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Published in:
Neuroscience and Biobehavioral Reviews

DOI:
[10.1016/j.neubiorev.2021.11.036](https://doi.org/10.1016/j.neubiorev.2021.11.036)

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
2022

[Link to publication in University of Groningen/UMCG research database](#)

Citation for published version (APA):

Visser, K., Koggel, M., Blaauw, J., van der Horn, H. J., Jacobs, B., & van der Naalt, J. (2022). Blood-based biomarkers of inflammation in mild traumatic brain injury: A systematic review. *Neuroscience and Biobehavioral Reviews*, 132, 154-168. <https://doi.org/10.1016/j.neubiorev.2021.11.036>

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Review article

Blood-based biomarkers of inflammation in mild traumatic brain injury: A systematic review

Koen Visser^{a,*}, Milou Koggel^b, Jurre Blaauw^a, Harm Jan van der Horn^a, Bram Jacobs^a, Joukje van der Naalt^a

^a Department of Neurology, University of Groningen, University Medical Center Groningen, Hanzeplein 1, 9713 GZ, Groningen, the Netherlands

^b Faculty of Science, Department of Biology, Utrecht University, Padualaan 8, 3584 CH, Utrecht, the Netherlands



ARTICLE INFO

Keywords:

Mild traumatic brain injury
Inflammation
Biomarkers
Cytokines
Diagnosis
Prognosis
Inflammatory markers
Neuroinflammation

ABSTRACT

VISSER, K., M. Koggel, J. Blaauw, H.J.v.d. Horn, B. Jacobs, and J.v.d. Naalt. Blood based biomarkers of inflammation in mild traumatic brain injury: A systematic review. *NEUROSCI BIOBEHAV REV* XX(X) XXX-XXX, 2021. – Inflammation is an important secondary physiological response to traumatic brain injury (TBI). Most of the current knowledge on this response is derived from research in moderate and severe TBI. In this systematic review we summarize the literature on clinical studies measuring blood based inflammatory markers following mild traumatic brain injury (mTBI) and identify the value of inflammatory markers as biomarkers. Twenty-three studies were included. This review suggests a distinct systemic inflammatory response following mTBI, quantifiable within 6 h up to 12 months post-injury. Interleukin-6 is the most promising biomarker for the clinical diagnosis of brain injury while interleukin-10 is a potential candidate for triaging CT scans. The diagnostic and prognostic utility of inflammatory markers may be more fully appreciated as a component of a panel of biomarkers. However, discrepancies in study design, analysis and reporting make it difficult to draw any definite conclusions. For the same reasons, a meta-analysis was not possible. We provide recommendations to follow standardized methodologies to allow for reproducibility of results in future studies.

1. Introduction

It is estimated that around half of the global population will experience a traumatic brain injury (TBI) during their lifetime and TBI is foreseen to be within the top three causes of neurodisability up to 2030 (Maas et al., 2017; WHO, 2006). Mild traumatic brain injury (mTBI) constitutes 80–90 % of all TBI cases of which more than 20–30 % suffer from persistent symptoms affecting daily functioning up to six months or later after injury (Bazarian et al., 2005; McInnes et al., 2017). Although, most individuals with mTBI fully recover, it is difficult to predict who will suffer from persistent symptoms.

The pathophysiology of TBI is complex, with the outcome being determined by an interaction between the primary injury and secondary injury responses that include: neuroinflammation, blood-brain-barrier (BBB) disruption, and metabolic disturbances (Dixon, 2017; van der Horn et al., 2019; Werner and Engelhard, 2007). Dysregulation of these secondary injury processes may contribute to persisting symptoms and unfavourable outcome following TBI.

To establish the diagnosis of mTBI, the main clinical tool used in the Emergency Department (ED) is head computed tomography (CT) to assess the presence of a structural intracranial lesion. However, CT lacks sensitivity for identifying more diffuse injuries such as traumatic axonal injury (TAI), which also can be found after mTBI. More advanced imaging modalities such as magnetic resonance imaging (MRI) have shown more specificity for detecting diffuse trauma-related brain damage. However, MRI is often not feasible to regularly assess in the acute phase. There is a need for more sensitive, readily available diagnostic tools at the ED to diagnose a mTBI and the possible presence of tissue damage.

Biomarkers are an established research topic which might bridge this gap. A biomarker is generally defined as an objective indicator of biological state observed from outside the patient – which can be measured accurately and reproducibly (e.g., concentrations of biochemicals measured in blood or using advanced neuroimaging, such as diffusion or functional MRI) (Strimbu and Tavel, 2010; van der Horn et al., 2019). Measuring biomarkers after TBI could potentially aid in diagnosing injury, monitoring disease progression, and predicting long-term

* Corresponding author at: Department of Neurology, University Medical Center Groningen, Hanzeplein 1, 9713 GZ, Groningen, the Netherlands.
E-mail address: k.visser.16@student.rug.nl (K. Visser).

outcome (Wang et al., 2018).

In mTBI, biochemical markers are frequently subjects of investigation, owing to the aforementioned limitations of routing clinical neuroimaging. A recent scoping review of biochemical biomarkers in TBI reported that amongst 1036 studies, 540 unique biomarker candidates were analysed (Edalatfar et al., 2021). However, only a handful of these markers have been approved for clinical use: S100 calcium-binding protein B (S100B) in the Scandinavian neurotrauma guidelines (Undén et al., 2013) and a combination panel of Ubiquitin Carboxy-terminal Hydrolase L1 (UCH-L1) and Glial Fibrillary Acidic Protein (GFAP) by the Federal Drug Authority (FDA) (Bazarian et al., 2018). In both cases, clinical use is restricted to the purpose of identifying patients with a low risk of intracranial injury, removing the need for a head CT scan. Large international collaborations such as the CENTER-TBI and TRACK-TBI are spearheading the hunt for biomarkers that can predict outcome or monitor disease progression in the medical clinic (Manley and Maas, 2013; Maas et al., 2015).

The multi-faceted pathophysiology of mTBI makes the process of finding useful biomarkers challenging. Most individual biomarkers often lack the necessary sensitivity and specificity required for daily clinical care. Therefore, studies are now investigating biomarker panels consisting of markers that cover possible active pathophysiological pathways in mTBI (Wang et al., 2018; Huie et al., 2019). Insights into these pathways may lead to better characterization of injury profiles, which may ultimately lead to individualized treatment strategies. However, currently little is known about which biochemical markers of secondary injury may contribute to such a panel.

Dysregulated neuroinflammation is a pertinent secondary injury process in mTBI, that has often been implicated in unfavourable outcome (Schimmel et al., 2017). Microglia and Astrocytes are the central immune effectors, that initiate neuroinflammation in a response to damage-associated molecular patterns (DAMPs) released by cells damaged after trauma. Active neuroinflammation consists of a complex cascade of many intertwined and parallel running pathways, that can be upregulated or downregulated according to many factors (See reviews by Simon et al., 2017; Xiong et al., 2018) for a comprehensive overview of the mechanisms of neuroinflammation in TBI). Dysregulation of these pathways can be damaging to healthy brain tissue, leading to secondary

injury after TBI. Inflammatory signalling molecules such as cytokines, chemokines, and acute phase proteins (APPs) are potential biochemical markers of this response (Thelin et al., 2017).

To accurately quantify the neuroinflammatory response, inflammatory markers are ideally measured close to their source of origin. In moderate and severe TBI, cerebrospinal fluid (CSF) or micro dialysate can be analysed, for example at the intensive care unit (Zeiler et al., 2017). This is not feasible in mild TBI as invasive techniques such as lumbar puncture to obtain CSF fluid are considered disproportionate to the severity of injury. Studies in mTBI are therefore limited to the measurement of inflammatory markers in less invasive fluid compartments such as blood. Studies have demonstrated that the concentration of blood-based inflammatory markers are much lower than concentrations identified in the CSF (Kossmann et al., 1995; Csuka et al., 1999). Furthermore, there are also numerous limitations inherent to the investigation of blood-based biomarkers in mTBI (see McDonald et al., 2021 for a detailed discussion). Additionally, many non-TBI related factors have a strong influence on the concentrations of inflammatory markers measured in the individual patient (Fig. 1).

A well-recognized limitation of inflammatory blood biomarkers is that they are non-specific to brain injury. Inflammation is present in response to almost any disease that involves cellular damage. For TBI specifically, extracranial injury is a major systemic source of inflammatory markers (McDonald et al., 2016) Systemic inflammation resulting from for instance extracranial injury, also has a direct influence on the magnitude of the neuroinflammatory response (Lassarén et al., 2021). Pre-clinical studies have reported that the presence of extracranial injury exacerbates the neuroinflammatory response after TBI (Shultz et al., 2015; Yang et al., 2016). These findings majorly limit the potential application of inflammatory markers in mTBI patients with concomitant extracranial injury. A major challenge is therefore to find methods of separating neuroinflammation from systemic inflammation, or to find markers that are specific to the neuroinflammatory response. Despite the many challenges for measuring blood-based inflammatory markers after TBI, important reviews of inflammation in moderate and severe TBI suggest that there is potential clinical utility for these markers (Zeiler et al., 2017; Woodcock and Morganti-Kossmann, 2013).

Systematic reviews can help appraise and interpret the vast amount

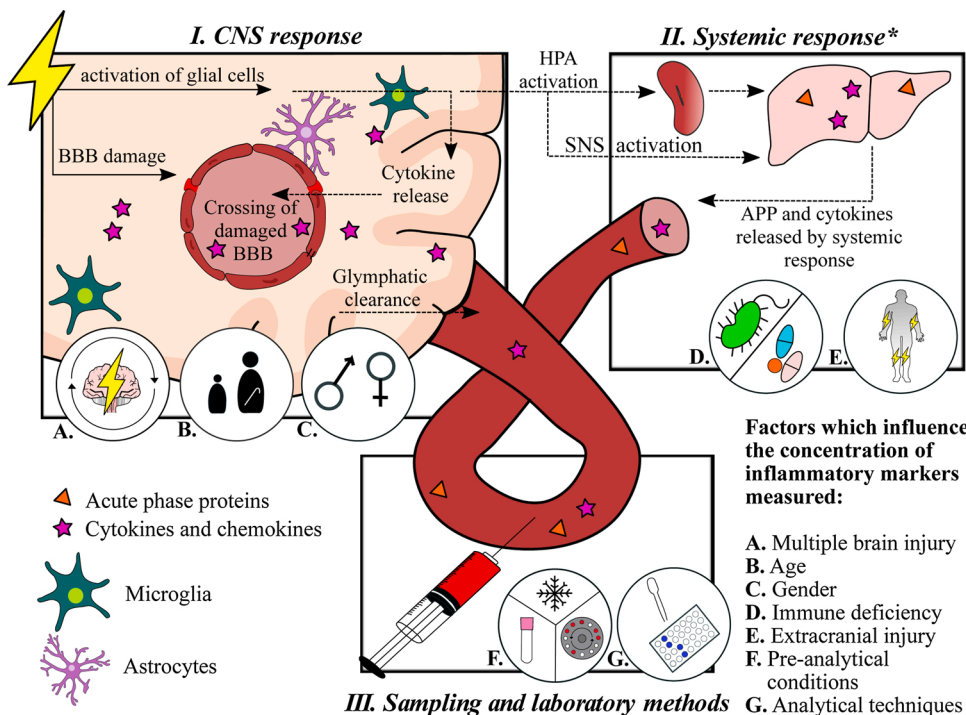


Fig. 1. Elements that determine the measured concentration of inflammatory markers in the blood after mTBI. I. Within hours after trauma, glial cells release inflammatory markers into the intracranial spaces. These markers enter the blood by the way of glymphatic clearance or leakage through the damaged blood brain barrier. II. Organs such as the liver and spleen release inflammatory markers into the systemic circulation as a response to HPA and SNS activation caused by the injured brain. III. Sampling and laboratory methods add further variance to the final concentrations of inflammatory markers measured.

* The extent of the systemic inflammatory response has a direct influence on the strength of the CNS response (Lassarén et al., 2021).

Note. Acute phase proteins (APP); Central nervous system (CNS); Traumatic brain injury (TBI); blood brain barrier (BBB); hypothalamic-pituitary-adrenal (HPA); Sympathetic nervous system (SNS).

of published TBI biomarker research, providing high quality evidence for the use of inflammatory markers in mTBI (Huie et al., 2021). We therefore aimed to systematically review the current knowledge on blood-based biomarkers of inflammation in mTBI. We focused on three key questions based on the common use cases of biomarkers in TBI. First, which inflammatory markers have been studied after mTBI and compared to blood levels in controls? Second, what is the ability of acute markers of inflammation to differentiate between patients with and without traumatic findings on conventional imaging (CT and MRI)? Finally, what is the ability of acute markers to predict long term functional outcome? In this review we will also summarize the most frequently used methods in studies for measuring inflammatory markers.

2. Materials and methods

This systematic review is reported according to the Preferred Reporting Items for Systematic Reviews and Meta Analyses (PRISMA) guidelines (Page et al., 2020). Prior to the commencement of this review a protocol was published to the PROSPERO database (registration number CRD42021227196).

2.1. Information sources and search strategy

The databases PubMed, PsychInfo (EBSCO host), EMBASE, and Web of Science were systematically searched using search terms related to mTBI, inflammatory blood biomarkers and observational studies. Results relating to animal studies were explicitly excluded in the search strategy. The search was last updated on the 5th of October 2021 and went as far back as data was available. Supplementary Material S1 provides the full search strategy used in each database.

Included articles were forward searched using Google scholar and their references were checked for additional studies which may have been missed by our database search.

2.2. Study selection

Citations identified by database searching were first uploaded into EndNote X9 (Philadelphia, PA) for automatic and manual de-duplication. Citation screening occurred in two stages: First unique citations were uploaded into Rayan QCRI (Ouzzani et al., 2016) for title and abstract screening. Next the full text of relevant articles were obtained and assessed against the study eligibility criteria. Both stages were performed by two reviewers (K.V. and M.K.). At each stage, the reviewers first independently assessed the studies, blinded to the decisions of the other. On completion discrepancies were discussed and any remaining conflicts were resolved by a third reviewer (J.v.d.N).

2.3. Eligibility criteria

To be eligible for inclusion in this review studies had to: Include patients with mTBI, measure a minimum of one inflammatory biomarker in blood (plasma or serum) and either assess its levels relative to controls, and/or in relation to conventional imaging findings, and/or with long-term outcomes (no restriction on the outcome measure was used). Additionally, studies had to be in English, peer reviewed, available in full text with, an observational or diagnostic accuracy design. Mild TBI is defined by the author's definition, however an initial Glasgow Coma Scale (GCS) score between 13–15 was a minimal requirement.

Studies were ineligible if they included non-human subjects or children with non-accidental TBI. There was no restriction on age. Studies also measuring markers of inflammation in subjects with more severe TBI or in fluid compartments other than blood were excluded if they did not provide a separate analysis of mTBI patients or blood compartment data. Studies with overlapping populations were included

if they had <50 % overlap or measured separate outcomes or biomarkers.

2.4. Assessment of methodological quality

The New-Castle Ottawa scale for observational studies (NOS) was used to assess the bias of observational studies (Wells et al., 2000). The Quality Assessment of Diagnostic Accuracy Studies 2 (QUADAS-2) was used to assess the risk of bias in studies of diagnostic accuracy (Whiting et al., 2011). Both criteria were modified to match this review. One reviewer (K.V.) performed bias checking of the studies included in this review. A second reviewer (M.K.) checked for completeness. No study was removed on account of the quality assessment as this review is exploratory in nature and no clear standards are available yet.

2.5. Data extraction

Data extraction was carried out using a piloted data extraction form. One reviewer (K.V.) independently carried out data extraction. A second reviewer (M.K.) successively checked these data extraction forms for completeness. Data was retrieved for the following items: study design, first author, study setting, participant demographics (age, sex, comorbidities), GCS score, post traumatic amnesia (PTA), loss of consciousness (LOC), mechanism of injury, inclusion criteria (definition of mTBI), laboratory aspects of biomarker measurement (assay, limits of quantification, concentrations of markers, methods of analysis, sampling time, storage temperature), relationship between conventional imaging (head CT and/or MRI) and blood markers (imaging protocol, definition of positive imaging, cut-offs, sensitivity, specificity, ROC curves), relationship between outcome tools and markers (outcome tools used, time points, results, statistical analysis used), study recommendations/conclusions, study limitations.

2.6. Data synthesis

Initially we planned, as stated in our PROSPERO protocol, to carry out a meta-analysis of our findings. The heterogeneity in study outcomes, reporting and design made this infeasible. Data was therefore synthesized using a qualitative approach. Tables and figures were constructed for the main characteristics of the studies and for each key question of this review. Fig. 1 was made in Inkscape (v.1.1) the figures in Section 3 were constructed using Python (v.3.9.2) and the Matplotlib library (Caswell et al., 2018).

3. Results

3.1. Search results

Literature searching identified 1396 unique records, an additional 6 records were obtained through citation searches of the included articles (See Fig. 2). Full texts were obtained for 62 articles, of which 23 were included in this review. Two studies showed overlap, data of (Lagerstedt et al., 2018a) was also used for the analyses in (Lagerstedt et al., 2018b), we therefore only reported results from the former which focused on the non-overlapping sub cohort. Similarly, study cohorts from (Lagerstedt et al., 2020) and (Meier et al., 2020) were drawn from the same study population as (Posti et al., 2019) and (Nitta et al., 2019) respectively. However, both studies reported distinctly different outcomes and were included. The most common reason for article exclusion was incorrect study type, being conference abstracts. Some articles (Huie et al., 2019; Posti et al., 2020) lacked stratification of their analyses to the mTBI cohort.

3.2. General characteristics of the included studies

The general characteristics of each included study are displayed in

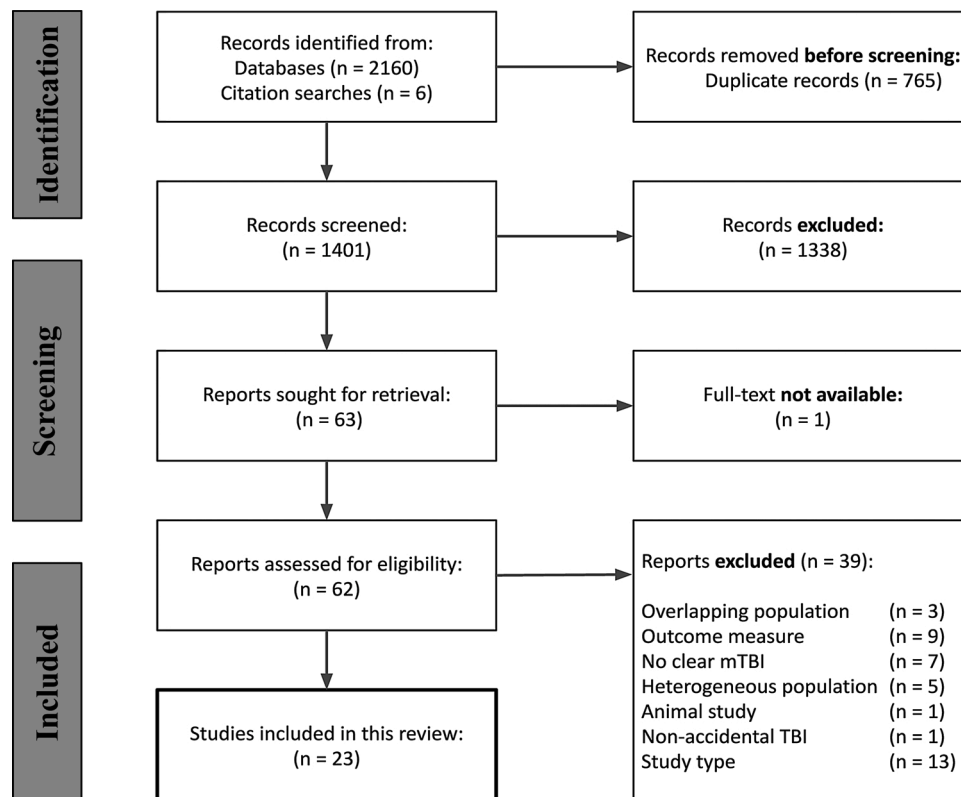


Fig. 2. PRISMA flow diagram showing each stage of the screening process for article inclusion.

Table 1. All studies were published in recent years, from 2014, with eighteen (78 %) being published from 2019 onwards. Population sizes ranged from 10 up to 1030 mTBI patients. Twelve (52 %) included less than 100 mTBI patients. Most study cohorts (n = 18) comprised mTBI patients seen at the ED, one study comprised military personnel (Edwards et al., 2020a), and four studies solely included athletes (Di Battista et al., 2019, 2020b; Meier et al., 2020; Nitta et al., 2019). Definitions of mTBI differed; ten studies defined mTBI solely on the GCS score, whereas others used published definitions; most commonly the WHO Collaborating Centre Task Force definition (Carroll et al., 2004). Most studies (n = 15) only considered mTBI patients with isolated head injury. The abbreviated injury score (AIS) and Injury Severity Score (ISS) was most often used to define extracranial injury.

A wide variety of inflammatory markers were measured between the studies. Marker selection was often based on evidence found in studies of other neurological conditions (for instance epilepsy or, stroke). Four studies took an exploratory approach and measured a large variety of markers to discover potentially novel markers (Anada et al., 2018; Carabias et al., 2020; Lagerstedt et al., 2018b; Sharma et al., 2017). Regarding the blood compartment in which the studies were performed eleven were done in plasma and twelve in serum. Multiplex was the most common assay technique. For further details regarding the collection, processing and storage of samples see Supplementary Material S4.

3.3. Timing of blood sampling, conventional imaging, and outcome measure(s)

The timing and number of blood draws varied between studies (Fig. 3). Ten studies sampled at only one time-point post injury compared to thirteen studies that longitudinally measured the inflammatory profile. Sampling intervals ranged from six hours after injury up to twelve months post-injury. Only two studies included a baseline, pre-injury measurement (Meier et al., 2020; Nitta et al., 2019). Computed tomography (CT) imaging was done within 24 h after injury in all

studies. Eleven studies also measured various outcomes at different time points (see Section 3.7. of this review).

3.4. Methodological quality of the included studies

Eighteen observational cohort studies were assessed using the Newcastle Ottawa scale (NOS) ranging from 0 (high bias) – 8 (low bias). No study scored a maximum of 8 points. Five of the sixteen studies scored 7. The lowest score was 2 which was obtained by two studies (Shetty et al., 2019; Su et al., 2014). See Supplementary Material S2 for the risk of bias of all individual studies. Five studies were assessed using the QUADAS-2 tool for studies of diagnostic accuracy (see Supplementary Material S3). Similarly, to the NOS scale all studies had a high risk of bias for at least one item. The lowest score was 2 (Posti et al., 2019).

3.5. The inflammatory response following mild traumatic brain injury

Fourteen studies measured levels of inflammatory markers in controls. Most studies 11/14 (79 %) considered only healthy controls. One study included only orthopaedic controls (Anada et al., 2018) and the remaining two studies included both (Vedantam et al., 2021; Xu et al., 2020). In almost all studies, controls were age and sex matched, with studies predominantly including male subjects (Table 2). In these studies, 26 different inflammatory markers were analysed (Fig. 3). In Section 3.3 the time intervals in which each study assessed the levels of biomarkers are reported.

Tumor necrosis factor alpha (TNF α) was the most frequently measured marker, but its concentration was only significantly elevated relative to controls in two out of eight studies (Chaban et al., 2020; Thompson et al., 2020). Interleukin-6 (IL-6) was the next most frequently measured marker. The concentration of IL-6 was significantly elevated compared to controls in six out of the seven studies in which it was measured. Significant elevations were quantified within six hours after mTBI (Nitta et al., 2019) and up to six months (Vedantam et al.,

Table 1
General characteristics and outcome parameters of the included studies.

Reference (Year)	Inclusion criteria	Poly-trauma/ ECI	Subjects (n), CT+ /MRI+	Diagnostic criteria for mTBI	Markers of inflammation analyzed	Blood compartment, assay (Manufacturer)	Study goals(s)	Bias (NOS)
(Anada et al., 2018)	Admission with TBI & age 18–50	X	*ED (10), CT+ (N.A.)	GCS 14–15	**CRP, SAA1 & A1A	Serum, ELISA (Abcam, USA)	Differentiating severity of TBI	6
(Carabias et al., 2020)	Admission with closed head injury, age 15–85 & indication for head CT	Yes	*ED (90), CT+ (N.A.)	GCS 13–15	**SAA1	Serum, ELISA (Anogen, Canada)	Differentiating severity of TBI and extracranial injury	5
(Chaban et al., 2020)	Age 16–59 & referral for head CT	Yes	ED (207), MRI+ (11.1 %)	WHO	**IL-1RA, IL-8, IL-9, IL-17, Eotaxin, IFN γ , IP10, MCP-1, MIP-1 β , TNF α , TARC	Plasma, Immunoplex (Bio-Rad, USA)	Temporal profile of inflammatory markers compared to controls & association between acute phase cytokine levels and demographic variables. Characterize inflammatory profiles after SRC & differentiating between MSK and SRC	7
(Di Battista et al., 2019)	No concussion within 6 months of enrollment	X	University Athletes (43), CT+ (N.A.)	Concussion in Sport Group Guidelines	**IFN γ , IL-8, TNF α , MPO, MCP-1, MCP-4, MIP-1 β , IP-10, CCL17, Eotaxin	Plasma, Multiplex (Meso Scale Diagnostics, USA)	Characterize profile of IL-6 after SRC while addressing confounders	6
(Di Battista et al., 2020b)	No concussion within 6 months of enrollment	X	University Athletes (41), CT+ (N.A.)	Concussion in sport group guidelines	IL-6	Plasma, Multiplex, (ProteinSimple, USA)	Characterize the relationship between cytokines and recovery in active-duty service members	7
(Edwards et al., 2020a)	Provider diagnosed concussion without other major injury	X	Military (45), CT+ (0 %)	Department of Defense Protocol	IL-6, IL-10, TNF α	Serum, Simoa (Quanterix, USA)	Differentiating mTBI patients with and without CT and MRI findings	5
(Edwards et al., 2020b)	Age 18–96, GCS > 13 & Initial visit <24 -hs post-injury	N.A.	ED (250), CT+ (25.6 %) & MRI+ (32 %)	GCS 13–15	IL-6, IL-10, TNF α	Plasma, Simoa (Quanterix, USA)	The relationship between serum G-CSF and serum TNF α	6***
(Huang et al., 2020)	Closed TBI, positive head CT & admission < 24 h	X	*Admission or ICU (20), CT+ (100%)	GCS 13–15	TNF α	Serum, ELISA (Xitang Biological co., China)	Differentiating mTBI patients with negative or positive head CT	5
(Lagerstedt et al., 2018a)	GCS < 15, age > 14, blood sample < 6 h & CT < 24 h	Yes	ED (109), CT+ (16 %)	GCS 15	IL-10	Serum, Multiplex (Meso Scale Diagnostics, USA)	Differentiating mTBI patients with negative or positive head CT	4***
(Lagerstedt et al., 2018b)	GCS 15 & either: headache; nausea; or vomiting	Yes	Admission (133), CT+ (16.5 %)	GCS 15	**MCP-1, MIP1- α , IL-10	Plasma, Multiplex (Meso Scale Diagnostics, USA)	The utility of IL-10 as a prognostic marker	4***
(Lagerstedt et al., 2020)	Age \geq 18, admission <24 h, indication for CT, outcome data at 6–12 months	Yes	*ED (49), CT+ (42.6%)	GCS 13–15	IL-10	Serum Multiplex (Meso Scale Diagnostics, USA)	Differentiating concussed athletes from non-concussed athletes.	4
(Meier et al., 2020)	SRC within study timeframe	X	University athletes, (106), CT+ (N.A.)	CDC	IL-6, IL-1RA, CRP	Serum Multiplex (Meso Scale Diagnostics, USA)	The relationship between acute inflammatory markers and symptom recovery in SRC	7
(Nitta et al., 2019)	SRC within study timeframe	X	University Athletes (41), CT+ (N.A.)	CDC	**IL-6, IL-1RA, IL-10, TNF α , CRP, IFN γ	Serum, Multiplex (Meso Scale Diagnostics, USA)	Differentiate pediatric concussion with persistent symptoms and without & predicting persistent symptoms	7
(Parkin et al., 2019)	Age 5–18 & Initial visit < 48 h post-injury	X	ED (18), CT+ (0%)	Berlin/ Zurich concussion in sports statements	IL-8, IL-1 β , IL-6, IL-10, TNF α	Plasma, Multiplex (R&D Systems, USA)	Differentiating mTBI patients with negative or positive head CT	5
(Posti et al., 2019)	Triaged for head CT after head injury & age \geq 16	Yes	*ED (93), CT+ (39.8 %)	GCS 13–15	IL-10	Plasma, Multiplex (Meso Scale Diagnostics, USA)	Differentiating between complicated and uncomplicated mTBI	2***
(Sharma et al., 2017)	Initial visit <24 h post injury, One symptom of head trauma & initial GCS >13	Yes	ED (92), CT+ (14.1 %)	GCS 14–15	**CRP	Serum, Multiplex (Meso Scale diagnostics, USA)	Characterizing the relationship between hsCRP and mTBI	5***
(Shetty et al., 2019)	Initial visit < 30 days & negative neuroimaging.	X	ED (311), CT+ (0%)	ACRM	hsCRP	Serum, hsCRP reagent (Vitros Chemistry Products)	Predict persistent adverse outcomes	2
(Su et al., 2014)	Age 18–60, normal head CT, & GCS 13–15	X	ED (213), CT+ (0%)	WHO	hsCRP	Plasma, Immunoturbidimetric		3

(continued on next page)

Table 1 (continued)

Reference (Year)	Inclusion criteria	Poly-trauma/ ECI	Subjects (n), CT+/MRI+	Diagnostic criteria for mTBI	Markers of inflammation analyzed	Blood compartment, assay (Manufacturer)	Study goals(s)	Bias (NOS)
(Sun et al., 2019)	Initial visit within 1 week of injury & head CT	X	ED (95), CT+ (N.A.)	WHO	IL-1β, IL-6, IL-12, IL-4, IL-10, MCP-1, IL-8, IFNγ, TNFα	assay (Siemens Healthcare, Germany) Serum, Multiplex (Luminex, USA)	Characterize inflammatory profile after mTBI & the relationship with cognitive consequences	6
(Thompson et al., 2020)	Independently perform activities of daily life & understands English	X	ED (171), CT+ (32.5 %)	CDC	IL-2, IL-1β, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12, IFNγ, TNFα, Fractalkine	Plasma, Multiplex (BioRad, USA)	Differentiating mTBI patients from healthy controls in an old and young cohort	6
(Tylicka et al., 2020)	Age 7–17 & Initial visit <6 h post-injury.	N.A.	ED (29), CT+ (0%)	GCS 13–15	IL-8, IL-11	Plasma, ELISA (N.A.)	Differentiating children with and without loss of consciousness	4
(Vedantam et al., 2021)	Age 18–50 & negative head CT	Yes	ED (53), CT+ (0%)	GCS 13–15	IL-1β, IL-2, IL-4, IL-6, IL-10, IL12p70, IL-17a, IFNγ, TNFα	Plasma, Luminex Magpix (Luminex, USA)	Characterize inflammatory profile after mTBI & the relationship between neuropsychological outcomes	7
(Xu et al., 2020)	Admission at ED with indication for head CT	Yes	ED (1030), CT+ (N.A.)	GCS 13–15	hsCRP	Serum, Assay (Abbott Architect C8000)	Characterizing the profile of hsCRP after TBI & its utility as a prognostic biomarker	6

Note. American Congress of Rehabilitation Medicine (ACRM); Alpha-1-antichromotrypsin (A1A); C-C Motif Chemokine Ligand (CCL-17); Center for Disease Control and Prevention (CDC); C reactive protein (CRP); Computed tomography (CT); Emergency department (ED); Enzyme-linked immunosorbent assay (ELISA); Extra cranial injury (ECI); Glasgow coma score (GCS); Granulocyte-colony stimulating factor (G-CSF); High sensitivity C Reactive protein (hsCRP); Intensive care unit (ICU); Interferon gamma (IFNγ); Interferon gamma induced protein (IP); Interleukin (IL); Macrophage inflammatory protein (MIP); Magnetic resonance imaging (MRI); Monocyte Chemoattractant Protein (MCP); Myeloperoxidase (MPO); NOS: Newcastle Ottawa Scale (NOS); Not Available (N.A.); Receptor antagonist (RA); Serum amyloid a1 (SAA1); Sports related concussion (SRC); singular molecular array (Simoa); Thymus and activation-regulated chemokine (TARC); Traumatic brain injury (TBI); Tumor Necrosis Factor α (TNFα); World Health Organization (WHO).

* Study also included patients with moderate and severe TBI.
 ** Study used an exploratory approach to identify which markers to study.
 *** Bias assessed using the QUADAS-2 tool for studies of diagnostic accuracy.

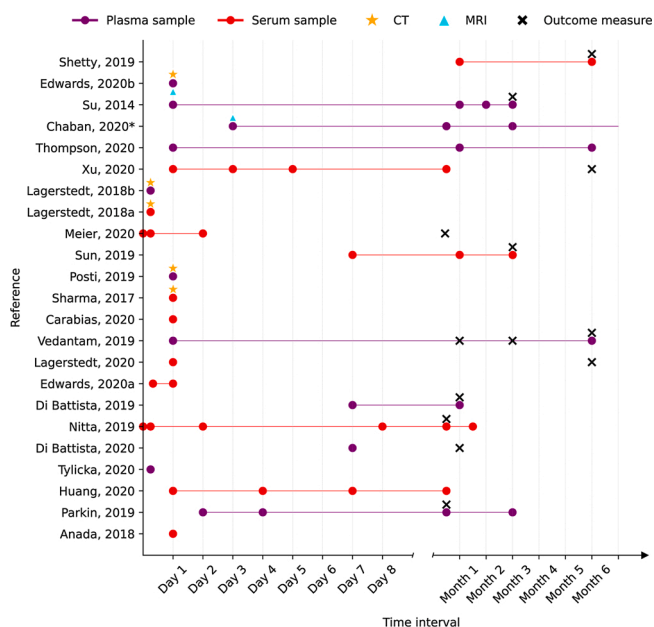


Fig. 3. The sampling time points for studies included in the review. Studies are listed in order of increasing sample size (bottom to top). Note. Computed tomography (CT); Magnetic resonance imaging (MRI). *Also sampled blood at 12 months.

2021). Interferon gamma-induced protein 10 (IP-10), thymus and activation-regulated chemokine (TARC), myeloperoxidase (MPO), and IL-11 were the only inflammatory markers that were not significantly different to control concentrations at any time point. However, apart

from IP-10 which was investigated in two studies (Chaban et al., 2020; Di Battista et al., 2019), TARC, MPO and IL-11 were only investigated once (Chaban et al., 2020; Di Battista et al., 2019; Tylicka et al., 2020).

Acute IL-6 levels had good predictive value (AUC 0.82 [95 % CI 0.72–0.90]) for differentiating military personnel with mTBI from non-mTBI (Edwards et al., 2020a). Similar results were found for differentiating between mTBI and non-mTBI athletes (AUC 0.79 [0.65–0.92]) (Nitta et al., 2019). This was further confirmed by one study (AUC 0.75 [SD 0.10]) who drew their cohort from the sample population as the previously mentioned study (Meier et al., 2020). Taking a multivariate approach one study reported that combining the concentrations of IL-6, TNFα and IL-10 slightly improved the diagnostic capability compared to IL-6 alone [AUC 0.82, CI 0.73–0.90] (Edwards et al., 2020a). Also, a combination panel consisting of IL-1β, IL-6 and Monocyte Chemoattractant Protein-1 (MCP-1) presented similar diagnostic capabilities (AUC = 0.83 [CI 0.76–0.89]) (Sun et al., 2019).

3.6. The relationship between acute markers of inflammation and conventional imaging

Five studies examined the ability of inflammatory markers to diagnose a positive head CT (Edwards et al., 2020b; Lagerstedt et al., 2018a, b; Posti et al., 2019; Sharma et al., 2017). In general, the visualizations of one or more traumatic abnormalities (for example, epidural haemorrhage, subdural haemorrhage, cerebral oedema, arterial dissection, and skull fractures) were used to classify a CT as positive. In the study by Posti et al. grading of CT scans was based on the Marshall criteria. The markers investigated included IL-6, IL-10, TNFα, and C-reactive protein (CRP) (Table 3). IL-10 was most frequently measured (n = 4) and showed AUC values ranging from 0.52–0.74 for predicting intracranial traumatic abnormalities. However, AUC values were higher for IL-6 and TNFα (0.87 and 0.75 respectively) which were only studied

Table 2
Biomarkers of inflammation in mTBI relative to controls.

Reference	mTBI (n), age & % female	Control type (n), age & % female	Time point(s)	Biomarkers significantly elevated compared to controls (p < 0.05)	Significant changes between time points (inflammatory profile)
(Anada et al., 2018)	10, (18–50), 50%	Healthy (10), N.A., N.A.	T1: <24 h	T1: CRP, SAA1, & A1A	N.A.
(Carabias et al., 2020)	90, 45 (IQR 31–62), 31%	Healthy (15), 40 (IQR 31–62), 47%	T1: <24 h	T1: SAA1	N.A.
(Chaban et al., 2020)	207, 32.4 (SD 13.2), 37 %	Healthy (82), 33.02 (SD 12.9), 44%	T1: <72h T2: 3m T3: 12m T1: <7d	T1, T2, & T3: IL-8, IFN γ , TNF α , MCP-1, MIP-1 β , Eotaxin, IL-17a, & IL-9 T1: MCP-4 & MIP-1 β	IL-17a showed a significant time-by-group effect. T1 \rightarrow T2, increase, T2 \rightarrow T3, no change
(Di Battista et al., 2019)	43, 21.2 (IQR 18.9–22.1), 51%	Healthy athletes (87), 21.2 (19.6–22.3), 47%	T2: <5d after medical clearance	T2: None	Levels of inflammatory markers had returned to baseline at medical clearance
(Di Battista et al., 2020b)	41, 20.9 (IQR 19.6–22.0), 49%	Healthy athletes (55), 21 (IQR 19–22), 62%	T1:<7d	T1: None	N.A.
(Edwards et al., 2020a)	45, 26.6 (SD 6.98), 0%	Healthy military (49), 26.4 (SD 5.75), 6.7 %	T1: <8h T2: 24 h after T1	T1: IL-6 T2: None	T1 \rightarrow T2: IL-6 decreased 66.7 % in concussed subjects, increased 34 % in healthy controls
(Huang et al., 2020)	20, 52.3 (SD 15.5), 10 %	Healthy (20), 48.4 (SD 9.58), 15 %	T1: <24 h T2: <4d T3: <7d T4: <14d	T1, T2, T3, & T4: None	No significant changes in the levels of TNF α within or between groups
(Meier et al., 2020)	106, 18 (SD 1.52), 0%	Contact controls (84), 18.37 (SD 1.68), 0%	T1: Baseline T2: <6 h T3: <2d	T2: IL-1RA & IL-6 T3: IL-1RA T1: None	IL-6 and IL-1RA peaked at T2 IL-6 returned to baseline by T3, IL-1RA had not.
(Nitta et al., 2019)	41, 17.8 (SD 1.79), 0%	Healthy athletes (43), 18.1 (SD 1.67), 0%	T1: Baseline T2: <6 h T3: <2d T4: <8d T5: <15d T6: <45d	T2: IL-6 & IL-1RA T1, T3, T4, T5, T6: None	IL-6 and IL-1RA peaked at T2 before returning to baseline by T3
(Sun et al., 2019)	95, 35.9 (SD 13.7), 42 %	Healthy (54), 35.74 (SD 11.5), 46 %	T1: <7d T2: 1 m T3: 3m	T1: MCP-1, IL-1 β , & IL-6 T2 & T3: None	T1 \rightarrow T2: MCP-1 (35 %) T2 \rightarrow T3: MCP-1 (29%), IL-1 β (-8.1%), IL-6 (-23%)
(Thompson et al., 2020)	Young TBI (96), 35.0 (SD 9.4), 38 % Old TBI (75), 66.5 (SD 8.9), 35 %	Healthy young (80), 35.1 (SD 10.0), 35 % Healthy old (62), 65.7 (SD 8.1), 36 %	T1: <24 h T2: 1 m T3: 6m	Young: T1: IL-1 β , IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12, IFN γ , TNF α T2: TNF α , IL-7, IL-8 T3: IL-5, IL-7, IL-8, IL-12, TNF α Old: T1: IL-6, IL-8, IL-10, Fractalkine T2: None T3: Fractalkine	No within group analysis of time.
(Tylicka et al., 2020)	29, (7–17), N.A.	Healthy (13), N.A., N.A.	T1: <6 h	T1: IL-11	N.A.
(Vedantam et al., 2021)	53, 26 (18–49), 38%	Orthopedic (24), 28 (20–50), 33% female	T1: <24 h T2: 6m T1: <24 h T2: 3d T3: 5d T4: 2w	T1: IL-2, IL-6 T2: IL-6	T1 \rightarrow T2: IL-1 β , IL-4, IL-6, IFN γ significantly decreased, IL-2 increased
(Xu et al., 2020)	171, N.A., N.A.	Orthopedic (19), NA, NA	T1: <24 h T2: 3d T3: 5d T4: 2w	T1, T2, T3, & T4: None	N.A.

Note. Alpha-1-antichromotrypsin (A1A); C reactive protein (CRP); Days (d); Hours (h); Interferon gamma (IFN γ); Interleukin (IL); Interquartile range (IQR); Macrophage inflammatory protein 1beta (MIP-1 β); Mild traumatic brain injury (mTBI); Months (m); Monocyte Chemoattractant Protein (MCP); Not Available (N.A.); Standard deviation (SD); Serum amyloid A1 (SAA1); Tumor necrosis factor alpha (TNF α); Weeks (w).

once (Edwards et al., 2020b). Values of sensitivity and specificity were only reported for IL-10. A sensitivity of 100 % was present with specificity ranging from 2.8 % to 27 % (Lagerstedt et al., 2018b; Posti et al., 2019). There were also slight differences in cut-offs reported: 0.12–0.16 pg/mL. Two studies reported a significantly higher level of IL-10 in CT positive patients compared to those who were CT negative (Lagerstedt et al., 2018a, b). While three studies reported no significant difference between imaging groups (Edwards et al., 2020b; Posti et al., 2019; Sharma et al., 2017).

These aforementioned studies also included non-inflammatory biomarkers in their analysis. These markers were combined with the inflammatory markers to form combination panels. Biomarker panels outperformed single markers in all cases. For example, a panel consisting of three biomarkers IL-10, Heart-Fatty Acid Binding Protein (H-FABP), and GFAP demonstrated a specificity of 69 % at a sensitivity of 95 % (Lagerstedt et al., 2018b). When sensitivity was locked at 100 % the same panel only had 50 % specificity (Lagerstedt et al., 2018a). Similarly, a biomarker panel including two biomarkers IL-10 and GFAP

reported 37.5 % specificity at 100 % sensitivity. The highest specificity, 87 % with a sensitivity of 85 %, was shown by a panel consisting of CRP, matrix metalloproteinase-2 (MMP-2), and Brain-type creatine kinase (CKBB) (Posti et al., 2019).

The study by Edwards et al. also included a cohort of MRI positive patients but the criteria for a positive MRI were not given (Edwards et al., 2020b). The markers IL-6, IL-10 and TNF α had AUC values of 0.69, 0.46 and 0.56 for discriminating between MRI positive and negative patients. The combination panel consisting of IL-6, IL-10, TNF α , and vascular endothelial growth factor (VEGF) had an AUC of 0.71, which was lower than when it was used to stratify between CT positive and negative patients (AUC = 0.92). Chaban et al. also performed MRI imaging (Chaban et al., 2020). However, instead of using biomarkers to diagnose MRI findings, multivariate regression models were constructed to investigate whether MRI findings (and other clinical factors) affected biomarker levels. The inflammatory markers IP-10, IL-9, Eotaxin and Macrophage inflammatory protein-1beta (MIP-1 β) were negatively associated with MRI findings; patients with a positive MRI finding had

Table 3

The association between markers of inflammation and traumatic abnormalities as found with head computed tomography.

Inflammatory marker (s)	n. of observations	AUC	Specificity & sensitivity
IL-10	4	0.52 – 0.74	Sens (100 %), spec (2.8 %–27 %)
IL-6	1	0.87	N.A.
TNF α	1	0.75	N.A.
CRP	1	0.70	N.A.
Panel (IL-10+IL-6+TNF α + VEGF)	1	0.92	N.A.
Panel (CRP + MMP-2 + CKBB)	1	0.96	Sens (85 %), Spec (87 %)
Panel (IL-10 + GFAP)	1	N.A.	Sens (100 %), Spec (38 %)
Panel (IL-10 + H-FABP + GFAP)	2	N.A.	Sens (95 %), Spec (69 %); Sens (100 %), Spec (50 %)

Note. Area under the receiver operating curve (AUC); Computed tomography (CT); Creatine kinase brain (CKBB); C-reactive protein (CRP); Glial fibrillary acid protein (GFAP); Heart fatty acid binding protein (H-FABP); Interleukin (IL); Matrix-metalloproteinase-2 (MMP-2); Not Available (N.A.); Sensitivity (Sens); Specificity (Spec); Tumor necrosis factor alpha (TNF α); Vascular endothelial growth factor (VEGF).

lower levels of these biomarkers.

3.7. The prognostic role of markers of inflammation for predicting (long-term) outcome

Eleven studies related levels of inflammatory markers to long-term outcomes. The outcomes assessed (functional, neuropsychological, recovery time) varied and so did the timing of the outcome measurements (Table 4). The time to medical clearance was the most frequently used outcome measure determined in studies with athletes (Di Battista et al., 2019, 2020b; Meier et al., 2020; Nitta et al., 2019). This term corresponds with return to play (RTP) as defined in the *Concussion in sport group guidelines* (McCrorry et al., 2017). Median RTP time for athletes varied from 11 to 27 days. Higher levels of IL-6 and IL-1RA were significantly associated with RTP when measured within six hours of trauma (Meier et al., 2020; Nitta et al., 2019). The concentrations of the chemokines MCP-4 and MIP-1 β measured within seven days of injury were also significantly correlated with RTP (Di Battista et al., 2019). IL-10 had no prognostic utility for predicting functional outcome using the Glasgow Outcome Scale Extended (GOSE) (specificity 0% at sensitivity 100 %) (Lagerstedt et al., 2020).

4. Discussion

With this systematic review we show that measuring blood based inflammatory markers in mTBI has only recently become a topic of interest, with 19/23 included studies (83 %) being published within the last three years. The novelty of this topic is reflected in the heterogeneity of studies, with differences in study design, the analytical methods and reporting of results. Nevertheless, there is evidence for a distinct (systemic) inflammatory response following mTBI, with increasing levels of inflammatory markers being present as early as six hours after injury and lasting up to twelve months in some cases. Furthermore, several acute inflammatory markers in mTBI have potential diagnostic and prognostic properties. It is however important to interpret these results within the current context, as the lack of standardisation does not allow for extrapolating of the reported findings to the general mTBI population, and any conclusions drawn by this review must be further validated.

4.1. The inflammatory response following mild traumatic brain injury

The concentration of IL-6 was most often elevated in the mTBI group

Table 4

Relationship between acute biomarkers of inflammation and long-term outcome.

Reference	Outcome(s) measured (population)	Outcome(s)	Conclusion(s)
(Di Battista et al., 2019)	RTP (athletes)	Time to RTP median 25 days (15–55)	Levels of MCP-4 and MIP-1 β measured within 7 days of injury were positively correlated with RTP (p = 0.007 & p < 0.001)
(Di Battista et al., 2020b)	RTP (athletes)	Time to RTP median 27 days (22–51)	Levels of IL-6 measured within 7 days showed no relationship with RTP (p = 0.508)
(Lagerstedt et al., 2020)	GOSE at six months (ED)	25 (51 %) complete recovery (GOSE = 8)	Levels of IL-10 were higher in incomplete recovery group (not significant. At 100 % sensitivity, 0% specificity, AUC 0.0 95 % CI 0.0–0.6)
(Meier et al., 2020)	RTP (athletes)	Time to RTP median 11 days (9–15)	Higher IL-1RA at 6 h post injury was associated with greater symptom duration (HR 0.60 [95% CI 0.38–0.95], p = 0.03)
(Nitta et al., 2019)	RTP (athletes)	All athletes had RTP within 15 days	Higher IL-6 at 6 h post injury were associated with a slower recovery rate (HR 0.61 [95% CI 0.38–0.96], p = 0.031)
(Parkin et al., 2019)	Persistent post concussive symptoms (children)	N.A.	TNF α measured within 1–4 days post injury were higher in children with PCS than those with normal recovery (p = 0.031)
(Shetty et al., 2019)	Time to recovery (ED)	Days of follow up lasted from 2 to 188 days	Initial hsCRP level (< 30 days) did not significantly influence time to recovery in a cox regression model (HR 0.94 [95% CI 0.79–1.13], p = 0.524)
(Su et al., 2014)	PCS, Persistent psychological, physiological problems, and cognitive impairment (ED)	3 months incidence of PCS was 16 %, persistent psychological problems 31 %, persistent cognitive impairment 25 % and physiological problems 25 %	Elevated baseline CRP Persistent postconcussion syndrome (OR 2.72 [95 % CI 1.61–4.59], p = 0.00) Persistent psychological problems (OR 1.54 [95% CI 1.06–2.22], p = 0.022) Persistent physiological problems (OR 1.33 [95 % CI 0.91–1.96], p = 0.146) Persistent cognitive impairment (OR 1.69 [95 % CI

(continued on next page)

Table 4 (continued)

Reference	Outcome(s) measured (population)	Outcome(s)	Conclusion(s)
(Sun et al., 2019)	Neuro-psychological tests (ED)	At 3 months PCS as measured using the RPQ were still significant compared with controls	1.14–2.51], $p = 0.01$ High MCP-1 within 7 days were associated with higher Digital symbol coding score at 3 months (β 0.214, $p = 0.009$)
(Vedantam et al., 2021)	Neuro-psychological tests (ED)		Higher IL-2 (24 h) more severe RPQ at 1 week ($p = 0.001$) lower IL-6 & IL-17a (24 h) more severe RPQ ($p = 0.035$, $p = 0.007$)
(Xu et al., 2020)	GOSE (ED)	N.A.	Levels of hsCRP at 2 weeks had an AUC of 0.928 for predicting favorable outcome and an AUC of 0.547 for predicting complete recovery

Note. Area under the curve (AUC); Confidence interval (CI); Emergency department (ED); Glasgow Outcome Scale Extended (GOSE); Hazard ratio (HR); High sensitivity C reactive protein (hsCRP); hr (hour); Interleukin (IL); Monocyte chemoattractant protein (MCP); Macrophage inflammatory protein (MIP); Not Available (N.A.); Odds ratio (OR); Post concussive symptoms (PCS); Return to play (RTP); Tumor necrosis factor (TNF).

compared to the control group. Peak levels of IL-6 were found in the acute phase (<24 h) post-trauma. IL-6 is one of the best characterized inflammatory markers in TBI and several studies in moderate and severe TBI have reported similar systemic elevations of IL-6 within the acute phase of moderate and severe TBI (Edalatfar et al., 2021; Hergenroeder et al., 2010; Lustenberger et al., 2016; Rodney et al., 2018; Yan et al., 2014). IL-6 is a pleiotropic cytokine, which is involved in promoting neuronal regeneration but also has a pro-inflammatory role by stimulating the systemic acute phase response following TBI (Morganti-Kossmann et al., 1997; Woodcock and Morganti-Kossmann, 2013).

A novel finding however is that the temporal profile of IL-6 points to a distinctly different inflammatory profile in sports related concussion (SRC) and military concussion versus the general unselected ED population with mTBI. Whereas the first group of athletes (Nitta et al., 2019; Meier et al., 2020) and military personnel (Edwards et al., 2020a) showed early acute elevations (<8 h) with a return to baseline within 48 h, the latter group (Vedantam et al., 2021) showed alterations lasting up to six months after injury. This is supported by a study of military personnel with moderate blast exposure (without diagnosis of mTBI) that showed significant acute elevations of IL-6 and TNF α compared to no blast controls which returned to baseline levels a day later (Gill et al., 2017). This finding would also explain why Di Battista and colleagues found no difference between levels of IL-6 measured in healthy athletes and athletes with SRC measured at a median of four days after injury (Di Battista et al., 2020b). However, as levels of IL-6 were not measured at very acute time points (<8 h) in the civilian ED populations, it might be possible that this population shows similar early elevations as athletes and military personnel. Nevertheless, the earlier resolution of the inflammatory response in sports-related and military mTBI is a significant finding and indicates that this category might concern patients with milder injuries compared to the ED population with mTBI.

The most frequently assessed inflammatory marker, TNF α , was only significantly elevated compared to controls in two out of the eight studies that measured it. This suggests that TNF α might contribute as a biomarker in mTBI but is not the most important marker. Other inflammatory cytokines were also reported to be elevated relative to

controls in at least a single study. However, no conclusions can be drawn as other studies reported contradicting results (Fig. 4). There was also a marked acute phase response with acute phase proteins (APPs) SAA1, A1A and CRP being elevated relative to controls within 24 h of TBI (Anada et al., 2018; Carabias et al., 2020). APPs are produced by the liver as a response to inflammatory cytokines, adrenergic activity, and hypothalamic-pituitary-axes (HPA) activation (Fig. 1) (Baumann and Gaudie, 1990; Purkayastha et al., 2019). However, there is still limited evidence regarding the temporal profile of the APPs after TBI and their relation to TBI severity.

It remains unclear if the acute findings of increased systemic markers are due to central nervous system or systemic inflammatory processes or both. There is clear evidence that cytokine production also occurs in response to extracranial injury (McDonald et al., 2016). Studies have demonstrated higher IL-6 levels in patients with concomitant extracranial trauma compared to isolated head injury (Hergenroeder et al., 2010). Similarly, APPs such as CRP have also found to be elevated in response to many other conditions such as infections, extracranial injury, and even psychological stress. Levels of CRP were also found to be significantly elevated in orthopaedic trauma controls as opposed to healthy controls (Anada et al., 2018; Xu et al., 2020). Conversely, Vedantam and colleagues reported that levels of IL-6 and IL-2 were significantly elevated in mTBI patients compared to orthopaedic controls which might point to the effects of the brain injury. Altogether, these results indicate that it is important to acquire data of additional extracranial injuries that occur, for example using the ISS.

Acute alterations of biomarkers are important as their assessment can help guide clinical decision making for example at the ED. However, many patients with mTBI are not seen at the ED in the acute phase but rather the general practitioner (especially relevant in rural settings). Therefore, measuring inflammatory markers sub acutely (<7 days) or in the chronic phase could also provide valuable information. Studies included in this review provide evidence for alterations in levels of inflammatory markers up to twelve months (Chaban et al., 2020; Thompson et al., 2020; Vedantam et al., 2021). However, in most cases a time course analysis of these inflammatory markers demonstrated a significant decrease in the chronic phase compared to their initial measurement. These findings suggest that the clinical utility of inflammatory markers may be limited to the acute and subacute phase. Future longitudinal studies on inflammatory markers are needed to investigate

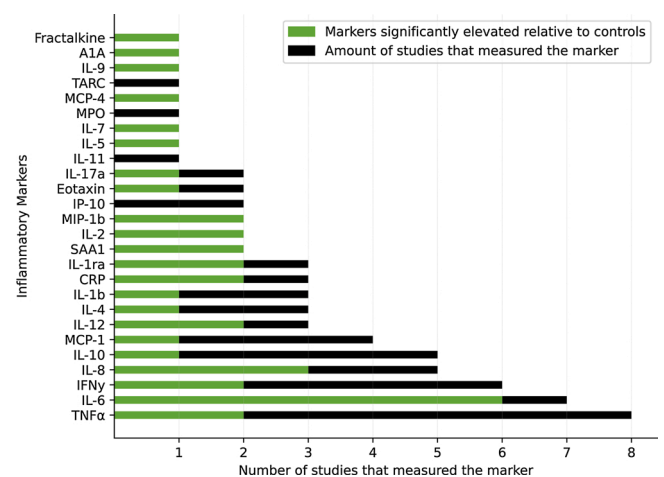


Fig. 4. Bar graph comparing the number of times a marker was measured compared with the number of times it was significantly elevated relative to levels in controls.

Note. Alpha-1-antichromotrypsin (A1A); C Reactive Protein (CRP); Interferon gamma (IFN γ); Interleukin (IL); Interferon gamma inducing protein (IP); Macrophage inflammatory protein (MIP); Monocyte chemoattractant protein (MCP); Myeloperoxidase (MPO); Tumor necrosis factor alpha (TNF α); Thymus and activation-regulated chemokine (TARC).

recovery in the light of protracted immune responses.

Most included studies reported significant elevations using group comparisons by comparing means or medians. Although this is useful in a research setting, it provides limited information on the clinical utility of the marker. The clinical validity is more accurately assessed using Receiver Operating Curves (ROC) curves and positive and negative predictive values (PPV's and NPVs) (Wang et al., 2018) which were presented in only four of the articles included in this review (Edwards et al., 2020a; Nitta et al., 2019; Meier et al., 2020; Sun et al., 2019). Their findings infer that IL-6 may have clinical utility for diagnosing TBI.

The concentrations of inflammatory markers may also be used to improve our understanding of the pathobiology of mTBI. Because, the inflammatory response consists of multiple complex inflammatory cascades, investigating multiple markers simultaneously could provide insight into the inflammatory response of an individual. A recommended approach for future studies is to investigate an unbiased panel of markers and apply dimensionality reduction techniques such as principal component analysis (PCA) to find markers which best represent the relevant inflammatory profile (Helmy et al., 2012). A good example reflecting this approach is the study of Huie and colleagues. In their study, 72 markers were investigated and PCA was carried out to identify a group of 21 largely inflammatory markers that best represented the biomarker profile following TBI (Huie et al., 2019).

4.2. The relationship between acute markers of inflammation and conventional imaging

Five studies examined the ability of acute levels of inflammatory markers to diagnose positive or negative head CT findings. The cytokine IL-10 was the most frequently studied marker for this purpose (Edwards et al., 2020b; Lagerstedt et al., 2018a, b; Posti et al., 2019; Sharma et al., 2017). IL-10 is an anti-inflammatory marker that has been well studied in acute brain injury including TBI but also stroke (for a review see (Garcia et al., 2017)). It is thought to act by attenuating the immune response, suppressing pro-inflammatory markers such as IL-6, IL-1 and TNF α , initiating immune recovery. Acute levels of IL-10, within 24 h of mTBI, showed specificities comparable to that of S100B – the currently best characterized biomarker in TBI for this purpose (Edalatfar et al., 2021; Mondello et al., 2018). The other reported biomarkers IL-6, TNF α and CRP also showed potential, however sensitivity and specificity values were not reported (Edwards et al., 2020b; Sharma et al., 2017).

Although promising, these findings demonstrate that the clinical utility of individual markers is limited so far as their low specificity will not lead to decreased use of head CTs in daily practice. The included studies also assessed combination panels with biomarkers reflecting cellular brain damage such as H-FABP, S100B and GFAP. The use of multiple biomarkers improved the diagnostic capability showing increased specificity for diagnosing positive head CT compared to single markers. The highest specificity (87 % at 85 % sensitivity) resulted from a three-marker panel consisting of CRP, MMP-2, and CKBB (Sharma et al., 2017). These findings were compared to a FDA approved panel of biomarkers consisting of GFAP and UCHL-1 with a specificity of 37 % at a sensitivity of 97 % (Bazarian et al., 2018). This provides evidence that the right combination of biomarkers might lead to combination panels that are highly sensitive and specific. Inflammatory markers, such as IL-10, may play an important role in the formation of these panels.

Although the panels of combined markers have shown good predictive value for findings on head CT, there is discussion on whether CT should be used as the golden imaging standard for diagnosing intracranial traumatic abnormalities. MRI is more sensitive for this purpose. Especially, considering that frequently present diffuse (axonal) injuries are not visualized by CT (Lunkova et al., 2021). Only a single study on inflammatory markers in mTBI also reported MRI findings (Edwards et al., 2020b). The AUC values of the biomarkers IL-6, TNF α were much lower for MRI positive patients (as compared to CT positive patients). Although, the levels of these biomarkers were still significantly elevated

in the MRI positive group compared to the CT negative and MRI negative control group (Edwards et al., 2020b). An explanation for this finding might be that the increased sensitivity of MRI leads to the detection of subtle intracranial injuries (e.g., micro-haemorrhages) that are less likely to result in a significant inflammatory response compared to the lesions detected with CT. For future studies it would be interesting to perform large observational studies to assess the correlation between levels of inflammatory markers and specific intracranial abnormalities as seen on conventional imaging. For example, questions to be answered are whether lesion type or location of injury within the brain impact the inflammatory response.

4.3. The prognostic role of markers of inflammation for predicting long term outcome

Return to play (RTP) was the most frequently investigated outcome measure, which was investigated in all four studies with athlete populations (Di Battista et al., 2019, 2020b; Meier et al., 2020; Nitta et al., 2019). Nitta and colleagues found that athletes with higher levels of IL-6 within six hours after injury showed protracted recovery (Nitta et al., 2019). Meier and colleagues showed similar trend, however the relationship between IL-6 levels and RTP was not statistically significant (Meier et al., 2020). These findings are conflicting with those of Di Battista and colleagues, who demonstrated no correlation between IL-6 and RTP (Di Battista et al., 2020b). A potential explanation for this difference is that in the latter study levels of IL-6 were measured sub-acutely, after the temporal peak of IL-6.

Other markers related to RTP were the chemokines MCP-1 and MIP-1 β and the anti-inflammatory cytokine IL-1RA which, when elevated, were significantly positively correlated with later RTP. A study of ED patients failed to significantly correlate high sensitivity CRP concentrations with time to recovery in a cox regression model (Shetty et al., 2019). This finding might imply that acute inflammatory markers are more sensitive in predicting recovery in athletes than in the general mTBI population. An explanation could be that the inflammatory response is milder in the relatively young and healthy athlete population compared to the average mTBI patient. On the other hand, assessing recovery using RTP does not guarantee that the athlete does not develop symptoms at a later stage as these are different outcome measures.

Cognitive functioning and functional recovery were also used as outcome measures. Elevations of CRP associated with persistent psychological impairments and increased levels of MCP with decreased information processing speed three months after trauma (Su et al., 2014; Sun et al., 2019). Further, increased IL-10 and IL-17A at six months post trauma were associated with increased frequency of post-traumatic stress disorder and executive function (Vedantam et al., 2021). Increased inflammation in the acute phase may be an important predictor of cognitive recovery following mTBI, while chronic levels of inflammatory markers could be used as a clinical tool for measuring the therapeutic effect of treatment and symptom resolution.

The ability of inflammatory markers to predict functional outcome using the Glasgow Outcome Scale (GOS) or Glasgow Outcome Scale Extended (GOSE) are less clear. Although these scales have been widely used in research, they lack sensitivity for subclinical functional alterations after mTBI because of a ceiling effect (Maas et al., 2017). Two included studies were unable to relate levels of inflammatory markers to recovery as all mTBI patients completely recovered according to the GOSE (Carabias et al., 2020; Huang et al., 2020). By dichotomizing GOSE scores into unfavorable outcome (GOSE < 5) and favorable outcome Xu and colleagues demonstrated that high sensitivity CRP levels measured at two weeks post-trauma may have potential as a prognostic marker (Xu et al., 2020).

4.4. Caveats related to measuring inflammatory markers

Despite the findings of this review there are some inherent

limitations of using inflammatory markers as biomarkers. In Fig. 1 and Section 1 of this review we briefly mentioned the complex interplay between the central nervous system and systemic inflammatory response, and external factors which may further influence this response. Therefore, we provided a discussion on some of these influencing factors in relation to the articles included in this review. The most important influencing factor, especially in the acute stage post-trauma, is extracranial injury (the potential influence of extracranial injury is previously discussed in Section 4.1. paragraph 4).

The inflammatory response is also sensitive to age. Advancing age has been shown to lead to dysregulation of the immune response resulting in a higher basal level of inflammatory markers (Shaw et al., 2013). This process has been called ‘immunosenescence’ (Goronzy and Weyand, 2013). It is also believed that microglia and astrocytes as the central immune effectors undergo immunosenescence, which lead to a heightened pro-inflammatory response in the older population (Niraula et al., 2017). Thompson and colleagues aimed to assess the effect of ageing on the inflammatory response after mTBI (Thompson et al., 2020). They found differences in IL-8, IL-6, and the chemokine fractalkine, however no analysis was done to determine if the age-related immune response influenced symptoms or recovery. Lagerstedt and colleagues also found that IL-10 was better at diagnosing cranial injuries in mTBI patients older than 65 years, compared to younger patients (Lagerstedt et al., 2018b).

Sex has also been shown to influence the inflammatory response (Thelin et al., 2017; Xiong et al., 2018). Women are generally thought to have a milder neuroinflammatory response after TBI compared to males (Di Battista et al., 2020a; Villapol et al., 2017). The mTBI populations of the included studies in this review comprise predominantly males. It may be important for future studies to perform subgroup analyses and compare the inflammatory response and clinical utility of markers in both male and female patients.

Prior brain injury might also influence the neuroinflammatory response (Di Battista et al., 2016; Rodney et al., 2020). It is believed that a brain injury might ‘prime’ microglia into a more active state much like ageing influences the inflammatory response. In an animal model of repetitive mTBI, mice that had experienced repetitive mTBI demonstrated worse cognitive recovery compared to mice exposed to a single mTBI (Mouzon et al., 2018). Furthermore, a study measuring concentrations of inflammatory markers in military veterans with a history of TBI found that the mean concentration of IL-6 was higher in veterans who had experienced multiple TBIs (Rodney et al., 2020). Repetitive concussions are especially relevant in the military and sports setting. A study measuring inflammatory markers in athletes with a history of concussion report higher concentrations of IL-1 β in athletes with a history of concussion and those without (O’Brien et al., 2021).

There are also technical limitations when measuring markers of inflammation. Studies have demonstrated that the measured levels of inflammatory markers are dependent on the collecting tube used, coagulation time, plasma or serum, storage temperature and number of freeze-thaw cycles (Hennø et al., 2017). There have been attempts made to define common data elements for the collection, preparation, and storage of biofluids for biomarkers (Manley et al., 2010). However only one study in this review abided by these common data elements (Xu et al., 2020).

A further notable limitation is related to the platform chosen for biomarker analysis (McDonald et al., 2021). A study comparing different Immunoplex assays from various manufacturers reported different levels of inflammatory markers (Numis et al., 2021). Although, variability between platforms is an important issue, for mTBI detectability concerns a bigger problem as the concentrations of inflammatory markers are often detected in the low picomolar range. Certain analytical platforms such as ELISA or Immunoplex have little sensitivity at the lower limits of this range (Lasseter et al., 2020). This often leads to unknown concentrations of markers as these are often below the limit of detection of the platforms. For example, in two studies included within

this review, the concentration of IL-6 was not detectable in a large portion of the study population (Chaban et al., 2020; Di Battista et al., 2019). The fact that concentrations of inflammatory markers at the lower limit of the spectrum are not reliably detected may artificially inflate the reported concentrations. Researchers may consider using platforms with greater sensitivity such as platforms applying single molecule array (SIMOA) technology (Lasseter et al., 2020).

4.5. Future perspectives

We report that certain markers of inflammation might improve the clinical utility of combination panels for mTBI. However, current evidence is confounded by heterogeneous study design, analysis, and reporting.

A first step towards improving the quality of evidence of markers of inflammation in mTBI would be the recruitment of study cohorts (for example using large multicentre initiatives, such as the CENTER-TBI and TRACK-TBI studies) which are representative and generalisable to the entire mTBI population (Mercier et al., 2017). Additionally, large multicentre studies may provide sample sizes which have the necessary statistical power to correct for the numerous factors that could influence concentrations of inflammatory markers such as age, gender, previous head injury, or the extent of extracranial injury.

The ability to distinguish neuroinflammation from systemic sources (e.g., from extracranial injury) would vastly improve the clinical utility of inflammatory markers after mTBI. Some solutions are currently being considered: (1) Statistical correcting for extracranial injuries or inclusion of orthopaedic injured patients in addition to healthy controls (Wilde et al., 2019), (2) Investigating the concentrations of inflammatory markers in cranially derived extracellular vesicles (EVs), that could directly represent the cranial inflammatory response (Puffer et al., 2020; Beard et al., 2021), and (3) Measuring microRNAs as a surrogate biochemical marker of the inflammatory response (Zhou et al., 2021; Puffer et al., 2020). Proteomic techniques could also be considered to potentially find candidate biomarkers which are specific to the neuroinflammatory response (Lindblad et al., 2021).

For future studies, we expect a shift away from traditional analytical platforms such as ELISA, Immunoplex, or SIMOA. Although some of these platforms, especially SIMOA, can solve the detectability issue of inflammatory markers, they are laboratory tests with the disadvantage of requiring several hours before a result is returned (Bazarian et al., 2021; Okonkwo et al., 2020). Point of Care (POC) platforms using electrochemical biosensors have recently been gaining traction (Pankratova et al., 2021). POC platforms can identify biomarker concentrations at the scene of injury, GP practice, or ED without requiring processing in a laboratory. It was recently reported that a POC test consisting of UCH-L1 and GFAP, demonstrated comparable sensitivities to laboratory-based platforms (Bazarian et al., 2021). Considering that certain inflammatory markers are expressed soon after injury, they may well be part of a POC combination platform that could be used in the early phase after mTBI.

Before inflammatory markers can be considered, high-quality evidence is needed for the role of and application of blood-based biomarkers in mTBI. To this end, we provide further recommendations for future studies (Table 5). These recommendations reiterate and complement recommendations found in key literature of biomarkers in TBI (Mondello et al., 2018; Wang et al., 2018; Huie et al., 2021).

4.6. Limitations

Our review has several limitations which are related to both the methodology of the review process and the included articles. First, there is a chance for reporting bias as only peer reviewed studies are included. Second, we had strict eligibility criteria which may have led to the exclusion of potentially relevant studies. Third, there was no unifying definition of mTBI, reporting of results or interval from trauma to

Table 5
Recommendations for future studies of inflammatory markers in (mild) TBI.

Recommendation	Reason
Include a wide range of patients with varying demographic factors	A good clinical biomarker should be able to use as a one-size-fits all approach. As the inflammatory response is influenced by demographic factors like age and sex, focusing on just a single demographic group might decrease the clinical utility of findings.
Include patients of varying severity	Future diagnostic criteria might include a biomarker. Research thus needs to focus on how the severity of TBI influences levels of these markers
Include multiple biomarkers; both novel and already well-established markers.	Taking an unbiased approach to biomarker discovery will help in identifying new markers. It will allow for the use of multivariate statistical techniques to form biomarker panels which have shown increased effectivity. (Panelomix, PCA, machine learning techniques).
Include multiple time points (acute, subacute, chronic)	There is still limited knowledge on the acute phase response of all markers. In addition, the inflammatory profile is constantly evolving across a continuum in time and knowing how this evolves will allow clinicians to make decisions on the effectivity of care.
Include well-defined outcome measure(s)	Studies prospectively measuring levels of inflammatory markers after mTBI should preferably measure outcomes at set time points post -trauma using a well-defined outcome measure (for example the GOSE).
Report all details related to collection, sampling, processing, and storage of blood samples.	It is not feasible to expect that all studies follow the same methods due to different laboratory procedures or sampling conditions. Reporting methods used for biospecimen preparation will allow the identified levels of inflammatory markers to be interpreted within the context in which they are assessed.
Report raw values of inflammatory markers	This facilitates comparisons between studies and pooling of results in meta-analyses. It also allows comparisons between performance of different assays.
Report cut-offs	Biomarker level cut-offs used for triaging patients for traumatic CT abnormalities or for unfavourable recovery will allow for better comparability between studies and give a better indication for the clinical use of inflammatory markers.
Report all relevant clinical characteristics	Reporting clinical characteristics like LOC, PTA is vital for TBI research as it will give other researchers a better understanding of the TBI population studied and allow for better comparability of cohorts. Clinical characteristics may have value in multivariate analysis. Furthermore, it is imperative to acquire data regarding poly-trauma (to account for extracranial injuries).
Analyse data with statistical techniques other than mere group comparisons	Group comparisons provide limited information about the diagnostic or prognostic utility of the inflammatory markers. Receiver operating curve (ROC) analysis with reporting of AUC values, sensitivity and specificity have better clinical value.

Note. Area under the receiver operating curve (AUC); Computed tomography (CT); Glasgow Outcome Scale Extended (GOSE); Loss of consciousness (LOC); Post traumatic amnesia (PTA); Principal component analysis (PCA); traumatic brain injury (TBI).

measurement of inflammatory marker making comparison difficult. For this last reason, we were unable to perform a meta-analysis which would have increased the objectivity of our findings. Finally, by including studies investigating cohorts drawn from the same population and counting these as unique (i.e., Nitta et al., 2019; Meier et al., 2020) certain statistics could be artificially inflated.

5. Conclusion

This review of the available relevant literature suggests a distinct inflammatory response following mTBI, which is quantifiable in blood within six hours and up to twelve months post trauma. There is reason to believe that the temporal expression of inflammatory markers is different in military/ sports related mTBI compared to civilian mTBI. When assessed in combination with well-established markers such as GFAP and UCH-L1, the early expression of inflammatory markers might contribute to the development of combination panels and POC devices which could diagnose TBI at the scene, especially relevant in military and sports settings. IL-6 holds the most promise for this purpose. Inflammatory markers such as IL-10 may also have capabilities for distinguishing between patients with and without imaging abnormalities. However, inflammatory markers may be better suited as part of a multiple biomarker panel including markers of other pathophysiological processes post-TBI. This review further suggests that inflammatory markers may also have potential for decisions regarding return to play for athletes or for predicting neuropsychological outcomes following mTBI. Before implementation of these biomarkers in clinical practice is possible, increased standardization of measuring techniques and study design must be done in large multicentre studies to provide high quality evidence. To this end, recommendations to aid and guide further research on this topic are provided.

Financial support

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Declaration of Competing Interest

The authors report no declarations of interest.

Acknowledgements

We would like to thank Karin Sijtsma, medical information specialist, University of Groningen, for her assistance in preparing the search strategy for this systematic review. We would also like to thank Isabel Dieleman, medical student, University of Groningen, for preparing Fig. 1 of this review.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.neubiorev.2021.11.036>.

References

- Anada, R.P., Wong, K.T., Jayapalan, J.J., Hashim, O.H., Ganesan, D., 2018. Panel of serum protein biomarkers to grade the severity of traumatic brain injury. *Electrophoresis* 39, 2308–2315. <https://doi.org/10.1002/elps.201700407>.
- Baumann, H., Gauldie, J., 1990. Regulation of hepatic acute phase plasma protein genes by hepatocyte stimulating factors and other mediators of inflammation. *Mol. Biol. Med.* 7, 147–159.
- Bazarian, J.J., McClung, J., Shah, M.N., Ting Cheng, Y., Flesher, W., Kraus, J., 2005. Mild traumatic brain injury in the United States, 1998–2000. *Brain Inj.* 19, 85–91. <https://doi.org/10.1080/02699050410001720158>.
- Bazarian, J.J., Biberthaler, P., Welch, R.D., Lewis, L.M., Barzo, P., Bogner-Flatz, V., Brolinson, P.G., Büki, A., Chen, J.Y., Christenson, R.H., 2018. Serum GFAP and UCH-

- L1 for prediction of absence of intracranial injuries on head CT (ALERT-TBI): a multicentre observational study. *Lancet Neurol.* 17, 782–789. [https://doi.org/10.1016/S1474-4422\(18\)30231-X](https://doi.org/10.1016/S1474-4422(18)30231-X).
- Bazarian, J.J., Welch, R.D., Caudle, K., Jeffrey, C.A., Chen, J.Y., Chandran, R., McCaw, T., Datwyler, S.A., Zhang, H., McQuiston, B., 2021. Accuracy of a rapid glial fibrillary acidic protein/ubiquitin carboxyl-terminal hydrolase L1 test for the prediction of intracranial injuries on head computed tomography after mild traumatic brain injury. *Acad. Emerg. Med* 28 (11), 1308–1317. <https://doi.org/10.1111/acem.14366>.
- Beard, K., Yang, Z., Haber, M., Flamholz, M., Diaz-Arrastia, R., Sandsmark, D., Meaney, D.F., Issadore, D., 2021. Extracellular vesicles as distinct biomarker reservoirs for mild traumatic brain injury diagnosis. *Brain communications* 3, fcab151. <https://doi.org/10.1093/braincomms/fcab151>.
- Carabias, C.S., Castaño-León, A.M., Blanca Navarro, B., Panero, I., Eiriz, C., Gómez, P.A., Egea, J., Lagares, A., 2020. Serum amyloid A1 as a potential intracranial and extracranial clinical severity biomarker in traumatic brain injury. *J. Intensive Care Med.* 35, 1180–1195. <https://doi.org/10.1177/0885066619837913>.
- Carroll, L.J., Cassidy, J.d., Holm, L., Kraus, J., Coronado, V.G., 2004. Methodological issues and research recommendations for mild traumatic brain injury: the WHO collaborating centre task force on mild traumatic brain injury. *J. Rehabil. Med.* 113–125.
- Caswell, T.A., Droettboom, M., Hunter, J., Firing, E., Lee, A., Klymak, J., Stansby, D., De Andrade, E.S., Nielsen, J.H., Varoquaux, N., 2018. *Matplotlib/Matplotlib V3.0.2*.
- Chaban, V., Clarke, G.J.B., Sk, sen, T., Islam, R., Einarsen, C.E., Vik, A., Damás, J.K., Mollnes, T.E., Häberg, A.K., Pischke, S.E., 2020. Systemic inflammation persists the first year after mild traumatic brain injury: results from the prospective trondheim mild traumatic brain injury study. *J. Neurotrauma* 37, 2120–2130. <https://doi.org/10.1089/neu.2019.6963>.
- Csuka, E., Morganti-Kossmann, M.C., Lenzlinger, P.M., Joller, H., Trentz, O., Kossmann, T., 1999. IL-10 levels in cerebrospinal fluid and serum of patients with severe traumatic brain injury: relationship to IL-6, TNF- α , TGF- β 1 and blood-brain barrier function. *J. Neuroimmunol.* 101, 211–221. [https://doi.org/10.1016/S0165-5728\(99\)00148-4](https://doi.org/10.1016/S0165-5728(99)00148-4).
- Di Battista, A.P., Rhind, S.G., Richards, D., Baker, A.J., Hutchison, M.G., 2016. Comparison of systemic inflammatory profiles in healthy athletes with and without a history of concussion. *Brain Inj.* 30, 707.
- Di Battista, A.P., Churchill, N., Rhind, S.G., Richards, D., Hutchison, M.G., 2019. Evidence of a distinct peripheral inflammatory profile in sport-related concussion. *J. Neuroinflammation* 16, 17. <https://doi.org/10.1186/s12974-019-1402-y>.
- Di Battista, A.P., Churchill, N., Rhind, S.G., Richards, D., Hutchison, M.G., 2020a. The relationship between symptom burden and systemic inflammation differs between male and female athletes following concussion. *BMC Immunol.* 21 <https://doi.org/10.1186/s12865-020-0339-3>.
- Di Battista, A.P., Rhind, S.G., Richards, D., Hutchison, M.G., 2020b. An investigation of plasma interleukin-6 in sport-related concussion. *PLoS One* 15, e0232053. <https://doi.org/10.1371/journal.pone.0232053>.
- Dixon, K.J., 2017. Pathophysiology of traumatic brain injury. *Phys. Med. Rehabil. Clin. N. Am.* 28, 215–225. <https://doi.org/10.1016/j.pmr.2016.12.001>.
- Edalatfar, M., Piri, S.M., Mehrabinejad, M.-M., Mousavi, M.-S., Meknatkhah, S., Fattahi, M.-R., Kavyani, Z., Hajighadery, A., Kaveh, M., Aryannejad, A., 2021. Blood biomarkers in traumatic brain injury: a systematic scoping review. *Neurocrit. Care* 1–14. <https://doi.org/10.1007/s12028-020-01173-1>.
- Edwards, K.A., Gill, J.M., Pattinson, C.L., Lai, C., Brière, M., Rogers, N.J., Milhorn, D., Elliot, J., Carr, W., 2020a. Interleukin-6 is associated with acute concussion in military combat personnel. *BMC Neurol.* 20, 209. <https://doi.org/10.1186/s12883-020-01760-x>.
- Edwards, K.A., Pattinson, C., L., r, Guedes, V.A., Peyser, J., Moore, C., ace, Davis, T., Devoto, C., Turtzo, L.C., Latour, L., Gill, J.M., 2020b. Inflammatory cytokines associate with neuroimaging after acute mild traumatic brain injury. *Front. Neurol.* 11, 348. <https://doi.org/10.3389/fneur.2020.00348>.
- Garcia, J.M., Stillings, S.A., Leclerc, J.L., Phillips, H., Edwards, N.J., Robicsek, S.A., Hoh, B.L., Blackburn, S., Doré, S., 2017. Role of interleukin-10 in acute brain injuries. *Front. Neurol.* 8, 244. <https://doi.org/10.3389/fneur.2017.00244>.
- Gill, J., Motamed, V., Osier, N., Dell, K., Arcurio, L., Carr, W., Walker, P., Ahlers, S., Lopresti, M., Yarnell, A., 2017. Moderate blast exposure results in increased IL-6 and TNF α in peripheral blood. *Brain Behav. Immun.* 65, 90–94. <https://doi.org/10.1016/j.bbi.2017.02.015>.
- Goronzy, J.J., Weyand, C.M., 2013. Understanding immunosenescence to improve responses to vaccines. *Nat. Immunol.* 14, 428. <https://doi.org/10.1038/ni.2588>.
- Helmy, A., Antoniadis, C.A., Guilfoyle, M.R., Carpenter, K.L., Hutchinson, P.J., 2012. Principal component analysis of the cytokine and chemokine response to human traumatic brain injury. *PLoS One* 7, e39677. <https://doi.org/10.1371/journal.pone.0039677>.
- Henno, L.T., Storfjord, E., Christiansen, D., Bergseth, G., Ludviksen, J.K., Fure, H., Barene, S., Nielsen, E.W., Mollnes, T.E., Brekke, O.-L., 2017. Effect of the anticoagulant, storage time and temperature of blood samples on the concentrations of 27 multiplex assayed cytokines—consequences for defining reference values in healthy humans. *Cytokine* 97, 86–95. <https://doi.org/10.1016/j.cyto.2017.05.014>.
- Hergenroeder, G.W., Moore, A.N., McCoy, J.P., Samsel, L., Ward, N.H., Clifton, G.L., Dash, P.K., 2010. Serum IL-6: a candidate biomarker for intracranial pressure elevation following isolated traumatic brain injury. *J. Neuroinflammation* 7, 1–13. <https://doi.org/10.1186/1742-2094-7-19>.
- Huang, X., Dai, Y., Ma, X., Wang, S., Xu, X., Pei, X., Li, R., Wang, H., 2020. Different changes in granulocyte-colony stimulating factor and its correlation with inflammatory biomarkers in patients after traumatic brain injury. *NeuroReport: Rapid Commun. Neurosci. Res.* 31, 293–299. <https://doi.org/10.1097/WNR.0000000000001397>.
- Huie, J.R., Diaz-Arrastia, R., Yue, J.K., Sorani, M.D., Puccio, A.M., Okonkwo, D.O., Manley, G.T., Ferguson, A.R., 2019. Testing a multivariate proteomic panel for traumatic brain injury biomarker discovery: a TRACK-TBI pilot study. *J. Neurotrauma* 36, 100–110. <https://doi.org/10.1089/neu.2017.5449>.
- Huie, J.R., Mondello, S., Lindsell, C.J., Antiga, L., Yuh, E.L., Zanier, E.R., Collaborative European NeuroTrauma Effectiveness Research in Traumatic Brain Injury (CENTER-TBI) Participants and Investigators, 2021. Biomarkers for traumatic brain injury: data standards and statistical considerations. *J. Neurotrauma* 38 (18). <https://doi.org/10.1089/neu.2019.6762>.
- Kossmann, T., Hans, V.H., Imhof, H.G., Stocker, R., Grob, P., Trentz, O., Morganti-Kossmann, C., 1995. Intrathecal and serum interleukin-6 and the acute-phase response in patients with severe traumatic brain injuries. *Shock* 4, 311–317. <https://doi.org/10.1097/00024382-199511000-00001>.
- Lagerstedt, L., Egea-Guerrero, J.J., Bustamante, A., Rodríguez-Rodríguez, A., Rahal, A.E., Quintana-Díaz, M., García-Armengol, R., Prica, C.M., Anderegg, E., Rinaldi, L., Sarrafzadeh, A., Schaller, K., Montaner, J., Sanchez, J.C., 2018a. Combining H-FABP and GFAP increases the capacity to differentiate between CT-positive and CT-negative patients with mild traumatic brain injury. *PLoS One* 13. <https://doi.org/10.1371/journal.pone.0200394>.
- Lagerstedt, L., Egea-Guerrero, J.J., Rodríguez-Rodríguez, A., Bustamante, A., Montaner, J., Rahal, A.E., Anderegg, E., Rinaldi, L., Sarrafzadeh, A., Schaller, K., Sanchez, J.C., 2018b. Early measurement of interleukin-10 predicts the absence of CT scan lesions in mild traumatic brain injury. *PLoS One* 13. <https://doi.org/10.1371/journal.pone.0193278>.
- Lagerstedt, L., Azurmendi, L., Tenovuo, O., Katila, A.J., Takala, R.S.K., Blennow, K., Newcombe, V.F.J., Maanpää, H.R., Tallus, J., Hossain, I., Gils, M.V., Menon, D.K., Hutchinson, P.J., Zetterberg, H., Posti, J.P., Sanchez, J.C., 2020. Interleukin 10 and heart fatty acid-binding protein as early outcome predictors in patients with traumatic brain injury. *Front. Neurol.* 11 <https://doi.org/10.3389/fneur.2020.00376>.
- Lassarén, P., Lindblad, C., Frostell, A., Carpenter, K.L.H., Guilfoyle, M.R., Hutchinson, P.J.A., Helmy, A., Thelin, E.P., 2021. Systemic inflammation alters the neuroinflammatory response: a prospective clinical trial in traumatic brain injury. *J. Neuroinflammation* 18, 221. <https://doi.org/10.1186/s12974-021-02264-2>.
- Lasseter, H.C., Provost, A.C., Chaby, L.E., Daskalakis, N.P., Haas, M., Jeromin, A., 2020. Cross-platform comparison of highly sensitive immunoassay technologies for cytokine markers: platform performance in post-traumatic stress disorder and Parkinson's disease. *Cytokine: X* 2. <https://doi.org/10.1016/j.cytex.2020.100027>.
- Lindblad, C., Pin, E., Just, D., Al Nimer, F., Nilsson, P., Bellander, B.M., Svensson, M., Pielh, F., Thelin, E.P., 2021. Fluid proteomics of CSF and serum reveal important neuroinflammatory proteins in blood-brain barrier disruption and outcome prediction following severe traumatic brain injury: a prospective, observational study. *Crit. Care* 25, 1–18. <https://doi.org/10.1186/s13054-021-03503>.
- Lunkova, E., Guberman, G.I., Ptiito, A., Saluja, R.S., 2021. Noninvasive magnetic resonance imaging techniques in mild traumatic brain injury research and diagnosis. *Hum. Brain Mapp.* 42 (16), 5477–5494. <https://doi.org/10.1002/hbm.25630>.
- Lustenberger, T., Kern, M., Relja, B., Wutzler, S., Störmann, P., Marzi, I., 2016. The effect of brain injury on the inflammatory response following severe trauma. *Immunobiology* 221, 427–431. <https://doi.org/10.1016/j.imbio.2015.11.011>.
- Maas, A.I., Menon, D.K., Steyerberg, E.W., Citerio, G., Lecky, F., Manley, G.T., Hill, S., Legrand, V., Sorgner, A., 2015. Collaborative European NeuroTrauma effectiveness research in traumatic brain injury (CENTER-TBI) a prospective longitudinal observational study. *Neurosurgery* 76, 67–80. <https://doi.org/10.1227/NEU.0000000000000575>.
- Maas, A.I., Menon, D.K., Adelson, P.D., Andelic, N., Bell, M.J., Belli, A., Bragge, P., Brazinova, A., Büki, A., Chesnut, R.M., 2017. Traumatic brain injury: integrated approaches to improve prevention, clinical care, and research. *Lancet Neurol.* 16, 987–1048. [https://doi.org/10.1016/S1474-4422\(17\)30371-X](https://doi.org/10.1016/S1474-4422(17)30371-X).
- Manley, G.T., Maas, A.I., 2013. Traumatic brain injury: an international knowledge-based approach. *J. Am. Med. Assoc.* 310, 473–474. <https://doi.org/10.1001/jama.2013.169158>.
- Manley, G.T., Diaz-Arrastia, R., Brophy, M., Engel, D., Goodman, C., Gwinn, K., Veenstra, T.D., Ling, G., Ottens, A.K., Tortella, F., 2010. Common data elements for traumatic brain injury: recommendations from the biospecimens and biomarkers working group. *Arch. Phys. Med. Rehabil.* 91, 1667–1672. <https://doi.org/10.1016/j.apmr.2010.05.018>.
- McCroory, P., Meeuwisse, W., Dvorak, J., Aubry, M., Bales, J., Broglio, S., Cantu, R.C., Cassidy, D., Echemendia, R.J., Castellani, R.J., 2017. Consensus statement on concussion in sport—the 5th international conference on concussion in sport held in Berlin, October 2016. *Br. J. Sports Med.* 51, 838–847. <https://doi.org/10.1136/bjsports-2017-097699>.
- McDonald, S.J., Sun, M., Agoston, D.V., Shultz, S.R., 2016. The effect of concomitant peripheral injury on traumatic brain injury pathobiology and outcome. *J. Neuroinflammation* 13, 1–14. <https://doi.org/10.1186/s12974-016-0555-1>.
- McDonald, S.J., Shultz, S.R., Agoston, D.V., 2021. The known unknowns: an overview of the state of blood-based protein biomarkers of mild traumatic brain injury. *J. Neurotrauma* 38 (19), 2652–2666. <https://doi.org/10.1089/neu.2021.0011>.
- McInnes, K., Friesen, C.L., MacKenzie, D.E., Westwood, D.A., Boe, S.G., 2017. Mild Traumatic Brain Injury (mTBI) and chronic cognitive impairment: a scoping review. *PLoS One* 12, e0174847. <https://doi.org/10.1371/journal.pone.0174847>.
- Meier, T.B., Huber, D.L., Bohorquez-Montoya, L., Nitta, M.E., Savitz, J., Teague, T.K., Bazarian, J.J., Hayes, R.L., Nelson, L.D., McCreary, M.A., 2020. A prospective study of acute blood-based biomarkers for sport-related concussion. *Ann. Neurol.* 87, 907–920. <https://doi.org/10.1002/ana.25725>.

- Mercier, E., Tardif, P.-A., Emond, M., Ouellet, M.-C., De Guise, É., Mitra, B., Cameron, P., Le Sage, N., 2017. Characteristics of patients included and enrolled in studies on the prognostic value of serum biomarkers for prediction of postconcussion symptoms following a mild traumatic brain injury: a systematic review. *BMJ Open* 7, e017848. <https://doi.org/10.1136/bmjopen-2017-017848>.
- Mondello, S., Sorinola, A., Czeiter, E., Vámos, Z., Amrein, K., Synnot, A., Donoghue, E., Sandor, J., Wang, K.K., Diaz-Arrastia, R., 2018. Blood-based protein biomarkers for the management of traumatic brain injuries in adults presenting to emergency departments with mild brain injury: a living systematic review and meta-analysis. *J. Neurotrauma* 38 (8), 1086–1106. <https://doi.org/10.1089/neu.2017.5182>.
- Morganti-Kossmann, M., Lenzlinger, P., Hans, V., Stahel, P., Csuka, E., Ammann, E., Stocker, R., Trentz, O., Kossmann, T., 1997. Production of cytokines following brain injury: beneficial and deleterious for the damaged tissue. *Mol. Psychiatry* 2, 133–136. <https://doi.org/10.1038/sj.mp.4000227>.
- Mouzon, B.C., Bachmeier, C., Ojo, J.O., Acker, C.M., Ferguson, S., Paris, D., Ait-Ghezala, G., Crynen, G., Davies, P., Mullan, M., 2018. Lifelong behavioral and neuropathological consequences of repetitive mild traumatic brain injury. *Ann. Clin. Transl. Neurol.* 5, 64–80. <https://doi.org/10.1002/acn3.510>.
- Niraula, A., Sheridan, J.F., Godbout, J.P., 2017. Microglia priming with aging and stress. *Neuropsychopharmacology* 42, 318–333. <https://doi.org/10.1038/npp.2016.185>.
- Nitta, M.E., Savitz, J., Nelson, L.D., Teague, T.K., Hoelzle, J.B., McCreary, M.A., Meier, T. B., 2019. Acute elevation of serum inflammatory markers predicts symptom recovery after concussion. *Neurology* 93, E497–E507. <https://doi.org/10.1212/WNL.0000000000007864>.
- Numis, A.L., Fox, C.H., Lowenstein, D.J., Norris, P.J., Di Germanio, C., 2021. Comparison of multiplex cytokine assays in a pediatric cohort with epilepsy. *Heliyon* 7, e06445. <https://doi.org/10.1016/j.heliyon.2021.e06445>.
- O'Brien, W.T., Symons, G.F., Bain, J., Major, B.P., Costello, D.M., Sun, M., Kimpton, J.S., Chen, Z., Brady, R.D., Mychasiuk, R., 2021. Elevated serum interleukin-1 β levels in male, but not female, collision sport athletes with a concussion history. *J. Neurotrauma* 38 (10), 1350–1357. <https://doi.org/10.1089/neu.2020.7479>.
- Okonkwo, D.O., Puffer, R.C., Puccio, A.M., Yuh, E.L., Yue, J.K., Diaz-Arrastia, R., Transforming Research and Clinical Knowledge in Traumatic Brain Injury (TRACK-TBI) Investigators, 2020. Point-of-care platform blood biomarker testing of glial fibrillary acidic protein versus S100 calcium-binding protein B for prediction of traumatic brain injuries: a transforming research and clinical knowledge in traumatic brain injury study. *J. Neurotrauma* 37 (23), 2460–2467. <https://doi.org/10.1089/neu.2020.7140>.
- Ouzzani, M., Hammady, H., Fedorowicz, Z., Elmagarmid, A., 2016. Rayyan—a web and mobile app for systematic reviews. *Syst. Rev.* 5, 210.
- Page, M.J., Moher, D., Bossuyt, P., Boutron, I., Hoffmann, T., Mulrow, C., Shamseer, L., Tetzlaff, J., Akl, E., Brennan, S.E., 2020. PRISMA 2020 Explanation and Elaboration: Updated Guidance and Exemplars for Reporting Systematic Reviews. <https://doi.org/10.1136/bmj.n160>.
- Pankratova, N., Jović, M., Pfeifer, M.E., 2021. Electrochemical sensing of blood proteins for mild traumatic brain injury (mTBI) diagnostics and prognostics: towards a point-of-care application. *RSC Adv.* 11, 17301–17319. <https://doi.org/10.1039/D1RA00589H>.
- Parkin, G.M., Clarke, C., Takagi, M., Harp, S., Babl, F.E., Davis, G.A., Anderson, V., Ignjatovic, V., 2019. Plasma tumor necrosis factor alpha is a predictor of persisting symptoms post-concussion in children. *J. Neurotrauma* 36, 1768–1775. <https://doi.org/10.1089/neu.2018.6042>.
- Posti, J.P., Takala, R.S.K., Lagerstedt, L., Dickens, A.M., Hossain, I., Mohammadian, M., Ala-Seppälä, H., Frantzen, J., Van Gils, M., Hutchison, P.J., Katila, A.J., Maanpää, H.R., Menon, D.K., Newcombe, V.F., Tallus, J., Hrusovsky, K., Wilson, D. H., Gill, J., Sanchez, J.C., Tenovuo, O., Zetterberg, H., Blennow, K., 2019. Correlation of blood biomarkers and biomarker panels with traumatic findings on computed tomography after traumatic brain injury. *J. Neurotrauma* 36, 2178–2189. <https://doi.org/10.1089/neu.2018.6254>.
- Posti, J.P., Takala, R.S., Raj, R., Luoto, T.M., Azurmendi, L., Lagerstedt, L., Mohammadian, M., Hossain, I., Gill, J., Frantzen, J., 2020. Admission levels of interleukin 10 and amyloid beta 1-40 improve the outcome prediction performance of the helsinki computed tomography score in traumatic brain injury. *Front. Neurol.* 11:549527 <https://doi.org/10.3389/fneur.2020.549527>.
- Puffer, R.C., Garcia, L.M.C., Himes, B.T., Jung, M.Y., Meyer, F.B., Okonkwo, D.O., Parney, I.F., 2020. Plasma extracellular vesicles as a source of biomarkers in traumatic brain injury. *J. Neurosurg.* 134 (6), 1921–1928. <https://doi.org/10.3171/2020.4.JNS.20305>.
- Purkayastha, S., Stokes, M., Bell, K.R., 2019. Autonomic nervous system dysfunction in mild traumatic brain injury: a review of related pathophysiology and symptoms. *Brain Inj.* 33, 1129–1136. <https://doi.org/10.1080/02699052.2019.1631488>.
- Rodney, T., Osier, N., Gill, J., 2018. Pro- and anti-inflammatory biomarkers and traumatic brain injury outcomes: a review. *Cytokine* 110, 248–256. <https://doi.org/10.1016/j.cyt.2018.01.012>.
- Rodney, T., Taylor, P., Dunbar, K., Perrin, N., Lai, C., Roy, M., Gill, J., 2020. High IL-6 in military personnel relates to multiple traumatic brain injuries and post-traumatic stress disorder. *Behav. Brain Res.* 392 <https://doi.org/10.1016/j.bbr.2020.112715>.
- Schimmel, S.J., Acosta, S., Lozano, D., 2017. Neuroinflammation in traumatic brain injury: a chronic response to an acute injury. *Brain Circ.* 3, 135–142. https://doi.org/10.4103/bc.bc_18_17.
- Sharma, R., Rosenberg, A., Bennett, E.R., Laskowitz, D.T., Acheson, S.K., 2017. A blood-based biomarker panel to risk-stratify mild traumatic brain injury. *PLoS One* 12, e0173798. <https://doi.org/10.1371/journal.pone.0173798>.
- Shaw, A.C., Goldstein, D.R., Montgomery, R.R., 2013. Age-dependent dysregulation of innate immunity. *Nat. Rev. Immunol.* 13, 875–887. <https://doi.org/10.1038/nri3547>.
- Shetty, T., Cogsil, T., Dalal, A., Kim, E., Halvorsen, K., Cummings, K., Nguyen, J.T., 2019. High-sensitivity C-reactive protein: retrospective study of potential blood biomarker of inflammation in acute mild traumatic brain injury. *J. Head Trauma Rehabil.* 34, E28–E36. <https://doi.org/10.1097/HTR.0000000000000450>.
- Shultz, S.R., Sun, M., Wright, D.K., Brady, R.D., Liu, S., Beynon, S., Schmidt, S., Kaye, A. H., Hamilton, J.A., O'Brien, T.J., Grills, B.L., McDonald, S.J., 2015. Tibial fracture exacerbates traumatic brain injury outcomes and neuroinflammation in a novel mouse model of multitrauma. *J. Cereb. Blood Flow Metab.* 35, 1339–1347. <https://doi.org/10.1038/jcbfm.2015.56>.
- Simon, D.W., McGeachy, M.J., Bayir, H., Clark, R.S., Loane, D.J., Kochanek, P.M., 2017. The far-reaching scope of neuroinflammation after traumatic brain injury. *Nat. Rev. Neurol.* 13, 171–191. <https://doi.org/10.1038/nrneuro.2017.13>.
- Strimbu, K., Tavel, J.A., 2010. What are biomarkers? In: *Current Opinion in HIV and AIDS*, 5, pp. 463–466. <https://doi.org/10.1097/COH.0b013e32833ed177>.
- Su, S.H., Xu, W., Li, M., Zhang, L., Wu, Y.F., Yu, F., Hai, J., 2014. Elevated C-reactive protein levels may be a predictor of persistent unfavourable symptoms in patients with mild traumatic brain injury: a preliminary study. *Brain Behav. Immun.* 38, 111–117. <https://doi.org/10.1016/j.bbi.2014.01.009>.
- Sun, Y., Bai, L., Niu, X., Wang, Z., Yin, B., Bai, G., Zhang, D., Gan, S., Sun, C., Wang, S., Zhu, F., Zhang, M., 2019. Elevated serum levels of inflammation-related cytokines in mild traumatic brain injury are associated with cognitive performance. *Front. Neurol.* 10, 1120. <https://doi.org/10.3389/fneur.2019.01120>.
- Thelin, E.P., Tajsic, T., Zeiler, F.A., Menon, D.K., Hutchinson, P.J., Carpenter, K.L., Morganti-Kossmann, M.C., Helmy, A., 2017. Monitoring the neuroinflammatory response following acute brain injury. *Front. Neurol.* 8, 351. <https://doi.org/10.3389/fneur.2017.00351>.
- Thompson, H.J., Marthas, S.R., Wang, J., Becker, K.J., 2020. Impact of age on plasma inflammatory biomarkers in the 6 months following mild traumatic brain injury. *J. Head Trauma Rehabil.* 35, 324–331. <https://doi.org/10.1097/HTR.0000000000000606>.
- Tylicka, M., Matuszczak, E., Hermanowicz, A., Dębek, W., Karpińska, M., Kamińska, J., Koper-Lenkiewicz, O.M., 2020. BDNF and IL-8, but not UCHL-1 and IL-11, are markers of brain injury in children caused by mild head trauma. *Brain Sci.* 10 <https://doi.org/10.1097/HTR.0000000000000606>.
- Udén, J., Ingebrigtsen, T., Romner, B., 2013. Scandinavian guidelines for initial management of minimal, mild and moderate head injuries in adults: an evidence and consensus-based update. *BMC Med.* 11, 1–4. <https://doi.org/10.1186/1741-7015-11-50>.
- van der Horn, H.J., Out, M.L., de Koning, M.E., Mayer, A.R., Spikman, J.M., Sommer, I. E., van der Naalt, J., 2019. An integrated perspective linking physiological and psychological consequences of mild traumatic brain injury. *J. Neurol.* 1–10. <https://doi.org/10.1007/s00415-019-09335-8>.
- Vedantam, A., Brennan, J., Levin, H.S., McCarthy, J.J., Dash, P.K., Redell, J.B., Yamal, J.-M., Robertson, C.S., 2021. Early versus late profiles of inflammatory cytokines after mild traumatic brain injury and their association with neuropsychological outcomes. *J. Neurotrauma* 38, 53–62. <https://doi.org/10.1089/neu.2019.6979>.
- Villapol, S., Loane, D.J., Burns, M.P., 2017. Sexual dimorphism in the inflammatory response to traumatic brain injury. *Glia* 65, 1423–1438. <https://doi.org/10.1002/glia.23171>.
- Wang, K.K., Yang, Z., Zhu, T., Shi, Y., Rubenstein, R., Tyndall, J.A., Manley, G.T., 2018. An update on diagnostic and prognostic biomarkers for traumatic brain injury. *Expert Rev. Mol. Diagn.* 18, 165–180. <https://doi.org/10.1080/14737159.2018.1428089>.
- Wells, G.A., Shea, B., O'Connell, D., Peterson, J., Welch, V., Losos, M., Tugwell, P., 2000. The Newcastle-Ottawa Scale (NOS) for Assessing the Quality of Nonrandomised Studies in Meta-Analyses. Oxford.
- Werner, C., Engelhard, K., 2007. Pathophysiology of traumatic brain injury. *BJA: Br. J. Anaesthesia* 99, 4–9. <https://doi.org/10.1093/bja/aem131>.
- Whiting, P.F., Rutjes, A.W., Westwood, M.E., Mallett, S., Deeks, J.J., Reitsma, J.B., Leeflang, M.M., Sterne, J.A., Bossuyt, P.M., 2011. QUADAS-2: a revised tool for the quality assessment of diagnostic accuracy studies. *Ann. Intern. Med.* 155, 529–536.
- WHO, 2006. Neurological Disorders: Public Health Challenges. World Health Organization.
- Wilde, E.A., Ware, A.L., Li, X., Wu, T.C., McCauley, S.R., Barnes, A., Newsome, M.R., Biekman, B.D., Hunter, J.V., Chu, Z.D., Levin, H.S., 2019. Orthopedic injured versus uninjured comparison groups for neuroimaging research in mild traumatic brain injury. *J. Neurotrauma* 36 (2), 239–249. <https://doi.org/10.1089/neu.2017.5513>.
- Woodcock, T., Morganti-Kossmann, C., 2013. The role of markers of inflammation in traumatic brain injury. *Front. Neurol.* 4, 18. <https://doi.org/10.3389/fneur.2013.00018>.
- Xiong, Y., Mahmood, A., Chopp, M., 2018. Current understanding of neuroinflammation after traumatic brain injury and cell-based therapeutic opportunities. *Chin. J. Traumatol.* 21, 137–151. <https://doi.org/10.1016/j.cjtee.2018.02.003>.
- Xu, L., Yue, J.K., Korley, F.K., Puccio, A.M., Yuh, E.L., Sun, X., Rabinowitz, M., Vassar, M., Taylor, S.R., Winkler, E.A., Puffer, R., Deng, H., McCreary, M., Stein, M.B., Robertson, C.S., Levin, H., Dikmen, S., Temkin, N., Giacino, J.T., Mukherjee, P., Wang, K.K.W., Okonkwo, D.O., Markowitz, A.J., Jain, S., Manley, G.T., Diaz-Arrastia, R., 2020. High-sensitivity C-reactive protein is a prognostic biomarker of 6-month disability after traumatic brain injury: results from the TRACK-TBI study. *J. Neurotrauma* 38 (1), 918–927. <https://doi.org/10.1089/neu.2020.7177>.
- Yan, E.B., Satgunaseelan, L., Paul, E., Bye, N., Nguyen, P., Agyapomaa, D., Kossmann, T., Rosenfeld, J.V., Morganti-Kossmann, M.C., 2014. Post-traumatic hypoxia is associated with prolonged cerebral cytokine production, higher serum biomarker levels, and poor outcome in patients with severe traumatic brain injury. *J. Neurotrauma* 31, 618–629. <https://doi.org/10.1089/neu.2013.3087>.

- Yang, L., Guo, Y., Wen, D., Yang, L., Chen, Y., Zhang, G., Fan, Z., 2016. Bone fracture enhances trauma brain injury. *Scand. J. Immunol.* 83, 26–32. <https://doi.org/10.1111/sji.12393>.
- Zeiler, F.A., Thelin, E.P., Czosnyka, M., Hutchinson, P.J., Menon, D.K., Helmy, A., 2017. Cerebrospinal fluid and microdialysis cytokines in severe traumatic brain injury: a scoping systematic review. *Front. Neurol.* 8, 331. <https://doi.org/10.3389/fneur.2017.00331>.
- Zhou, Q., Yin, J., Wang, Y., Zhuang, X., He, Z., Chen, Z., Yang, X., 2021. MicroRNAs as potential biomarkers for the diagnosis of Traumatic Brain Injury: a systematic review and meta-analysis. *Int. J. Med. Sci.* 18 (1), 128. <https://doi.org/10.7150/ijms.48214>.