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Exploring the potential for biomarkers to aid forensic diagnosis of traumatic brain injury (TBI) – a systematic literature review and meta-analysis

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Declaration

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Contents

Abstract

Background: Traumatic brain injury (TBI) is a prevalent condition worldwide. Understanding its pathophysiology is imperative for clinical diagnosis, treatment, and cause of death determination. Biomarkers could offer potential insight. Proteins involved in neuroinflammation, such as systemic inflammatory biomarkers interleukin (IL)-1β, IL-6, and IL-10 and astroglia-associated biomarkers S100 Calcium-Binding Protein B (S100β) and Glial Fibrillary Acidic Protein (GFAP), have been assessed as potential TBI biomarkers. The aim of this review was to evaluate recent articles that investigated these biomarkers in relation to TBI and relate this to a forensic diagnostic context.

Methods: This review included 44 peer-reviewed articles from three major literature databases published from 2018 onwards, that investigated either IL-1β, IL-6, IL-10, GFAP, S100β, or a combination thereof, in relation to TBI. Studies conducted in a clinical or forensic setting were included. A meta-analysis was conducted on a subset of these studies.

Results: Majority of the biomarkers were elevated in TBI versus control groups. The most promising biomarkers were GFAP and S100β, which in addition to being elevated also correlated with unfavourable outcomes and TBI severity. GFAP alone was increased in TBI patients with positive CT scans. The ILs had inconclusive results due to minimal studies and inconsistent study designs. A wide range of biomarker expression levels were noted across all articles (from 0.01 to 1.5 million pg/mL). The meta-analysis yielded a pooled effect size of 0.97.

Discussion: Inconsistencies in results could potentially be explained by heterogenous TBI and control groups, various body specimens, and different immunoassays used. Thus, each biomarker should be investigated systematically whilst keeping other variables consistent to ensure definitive conclusions. Overall, none of the proteins could function as biomarkers of TBI alone. However, the meta-analysis did indicate a moderately significant association between biomarker levels and TBI occurrence. Future studies are needed to corroborate the findings.

Acknowledgements

I would like to express my deepest gratitude to my primary supervisor, Dr Shameemah Abrahams, as well as my co-supervisors, Associate Professor Laura Heathfield and Dr Itumeleng Molefe. Their guidance and insightful feedback were an immense help throughout this entire process. Dr Shameemah Abrahams in particular went above and beyond in providing me with expertise and encouragement as well as answering my every question, for which I am really thankful. I would also like to extend my gratitude to the other staff and students in the Division of Forensic Medicine and Toxicology at UCT for their insight and continuous motivation.

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Abbreviations

AC	All controls
AMI	Acute myocardial infarction
aTBI	Acute traumatic brain injury
AUC	Area under the curve
BBB	Blood brain barrier
CB	Cerebellum
CC	Community controls
CDC	Centers for Disease Control and Prevention
CLC	Contralateral cortical area
CNS	Central nervous system
CSF	Cerebrospinal fluid
CT	Computed tomography
d	Day/s
DAMPs	Damage-associated molecular patterns
DCH	Diffuse cerebral hypoxia
dTBI	Delayed TBI
ELISA	Enzyme-linked immunoassay
EV	Extracellular vesicles
FMP	Forensic medical practitioner
GCS	Glascow coma scale
GFAP	Glial fibrillary acidic protein
GOS	Glasgow Outcome Scale
GOS-E/GOSE	Glasgow Outcome Scale - Extended
h	Hour/s
HC	Healthy controls
IA	Immunoassay
ICH	Intracerebral haemorrhage
IHC	Ipsilateral hippocampus
IL	Interleukin
IL-10	Interleukin-10
IL-1β	Interleukin-1 beta
IL-6	Interleukin-6
isTBI	Isolated severe TBI
ITT	Isolated torso trauma
LMIC	Low- or Middle-Income Country

mmTBI	Mild to moderate TBI
moTBI	Moderate TBI
MRI	Magnetic resonance imaging
mRS	Modified Rankin Scale
msTBI	Moderate to severe TBI
mTBI	Mild TBI
ns	Not significant
OC	Orthopaedic controls
PCZ	Pericontusional zone
PMI	Post-mortem interval
PMR	Post-mortem redistribution
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
PT	Polytrauma
RTA	Road traffic accident
S100β	S100 Calcium-Binding Protein β
saTBI	Subacute TBI
sTBI	Severe TBI
SNC	Scandinavian Neurotrauma Committee
SRM	Salt River Mortuary
TBI	Traumatic brain injury
TC	Trauma controls
USA	United States of America

1. Introduction

Traumatic brain injury (TBI) is an injury to, or pathology of, the brain and surrounding structures, resulting in a disruption to normal brain function. It can be caused either by direct mechanical impact to the head or by acceleration/deceleration forces (DiSabato, Quan & Godbout, 2016; Centers for Disease Control and Prevention (CDC), 2022; Reeder et al., 2023).

TBI is prevalent worldwide, with approximately 64–74 million new cases occurring annually. Dewan et al. (2018) have reported TBI incidences in continents such as North America (1299 cases per 100 000 people), Europe (1012 cases per 100 000 people), and Africa (801 cases per 100 000 people) (Dewan et al., 2018). However, it is possible that these figures are skewed by poor data capturing or underreporting of TBI cases, particularly in low- or middle-income countries (LMIC) where TBI incidence is believed to be highest (Dewan et al., 2018; Abio et al., 2021).

South Africa is one LMIC where TBI incidences are thought to be underreported. Rampant levels of violence in the country result in a significant burden of injury morbidity and mortality. These injuries are predominantly caused by interpersonal violence and road traffic accidents (RTAs) (Norman et al., 2007), which notably are also some of the leading causes of TBI (CDC, 2022). Furthermore, a study from 2017 conducted at Salt River Mortuary (SRM) in Cape Town investigated homicide cases caused by blunt force trauma, reporting that 93% of such cases involved the head region (Clark, Mole & Heyns, 2017). Thus, it is anticipated that a significant portion of the trauma cases experienced in South Africa involve TBI, which could potentially lead to a fatal outcome.

All suspected unnatural deaths in South Africa are mandated to be investigated by the forensic pathology services (*Inquests Act, No. 58 of 1959*). Fatal TBI cases will therefore be examined in the mortuary, where forensic medical practitioners (FMPs) will perform an autopsy to elucidate cause of death. However, in cases of mild TBI (mTBI) it can be challenging to ascertain the extent to which TBI contributed to the death. Unless there are concomitant pathological signs of TBI (such as contusions, lacerations, and bleedings), clinically mild or microscopic TBI or the presence of other competing fatal non-neurologic injuries may hinder the diagnosis of TBI as contributory to the cause of death. Furthermore, concomitant signs of TBI are heterogenous and may vary in severity, affecting their reliability (Li et al., 2009; Zwirner, Bohnert, et al., 2021).

To address both the severe global impact of TBI (by improving diagnostic, prognostic, and therapeutic tools) and the challenges of diagnosing TBI in forensic settings, it is imperative

that its mechanism be fully understood. TBI has a complex and heterogenous pathophysiology which consists of two distinct phases; primary and secondary brain damage. Primary brain damage is the immediate mechanical injury or trauma sustained upon impact, causing stretching, compression, tearing, and/or death of the nervous tissue (Han et al., 2014; Ladak, Enam & Ibrahim, 2019). Secondary brain damage encompasses any pathological cellular processes or biochemical changes that result from the initial (primary) mechanical injuries. This is because primary damage to nervous tissue can trigger the release of neurotransmitters, initiating (secondary) processes such as neuroinflammation, glutamate excitotoxicity, and membrane depolarization, that can ultimately lead to secondary cell death (Xiong, Mahmood & Chopp, 2018; Ladak, Enam & Ibrahim, 2019; Mafuika et al., 2022).

Knowledge of these pathophysiologic processes has enabled researchers to investigate various methods related to the diagnosis, prognosis, and treatment of TBI. One notable method is the use of biomarkers. Biomarkers have been shown to serve as reliable and objective indicators of other pathogenic processes (Califf, 2018) and have been used previously to aid in cause of death determinations (Belsey & Flanagan, 2016; Zwirner et al., 2022). Biomarkers have also been used to determine cause of death in cases of hypothermia, myocardial infarction, and drowning, which all present with non-specific pathological findings at autopsy (Zwirner et al., 2022). Since mTBI can also present with non-specific pathology, it is hypothesised that biomarkers could facilitate investigations into TBI-associated pathophysiological changes that are not always clear in the gross morphology. Additionally, biomarkers could be used as adjuncts to other methods, such as gross brain dissection or histopathology, to support the diagnosis of TBI as a sole or contributory cause of death by serving as promising objective measures (Ondruschka, Sieber, et al., 2018; Zwirner et al., 2022).

Neuroinflammation, which plays a large role in TBI pathophysiology (Rodney, Osier & Gill, 2018), has been postulated to involve pathways that could provide information relating to TBI (Zetterberg, Smith & Blennow, 2013). Indeed, research has investigated potential TBI biomarkers involved in neuroinflammation for their ability to: (i) diagnose TBI in both clinical and forensic settings, (ii) differentiate between TBI severities, (iii) predict whether computed tomography (CT) scans of these patients will show intracranial lesions, and (iv) predict injury outcome. The vast majority of these studies have investigated biomarkers in clinical settings (Posti et al., 2019; Lagerstedt et al., 2020; Schindler et al., 2020; Beard et al., 2021). However, there is a growing number of studies being conducted in post-mortem settings (Ondruschka, Schuch, et al., 2018; Sieber et al., 2018; Zwirner, Bohnert, et al., 2021) to aid in both determining TBI as the sole or contributory cause of death and/or estimating time since death. Numerous studies have measured biomarkers in various bodily fluids (Edwards et al., 2020;

Schindler et al., 2020; To et al., 2023). Extracellular vesicles (Puffer et al., 2020; Beard et al., 2021), exosomes (Mondello et al., 2020), and post-mortem brain tissue (Trautz et al., 2019; Zwirner, Lier, et al., 2021) have been assessed to a lesser extent. Post-mortem brain tissue in particular is thought to be beneficial for TBI investigations as it allows for direct exploration into the diseased tissue and enables a more comprehensive understanding of TBI (Lewis, 2002).

Cytokines involved in systemic inflammatory pathways, including proteins within the interleukin (IL) family (e.g., IL-1β, IL-6, and IL-10), and proteins involved within brain-specific inflammation, including astroglia-associated proteins (e.g., S100 calcium-binding protein β (S100β) and glial fibrillary acidic protein (GFAP)), comprise some of the commonly investigated biomarkers related to TBI inflammation. The proteins IL-1β, IL-6, and IL-10 have been shown to markedly increase in TBI patients compared to healthy individuals (Kumar, Boles & Wagner, 2015). Both IL-1β and IL-6 play a central role in coordinating neuroinflammatory cascades (Thome et al., 2020) and are expressed at the injury site following TBI. Indeed, IL-1β has been termed “one of the most important pro-inflammatory cytokines” (Ozen et al., 2020) and IL-6 as “one of the best characterised inflammatory markers in TBI” (Visser et al., 2022). IL-10 has been reported to be a crucial anti-inflammatory cytokine with a primary role of protection against traumatic insults (Tsitsipanis et al., 2023).

However, these biomarkers are not exclusive to the brain and thus may be elevated following other injuries, underscoring the need for more brain-specific biomarkers. Astroglia-associated biomarkers S100β and GFAP have been extensively studied in the literature due to their unique origin from glial cells in the central nervous system (CNS) (Mafuika et al., 2022) and have shown the most promising results (Abdelhak et al., 2022; Janigro et al., 2022). S100β has therefore been recommended for use as a biomarker for mild, low-risk TBI by the Scandinavian Neurotrauma Committee (SNC) (Faisal et al., 2023).

The aforementioned biomarkers have demonstrated promising results in research thus far. Considering this and the primary focus of this review being neuroinflammation following TBI, the biomarkers IL-1β, IL-6, IL-10, S100β, and GFAP were chosen for investigation. Incorporating both systemic inflammatory biomarkers as well as astroglia-associated biomarkers could provide a comprehensive understanding of neuroinflammation following TBI, as research has demonstrated that these ILs are interconnected with S100β and GFAP in these pathways (Burda, Bernstein & Sofroniew, 2016). The recent findings of these biomarkers, their roles, and their interactions with one another following TBI could aid in the diagnosis, prognosis, and treatment of this injury. Therefore, the aim of this review was to systematically evaluate the literature on the systemic inflammatory biomarkers IL-1β, IL-6, and

IL-10 and astroglia-associated biomarkers S100β and GFAP in TBI cases. Additionally, we aimed to report on the neuroinflammatory pathways in which these biomarkers are involved.

The objectives of this review were to: (i) highlight significant concentration differences in biomarkers between TBI cases and controls, (ii) compare the reported biomarker concentrations obtained among studies, (iii) identify the ability of biomarkers to differentiate between TBI severities, outcome, and clinical presentation, (iv) relate the information obtained to a forensic setting, and (v) provide a holistic overview of the overlapping neuroinflammatory pathways involving IL-1β, IL-6, IL-10, S100β, and GFAP.

2. Methods

2.1. Study design and search terms

This systematic review was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (Page et al., 2021). The databases used to perform the literature search were PubMed, ScienceDirect, and Web of Science. General search terms used included: "**traumatic brain injury**", "**TBI**", "**neuroinflammation**", "**biomarker**", "**IL-1β**", "**IL-6**", "**IL-10**", "**GFAP**", and "**S100β**". A full list of search terms is available in the appendix (Appendix A, Table A1). Additional studies were identified by handsearching eligible studies in the reference lists of the included articles.

2.2. Eligibility criteria

Articles eligible for this systematic review were peer-reviewed journal articles (including randomised controlled clinical trials, case studies, original research articles) that were either written in, or translated into, English. Only articles published from 2018 onwards were included, as the popularity of the term "neuroinflammation" grew rapidly around this time and publications using this term began to surge (Galea & Graeber, 2023). Studies conducted in both clinical and post-mortem settings were included.

In this review, TBI referred to an anatomical and/or functional injury to the brain, resulting either from an external impact to the head region or from acceleration/deceleration forces (Galgano et al., 2017; CDC, 2022). Fatal and non-fatal TBI cases were included, with the former defined as TBI being causative of or contributory to death. The articles needed to have investigated one or more of the following biomarkers: IL-1β, IL-6, IL-10, GFAP, or S100β, and were required to have at least one concentration measurement of the biomarker within seven days following TBI. This enabled inter-study comparisons of biomarker measurements within a similar timeframe.

Exclusion criteria included studies on animals or cell lines and on unique populations, such as paediatric patients, military personnel, or athletes. This is because TBI in children younger than 16-years-old show a different physiological profile compared to adults (Figaji, 2017) and the circumstances of TBI in military personnel and athletes are not always representative of the general population (Reid & Velez, 2015; Ntikas et al., 2024). Articles were also excluded if the studies were assessing methodology or biomarker stability, e.g.: novel methods for biomarker detection, method performance evaluations, or biomarker stability in body fluid

samples without focus on the effect of potential biomarkers of TBI and related pathology, which was the aim of this review.

2.3. Screening and evaluation of studies

The aforementioned search terms were used to identify potential articles, which were then uploaded onto EndNote (version 21, Clarivate). EndNote was used to remove duplicate articles, the outcome of which was verified manually. The resulting articles were screened by title and then by abstract. The full texts of the screened articles were sought for retrieval and assessed for eligibility, based on the inclusion and exclusion criteria. Searching, screening, and data collection were carried out by one researcher. A second reviewer (SA) then verified these inclusions by checking the screening of the studies. They agreed with all inclusions.

Final articles chosen for review were evaluated according to the five outcome variables specified in Table 1. Outcome variable number 3 was most often assessed using the using the Glasgow Coma Scale (GCS), and number 5 was assessed using either the Glasgow Outcome Scale (GOS), Glasgow Outcome Scale-Extended (GOS-E), or Modified Rankin Scale (mRS). Further details on the outcome variables GCS, GOS, GOS-E, and mRS are provided in Table 2.

Table 1: Descriptions of the five outcome variables utilised for evaluating the chosen articles for review.

Outcome variable	Explanation
1	The concentration difference of the investigated biomarker in TBI cases compared to controls.
2	The specific mean or median value reported for the biomarker. AUC values or other measurements were utilised if exact values were missing. Reported values were compared across studies for each biomarker.
3	The ability of the biomarker to differentiate between TBI severities.
4	The ability of the biomarker to differentiate between intracranial lesion presence (CT-positive) and absence (CT-negative) on CT scans.
5	The ability of the biomarker to anticipate TBI prognosis, by predicting favourable versus unfavourable outcomes, complete versus incomplete recoveries, or life versus death.

TBI: Traumatic brain injury. *AUC:* Area under the curve. *CT:* Computed tomography.

Table 2: Details on the GCS, GOS, GOS-E, and mRS metrics used to classify TBI severity and/or prognosis.

Metric used	Explanation
GCS	Measures the level of impaired consciousness by assessing three functions: eye opening, verbal response, and motor response. Each function is graded individually and then a cumulative score (from 3–15) is calculated to summarise the severity of an individual's consciousness level (Corrigan, Harrison-Felix & Haarbauer-Krupa, 2018; Jain & Iverson, 2022).
13–15	Mild TBI.
9–12	Moderate TBI.
3–8	Severe TBI.
GOS	Measures the impact of injury on function across integral aspects of life (Thelin et al., 2019; Wilson et al., 2021).
1	Dead.
2	Vegetative state.
3	Severe disability.
4	Moderate disability.
5	Good recovery.
1–3	Unfavourable outcome.
4–5	Favourable outcome.
= 1	Dead.
2–5	Alive.
GOS-E	Standard metric for evaluating functional outcome following TBI. Rated from 1–8 (Posti et al., 2020; Johnson et al., 2022; Yue et al., 2023).
1	Dead.
2	Vegetative state.
3	Lower severe disability.
4	Upper severe disability.
5	Lower moderate disability.
6	Upper moderate disability.
7	Lower good recovery.
8	Upper good recovery.
1–4	Death or severe disability/unfavourable outcome.
5–8	Moderate disability or good recovery/favourable outcome.
< 8	Incomplete recovery.
= 8.	Complete recovery.
mRS	Assesses the level of disability of an individual by encompassing a diverse array of functional states (Saver et al., 2010; Lewis et al., 2019; Pożarowszczyk et al., 2023).
0	No disability.
1	No significant disability.
2	Slight disability.
3	Moderate disability.
4	Moderately severe disability.
5	Severe disability.
6	Death.
≤ 3	Good outcome.
≥ 4	Poor outcome.

GCS: Glasgow Coma Scale. *GOS:* Glasgow Outcome Scale. *GOS-E:* Glasgow Outcome Scale Extended. *mRS:* Modified Rankin Scale. *TBI:* Traumatic brain injury.

2.4. Quality appraisal of included studies

The quality of the included studies was assessed using the 'quality assessment with diverse studies' (QuADS) appraisal tool (Appendix B), which investigates the methodological and reporting quality of mixed- and multi-method studies. The tool consists of 13 items, each of which is scored from a value of 0 (not at all) to 3 (performed well). Each factor is considered individually when assessing a specific study and no one criterion is considered more important than another (Harrison et al., 2021). However, a summation of scores will be calculated to provide a general overview, with a maximum achievable score being 39.

2.5. Data analysis

Not all studies reported on the measured values for the specific biomarker(s) they were investigating nor calculated the mean/median values for their case and control groups. Where possible, supplementary material was consulted to locate missing values. Among the studies that did report biomarker levels, the majority used consistent units although a few had used different units. Units were standardised to align with those predominantly used for that biomarker in the literature, enabling inter-study comparisons. Descriptive statistics on all 44 included studies and the graphical representations thereof were performed using Microsoft Excel 2021 (version 2108, Microsoft Corporation), GoodNotes (version 6, Steven Chan), and Microsoft PowerPoint 2021 (version 2108, Microsoft Corporation).

A meta-analysis was conducted on a subset of studies that had either reported mean values for their TBI and control groups or had available supplementary data with which to calculate these values. Forest plots, using the standardised mean difference, were generated using R Statistical Software (version 4.3.2; R Core Team, 2022) with the package {meta} (Sara Balduzzi, Gerta Rücker, & Guido Schwarzer, 2019). A random-effects model was used due to heterogeneity within and between studies, and a restricted maximum likelihood estimator was used to measure the heterogeneity variance t^2 (Viechtbauer, 2005). To calculate the confidence interval around the pooled effect, Knapp-Hartung adjustments were used (Knapp & Hartung, 2003). Finally, the I^2 statistic was calculated to report the between-study heterogeneity. Values of 25%, 50%, and 75% correlate to low, moderate, and substantial heterogeneity, respectively (Harrer et al., 2021).

2.6. Assessment of certainty of evidence

The certainty of evidence of the included articles was assessed using the grading of recommendations, assessment, development and evaluation (GRADE) approach (Guyatt et al., 2008). This included assigning the included studies an initial certainty of evidence rating based on their study design, and then downgrading or upgrading this rating when assessing additional criteria. The rating was downgraded if there was risk of bias, inconsistency, indirectness, imprecision, and/or publication bias. The rating was upgraded if there was a large magnitude of an effect, a dose-response gradient, and/or an effect of plausible residual confounding factors. This produced a final certainty of evidence rating.

3. Results

3.1. Included studies

The initial literature search identified a total of 316 articles from the three databases. After the removal of duplicates, 221 articles were screened. Among these, 114 articles were excluded based on title and a further 48 were excluded based on abstract, meeting one or more of the exclusion criteria. The remaining 59 articles were assessed for eligibility, where 25 articles were excluded for not meeting the inclusion criteria. Handsearching led to the inclusion of an additional 10 articles, culminating in a total of 44 articles in this systematic review (Figure 1).

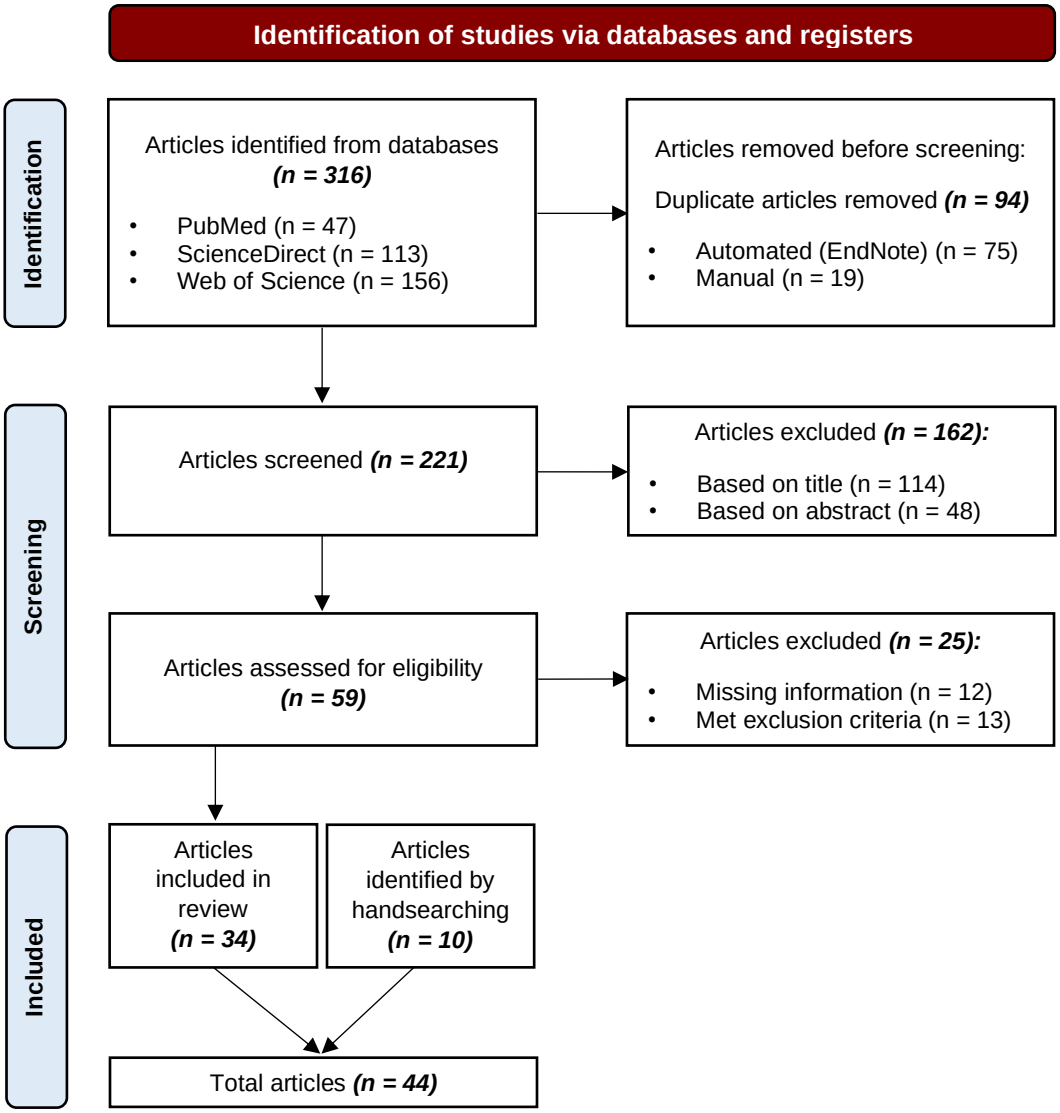

Figure 1: Article identification, screening, and inclusion for this systematic review. [Adapted from (Page et al., 2021)].

3.2. Study characteristics

The 44 included studies were conducted across 14 different countries worldwide with a predominant geographical distribution in the Global North, notably in the United States of America (USA) [12], Germany [11], and Finland [5]. The remaining countries were Hungary [2], Sweden [2], Italy [2], Denmark [2], Spain [1], Norway [1], Japan [1], Indonesia [1], France [1], Czech Republic [1], and Australia [1] (Figure 2).

Within the last five years, the 44 included articles were predominantly published in 2020 and 2021, with the least number of articles being published in 2023 (Appendix C, Figure C1). These articles were published in peer-reviewed journals covering multiple disciplines, such as neurology, medicine, and law and/or forensics (Appendix C, Figure C2). The most prevalent journals were the 'Journal of Neurotrauma' (Impact Factor: 4.2), 'Frontiers in Neurology' (Impact Factor: 3.4), and 'International Journal of Legal Medicine' (Impact Factor: 2.8).

Analysing broad trends among the 44 included articles revealed that GFAP was the most extensively studied biomarker, appearing in 30 studies, followed by S100β in 21 studies. Both IL-10 and IL-6 were assessed to a lesser extent (seven and eight studies, respectively), and IL-1β was the least frequently examined biomarker with only one study (Appendix C, Figure C3).

With regards to the type and frequency of biological samples collected, serum was the most commonly examined, closely followed by plasma (Appendix C, Figure C4). Less than five studies analysed cerebrospinal fluid (CSF), brain tissue, extracellular vesicles, exosomes, urine, saliva, and vitreous humour.

For measurement of biomarker concentration, the vast majority of studies utilised an electrochemiluminescence immunoassay (IA), an enzyme-linked immunosorbent assay (ELISA), or a multiplex IA (Appendix C, Figure C5). The remaining studies utilised various IA techniques, ranging from chemiluminescence to multiplex systems.

The timeframe within which samples were collected following occurrence of TBI was most frequently within 24 hours after injury, followed by six hours. Certain studies provided only the time elapsed from hospital admission to sample collection and not necessarily time of injury, so this was reported as such (Appendix C, Figure C6).

The above study characteristics were also summarised and reported as an infographic (Figure 3), illustrating the main findings.

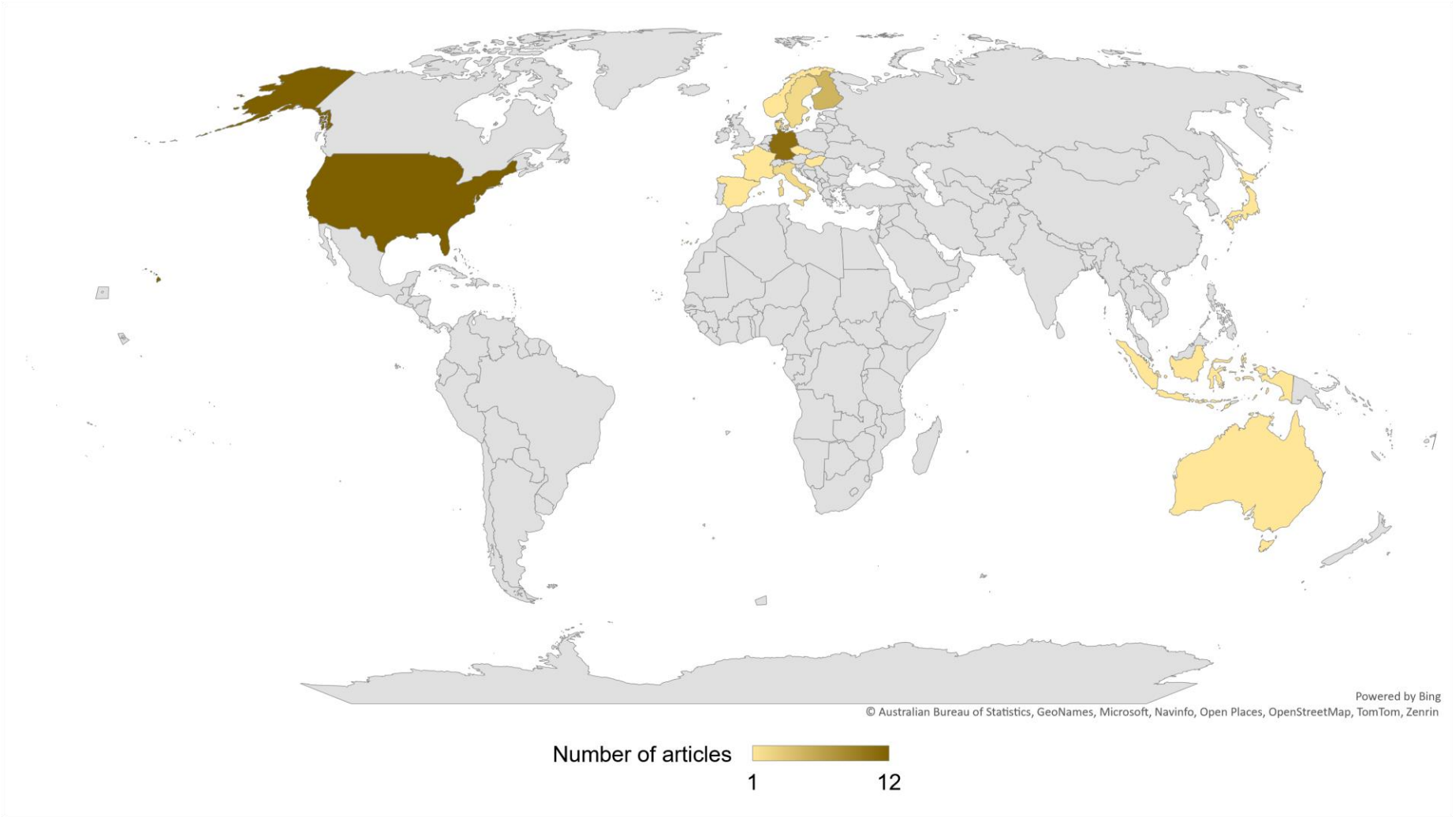

Figure 2: Global geographical distribution of the 44 included articles by country. Majority of the studies were conducted in the Global North. Created using Microsoft Excel 2021 (version 2108, Microsoft Corporation).

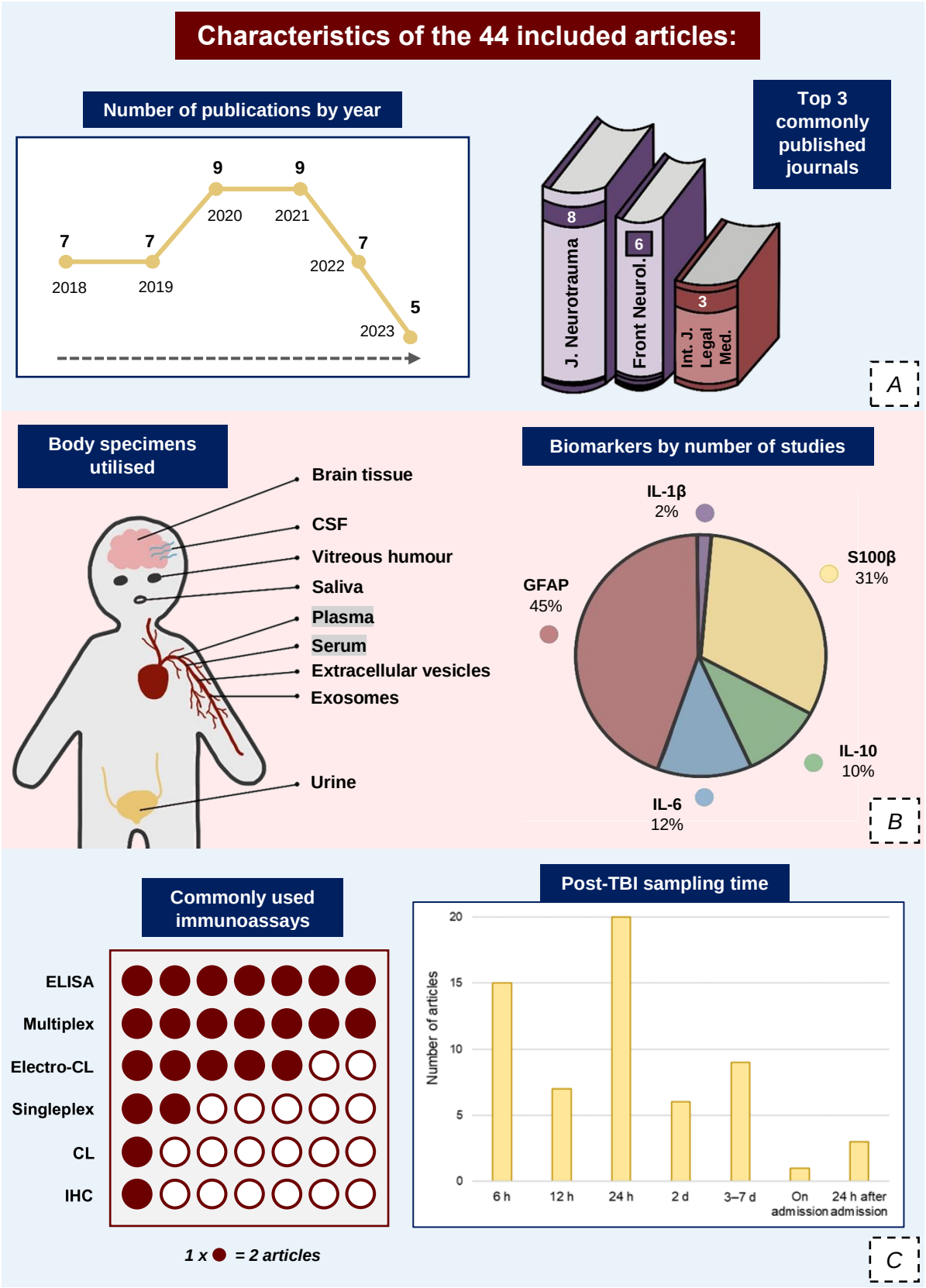

Figure 3: Summary of the main study characteristics for the 44 included studies. Only the predominant journals and assays were included.

A: (left) ‘Number of publications by year’: the numbers above the dates indicate how many articles were published each year.

(right) ‘Top 3 commonly published journals’: the predominant journals were illustrated. *J. Neurotrauma:* Journal of Neurotrauma. *Front Neurol.:* Frontiers in Neurology. *Int. J Legal Med.:* International Journal of Legal Medicine. The colours of the journals correlate to the disciplines covered. *Dark purple:* neurology, *red:* law and/or forensics. The numbers above the journals indicate the number of articles published in those journals.

B: (left) ‘Body specimens utilised’: all body tissues and fluids collected were illustrated. The most frequently collected were plasma and serum. *CSF:* cerebrospinal fluid.

(right) ‘Biomarkers by number of studies’: the percent of studies assessing each biomarker.

C: (left) ‘Commonly used assays’: the predominant immunoassays performed. Each well represents two articles that utilised those assays. *ELISA:* Enzyme-linked immunosorbent assay. *Electro-CL:* Electrochemiluminescence. *CL:* Chemiluminescence. *IHC:* Immunohistochemistry.

(right) ‘Post-TBI sampling time:’ the time within which the sample was collected following TBI. *h:* hours. *d:* days.

3.3. Main findings of the investigated biomarkers

The combined results of the 44 included articles investigating IL-1β, IL-6, IL-10, S100β, and GFAP are summarised in Table 3. The trends displayed by the results for each biomarker were arranged according to the following:

i. Whether that biomarker was reported to have an increase or decrease in its levels in TBI patients compared to controls. This included studies who compared various TBI patient groups (such as those only assessing a specific severity of TBI, those looking at isolated TBI, or those looking at a combination) to various control groups (including healthy controls, orthopaedic controls, and polytrauma controls).

ii. If the values reported by each paper for a specific biomarker aligned with one another or whether they varied widely. For example, certain biomarkers obtained values ranging from the single digits to the millions.

iii. The ability of the biomarker to distinguish between TBI patients that had positive CT scans compared to those whose CT scans were negative, i.e.: between those that had intracranial lesions and those that did not. Again, the included studies had used various TBI groups, some consisting of only one or two of the three TBI severities and others only using isolated or non-isolated cases of TBI.

iv. Whether the biomarker could differentiate between mild, moderate, and severe TBI. This outcome variable was slightly challenging to assess, as many studies grouped the TBI severities differently. Certain studies had compared biomarker levels in mild cases of TBI to a combined group of moderate and severe TBI cases,

whereas others had compared mild and moderate TBI to biomarker levels in severe TBI.

v. If the biomarker was able to show a correlation relating to the patient outcome following TBI. Patient outcomes were assessed differently amongst studies, with some comparing favourable versus unfavourable outcomes, others incomplete versus complete recoveries, and still others outcomes of death versus survival.

For the above outcome variables, the most common finding was reported in Table 3, even if there were a limited number of studies investigating the biomarker in that regard. However, the actual results were more nuanced and are discussed in greater detail for each individual biomarker following the table.

Table 3: Overall findings for each of the investigated biomarkers (IL-1β, IL-6, IL-10, S100β, GFAP) from the 44 included studies.

Biomarker	Values of TBI group compared to control group	Concordance in reported values	Distinguish presence and absence of intracranial lesions	Differentiate between TBI severities	Correlate with TBI outcome
IL-1β	Decreased[1]	N/A	N/A	N/A	N/A
IL-6	Increased	Inconsistent	Yes[1]	N/A	Yes[1]
IL-10	Increased	Inconsistent	No	Inconclusive	Yes
S100β	Increased	Inconsistent	No	Yes	Yes
GFAP	Increased	Inconsistent	Yes	Yes	Yes

Note: A “yes” or a “no” were recorded if the only study available reported significant results or if the majority of the studies obtained significant results.

N/A: No studies had assessed the biomarker with regards to the outcome variable, thus there was no data to report.
Inconsistent: Wide ranges of values were reported by the articles investigating that biomarker.
Inconclusive: An almost equivalent number of studies had found a significant difference in biomarker levels as those that did not.

[1] Only one study assessed the biomarker in this regard, therefore the results are based on that study only.

3.3.1. IL-1β

Of the articles included in this review, only one had investigated IL-1β as a potential biomarker of TBI (Appendix D, Figure D1) (To et al., 2023). This sole article investigated whether plasma IL-1β levels would be increased or decreased in TBI patients compared to healthy controls. The authors observed a statistically significant increase of IL-1β in the control group of more than four-fold (To et al., 2023). This study did not report on any of the other outcome variables discussed in this review.

3.3.2. IL-6

Eight articles reporting on IL-6 were included in this review (Appendix D, Figure D2). Five had investigated plasma or serum IL-6 levels in a clinical setting (Lewis et al., 2019; Edwards et al., 2020; Schindler et al., 2020; Beard et al., 2021; To et al., 2023), whilst the remaining three articles assessed the biomarker in a post-mortem setting (Ondruschka, Schuch, et al., 2018; Trautz et al., 2019; Zwirner, Bohnert, et al., 2021).

Two of the three studies that assessed IL-6 in a post-mortem setting aimed to identify whether there was a significant difference in IL-6 levels between TBI cases and controls (Ondruschka, Schuch, et al., 2018; Zwirner, Bohnert, et al., 2021). Both Ondruschka et al. (2018) and Zwirner et al. (2021) observed a significant increase in CSF IL-6 levels in their TBI group compared to their respective control groups, with Ondruschka et al. (2018) also demonstrating this increase in serum IL-6. Ondruschka et al. (2018) further compared serum and CSF IL-6 levels in their TBI group to different control subgroups, finding significant elevations in almost all the TBI groups.

The third post-mortem study investigated potential associations between the spatial expression of IL-6 in various brain regions and cause of death related to TBI, as well as to post-TBI survival times. The authors assessed neuronal and glial expression of IL-6 in the pericontusional zone (PCZ; the region of the brain exhibiting macro- or microscopic evidence of TBI), the contralateral cortex (CLC; the contralateral cortical area to the PCZ), ipsilateral hippocampus (IHC), or ipsilateral cerebellum (CB). The authors observed greater expression of neuronal IL-6 in the PCZ of those who suffered from subacute deaths following TBI (survival time greater than two hours but less than three days) compared to acute TBI sufferers (survival time less than two hours) and controls, respectively. No statistically significant difference in neuronal IL-6 expression was found between acute or subacute TBI deaths in the CLC, IHC, or CB compared to controls. No significant difference was found for glial IL-6 expression across all groups (Trautz et al., 2019).

A significant increase in plasma and extracellular vesicle IL-6 levels was observed in the two clinical studies that had compared TBI patients to healthy and/or orthopaedic controls (Beard et al., 2021; To et al., 2023). Schindler et al. (2020) used slightly different TBI and control groups to investigate differences in IL-6 levels. Their study groups consisted of isolated severe TBI patients (isTBI), severe TBI patients also suffering from polytrauma (sTBI + PT), and polytrauma patients without TBI (PT). The PT group consistently had increased serum IL-6 levels compared to the isTBI group over five days, and the sTBI + PT group also had significantly elevated levels on the first day compared to the isTBI group.

Only one study investigated whether IL-6 could serve as a prognostic biomarker of TBI. It observed that plasma levels of IL-6 were increased in patients who suffered an unfavourable outcome versus a favourable outcome following TBI (Lewis et al., 2019).

One study assessed IL-6 as a biomarker to differentiate patients with and without neuroimaging findings. Edwards et al. (2020) compared plasma IL-6 levels in mTBI patients among those with positive CT scans (CT+; indicating neuronal injuries), those with positive magnetic resonance imaging (MRI) scans but negative CT scans (MRI+; indicating mild neuronal injuries), and those with negative CT and MRI scans (MRI-/CT-; indicating an absence of intracranial injuries). The CT+ group had significantly elevated IL-6 levels compared to both the MRI+ group and the MRI-/CT- group, and the MRI+ group had increased levels compared to the MRI-/CT- group (Edwards et al., 2020).

Comparison of the IL-6 values obtained by the studies returned a large range of values with minimal overlap. Certain studies reported single-digit IL-6 values, whilst others reported values in the thousands. Even when comparing similar studies that used similar techniques discrepancies were observed. Both Lewis et al. (2019) and Beard et al. (2021) investigated plasma IL-6 levels within 48 hours of TBI and performed an ELISA, however a difference of 20-fold in IL-6 levels was reported in the TBI groups in the respective studies. The highest IL-6 values overall were reported by Ondruschka, Schuch, et al. (2018) in CSF and serum.

3.3.3. IL-10

A review of the recent literature returned seven studies that met the inclusion criteria for investigating IL-10 as a potential TBI biomarker (Appendix D, Figure D3). All seven of these were clinical studies, with four having assessed plasma IL-10 levels (Lewis et al., 2019; Posti et al., 2019; Edwards et al., 2020; Beard et al., 2021) and three having investigated serum IL-10 levels (Lagerstedt et al., 2020; Schindler et al., 2020; Koivikko et al., 2022). One of the plasma studies also measured IL-10 levels in extracellular vesicles (Beard et al., 2021).

Three studies examined the difference in IL-10 levels in TBI patients compared to controls (Schindler et al., 2020; Beard et al., 2021; Koivikko et al., 2022). Beard et al. (2021) observed a significant elevation in plasma IL-10 in their TBI group (consisting solely of mild cases) versus their control group (consisting of both healthy and orthopaedic controls). No increase was observed when assessing IL-10 levels in extracellular vesicles between both groups (Beard et al., 2021). Koivikko et al. (2022) compared TBI subgroups to orthopaedic controls and observed a significant elevation in moderate and severe TBI cases but not in mTBI cases. Schindler et al. (2020) reported conflicting results, as their control group of polytrauma patients had increased serum IL-10 levels compared to isTBI cases.

The remaining articles investigated IL-10 as a means to differentiate between either CT-positive and CT-negative scans (Posti et al., 2019; Edwards et al., 2020), favourable and unfavourable outcome (Lewis et al., 2019; Lagerstedt et al., 2020), or TBI severities (Koivikko et al., 2022). Edwards et al. (2020) and Posti et al. (2019) both reported no significant difference in plasma IL-10 levels in mTBI patients who were CT-positive versus those who were CT-negative. However, Posti et al. (2019) did observe a significant difference between those with intracranial lesions and those without, though they had grouped mild, moderate, and severe TBI patients together.

Lewis et al. (2019) and Lagerstedt et al. (2020) found a significant elevation in IL-10 levels in TBI patients with an unfavourable outcome compared to those with a favourable outcome. However, Lagerstedt et al. (2020) reported that IL-10 levels could not differentiate between those who had an incomplete recovery versus those who recovered completely.

Koivikko et al. (2022) was the sole study to compare concentrations of IL-10 between TBI severities. They reported that mTBI cases had significantly lower serum IL-10 levels compared to both moderate and severe TBI, respectively, but that moderate and severe TBI did not have statistically significant differences when compared to each other (Koivikko et al., 2022).

Finally, when comparing the values of IL-10 reported, the majority of the results were in agreement with each other. Posti et al. (2019), Beard et al. (2021), and Koivikko et al. (2022) obtained similar results, ranging from decimal values to single-digits. However, compared to these studies Lewis et al. (2019) reported significantly elevated values that spanned a much broader range, observing a maximum difference of over 600-fold when compared to Posti et al. (2019).

3.3.4. S100β

In total, 21 articles assessing S100β were identified for this review (Appendix D, Figure D4). Four of these were conducted in a post-mortem setting (Sieber et al., 2018; Zwirner, Bohnert, et al., 2021; Lanzilao et al., 2023; Olczak et al., 2023) and the remaining 17 were conducted in a clinical setting (Posti et al., 2019; Thelin et al., 2019; Janigro et al., 2020; Lagerstedt et al., 2020; Okonkwo et al., 2020; Schindler et al., 2020; Biberthaler et al., 2021; Haselmann et al., 2021; Oris et al., 2021; Seidenfaden et al., 2021, 2022; Vedin et al., 2021; Wijanarko et al., 2021; Gardner et al., 2022; Koivikko et al., 2022; Richter et al., 2023; Trnka et al., 2023).

The four post-mortem studies investigated S100β concentration differences between fatal TBI cases and controls in various body fluids. Olczak et al. (2023) reported an elevation in serum S100β (but not in urine S100β) in TBI cases compared to cardiopulmonary controls; Lanzilao et al. (2023) observed a significant increase in vitreous humour S100β levels in severe fatal TBI cases compared to mild-to-moderate TBI cases (not fatal); and Zwirner et al. (2021) and Sieber et al. (2018) observed an elevation in CSF S100β in their TBI groups compared to non-TBI controls, even in TBI cases with very short survival times. Sieber et al. (2018) additionally illustrated this increase compared to each of their subgroups, consisting of isolated torso trauma (ITT), diffuse cerebral hypoxia (DCH), and acute myocardial infarction (AMI). This demonstrated that even in fatal cases of torso trauma, CSF S100β levels were not as elevated as they were in fatal TBI cases. The authors did note however that the release of S100β is not specific to TBI cases as the DCH group also demonstrated high levels of CSF S100β, potentially resulting from hypoxia-induced cell damage. However, Sieber et al. (2018) found no significant elevation in serum S100β when comparing the same TBI and control groups to each other.

Five clinical studies investigated S100β levels in TBI patients compared to healthy or orthopaedic controls. Of these, four reported a significant elevation of S100β in their TBI group or in the majority of their TBI subgroups (Janigro et al., 2020; Okonkwo et al., 2020; Gardner et al., 2022; Koivikko et al., 2022). The only comparisons with no statistically significant difference were between the TBI group and an orthopaedic control group (both with an age range of 65–90 years) in the study by Gardner et al. (2023), and the mTBI group versus orthopaedic controls in the study by Koivikko et al. (2022). The fifth study reported conflicting results with no reported significant difference between their TBI and control groups (Schindler et al., 2020). The control group in this study consisted of patients suffering from polytrauma.

Five studies evaluated the effectiveness of S100β in discriminating CT-positive and CT-negative scans in TBI patients, with conflicting results. Two studies demonstrated significantly elevated S100β levels in CT-positive TBI patients compared to CT-negative TBI patients

(Okonkwo et al., 2020; Gardner et al., 2022). Two other studies observed no significant difference in S100β levels in their CT-positive versus CT-negative groups (Biberthaler et al., 2021; Haselmann et al., 2021). The last study investigating this reported only a significant elevation in the CT-positive TBI group when comparing S100β levels amongst isolated cases of mTBI (Posti et al., 2019).

Two clinical studies (Biberthaler et al., 2021; Koivikko et al., 2022) and one post-mortem one (Lanzilao et al., 2023) had investigated whether S100β levels could be used to distinguish between mild, moderate, and severe TBI. However, the TBI cases were grouped slightly differently in all three of these studies, making drawing comparisons challenging. Koivikko et al. (2022) compared S100β levels amongst mild, moderate, and severe TBI and observed significantly lower levels in the mild group compared to the other two. They did not find a significant difference between their moderate and severe TBI group. Biberthaler et al. (2021) reported statistically significant elevations in their mTBI group compared to their combined group of moderate and severe TBI cases. Lanzilao et al. (2023) had combined mild and moderate cases of TBI and compared the S100β levels of this group to their severe TBI group, finding significantly elevated S100β levels in the latter.

Three studies assessed S100β as a potential TBI prognosis biomarker, with two of these having conducted statistical testing to determine if their findings were significant. Both these studies observed increased serum S100β levels in an unfavourable versus a favourable outcome following TBI (GOS 1–3 versus 4–5, or GOS-E 1–4 versus 5–8) (Thelin et al., 2019; Lagerstedt et al., 2020). Thelin et al. (2019) also demonstrated elevated serum S100β levels in those whose eventual outcome following TBI was death (GOS 1), compared to those who remained alive (GOS 2–5). Lagerstedt et al. (2020) did not find a statistically significant difference between those who had an incomplete recovery (GOS-E 1–7) compared to those who had a complete recovery (GOS-E 8).

Comparison of the reported S100β levels across studies returned inconsistent results. Overall, three broad ranges of values were reported, however these ranges varied widely. The lowest range included values from 0.001 ng/mL to 4 ng/mL, with the vast majority of the studies observing values within this range (Posti et al., 2019; Janigro et al., 2020; Okonkwo et al., 2020; Schindler et al., 2020; Biberthaler et al., 2021; Haselmann et al., 2021; Wijanarko et al., 2021; Gardner et al., 2022; Koivikko et al., 2022; Olczak et al., 2023; Oris et al., 2023; Richter et al., 2023). The second range was over 10 000-fold larger than the first, and included values from 10 ng/mL to 800 ng/mL (Sieber et al., 2018; Thelin et al., 2019; Seidenfaden et al., 2021, 2022; Vedin et al., 2021; Olczak et al., 2023; Trnka et al., 2023). Finally, the third range, of

within which the least number of studies reported values, contained values from 2000 ng/mL up to 12 500 ng/mL (Sieber et al., 2018; Thelin et al., 2019).

3.3.5. GFAP

GFAP was the predominantly investigated TBI biomarker in this review, with 30 articles assessing the protein (Appendix D, Figure D5). Among them, six studies were focused on post-mortem investigations, whilst the remaining 24 were conducted in a clinical setting.

Three post-mortem studies investigated GFAP levels in various body fluids in TBI cases compared to controls, whilst the remaining three assessed GFAP expression in brain tissue. The body fluids investigated were CSF (Ondruschka, Sieber, et al., 2018; Zwirner, Bohnert, et al., 2021), serum (Ondruschka, Sieber, et al., 2018; Olczak et al., 2023), and urine (Olczak et al., 2023). All three studies observed statistically significant elevations in GFAP levels in their TBI cases. Ondruschka, Sieber, et al. (2018) additionally compared various control subgroups, and observed that the TBI group still displayed increased serum and CSF GFAP levels, with the vast majority of these differences being statistically significant. With regards to the studies investigating GFAP expression in post-mortem brain tissue, both Trautz et al. (2019) and Zwirner, Lier, et al. (2021) examined the PCZ, CLC, IHC, and CB in acute and subacute TBI cases as well as controls. However, neither study had promising results. Trautz et al. (2019) found no statistically significant difference in the immunohistochemical stains of these brain regions in either of their TBI groups compared to controls. Likewise, Zwirner, Lier, et al. (2021) reported only minimal expression, and even discussed how it was possible that the GFAP immunopositivity was a processing artefact. However, the study conducted by Neri et al. (2018) yielded contradictory findings. These authors reported GFAP brain tissue immunopositivity in up to 33% of cells within one day of trauma, and up to 70% of cells 3–7 days after the TBI was sustained. This increase was significant compared to controls (Neri et al., 2018).

The clinical setting GFAP studies comprised eight articles that analysed concentration differences in TBI and control groups. All except one of these studies reported significantly elevated GFAP concentrations in their respective TBI groups. This was done in various body fluids, such as plasma (Okonkwo et al., 2020; Beard et al., 2021; Clarke et al., 2021; Gardner et al., 2022; Halbgebauer et al., 2022) and serum (Castaño-Leon et al., 2022), as well as extracellular vesicles (Puffer et al., 2020; Beard et al., 2021). These studies had also used varying severities of TBI and different control groups, such as healthy controls and orthopaedic controls. The only study that did not observe an elevation was conducted by Schindler et al. (2020), where the polytrauma group had increased GFAP levels compared to the isTBI group.

Eight studies investigated GFAP in CT-positive and CT-negative TBI patients, with all observing significantly elevated GFAP levels in the former (Bazarian et al., 2018; Gardner et al., 2018, 2022; Gill et al., 2018; Posti et al., 2019; Huebschmann et al., 2020; Okonkwo et al., 2020; Biberthaler et al., 2021). Three studies assessed the ability of GFAP to distinguish between the different TBI severities. Mostly promising results were reported, however the TBI groups were slightly different in each study. Nakamura et al. (2022) reported an elevation in GFAP in severe TBI patients compared to controls (a group that consisted of mild-to-moderate TBI patients as well as patients without TBI). Biberthaler et al. (2021) had combined moderate-to-severe TBI patients in one group and compared this to mTBI patients, observing an increase in plasma GFAP in the former. The third study again grouped the TBI severities differently, this time comparing mild, moderate, and severe TBI patients to each other. The authors reported that the severe group had elevated serum GFAP compared to the mild group, but no other significant comparison was found, making it difficult to differentiate moderate TBI sufferers from the other two (Castaño-Leon et al., 2022).

With regards to predicting the outcome of TBI, GFAP was assessed as a potential biomarker in six studies who conducted statistical testing on their results. Serum and plasma GFAP levels were elevated in those who suffered an unfavourable outcome after TBI versus a favourable outcome (Hossain et al., 2019; Thelin et al., 2019; Huebschmann et al., 2020; Lagerstedt et al., 2020; Lewis et al., 2020; Korley et al., 2022). Serum and plasma GFAP levels were also significantly higher in those who died months after TBI compared to those who survived (Thelin et al., 2019). However, no difference in GFAP levels was observed between those who had a complete versus incomplete recovery following TBI (Hossain et al., 2019; Lagerstedt et al., 2020).

Comparison of the actual GFAP values reported by studies returned a very wide range of results. Certain studies reported values that ranged from single-digits to double-digits (Bazarian et al., 2018; Schindler et al., 2020; Ward et al., 2020; Beard et al., 2021; Biberthaler et al., 2021). Other studies had values ranging from the hundreds to the thousands (Gardner et al., 2018, 2022; Gill et al., 2018; Ondruschka, Sieber, et al., 2018; Hossain et al., 2019; Posti et al., 2019; Thelin et al., 2019; Huebschmann et al., 2020; Lewis et al., 2020; Mondello et al., 2020; Okonkwo et al., 2020; Puffer et al., 2020; Biberthaler et al., 2021; Clarke et al., 2021; Seidenfaden et al., 2021; Nakamura et al., 2022; Olczak et al., 2023). There were also two studies that had reported extremely high values, ranging from the hundred-thousands to the millions (Thelin et al., 2019; Zwirner, Bohnert, et al., 2021).

3.4. Quality of the included studies

The cumulative QuADS scores of each of the 44 included studies ranged from 23–34 (59–87 %). Majority of the articles received lower ratings for “evidence that the research stakeholders have been considered in research design or conduct”, “thorough discussion of strengths and limitations”, “detailed evidence of consideration of the sample required to address the research aims”, and “detailed explanation of rationale for choice of data collection tools”. The articles received high ratings for “the study design is appropriate to address the stated research aim/s”, “the format and content of data collection tool is appropriate to address the stated research aim/s”, and “the method of analysis was appropriate to answer the research aim/s”. The results are tabulated and represented graphically in the appendix (Appendix B, Table B2 and Figure B1).

3.5. Meta-analysis

Only five of the 44 included articles were eligible for the pooled effect analysis (Figure 4) (Janigro et al., 2020; Puffer et al., 2020; Clarke et al., 2021; Olczak et al., 2023; To et al., 2023). The predominant biomarker included was GFAP, appearing three times. Both IL-6 and S100β appeared once. The biomarkers IL-1β and IL-10 were not investigated in these five studies.

The line of no effect passed through zero and intersected the confidence interval of the study by Olczak et al. (2023). The pooled effect size (0.97) and its confidence interval [0.20; 1.75] did not intersect with the line of no effect. The I^2 value for study heterogeneity was 64%, indicating that the extent to which effect sizes vary was moderate to substantial. This variability was also observed in the individual effect sizes of the articles which varied from 0.35 to 2.25.

The studies with wider confidence intervals and lower weights were also lowest in precision (Janigro et al., 2020; Puffer et al., 2020). This could be explained by their small sample sizes. Puffer et al. (2020) had only 12 participants in their TBI group and eight in their control group and Janigro et al. (2020) had 14 and 15, respectively. The studies with the highest weights (Clarke et al., 2021; Olczak et al., 2023) also had the largest sample sizes.

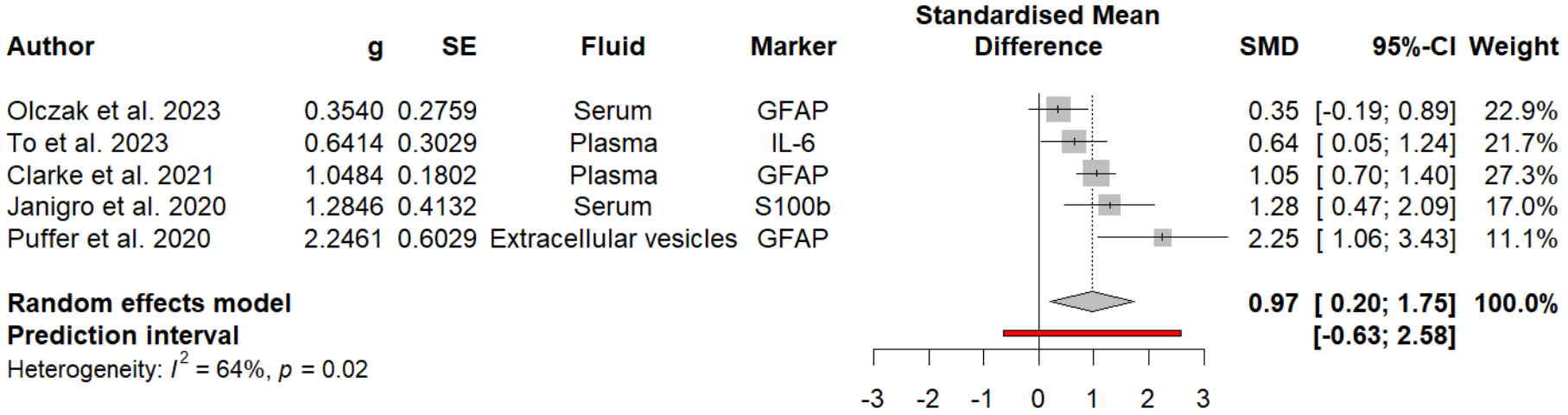

Figure 4: Forest plot demonstrating the effect sizes of the five studies that reported mean values of their investigated biomarker, using the standardised mean difference.

GFAP: Glial fibrillary acidic protein. *IL-6:* Interleukin-6. *S100β:* S100 calcium-binding protein β.

3.6. Certainty of evidence of the included studies

The initial certainty of evidence rating given to the included studies was “low” as their study design was observational (Guyatt et al., 2008). Using the QuADS tool to appraise the quality of the studies indicated no significant issues, thus no downgrade was applied here. The I^2 value for study heterogeneity was 64% (indicating moderate to substantial heterogeneity), so the certainty of evidence rating was downgraded by one level, to “very low”. No significant indirectness was ascertained as the included studies did address the research question of this systematic review. There was a wide prediction interval as observed on the meta-analysis, resulting in another downgrade, however in this case the rating remained on “very low” as a lower level does not exist. A funnel plot was produced using the five studies included in the meta-analysis and indicated a somewhat symmetrical distribution around the mean, indicating no downgrade.

For the criteria that could potentially upgrade the rating, only a dose-response gradient was observed in certain studies (in five out of the six studies that had assessed this outcome) where those with severe TBI had increased levels of biomarkers compared to those with mild TBI. However, the final certainty of evidence rating remained on “very low”. This decision was influenced by the presence of more factors contributing to downgrade the rating than to upgrade it. Additionally, the limited scope of the meta-analysis, which contained only five studies, may have influenced the results.

4. Discussion

Overall, minimal studies were conducted on the biomarkers of systemic inflammation compared to the brain-specific, astroglia-associated biomarkers. There was a notable paucity of data involving IL-1β specifically. This makes it challenging to draw conclusions from the articles discussed, as further studies are needed to corroborate the results obtained. Despite the minimal studies, IL-6 demonstrated promising results as a potential TBI biomarker. Both S100β and GFAP were also overwhelmingly promising, with GFAP demonstrating potential in relation to all the outcome variables.

4.1. Systemic inflammatory biomarkers

The sole study investigating IL-1β reported a significant decrease in plasma levels in TBI patients compared to controls (Table 3). The authors postulated this was due to the temporal expression of IL-1β, as plasma was only obtained from TBI patients a few days after hospital admission. As such, IL-1β levels could have peaked and resolved prior to sampling (To et al., 2023). However, this does not explain the statistically significant difference between the TBI and control group, which should have then been similar. Overall, the findings of one article are not sufficient to draw any conclusions about the potential use of IL-1β as a TBI biomarker in forensic settings.

Unlike IL-1β, a greater number of studies investigated IL-6 and IL-10. The protein IL-6 showed potential as a TBI biomarker in both clinical and post-mortem settings (Ondruschka, Schuch, et al., 2018; Beard et al., 2021; Zwirner, Bohnert, et al., 2021; To et al., 2023) as it was able to differentiate between cases and controls. Furthermore, Trautz et al. (2019) reported an increase in neuronal IL-6 expression in the PCZ in those who suffered fatal TBI with extended durations of survival following trauma. These results were encouraging. Conversely, IL-10 returned inconsistent results and did not show promise as a biomarker of TBI. Neither of these cytokines were able to distinguish TBI from polytrauma cases (Schindler et al., 2020), which could be attributed to their systemic inflammatory roles and resulting lack of specificity to the brain. As a result, if IL-6 were to be implemented as a biomarker of TBI its levels would need to be interpreted within the context of other body injuries sustained. It might be more informative if used alongside brain-specific biomarkers, such as those associated with astroglia.

The ability of IL-6 and IL-10 to differentiate between TBI severities had not been assessed in this review, thus no conclusions could be reached. However, IL-6 has previously been shown to correlate with the extent of tissue trauma (Jawa et al., 2011) and did show promise in

distinguishing TBI cases from controls. It is probable that IL-6 levels would increase with increasing TBI severity and therefore could function as an indicator of severity.

For biomarkers of TBI prognosis and CT scans, no conclusion could be reached regarding IL-10 (Table 3) (Lewis et al., 2019; Posti et al., 2019; Edwards et al., 2020; Lagerstedt et al., 2020). Minimal studies and conflicting results made it seem unreliable, thus it cannot be used to identify intracranial lesions. However, IL-6 showed promise in both regards (Lewis et al., 2019; Edwards et al., 2020). It has the potential to be used in clinical settings to indicate the presence of intracranial injuries, which could aid in triaging patients in emergency rooms or in reducing the number of CT scans needed. Furthermore, the elevation of IL-6 in CT-positive versus MRI-positive scans and in MRI-positive scans versus controls (Edwards et al., 2020) indicated that IL-6 can indicate of extent of the intracranial injury as well as discern very mild intracranial injuries only determined by MRI. IL-6 could also predict the probable outcome following TBI, thus aiding medical professionals in implementing the necessary treatment or care for their patients.

Values obtained for the cytokines demonstrated more concordant results for IL-10 than IL-6 (Table 3). Discrepancies in the IL-6 measurements were unlikely to be attributed to severity of TBI investigated, body fluid or tissue used, sample collection time, or technique used to measure the biomarker levels, as similar studies returned vastly different results. Conversely, for IL-10 similar studies had returned similar values (Posti et al., 2019; Beard et al., 2021). The main difference observed was the difference in sample collection times, suggesting temporal-related changes in IL-10 expression. The highest IL-6 values were reported by a forensic study (Ondruschka, Schuch, et al., 2018), which was anticipated as TBI was fatal and thus more severe. In this study, IL-6 was more elevated in CSF than serum, indicating recruitment of peripheral immune cells to the site of TBI (Gyoneva & Ransohoff, 2015) and inflammation of the CNS (Ungureanu et al., 2021).

Overall, insufficient literature and inconsistent results relating to IL-1β and IL-10, respectively, made it challenging to draw definitive conclusions. The results from this review demonstrated that these ILs could not be classified as potential biomarkers of TBI as of yet. Promising results were obtained for IL-6, indicating its potential in distinguishing TBI cases from controls, differentiating CT scans, and predicting TBI prognosis. Further research should investigate ranges of TBI cases and severities, control types, assays, and body specimens to support or refute the conclusions made. Studies are also needed to identify a range of values for each biomarker in each body fluid to ensure consistency in results. Finally, IL-1β, IL-6, and IL-10 are all biomarkers of systemic inflammation and as such can only provide an indication of

inflammation in the brain region. They cannot definitely ascertain its presence and as such need to be interpreted with caution.

4.2. Brain-specific biomarkers

S100β and GFAP were able to distinguish TBI cases from controls, with GFAP in particular exhibiting significant potential (Table 3). Both astroglia-associated biomarkers could therefore be used to indicate the presence of TBI in clinical and forensic settings. However, similarly to the ILs, S100β and GFAP could not differentiate TBI from polytrauma (Schindler et al., 2020). Despite being primarily located in the brain, both proteins are also found elsewhere in the body (Hainfellner et al., 2001; Sorci et al., 2013) thus it is not surprising that other forms of physical trauma might also trigger their release. These biomarkers could therefore be used to provide indications of TBI, but not conclusively determine its presence.

While GFAP had also exhibited promising results in differentiating CT-positive and -negative scans in TBI patients, S100β did not (Table 3). Given the localization of GFAP in the brain it does follow that its levels correlate with the presence of intracranial lesions, as this biomarker would be directly affected by TBI. It is therefore unusual that S100β did not show the same potential, especially since its use has been recommended by the SNC to rule out traumatic intracranial hemorrhage (Faisal et al., 2023). Although in this review only minimal studies had assessed its ability in this regard. As a result of the findings of this review, GFAP alone could function as a biomarker of intracranial injury in clinical and forensic settings. Similarly to IL-6, this could include using GFAP levels in triage situations; to replace CT scans which can be costly, time-consuming, and expose patients to radiation (Hossain et al., 2024); or to indicate which patients might require MRI scans.

Minimal studies had assessed both GFAP and S100β as biomarkers of TBI severity, although the results thus far exhibit promise (Table 3). Both biomarkers were able to distinguish between severities, although often moderate TBI cases had been grouped together with either mTBI or severe TBI. Therefore, GFAP and S100β could be used to differentiate milder cases of TBI from those that are more severe with more confidence than cases of TBI that are closer in severity. This might be useful when severe TBI cases need to be identified as quickly as possible in clinical settings, or when confirming TBI as the cause of death compared to it merely being present at time of death in forensic settings.

Both S100β and GFAP were shown to differentiate between unfavourable and favourable outcomes following TBI, similarly to IL-6 (Table 3). These biomarkers could therefore inform

medical professionals about the likely prognosis of the injury. However, additional studies are needed to confirm these findings particularly for S100β where research was scarce.

The reported values for S100β and GFAP were inconsistent and highly variable (Table 3). This could not fully be explained by the study techniques however, as controlling for similar methods still returned vastly different values for both biomarkers. The most elevated S100β and GFAP levels were obtained in CSF in post-mortem studies (Sieber et al., 2018; Zwirner, Bohnert, et al., 2021). This was anticipated as S100β and GFAP are primarily expressed in the brain and thus would have elevated levels in the adjacent fluid. However, Thelin et al. (2019) also reported extremely high values of GFAP in serum. As GFAP levels are not usually measurable in healthy individuals (Brunkhorst, Pfeilschifter & Foerch, 2010), this indicates loss of the structural integrity of astrocytes and translocation of GFAP from brain tissue to the bloodstream. The mechanism of how astroglia-associated biomarkers reach the bloodstream has not yet been fully elucidated, although two potential processes could be via increased permeability of the blood brain barrier (BBB) following damage or via the glymphatic system (Plog et al., 2015; Janigro et al., 2022). This is an area for future research.

Both S100β and GFAP demonstrated more promise as biomarkers of TBI than the ILs, potentially due to their localization being closer to the site of injury. These biomarkers exhibited significant elevations in TBI cases compared to controls and could differentiate between TBI severities and various outcomes. Though not unanimous, these findings were observed in the majority of studies assessed. GFAP could also distinguish CT-positive and CT-negative patients, unlike S100β which had inconsistent findings. Both biomarkers returned large discrepancies in reported values, indicating the need for further research in this area. Additional research is required overall to corroborate the aforementioned promising findings and to investigate those areas where minimal research has been done.

4.3. The forensic diagnostic context

The biomarkers in this review demonstrated significant differences between TBI cases and controls in the majority of studies assessed. However, only IL-6, S100β, and GFAP showed potential to be used in a forensic diagnostic context to assist FMPs in classifying TBI as a sole or contributory cause of death. Insufficient data for IL-1β and inconsistent findings for IL-10 prevented any assessment regarding their suitability as potential biomarkers of TBI. Additional studies examining these two in particular are required prior to their use as biomarkers.

The post-mortem studies for IL-1β and IL-10 were non-existent and for IL-6, S100β, and GFAP were minimal. Nevertheless, these studies provided valuable insights into the body specimens

used. Firstly, CSF consistently returned the most elevated biomarker measurements and was occasionally the only fluid with significant results (Sieber et al., 2018). This could be due to its composition being highly regulated (Sakka, Coll & Chazal, 2011) and/or because its anatomical location ensures that it is protected and undergoes minimal early post-mortem changes (Bohnert et al., 2019). CSF could therefore be one of the most informative and reliable body fluids to use in a forensic setting. Secondly, newly-investigated body fluids such as vitreous humour are promising and warrant further attention (Lanzilao et al., 2023). Similarly to CSF, vitreous humour is well-protected, shielded from contaminants, and easy to access during routine autopsy (Focardi et al., 2020), therefore further studies should be conducted using this fluid type. Finally, post-mortem brain tissue could offer investigations into the spatial expression of the biomarkers (Trautz et al., 2019) and should be explored in greater depth.

No one biomarker will ever be able to definitively prove TBI. Thus, it is not unexpected that none of the biomarkers could distinguish TBI patients from polytrauma patients (Schindler et al., 2020). Nevertheless, IL-6, S100β, and GFAP still showed considerable promise in differentiating TBI cases from controls in both clinical and post-mortem studies. With further research confirming the findings in this review, these biomarkers could be used to complement other routine forensic methods to assist in diagnosis of TBI as a sole or contributory cause of death. Additionally, these biomarkers could potentially be used to provide estimations of survival times following TBI. IL-6 specifically had greater expression in those who survived slightly longer after injury that those who died within a few hours (Trautz et al., 2019).

S100β and GFAP were also shown to differentiate between TBI severities in clinical studies. In a forensic diagnostic setting, biomarker expression levels of mild, moderate, and severe TBI could aid in its confirmation as the sole or contributory cause of death or indicate its presence in those who died from an unrelated cause. For example, a post-mortem study by Lanzilao et al. (2023) had classified severe TBI cases as those who died from TBI and mild-to-moderate TBI cases as those who died with TBI (from unrelated causes). Similarly, S100β and GFAP could indicate which mortuary cases potentially suffered fatal TBI and which merely had TBI present at death, thereby aiding FMPs in cause of death determination.

Both IL-6 and GFAP were able to differentiate between CT-positive and CT-negative scans in clinical settings, though this might not be applicable in all forensic diagnostic contexts. In South Africa for example, low dosage X-ray (Lodox) machines are used in mortuaries across the country to scan the deceased (Spies, Steyn & Brits, 2021; Horn-Lodewyk et al., 2022) and thus will detect any intracranial injuries. The FMPs will also personally observe any intracranial injuries during routine autopsy. Similarly, the ability of IL-6 and GFAP to predict TBI prognosis clinically might not have direct applications in forensic settings, as the death of the decedent

prevents the TBI from manifesting further. Nevertheless, these biomarkers could still contribute to understanding the circumstances surrounding death and aid in the interpretation of autopsy findings.

4.4. Inflammation in the brain

Before the roles of the systemic inflammatory biomarkers and brain-specific, astroglia-associated biomarkers assessed in this review can be elucidated, the proteins themselves need to be discussed. The interleukins IL-1β, IL-6, and IL-10 are involved in modulating the immune response (Justiz Vaillant & Qurie, 2023). They are a type of cytokine, which are proteins produced by various cells such as macrophages, fibroblasts, and lymphocytes, that are involved in the inflammatory response to pathogens or injury. Both IL-1β and IL-6 are pro-inflammatory cytokines, whereas IL-10 is an anti-inflammatory cytokine. The levels of these cytokines in healthy patients are negligible (Kumar, Boles & Wagner, 2015) and the studies in this review have shown them to be significantly up- or down-regulated following TBI in most instances.

Both GFAP and S100β are proteins predominantly found in astrocytes (Yang & Wang, 2015; Michetti et al., 2019); a type of glial cell in the CNS that provides structural and functional support for neurons. GFAP is a major component of the intermediate filament network in the cytoskeleton of astrocytes and thus contributes to the shape and structure of these cells (Yang & Wang, 2015). S100β is usually located intracellularly and is responsible for regulating various cellular activities and relaying signals from second messengers (Michetti et al., 2019).

Overall, TBI results in an immediate reaction of the body to the injury, triggering the activation of glial cells (including astrocytes), recruitment of leukocytes, and upregulation of various cytokines and chemokines (Ondruschka, Schuch, et al., 2018) (Figure 5). The physical damage caused by TBI results in the injured cells releasing damage-associated molecular patterns (DAMPs). These DAMPs bind to receptors on resident CNS immune cells, such as microglia and astrocytes, which initiate neuroinflammatory cascades. The DAMP molecules also cause peripheral immune cells to be recruited to the site of injury if the TBI compromised the integrity of the blood-brain barrier (Loane & Kumar, 2016; Simon et al., 2017; Visser et al., 2022). Activation of microglia and astrocytes results in the subsequent release of cytokines, such as IL-1β, IL-6, and IL-10 (resulting in their upregulation), and chemokines. Chemokines are responsible for the continued recruitment of systemic immune cells towards the site of injury, including lymphocytes, neutrophils, and monocyte-derived macrophages, which need to cross the BBB to get there (Simon et al., 2017; Xiong, Mahmood & Chopp, 2018).

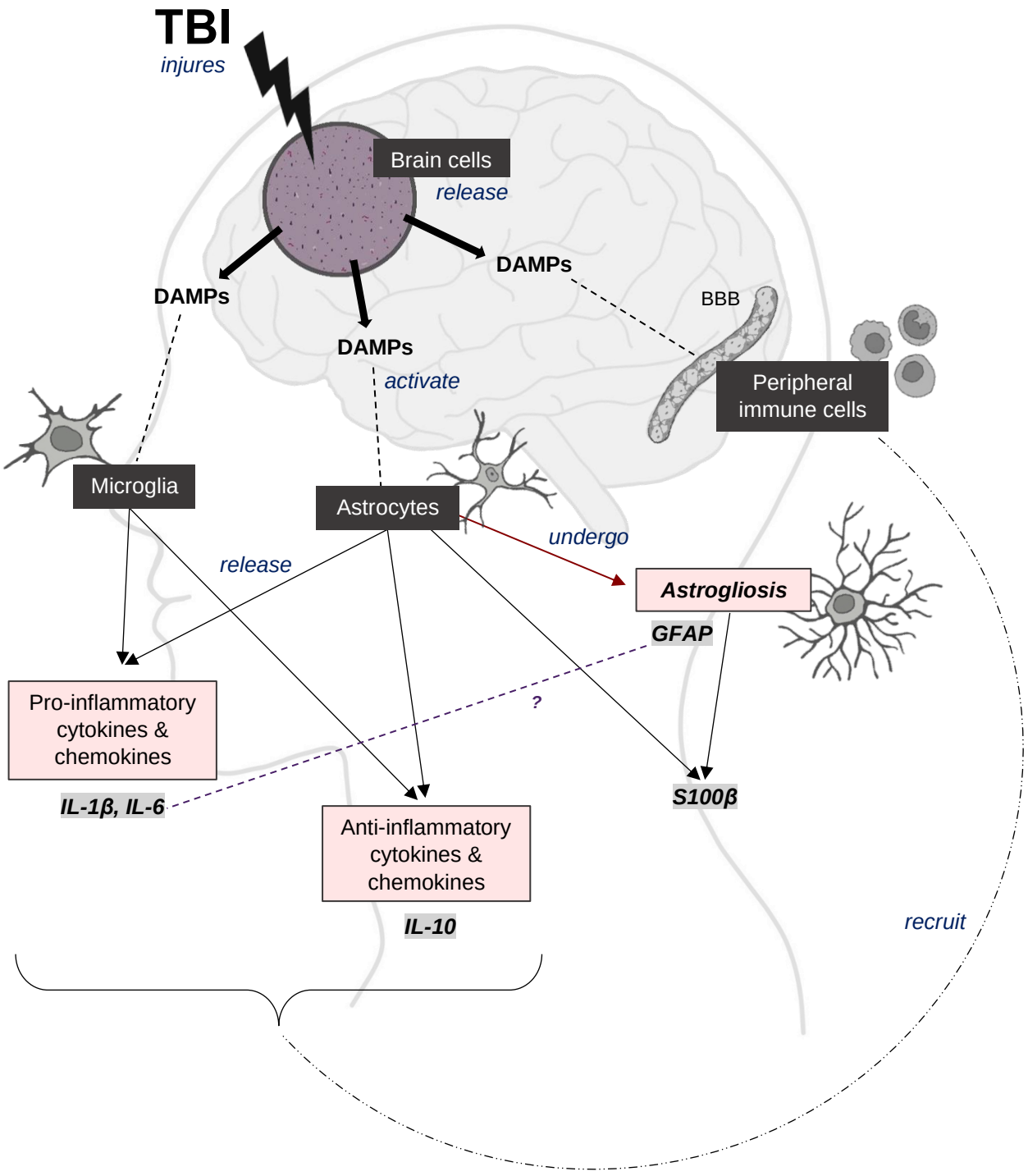

Figure 5: The possible interplay of the neuroinflammatory pathways involving the astroglia-associated biomarkers, GFAP and S100β, and the systemic inflammatory biomarkers, IL-1β, IL-6, and IL-10.

BBB: Blood brain barrier. *TBI:* Traumatic brain injury. *IL-1β:* Interleukin 1 beta. *IL-6:* Interleukin 6. *IL-10:* Interleukin 10. *S100β:* S100 calcium-binding protein β. *GFAP:* Glial fibrillary acidic protein. *DAMPs:* Damage-Associated Molecular Patterns

In addition to activated astrocytes releasing cytokines and chemokines, they also undergo astrogliosis. This involves the proliferation and enlargement of astrocytes, a process in which GFAP is rapidly upregulated to support the astrocytic cytoskeleton (Yang & Wang, 2015; Burda, Bernstein & Sofroniew, 2016; Trautz et al., 2019). This could explain the marked increase in GFAP observed in TBI patients compared to controls. Furthermore, damaged astrocytes release GFAP and S100β directly into the bloodstream and CSF (Goyal et al., 2013; Burda, Bernstein & Sofroniew, 2016). Certain studies have shown that IL-6 might also play an indirect role in GFAP upregulation through different pathways, however some studies have reported that no association exists (Mafuika et al., 2022).

Neuroinflammation is a complex process involving numerous pathways and various role-players. This review only focuses on a few of these role-players as a proxy to identify and diagnose TBI. The upregulation of IL-1β, IL-6, IL-10, S100β, and GFAP in the neuroinflammatory process provides an explanation for the measurable biomarker concentration difference between TBI cases and controls. Assessing this combination of systemic inflammatory biomarkers and brain-specific biomarkers provides a more holistic overview of the pathways in which they are involved.

In this review, S100β and GFAP were shown to be more promising biomarkers of TBI than the interleukins. This could be attributed to the localization of the astroglia-associated biomarkers to the brain and this being closer to the site of injury. It could also indicate the state of permeability of the BBB, which correlates with increasing TBI severity. However, the minimal studies on IL-1β, IL-6, and IL-10 make it challenging to compare these groups of biomarkers.

4.5. Meta-analysis

Minimal studies had reported mean biomarker values, therefore only five were included in the meta-analysis. The vast majority had reported medians, due to non-normally distributed data, or no values at all (instead using a different metric, such as area under the curve (AUC) values). There was also a paucity of post-mortem data in the meta-analysis, requiring further research in this area. The pooled effect size (Figure 4) indicated a statistically significant association between biomarker levels and TBI, although too few studies were included to draw definitive conclusions. Notably, the study by Olczak et al. (2023) was intersected by the line of no effect, indicating no clear difference in the biomarker levels between their TBI and control group.

Moderate to substantial heterogeneity was observed, which could potentially be explained by the studies having assessed their TBI and control groups slightly differently. For example,

Clarke et al. (2021) had investigated plasma GFAP at 24 hours following injury, Olczak et al. (2023) was a post-mortem study that had used only cases of fatal TBI, Janigro et al. (2020) only used mTBI cases, and Puffer et al. (2020) and To et al. (2023) had a combined TBI group consisting of various severities. Additional studies are needed to conduct a more robust meta-analysis.

4.6. Quality appraisal

Quality appraisal of the included studies revealed several areas with low ratings. Firstly, research stakeholders were not considered in research design or conduct, however this was likely due to their absence. The strengths and limitations of these included studies could have been discussed in greater detail, or even mentioned at all in some articles. More thorough reflection on the methodological robustness and potential weaknesses within the research would be useful, both to enhance transparency and trustworthiness and to inform future studies. Additionally, more detailed evidence could be provided for consideration of the sample and for choice of data collection tools, again to guide further research.

The areas with high ratings indicated that the studies had used the most suitable methodological approach and data collection tool in addressing their aims and that the method of analysis was ideal. This adds strength to the validity and overall credibility of the findings. The detailed descriptions of the data collection procedures enable reproducibility and allow for a clearer assessment of study quality.

4.7. Limitations and strengths

The strengths of this review include that the studies were obtained by a non-biased and rigorous identification method by adhering to PRISMA guidelines. The stringent inclusion criteria for the systematic review and meta-analysis ensured that the studies were of a high quality, had been published in reputable journals, and were available in well-known databases. Certain studies had provided units that were suitable for a meta-analysis, which provided new insights into collated results. Moreover, the journals the studies were published in covered a wide range of disciplines, ensuring comprehensive information on each biomarker.

Due to the study design of the systematic review, reporting bias is a limitation as there exists a tendency for studies reporting on significant results to be preferentially published in peer-reviewed journals. Negative findings add a different perspective and as such the conclusions made in this review may be slightly skewed. This is further influenced by the exclusion of grey

literature in this study, which could result in the data not being fully saturated. Only findings of the adult TBI population were assessed in this review, which limits translation to the paediatric TBI population. The study characteristics of the included articles demonstrated that the vast majority were conducted in a select few countries predominantly located in the Global North. As such, it is unclear whether the results of these studies can be translated globally to other diverse populations, such as African countries, which might reflect a different genetic makeup, potentially influencing biomarker expression.

A further limitation was the minimal number of studies obtained for this review. Certain biomarkers had a complete paucity of data in relation to specific outcome variables, making it challenging to draw conclusions. There were also minimal post-mortem studies investigating the biomarkers, thus clinical findings needed to be extrapolated to a forensic diagnostic setting. Additionally, articles had inconsistently reported their findings (and occasionally had not reported values) making certain inter-study comparisons challenging. The meta-analysis was limited by this inconsistent and heterogenous study design across studies, which resulted in only five studies being included. The lack of adequate studies restricted the meta-analysis to one forest plot and prevented the assessment of smaller groups comprising similar studies (such as those that had investigated the same body specimen or biomarker). It was also unclear whether these included articles had used samples from the same TBI population, which could have led to an over-representation of certain biomarker values, further influencing the results. Finally, the certainty of evidence rating was “very low” which could raise concerns regarding the reliability of the evidence ascertained from the included studies.

4.8. Future recommendations

There is an urgent need for additional studies on the biomarkers IL-1β, IL-6, IL-10, S100β, and GFAP, particularly on the interleukins. Additional studies would contribute to the knowledge provided by this review, by corroborating or contradicting the findings reported. This could give a clearer indication of the use of IL-1β, IL-6, IL-10, S100β, and GFAP as TBI biomarkers. Thus far, only IL-6, S100β, GFAP demonstrated promising results, however even certain findings of these biomarkers were inconsistent or minimally reported.

With regards to the outcome variables measured, future studies should focus on comparing a combined TBI group to a combined control group (and reporting the values obtained for each), prior to dividing them into subgroups and individually comparing them to each other. This was not always performed in the appraised studies. The use of different control groups would also be an important avenue to explore, involving both healthy and trauma controls. The latter could

include various types of traumas to provide clarity on whether biomarkers can differentiate these cases from TBI, as often they are unable to. Research could also focus on generating an expected range of values for each biomarker in various body fluids, as large discrepancies in values were noted amongst studies.

Notably, TBI research needs to be conducted in additional countries, particularly LMIC ones where TBI incidences are postulated to be highest. These findings could demonstrate how biomarkers of TBI behave in different populations and could help ensure that diagnosis and treatment of TBI is more effective and efficient. Larger sample sizes in studies are also imperative, so as to provide statistically significant and robust results that can be applied to a wider population.

Another important focus area for future research is a forensic setting, as minimal post-mortem studies were included in this review. Though fatal TBI and clinical TBI possess some similarities, they also vary. Biomarkers need to be thoroughly vetted in post-mortem specimens and examined in conjunction with specific post-mortem phenomena, such as post-mortem redistribution (PMR). Post-mortem brain tissue specifically should be further researched, based on the unique benefits it can provide (Lewis, 2002), as well as other easily obtainable specimens during routine autopsy such as urine and vitreous humour.

Finally, biomarkers should be investigated in panels instead of individually. Combining different biomarkers involved in the various pathways of neuroinflammation could provide a more holistic and accurate view of TBI. Certain studies have already begun evaluating them, signalling a promising step in the right direction (Zwirner, Bohnert, et al., 2021).

4.9. Conclusion

TBI poses a significant challenge worldwide, with case numbers increasing annually. Biomarkers have been evaluated in the literature for their ability to diagnose TBI, differentiate between TBI severities, predict whether CT scans of TBI patients will show intracranial lesions, and indicate injury outcome. Neuroinflammation plays a crucial role in TBI development, thus biomarkers involved in these pathways have been postulated to be useful. This systematic review aimed to compare the current literature on five potential TBI biomarkers involved in neuroinflammation; IL-1β, IL-6, IL-10, S100β, and GFAP.

The findings of this review illustrated that there is a paucity of research on these biomarkers, specifically the interleukins. The minimal studies and conflicting results made it challenging to draw definitive conclusions. Heterogenous study designs and reporting of results added to the

challenge, further resulting in a meta-analysis that could only be conducted on five studies. Nevertheless, the overall findings, meta-analysis results, and quality appraisal of the studies indicated that certain biomarkers show promise in diagnosing TBI and could potentially be implemented in a forensic context in certain settings. In particular, GFAP demonstrated the greatest diagnostic and discriminatory ability, followed by S100β. With additional research to confirm these findings GFAP could potentially be utilised as a TBI biomarker. Further studies also need to focus on the lesser-investigated systemic inflammatory biomarkers; IL-1β, IL-6, and IL-10, and body specimens; vitreous humour, urine, and post-mortem brain tissue. Notably, disparities in certain findings will need to be resolved.

Therefore, additional research is required before a definitive conclusion can be made regarding the potential of these biomarkers to aid forensic diagnosis of TBI. Thus far only GFAP has demonstrated promising results, although further studies are still required. Studies conducted in a forensic setting specifically would greatly advance our understanding of TBI in this regard. Nevertheless, progress on potential biomarkers of TBI is being made in the right direction.

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Appendix

Appendix A: Databases and search terms

Table A1: Databases, filters, and search terms used for this systematic review, with number of articles returned.

Data-base	Filters used	*IL-1β*	*IL-6*	*IL-10*	*S100β*	*GFAP*	Total number
PubMed	2018-2023. Clinical trial and RCT.	(Interleukin-1 OR IL-1) AND (traumatic brain injury OR TBI).	(Interleukin-6 OR IL-6) AND (traumatic brain injury OR TBI).	(Interleukin-10 OR IL-10) AND (traumatic brain injury OR TBI).	(s100b OR S100 calcium-binding protein B) AND (traumatic brain injury OR TBI).	(GFAP OR glial fibrillary acidic protein) AND (traumatic brain injury OR TBI).	***47 articles***
		3 results.	*13 results.*	*5 results.*	*12 results.*	*14 results.*	
ScienceDirect	2018-2023. Research articles only.	(IL-1 OR interleukin-1) AND (traumatic brain injury OR TBI) AND (biomarker). *Title, abstract, keywords:* IL-1 and TBI.	(IL-6 OR interleukin-6) AND (traumatic brain injury OR TBI) AND (biomarker). *Title, abstract, keywords:* IL-6 and TBI.	(IL-10 OR interleukin-10) AND (traumatic brain injury OR TBI) AND (biomarker). *Title, abstract, keywords:* IL-10 and TBI.	(s100b OR S100 calcium-binding protein B) AND (traumatic brain injury OR TBI) AND (biomarker). *Title, abstract, keywords:* S100b and TBI.	(gfap OR glial fibrillary acidic protein) AND (traumatic brain injury OR TBI) AND (biomarker). *Title, abstract, keywords:* GFAP and TBI.	***113 articles***
		33 results.	*28 results.*	*7 results.*	*14 results.*	*31 results.*	
Web of Science	2018-2023. Refined by article.	*All fields:* (traumatic brain injury) AND (IL-1b) OR (interleukin-1 beta). *Keywords:* must include (interleukin 1 beta), (TBI). Should include (biomarker), (neuroinflammation). Must not include (athletes), (dogs), (mice), (military), (porcine), (rats), (sports), (swine).	*All fields:* (traumatic brain injury) AND (IL-6) OR (interleukin-6). *Keywords:* must include (interleukin-6), (TBI), (biomarker). Should include (IL-6), (neuroinflammation).	*All fields:* (traumatic brain injury) AND (IL-10) OR (interleukin-10). *Keywords:* must include (interleukin-10), (TBI), (biomarker). Should include (IL-10), (neuroinflammation).	*All fields:* (traumatic brain injury) AND (s100b) OR (s100 calcium-binding protein B). *Keywords:* must include (s100B), (TBI), (biomarker). Should include (s100 calcium-binding protein B), (inflammation).	*All fields:* (traumatic brain injury) AND (GFAP) OR (glial fibrillary acidic protein). *Keywords:* must include (GFAP), (TBI), (biomarker). Should include (brain injury), (inflammation). Must not include (athletes), (sport), (military), (treatment), (paediatric), (rats), (mice), (swine), (porcine).	***156 articles***
		10 results.	*18 results.*	*9 results.*	*65 results.*	*54 results.*	
Total number		***46 articles***	***59 articles***	***21 articles***	***91 articles***	***99 articles***	***n = 316***

Appendix B: The QuADS tool and results of the included studies

Table B1: The QuADS criteria and scoring system.

QuADS criteria	Scoring			
	0	**1**	**2**	**3**
(1) Theoretical or conceptual underpinning to the research	No mention at all.	General reference to broad theories or concepts that frame the study.	Identification of specific theories or concepts that frame the study and how these informed the work undertaken.	Explicit discussion of the theories or concepts that inform the study, with application of the theory or concept evident through the design, materials and outcomes explored.
(2) Statement of research aim/s	No mention at all.	Reference to what the sought to achieve embedded within the report but no explicit aims statement.	Aims statement made but may only appear in the abstract or be lacking detail.	Explicit and detailed statement of aim/s in the main body of report.
(3) Clear description of research setting and target population	No mention at all.	General description of research area but not of the specific research environment.	Description of research setting is made but is lacking detail.	Specific description of the research setting and target population of study.
(4) The study design is appropriate to address the stated research aim/s	No research aim/s stated or the design is entirely unsuitable.	The study design can only address some aspects of the stated research aim/s.	The study design can address the stated research aim/s but there is a more suitable alternative that could have been used or used in addition.	The study design selected appears to be the most suitable approach to attempt to answer the stated research aim/s.
(5) Appropriate sampling to address the research aim/s	No mention of the sampling approach.	Evidence of consideration of the sample required.	Evidence of consideration of sample required to address the aim.	Detailed evidence of consideration of the sample required to address the research aim/s.
(6) Rationale for choice of data collection tool/s	No mention of rationale for data collection tool used.	Very limited explanation for choice of data collection tool/s.	Basic explanation of rationale for choice of data collection tool/s.	Detailed explanation of rationale for choice of data collection tool/s.
(7) The format and content of data collection tool is appropriate to address the stated research aim/s	No research aim/s stated and/or data collection tool not detailed.	Structure and/or content of tool/s suitable to address some aspects of the research aim/s or to address the aim/s superficially.	Structure and/or content of tool/s allow for data to be gathered broadly addressing the stated aim/s but could benefit from refinement.	Structure and content of tool/s allow for detailed data to be gathered around all relevant issues required to address the stated research aim/s.

(8) Description of data collection procedure	No mention of the data collection procedure.	Basic and brief outline of data collection procedure.	States each stage of data collection procedure but with limited detail or states some stages in detail but omits others.	Detailed description of each stage of the data collection procedure, including when, where and how data was gathered such that the procedure could be replicated.
(9) Recruitment data provided	No mention of recruitment data.	Minimal and basic recruitment data.	Some recruitment data but not a complete account.	Complete data allowing for full picture of recruitment outcomes.
(10) Justification for analytic method selected	No mention of the rationale for the analytic method chosen.	Very limited justification for choice of analytic method selected.	Basic justification for choice of analytic method selected.	Detailed justification for choice of analytic method selected.
(11) The method of analysis was appropriate to answer the research aim/s	No mention at all.	Method of analysis can only address the research aim/s basically or broadly.	Method of analysis can address the research aim/s but there is a more suitable alternative that could have been used or used in addition to offer a stronger analysis.	Method of analysis selected is the most suitable approach to attempt answer the research aim/s in detail.
(12) Evidence that the research stakeholders have been considered in research design or conduct.	No mention at all.	Consideration of some the research stakeholders.	Evidence of stakeholder input informing the research.	Substantial consultation with stakeholders identifiable in planning of study design and in preliminary work.
(13) Strengths and limitations critically discussed	No mention at all.	Very limited mention of strengths and limitations with omissions of many key issues.	Discussion of some of the key strengths and weaknesses of the study but not complete.	Thorough discussion of strengths and limitations of all aspects of study including design, methods, data collection tools, sample & analytic approach.

Table B2: Quality assessment of the 44 included articles, using the QuADS tool (Harrison et al., 2021).

Studies	Criteria													Total
	1	2	3	4	5	6	7	8	9	10	11	12	13	
Wijanarko et al., 2021	2	1	3	3	3	2	3	1	2	1	2	0	0	23
Trnka et al., 2023	2	2	2	3	2	1	3	2	2	2	3	0	0	24
Ward et al., 2020	2	2	2	3	1	1	3	2	2	1	3	0	2	24
Zwirner, Lier, et al., 2021	2	1	1	3	1	2	3	2	1	2	3	0	3	24
Lewis et al., 2019	1	1	3	3	1	2	3	3	2	3	3	0	1	26
Gill et al., 2018	2	2	2	3	2	2	3	2	2	2	3	0	1	26
Lanzilao et al., 2023	2	2	3	3	2	2	3	2	1	1	3	0	2	26
Zwirner, Bohnert, et al., 2021	2	3	2	3	2	1	3	2	0	3	3	0	2	26
Lewis et al., 2020	1	1	3	3	2	2	3	2	2	3	3	0	2	27
Haselmann et al., 2021	2	3	2	3	2	2	3	3	2	2	3	0	0	27
Janigro et al., 2020	3	2	3	3	2	2	3	2	2	1	3	0	1	27
Posti et al., 2019	2	2	2	3	1	2	3	3	2	2	3	0	2	27
Vedin et al., 2021	3	3	2	3	2	1	3	3	2	2	3	0	0	27
Neri et al. 2018	3	2	3	3	2	2	3	3	1	2	3	0	0	27
Biberthaler et al., 2021	2	3	3	3	2	2	3	3	2	1	3	0	1	28
Koivikko et al., 2022	2	2	3	3	2	2	3	2	2	2	3	0	2	28
Puffer et al., 2020	2	2	2	3	2	2	3	3	2	1	3	0	3	28
Schindler et al., 2020	2	3	2	3	2	2	3	3	2	1	3	0	2	28
Seidenfaden et al., 2021	1	3	3	3	2	2	3	2	3	1	3	0	2	28
Sieber et al., 2018	3	3	3	3	2	2	3	2	1	1	3	0	2	28
Trautz et al., 2019	2	2	2	3	2	1	3	3	2	2	3	0	3	28
Castaño-Leon et al., 2022	1	3	3	3	2	2	3	2	2	3	3	0	2	29
Mondello et al., 2020	2	2	2	3	2	2	3	3	2	3	3	0	2	29
Nakamura et al., 2022	2	2	3	3	2	2	3	2	3	2	3	0	2	29
Olczak et al., 2023	3	3	2	3	2	2	3	3	2	1	3	0	2	29
Oris et al., 2021	2	2	3	3	2	2	3	2	3	3	3	0	1	29
Richter et al., 2023	2	2	3	3	2	2	3	2	2	3	3	0	2	29
Hossain et al., 2019	2	3	3	3	2	2	3	2	2	3	3	0	2	30
To et al., 2023	3	3	3	3	2	2	3	3	1	3	3	0	1	30
Edwards et al., 2020	3	3	3	3	2	2	3	2	2	2	3	0	2	30
Halbgebauer et al., 2022	2	3	3	3	2	2	3	3	2	2	3	0	2	30
Lagerstedt et al., 2020	2	3	3	3	2	2	3	2	2	3	3	0	2	30

Okonkwo et al., 2020	2	2	2	3	2	2	3	3	3	2	3	0	3	30
Ondruschka, Sieber, et al., 2018	3	3	1	3	2	3	3	3	2	3	3	0	3	32
Beard et al., 2021	3	3	3	3	2	3	3	3	2	3	3	0	1	32
Clarke et al., 2021	2	3	3	3	2	2	3	3	3	3	3	0	2	32
Gardner et al., 2018	3	3	3	3	3	2	3	3	2	2	3	0	2	32
Huebschmann et al., 2020	2	3	3	3	3	2	3	3	3	2	3	0	2	32
Seidenfaden et al., 2022	2	3	3	3	2	2	3	3	3	2	3	0	3	32
Thelin et al., 2019	2	3	3	3	2	2	3	3	2	3	3	0	3	32
Korley et al., 2022	2	3	3	3	2	3	3	3	3	3	3	0	3	34
Ondruschka, Schuch, et al., 2018	3	3	3	3	2	3	3	3	3	3	3	0	2	34
Bazarian et al., 2018	2	3	3	3	2	3	3	3	3	3	3	0	3	34
Gardner et al., 2022	3	3	3	3	2	3	3	3	2	3	3	0	3	34

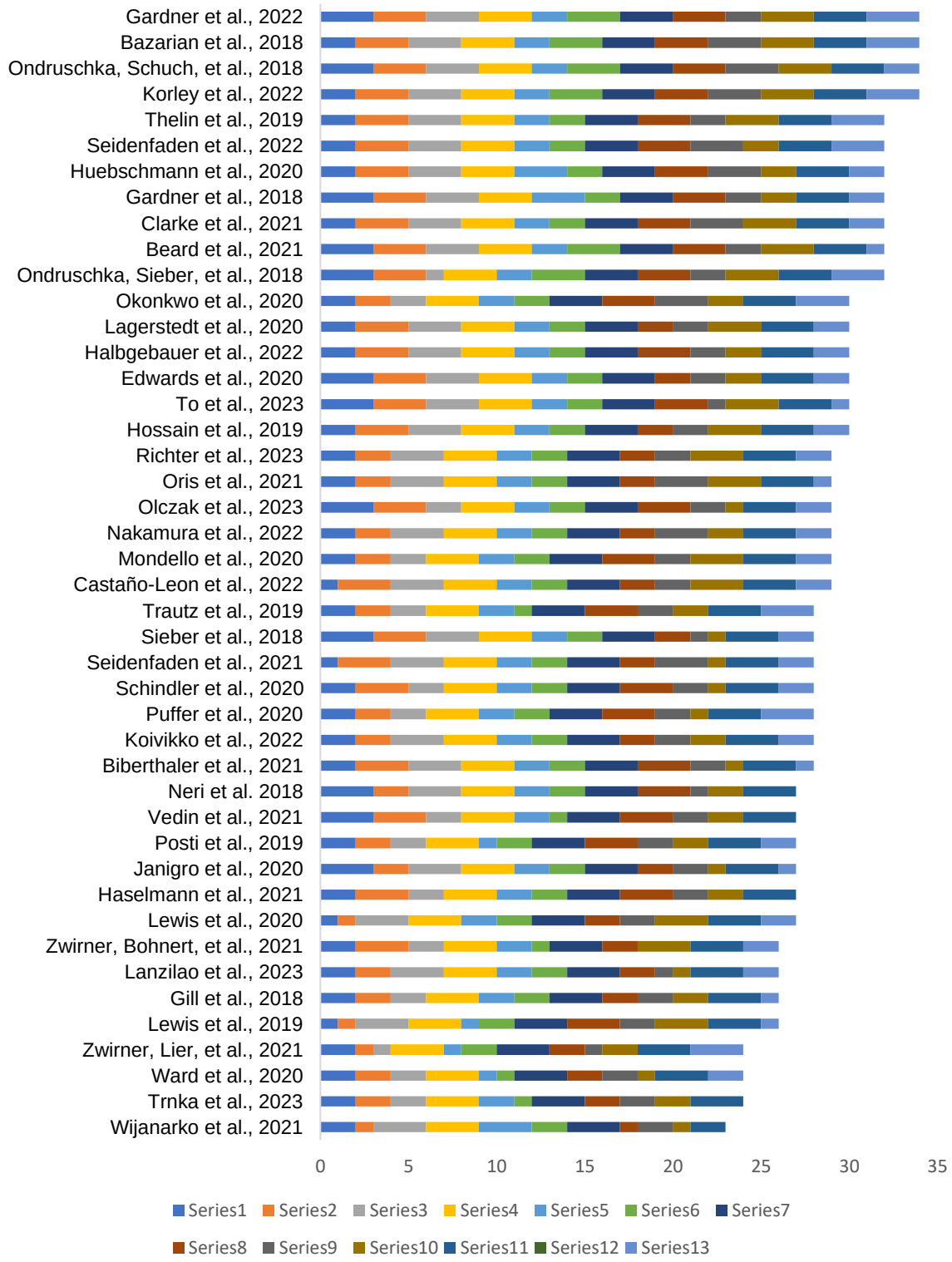

Figure B1: Quality assessment of the 44 included articles, using the QuADS tool (Harrison et al., 2021) demonstrated graphically.

The term “series” refers to the corresponding criteria, so “series1” responds to “Criteria 1” in Table B2.

Appendix C: Study characteristics

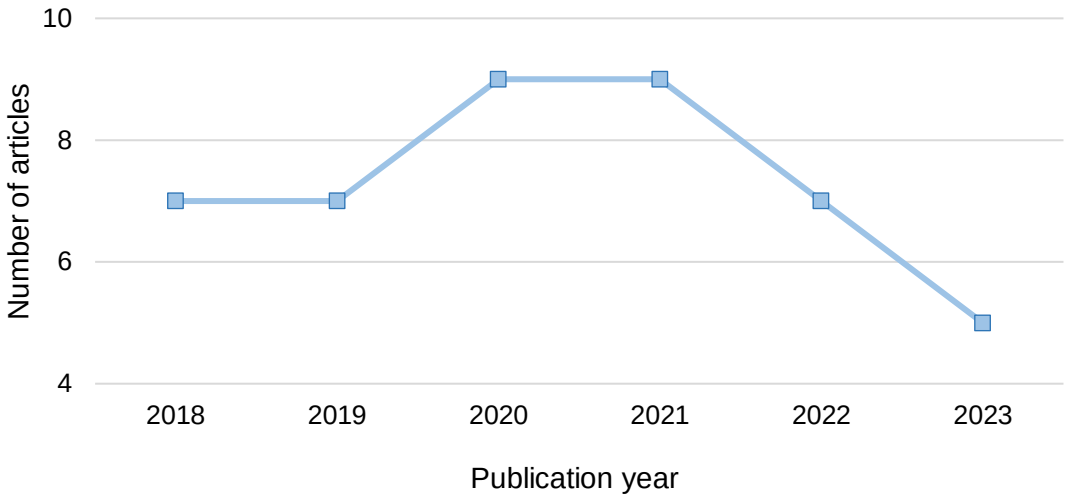

Figure C1: Year of publication for each of the final 44 articles selected for this review.

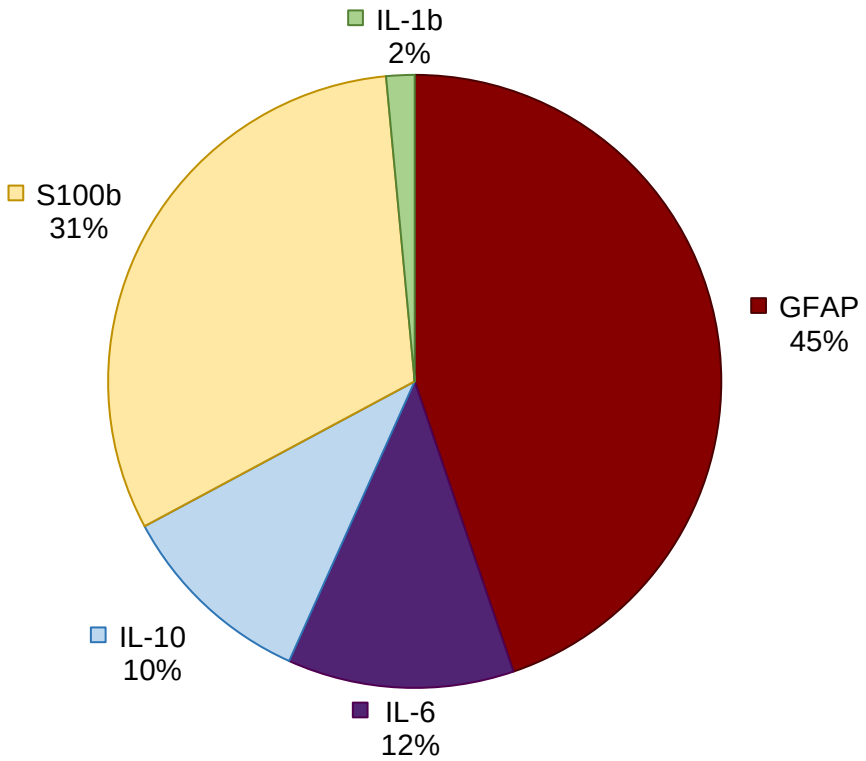

Figure C3: The frequency the biomarkers IL-1β, IL-6, IL-10, S100β, and GFAP were investigated within the 44 studies included in this systematic review.

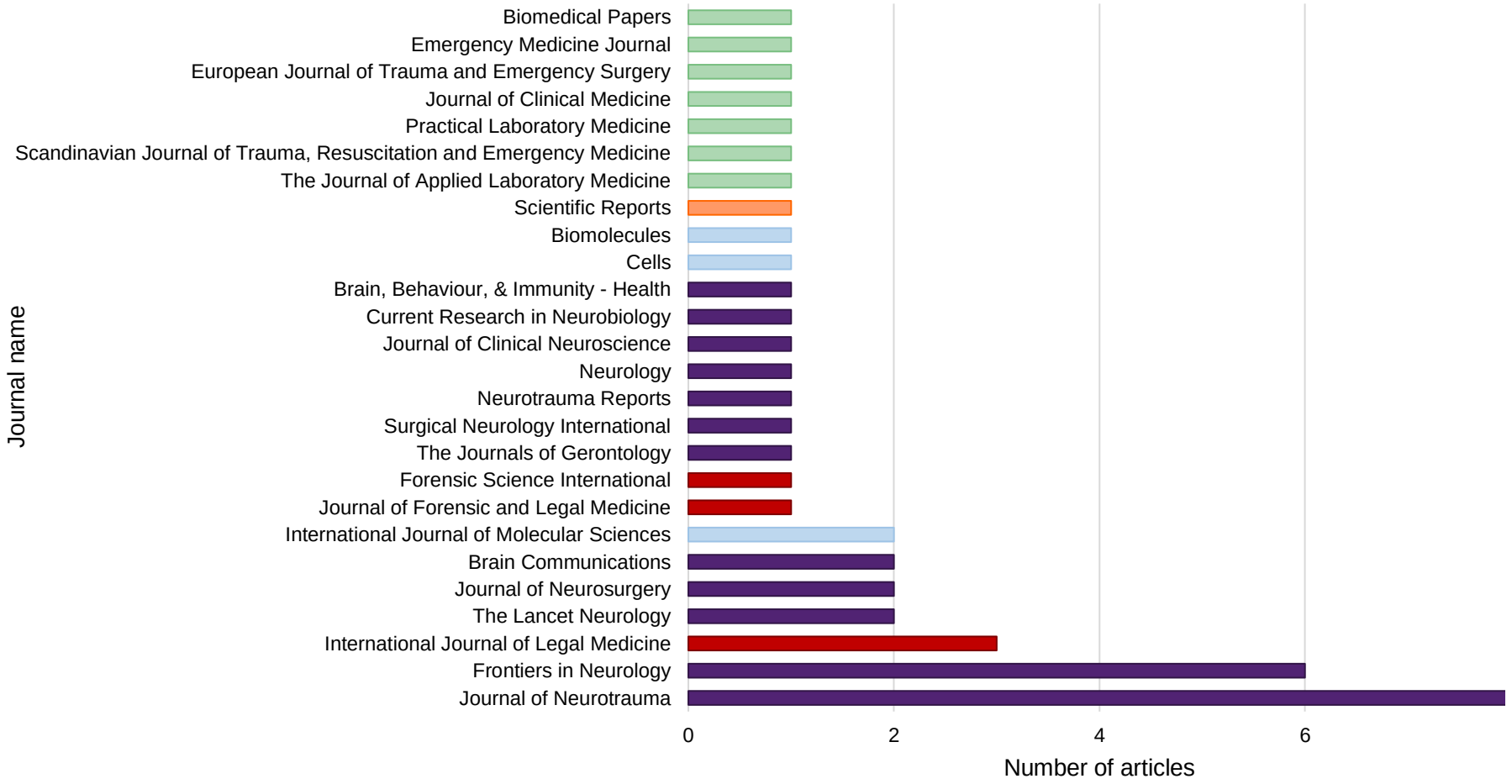

Figure C2: The titles of the various journals the 44 included articles were published in, with their separate disciplines colour-coded. *Light green:* medicine, *orange:* science, *light blue:* molecular biology, *dark purple:* neurological, *red:* law and/or forensics.

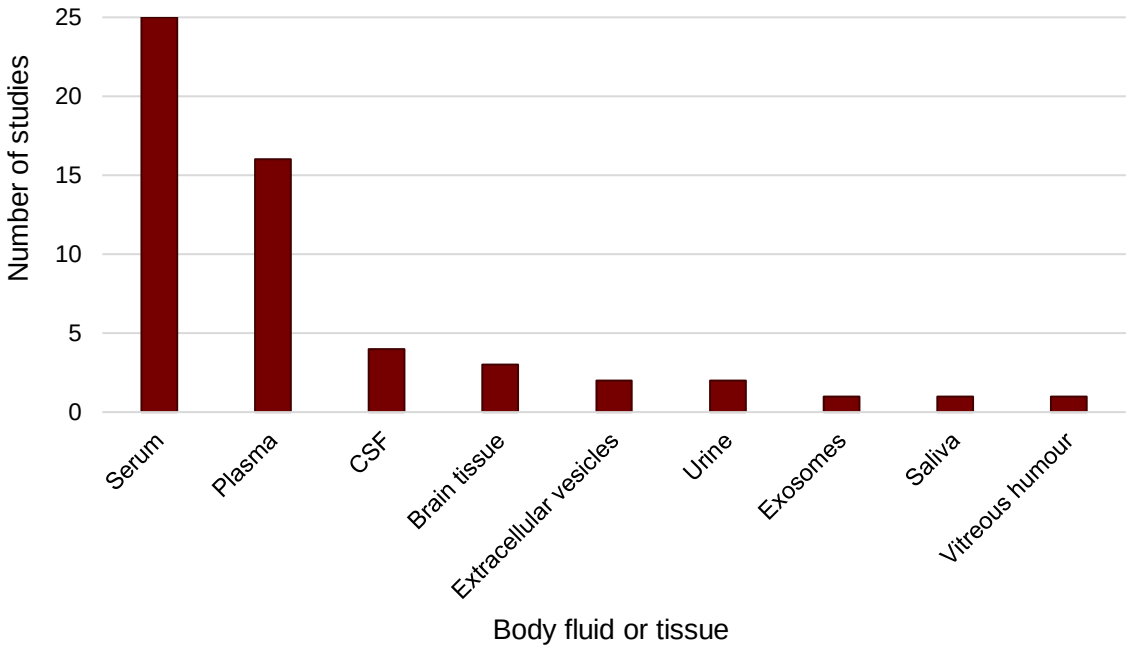

Figure C4: The frequency of the biological sample type collected to assess the biomarkers within the 44 included studies. *CSF:* Cerebrospinal fluid.

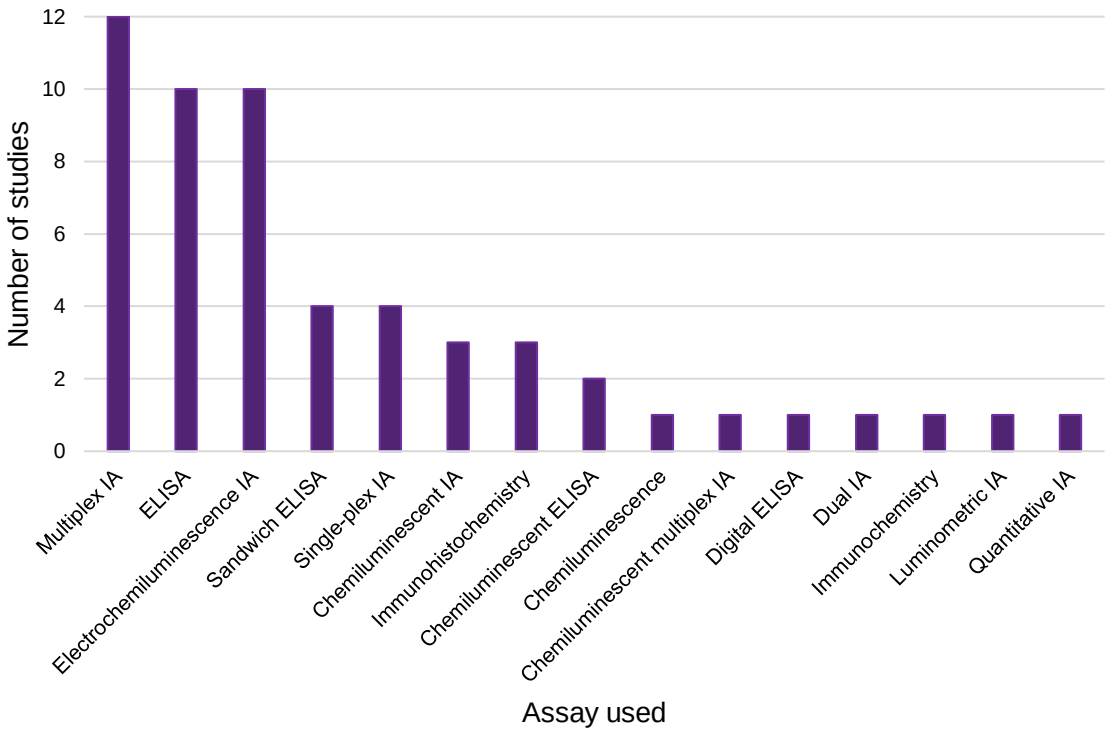

Figure C5: The distribution of various assays used to measure biomarker concentration across the 44 included articles. *IA:* immunoassay, *ELISA*: enzyme-linked immunosorbent assay.

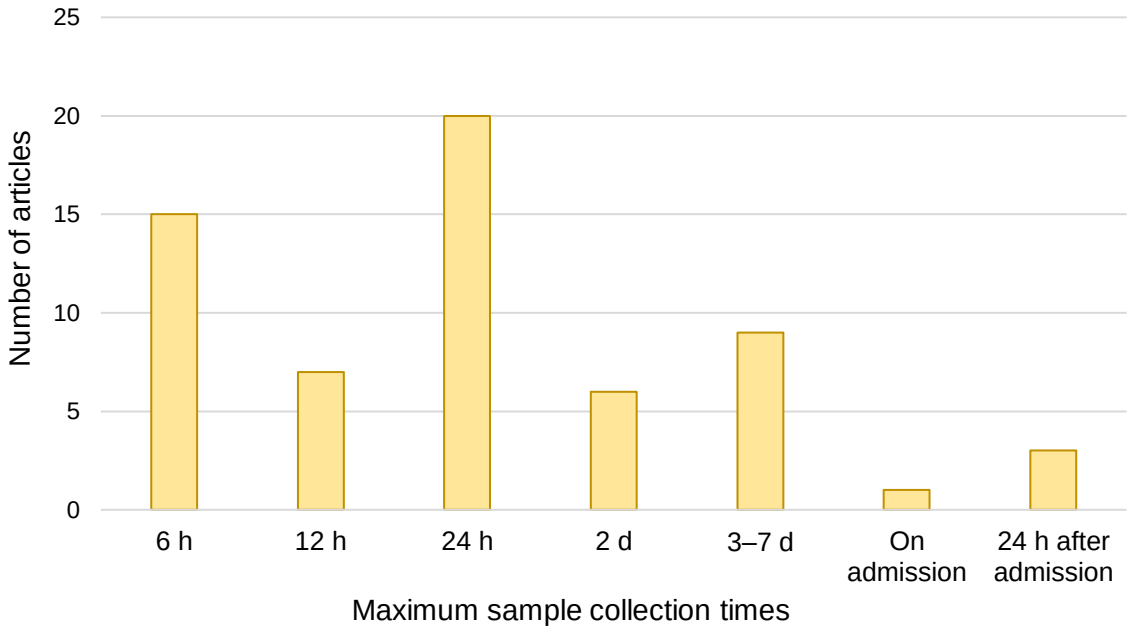

Figure C6: Time elapsed between TBI occurrence and collection of the body fluid or tissue, i.e.: the times within which the samples were collected following TBI. *h*: hours, *d*: days.

Appendix D: Summary of the findings for each biomarker

Table D1: Previous studies' findings of expression of IL-1(β) in humans with TBI compared to those without TBI.

Article	Body fluid/tissue	Sample taken/survival time	Assay	Study groups *(n)*	Results (pg/mL)	P-value
To et al. (2023)	Plasma	After admission (median of 4 days).	Multiplex immunoassay.	TBI patients *(29).*	0.35	**p < 0.05**
				HC *(19).*	1.35	

HC = healthy controls.

Table D2: Previous studies' findings of expression of IL-6 in humans with TBI compared to those without TBI.

Article	Body fluid/ tissue	Sample taken/ survival time	Assay	Study groups *(n)*	Results (pg/mL)	Extra data	P-value
To et al. (2023)	Plasma	After admission (median of 4 days).	Multiplex immunoassay.	TBI patients *(29)* Healthy controls *(19)*	0.1 0		**< 0.05**
Edwards et al. (2020)	Plasma	Within 24 hours of injury.	Multiplex immunoassay.	mTBI CT+ *(64)* mTBI MRI+/CT- *(80)* mTBI MRI-/CT- *(106)*		CT+ vs controls: 86.9%* MRI+ vs controls: 69.4%* MRI+ vs CT+: 69%*	CT+ > MRI+: **p < 0.01** MRI+ > MRI-/CT-: **p < 0.05** CT+ > MRI-/CT-: **p < 0.001**
Lewis et al. (2019)	Plasma	24–48 hours after injury.	ELISA.	TBI patients *(76):* - mRS ≤ 3 *(33)* - mRS ≥ 4 *(43)*	 133 190		**p < 0.05**
Beard et al. (2021)	Plasma	Within 24 hours of injury.	Digital ELISA.	mTBI *(47)* Controls *(46)*		86%*	**p < 0.0001**
	Extracellular vesicles	Within 24 hours of injury.	Digital ELISA.	mTBI *(47)* Control *(46)*		65%*	**p < 0.01**
Schindler et al. (2020)	Serum	Daily, on day 1–5 after injury.	ELISA.	Isolated sTBI (isTBI) *(26)* sTBI + polytrauma (PT+TBI) *(35)* Polytrauma, no TBI (PT) *(43)*	98 *(1 d)* 267 *(admission)* 400 *(1 d)*		PT > isTBI: **p < 0.001** *(1-3 d)* PT > isTBI: **p < 0.05** *(4-5 d)* PT+TBI > isTBI: **p < 0.05** *(1 d)* PT > PT+TBI: **p < 0.05** *(2 d)*

Zwirner, Bohnert, et al. (2021)	CSF (suboccipital subarachnoid space).	Survival time <2 hours post-TBI.	Quantitative immunoassay.	TBI group *(30)* Non-TBI controls *(70)*		88%*	N/A.
Ondruschka, Schuch, et al. (2018)	CSF (suboccipital subarachnoid space).	PMI** range: 5–148 hours.	Electrochemi-luminescence immunoassay.	TBI group *(46)* All controls *(49):* - ITT *(18)* - DCH *(16)* - AMI *(15)*	5 555 . 122 43 53		TBI vs controls: **< 0.001** **< 0.05** **< 0.001** **< 0.001**
	Serum (femoral veins).	PMI** range: 5–148 hours.	Electrochemi-luminescence immunoassay.	TBI group *(46)* All controls *(49):* - ITT *(18)* - DCH *(16)* - AMI *(15)*	1 091 804 240 294		TBI vs controls: **< 0.05** ns. **< 0.05** **< 0.05**
Trautz et al. (2019)	Post-mortem brain tissue.	PMI** <6 days.	Immuno-histochemistry.	aTBI *(26)* saTBI *(16)* Controls *(21)*			saTBI > controls: **p < 0.001** (PCZ) saTBI > aTBI: **p < 0.05** (PCZ) aTBI/saTBI vs controls: ns (CLC/HC/CB) aTBI/saTBI vs controls: ns (PCZ)

CSF = Cerebrospinal fluid.
PMI = Post-mortem interval.
ITT = Isolated Torso Trauma.
DCH = Diffuse Cerebral Hypoxia.
AMI = Acute Myocardial Infarction.

ns = Not significant.
mTBI = Mild TBI.
ELISA = Enzyme-linked immunosorbent kit.
sTBI = Severe TBI.
d = Day/s.
aTBI = Acute death from TBI (survival time <2 hours following TBI).
saTBI = Subacute death from TBI (survival time 2h – 3d following TBI).

*AUC value.
**Here defined as the duration between the time of death and the freeze-storage of the samples.

Table D3: Previous studies' findings of expression of IL-10 in humans with TBI compared to those without TBI.

Article	Body fluid/ tissue	Sample taken/ survival time	Assay	Study groups *(n)*	Results (pg/mL)	Extra data	P-value
Edwards et al. (2020)	Plasma	Within 24 hours of injury.	Multiplex immunoassay.	mTBI CT+ *(64)*		CT+ vs controls: 64%*	ns.
				mTBI MRI+/CT- *(80)*		MRI+ vs controls: 46.3%*	
				mTBI MRI-/CT- *(106)*		MRI+ vs CT+: 64%*	
Lewis et al. (2019)	Plasma	24–48 hours after injury.	ELISA.	TBI patients *(76):*			
				- mRS ≤ 3 *(33)*	33		**p < 0.05**
				- mRS ≥ 4 *(43)*	49		
Posti et al. (2019)	Plasma	Within 24 hours of admission.	ELISA.	TBI all severities *(160):*			
				- Non-isolated, CT+ *(40)*	1.10	All CT+ > all CT-: 67.6%*	All CT+ > all CT-: **p < 0.001**
				- Non-isolated, CT- *(26)*	0.79		
				- Isolated, CT+ *(55)*	0.86	Isolated, CT+ > isolated, CT-: 72.1%*	Isolated, CT+ > isolated, CT-: **p < 0.001**
				- Isolated, CT- *(39)*	0.37		
				mTBI (93)			
				- Non-isolated, CT+ *(18)*	1.10	mCT+ > mCT-: 58.3%*	All CT+ > all CT-: ns
				- Non-isolated, CT- *(20)*	0.73		
				- Isolated, CT+ *(19)*	0.41	Mild isolated, CT+ > mild isolated, CT-: 51.5%*	Isolated, CT+ > isolated, CT-: ns
				- Isolated, CT- *(36)*	0.34		

Beard et al. (2021)	Plasma	Within 24 hours of injury.	Digital ELISA.	mTBI *(47)*	1.17	69%*	TBI > controls: **p < 0.001**
				Controls *(46)*	0.84		
	Extracellular vesicles	Within 24 hours of injury.	Digital ELISA.	mTBI *(47)*	0.61	53%*	TBI > controls: ns
				Controls *(46)*	0.58		
Koivikko et al. (2022)	Serum	Within 24 hours of admission.	Single-plex immunoassay.	All TBI *(189):*			mTBI < moTBI: **p < 0.001** mTBI < sTBI: **p < 0.0001**
				- mTBI *(108)*	0.436		
				- moTBI *(48)*	1.41		moTBI vs sTBI: ns
				- sTBI *(33)*	1.38		moTBI/sTBI > OC: **p < 0.001**
				OC *(40)*	0.51		mTBI vs OC: ns
Lagerstedt et al. (2020)	Serum	Within 24 hours of injury.	Single-plex immunoassay.	All TBI *(88):*			Unfavourable > favourable outcome: **p < 0.001**
				- Unfavourable outcome *(28)*			
				- Favourable outcome *(60)*			
				mTBI *(49):*			Incomplete > complete recovery: ns
				- Incomplete recovery *(24)*			
				- Complete recovery *(25)*			
Schindler et al. (2020)	Serum	Daily, on day 1–5 after injury.	ELISA.	Isolated sTBI (isTBI) *(26)*	31		PT > isTBI: **p < 0.01** *(1 d)*
				sTBI + polytrauma (PT+TBI) *(35)*	111		PT+TBI > isTBI: **p < 0.05**
				Polytrauma, no TBI (PT) *(43)*	148		

mTBI = Mild TBI.
ns = Not significant.
mRS = Modified Rankin Scale. Values ≤ 3 indicate a good outcome. Values ≥ 4 indicate a poor outcome.
moTBI = Moderate TBI.
sTBI = Severe TBI.
OC = Orthopaedic controls.

*AUC value.

Table D4: Previous studies' findings of expression of S100β in humans with TBI compared to those without TBI.

Article	Body fluid/ tissue	Sample taken/ survival time	Assay	Study groups *(n)*	Results (ng/mL)	Extra data	P-value
Posti et al. (2019)	Plasma	Within 24 hours of admission.	ELISA.	TBI all severities *(160):* - Non-isolated, CT+ *(40)* - Non-isolated, CT- *(26)* - Isolated, CT+ *(55)* - Isolated, CT- *(39)* mTBI *(93):* - Non-isolated, CT+ *(18)* - Non-isolated, CT- *(20)* - Isolated, CT+ *(19)* - Isolated, CT- *(36)*	0.122 0.0811 0.0835 0.0859 0.0923 0.0811 0.0514 0.08346	All CT+ > all CT-: 58.4%** Isolated, CT+ > isolated, CT-: 46.6%** mCT+ > mCT-: 56.9%** Mild isolated, CT+ > mild isolated, CT-: 68.9%**	All CT+ > all CT-: ns Isolated, CT+ > isolated, CT-: ns mCT+ > mCT-: ns Mild isolated, CT+ > mild isolated, CT-: **p = 0.022**
Gardner et al. (2022)	Plasma	Within 24 hours of injury.	Electrochemi-luminescence immunoassay.	All TBI *(2151):* - CT+ *(461, 391, 171)* - CT- *(1690, 1760, 1980)* OC *(242)* HC *(209)*	*17-39 yrs*: 0.131, 0.202, 0.106, 0.088, 0.041 *40-64 yrs*: 0.135, 0.178, 0.107, 0.084, 0.051 *65-90 yrs*: 0.128, 0.149, 0.109, 0.096, 0.043	CT+ vs CT-: *17-39 yrs* 69.5%**, *40-64 yrs* 68.3%**, *65-90 yrs* 59.5%** TBI vs OC: *17-39 yrs* 65.2%**, *40-64 yrs* 69.2%**, *65-90 yrs* 58.1%** TBI vs HC: *17-39 yrs* 90.2%**, *40-64 yrs* 86.1%**, *65-90 yrs* 84.4%**	CT+ > CT-: **p < 0.001** *(17-64 yrs)* TBI > OC: **p < 0.001** *(17-64 yrs)* TBI > HC: **p < 0.001** *(all yrs)* TBI > OC: ns (65-90 yrs)

Haselmann et al. (2021)	Plasma	Within 24 hours of injury.	Electrochemi-luminescence immunoassay.	All TBI *(51):*	2.823	Plasma vs serum: r^2 = 0.99	CT+ vs CT-: ns
				- mTBI *(27)*	0.943		
				- mTBI CT+ *(9)*	2.069		
				- mTBI CT- *(19)*	0.469		
	Serum	Within 24 hours of injury.	Electrochemi-luminescence immunoassay.	All TBI *(51):*	2.760		
				- mTBI *(27)*	0.972		
				- mTBI CT+ *(9)*	2.146		
				- mTBI CT- *(19)*	0.477		
Okonkwo et al. (2020)	Serum	Within 24 hours of injury.	Electrochemi-luminescence immunoassay.	All TBI *(1287):*	0.12 *	CT+ vs CT-: 67%**	TBI vs OC: **< 0.001**
				- CT+ *(549)*	0.17 *		CT+ vs CT-: **< 0.001**
				- CT- *(810)*	0.10 *		
				OC *(122)*	0.08 *		CT+ vs OC: **< 0.001**
Biberthaler et al. (2021)	Serum	Within 6 hours of injury.	Electrochemi-luminescence immunoassay.	TBI *(109):*	0.15	mTBI vs msTBI: 83%**	msTBI > mTBI: **p < 0.0001**
				- mTBI *(102)*	0.14		
				- msTBI *(7)*	0.60		
		Within 2 hours of injury.	Electrochemi-luminescence immunoassay.	TBI *(20):*	0.25	CT+ vs CT-: 60%**	CT+ vs CT-: ns
				- CT+ *(3)*	0.17		
				- CT- *(17)*	0.29		
Koivikko et al. (2022)	Serum	Within 24 hours of admission.	ELISA.	All TBI *(189):*			mTBI < moTBI: **p < 0.001** mTBI < sTBI: **p < 0.0001** moTBI vs sTBI: ns moTBI/sTBI > OC: **p < 0.001**
				- mTBI *(108)*	0.07805		
				- moTBI *(48)*	0.16824		
				- sTBI *(33)*	0.18445		
				OC *(40)*	0.08510		mTBI vs OC: ns

Lagerstedt et al. (2020)	Serum	Within 24 hours of injury.	ELISA.	All TBI *(88):* - Unfavourable outcome *(28)* - Favourable outcome *(60)* mTBI *(49):* - Incomplete recovery *(24)* - Complete recovery *(25)*			Unfavourable > favourable outcome: **$p \leq 0.001$** Incomplete > complete recovery: ns
Richter et al. (2023)	Serum	Within 24 hours of injury.	Electrochemi-luminescence immunoassay.	msTBI, CT+ *(434):* - Favourable outcome *(131)* - Unfavourable outcome *(262)* msTBI, CT- *(438):* - Favourable outcome *(247)* - Unfavourable outcome *(143)*	0.25 0.62 0.23 0.49		N/A.
Schindler et al. (2020)	Serum	Daily, on admission and day 1–5 after injury.	ELISA.	Isolated sTBI (isTBI) *(26)* sTBI + polytrauma (PT+TBI) *(35)* Polytrauma, no TBI (PT) *(43)*	0.071 *(on admission)* 0.333 *(on admission)* 0.495 *(on admission)*		ns.
Seidenfaden et al. (2022)	Serum	Within 6 hours of injury. Pre-hospital and in-hospital values.	Chemi-luminescent immunoassay.	TBI patients *(592):* - mTBI *(566)* - moTBI *(20)* - sTBI *(6)*	*Pre-hospital* 290 510 810	*In-hospital* 170 330 440	N/A.

Seidenfaden et al. (2021)	Serum	Within 6 hours of injury. Pre-hospital and in-hospital values.	Chemi-luminescent immunoassay	mTBI *(566)* - Pre-hospital samples - In-hospital samples			. 290 170				Pre-hospital > in-hospital values: **p < 0.001**
Thelin et al. (2019)	Serum	Days 1–5 after injury.	Luminometric immunoassay & electrochemi-luminescence immunoassay.	TBI *(172):* - GOS 1 - GOS 3 - GOS 4 - GOS 5	*1 d* 2000 330 210 110	*2 d* 1300 220 350 120	*3 d* 1330 260 140 190	*4 d* 270 150 90 50	*5 d* 580 90 60 70	GOS1-3 vs 4-5: 70.8%** GOS 1 vs 2-5: 82.2%**	. GOS1-3 vs 4-5: **p < 0.001** GOS 1 vs 2-5: **p < 0.001**
Oris et al. (2021)	Serum	Within 3 hours of injury.	Electrochemi-luminescence immunoassay.	mTBI *(1172):* - 65–79 years *(476)* - 80–89 years *(491)* - >90 years *(205)*			0.23 0.184 0.255 0.318				**p < 0.001**
Wijanarko et al. (2021)	Serum	Within 3 hours of injury, and then 27 hours after injury.	ELISA.	mTBI (22)	*< 3 h* 0.0285		*3–27 h* 0.0223				**p = 0.004**
Trnka et al. (2023)	Serum	Within 3 hours of injury, then after 8 h, 24 h, 72 h.	Electrochemi-luminescence immunoassay.	TBI patients *(124):* - With polytrauma (PT+) *(33)* - No polytrauma (PT-) *(91)*	*3 h* 546 1896 1180	*8 h* 178 663 293	*24 h* 130 250 185	*72 h* 85 148 115			Overall TBI *3 h* value > *8 h / 24 h / 72 h:* **p < 0.001** PT+ > PT-: **p < 0.001** *(3 h)* PT+ > PT-: ns *(8 h, 24 h, 72 h)*

Janigro et al. (2020)	Serum	Within 6 hours of injury.	Chemi-luminescence & automated sandwich ELISA.	TBI patients *(15)*	0.502	94%**	TBI vs controls (serum and saliva): **< 0.01**
				Controls *(15)*	0.058		
	Saliva	Within 6 hours of injury.	Chemi-luminescence and automated sandwich ELISA.	TBI patients *(15)*	3.620	75%**	
				Controls *(15)*	0.849		
Vedin et al. (2021)	Serum	Within 6 hours of injury.	Electrochemi-luminescence immunoassay.	Isolated TBI (Population 1) *(243):*	130		Population 2 overall > Population 1 with ICH: ns
				- With ICH *(13)*	180		
				- Without ICH *(230)*	120		
				Isolated TBI with ICH (Population 2) *(13):*	220		
		At 2, 4, 6, 8, 12, 24, 48 h after injury.		Isolated TBI with ICH (Population 2) *(13)*			
	Urine	Within 6 hours of injury.	Electrochemi-luminescence immunoassay.	Isolated TBI (Population 1) *(243):*	70		Population 1 with ICH > Population 2 overall: **p = 0.010**
				- With ICH *(13)*	80		
				- Without ICH *(230)*	70		
				Isolated TBI with ICH (Population 2) *(13)*	39		
		At 2, 4, 6, 8, 12, 24, 48 h after injury.		Isolated TBI with ICH (Population 2) *(13)*			

Olczak et al. (2023)	Serum (femoral vein puncture)	12–24 hours after death.	ELISA.	Fatal sTBI *(40)*	19.64		**p < 0.005**
				Cardiopulmonary death controls *(20)*	7.23		
	Urine (aspirated directly from bladder)	12–24 hours after death.	ELISA.	Fatal sTBI *(40)*	0.45		ns
				Cardiopulmonary death controls *(20)*	0.05		
Sieber et al. (2018)	Serum (femoral veins).	PMI* range: 5–148 hours.	Electrochemi-luminescence immunoassay.	TBI group *(45):*			ns
				- aTBI *(23)*	132		
				- saTBI *(11)*	347		
				- dTBI *(11)*	211.1		
				All controls *(49):*			
				- ITT *(18)*	51.7		
				- DCH *(15)*	281.5		
				- AMI *(14)*	99		
	CSF (suboccipital subarachnoid space).	PMI* range: 5–148 hours.	Electrochemi-luminescence immunoassay.	TBI group *(45):*			TBI vs controls: **< 0.001**
				- aTBI *(23)*	5946		
				- saTBI *(11)*	12 372		TBI vs DCH/AMI: **< 0.05**
				- dTBI *(11)*	8320		
				All controls *(49):*			TBI vs ITT: **< 0.001**
				- ITT *(18)*	1890.5		
				- DCH *(15)*	3247		aTBI vs DCH/ ITT: **< 0.01**
				- AMI *(14)*	4308		aTBI vs sTBI: **< 0.05**

Zwirner, Bohnert, et al. (2021)	CSF (suboccipital subarachnoid space)	Survival time <2 hours post-TBI.	Quantitative immunoassay.	TBI group *(30)* Non-TBI controls *(70)*		78%**.	N/A.
Lanzilao et al. (2023)	Vitreous humour (both eyes)	Within 48 hours of death (<6 hours after TBI).	Immunochemistry.	mmTBI *(10)* sTBI *(20)*		68%**	**< 0.05**

ELISA = Enzyme-linked immunosorbent kit.
PMI = Post-mortem interval.
aTBI = Acute death from TBI (survival time <2 hours following TBI).
saTBI = Subacute death from TBI (survival time 2h – 3d following TBI).
dTBI = Delayed death from TBI (survival time 3–19 d following TBI).
ITT = Isolated Torso Trauma.
DCH = Diffuse Cerebral Hypoxia.
AMI = Acute Myocardial Infarction.
ns = Not significant.
CSF = Cerebrospinal fluid.
OC = Orthopaedic controls.
mmTBI = Mild to moderate TBI.
sTBI = Severe TBI.
HC = Healthy controls.
mTBI = Mild TBI.
moTBI = Moderate TBI.
GOS = Glasgow Outcome Scale.
h = Hours.
ICH = Intracerebral haemorrhage.

*Here defined as the duration between the time of death and the freeze-storage of the samples.
**AUC values.

Table D5: Previous studies' findings of expression of GFAP in humans with TBI compared to those without TBI.

Article	Body fluid/ tissue	Sample taken/ survival time	Assay	Study groups *(n)*	Results (pg/mL)			Extra data	P-value
					0 h	*24 h*	*5 d*		
Halbgebauer et al. (2022)	Plasma.	0** h, 24 h, 5 d, 10 d after injury.	Single-plex immuno-assay	Severe trauma patients, with msTBI *(19)* Severe trauma patients, no TBI *(13)* HC *(13)*	15,768	25,887 174	7892	TBI vs non-TBI: 89%*	All severe trauma patients > HC (0 h, 24 h, 5 d): **p < 0.01** TBI > non-TBI (0 h): **p < 0.001** TBI > non-TBI (24 h & 5 d): **p < 0.01**
Okonkwo et al. (2020)	Plasma.	Within 24 hours of injury.	Sandwich ELISA.	TBI patients *(1359):* - CT+ *(549)* - CT- *(810)* OC (122)		336 1358 116 13		CT+ vs CT-: 85%*	TBI > OC: **p < 0.001** CT+ > CT-: **p < 0.001** CT+ > OC: **p < 0.001**
Biberthaler et al. (2021)	Plasma	Within 6 hours of injury.	Dual immuno-assay.	Total TBI patients *(109):* - mTBI *(102)* - msTBI *(7)*		**73** 64 764		mTBI vs msTBI: 91%*	msTBI > mTBI: **p < 0.0001**
	Plasma	Within 2 hours of injury.	Dual immuno-assay.	TBI patients *(20):* - CT+ *(3)* - CT- *(17)*		77 833 56		CT+ vs CT-: 97%*	CT+ > CT-: **p = 0.010**

Korley et al. (2022)	Plasma	Within 24 hours of injury.	Sandwich ELISA & chemi-luminescent immunoassay.	All TBI *(1696):*			GOSE 1-4 vs 5-8: 86%*	GOSE 1 vs 2-8: **p < 0.0001**
				- GOSE = 5-8 *(1461)*	356			
				- GOSE = 2-4 *(235)*	3998		GOSE 1 vs 2-8: 87%*	GOSE 2-4 vs 5-8: **p < 0.0001**
				- GOSE = 1 *(120)*	8680		GOSE 1-7 vs 8: 62%*	
Clarke et al. (2021)	Plasma	Within 24 hours of injury and again at 72 hours post injury.	Multiplex immunoassay.		*24 h*	*72 h*		mTBI > AC: **p < 0.001** (24 h) & **p = 0.004** (72 h)
				mTBI patients *(207):*	636.4	291.4		mTBI+ > mTBI-: **p = 0.01** (24 h) & **p = 0.04** (72 h)
				- mTBI+ *(76)*	836.1	432.6	mTBI vs controls: 92%* *(24 h)*	mTBI+/mTBI- > CC: **p < 0.0001** (24 h)
				- mTBI- *(131)*	495.5	215.7		
				AC *(136)*	38.2	30.1		mTBI+/mTBI- > TC: **p < 0.0001** (24 h) & **p = 0.008** (72 h)
				- CC *(84)*	38.1		mTBI vs controls: 74%* *(72 h)*	
				- TC *(52)*	41.1	30.1		mTBI+ > TC: **p = 0.03** (72 h)
Hossain et al. (2019)	Plasma	Within 24 hours of admission.	Multiplex immunoassay.	GOSE 8	612		GOSE 8 vs <8: 60%*	GOSE 8 vs GOSE<8: ns
				GOSE < 8	1467			
				GOSE 5–8	875		GOSE 5–8 vs 1–4: 75.5%*	GOSE 5–8 vs 1–4: **p = 0.002**
				GOSE 1–4	4867			

Gardner et al. (2018)	Plasma	Within 24 hours of injury.	Sandwich ELISA.	<40 yrs *(79):*	150		
				- CT+ *(18)*	1550		<40 yrs vs 40–59 yrs vs ≥60 yrs: **p = 0.032**
				- CT- *(61)*	110		
				40–59 yrs *(60):*	280		
				- CT+ *(20)*	2220		CT+ > CT-: **p < 0.001** (<40 yrs)
				- CT- *(40)*	150		
				≥60 yrs *(30):*	460		CT+ > CT-: **p < 0.001** (40–59 yrs)
				- CT+ *(18)*	670		
				- CT- *(12)*	220		CT+ > CT-: **p = 0.038** (≥60 yrs)
Gill et al. (2018)	Plasma	Within 48 hours of injury.	Multiplex immunoassay.	mTBI (277)			
				- MRI+, CT+ (69)	2853	mTBI vs HC: 93%*	HC < MRI+, CT+/ MRI+, CT-/ MRI-, CT-: **p < 0.001**
				- MRI+, CT- (28)	2098	CT+ vs CT-: 77%*	
				- MRI-, CT- (177)	266.2	MRI+ vs MRI-: 80%	MRI+, CT+ > MRI+, CT-: **p < 0.01**
				HC (49)	56.18	CT-MRI- vs CT-MRI+: 74%*	MRI+, CT+ > MRI-, CT-: **p < 0.001**

Gardner et al. (2022)	Plasma	Within 24 hours of injury.	ELISA & chemi-luminescent immunoassay.		*17-39 yrs*	*40-64 yrs*	*65-90 yrs*		*17-39 yrs*	*40-64 yrs*	*65-90 yrs*	CT+ > CT-: **p < 0.001** (all ages)
				All TBI *(2151):*	378	503.4	618.8	CT+ vs CT-	87.4%*	85.4%*	83.5%	
				- CT+ *(461, 391, 171)*	1612.7	1497.2	1162					
				- CT- *(1690, 1760, 1980)*	122.4	159	115.2	TBI vs OC	89.9%*	91.1*	94.8%*	TBI > OC/HC: **p < 0.001** (all ages)
				OC *(242)*	8.4	14	26.6					
				HC *(209)*	5	10	28.5	TBI vs HC	95.4%*	95.3%*	96%*	
Posti et al. (2019)	Plasma	Within 24 hours of admission.	Multiplex immunoassay.	TBI all severities *(160):*								
				- Non-isolated, CT+ *(40)*		5890		All CT+ > all CT-: 82.2%*				All CT+ > all CT-: **p < 0.001**
				- Non-isolated, CT- *(26)*		1140						
				- Isolated, CT+ *(55)*		6840		Isolated, CT+ > isolated, CT-: 95.9%*				Isolated, CT+ > isolated, CT-: **p < 0.001**
				- Isolated, CT- *(39)*		204						
				mTBI *(93):*								
				- Non-isolated, CT+ *(18)*		1830		mCT+ > mCT-: 72%*				mCT+ > mCT-: **p < 0.001**
				- Non-isolated, CT- *(20)*		1140						
				- Isolated, CT+ *(19)*		604		Mild isolated, CT+ > mild isolated, CT-: 74.9%*				Mild isolated, CT+ > mild isolated, CT-: **p = 0.003**
				- Isolated, CT- *(36)*		186.64						

Beard et al. (2021)	Plasma	Within 24 hours of injury.	Digital ELISA.	mTBI *(47)* Controls *(46)*		91%*	**p < 0.0001**
	Extracellular vesicles	Within 24 hours of injury.	Digital ELISA.	mTBI *(47)* Controls *(46)*		70%*	**p < 0.001**
Huebschmann et al. (2020)	Plasma	Within 12 hours of injury.	Single-plex immunoassay.	Total mTBI *(121):* - CT+ *(22)* - CT- *(70)* - Good outcome *(31)* - Poor outcome *(76)*	635.9 1509.2 459.1 369.8 1509.2	CT+ vs CT-: 77.8%* Good vs poor outcome: 65.3%*	Total plasma values > serum: **p < 0.001** CT+ > CT- (plasma & serum): **p < 0.001**
	Serum	Within 12 hours of injury.	Multiplex immunoassay.	Total mTBI *(121):* - CT+ *(22)* - CT- *(70)* - Good outcome *(31)* - Poor outcome *(76)*	497 1108.2 344.5 283.1 551.6	CT+ vs CT-: 81.4%* Good vs poor outcome: 69%*	Poor > good outcome (plasma & serum): **p = 0.002**
Nakamura et al. (2022)	Serum	On admission.	ELISA.	Severe TBI *(41)* Non-severe TBI *(138)*	6000 149	81%*	**< 0.0001**

Bazarian et al. (2018)	Serum	Within 12 hours of injury.	Chemi-luminescent ELISA.	Total TBI patients (1959)	51.9		
				- CT+ (125)	171.7		CT+ > CT-: **p < 0.0001**
				- CT- *(1843)*	44.6		
Castaño-Leon et al. (2022)	Serum	Within 24 hours of injury.	Multiplex immuno-assay.	Total TBI patients *(87):*	12 224.28		
				- mTBI *(23)*	7145		All TBI/mTBI/ moTBI/sTBI > HC: **p < 0.001**
				- moTBI *(16)*	5302	GOSE 1-3 vs 4-8: 78%*	
				- sTBI *(48)*	17 325		
				HC (17)	91.35		mTBI < sTBI: **p = 0.008**
Lagerstedt et al. (2020)	Serum	Within 24 hours of injury.	Multiplex immunoassay.	All TBI *(88):*			
				- GOSE ≤ 4 *(28)*			GOSE ≤4 > GOSE ≥5: **p ≤ 0.001**
				- GOSE ≥ 5 *(60)*			
				mTBI *(49)*			
				- GOSE ≤ 7 *(24)*			GOSE ≤7 > GOSE =8: ns
				- GOSE = 8 *(25)*			

Richter et al. (2023)	Serum	Within 24 hours of injury.	Multiplex immunoassay.	msTBI, CT+ *(434):*		N/A.
				- Favourable outcome *(131)*	15 000	
				- Unfavourable outcome *(262)*	39 000	
				msTBI, CT- *(438)*		
				- Favourable outcome *(247)*	9600	
				- Unfavourable outcome *(143)*	27 000	
Schindler et al. (2020)	Serum	Daily, on day 1–5 after injury.	ELISA.	Isolated sTBI (isTBI) *(26)*	5.82 *(day 1)*	PT > isTBI: **p = 0.038** *(1 d)*
				sTBI + polytrauma (PT+TBI) *(35)*		PT > isTBI: **p < 0.05** *(2 d)*
				Polytrauma (PT), no TBI *(43)*	23.80 *(day 1)*	PT > isTBI: **p < 0.05** *(3 d)*
Seidenfaden et al. (2021)	Serum	Within 6 hours of injury. Obtained pre- & in-hospital values.	ELISA.	mTBI *(566):*		ns
				- Pre-hospital samples	1780 *(highest)*	
				- In-hospital samples	2340 *(highest)*	

Thelin et al. (2019)	Serum	Daily, on day 1–5 after injury.	Multiplex immunoassay.	TBI *(172)*	*1 d*	*2 d*	*3 d*	*4 d*	*5 d*	GOS1-3 vs 4-5: 72.4%*	.
				- GOS 1	235687	402995	86914	10267	21376		GOS1-3 vs 4-5: **p < 0.001**
				- GOS 3	40554	22145	35762	21041	4892		
				- GOS 4	33646	12128	19079	4167	4643	GOS 1 vs 3-5: 81.4%*	GOS 1 vs 3-5: **p < 0.001**
				- GOS 5	11422	8783	7112	3867	1662		
Puffer et al. (2020)	Extracellular vesicles	Within 24 hours of injury.	ELISA.	GCS = 15 (8)			207.8				**p = 0.02**
				GCS ≤ 14 (12)			2204.2				
Lewis et al. (2020)	Serum	Within 6 hours of injury.	Sandwich ELISA.	CT+ unfavourable outcome (5)			5237				**p = 0.026**
				CT+ favourable outcome (140)			284				

Ward et al. (2020)	Serum	Within 12 hours of injury.	Chemiluminescent ELISA.	All TBI *(1959):*	24.3	All TBI vs <65 yrs vs ≥65 yrs: **p < 0.001**
				<65 yrs *(1455)*	17.7	
				- CT+ *(70)*	167.1	CT+ <65 yrs vs CT+ ≥65 yrs: ns
				- CT- *(1294)*	16.2	
				≥65 yrs *(504):*	40.6	
				- CT+ *(35)*	102.3	CT- <65 yrs vs CT- ≥65 yrs: **p < 0.001**
				- CT- *(420)*	38.6	
Mondello et al. (2020)	Exosomes	Daily, on day 1–5 after injury.	Multiplex immunoassay.	msTBI *(21)*	*1 d*: 10 944; *2 d*: 9112; *3 d*: 3698; *4 d*: 2938; *5 d*: 2179	Serum > exosomes: **p < 0.001** (Day 1–5, respectively)
	Serum	Daily, on day 1–5 after injury.	Multiplex immunoassay.	msTBI *(21)*		
Olczak et al. (2023)	Urine (aspirated directly from bladder)	12–24 hours after death.	ELISA.	Fatal sTBI *(40)*	2520	**p < 0.0001**
				Cardiopulmonary death controls *(20)*	990	
	Serum (femoral vein puncture)	12–24 hours after death.	ELISA.	Fatal sTBI *(40)*	43 410	**p < 0.05**
				Cardiopulmonary death controls *(20)*	1800	

Ondruschka, Sieber, et al. (2018)	CSF (suboccipital subarachnoid space).	PMI range: <148 hours.	Chemiluminescent multiplex immunoassay.	TBI group *(42):* - aTBI *(21)* - saTBI *(10)* - dTBI *(11)* All controls *(42):* - ITT *(14)* - DCH *(14)* - AMI *(14)*	 501 000 1 578 000 440 000 77 000 733 000 136 000	TBI vs controls: 79%*	All TBI fatalities > all control fatalities: **p < 0.05** All TBI > ITT: **p < 0.05**
	Serum (femoral vein).	PMI range: <148 hours.	Chemiluminescent multiplex immunoassay.	TBI group *(42):* - aTBI *(21)* - saTBI *(10)* - dTBI *(11)* All controls *(42):* - ITT *(14)* - DCH *(14)* - AMI *(14)*	 5330 2800 1230 540 290 360	TBI vs controls: 78%*	All TBI fatalities > all control fatalities: **p < 0.05** All TBI > ITT: **p < 0.05** All TBI > DCH: **p < 0.05** All TBI > AMI: **p < 0.05**

Zwirner, Bohnert, et al. (2021)	CSF (sub-occipital sub-arachnoid space).	Survival time <2 hours post-TBI.	Quantitative immune-assay.	TBI group *(30)* Non-TBI controls *(70)*				77%*	N/A.
Neri et al. (2018)	Brain tissue.	Different times of death.	Immuno-histochemistry.	Almost immediate *(25)* 1 day *(24)* 3 days *(24)* 7 days *(24)* Controls *(25)*				+/- ^ ++ ^ +++ ^ +++ ^ +/- ^	GFAP immunopositivity 1–7 days of survival > immediate death and controls: **p < 0.05** 1 vs 3 vs 7 days: **p < 0.001**
Trautz et al. (2019)	Post-mortem brain tissue.	PMI*** <6 days.	Immuno-histochemistry.	aTBI *(26)* saTBI *(16)* Controls *(21)*					aTBI/saTBI vs controls: ns (PCZ/CLC/HC/CB)
Zwirner, Lier, et al. (2021)	Post-mortem brain tissue.	PMI*** <6 days.	Immuno-histochemistry.		PCZ (%)^	CLC (%)^	IHC (%)^	CB (%)^	
				aTBI *(24)*	0	0	0	0	
				saTBI *(15)*	13	0	17	14	
				Controls *(16)*	0	0	0	6	

* AUC value.
** 0 hours: on admission to hospital, which occurred <4 hours following injury.
*** Here defined as the duration between the time of death and tissue storage.
^ Study counted the number of GFAP-positive astrocytes.

CSF = Cerebrospinal fluid.
ELISA = Enzyme-linked immunosorbent kit.
msTBI = Moderate to severe TBI.
h = Hours.
HC = Healthy controls.
OC = Orthopaedic controls.
mTBI = Mild TBI.
moTBI = Moderate TBI.
sTBI = Severe TBI.
AC = All controls.
CC = Community controls.
TC = Trauma controls.
PCZ = Pericontusional zone.
CLC = Contralateral cortical area.
IHC = Ipsilateral hippocampus.
CB = Ipsilateral cerebellum.
yrs = Years.
GOS-E = Glasgow Outcome Scale – Extended.
PMI = Post-mortem interval.
aTBI = Acute death from TBI (survival time <2 hours following TBI).
saTBI = Subacute death from TBI (survival time 2h – 3d following TBI).
dTBI = Delayed death from TBI (survival time 3–19 d following TBI).
ITT = Isolated Torso Trauma.
DCH = Diffuse Cerebral Hypoxia.
AMI = Acute Myocardial Infarction.

Appendix E: Data management plan and ethical approval