena oli, että biomarkkereilla pystyttiin erottamaan lievät aivovammat vaikeammista, mutta lieviä aivovammoja ei pystytty erottamaan ortopedisista kontrollipotilaista. Toisessa osatyössä biomarkkerit pystyivät erottamaan vaikeimmin vammautuneet potilaat, mutta biomarkkerien kykyä erottaa eri leesioita ei voitu arvioida riittämättömän potilasmäärän vuoksi. Kolmannen osatyön poikkeavilla biomarkkeritasoilla vaikutti olevan yhteys epileptisiin kohtauksiin/status epileptikukseen sekä dekompressiiviseen hemikraniektomiaan, mutta löydös saattaa johtua potilaiden vakavammasta aivovammasta.

AVAINSANAT: Akuuttidiagnostiikka, kuvantaminen, tapaturmainen aivovamma, vakavuuden arviointi, veren biomarkkerit

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Abbreviations

Aβ40	β-amyloid 1–40
Aβ42	β-amyloid 1–42
APP	β-amyloid precursor protein
AUC-ROC	Area Under the Receiver Operating Characteristic Curve
CBI-M	Clinical, biomarker, imaging, and modifier
CDEs	Common Data Elements
CE	Conformité Européenne (European Conformity)
CL1	Cluster 1
CL2	Cluster 2
CL3	Cluster 3
CNS	Central nervous system
CRASH	Corticoid Randomization after Significant Head Injury
CT	Computed tomography
CT-neg	CT-negative cluster
CT-pos	CT-positive cluster
DAI	Diffuse axonal injury
DOACs	Direct-acting oral anticoagulants
DW-MRI	Diffusion-weighted magnetic resonance imaging
ECMO	Extracorporeal membrane oxygenation
ED	Emergency Department
EDH	Epidural haematoma
EEG	Electroencephalogram
FDA	US Food and Drug Administration
GCS	Glasgow Coma Scale
GFAP	Glial fibrillary acidic protein
GOSE	Glasgow Outcome Scale extended
HCTS	Helsinki CT score
HDI	Human development index
H-FABP	Heart fatty acid-binding protein
ICH	Intracerebral haematoma
ICP	Intracranial pressure

ICU	Intensive care unit
IMPACT	International Mission for Prognosis and Analysis of Clinical Trials
IL-10	Interleukin-10
IQR	Interquartile range
ISS	Injury Severity Score
IVH	Intraventricular haemorrhage
mTBI	Mild traumatic brain injury
moTBI	Moderate traumatic brain injury
MRI	Magnetic resonance imaging
NfL	Neurofilament light
NICE	National Institute for Health and Care Excellence
NIH-NINDS	National Institutes of Health–National Institute of Neurological Disorders and Stroke
N4PA	Human Neurology 4-Plex A
NSE	Neuron-specific enolase
pAUC	Partial area under the ROC curve
PTA	Post-traumatic amnesia
OHCA	Out-of-hospital cardiac arrest
S100B	S100 calcium-binding protein B
SAH	Subarachnoid haemorrhage
SD	Standard deviation
SDH	Subdural haematoma
SE	Status epilepticus
sTBI	Severe traumatic brain injury
TBI	Traumatic brain injury
tICH	Traumatic intracerebral haematoma
tSAH	Traumatic subarachnoid haemorrhage
tSDH	Traumatic subdural haematoma
t-tau	Total tau
UCH-L1	Ubiquitin C-terminal hydrolase-L1

List of Original Publications

This dissertation is based on the following original publications, which are referred to in the text by their Roman numerals:

I Koivikko P, Posti JP, Mohammadian M, Lagerstedt L, Azurmendi L, Hossain I, Katila AJ, Menon DK, Newcombe VF, Hutchinson P, Maanpää HR, Tallus J, Zetterberg H, Blennow K, Tenovuo O, Sanchez JC, Takala RSK. Potential of heart fatty-acid binding protein, neurofilament light, interleukin-10 and S100 calcium-binding protein B in the acute diagnostics and severity assessment of traumatic brain injury. *Emergency Medicine Journal*, 2022; Mar 39(3):206–212.

II Koivikko P, Katila AJ, Takala RSK, Hossain I, Luoto TM, Raj R, Koivisto M, Tenovuo O, Blennow K, Hutchinson P, Maanpää HR, Mohammadian M, Newcombe VF, Sanchez JC, Tallus J, van Gils M, Zetterberg H, Posti JP. Blood biomarkers to identify patients with different intracranial lesion combinations after traumatic brain injury. *Brain and Spine*, 2025; Jan 31(5):104195.

III Koivikko P, Posti JP, Hossain I, Tenovuo O, Hutchinson P, Katila AJ, Maanpää HR, Menon DK, Newcombe VF, Sanchez JC, Tallus J, van Gils M, Zetterberg H, Takala RSK. Blood biomarker outliers and clinical events during intensive care in patients with traumatic brain injury. *Brain and Spine, 2026;* May 1(6):106078.

1 Introduction

Traumatic brain injury (TBI) is a problem affecting millions of people worldwide. While most TBIs are mild and patients generally recover well, more severe forms have high mortality rates and require intensive care. Rehabilitation periods can be long, and the loss of work years is substantial. Survivors often suffer from a range of neurophysiological problems. Half the world's population is estimated to experience TBI at least once (Maas et al., 2017).

The diagnosis of TBI remains challenging, as it is based on clinical neurological signs, the Glasgow Coma Scale (GCS), and a head computed tomography (CT) scan. Clinical severity has typically been assessed using the GCS. Mild TBI (mTBI), the most common form of TBI, is hard to diagnose in cases where neurological symptoms are minimal and CT findings are negative. As CT scans may not contribute meaningfully to diagnosis, patients may receive unnecessary radiation. In moderate TBI (moTBI) or severe TBI (sTBI), the neurological signs and CT scan findings are more pronounced, making diagnosis easier (Korhonen et al., 2024).

However, TBI is a complex injury, and different pathophysiological mechanisms may cause similar neurological signs; GCS alone does not provide sufficient guidance for treatment (Godoy et al., 2024). International collaborations have been initiated to address this issue, and approaches to characterising TBI have been proposed to support more individualised methods where GCS, along with pupillary reactivity, is still a component, together with imaging methods, blood biomarker analysis, and psychosocial and environmental modifiers. However, substantial further research is needed to achieve this goal (Manley et al., 2025).

A major challenge in TBI research is the heterogeneity of the injuries (Richter et al., 2023). Blood biomarkers released from different brain structures have been studied to aid diagnosis and treatment decisions (Lagerstedt et al., 2018a), as they have shown potential to add value to clinical variables and imaging results (Posti & Tenovuo, 2022).

This thesis investigated eight blood biomarkers: β-amyloid 1–40 (Aβ40), β-amyloid 1–42 (Aβ42), glial fibrillary acidic protein (GFAP), heart fatty acid-binding

protein (H-FABP), interleukin-10 (IL-10), neurofilament light (NfL), S100 calcium-binding protein B (S100B), and total tau (t-tau), in all severities of TBI. Their utility in acute diagnostics, severity assessment, monitoring during intensive care unit (ICU) treatment, and in prognosis was assessed.

2 Review of the Literature

2.1 Traumatic brain injury

2.1.1 Definition

"TBI is defined as an alteration in brain function, or other evidence of brain pathology, caused by an external force" (Menon et al., 2010). This includes loss of consciousness, memory loss before or after injury, neurological deficits, or altered mental state at the time of the injury. Confounding factors (e.g., intoxication, medication, or pain), along with focal motor deficits from peripheral nerve injury, may complicate diagnostics (Menon et al., 2010). TBI is characterised as an acute condition (Maas et al., 2022) and as a chronic disease with an increased risk of late-onset neurodegeneration (Blennow et al., 2012; Maas et al., 2022).

2.1.2 Epidemiology

The injury burden of TBI worldwide is very high, with the global incidence of TBI being approximately 50 million patients yearly (Feigin et al., 2013; Maas et al., 2022). According to estimates, half the world's population will experience TBI at some point. The global economic burden of TBI is approximately 400 million US dollars annually (Maas et al., 2017).

The incidence rate in Europe is estimated at 2.5 million new TBI cases per year (Maas et al., 2017) and 262 per 100,000 hospitalised patients (Peeters et al., 2015). In the USA, the incidence of new TBIs is close to 3.5 million patients (Coronado et al., 2012), with an age-adjusted incidence of 787 TBIs per 100,000 individuals per year (Taylor et al., 2017). The numbers are much lower in South Korea, with 476 new patients per 100,000 people per year (Kim et al., 2020). In Europe, within the adult population, TBI is more prevalent in patients younger than 25 or older than 75. Males are more often affected (Peeters et al., 2015). In Finland, 69/100,000 working-aged TBI-related hospital admissions occurred between 2004 and 2018, the majority of these patients being men (Posti et al., 2022). These numbers, however, may underestimate the incidence of TBI as the diagnostic criteria vary and not all patients,

especially those with mTBI, seek hospital care (Langer et al., 2020; Menon et al., 2025).

Reliable data from low- or middle-income countries is not widely available, as most countries lack centralised trauma registries. However, the estimates are that in Sub-Saharan regions, 14 million persons per year will have TBI by 2050 (Buh et al., 2023); in India, the estimate is that TBI-related injuries kill one person every three minutes (Singh et al., 2023). In Cameroon, patients with TBI were younger than in Europe, mostly 15–45-year-old males with poor education and a traffic accident as the main cause (Buh et al., 2023).

The causes of TBI vary between continents. Road traffic accidents are the main cause of injury in low-income countries (Buh et al., 2023; Maas et al., 2022), whereas in middle- or high-income countries, where road traffic safety is better, falls cause most of the accidents, especially with geriatric patients (Maas et al., 2022; Peeters et al., 2015).

Retrospective studies have been used to estimate the worldwide TBI burden, but because different methods and procedures were used at different times and places, the characterisation may be unreliable (Corrigan et al., 2025).

Mortality from sTBI is high. In the US, in 2019, more than 60,000 deaths were associated with TBI, with suicide (35.5%) being the leading cause, followed by unintentional falls (29.9%) and road traffic accidents (17%). Most deaths occurred in patients over age 75. Males were more likely to suffer from TBI and deaths than females (Traumatic Brain Injury-Related Deaths by Age Group, Sex, and Mechanism of Injury, Surveillance Report, 2025). In Finland, between 2005 and 2020, more than 14,000 TBI-related deaths, corresponding to 20.5/100,000 person-years, occurred. Falls were the main cause of fatal TBI, and males were affected more than females. In Finland, TBI caused 1.8% of all deaths, but with younger adults aged 16–19, the number was much higher at 17% (Posti et al., 2023). In low-income countries, resources may contribute to outcomes, as patients without funding are more likely to leave the hospital against medical advice; thus, the prognosis may worsen (Buh et al., 2023).

2.1.3 Pathophysiology

In TBI, an external blow to the head or acceleration or deceleration forces cause the primary injury; the secondary injuries are neurochemical and metabolic disturbances triggered by the primary structural injury (Orr et al., 2024). The primary traumatic brain injury is divided into two categories: focal injury and diffuse injury. Contact forces mainly cause focal injuries, including contusions, haematomas, haemorrhages, and hypoxic–ischemic injuries. Diffuse injury includes diffuse axonal injury (DAI), oedema, hypoxic–ischemic injuries, and vascular injuries (Rao &

Lyketsos, 2002). Rotational acceleration can result in focal and diffuse injuries (Orr et al., 2024). DAI, axonal tearing caused by rotational acceleration (Rao & Lyketsos, 2002), has been reported to occur in all severities of TBI (Johnson et al., 2013) and has been diagnosed in 72% of patients with moderate or severe TBI, who typically have both focal and diffuse injuries (Skandsen et al., 2010). The extent of DAI in mTBI, however, seems to be unclear.

Primary TBI is irreversible due to direct structural damage to the cellular and tissue components of the central nervous system. Therapeutic treatments aim to limit the secondary injuries (Warner et al., 2007), including structural, metabolic, inflammatory, and neurovascular changes, as well as malfunctioning neurotransmitter networks (Orr et al., 2024; Posti & Tenovuo, 2022).

The secondary injuries cause rapid neuroinflammatory brain responses that might lead to chronic inflammation, with inflammation mediators travelling through the bloodstream, affecting not only the brain but also peripheral organs (Anthony et al., 2012), including the liver, kidneys, cardiovascular, respiratory, gastrointestinal, and endocrine systems (Sabet et al., 2021). The injured brain structures may lead to oedema, causing high intracranial pressure (ICP) and low cerebral perfusion pressure, resulting in life-threatening brain ischemia (Maas et al., 2017; Shahrokhi et al., 2010). Systemic hypotension and hypoxia increase morbidity and mortality (Spaite et al., 2017). The behavioural and cognitive changes in patients with TBI are due to neuroinflammatory brain responses (Orr et al., 2024). Secondary damage is long-lasting and can persist for years (Sabet et al., 2021). TBI may cause progressive neurodegeneration (L. Wilson et al., 2017), but the mechanism underlying this process remains poorly understood (Tenovuo et al., 2021).

2.2 TBI severity assessment

The severity of TBI in the acute phase is typically assessed with GCS and post-traumatic amnesia (Bazarian et al., 2025; Tenovuo et al., 2021). These, however, do not correlate well with the pathophysiology or long-term outcome from TBI. The GCS at different time points can have a different significance and thus can give a false assessment when only evaluated once and should not be used to characterise the whole clinical situation. The severity may also change due to possible neuroworsening (Tenovuo et al., 2021). Amnesia may be difficult to objectively verify due to factors like the patient's young or old age, pre-injury intoxication, cognitive impairment, or medical interventions like sedation (Menon et al., 2025).

2.2.1 Glasgow Coma Scale

GCS was introduced more than 50 years ago, and the severity of TBI is still usually assessed using its three components: eye opening, verbal response, and best motor response (Teasdale & Jennett, 1974). Each component is graded, and the sum scale ranges from 3 to 15 (**Table 1)**. TBI is graded into three groups by GCS: mTBI (GCS 13–15), moTBI (GCS 9–12), and sTBI (GCS 3–8) (Teasdale et al., 2014). As simple and useful as the GCS method is, it also oversimplifies the severity assessment of TBI and, by itself, does not provide sufficient information about the biology and pathoanatomical injury underlying TBI. Additionally, the severity assessment using GCS is estimated at only a single point in time without considering the continuum of TBI. Confounders (e.g., intoxication) may lead to incorrect interpretations of the patients' neurological condition (Manley et al., 2025; V. Newcombe et al., 2025). The variability of the assessment for GCS has also been a confounding factor; training and repeated assessment provide more reliable results (Teasdale et al., 2014).

Table 1. Glasgow Coma Scale. Modified from Teasdale et al. (2014).

Glasgow Coma Scale

Eye opening response	Spontaneously	4
	To speech	3
	To pressure	2
	No response	1
Best verbal response	Oriented	5
	Confused	4
	Words	3
	Sounds	2
	No response	1
Best motor response	Obeys commands	6
	Localises pain	5
	Flexion withdrawal from pain	4
	Abnormal flexion	3
	Extension	2
	No response	1
Total score	Normal response	15
	Unresponsive	3

2.2.2 CBI-M: four-pillar model

A more precise and individualised evaluation of TBI is needed, as the current method, based on GCS, is not accurate enough (Manley et al., 2025). The US National Institutes of Health–National Institute of Neurological Disorders and Stroke (NIH-NINDS) launched an international consensus initiative in 2023; in 2024, six working groups were formed. The goal is to develop a new and more individualised TBI classification system (V. Newcombe et al., 2025). Because the severity of TBI has typically been assessed using the GCS, which captures only a single time point along the continuum and does not account for pathoanatomic injuries, a new model was proposed. This clinical, biomarker, imaging, and modifier (CBI-M) model (**Figure 1**) incorporates a clinical, a biomarker, an imaging, and a modifier pillar to provide a multidimensional characterisation of TBI at the acute phase (Manley et al., 2025).

GCS is still recommended as the starting point for the clinical pillar with a few modifications or additions. Pupil reactivity has to be checked separately for each patient, not just as part of the GCS system (Menon et al., 2025). Visual inspection is imprecise and prone to interobserver variability, whereas automated pupillometry provides accurate and comparable results (Mathur et al., 2024). Loss of consciousness needs to be evaluated and possible confounders identified. Additional patient-related parameters (e.g., age, frailty, and comorbidities) should be considered to obtain a full picture of TBI (Menon et al., 2025).

Computed tomography is used worldwide to characterise TBI at the acute phase, but the reporting systems and terminology used vary. There are no agreed-upon methods on how to use the CT findings with the other available data (i.e., medical, psychosocial, or environmental). As part of the new TBI characterisation agenda, new methods for neuroimaging were considered. The working group proposed updates to definitions, standardising terminology, and integrating the imaging findings into other clinical or patient data to aid in patient management and prognostication. Also, conversations about the pathoanatomical features of TBI with the patients and their families are important and should be considered (Mac Donald et al., 2025).

Blood biomarkers may provide more individualised diagnostics and treatment planning for TBI and thus have been included in the new classification proposal. Biomarkers may also assist in developing targeted therapies, as they reflect the underlying pathobiological processes. Potential biomarkers for different time points in characterisation were identified to be GFAP, ubiquitin C-terminal hydrolase-L1 (UCH-L1), and S100B for the acute phase; NfL, GFAP, and S100B for the subacute phase; and NfL, GFAP, and p-tau for the chronic phase (Bazarian et al., 2025).

Psychosocial and environmental factors may play an important role in the early assessment and later, in the prognosis of TBI. These include individual factors, such

as age, ethnicity, employment, culture, injury-related factors, such as the cause of injury, psychological trauma, extracranial injury, secondary brain injury, and substance abuse, along with society-related factors, such as racism, family support, system of care, and access to it. These aspects have been considered modifiers in the CBI-M model (Nelson et al., 2025).

Planning for the new classification system is underway, but implementing the new procedures is a crucial step in launching the new classification system. An implementation agenda was planned, which included a method for identifying the right research questions and a way to translate research knowledge into clinical practice. Clinical guidelines based on systematic reviews, collaboration between research groups, working closely with hospitals to implement clinical procedures, and systems to evaluate the CBI-M model were suggested (Bragge et al., 2025).

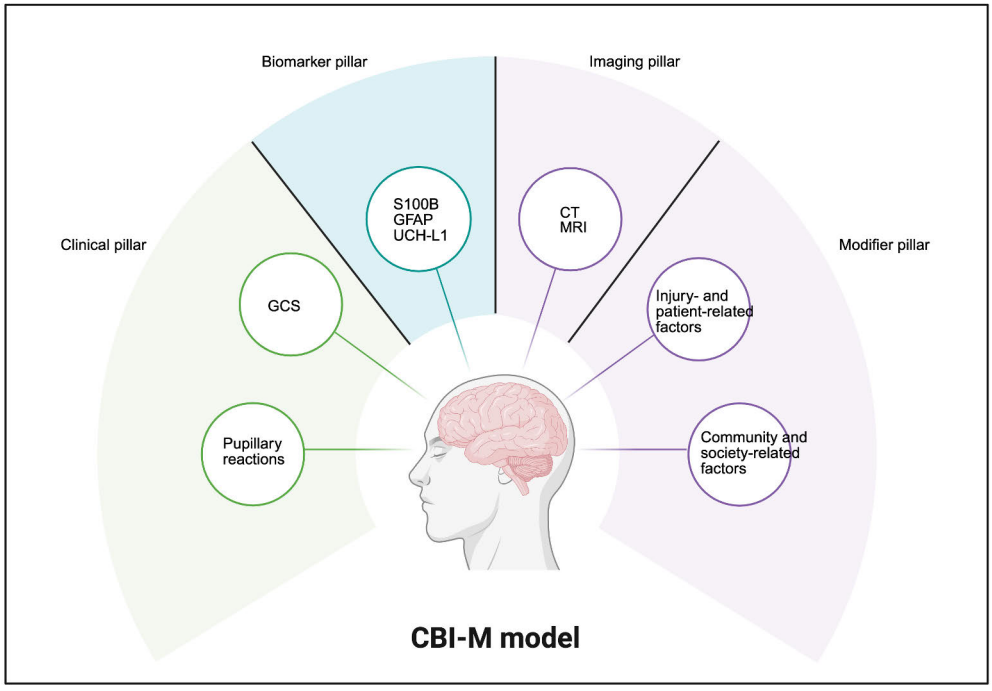

Figure 1. Proposed CBI-M-model for characterisation of TBI, including clinical, biomarker, imaging, and modifier pillars. Created in https://BioRender.com.

TBI is a continuous process, so follow-up for up to 14 days has been recommended for discharged patients; this also poses logistical issues, but recording the symptom severity from all patients during that time was beneficial (Menon et al., 2025). The suggested follow-up method is described in **Figure 2**, modified from (Menon et al., 2025).

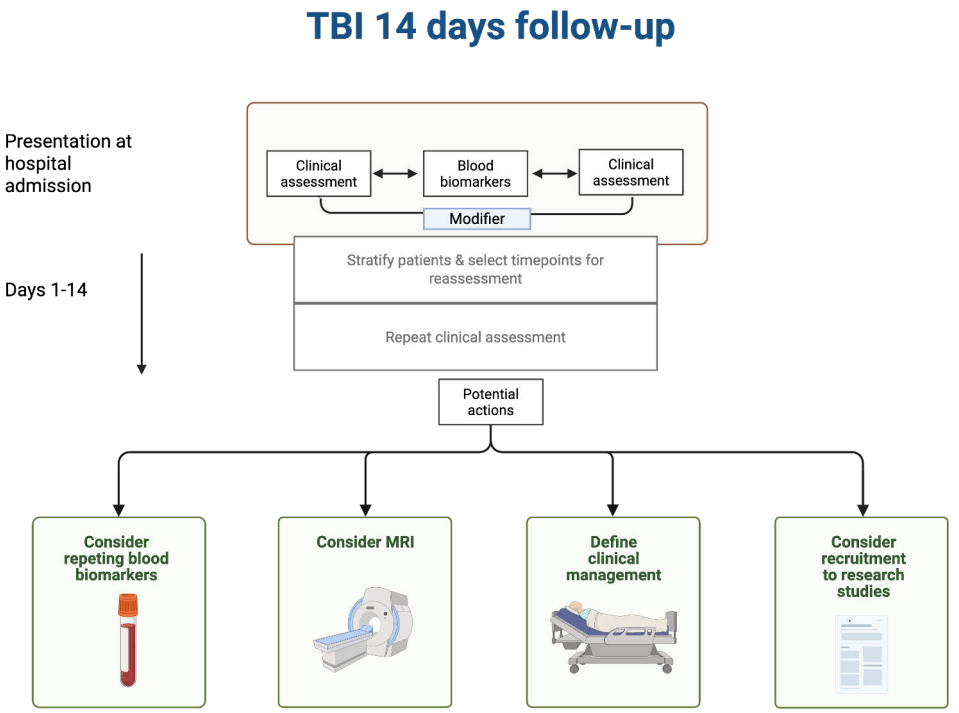

Figure 2. Proposed 2-week follow-up period for TBI, modified from Menon et al. (2025). Created in https://BioRender.com.

2.3 Imaging methods

2.3.1 Computed tomography

Head CT scanning was introduced almost 40 years ago and significantly impacted the small number of TBI patients who need neurosurgery (Tenovuo et al., 2021). In Europe, patients with suspected TBI are assessed according to the National Institute for Health and Care Excellence (NICE) guideline, Head Injury: Assessment and early management (NG232), which outlines the need for a head CT scan (**Table 2;** NICE guideline, 2023). Head CT is used in the emergency department (ED) to diagnose TBI, as CT scanning is a fast and accessible imaging method. The more severe injuries usually have pathological findings on CT, but, for example, DAI is mostly not visible on CT scans (Johnson et al., 2013), and fewer than 10% of patients with mTBI are estimated to have visible intracranial lesions on head CT scans (Easter et al., 2015). Thus, CT scanning exposes many patients to unnecessary imaging and radiation (D. Whitehouse et al., 2025).

Table 2. Criteria for scanning a head CT, according to the NICE guidelines. Modified from the guideline (NICE guideline, 2023).

For people aged ≥ 16 with a head injury, do a CT head scan within one hour of identifying any of the following risks:
• a GCS score of ≤ 12 on initial assessment in the ED • a GCS score of ≤ 15 at two hours after the injury on assessment in the ED • suspected open or depressed skull fracture • any sign of basal skull fracture (haemotympanum, 'panda' eyes, cerebrospinal fluid leakage from the ear or nose, Battle's sign) • post-traumatic seizure • focal neurological deficit • more than one episode of vomiting
For people aged ≥ 16 with a loss of consciousness or amnesia after the injury, do a CT head scan within eight hours of the head injury or within one hour if a patient presenting more than eight hours after the injury with any of the following risks:
• age 65 or over • any current bleeding or clotting disorders • dangerous mechanism of injury (a pedestrian or cyclist struck by a motor vehicle, an occupant ejected from a motor vehicle, or a fall from a height of more than one metre or five stairs) • more than 30 minutes of retrograde amnesia of events immediately before the head injury
For patients with a head injury and anticoagulant treatment (vitamin K antagonists, DOACs, heparin, and low molecular weight heparins) or antiplatelet treatment (excluding aspirin monotherapy) without any other indications for a CT head scan, consider doing a CT head scan with either of the following:
• within eight hours of the injury (e.g., if risk assessment is difficult or if the patient might not return to the ED in case of deterioration) or • within the hour if they present more than eight hours after the injury

CT = computed tomography, DOACs = direct-acting oral anticoagulants, ED = emergency department

2.3.2 CT abnormalities and lesion types

TBI can cause several types of brain lesions or combinations (**Figure 3)**. Clinically, the most important CT abnormalities are mass lesions, epidural haematoma (EDH), subdural haematoma (SDH), traumatic subarachnoid haemorrhage (tSAH), intraventricular haemorrhage (IVH), contusions, traumatic axonal injuries, midline shifts, or cisternal compressions (D. Whitehouse et al., 2025).

The terminology for characterising and reporting CT scan findings in TBI is standardised to ensure comparable results. These pathoanatomical terms and definitions, referred to as Common Data Elements (CDEs), provided information on common and uncommon lesions in a large study and, for example, demonstrated that some lesions tend to coexist. Also, clinical predictive models could be built based on CDEs (Vande Vyvere et al., 2020).

Acute-phase head CT scans of over 4000 patients with all severities of TBI from several European centres have been collected to evaluate the imaging pathophysiology of TBI. Regardless of the severity of TBI, the most common injuries identified were tSAH (45.3%), skull fractures (37.4%), contusions (31.3%), and SDH (28.9%), with a threefold higher frequency in mo/sTBI than in mTBI (Vande Vyvere et al., 2024). Biomarkers and their relation to different lesion types have been studied, but the results remain unclear, possibly due to the complexity and heterogeneity of TBI (Whitehouse et al., 2022a). The association between the biomarkers and the lesions appears to depend more on the extent and number of lesions than on lesion type (Whitehouse et al., 2022b).

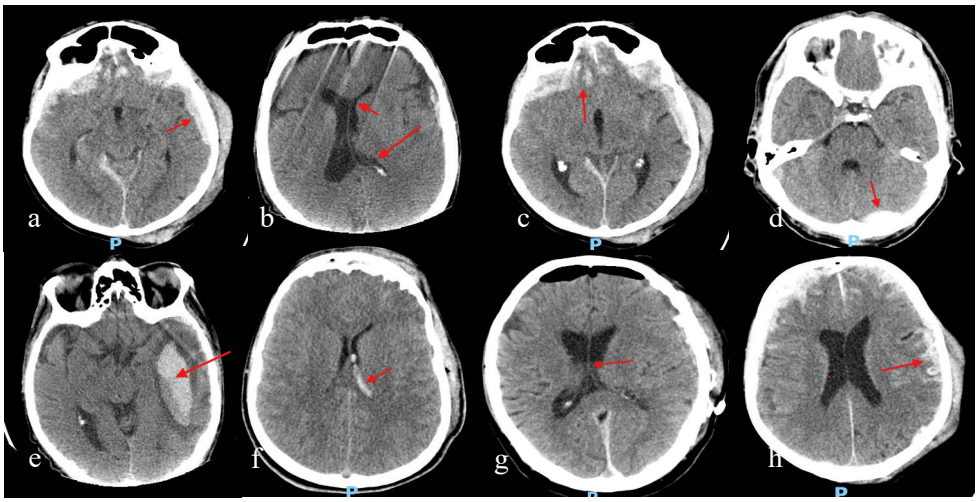

Figure 3. Different lesion types: a) acute subdural haematoma, b) cisternal compression, c) contusion, d) epidural haematoma, e) intracerebral haemorrhage, f) intraventricular haemorrhage, g) midline shift, h) traumatic subarachnoid haemorrhage. De-identified patient head CT scans from the study.

2.3.3 Other imaging methods

Structural magnetic resonance imaging (MRI) has far greater sensitivity for detecting structural abnormalities than CT (Dabas et al., 2024), with an estimated 29% of CT-negative patients showing MRI-positive findings (Yue et al., 2020). MRI, however, is more time-consuming than CT, which is not optimal at the acute phase of TBI. Also, MRI equipment or MRI-compatible ventilators, which are often needed for more severe TBI, may be unavailable. MRI is more suitable in the subacute phase of TBI or chronic mTBI with enduring symptoms (MacDonald et al., 2025).

In research, diffusion-weighted magnetic resonance imaging (DW-MRI) has been used to examine the association between acute-phase GFAP and white-matter

microstructural disruption at two weeks post-injury (Huibregtse et al., 2025). DAI changes in patients with mTBI more than three months after the injury appeared to correlate with the admission NfL levels (Hossain et al., 2023a). However, at present, DW-MRI is used only for research and has no clinical value.

2.4 Intracranial pressure

High ICP in the acute phase may indicate the severity of TBI; however, increasing ICP at a later phase may indicate secondary brain injury. Elevated ICP may result in fatal injuries, causing altered cerebral structures, compromising the cerebral blood flow (Maas et al., 2017). Raised ICP has been identified as an independent predictor of mortality (Badri et al., 2012). Continuous invasive ICP monitoring has long been the cornerstone of neurocritical care for sTBI (Carney et al., 2017; Glushakova et al., 2020), with studies reporting improved survival (Alali et al., 2013; Dawes et al., 2015), possibly due to effective treatment based on observed ICP levels (Robba et al., 2021).

2.5 Neurosurgical interventions

Neurosurgical interventions for patients with TBI are usually due to elevated ICP. Invasive ICP monitoring in the ICU is the gold standard for monitoring the ICP level; craniotomy is used to evacuate mass lesions, and decompressive hemicraniectomy may be warranted to reduce the ICP level (Hossain et al., 2023b). Previous studies have found that 0.9% of patients with mTBI (Easter et al., 2015) and about 25% of patients with moTBI require urgent neurosurgical intervention (Clark et al., 2022; Godoy et al., 2016). The type of emergency surgery most frequently performed was evaluated in a worldwide study, including patients from 57 countries. Elevation of a skull fracture in mTBI was the most common (45%) type of surgery in low human development index (HDI) countries, whereas evacuation of supratentorial EDH was the most common in medium- and high-HDI countries in mo/sTBI, 31% and 32%, respectively. Evacuation of supratentorial acute SDH in mo/sTBI (47%) was the most performed operation type in very high HDI countries. The median time from injury to operation was 13 h, with an overall mortality of 18% (Clark et al., 2022).

2.6 Electroencephalogram

TBI injures neurons causing electrophysiologic changes that may lead to seizures (Schmitt & Dichter, 2015). Studies report that early posttraumatic seizures occur in 0.4%–8.6% of patients with TBI, depending on geographic location, definition of

seizures, and methods of collecting and reporting the data (DeGrauw et al., 2018; Majidi et al., 2017; Sødal et al., 2022; Thapa et al., 2010). Posttraumatic seizures have been associated with increased admission to the ICU, along with prolonged ventilator and ICU treatments. Patients also had poorer outcomes than those without seizures. Although in-hospital mortality was not elevated, the mortality was elevated during 24-month surveillance (Laing et al., 2022).

The seizures or status epilepticus (SE) may be non-convulsive and remain undetected without diagnostic tools; continuous electroencephalogram (EEG) is non-invasive and detects real-time changes in the brain electrographic patterns and is used to detect seizures (Bitar et al., 2024; Taccone et al., 2026). EEG is recorded by placing electrodes on the patients' scalp, obtained electrical signals from cerebral cortex are converted to digital data and transferred to computer for analysis (Schmitt & Dichter, 2015). Combining clinical parameters with EEG monitoring predicts the neurological outcome better than clinical features only (Verboom et al., 2025).

2.7 Overall injury severity assessment

The Injury Severity Score (ISS) is a widely used medical scoring system developed to systematically and comparably assess the severity of injuries in patients with multiple traumas. ISS includes six body regions: head and neck, face, chest, abdomen, pelvis, and extremities and external soft tissue (Baker et al., 1974). All six parts of the body are evaluated; injuries are rated with an abbreviated severity score (AIS) from one (minor) to six (unsurvivable), and the three most seriously injured parts are taken into the calculations, with 75 being the highest possible score. If the AIS of six is estimated to any part of the body, the ISS is automatically 75. ISS has been associated with morbidity and mortality (Balogh et al., 2000; Osler et al., 1997). However, it fails to account for multiple injuries to the same body component, diminishing its utility (Osler, 1993), and multitrauma patients have a risk of being underdiagnosed (Schröter et al., 2019).

2.8 Outcome

Research and interviews of the patients have shown that patients diagnosed with mTBI are often discharged with their condition underestimated, without a plan for their rehabilitation or follow-up, leaving them often feeling that the diagnosis of "mild" has left them to struggle alone (Manley et al., 2025). They are suspected to recover and rapidly return to normal (Maas et al., 2023), but research indicates that mTBI may have substantial long-term deficits (Tenovuo et al., 2021), which have been associated with the extent of DAI (Tenovuo et al., 2021; van Eijck et al., 2018).

Studies show worse outcomes in patients with mTBI without pathologic findings on head CT scan if the biomarker values are elevated (L. Wilson et al., 2024). Also, concussion, typically considered a form of mTBI, may cause long-term problems (Tagge et al., 2018). On the contrary, patients with a diagnosis of sTBI may experience a self-fulfilling prognosis if they are categorised as "hopeless" cases and the treatment is withdrawn too early (Manley et al., 2025).

Several prediction models concerning the outcome of TBI have been studied. However, in a systematic comparison, the only models even moderately acceptable in mo/sTBI for predicting the mortality from two weeks to six months after the injury and the functional outcome after six months have been CRASH and IMPACT (Muehlschlegel et al., 2024). Outcome prediction is important to patients, their families, and the healthcare professionals making decisions about the care. About half the patients with mTBI do not recover to their pre-injury state within six months of the accident (Maas et al., 2022). A model for the prognosis from mTBI has a moderate ability to predict Glasgow Outcome Scale extended (GOSE) but poor prognostic capability on persistent post-concussion symptoms (Mikolić et al., 2023).

Post-traumatic amnesia (PTA) at the acute phase has been identified as an effective clinical predictor of long-term cognitive outcomes (Königs et al., 2012), even if the estimation of PTA might be inaccurate due to retrospective evaluation (Friedland & Swash, 2016). Parameters associated with poor outcome in moTBI are low GCS, old age, SDH, hypoxia and/or hypotension, intoxication, or prior disability (Einarsen et al., 2018). mTBI and sTBI with DAI appeared to have worse outcomes if the lesions were in the dorsolateral brainstem (Skandsen et al., 2010).

2.8.1 Glasgow Outcome Scale extended

GOSE is a hierarchical scale used to evaluate a patient's functional outcome following a TBI based on the lowest available outcome category. Consciousness, independence, and the ability to participate in work, family life, and hobbies, for example, are evaluated, and patients are categorised into eight categories **(Figure 4)**. GOSE 8 means complete recovery to their condition before the injury (J. T. L. Wilson et al., 1998; L. Wilson et al., 2021).

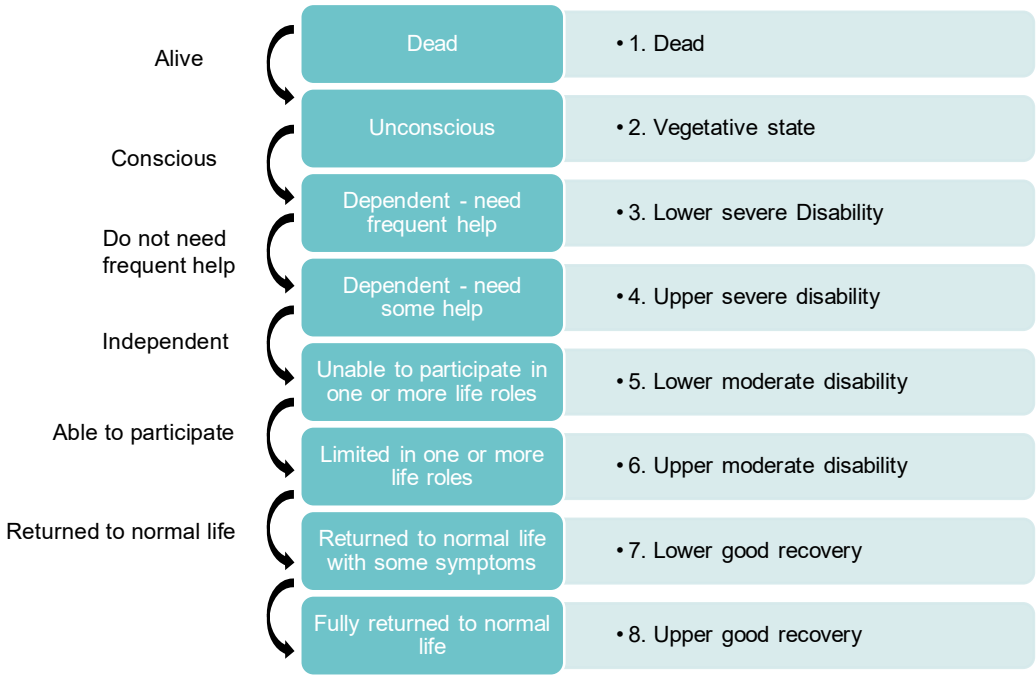

Figure 4. GOSE evaluation scale. Modified from L. Wilson et al. (2021).

2.9 CT scoring systems

Several CT scoring systems have been developed and used to evaluate patients' head CT scans, which may then be used for diagnostics, patient care, and prognosis.

2.9.1 Marshall CT classification

The Marshall CT classification (Marshall et al., 1992), **Table 3,** is the most widely used CT classification system. It categorises the findings into levels of diffuse or focal lesions based on basal cistern compression and midline shift or whether the lesion exceeds 25cm^3. However, the Marshall classification does not account for the influence of DAI or raised ICP (Mondello et al., 2011). The Marshall CT classification was not developed as a prognostic tool, and was later outperformed by the Helsinki CT score (Raj et al., 2014) as an outcome predictor (Thelin et al., 2017a).

Table 3. Marshall CT classification system. Modified from Marshall et al. (1992).

Injury type	CT scan	Scoring
Diffuse injury I (no visible pathologic change)	No visible intracranial pathology seen on CT	1 point
Diffuse injury II	Cisterns are present with shift 0–5 mm and/or Lesion densities present No high or mixed density lesion > 25 ml May include bone fragments and foreign bodies	2 points
Diffuse injury III (swelling)	Cisterns compressed or absent with 0–5 mm No high or mixed density lesion > 25 ml	3 points
Diffuse injury IV (shift)	Shift > 5 mm No high or mixed density lesion > 25 ml	4 points
Evacuated mass lesion	Any surgically evacuated lesion	5 points
Non-evacuated mass lesion	High or mixed density lesion > 25 ml, not surgically evacuated	6 points
Brain dead	No brainstem reflexes Flaccidity Fixed and nonreactive pupils No spontaneous respirations with a normal PaCO2 Spinal reflexes permitted	

2.9.2 Helsinki computed tomography score

Helsinki CT score (HCTS) is a validated prognostic method for TBI (**Table 4**). The CT scan features used for HCTS are mass lesion type (SDH, ICH, EDH), mass lesion size > 25 cm^3, presence of intraventricular haemorrhage, and the status of suprasellar cisterns (normal, compressed, obliterated). The range is -3 to 14 (Raj et al., 2014; Vehviläinen et al., 2022). HCTS provides more precise information on clinically meaningful injuries and has better prognostic value for outcomes than the Marshall CT classification system but is more complex to use (Thelin et al., 2017a).

Table 4. Helsinki computed tomography score. Modified from Raj et al. (2014).

Variable	Scoring
Mass lesion types	
Subdural haematoma	2 points
Intracerebral haematoma	2 points
Epidural haematoma	-3 points
Mass lesion size > 25 cm³	2 points
Intraventricular haemorrhage	3 points
Suprasellar cisterns	
Normal	0 points
Compressed	1 point
Obliterated	5 points
Sum score	-3 to 14 points

2.10 Blood biomarkers in TBI research

2.10.1 Overview

A biomarker should be a sign of normal biological process, a pathogenic process, or a pharmacological reaction to therapeutic intervention, and should be objectively measured and evaluated (Atkinson et al., 2001). Blood proteomic biomarkers, referring to proteins found in blood (Kratka et al., 2025) have been studied to aid the characterisation of TBI in the acute, subacute, and chronic phases, to reduce unnecessary radiation exposure with fewer CT scans, and detect and treat secondary injury, treatment efficacy, and outcome prediction (Bazarian et al., 2025; Hossain et al., 2024). Also, identifying TBI in cases of polytrauma and evaluating the need for further imaging (e.g., MRI) would be helpful (Hossain et al., 2024). Biomarkers have drawbacks: they are not brain-specific, and factors such as intoxication and age elevate their levels (Hossain et al., 2024; Posti & Tenovuo, 2022; D. Whitehouse et al., 2025), limiting their usefulness. However, blood biomarkers have been suggested as one part of a new CBI-M four-pillar method to characterise TBI and make the characterisation more individualised (Bazarian et al., 2025). **Figure 5** shows the expression of the studied biomarkers in neuronal cells following TBI. **Table 5** describes their characteristics.

Table 5. Properties of the studied biomarkers.

Biomarker	Mass (kDa)	Source	Location	Other sources	Half-life (h)	Peak (h)	Confounders
β-amyloids		APP/Axons	Endoplasmic reticulum			24	Age increases levels
GFAP	50	Astrocytes	Cytoplasm	Schwann cells, chondrocytes, enteric glial cells, liver, pancreas	24–48	20–24	Age increases levels, extracerebral sources
H-FABP	15	Neurons	Cytoplasm	Heart, kidneys, mammary glands		4–6	Extracerebral sources
IL-10		Glial cells				24	
NfL	68	Neurons	Myelinated axons	Peripheral axons	Unknown	Unknown	Age increases levels, peripheral nerve injury
S100B	11	Astrocytes	Cytoplasm	Adipocytes, muscle, chondrocytes, enteric glial cells	0.5–2	< 6	Age increases levels, short half-life, extracerebral sources
Tau proteins	33–46	Neurons	Axon terminals, unmyelinated axons	Astrocytes and oligodendrocytes, peripheral nervous system, kidneys	Unknown	12–24	Age increases levels, extracerebral sources
UCH-L1	25	Neurons	Cytoplasm	Testis, ovary, kidney	8	7–9	Short half-life, extracerebral sources

APP = β-amyloid precursor protein; β-amyloids = Aβ40 and Aβ42; GFAP = glial fibrillary acidic protein; H-FABP = heart fatty acid-binding protein; IL-10 = interleukin-10; NfL = neurofilament light; S100B = S100 calcium-binding protein B. Modified from (Posti & Tenovuo, 2022).

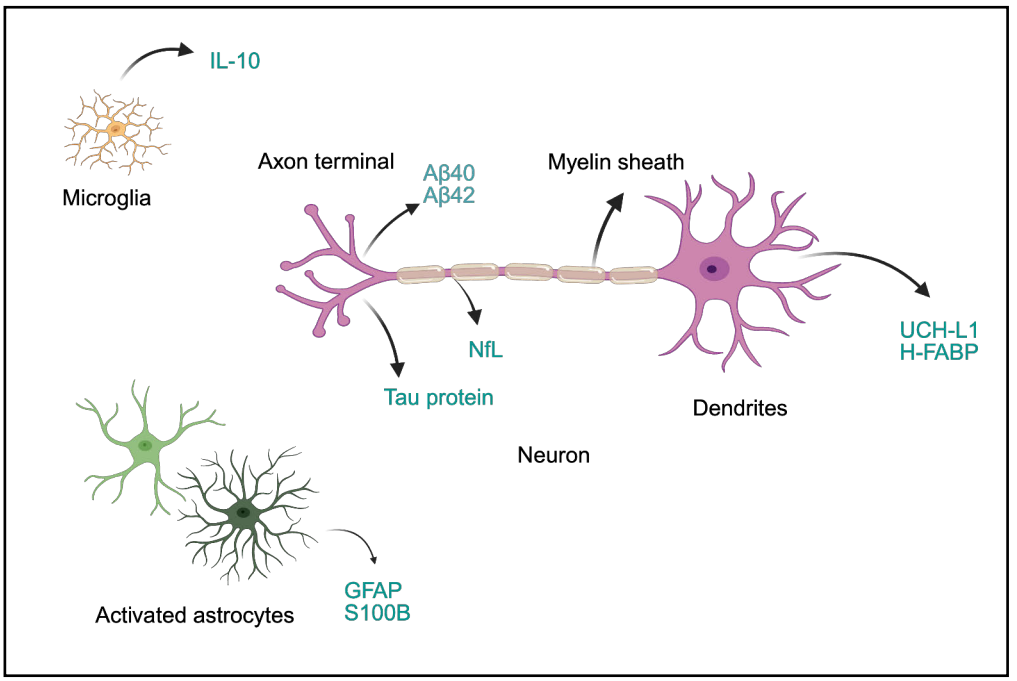

Figure 5. Biomarker expression in brain cells after traumatic brain injury. Author's own drawing. Aβ40 = β-amyloid 1–40; Aβ42 = β-amyloid 1–42; GFAP = glial fibrillary acidic protein; H-FABP = heart fatty acid-binding protein; IL-10 = interleukin-10; NfL = neurofilament light; S100B = S100 calcium-binding protein B; UCH-L1 = ubiquitin C-terminal hydrolase-L1. Created in https://BioRender.com.

2.10.2 β-amyloid isoforms 1–40 (Aβ40) and 1–42 (Aβ42)

β-amyloid precursor protein (APP) is synthesised in the endoplasmic reticulum, and mature APP is then converted to Aβ40 and Aβ42 by proteolysis in the plasma membrane (Chen et al., 2017; Haass et al., 2012), but Aβ40 and Aβ42 can also be the products of normal metabolism (Shahim et al., 2017a). Aβ40 and Aβ42 have been thought to represent axonal injury (Johnson et al., 2013), and the baseline levels increase with age (Toledo et al., 2011). Biomarker levels have been suggested to rise within 24 h (Hossain et al., 2024); however, contradictory results have been reported (Lippa et al., 2019). Aβ40 and Aβ42 are less studied as acute-phase biomarkers in TBI. They have been evaluated in CT-negative and CT-positive patients with TBI, in which Aβ42 levels did not differ, but Aβ40 levels were significantly higher in CT-positive than in CT-negative patients across all severities of TBI (Posti et al., 2019). Aβ40 and Aβ42 levels in microdialysis samples from patients with sTBI have been reported to show higher levels in diffuse injury than in focal lesions (Marklund et al., 2014). Levels of Aβ40 or Aβ42 did not sufficiently aid in diagnostics or outcome prediction in mTBI (Hossain et al., 2020).

2.10.3 Glial fibrillary acidic protein (GFAP)

The primary structural protein filament in the astroglial cytoskeleton is glial fibrillary acidic protein (GFAP), which is also found in nonmyelinated Schwann cells and enteric glial cells (Petzold et al., 2004; Yang & Wang, 2015) and contributes to blood–brain barrier integrity (Eng et al., 2000). GFAP can be detected in the blood one hour post-injury (Papa et al., 2016). The estimated half-life is 24–48 h, with a peak occurring within 20–24 h (Thelin et al., 2017b; Welch et al., 2017). GFAP levels increase with age (Eng et al., 2000; Gardner et al., 2018).

GFAP and ubiquitin C-terminal hydrolase L1 (UCH-L1) have been combined in an assay to exclude the need for a head CT scan for patients with suspected mTBI if a blood sample is taken within 12 h of the injury. The assay has been approved by the US Food and Drug Administration (FDA) and has the European Conformity (CE) marking (Bazarian et al., 2018; Kobeissy et al., 2024). The combination assay also detected patients whose scans remained negative, suggesting that it may detect subtle injuries not visible on CT scans (Bazarian et al., 2018). Almost 50% of intoxicated patients had CT-positive scans, and the combination assay could discriminate these from CT-negative intoxicated patients (Harris et al., 2025). GFAP also appears able to detect brainstem injury in mo/sTBI with 94% sensitivity, possibly aiding in decision-making when considering the need for an MRI (Richter et al., 2022). According to the NICE CT guideline (NICE, 2023), when evaluating patients with TBI, GFAP showed a strong ability to detect clinically meaningful CT abnormalities up to 24 h post-injury in the medium-risk patients (D. Whitehouse et al., 2025). White matter microstructural disruption at two weeks post-injury has been associated with elevated admission levels of GFAP, thus suggesting that admission GFAP could predict disruption in white matter connectivity at a later time (Huibregtse et al., 2025). GFAP discriminates between favourable and unfavourable outcomes in mTBI (Hossain et al., 2019).

2.10.4 Heart fatty acid-binding protein (H-FABP)

H-FABP is expressed in the brain's neuronal cytoplasm (Wunderlich et al., 2005) and has shown potential in diagnosing mTBI (Lagerstedt et al., 2017). However, as H-FABP is also expressed in the heart (Viswanathan et al., 2010), kidneys, and mammary glands (Goel et al., 2020), its performance as a specific marker of brain injury remains undetermined. H-FABP begins to rise within one hour and peaks at 4–6 h (Goel et al., 2020). In an outlier analysis for mo/sTBI, extracranial injuries accounted for all outlier-type H-FABP levels (Korhonen et al., 2024); another study found significantly higher levels of H-FABP in patients with TBI with extracranial injuries than in patients with isolated TBI (Niiranen et al., 2023). Acute-phase H-

FABP levels have been observed to predict mortality and outcome in sTBI (Walder et al., 2013).

2.10.5 Interleukin-10 (IL-10)

IL-10 is an anti-inflammatory cytokine expressed in response to inflammatory cytokines, and can be detected about 24 h after stimulation. IL-10 is considered the most fundamental immunosuppressive cytokine in the central nervous system (CNS). Its function is important for maintaining homeostasis and regulating neuroinflammatory injury; however, the role of IL-10 remains poorly understood (Burmeister & Marriott, 2018). The association between IL-10 and the severity of TBI is unclear (Garcia et al., 2017; Lagerstedt et al., 2018b); however, it discriminates between CT-positive and CT-negative patients with mTBI (Khosh-Fetrat et al., 2023; Lagerstedt et al., 2018b). IL-10 levels increase after TBI and may predict mortality (Garcia et al., 2017). An earlier study suggests IL-10 is associated with in-hospital mortality in sTBI (Schneider Soares et al., 2012).

2.10.6 Neurofilament light (NfL)

NfL is a marker of myelinated axonal injury (Zetterberg et al., 2013) and a structural component of neurofilaments, found especially in the axons of neurons (Petzold, 2022). NfL levels start rising on the day of the injury, continue rising for two to three weeks, and may remain elevated for several months (Bazarian et al., 2025). Old age or peripheral nerve injury from extracerebral injury may also cause elevated NfL levels and are confounding factors to NfL levels (Posti & Tenovuo, 2022). Admission levels of NfL in mTBI might predict the presence of DAI at a later phase (Hossain et al., 2023a), but with sTBI, NfL had 100% discrimination between patients with DAI and controls (Ljungqvist et al., 2017). In contact athletes with mTBI, NfL was elevated after long periods of repetitive hits to the head (Shahim et al., 2017b). High admission levels of NfL with mTBI have been associated with unfavourable outcomes (Hossain et al., 2019). Consistently high levels of NfL in cases of moderate to severe TBI seem to be associated with worse outcomes (V. F. J. Newcombe et al., 2022; Tuure et al., 2024).

2.10.7 S100 calcium-binding protein B (S100B)

S100B, expressed in astrocytes (Donato et al., 2009; Thelin et al., 2017b), is the most studied blood biomarker in TBI. S100B is not brain-specific and is expressed, for example, in muscles and chondrocytes (Posti & Tenovuo, 2022). Thus, the S100B levels in the bloodstream increase with extracranial trauma and physical exercise

(Hasselblatt et al., 2004; Kahouadji et al., 2020). S100B peaks within six hours with a half-life between 30 minutes to two hours (Posti & Tenovuo, 2022). S100B is beneficial in ruling out intracranial lesions in certain adult patients with mTBI, and Scandinavian guidelines recommend discharging patients without a head CT scan if they have minimal or mild TBI without additional risks, with the level of S100B < 0.10 µg/L within six hours of injury (J. Undén et al., 2013). It seems the clearance of S100B from extracranial origin is faster than that from TBI (Thelin et al., 2017c). S100B cannot be used in polytrauma (Kahouadji et al., 2020), in children, or six hours post-injury (J. Undén et al., 2013), limiting its usefulness in clinical settings. Higher levels of S100B have been observed in older (> 65 years) patients (Calcagnile et al., 2013), but contradictory findings have also been reported where age, sex, or GCS score did not seem to influence S100B levels in mTBI (Kahouadji et al., 2020). The results indicate that alcohol does not affect the level of S100B (Calcagnile et al., 2013).

2.10.8 Total tau (t-tau)

Tau is a microtubule-stabilising protein, mainly expressed in the CNS's distal parts of unmyelinated axons (Avila et al., 2004; Binder et al., 1985; Khan & Bloom, 2016); however, it is also in astrocytes, oligodendrocytes, the peripheral nervous system, and the kidneys (Posti & Tenovuo, 2022). Tau has six isoforms in the CNS (Hanes et al., 2009), but most studies have been conducted with total tau (Hossain et al., 2020). The half-life of tau is unknown, but the peak level seems to be at 12–24 h (Posti & Tenovuo, 2022). In the acute phase of TBI, tau has been detected in patients with CT abnormalities (Posti et al., 2019; Rubenstein et al., 2017) and in patients with DAI (Tomita et al., 2019). Admission levels of t-tau are associated with the outcome of mTBI but could not predict total recovery (Hossain et al., 2020).

2.10.9 Ubiquitin C-terminal hydrolase-L1 (UCH-L1)

UCH-L1 in the CNS is primarily expressed in neuronal cell bodies, reflecting neuronal injury (Wang et al., 2021), but can also be detected in the testis, ovaries, and kidneys (Meyer-Schwesinger et al., 2009; Wilkinson et al., 1989). UCH-L1 can be detected within one hour of brain injury, peaks at eight hours post-injury, and declines over 48 h (Papa et al., 2016). Rodent studies have shown the importance of the ubiquitin-proteasome pathway as its dysfunction appears to worsen the secondary injury in TBI (Sokka et al., 2007). The FDA has approved UCH-L1 with GFAP to help determine the need for a head CT scan after mTBI (Bazarian et al., 2018). In sTBI, biomarker levels have been compared with head CT findings, and higher levels of UCH-L1 were found in diffuse injury rather than in mass lesions

(Mondello et al., 2011). UCH-L1 has been a strong predictor of death at six months (Mondello et al., 2011).

2.11 Single biomarker vs biomarker panel

TBI damages several structures in the brain, leading to the release of multiple biomarkers from these structures, raising the hypothesis that a biomarker panel might be superior to single biomarkers for diagnosing the injury (Lagerstedt et al., 2018a). Studies have evaluated the ability of biomarker panels to discriminate between CT-positive and CT-negative patients with TBI, where biomarker panels have been shown to outperform a single biomarker's performance (Lagerstedt et al., 2018a; Posti et al., 2019). Biomarker panels have also been studied in prediction models (Richter et al., 2023; E. Thelin et al., 2019). A combination assay of GFAP and UCH-L1 has been approved in an FDA guideline to eliminate the need for a head CT scan for mTBI, provided that a blood sample was taken within 12 h of the injury (Bazarian et al., 2018). However, recent research has shown that single biomarkers perform as well as multiple biomarkers and that using more than one may represent a waste of resources (Czeiter et al., 2020; Manley et al., 2025).

2.12 PanelomiX

PanelomiX is a web-based tool enabling fast screening of large numbers of biomarkers to combine panels with biomarkers of different properties. The method searches for the best panel based on the user's preset definitions and targets. Cross-validation is performed on a test set to evaluate the model's stability and performance. Results are reported as the partial area under the ROC curve (pAUC) at the prespecified specificity or sensitivity. PanelomiX can be used in complex diseases where the properties of a single biomarker may be inadequate, and a combination of different properties in several biomarkers might be beneficial. The purpose is for the panel to achieve, for example, increased specificity and/or sensitivity compared to a single biomarker (Robin, 2019; Robin et al., 2013).

2.13 Blood biomarkers in other research or use

The biomarkers studied in the context of TBI have been researched for several other conditions. Elevated NfL values have been detected in the presymptomatic phase of several degenerative neurological conditions, such as frontotemporal lobar degeneration (Gendron et al., 2022), Huntington's disease (McColgan et al., 2022), and in patients with Diffuse Lewy Body dementia (Pilotto et al., 2021). NfL appears to have value as an outcome predictor after non-traumatic SAH (Garland et al., 2021;

Labib et al., 2024) and in brain injury after out-of-hospital cardiac arrest (OHCA), with an initial shockable rhythm (Wihersaari et al., 2021), outperforming the currently used clinical standard neuron-specific enolase (NSE) at 24 h (Wihersaari et al., 2022). GFAP and tau also showed excellent prognostic properties in brain injury after OHCA at 48 and 72 h, but they did not outperform NSE (Humaloja et al., 2022). S100B could detect intracranial complications in patients undergoing venovenous extracorporeal membrane oxygenation (ECMO) (Walther et al., 2024). Preclinical models suggest a weak neuroprotective effect of IL-10 after ICH, but in clinical studies, its role remains unclear. However, IL-10 levels have been associated with the extent of brain injury and prognosis (Garcia et al., 2017).

2.14 Preclinical studies

Numerous randomised clinical trials on TBI models have been studied with little success. Trials of pharmacological treatments have been completely unsuccessful, as the transition from rodents to humans has proven difficult (Bragge et al., 2016). It is suspected that the physiology and TBI severity between rodents and humans may be too different, rendering the rodent models inadequate to mimic human TBI (Yamamoto et al., 2018). Previous drug trials have extrapolated data from preclinical studies into clinically crucial aspects of patient care, which might not be optimal. Clinically relevant actions are being considered for implementation in the pre-clinical phase to improve transition into the clinical phase. Blood biomarkers have been proposed as a tool to align preclinical rodent data with clinical human TBI data. Several preclinical studies on GFAP, UCH-L1, NfL, t-tau, and p-tau have been conducted, showing results similar to those in human TBI; however, larger studies are warranted to provide further information (Lisi et al., 2025).

3 Aims of the study

This study investigated blood biomarkers in TBI, focusing on acute diagnosis, treatment monitoring, and outcome prediction. The specific objectives of the studies were to:

1. Evaluate the association of the biomarker levels and the severity of TBI and to assess whether biomarkers or their combinations could distinguish patients with mTBI, with or without CT-positive results, from orthopaedic controls (Study I).
2. Investigate whether biomarkers of different cellular origins in the brain can differentiate various lesion types and their combinations on head CT scans (Study II).
3. Study whether the biomarker outlier levels are associated with clinically significant events and GOSE in ICU-treated patients with TBI (Study III).

4 Materials and Methods

This study was part of the EU-funded TBIcare project (Evidence-based Diagnostic and Treatment Planning Solution for Traumatic Brain Injuries). Studies I and II were part of a prospective observational single-centre study; Study III was a retrospective analysis from the same prospective observational study. Ethical approval for the study was obtained from Southwest Finland Hospital District Research Ethics Committee (decision 68/180/2011).

4.1 Study population

All severities of patients with TBI (N = 200) were recruited at Turku University Hospital between November 2011 and October 2013. Information regarding the study was provided verbally and in writing to all patients or their representatives, and written consent was obtained.

4.1.1 Inclusion criteria

Inclusion criteria for the TBI group were age ≥18 and a clinical diagnosis of TBI, with indications for acute head CT, according to National Institute for Health and Care Excellence criteria (Eades, 2014).

The patients in the orthopaedic control group in Study I were ≥18 with an acute severe injury to the extremities or trunk but no head injury.

4.1.2 Exclusion criteria

The exclusion criteria in the TBI group included head injury with no indication for a head CT, blast-induced or penetrating injuries, prior neurological disease resulting in an inability to live independently, injuries occurring more than two weeks prior, chronic SDH, non-fluency in Finnish, or lack of obtained consent.

For the orthopaedic control group in Study I, the exclusion criteria were an earlier TBI or degenerative neurological disease, multiple injuries resulting in intensive care, or trivial injuries that did not require acute care or follow-up.

4.2 Study design

Characteristics of all the studies are listed in **Table 6**.

Table 6. Characteristics of the studies.

	Study I	Study II	Study III
Patients (N)	189 TBI, 40 orthopaedic controls	130 TBI	70 TBI, ICU-treated patients
Severity of TBI	All severities	All severities	All severities
Blood sampling	Admission	Admission	Admission, days 1, 2, 3, and 7
Study design	Single biomarkers vs combinations	CT-pos vs CT-neg patients	Biomarker outliers vs non-outliers
Studied biomarkers	H-FABP, IL-10, NfL, S100B	Aβ40, Aβ42, GFAP, H-FABP, IL-10, NfL, S100B, t-tau	GFAP, IL-10, NfL, t-tau
CT scans	Marshall	HCTS	Marshall, HCTS

Aβ40 = β-amyloid 1–40; Aβ42 = β-amyloid 1–42; CT = computed tomography; GFAP = glial fibrillary acidic protein; HCTS = Helsinki CT score; H-FABP = heart fatty acid-binding protein; ICU = intensive care unit; IL-10 = interleukin-10; NfL = neurofilament light; S100B = S100 calcium-binding protein B; TBI = traumatic brain injury; t-tau = total tau.

4.2.1 Study I

The demographics of Study I patients are summarized in **Table 7**. Admission blood samples and head CT scans were acquired from all patients within 24 h of hospitalisation. H-FABP, IL-10, NfL, and S100B levels were analysed from the blood plasma samples; the head CT scans were evaluated using the Marshall scoring system. The association between biomarker levels and the severity of TBI was examined, as well as the biomarkers' ability to distinguish patients with TBI with CT-positive scans from those with CT-negative scans. PanelomiX was used to study biomarker combinations' ability to distinguish mTBI from orthopaedic controls compared to single biomarkers.

Table 7. Demographic and clinical characteristics of all patients with TBI and orthopaedic controls in Study I.

Variable	TBI (n = 189)	Controls (n = 40)	P-value	TBI CT+ (n = 112)	TBI CT- (n = 77)	P-value
Age (years)	49 ± 20	52 ± 19	0.351[a]	53 ± 20	42 ± 18	**<0.001[a]**
Sex, n (%)						
Male	135 (71.4)	18 (45)	0.431[b]	86 (76.8)	49 (63.6)	**0.049[b]**
Female	54 (28.6)	22 (55)		26 (23.2)	28 (36.4)	
Severity, n (%)						
Mild (GCS 13–15)	108 (57.1)			41 (36.6)	67 (87)	
Moderate (GCS 9–12)	48 (25.4)			42 (37.5)	6 (7.8)	
Severe (GCS 3–8)	33 (17.5)			29 (25.9)	4 (5.2)	
Injury Severity Score (median [IQR])	13 (16)			17 (16)	6 (11)	**<0.001[c]**
CT findings (Marshall Grade), n (%)						
No visible pathology	77 (40.7)			0 (0)	77 (100)	
Diffuse injury	37 (19.6)			37 (33)		
Diffuse injury with swelling	6 (3.2)			6 (5.4)		
Diffuse injury with shift	2 (1.1)			2 (1.8)		
Evacuated mass lesions	37 (19.6)			37 (33)		
Unevacuated mass lesions	30 (15.9)			30 (26.8)		
Pupil reactivity, n (%)						
Unreactive	17 (9)			15 (13.4)	2 (2.6)	**0.009 [b]**
Sluggish	6 (3.2)			5 (4.5)	1 (1.3)	
Reactive	154 (81.5)			82 (73.2)	72 (93.5)	
Missing data	12 (6.3)			10 (8.9)	2 (2.6)	

[a] Student t-test significance; [b] chi-squared test significance; [c] Mann–Whitney U test significance. Marshall grade I = CT-negative (no visual pathology), Marshall grade II–VI = CT-positive (pathological findings in CT)

4.2.2 Study II

Study II included 130 patients with all severities of TBI, of whom 75 were CT-positive, and 55 were CT-negative. Demographics are presented in **Table 8**. The CT-positive patients were divided into three clusters based on lesion type according to the Helsinki CT scoring system **(Figure 6)**. Cluster 1 (CL1) included patients with contusions and focal lesions only, measuring < 25 cm^3, with normal suprasellar cisterns. Cluster 2 (CL2) and cluster 3 (CL3) included patients with mixed lesions over 25 cm^3 in volume, traumatic subdural haematomas (tSDH), and/or tICH. All patients with EDH were included in CL2. The most severely injured cluster was CL3,

including the patients with IVH, except for the two patients who also had EDH and were thus included in CL2. The CT-negative patients with TBI served as a reference cluster in this study. Admission day head CT scans and blood samples were used, and Aβ40, Aβ42, GFAP, H-FABP, IL-10, NfL, S100B, and t-tau were analysed from the blood plasma samples. The biomarkers were utilised to assess whether admission levels can differentiate individuals with various combinations of traumatic intracranial abnormalities from those with negative head CT scans. The purpose was also to study whether different injuries and their combinations could be separately detected using blood biomarkers.

Table 8. Demographics of Study II patients.

Variable	CL1, n = 9	CL2, n = 47	CL3, n = 19	CT-neg, n = 55	p-value
Age (years, mean±SD)	48.33±22.71	53.49±19.47	45.42±20.75	43.67±18.21	0.082
Sex (male/female), n(%)	7 (78) / 2 (22)	39 (83) / 8 (17)	15 (79) / 4 (21)	34 (62) / 21 (38)	0.0971
GCS [median (IQR)]	15 (13–15)	14 (9–15)	9 (4–11)	15 (14–15)	**< 0.0001**
mTBI, n(%)	7 (77.8)	29 (61.7)	3 (15.8)	51 (92.7)	
moTBI, n(%)	0 (0.0)	7 (14.9)	7 (36.8)	3 (5.5)	
sTBI, n(%)	2 (22.2)	11 (23.4)	9 (47.4)	1 (1.8)	
ISS [median (IQR)]					
total	6 (4–22)	16 (9–25)	25 (9–27)	3 (1–9)	**< 0.0001**
cranial	4 (4–16)	9 (4–16)	16 (9–25)	1 (1–4)	**< 0.0001**
extracranial	2 (0–16)	0 (0–8)	2 (0–9)	0 (0–8)	0.811
Extracranial injury, n(%)	3 (33)	18 (38)	7 (37)	19 (35)	
Pupil reactivity, n(%)					
Unreactive/reactive	0 (0) / 9 (100)	4 (10) / 36 (90)	5 (29) / 12 (71)	1 (2) / 53 (98)	**0.0065**
Outcome, n(%)					
Favourable (GOSE 5–8)	7 (78)	27 (57)	9 (47)	51 (93)	**< 0.0001[a]**
Unfavourable (GOSE 1–4)	2 (22)	20 (43)	10 (53)	4 (7)	
Complete (GOSE 8)	1 (11)	7 (15)	0 (0)	23 (42)	**0.0002[b]**
Incomplete (GOSE 1–7)	8 (89)	40 (85)	19 (100)	32 (58)	

EDH = epidural haematoma, GCS = Glasgow Coma Scale, GOSE = Glasgow Outcome Scale - extended, ISS = injury severity score, IVH = intraventricular haemorrhage, IQR = interquartile range, SD = standard deviation, SDH = subdural haematoma, TBI = traumatic brain injury, tICH = traumatic intracerebral haematoma. CL1: Focal contusions; CL2: Mixed lesions (SDH and tICH) and EDH; CL3: Mixed lesions (SDH and tICH) and IVH; CT-neg: CT-negative cluster. [a]GOSE 1–4 vs 5–8. [b]GOSE 1–7 vs 8. P-value < 0.05 was considered statistically significant.

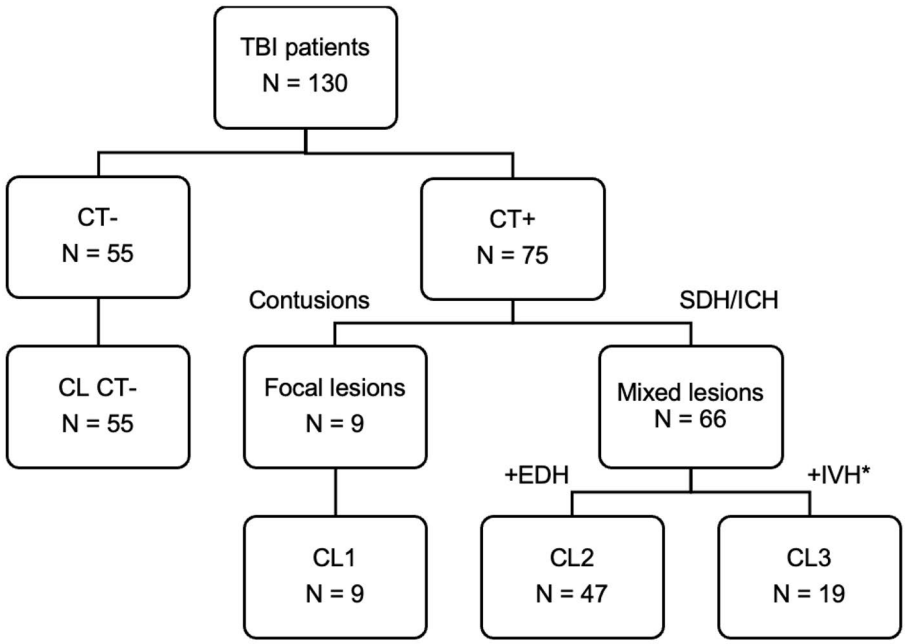

Figure 6. Categorization of the patients into clusters according to HCTS in Study II. *Two patients had both EDH and IVH and were included in C2. CL1 = Cluster 1, CL2 = Cluster 2, CL3 = Cluster 3, CL CT- = CT-negative cluster, CT = computed tomography, CT- = CT-negative patients, CT+ = CT-positive patients, EDH = epidural haematoma, IVH = intraventricular haemorrhage, SDH = subdural haematoma, TBI = traumatic brain injury, tICH = traumatic intracerebral haematoma. CL1: Focal contusions; CL2: Mixed lesions (SDH and tICH) and all EDH's; CL3: Mixed lesions (SDH and tICH), all patients had IVH, none had EDH.

4.2.3 Study III

In Study III, all patients were treated in the ICU at Turku University Hospital, and the severity of TBI ranged from mild to severe. A head CT scan was taken within 24 h of admission, and Marshall and Helsinki CT scores were evaluated from the scans. Blood samples were collected from patients on admission and on days 1, 2, 3, and 7. GFAP, IL-10, NfL, and t-tau levels were analysed from plasma samples. The biomarkers were screened for outliers. Outliers were defined as biomarker values lower than Q1 − 1.5 × IQR or higher than Q3 + 1.5 × IQR on any given day or for any biomarker. The patients were classified as outlier and non-outlier groups. The association between biomarker outliers and clinically significant events, pathological EEG activities, raised ICP, the need for neurosurgical intervention, and in-hospital mortality/poor outcome was evaluated. Patient demographics are presented in **Tables 9** and **10**.

Table 9. Demographics of Study III ICU patients.

Variable	Outlier group (N = 39)	Non-outlier group (N = 31)	p-value
Age, years, mean (SD)	51.17 (20.25)	47.29 (18.19)	0.407[a]
Sex, N (%, Female)	6 (15.38)	4 (12.9)	0.768[b]
GCS severity, N (%)			0.467[b]
Mild (13–15)	13 (33.33)	15 (48.39)	
Moderate (9–12)	11 (28.21)	10 (32.26)	
Severe (3–8)	15 (38.46)	6 (19.35)	
Marshall CT score, N (%)			0.348[c]
1	3 (7.69)	6 (19.35)	
2	7 (17.95)	9 (29.03)	
3	5 (12.82)	3 (9.68)	
4	0	0	
5	19 (48.72)	9 (29.03)	
6	5 (12.82)	4 (12.9)	
Helsinki CT score, N (%)			0.337[c]
-3	0	1 (3.23)	
-2	0	0	
-1	0	1 (3.23)	
0	3 (7.69)	3 (9.68)	
1	0	0	
2	1 (2.56)	3 (9.68)	
3	1 (2.56)	3 (9.68)	
4	3 (7.69)	4 (12.9)	
5	7 (17.95)	3 (9.68)	
6	4 (10.26)	1 (3.23)	
7	4 (10.26)	0	
8	2 (5.13)	0	
9	1 (2.56)	0	
10	4 (10.26)	2 (6.45)	
11	0	0	
12	1 (2.56)	1 (3.23)	
13	0	0	
14	0	0	
Missing	8 (20.51)	9 (29.03)	

[a]Student's t-test; [b]chi-square test; [c]Fisher's exact test. GCS = Glasgow Coma Scale, CT = computed tomography.

Table 10. Demographics of Study III ICU patients.

Variable	Outlier group (N = 39)	Non-outlier group (N = 31)	p-value
Pupil reactivity, N (%)			0.228[c]
Reactive	23 (58.97)	20 (64.52)	
Sluggish	1 (2.56)	1 (3.23)	
Unreactive	12 (30.77)	4 (12.9)	
Missing	3 (7.69)	6 (19.35)	
ISS total, median (IQR)	25 (18)	17 (16)	**0.002[d]**
ISS cranial, median (IQR)	16 (16)	9 (12)	**0.03[d]**
ISS extracranial, median (IQR)	8 (18)	2 (8)	0.242[d]
Neurosurgery, N (%)			0.178[c]
ICP	6 (15.38)	5 (16.13)	
Craniotomy	13 (33.33)	9 (29.03)	
Hemicraniectomy	6 (15.38)	0	
Ventriculostomy, trepanation	3 (7.69)	3 (9.68)	
No operations	11 (28.21)	14 (45.16)	
GOSE, N (%)			**0.033[c]**
1	13 (33.33)	3 (9.68)	
2	0	0	
3	6 (15.38)	2 (6.45)	
4	5 (12.82)	2 (6.45)	
5	5 (12.82)	5 (16.13)	
6	4 (10.26)	5 (16.13)	
7	5 (12.82)	6 (19.35)	
8	1 (2.56)	5 (16.13)	
Missing	0	3 (9.68)	
EEG, N (%)			**0.037[c]**
No seizures or SE	32 (82.05)	31 (100)	
Seizures	5 (12.82)	0	
Status epilepticus	2 (5.13)	0	
ICP burden, N (%)			0.186[b]
<20 mmHg	29 (74.36)	27 (87.1)	
>20 mmHg	10 (25.64)	4 (12.9)	
In-hospital mortality, N (%)			**0.018[c]**
Yes	10 (25.64)	1 (3.23)	
No	29 (74.36)	30 (96.77)	
Presence of major extracranial injury, N (%)			0.156[b]
Yes	23 (58.97)	13 (41.94)	
No	16 (41.03)	18 (58.06)	

[b]chi-square test; [c]Fisher's exact test; [d]Mann–Whitney U test. EEG = electroencephalogram, GOSE = Glasgow Outcome Scale extended, ICP = intracerebral pressure, ISS = injury severity score, SE = status epilepticus.

4.3 Biomarker analysis

Blood samples from the patients were collected within 24 h of hospital admission, between 8 a.m. and 10 p.m. (convenience sampling); in Study III, additional samples

were drawn on days 1, 2, 3, and 7. The blood samples were centrifuged, and plasma was stored at -70 °C for analysis. The plasma samples were analysed in laboratories in Geneva and Gothenburg. Unfortunately, for some patients, there was insufficient frozen plasma for all the biomarker analyses, which explains the missing data for those patients.

Table 11 describes the analysis methods for the biomarkers. The lower limit of detection (LLoD), lower limit of quantification (LLoQ), and calibration ranges were determined. For H-FABP and S100B, LLoQ has not been established. No samples were below the LLoDs and LLoQs. All the kits were used according to the manufacturer's recommendations. The measurements were performed by board-certified laboratory technicians, blinded to clinical data, using a single batch of reagents in a single round of experiments. Intra-assay coefficients of variation were monitored using high- and low-quality control samples that were common across plates fell below 10% for all analytes.

Table 11. Biomarker analysis methods.

Biomarker	LLoD	LLoQ	Calibration range	Assay
Aβ40 pg/ml	0.045	0.142	0–90.0	Duplex immunoassay[a]
Aβ42 pg/ml	0.142	0.69	0–11.0	Duplex immunoassay[a]
GFAP pg/ml	0.221	0.467	0.987–725	N4PA on an HD-1 Single molecule array[a]
H-FABP ng/ml	0.103	NA	0.137–100	K151HTD kit[b]
IL-10 pg/ml	0.04	0.298	0.0774–317	K151QUD kit[b]
NfL pg/ml	0.104	0.241	0.533–453	N4PA on an HD-1 Single molecule array[a]
S100B pg/ml	2.7	NA	2.7–2000	EZHS100B-33K kit[c]
t-tau pg/ml	0.024	0.053	0.136–112	N4PA on an HD-1 Single molecule array[a]

NA = Not available, N4PA = Human Neurology 4-Plex A assay. [a]Simoa assay by Quanterix, Billerica, MA, USA. [b]Meso Scale Diagnostics, Rockville, MD, USA. [c]Millipore, Billerica, MA, USA.

4.4 Head computed tomography scans

The head CT scans were taken within 24 h of hospital admission. Experienced neuroradiologists and neurosurgeons blinded to the study analysed the CT scans. Discrepancies in results were resolved through group discussions. For Study I, the Marshall grading system was used to assess the scans (Marshall et al., 1991). For Study II, outcome-weighted HCTS was used to classify the scans (Raj et al., 2014). For Study III, Marshall and HCTS were evaluated.

4.5 Severity assessment

The severity of TBI was assessed using the lowest GCS before possible intubation at the scene of the accident or at the ED. GCS 13–15 was classified as mTBI, GCS 9–12 as moTBI, and GCS 3–8 as sTBI.

4.6 Injury severity assessment

ISS was calculated for each patient to evaluate the extent of extracranial injuries. In addition to the total injury severity score, extracranial and cranial ISS were also evaluated separately in Studies II and III. Extracranial ISS greater than four was classified as a major extracranial injury to ensure that all injuries more severe than minor bruises were accounted for, as they may be a source of biomarker release.

4.7 Clinical outcome events

In Study III, the association of clinically significant events and biomarker outliers was studied. The events evaluated in ICU-treated patients were epileptic events (seizures or SE), high ICP, neurosurgical interventions, and poor outcome (GOSE 1–3).

4.8 Intracranial pressure

ICP was monitored in Study III in ICU-treated patients using continuous intraparenchymal methods, either the Neurovent-PTO (Raumedic AG, Helmbrecht, Germany) or the Codman® ICP monitoring system (DePuy Synthes, Wokingham, UK). The electronic patient record system in the ICU (Centricity ™ Critical Care 9.9 SP1, General Electric Company) was used to record the ICP values, which are transferred to the patient electronic data system once a minute. The cut-off value for increased ICP was 20 mmHg, and the time spent above 20 mmHg for each patient was calculated as ICP burden.

4.9 Electroencephalogram

In Study III, the ICU patient data were searched for electroencephalogram reports, and the patients were divided into three groups: no seizures, seizures, and SE; the biomarker outliers' association with the different groups was studied.

4.10 Neurosurgical interventions

Study III examined neurosurgical interventions performed in ICU patients. The procedures were divided into four categories according to their severity: 0 = no

surgery, 1 = inserting an ICP catheter, 2 = ventriculostomy or burr hole for acute SDH, 3 = craniotomy, 4 = decompressive hemicraniectomy, to evaluate the association between biomarker outliers and the need for neurosurgical intervention.

4.11 Glasgow Outcome Scale extended

The outcome was evaluated for most of the patients between six and 12 months post-injury. An experienced neurologist performed the assessment using the Glasgow Outcome Scale Extended (GOSE).

4.12 Statistical analysis

All available data from the studies were used without prior sample size estimation.

Data for Study I were analysed with IBM SPSS Statistics version 24 (IBM Corporation, Armonk, New York, USA). The patient demographics are displayed as mean ± SD. The elapsed time from injury to blood sampling is presented as median (IQR). The Kolmogorov–Smirnov test was used to assess the normality of the biomarkers. As the biomarker levels were not normally distributed, nonparametric tests were used, and the results were presented as medians (IQR). Correlations between biomarker levels and gender was assessed using Spearman's rank correlation and the correlations with age using Pearson's correlation for all severities of TBI. The levels of the biomarkers across severity levels were compared using the Mann–Whitney U test. It was also used to compare the biomarker levels between patients with mTBI admitted to the hospital and those discharged from the emergency department. Correction for multiple testing was not used. The threshold for statistical significance was set at $p < 0.05$.

The neurological symptoms for patients with mTBI may be very subtle and do not fulfil the head CT criteria. Thus, the area under the receiver operating characteristic (ROC) (pROC package for S+ version 8.1 [TIBCO, Software Inc.]) (Robin et al., 2013) curve (AUC) assessed the biomarkers' diagnostic capacity in distinguishing between orthopaedic controls and all patients with mTBI, as well as separately in patients with mTBI with or without CT abnormalities. An AUC < 0.7 was considered poor, AUC 0.8–1.0 good, and AUC 0.7–0.8 adequate. All tests were two-tailed. A clinically meaningful subset of the AUC curves (sensitivity range of 90%–100%) was compared using partial AUC (pAUC). Its value describes the ROC curve's pre-defined range of interest, omitting areas with low sensitivity or specificity.

Study I used PanelomiX (Robin et al., 2013) to evaluate biomarker combinations. It uses iterative permutation-response calculations. Iteratively, 2% increment quantiles were used to modify each molecule's cut-off values. The

specificity (SP) was calculated after each iteration using a sensitivity (SE) set between 90% and 100% to minimise the false negative cases of mTBI patients.

In each model, a maximum of three biomarkers or clinical parameters were examined. The model's performance was assessed using ROC analysis and cross-validation. Only patients with all evaluated parameters were included in the study when assessing a combination of biomarkers. The panel testing did not include any patients with missing data. The threshold, which was determined by setting the sensitivity above 90%, was cross-tabulated with the index test findings.

Data for Study II were analysed using the SAS System, version 9.4 for Windows (SAS Institute Inc., Cary, NC). The elapsed time from injury to blood sampling is presented as median (IQR). The mean and standard deviation (SD) were used to summarise continuous data with a normal distribution, while the median (IQR) was used to summarise other continuous variables. Frequencies and percentages were used to characterise categorical variables. One-way ANOVA or the nonparametric Kruskal–Wallis test was used to assess the differences between the continuous background variables or demographic factors and the clusters. Fisher's exact test or the chi-squared test was used with categorical variables. The normality of the biomarkers was assessed with the Kolmogorov–Smirnov test. Biomarker levels between the clusters were compared using the Kruskal–Wallis test. The p-values for multiple testing were corrected with a false discovery rate (FDR). A corrected p-value < 0.05 was considered statistically significant. Spearman correlation was used to analyse biomarker correlations with HCTS, GOSE, and age. AUROC curves were used to analyse the biomarkers' capacity to distinguish the clusters from CT-negative patients, where an AUC of 0.8–1.0 was considered good, AUC of 0.7–0.8 adequate, and AUC < 0.7 poor (Safari et al., 2016).

In Study III, patients with biomarker values below Q1 – 1.5 × IQR or above Q3 + 1.5 × IQR for any biomarker on any day of post-injury blood sampling were considered outliers (Korhonen et al., 2024). Categorical variables are reported as frequency and percentage, whereas continuous variables are reported as mean (SD) or median (interquartile range) (IQR). The outlier and non-outlier groups were compared using Fisher's exact test on the EEG data (seizures or SE) and the ICP data. Outliers were assessed using neurosurgical operation type in a multinomial logistic regression model, including age and sex. The association between in-hospital mortality or severe disability (GOSE 1–3) and outliers were assessed with logistic regression. A p-value less than 0.05 was considered statistically significant. Data were analysed using R statistical software (v4.5.1) (R Core Team, 2021).

5 Results

Most patients with TBI in each study group were male. Admission blood samples were obtained within 24 h of hospital admission, with a longer delay among those patients who did not arrive at the hospital on the day of the accident. In Study I, the median (IQR) time from injury to blood sampling was 15.6±12.4 h among patients whose time of injury was known (N = 84). For 105 patients with TBI and 33 orthopaedic controls, the time of injury was estimated with the information available in the patient records. Of those, 40 TBI and 21 controls were sampled within 24 h of the injury, whereas 65 TBI and 12 controls were sampled more than 24 h post-injury. Seven controls had no records of injury time; thus, estimation was impossible. In Study II, the time delay from injury to blood sampling was 11 h (4–18.5) for the patients (n = 56, 43%) whose injury time was known. The time of injury was unknown for 74 patients, 28 of those who were sampled within 24 h and 46 after 24 h of the injury.

5.1 Severity assessment and diagnostics (Study I)

The association between single biomarkers and the severity of TBI was studied **(Table 12** and **Figure 7)**. The results indicate significant differences in all individual biomarkers between mTBI and mo/sTBI, but no difference between patients with moTBI and sTBI.

There appeared to be no correlation with age or gender with the levels of IL-10 or S100B with any severity of TBI. In mTBI, H-FABP ($r = 0.300$, $p = 0.002$) and NfL ($r = 0.315$, $p = 0.002$) levels correlated positively with age, and males had higher NfL values than females, 14.40 (IQR 8.5, 19.95) vs 8.80 (IQR 6.7, 15.75), $p = 0.04$. No correlation was found with gender and H-FABP.

Lower levels of H-FABP, IL-10, and NfL were measured in discharged patients with mTBI compared to those admitted to the ward.

Table 12. Levels of single biomarkers in patients with TBI.

	Mild (N = 104)[a]	Moderate (N = 47)[a]	Severe (N = 33)[a]	P-value
IL-10[b]	0.436 (0.25, 0.89)	1.41 (0.67, 5.36)	1.38 (0.62, 4.33)	<0.0001
H-FABP[b]	5.17 (3.78, 10.41)	8.67 (5.47, 21.25)	12.66 (8.37, 46.11)	<0.0001
S100B[b]	78.05 (44.36, 114.39)	168.24 (63.14, 278.95)	184.45 (69.02, 498.87)	<0.0001
NfL [b]	12.35 (7.52, 19.02)	70.95 (49.75, 154.70)	79.4 (41.7, 179)	<0.0001

[a]For NfL, N(mTBI) = 98, N(moTBI) = 46, N(sTBI) = 31. [b]H-FABP (ng/ml), IL-10, S100B and NfL (pg/ml). All biomarker levels are reported as median (IQR).

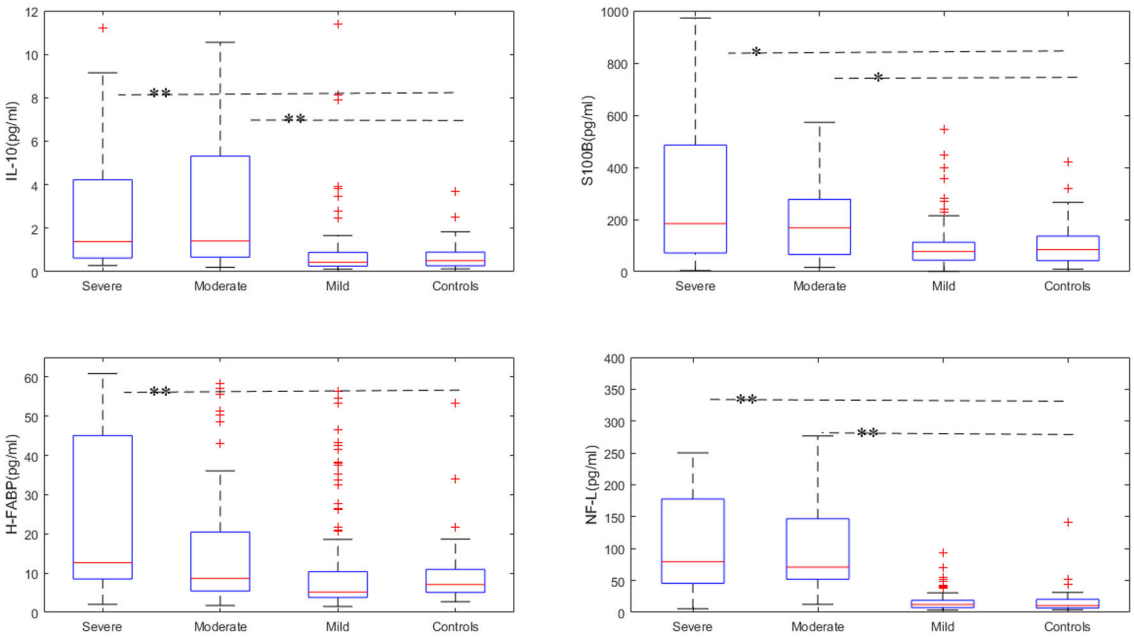

Figure 7. Box plot figures of patients with TBI and orthopaedic patients. IL-10: ** Difference between the patients with mTBI and moTBI ($p < 0.001$) and between mTBI and sTBI ($p < 0.0001$). S100B: * Difference between the patients with mTBI and moTBI ($p < 0.001$) and between mTBI and sTBI ($p < 0.0001$). H-FABP: ** Difference between the patients with mTBI and moTBI ($p < 0.001$) and between mTBI and sTBI ($p < 0.0001$). NfL: ** Difference between the patients with mTBI and moTBI ($p < 0.001$) and between mTBI and sTBI patients ($p < 0.0001$). Correction for multiple testing was not performed. The number of multiple tests requiring correction was 12 (number of biomarkers x number of groups, hence 4 x 3). If adjusted for this number, P-values below 0.05/12 would be considered statistically significant. Since our P-values are below 0.001 or 0.0001, all these results would still be statistically significant after correcting for multiple comparisons. Nevertheless, all P-values below 0.001 or 0.0001 are presented as $P < 0.001$ and $P < 0.0001$, respectively.

The biomarkers' ability to distinguish between patients with mTBI and orthopaedic controls was evaluated. PanelomiX, with sensitivity set to > 90%, was used to calculate the results in **Table 13.** None of the single biomarkers could distinguish patients with mTBI from the orthopaedic controls. The patients with mTBI were dichotomised into CT-negative and CT-positive groups; however, the biomarkers could not differentiate the subgroups from the controls.

Table 13. Individual biomarkers' ability to discriminate between patients with mTBI (n = 94, CT-positive and CT-negative) and orthopaedic controls (n = 39) with sensitivity set to >90%.

	AUC (95% CI)	pAUC (95% CI)	Threshold	SE (%) (95% CI)	SP (%) (95% CI)
H-FABP*	0.592 (0.495–0.688)	0.2 (0.0–0.8)	53.31	98.9 (96.8–100.0)	2.6 (0.0–7.7)
IL-10*	0.544 (0.438–0.649)	0.3 (0.0–1.2)	83.70	100.0 (100.0–100.0)	2.6 (0.0–7.7)
S100B*	0.527 (0.413–0.642)	0.7 (0.1–1.7)	244.90	94.7 (89.5–98.9)	10.3 (2.6–20.5)
NfL *	0.526 (0.416–0.636)	0.4 (0.0–1.4)	4.2	97.9 (94.7–100.0)	2.6 (0.0–7.7)

*H-FABP (ng/ml), IL-10, S100B and NfL (pg/ml). When the SE is set to > 90%, the examination area of the ROC curve covers only the range established between 90% to 100% SE. According to that, the pAUC values ranged from 1% to 10%, with 10 indicating a perfect partial ROC curve and 5 indicating non-relevant discrimination. SE = sensitivity, SP = specificity, Threshold = Biomarker concentration.

Biomarker panels were also evaluated with PanelomiX, with sensitivity set to > 90%, to assess if combinations of biomarkers could distinguish patients with mTBI from orthopaedic controls. The results from best panels, combinations of three biomarkers, are presented in **Table 14** indicating that none of the combinations could distinguish mTBI from orthopaedic controls.

Table 14. PanelomiX: Best biomarker panels to discriminate patients with mTBI (CT-positive and CT-negative) and orthopaedic controls with sensitivity set to > 90% (n(mTBI) = 94, n(mTBI, CT-negative) = 58, n(mTBI, CT-positive) = 36, n(orthopaedic controls) = 39).

	Number of biomarkers	IL-10 (pg/ml)	H-FABP (ng/mL) S100B (pg/ml)	NfL (pg/mL)	No of biomarkers needed to be +	Sensitivity (%) (95% CI)	Specificity (%) (95% CI)	pAUC (%) (95% CI)	p
mTBI vs controls	3	IL-10 (<0.359)	H-FABP (<4.66)	NfL (>11.8)	1	90.4 (84.0–95.7)	33.3 (20.5–48.7)	1.7 (0.8–3.2)	0.1494
mTBI (CT-) vs controls	3	IL-10 (<0.274)	H-FABP (<4.06)	NfL (>10)	1	91.4 (82.8–98.3)	30.8 (17.9–46.2)	1.8 (0.7–3.5)	0.32993
mTBI (CT+) vs controls	3	IL-10 (<0.269)	S100B (<47.9)	NfL (>12)	1	91.7 (80.6–100.0)	33.3 (17.9–48.7)	2.0 (0.7–3.9)	0.52813

When sensitivity is set to > 90%, the examination area of the ROC curve covers only the range from 90% to 100% SE. According to that, pAUC values that are displayed in this manuscript range from 1% to 10%, with 10 being a perfect partial ROC curve and 5 being a non-relevant discrimination. CT- = CT-negative, CT+ = CT-positive.

5.2 Evaluation of CT findings with biomarkers (Study II)

CT-positive patients were divided into three clusters, according to HCTS, and the CT-negative patients were used as controls **(Figure 6)**.

The CT-negative cluster was the least injured with the lowest ISS value, while CL3 was the most injured with the highest ISS, with medians (IQR) of 3 (1–9) and 25 (9–27) ($p < 0.0001$), respectively **(Tables 8** and **15)**. The number of patients with extracranial injury was similar across groups, ranging from 33% to 38% in each cluster. CL3 included the most severely injured patients, mostly mo/sTBI, n = 16 (84%). The CT-negative cluster was mostly mTBI and had the most favourable outcome (GOSE 5–8), n = 51 (93%).

Table 15. Comparison of the patient clusters.

Variable	p-value CL1 vs CL2	p-value CL1 vs CL3	p-value CL2 vs CL3	p-value CL1 vs CT-neg	p-value CL2 vs CT-neg	p-value CL3 vs CT-neg
GCS	0.270	**0.030**	**0.018**	0.265	**< 0.0001**	**< 0.0001**
ISS						
total	0.262	0.088	0.124	0.134	**< 0.0001**	**< 0.0001**
cranial	0.095	**0.016**	0.123	**0.003**	**< 0.0001**	**< 0.0001**
HCTSsum	**0.0031**	**< 0.0001**	**0.0013**			

CT-neg = CT-negative cluster, GCS = Glasgow Coma Scale, HCTS = Helsinki CT score, ISS = injury severity score. ISS cranial: the injury severity score due to the brain injury; ISS total: the injury severity score of the patient, including all the injuries (cerebral and external injuries). CL1: Focal contusions; CL2: Mixed lesions (SDH and tICH) and EDH; CL3: Mixed lesions (SDH and tICH) and IVH. P-value < 0.05 was considered statistically significant.

Biomarkers could distinguish between the CT-positive and CT-negative clusters with varying degrees (**Figures 8 a–c**). NfL and t-tau distinguished CL1 from the CT-negative cluster with AUCs of 0.737 and 0.771, respectively. GFAP, NfL, and t-tau distinguished between CL2 and the CT-negative cluster with AUCs of 0.840, 0.839, and 0.781, respectively. CL3, with the most severely injured patients, could be distinguished from the CT-negative cluster by all the studied biomarkers, with GFAP and NfL having the highest AUCs of 0.936 and 0.912, respectively.

The biomarker levels were compared across clusters. Aβ40, Aβ42, and GFAP differed between CL1, CL2, and CL3 with $p = 0.036$, 0.048, and 0.047, respectively.

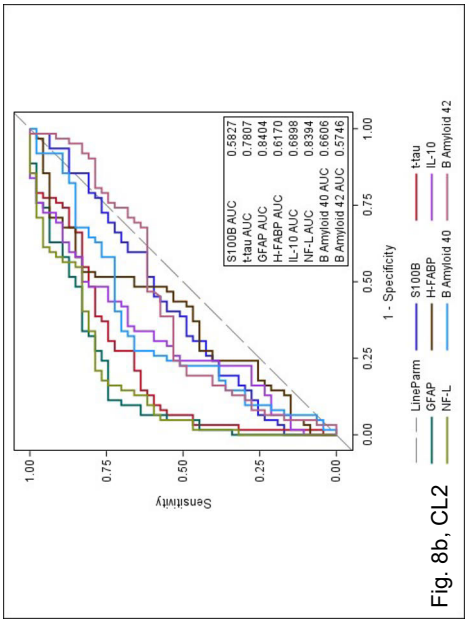

Fig. 8b, CL2

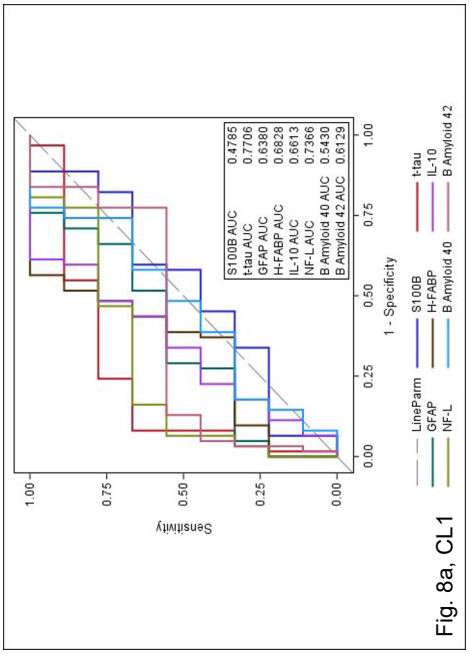

Fig. 8a, CL1

Figure 8 a-c. The ability of the biomarkers to distinguish between CT-negative and CT-positive clusters, CL1, CL2, and CL3, respectively, is shown in the AUC-ROC curves.

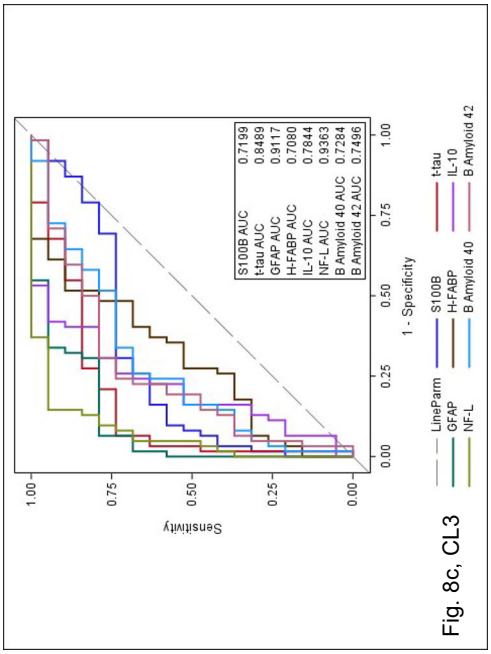

Fig. 8c, CL3

Table 16. Biomarker correlations between the CT-positive clusters 1–3 with the sum of HCTS, age, and GOSE.

Spearman Correlation Coefficients, N = 75
Prob > |r| under H0: Rho = 0

	HCTS score	S100B	t-tau	GOSE	Age	GFAP	H-FABP	IL-10	NfL	B-Amyloid 1_40	B-Amyloid 1_42
HCTS sum p-value	1.000	0.382 **0.0007**	0.415 **0.0002**	-0.371 **0.0010**	0.204 0.0786	0.491 **< 0.0001**	-0.0274 0.815	0.285 **0.0131**	0.442 **< 0.0001**	0.277 **0.0161**	0.103 0.382
S100B p-value	0.382 **0.0007**	1.000	0.728 **< 0.0001**	-0.433 **0.0001**	0.116 0.321	0.743 **< 0.0001**	0.401 **0.0004**	0.543 **< 0.0001**	0.483 **< 0.0001**	0.361 **0.0014**	0.179 0.125
t-tau p-value	0.415 **0.0002**	0.728 **< 0.0001**	1.000	-0.382 **0.0007**	0.0216 0.854	0.850 **< 0.0001**	0.247 **0.0323**	0.496 **< 0.0001**	0.708 **< 0.0001**	0.192 0.0991	0.181 0.121
GOSE p-value	-0.371 **0.0010**	-0.433 **0.0001**	-0.382 **0.0007**	1.000	-0.377 **0.0009**	-0.401 **0.0004**	-0.209 0.0714	-0.320 **0.0051**	-0.409 **0.0003**	-0.252 **0.0293**	-0.229 **0.0480**
Age p-value	0.204 **0.0786**	0.116 0.321	0.022 0.854	-0.377 **0.0009**	1.000	0.184 0.115	0.135 0.247	0.0789 0.501	0.315 **0.0059**	0.101 0.388	0.0871 0.457
GFAP p-value	0.491 **< 0.0001**	0.743 **< 0.0001**	0.850 **< 0.0001**	-0.401 **0.0004**	0.184 0.115	1.000	0.141 0.229	0.454 **< 0.0001**	0.708 **< 0.0001**	0.244 **0.0349**	0.159 0.174
H-FABP p-value	-0.0274 0.815	0.401 **0.0004**	0.247 **0.0323**	-0.209 0.0714	0.135 0.247	0.141 0.229	1.000	0.448 **< 0.0001**	0.201 0.0833	0.0749 0.523	0.258 **0.0252**
IL-10 p-value	0.285 **0.013**	0.543 **< 0.0001**	0.496 **< 0.0001**	-0.320 **0.0051**	0.0789 0.501	0.454 **< 0.0001**	0.448 **< 0.0001**	1.000	0.437 **< 0.0001**	0.122 0.296	-0.00307 0.979
NfL p-value	0.443 **< 0.0001**	0.483 **< 0.0001**	0.708 **< 0.0001**	-0.409 **0.0003**	0.315 **0.0059**	0.708 **< 0.0001**	0.201 0.0833	0.437 **< 0.0001**	1.000	0.218 0.0598	0.144 0.217
Aβ40 p-value	0.277 **0.016**	0.361 **0.001**	0.192 0.0991	-0.252 **0.0293**	0.101 0.388	0.244 **0.0349**	0.0749 0.523	0.122 0.296	0.218 0.0598	1.000	0.313 **0.0062**
Aβ42 p-value	0.103 0.382	0.179 0.125	0.181 0.121	-0.229 **0.0480**	0.0871 0.457	0.159 0.174	0.258 **0.0252**	-0.00307 0.979	0.144 0.217	0.313 **0.0062**	1.000

Statistically significant values ($p < 0.05$) are highlighted. GOSE = Glasgow Outcome Scale – extended, HCTS = Helsinki CT score.

Table 16 demonstrates the correlations between different biomarkers, age, the HCTS sum, and GOSE. There was a significant positive correlation with the levels of Aβ40, GFAP, NfL, t-tau, IL-10, and S100B, and a negative correlation with GOSE. Age, H-FABP, and Aβ42 levels demonstrated a weak association with the HCTS sum. Age significantly correlated positively with NfL and negatively with GOSE.

5.3 Clinical events in ICU and biomarker outliers (Study III)

No significant difference was observed in GCS, pupillary reactions, or head CT scans between the outlier and non-outlier groups. However, the patients in the outlier group were more severely injured, with a significant difference between the outlier and non-outlier groups in total and cranial injury severity scores (ISS), $p = 0.002$ and 0.03, respectively. Extracranial injuries did not differ between the groups.

All studied biomarkers (GFAP, IL-10, NfL, t-tau) exhibited outliers presented in a heat map according to sampling dates **(Figure 9)**. All outliers were above Q3 + 1.5 × IQR **(Table 17)**.

Table 17. Plasma concentrations of biomarker outliers.

Biomarker	Admission median (IQR)	Day 1 median (IQR)	Day 2 median (IQR)	Day 3 median (IQR)	Day 7 median (IQR)
GFAP (ng/ml)	25.90 (66.52)	17.02 (31.03)	20.49 (49.83)	10.83 (39.61)	3.29 (3.74)
IL-10 (pg/ml)	1.78 (5.06)	1.11 (2.60)	0.80 (1.51)	0.71 (1.02)	1.22 (1.11)
NfL (pg/ml)	71.64 (139.76)	84.26 (98.71)	106.94 (104.38)	93.23 (80.26)	389.26 (332.76)
t-tau (pg/ml)	28.24 (49.70)	1.99 (2.29)	1.43 (2.18)	1.34 (1.12)	0.93 (0.90)

GFAP: N(admission) = 3, N(D1) = 7, N(D2) = 6, N(D3) = 4, N(D7) = 4; IL-10: N(admission) = 9, N(D1) = 6, N(D2) = 6, N(D3) = 4, N(D7) = 4; NfL: N(admission) = 7, N(D1) = 5, N(D2) = 5, N(D3) = 5, N(D7) = 2; t-tau: N(admission) = 4, N(D1) = 4, N(D2) = 5, N(D3) = 6, N(D7) = 3. IQR = interquartile range.

Figure 9. Biomarker outliers by sampling dates.

All patients with seizures or status epilepticus (SE) were in the outlier group (p = 0.037). No difference in ICP burden was observed between the study groups nor in the total number of neurosurgical procedures (**Table 10**). However, **Table 18** demonstrates a strong positive association between hemicraniectomy and the outlier group (p < 0.0001). Several patients (N = 9, 12.9%) underwent multiple neurosurgical interventions. Craniotomy was performed on five patients (71.4%) who later experienced epileptic seizures; however, one patient with SE also required decompressive hemicraniectomy. The outlier group had a significantly higher rate of in-hospital mortality or poor outcome (GOSE 1–3) than the non-outlier group, p = 0.012.

Table 18. Outlier association with neurosurgical procedures.

Surgery type	Predictor	Estimate	Std. Error	Odds Ratio	p-value
Craniotomy	Any_iqr	0.57	0.61	1.77	0.35
	Age	0.0091	0.017	1.01	0.60
	Sex=male	-0.76	1.00	0.469	0.45
Hemicraniectomy	Any_iqr	18.66	0.85	>> 100,000	**< 0.0001**
	Age	-0.048	0.029	0.95	0.097
	Sex=male	-2.61	1.31	0.074	**0.047**
Ventriculostomy	Any_iqr	0.29	0.92	1.33	0.76
	Age	-0.022	0.025	0.98	0.37
	Sex=male	11.72	0.66	>> 100,000	**< 0.0001**
ICP monitoring	Any_iqr	0.68	0.77	1.97	0.38
	Age	-0.033	0.021	0.97	0.11
	Sex=male	-0.64	1.12	0.53	0.57

Any_iqr = any outlier biomarker at any sampling day. The significant results are bolded.

6 Discussion

6.1 Results

The main results from Study I were that S100B, H-FABP, NfL, and IL-10 levels increase with the severity of brain injury. mTBI and the orthopaedic controls could not be distinguished with S100B, H-FABP, NfL, and IL-10 or their combinations. In Study II, all biomarkers distinguished the most severely injured patient cluster (CL3), but brain lesion combinations could not be identified using these biomarkers. The main finding from Study III was that all epileptic seizures only occurred in the outlier group. Decompressive hemicraniectomies were also performed only on patients in the outlier group, who were also more likely to have a poor outcome (GOSE 1–3) than patients in the non-outlier group.

6.2 Biomarkers in acute diagnostics and severity assessment (Study I)

Significantly higher levels of S100B have been reported in CT-positive patients with mTBI than in CT-negative patients (Lagerstedt et al., 2018a). Scandinavian guidelines advise using S100B obtained less than six hours post-injury to assist in determining the need for a head CT scan (J. Undén et al., 2013). External cohorts have validated the proposed method (L. Undén et al., 2015). In Study I, however, S100B could not distinguish between CT-positive and CT-negative study patients, nor head injury patients, from the orthopaedic controls. These findings are similar to previous studies (Ohrt-Nissen et al., 2011). The results are probably because S100B is not brain-specific, and the time of admission blood sampling for most patients exceeded the recommended six hours. The analysis used in Study I for S100B was Millipore rather than the previously used Elecsys, which may have influenced the results. If S100B is unavailable within six hours of trauma or in cases of extracranial injuries, a head CT scan should be performed according to Scandinavian standards.

CT-positive patients with mTBI have significantly higher H-FABP levels than CT-negative patients (Lagerstedt et al., 2018a; Posti et al., 2019). In Study I, no difference was observed in the level of H-FABP between orthopaedic controls and patients with mTBI, with or without CT abnormalities. H-FABP has rapid kinetics

(Lippi et al., 2014), thus necessitating blood samples to be drawn promptly post-injury; however, sampling was mostly delayed beyond this timing. H-FABP is not brain-specific, and high levels have been detected in polytrauma patients compared to patients with isolated TBI (Walder et al., 2013). Increased levels of H-FABP were also detected in the orthopaedic control patients. Orthopaedic trauma and TBI may cause an additive increase in biomarker levels.

Research shows that CT-positive patients with mTBI have significantly higher levels of NfL than CT-negative patients (Posti et al., 2019). In Study I, admission NfL levels did not differ between CT-positive or CT-negative patients with mTBI and orthopaedic controls. Previous studies have shown that NfL has a very long half-life, and blood samples (Thelin et al., 2017b) might be more advantageous at later time points.

The biomarker levels of the orthopaedic controls and mTBI patients with or without abnormal CT findings did not differ, suggesting that IL-10 also rises in orthopaedic trauma.

TBI is a complex injury, including multiple structures. Biomarker panels have been studied to achieve better precision than with individual biomarkers alone, as they represent different brain structures. CT-negative and CT-positive mTBIs (Lagerstedt et al., 2018a; Posti et al., 2019), as well as all severities of TBIs (Posti et al., 2019), have been distinguished with biomarker panels. H-FABP, S100B, and tau combined in a panel best discriminated CT-positive patients with mTBI from CT-negative patients, whereas GFAP, H-HABP, and IL-10 were the best combination for patients with all severities of TBI (Posti et al., 2019). In Study I, the biomarker panels' ability to distinguish orthopaedic controls from patients with mTBI, with or without CT findings in the acute phase, was evaluated. None of the biomarkers or their combination panels could distinguish between patients with mTBI and orthopaedic controls. The hypothesis was that the patients, most of whom had a GCS 15, had only minimal head injury; thus, this study should be repeated with patients with a GCS of 13–14, who have an indication for a head CT.

Age did not affect S100B levels in this study of patients older than 65 with mTBI. This conflicts with a previous study (Calcagnile et al., 2013), which used a cut-off point of age 65, whereas Study I did not. Prior neurological conditions have been shown to affect the results (Calcagnile et al., 2013). NfL has been reported to increase with age (Iverson et al., 2019), and higher levels of NfL and H-FABP were observed in older patients with mTBI in this study. Certain TBI biomarkers will likely require age-related cut-offs for increased levels. Only NfL levels were affected by gender in mTBI, with females having lower biomarker levels than males.

The studied biomarkers or their panels could neither distinguish patients with mTBI from orthopaedic controls nor aid in decision-making concerning the requirement for head CT scanning. This research emphasises the importance of

evaluating the applicability and dependability of numerous diagnostic biomarkers in a range of patient demographics and at different intervals following TBI.

6.3 Identification of intracranial lesion combinations with blood biomarkers (Study II)

Intracerebral pathologies are known to elevate biomarker levels, but the underlying pathophysiology remains poorly understood. Previous studies have focused on admission biomarkers' ability to identify CT-positive patients, but only a few have examined the relevance of different lesion types (Undén et al., 2009; Whitehouse et al., 2022b; Wolf et al., 2015).

Head CT pathology assessed with a Marshall CT score was examined in a CENTER-TBI study, where large intraparenchymal haemorrhage, oedema, and IVH elevated the biomarker values the most; however, the extent and number of lesions were considered more important than the lesion type. TBI is typically characterised by multiple injured structures and lesions, and the number of patients with a single specific lesion was small, making lesion-based diagnostics unsuccessful (Whitehouse et al., 2022b).

In Study II, the patients were divided into three clusters according to outcome-weighted HCTS (Raj et al., 2014), unlike the above study (Whitehouse et al., 2022b). The clinical diagnostic significance of biomarkers with different lesion combinations was evaluated. All other biomarkers, except Aβ42 and H-FABP, showed a substantial positive correlation with the HCTS sum, while GOSE showed a negative correlation.

EDH has been reported to have a better prognosis, while SDH, traumatic ICH, tSAH, and IVH have been linked to unfavourable outcomes (Maas et al., 2007). The HCTS classification does not include tSAH, as it is not an independent predictor (Raj et al., 2014). As most of the study patients with mixed lesions had SDH, tICH, or both, they were categorised into groups according to whether they had IVH or EDH. According to Whitehouse et al. (2022b), biomarker levels indicated the extent of injury rather than serving as precise lesion markers. This aligns with our findings, as the most injured cluster, CL3, was distinguished from the CT-negative cluster by all studied biomarkers with an AUC > 0.7. In CL3, 84% of patients had moTBI or sTBI, while almost all patients (93%) in the CT-negative cluster had mTBI. Only GFAP and NfL showed good discriminatory capacity, with AUCs of 0.84 and 0.839, respectively, for separating CL2 from the CT-negative cluster, whereas t-tau had an acceptable AUC of 0.781. The discriminatory ability of other biomarkers was poor.

The patients in CL1 only had focal contusions, and NfL and t-tau were the only biomarkers to reach an AUC of > 0.7. The number of patients in this cluster is

relatively small, though, and definite conclusions should not be drawn from these results alone.

The severity of extracranial injuries was estimated using ISS scores because the biomarkers are not brain-specific. In each cluster, the percentage of patients with significant extracranial injuries ranged from 33% to 38%. CL1 had somewhat higher extracranial ISS values than CL2, CL3, and the CT-negative cluster, which all had similar ISS scores.

Aβ40 levels differed most between the CT-positive clusters. CL3 had higher GFAP, Aβ40, and Aβ42 values than CL1. Unlike its surprisingly weak performance in identifying patients with focal lesions, GFAP's diagnostic value was highest in detecting patients with mixed intracranial damage. In the literature, GFAP levels have been linked to the degree of intracranial injury and the clinical severity of TBI (Bazarian et al., 2018; Posti et al., 2016), which increases with age (Gardner et al., 2018). The GFAP results and variations in biomarker levels of S100B, Aβ40, and Aβ42 compared to CL3 may be explained by the fact that the patients in CL1 had only very minor localised lesions. Because only nine patients were in cluster CL1, there is significant diversity among them, which could skew the results.

Injury type affects the S100B levels; however, results have been somewhat contradictory. High S100B levels have been associated with cerebral oedema, and significantly lower values were found with EDH, SDH, SAH, and contusions, with the lowest levels in concussion (Wolf et al., 2015). S100B is raised by IVH (Whitehouse et al., 2022b), which aligns with our results from CL3.

When predicting CT pathology, GFAP outperforms several biomarkers (e.g., S100B, neuron-specific enolase, UCHL-1, NfL, and t-tau), their combinations, or GCS. Thus, GFAP has been recommended to be added to an assay to be utilised in the clinical context of TBI triage and decision-making (Czeiter et al., 2020).

SDH and tICH are the most common traumatic intracranial mass lesions. Large studies show that the initial aggressive operational treatment does not yield better outcomes than initial conservative treatment with SDH (van Essen et al., 2022). While conservative initial therapy was associated with improved outcomes in mTBI, early operation seemed favourable for large tICH in moTBI (van Erp et al., 2023). Currently, the decision to operate is surgical and based on clinical and imaging findings. The number of patients in our study was too small to examine the relationship between biomarkers and surgical decision-making.

The findings showed that the biomarkers studied could distinguish between the most severely injured cluster with mixed lesions, and IVH, and the CT-negative cluster with TBI. Traumatic intracranial pathologies can occur in various combinations; because of this wide range, not all may raise biomarker levels to diagnostic levels, particularly in patients who are still conscious while examined in the ED. A larger group of patients warrants further study.

6.4 Are the clinically significant events in the ICU associated with biomarker outliers? (Study III)

Primary brain injury triggers the secondary injuries – the main targets of TBI treatment (Orr et al., 2024). The development of secondary injuries is difficult to predict (Lazaridis et al., 2019). This study focused on the biomarker outlier levels and their possible association with clinically significant situations linked to secondary injuries during the first week of ICU treatment.

Blood biomarkers are not brain-specific, and their levels may be elevated not just due to TBI but also to e.g., extracranial injuries, old age, and prior neurological conditions. GFAP increases with age (Eng et al., 2000; Gardner et al., 2018), whereas NfL and tau increase in extracerebral injuries (Posti & Tenovuo, 2022). In TBI, early hypotension, alone or combined with hypoxia, elevates S100B, tau, and NfL (Robba et al., 2024).

Biomarker outliers have been studied in admission blood samples for GFAP, NfL, IL-10, and t-tau in patients with mo/sTBI (Korhonen et al., 2024). Seriously injured patients with TBI had markedly high values of GFAP, whereas patients with severe extracranial injuries had high values of IL-10. NfL values were elevated by old age and pre-existing neurological conditions, which also elevated t-tau values. In orthopaedic controls, multi-trauma caused high values of IL-10, whereas old age and pre-existing neurological conditions explained high levels of GFAP and NfL. Multi-trauma led to elevated t-tau in a few patients, but the cause of t-tau outliers remained mostly unknown. Poor outcome was associated with high GFAP levels (Korhonen et al., 2024).

Patients in this study were treated in the ICU but represented all severities of TBI. This profile is consistent with previous studies indicating that, among the patients with TBI treated in the ICU, fewer than half are sTBI and more than a third are mTBI with severe extracranial injuries (Huijben et al., 2020) or are at risk of neurological or clinical decline (Bonow et al., 2019). The patients initially categorised as mTBI in Study III were treated in the ICU due to severe extracerebral injuries or neuroworsening after the initial severity categorisation. The GCS and extracranial ISS between the outlier and non-outlier groups were similar. Total ISS and cranial ISS, however, differed significantly, with the difference in the total ISS score likely due to cranial ISS, suggesting that the patients in the outlier group had a more severe brain injury.

The reported incidence of early posttraumatic seizures after TBI varies considerably from 0.4% in the USA (DeGrauw et al., 2018; Majidi et al., 2017) up to 5.6% in Norway (Sødal et al., 2022) and 8.6% in India (Thapa et al., 2010). The large gap may reflect differences in the definitions, data collection, or reporting methods. Posttraumatic seizures after TBI cause increased admission to the ICU and lengthen the ICU stay; the patients also seem to have a poor outcome, including

increased long-term mortality (Laing et al., 2022). Alcohol abuse, SDH, and brain contusions have been identified, among others, as independent risks of seizures after mo/sTBI (Majidi et al., 2017; Wiedemayer et al., 2002); however, TBI severity may also participate in the occurrence of seizures (Laing et al., 2022). A significant difference was observed between the outlier and non-outlier groups in the occurrence of epileptic activity in our study. None of the patients with seizures or SE were in the non-outlier group, indicating that epileptic activity may reflect the severity of TBI. The outlier group also had a poorer outcome than the non-outlier group. This suggests that those in the outlier group were more severely injured than those in the non-outlier group, which is consistent with previous studies (Laing et al., 2022).

The standard of care for TBI with suspected high intracranial pressure is invasive ICP monitoring, which is associated with better outcomes with severe TBI (Slot et al., 2025). The association between ICP burden and biomarker outlier levels was examined, but no difference in the ICP burden between the outlier and non-outlier groups was observed. However, this may be due to the urgent need to treat high ICP, as ventriculostomies and decompressive hemicraniectomies were performed in the outlier group to lower the ICP.

The neurosurgical operations required for the patients were categorised from minimal to major operations, ranging from insertion of an ICP monitoring catheter to decompressive hemicraniectomy. The outlier status had a strong positive association with decompressive hemicraniectomy, but given the number of patients in the study, interpretation should be made cautiously. A borderline association with age was observed for hemicraniectomy ($p = 0.097$); otherwise, age did not show a significant association with operations. None of the predictors reached significance with craniotomy.

A significant difference was observed between the outlier and non-outlier groups in in-hospital mortality and poor outcome, as patients in the outlier group were significantly more likely to have a poor outcome (GOSE 1–3) than those in the non-outlier group. This aligns with previous findings that high levels of biomarkers are associated with the severity of TBI (Whitehouse et al., 2022b).

An association appeared to exist between biomarker outliers and epileptic features, decompressive hemicraniectomy, and poor outcome. However, this finding may reflect the greater baseline severity of TBI in the outlier group.

6.5 Methodological considerations

Sample size estimation was not performed before the studies, as these studies were a part of a larger study, and all available data was used. The blood samples for biomarker evaluation were collected from patients within 24 h of admission. However, sampling was not always conducted within 24 h of the injury, as patients

may not arrive at the hospital immediately post-injury, and blood samples were collected only during the day. This may affect the results, as the optimal timing of sampling varies across biomarkers, and some of our admission samples were collected beyond this window. In Study III, the injury time was adjusted for, and the sampling days were calculated from the injury.

None of the studied biomarkers is brain-specific, which may lead to an unknown rise in their levels from other sources.

In Study II, CT-negative patients were used as controls. While the CT scans remained negative, the patients were diagnosed with TBI with unknown levels of released biomarkers due to, for example, DAI. The interest in Study II was in the biology underlying the lesions identified on CT scans and their association with biomarker levels. However, due to the complexity of TBI, the sample size was too small, and the study would require a much larger patient population.

In Study III, ICU-treated patients were examined to clarify the possible association between biomarker outliers and clinically significant events during the ICU treatment. The exact timing of blood sampling was unknown, limiting the possibility for further analysis. The increased ICP episodes were not assessed separately; instead, the cumulative ICP burden was calculated over the entire ICU stay, as this is associated with increased six-month mortality (Güiza et al., 2015).

Control head CT scans were performed on a subset of deteriorating patients, but not for all patients. The control scans were not analysed according to the Marshall CT score or HCTS, further complicating the analysis of possible deterioration of the patients. During ICU stay, seizures are difficult to diagnose as the seizures are commonly nonconvulsive and may vary from periodic electrographic seizures to status epilepticus. Once a day, obtained biomarkers likely cannot predict single epileptic seizures. An EEG is always needed to confirm epileptic seizures or SE (Alkhachroum et al., 2022). An association appears to exist between the biomarker outliers and the clinical events; however, the biomarker outliers may have had more severe TBI, and the results reflect this.

6.6 Limitations of the studies

In Studies I and II, only admission blood samples were used, providing only a narrow window into the dynamic pathophysiological processes of TBI. All substudies used the same admission blood biomarker samples. The exact time of the accident was not known for all the patients, causing delays of unknown duration in admission and blood sampling. This may have influenced biomarker levels, especially those with a short half-life. However, this is the clinical reality and will likely remain a challenge following TBI. Also, the GCS-based TBI severity evaluation is arbitrary and does not accurately reflect the biological gravity of the trauma because it is defined at one

specific moment. The percentage of patients with mTBI was lower than in many other trials because the recruitment strategy preferred patients with mTBI who were admitted to the ward. Thus, the findings might be irrelevant to the mildest mTBI patients who are discharged from the emergency department, possibly even without a head CT scan.

In Study II, the biggest limitation was the small sample and cluster sizes. As TBI is a complex injury affecting multiple structures, the number of possible lesion combinations is considerable, requiring a sample of patients for robust results. Seeing the biomarker levels in isolated lesions would have been intriguing; however, not enough information was available. Another limitation was that clustering was not performed based on the severity of TBI, which may have led to variability in biomarker levels across clusters. Moreover, the CT-negative patients with TBI served as the reference cluster. While CT-negative, they still had TBI and may have had brain tissue injury with an unknown amount of biomarker release.

In Study III, daily blood biomarker samples were collected in addition to admission samples (days 1, 2, 3, and 7); however, the samples were collected only once a day and a few follow-up samples were missing for some patients. In this study, the number of patients remained relatively small, limiting the possibilities to study individual biomarkers or days; instead, group analysis was conducted. Also, the results for decompressive hemicraniectomy should be interpreted cautiously due to the small sample size, as the data can only show association but lack the power to predict. The effects of the neurosurgical procedures on the biomarker levels remain unknown. The severity of TBI, initially classified as m/mo/sTBI, was not considered in this analysis. This was a retrospective evaluation from a prospective observational study; the analysis depended on the recorded data that was available.

6.7 Clinical implications and future studies

In Study I, we demonstrated the biomarker levels were significantly lower in patients with mTBI than in those with mo/sTBI, suggesting that levels increase with increasing severity. Biomarkers could not distinguish mTBI and orthopaedic controls, indicating that biomarkers alone are insufficient to diagnose mTBI with the presence of an orthopaedic trauma. However, the new characterisation of TBI is under evaluation, as the severity assessment based on GCS is not accurate enough, and more individualised characterisation, based on pathoanatomical features, among others, has been proposed.

The findings in Study II showed that the extent and severity of the lesions seemed more important than the lesion type. However, because the number of patients in the study was small compared to the possible combinations of lesions, the study should

be conducted on a larger scale to identify associations between lesion types and biomarker levels.

In Study III, the association between biomarker outliers and clinically significant outcome events (i.e., epileptic seizures, decompressive hemicraniectomy, and poor outcome) appeared to exist. However, the result may be due to the more severe TBI in the outlier group. Thus, the experiment should be reproduced on a larger scale to distinguish whether the association is caused by the clinical events or baseline severity of TBI. A robust procedure for timing and the number of blood samples per day, as well as control CT scans, should be planned. The effects of confounding factors should be minimised.

7 Summary and Conclusions

1. S100B, H-FABP, NfL, and IL-10 levels were significantly higher in patients with mo/sTBI than in patients with mTBI. None of the biomarkers, alone or in combination, could distinguish patients with mTBI, with or without CT findings, from orthopaedic patients, suggesting that biomarkers alone are insufficient for diagnosing acute-phase mTBI in trauma patients.
2. Admission levels of Aβ40, Aβ42, GFAP, H-FABP, IL-10, NfL, S100B, and t-tau were compared with head CT scans obtained at admission. Patients were divided into clusters based on HCTS. All biomarkers could distinguish CL3, the most severely injured cluster; however, isolated lesions or lesion combinations could not be distinguished using biomarkers.
3. The association between outlier levels of GFAP, NfL, IL-10, and t-tau with clinically meaningful events and GOSE was examined in ICU-treated patients with TBI. Outlier biomarker levels were associated with epileptic seizures or SE, decompressive hemicraniectomy, and poor outcomes. However, these results may reflect the greater baseline severity of TBI.

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