MILD TRAUMATIC BRAIN INJURY AND NEUROINFLAMMATION

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By

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Abstract

Despite being a common problem, there are many gaps in the understanding of mild traumatic brain injury (mTBI). Its pathophysiology is unclear, diagnostic criteria are variable, and the associated symptomatology is non-specific. As a result, there are challengesassociated with precise mTBI diagnosis and treatment. The dissertation seeks to identify distinctive features, both clinical and pathophysiological, exclusively associated with mTBI. In addition, the neuroinflammatory component of mTBI is explored in detail in the context of inflammatory cytokines' potential use as prognostic biomarkers and development of a targeted treatment. Three studies were conducted to explore mTBI.

We conducteda retrospective chart review to identify the clinical presentation exclusively associated with mTBI that sets it apart from other similar conditions. This was accomplished through symptomatology comparison between thepatients with head injuries that meet the ACRM (1993) criteria for mTBI diagnosis vs. those who do not. The results of this study showed that 20.5% of patients with chronic post-concussive symptoms do not meet the ACRM (1993) criteria of mTBI despite sustaining a head injury. In addition,no symptom specific differences were found between the two populations.

A detailed systematic review and meta-analysis werealso conducted to identify the common inflammatory cytokines associated with mTBI and to explore their potential use as prognostic biomarkers. The results show significantly elevated blood IL-6, IL-1RA, IFN-γ (at <24 hrs.) and MCP-1/CCL2 (within a week)levels in patientswith mTBI compared to healthy controls in majority of the included studies. A meta-analysis was further conducted that supported these findings by showing significantly elevated IL-6, MCP-1/CCL2, and IL-1β levels in patientswith mTBI in the acute stages (<7 days). In

addition, elevated IL-6, TNF-α, IL-1RA, IL-10, and MCP-1/CCL2 levels were associated with poor prognosis in patients with mTBI.

In addition, a systematic review was conducted to identifythe inflammatory cytokines associated with adverse psychological outcome in population with mTBI. The results show that IL-6, TNF-α, IL-10, and CRP are associated with PTSD and/or depression in the population with mTBI, particularly in the chronic stages.

Collectively, these studies show that all symptomatic patients with head trauma, whetheror notthey meet the subjective criteria of mTBI, should be managed and offered early rehabilitation to avoid long tern adverse consequences. In addition, this thesis supports the neuroinflammatory hypothesis of mTBI and identifies inflammatory cytokines that could be potentially utilized as prognostic biomarkers and for the development of mTBI treatment.

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Table of Contents

List of Tables

List of Figures

Chapter 2

Chapter 3

Chapter 4

List of Abbreviations

HiBRID: Head Injury but Brain Injury Debatable

ICD-10: International Statistical Classification of Diseases and RelatedHealth Problems, 10th Revision

IFN-γ: Interferon- γ

IL-1: Interleukin-1

IL-10: Interleukin-10

IL-1RA: Interleukin-1 Receptor Antagonist

IL-1β: Interleukin-1 beta

IL-4: Interleukin-4

IL-6: Interleukin-6

IL-8: Interleukin-8

K: Kappa

LOC: Loss of Consciousness

MCP-1/CCL-2: Monocyte Chemoattractant Protein-1/C-C Motif Chemokine Ligand 2

MCP-4: Monocyte Chemoattractant Protein-4

MINORS: Methodological Index for Non-randomized Studies

MIP-1β: Macrophage Inflammatory Protein-1β

miRNA: microRNA

MRI: Magnetic Resonance Imaging

mTBI: Mild Traumatic Brain Injury

MVA: Motor Vehicle Accident

n: Number of Participants

NOS: Newcastle-Ottawa Quality Assessment ScaleOI: Orthopedic Injury

PCL- C: Post-traumatic Stress Checklist-Civilian Form

PCL-M: PTSD Checklist Military Version

PCS: Post-Concussion Syndrome

PCSx: Post-Concussion Symptoms

Pg/ml: Picogram per ml

PHQ-9: 9-item Patient Health Questionnaire Depression Scale

PRISMA: Preferred Reporting Items for Systematic Reviews and Meta-analyses

PTA: Post-traumatic Amnesia

PTSD: Post-traumatic stress disorder

QIDS: Quick Inventory of Depressive Symptomatology

R-AMSTAR: Revised Assessment of Multiple Systematic Reviews

RCT: Randomized Controlled Trial

REB: Research Ethics Board

RevMan: Review Manager

RPQ: Rivermead Patient Questionnaire

SD: Standard Deviation

SEM: Standard Error of Mean

SMD: Standard Mean Difference

SPECT:Single Photon-Emission Computed Tomography

Std: Standardized

TBI: Traumatic Brain Injury

TNF-α: Tumor Necrosis Factor-alpha

U.S.: United States

UCH-L1:C-terminal hydrolase L1

WHO: World Health Organization

χ^2: Pearson's Chi-square test

Declaration of Academic Achievement

Chapter 2: All data presented in this chapter was collected and analyzed byS. Malik and R. Ahmed. T. Gambale aided with aspects of data collection and analyses.M. Rathbonecontributed to the project designand aided with formation of the initial research questions. S. Malik and R. Ahmed wrote the first draft of the manuscript and prepared all the figures. T. Gambaleand M. Rathbone reviewed the manuscript and made contributions for improvement.

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Chapter 4:S. Malik defined the research of interest and were involved in topic selection. S. Malik and O. Alnaji carried out the literature search. S. Malik, O. Alnaji and M. Malik collected the data. F. Farrokhyar, S. Malik, and O. Alnaji performed the meta-analysis. S. Malik wrote the first draft of the manuscript and prepared all the figures. M. Malik, O. Alnaji, T. Gambale, F. Farrokhyar, and M. Rathbone reviewed the manuscript and made contributions for improvement. All authors helped to revise the paper. All authors contributed to the article and approved the submitted version.

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Chapter 5: M.Rathbone, S.Malik, and O.Alnaji contributed to conceptualization and project design.All data presented in this chapter was collected and analyzed by S.Malik and O.Alnaji. The original draft was prepared by S.Malik,O. Alnaji, andM. Malik. T. Gambaleand M. Rathbone reviewed the manuscript and made contributions for improvement. All authors helped to revise the paper. All authors contributed to the article and approved the submitted version.

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Chapter 1

Introduction

1.1. Epidemiology

The global surge in traumatic brain injuries (TBI) is a mounting public health concern [1–4]. TBI is defined as an alteration in brain function, or any evidence of brain pathology, caused by an external force [5].Globally, 100–749 per 100,000individuals (or approximately 69 million people) suffer from TBI each year, with its greatest incidence in North America and Europe (1299 and 1012 cases per 100 000, respectively) [3]. In the United States, approximately 2.8 million individualssustained a TBI in 2013 alone [4]. It is estimated that 47.4% (11.4 million) of individuals (greater than 40 years of age) with a history of head injury are living with a TBI related disability in the United States [6].

Mild traumatic brain injuries (mTBI) are the most common type of all brain injuries, andaccount for 70-90% of all TBIs [7–9]. According to the World Health Organization (WHO) Collaborating Centre Task Force, 1 to 3 of every 1000 persons around the world are treated annually for mTBIs [10]. In the US, mTBI prevalence ranges from 3.6% to 18.3% in children and adolescents alone [11]. The Public Health Agency of Canada reported more than 5 million emergency department (ED) visits for head trauma between 2002 and 2017, 70% (about 3.5 million) of which were due to mTBI [12,13]. In Ontario, about 1% of the adult population or about 150,000 residents are diagnosed with mTBI annually and an excess of about $110 million is spent on mTBI related healthcare [14,15].

These numbers represent only a fraction of mTBIs, as they are highly underestimated, because most mTBIs, being “milder injuries”, are not commonly reported [14,16]. In addition, up to 50% of the patients sustaining mTBI receive an inaccurate diagnosis, especially in the event of multisystem trauma[17,18]. Due to underreporting and inaccurate diagnosis, a vast majority of patients with mTBI do not receive timely and appropriate medical care.Furthermore, mTBI results in persistent symptoms in 13 to 62 percent of patients[13]. At 6 months, functional disability is reported in about 35% of the population with mTBI[19]. To overcome this issue, more advanced techniques are required to diagnose mTBIs accurately and to manage mTBIs appropriately.

1.2. mTBI Diagnostic Criteria:

mTBI is a clinical diagnosis, mostly reliant on the presence of certain subjective symptoms immediately after a head injury. Historically, the most common tool used for mTBI diagnosis is the Glasgow Coma Scale (GCS) score, a calculation based on the level of consciousness. Currently, mTBI is diagnosed using one of many clinical criteria that are highly variable. Despite some common features, many distinct differences exist between these criteria. The current neuroimaging tools, such as CT and MRI, also lack the ability to detect or assess mTBI. Hence, it is difficult to diagnose mTBI accurately.

One of the most widely accepted definitions of mTBI is the American Congress of Rehabilitation Medicine’s (ACRM 1993) criteria [20]. According to the ACRM (1993), mTBI is a physiological disruption of brain function due to trauma, which includes the

head being struck, the head striking an object, and the brain undergoing an acceleration or deceleration movement (i.e., whiplash) without direct external trauma to the head. The disruption of brain function is manifested by at least one of the following: (1) any period of loss of consciousness (LOC) or a Glasgow Coma Scale (GCS) score between 13-15 that does not exceed 30 minutes post-injury; (2) any post-traumatic amnesia (PTA) for events immediately before or after the injury that does not exceed 24 hours; (3) any alteration of mental state at the time of the injury (e.g. feeling dazed, disoriented or confused); and (4) transient or non-transient focal neurologic deficit(s). In addition, the head trauma is accompanied bynormal findings on imaging i.e., computed tomography (CT), magnetic resonance imaging (MRI), electroencephalogram (EEG), and a normal routine neurological evaluation.

Similarly, The Centers for Disease Control and Prevention (CDC) report to Congress proposes a standard conceptual definition of mTBI [21]. It defines mTBI as a head injury due to a blunt trauma or acceleration/deceleration forces that results in one or more of the following: (i) Any period of observed or self-reported: transient confusion, disorientation, impaired consciousness; memory impairment around the time of injury; LOC lasting less than 30 minutes; (ii) Any observed signs of neurological or neuropsychological dysfunction (e.g., seizures acutely following a head injury). This definition also encompasses specific symptoms that are helpful to diagnose mTBI in certain populations i.e., infants, children, and adults, provided there is a LOC or altered consciousness. This conceptual definition of mTBI also provides the basis for three

operational definitions to consistently identify mTBI cases from interviews and surveys, healthcare administrative data sets, and clinical records.

Additionally, the WHO (World Health Organization) Collaborating Centre for Neurotrauma Task Force also provides an operational definition for mTBI diagnosis, that is derived from both the ACRM (1993) and CDC diagnostic criteria [22]. According to WHO, mTBI is an acute brain injury due to mechanical energy from external physical forces. This includes: (i) one or more of the following: confusion or disorientation, LOC for 30 minutes or less, PTA for less than 24 hours, and/or other transient neurological abnormalities such as focal signs, seizure, and intracranial lesion not requiring surgery; (ii) GCS score of 13-15 after 30 minutes post-injury or upon presentation to health care. These manifestations must not be due to drugs, alcohol, medications, other injuries or treatment for other injuries (e.g., systemic injuries, facial injuries or intubation), caused by other problems (e.g., psychological trauma, language barrier or coexisting medical conditions) or caused by penetrating craniocerebral injury.

It is interesting to note that what may constitute an mTBI according to one criterion may not be considered an mTBI according to a different criterion, underscoring the inherent variability in the current subjective diagnostic criteria (Table 1). For example, feeling dazed immediately following a head injury or body blow would constitute an mTBI according to the ACRM (1993) criterion, but not according to the CDC and WHOcriteria. Similarly, only transient symptoms are considered by CDC and

WHO for mTBI diagnosis, whereas the ACRM (1993) also takes permanent focal neurological defects into account [23]. Additionally, the ACRM (1993) requires GCS score to be between 13-15 within 30 minutes post-injury, whereas the WHO also considers GCS scoreof 13-15 at 30 minutes post-injury upon presentation to health care provider [22]. Furthermore, unlike both the ACRM (1993) and CDC, the WHO criterion does not clearly define what constitutes an acute brain injury and does not consider retrograde amnesia a basis for an mTBI diagnosis[24].

	ACRM (1993)	**CDC (2003)**	**WHO (2005)**
Focal Neurological Deficits	Yes (T/P)	Yes (?)	Yes (T)
Loss of Consciousness	Yes	Yes	Yes
Retrograde Amnesia	Yes	Yes	No
Post-traumatic Amnesia < 24 hours	Yes	Yes	Yes
GCS 13-15 (30 mins or later)	Yes	Yes	Yes
Confusion/Disorientation	Yes	Yes	Yes
Dazed	Yes	No	No

Table 1: Comparison of mTBI Diagnostic Criteria

In addition, specific criteria exist for diagnosing concussions/mTBIs that occur inspecific contexts, such as in sports and military populations, further adding to the variability in the diagnostic criteria[25,26]. mTBIs are sometimes also referred to as "concussions", especially in the sports population.There is an ongoing debate whether both these injuries are equivalent. However, for the purpose of this thesis, we will be using the terms "mTBI" and "concussion" interchangeably.It should also be noted that the ACRM recently updated their guidelines in 2023[27]. The new ACRM (2023)criteria is

more inclusive than any listed above. However, this updated criterion was unavailable at the time the research mentioned in this thesis was carried out and published. Hence, for the purpose of this thesis, we will be using the ACRM (1993) criteria.

This prevalence of multiple diagnostic criteria and the discordance between them lead to inaccurate or missed mTBI diagnosis, resulting in underestimation of the incidence of mTBI[18]. Accurate mTBI diagnosis is the first step towards providing timely and appropriate medical care. The need for a standardized mTBI diagnostic tool that overcomes the inconsistency between various prevalent diagnostic criteria is evident.

1.3. Post-Concussion Symptoms:

mTBI usually leads to a combinationof cognitive, emotional, and physical symptoms, collectively referred to as Post-Concussion Symptoms (PCSx).These symptoms include headaches, dizziness, fatigue, irritability, emotional lability, depression and/or anxiety, sleep disturbances, impaired memory and concentration, nausea/vomiting, tinnitus, noise and light sensitivity, emotional lability, and visual changes. In the majority of the patients, these symptoms resolve within days to weeks without any intervention. However, a small subset continues to have persistent symptoms for months to years, sometimes lasting for a lifetime[28–30].

Post-concussive symptoms are also referred to as Post-Concussion Syndrome. The two most common diagnostic criteria used for Post-Concussion Syndrome are WHO

International Statistical Classification of Diseases and Related Health Problems 10th Revision (ICD-10) and the Diagnostic and Statistical Manual of Mental Disorders 4th Edition (DSM-IV) [31,32]. Similar to mTBI diagnostic criteria, there are many overlaps between the two criteria. For instance, both criteria require a significant head trauma and at least three of the various post-concussive symptoms are required for diagnosis. Despite similarities, there are some major differences between the two. For example, the DSM-IV criteriarequire a quantifiable defect in cognitive functioning, however the CD-10 does not. The latest diagnostic criterion for Post-Concussion Syndromeis outlined in the DSM-5 [33]. Despite being the most recent, DSM-5 does not recognize Post-Concussion Syndromeas a specific diagnosis. It rather refers to it as 'Major or Mild Neurocognitive Disorder' based ondecline in cognitive functioning. It does not take into accountany other post-concussive symptoms outlined in either DSM-IV or ICD-10. These conflicting diagnostic criteria lead to challenges with accurate diagnosis of symptoms associated with mTBI.

The word "syndrome" implies that various post-concussive symptoms are tied to a common pathology and that these symptomsalways occur together, which is not always true. Hence, researchers and clinicians are drifting away from using this term.For the purpose of this thesis, we will refer to the symptoms associated with an mTBI as post-concussive symptoms.

1.4. Non-Specific Post-Concussive Symptoms:

The term "Post-Concussive Symptoms"suggests that these symptoms are seen only after a concussion; however, all traumatic head injuries – whether mild, moderate, or severe – have a potential to induce these post-concussive symptoms. As a matter of fact, post-concussion like symptoms arenot exclusive to head injured patients alone as they are highly prevalent in the general population [34–36]. They are also seen in other clinical groups, such as those presenting with post-traumatic stress disorder [37,38], depression [39], anxiety [40], whiplash injuries [41], andchronic pain [42]. Interestingly, similar incidence and prevalence of post-concussive symptoms have been observed in both head injured mTBI and non-head injured controls [43–45]. A prospective longitudinal study by Meares et al. [2008] found a similar incidence rate of acute post-concussive symptoms in mTBI (43.4%) and non-brain injured trauma patients (43.5%) [43]. Similarly, Dean et al. [2011] found no significant difference in the prevalence of post-concussive symptoms in subjects with (31%) or without a head injury (34%), reinforcing the notion of the non-specificity of post-concussive symptoms [44]. There is an ongoing debate over whether post-concussive symptoms are due to a mechanical brain injury itself or other external factors [46].

In addition to non-definitive diagnostic criteria, the non-specificity of symptoms adds to the challenges associated with defining mTBI and the associated post-concussion symptoms.

1.5. Pathophysiology:

The exact pathophysiology that underlies mTBI is unclear. However, the knowledge of metabolic, structural, and inflammatory processes associated with mTBI is rapidly evolving in the last two decades.

mTBI is a mechanical injury that results in stretching and shearing of plasma membranes of central neurons, a process called "mechanoporation" [47]. This leads to potassium (K^+) outflux, causing diffuse membrane depolarization [48,49]. The membrane depolarization triggers the release of excitatory neurotransmitters, such as glutamate, that further promote and maintain the positive feedback loop of membrane depolarization and excitability. Glutamate eventually causes ionic imbalance by promoting K^+efflux via ligand-gated channels and sodium (Na^+) and calcium (Ca^{2+}) influx [50]. In order to restore ionic homeostasis, the ionic pumps go into an "overdrive" state, causing increased glucose utilization and diminishing energy reserves (ATP) [48,51]. This hyperglycolic and hypermetabolic state is noticed immediately (within seconds to minutes) following an mTBI[48].

The hyperglycolic state occurs in the setting of normal or reduced blood flow to the brain, causing an imbalance between the energy demand and supply, leading to an energy crisis [48,52]. In addition, the elevated intracellular Ca^{2+} levels eventually lead to mitochondrial dysfunction [50,51,53,54]. Thus, the initial hyperglycolic state is followed by a hypometabolic state that can persist for days [50]. This is a very vulnerable stage as any secondary trauma to brain at this point can lead to a much worse clinical outcome [55].

The neurometabolic changes are accompanied by microstructural changes within the brain. The mechanical trauma directly damages the microtubules and neurofilaments within the axons [56]. This damage is further exacerbated by elevated intracellular Ca^{2+} levels, disrupting the intra-axonal transport, hence causing beta-amyloid precursor protein (b-APP) accumulation and axonal swelling [50]. Elevated intracellular Ca^{2+} levels also promote proteolytic damage to other cytoskeletal structures [57]. These changes can eventually lead to axonal dysfunction and potential disconnection [51].

In addition, the blood brain barrier (BBB), an intricate capillary system that regulates exchange of blood products between the central nervous system and periphery, is also adversely affected. Despite limited data, evidence of reduced expression of junctional adhesion proteins and increased number of endothelial caveolae has been observed hours to days after TBI [58]. Radiographic evidence of BBB disruption is also observed in milder traumatic brain injuries [59], particularly in athletes with repetitive sub-concussive impacts [60]. As a matter of fact, BBB disruption is observed in 73 % of the patients presenting with post-concussive symptoms [59]. However, this breach in BBB integrity could be restored in days to weeks [61]. Despite evidence of BBB disruption, the extent and timeline of its course in mTBI is still unclear [48].

Neurotrauma also leads to inflammatory changes within the brain triggering neuroinflammation. Neuroinflammation is characterized by upregulation and activation of immune cells and release of both pro- and anti- inflammatory cytokines [62]. Neuro-inflammation is a key secondary injury mechanism responsible for symptoms seen after

TBI [63–65]. Our lab has identified that all major post-concussive symptoms are associated with elevated inflammatory cytokine levels [62]. Initially following the brain trauma, both pro- and anti- inflammatory cytokines work together to achieve cell protection, repair and regeneration. However, prolonged neuroinflammation can be detrimental, and may lead to abnormal brain function, resulting in prolonged symptoms [65].

The above-mentioned changes may result in cell death; however, it does not appear to be as common in mTBI compared to more severe forms of brain injury. Cell death is evident through diffuse volume loss in the limbic system, precuneal cortex and basal ganglia [66–71]. This brain atrophy, however, may be reversible up to one year [71].

1.6. Common Pathology between mTBI and Non-Brain Injured Patients:

With the prevalence of post-concussion-like symptoms seen across many situations that do not involve mechanical head trauma, it is feasible to theorize that there may be a common pathophysiology responsible for similar symptoms seen in both concussed and non-concussed populations.

Neuroinflammationis a key secondary mechanism associated with mTBI. It is also thought to be responsible for sickness-like behaviour seen in systemic infections and musculoskeletal trauma, such as whiplash and post-surgery [72–74]. The neuro-inflammation hypothesis posits whenever a trauma occurs, irrespective of the cause, the body launches an immune response that triggers neuroinflammation [62]. The cytokines produced either in response to brain or bodily insult, transport freely across body and brain [75]. As mentioned

earlier, neuroinflammation initially plays a role in defense and repair; however, its continued hyperexcitability leads to an altered brain function, which typically manifests as post-concussive symptoms [62,65]. The continuum of neuro-inflammation may explain the presence of persistent post-concussive symptoms in both concussed and non-concussed patients.

1.7. Prolonged Recovery and Associated Risks

The post-concussive symptoms observed after mTBI resolve within days or months. However, up to 56 % of patients with mTBI continue to have persistent symptoms that fail to resolve [30,76–78].

Research shows that just the head injury alone does not predict outcome [79]. Rather its complex interactions between various biological, social and psychological factors that shape outcome following mTBI [80–82]. Although this topic remains controversial, it has been shown that a few crucial factors associated with prolonged recovery. These include certain demographic factors such as young age [83,84], female gender [84–86] and years of education [87]. Similarly, certain comorbid conditions such as the history of prior mTBIs [87,88], migraine [89,90], poor sleep [91], mental health problems [80,85,92] andADHD [88,93] are associated with protracted recovery. In addition, certain acute mTBI symptoms such as LOC, PTA, disorientation, mental status change, post-traumatic headaches, fatigue, and low GCS score are predictors of prolonged recovery [84–86,94–96]. An increased number and severity of initial symptoms also predict poor recovery [83,84,86,94,97]. The extent to which

these predictors affect the outcome is presently unclear as the evidence is quite conflicting [84]. However, it's worth mentioning that mental health and psychological distress, both pre- and post- mTBI, appear to be the most robust and strongest predictors of prolonged recovery [98].

Prolonged recovery from mTBI often results in physical, psychosocial and economic strain on patients, resulting in impaired quality of life and increased burden on health care system. Hence it is critical to identify the patients at risk for prolonged recovery and deliver optimal medical care to avoid the associated burdens.

1.8. Aims

Despite significant research, there are many gaps in the understating of mTBI and associated symptoms. The literature presented above shows the challenges associated with precise mTBI diagnosis and defining associated symptoms. It also highlights the fact that post-concussive symptoms are highly non-specific to brain injuries, essentially indistinguishable from those seen after many other conditions. It also pinpoints to the possible common pathology underlying the non-specific symptoms seen across various conditions.

As mentioned earlier, studies have shown no differences in the clinical presentation of patients presenting with post-concussive symptoms, whether seen after a head injury or other conditions. Interestingly, in Dr. Rathbone's Concussion Clinic, it was observed that a subset of the head trauma patient population presents with post-

concussive symptoms despite not meeting the ACRM (1993) criteria for mTBI diagnosis. Their presenting symptoms appeared indistinguishable from those who met the criteria, especially in chronic stages. To define the specific symptomatology associated with mTBI, that sets it apart from sub-threshold head injuries, we conducted a retrospective chart review. We compared the chronic symptomatology between the head-injured patient groups meeting the ACRM (1993) criteria to those who do not (Chapter 2). We also determined the percentage of head-injured patients presenting with chronic post-concussive symptomatology despite not meeting the ACRM(1993) criteriafor mTBI diagnosis (Chapter 2).

As mentioned above, neuroinflammation appears to be a common theme associated with post-concussive symptoms, irrespective of the triggering cause, whether head trauma, systemic injuries, or infections. In the recent years, many studies have been conducted to explore the associations between theinflammatory cytokine levels and mTBI. In Chapter 3, we aimed to systematically review the literature and synthesize the data in form of meta-analysis to identify the common inflammatory cytokines associated with mTBI. Our primary aim is to compare the cytokine levels between the population with mTBI and healthy control groups. The secondary aim of this systematic review is to compare cytokine levels between the population with mTBI and trauma control groups. Finally, the last aim of this systematic review is to explore the associations between elevated post-mTBI cytokines and clinical outcomes and prognosis. This data would potentially help us identifythe cytokines commonly associated with mTBI and their

potential use as markers to monitor prognosis and treatment efficacy in patients with mTBI (Chapter 3).

Both pre- and post-injury mental health problems are among the strongest predictors of prolonged recovery after mTBI [98]. Since both mTBI and mental health problems have strong inflammatory component, we aimed to conduct a systematic review to identify the inflammatory cytokines commonly associated with poor emotional outcome in patients with mTBI (Chapter 4). We also comparedthe cytokines associated with poor emotional outcome in patients with mTBI to those associated with mental health problemsin the absence of head injury to support this association. This study wouldpotentially help usidentify patients at risk of poor emotional outcome, hence protracted recovery, following mTBI.

1.9. Objectives

The specific objectives of this thesis are as follows:

1. To define the specific symptomatology associated with mTBI, that sets it apart from the symptomatology associated with sub-threshold head injuries in thegeneral population.
2. To determine the percentage of head-injured patients presenting to aconcussion specialty clinic with chronic post-concussive symptomatologythat do not meet the ACRM (1993) diagnostic criteria for mTBI.
3. To find differences in the cytokine levels between the population with mTBI and healthy control groups.

4. To find differences in thecytokine levels between the population with mTBI and trauma control groups.

5. To explore the associations between elevated post-mTBI cytokine levels and clinical outcomes and prognosis.

6. To identify the common inflammatory cytokines associated with poor emotional outcome in patients with mTBI.

1.10. Hypotheses

Based on the literature reviewed, hypotheses for each objective are outlined below:

1. We hypothesize that there will be differences in the reported cognitive and emotional symptomatology betweenthe head trauma patients that meet or do not meet the ACRM (1993) criteria, especially in chronic stages.

2. We postulate that a significant proportion of head injured patients presenting with chronic post-concussive symptomswill not meet the ACRM (1993)criteria for mTBI diagnosis.

3. We hypothesize there will be differences in the cytokine levels between the patients with mTBI and healthy control groups.

4. We hypothesize there will be differences in cytokine levels between the patients with mTBI and trauma control groups.

5. We postulate that elevated levels of certain inflammatory blood cytokines following mTBI would correlate with poor outcome and prognosis.

6. We hypothesize that poor emotional outcome in patients with mTBI is associated with elevated levels of inflammatory cytokines that aresimilar to the cytokine elevations observed in patients with emotional symptoms without mTBI.

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Chapter 2

Do Concussed and Non-Concussed Head Trauma Individuals Have Similar Symptoms? A Retrospective Chart Review of Chronic Post-Concussive Symptomatology

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2.1. Abstract

Many patients with head trauma, presenting with prolonged post-concussion symptoms, do not meet the ACRM (1993) diagnostic criteria for mTBI. This population has not been extensively studied and its clinical characteristics are currently uncertain. A retrospective chart review was conducted to explore symptomatic differences between head trauma patients meeting the ACRM (1993) criteria for mTBI diagnosis and those who did not. Patient information was extracted from 161 charts, of which 128 subjects met the ACRM (1993) criteria for mTBI diagnosis (ACRM+PCSx), while 33 did not (non-ACRM+PCSx). These two groups were compared for demographic variables and symptomatology. This study found that 20.5% of subjects presenting with chronic post-concussion symptoms do not meet the ACRM (1993) criteria. No symptom-specific differences were found between the two populations in any of the categories tested. These results show that chronic post-concussion symptoms are similar in both mTBI and non-mTBI head trauma patients in the general population, suggesting a need for further research focusing on this group.

2.2. Introduction

Mild traumatic brain injuries (mTBI) or concussions account for approximately 80-90% of all TBIs [1]. The American Congress of Rehabilitation Medicine (ACRM) 1993 criteria, one of the most commonly used diagnostic criteria for mTBI, defines mTBI as a traumatically induced physiological disruption of brain function, manifested by at least

one of the following: (a) any period of loss of consciousness (LOC), (b) any loss of memory for events immediately before or after the accident, (c) any alteration of mental status (AMS) at the time of the accident (e.g., feeling dazed, disoriented, or confused) or (d) focal neurological deficit(s) that may or may not be transient. Mild traumatic brain injuries are differentiated from more severe TBI with the requirements of LOC to be approximately 30 minutes or less, an initial Glasgow Coma Scale (GCS) of 13–15 assessed after 30 minutes and post-traumatic amnesia (PTA) not greater than 24 hours. Patients who are diagnosed with mTBI may still have negative CT and MRI scans [2]. Although most patients recover from a mTBI within weeks [1,3], a notable proportion of individuals with mTBI (up to 56%) continue to have prolonged symptoms[4–7]. The constellation of physical, cognitive, and emotional symptoms commonly seen after a mTBI are collectively referred to as post-concussion symptoms. If the ICD-10 or DSM-IV criteria are used for these patients, they can be further classified as having "Post-concussion Syndrome" (PCS) or "Post-Concussional Disorder" (PCD) respectively. In this study, we simply compare the post-concussion symptoms (hereafter termed PCSx) and do not use ICD-10 or DSM-IV criteria.

In Hamilton, Ontario, a concussion specialty clinic specializes in the management of patients referred for prolonged post-concussive symptoms. Interestingly, in this clinic it was observed that a subset of the head trauma population reports an array of post-concussive symptoms despite not meeting the ACRM (1993) criteria for mTBI. To date, there is limited research exploring this group of patients from the general population and

their clinical outcomes. This population is often excluded from the studies [8,9] or sometimes grouped together with trauma controls [4,10]. Clinically, this population often goes unnoticed and does not receive appropriate care. This further adds to the challenges of acute identification and management of mTBI populations[11].

We hoped to investigate whether there are any differences between the chronic symptomatology of head injured patients who sustain a mTBI according to the ACRM (1993) criteria (ACRM+PCSx) and those who do not (non-ACRM+PCSx). We hypothesize that there will be differences in the reported symptomatology between the two populations, particularly in cognitive and emotional domains. Our secondary purpose is to determine the percentage of referred patients suffering from chronic post-concussive symptoms who do not meet the ACRM (1993) criteria (non-ACRM+PCSx) for mTBI. We hypothesize a significant proportion of patients with chronic symptoms presenting to the concussion specialty clinic have not sustained a mTBI according to the ACRM (1993) criteria.

2.3. Methodology

2.3.1. Consent and Confidentiality

This study was approved by the Hamilton Integrated Research Ethics Board, through which the waiver of consent was obtained.

2.3.2. Data Collection:

Using convenience sampling, patient records from a concussion clinic in Southern Ontario were retrospectively analyzed. All subjects were referred to the clinic for chronic post-concussion symptoms following a suspected mTBI. Data collection was limited to patient records with an initial consultation between January 1st, 2015, to December 31st, 2017. Data was extracted and recorded onto a data collection form (Appendix A) which included the following: demographic information, medical history, current medication(s), and mTBI-related information (ACRM criteria information, mechanism of injury, other systemic injuries sustained at the time of injury, therapies to treat systemic injuries, and symptoms following injury).

We collected data from charts on thirty-seven different symptoms (Appendix A). We later organized these symptoms using a modified version of Rivermead Post-Concussion Symptoms Questionnaire (RPQ) checklist template (Appendix B) [12]. This modified RPQ checklist had only 15 of the 16 symptoms found in the RPQ as "Frustrated or impatient" was not captured. To ensure that all the included subjects had chronic post-concussive symptoms (PCSx), we required them to have 3 or more symptoms from the checklist listed in Appendix B (not including the "Others" category) and required the initial head injury to precede symptoms by at least 4 weeks. In this study, we focused on post-concussive symptoms (PCSx) in general and did not use DSM-IV or ICD-10 to label patients as having *Post-Concussion Syndrome* (PCS) or*Post-Concussional Disorder (PCD)*.

Following data collection, collection forms were screened according to predefined inclusion and exclusion criteria. Data from subjects who met the eligibility criteria were inputted into two electronic databases (Excel and SPSS), where data auditing and analysis were performed.

2.3.3. Inclusion and Exclusion Criteria:

Participants were included if: (a) the initial consultation date was between January 1st, 2015 to December 31st, 2017 (b) age 19 – 55, (c) trauma occurred under any of the injury conditions specified by the ACRM (1993) (the head being struck; head striking an object; or the brain undergoing an acceleration/ deceleration movement, such as whiplash, without external trauma to the head), (d) presented with at least three or more symptoms (Appendix B excluding "Others"), (e) GCS score of 13- 15, if available,

Participants were excluded for the following: (a) Do not meet one or more of the inclusion requirements, (b) moderate or severe TBIs, (c) insufficient or incomplete information to diagnose or rule out a mTBI, (d) had positive Head CT scan (hemorrhages or abnormalities), if available, (e) positive focal neurological sign due to a secondary pathology, determined by our clinician, (f) had a time delay of <30 days between the injury and initial assessment (to ensure that only chronic PCSx patients were included).

Following screening for the inclusion and exclusion criteria, the participants were later categorized into the ACRM+PCSx and non-ACRM+PCSx groups. Participants in the ACRM group had at least one of the following symptoms immediately following head

trauma: a) LOC less than 30 minutes, b) post-traumatic amnesia (PTA) < 24 hours, c) altered mental status (AMS) evident by the presence (feeling dazed, confused, or disoriented) or presence of focal neurological signs at the time of injury. Thus, subjects in the non-ACRM groups had no LOC, PTA, signs of AMS or focal neurological signs immediately following the head trauma. If sufficient ACRM-related information was not available, charts were excluded from the study. All included participants had at least three chronic symptoms from the modified RPQ checklist listed in Appendix B (not including the "Others" category).

2.3.4. Statistical Analysis

Before data collection, the sample size was calculated using G* Power Software 3.1. A total sample size of n=88 was calculated using a significance level of α=0.05 and power (1- β) = 0.8. Baseline patient demographics were analyzed using descriptive statistics. Quantitative analysis of a descriptor was not performed when more than 20% of data was missing. Data from patients was inputted into two electronic databases (Excel and SPSS), where data auditing and analysis were performed.

Pearson's Chi-square test (χ^2) was used to compare each symptom category between the two groups. Fisher's Exact Test was used only when at least one of the cells in a chi-square contingency table had an expected frequency of less than 5. The uncorrected statistical significancelevel was set at $p<0.05$. No correction for multiple

comparisons was performed. All statistical analyses were performed using IBM SPSS Statistics Version 23.0.

2.4. RESULTS:

2.4.1. Participants:

A total of 508 patient charts were reviewed for eligibility. 161 (31.6%) met inclusion criteria, while 347 (68.3%) patients were excluded. Of the 161 patients included, 128 (79.5%) met the ACRM (1993) criteria while 33 (20.5%) were categorized as non-ACRM patients.

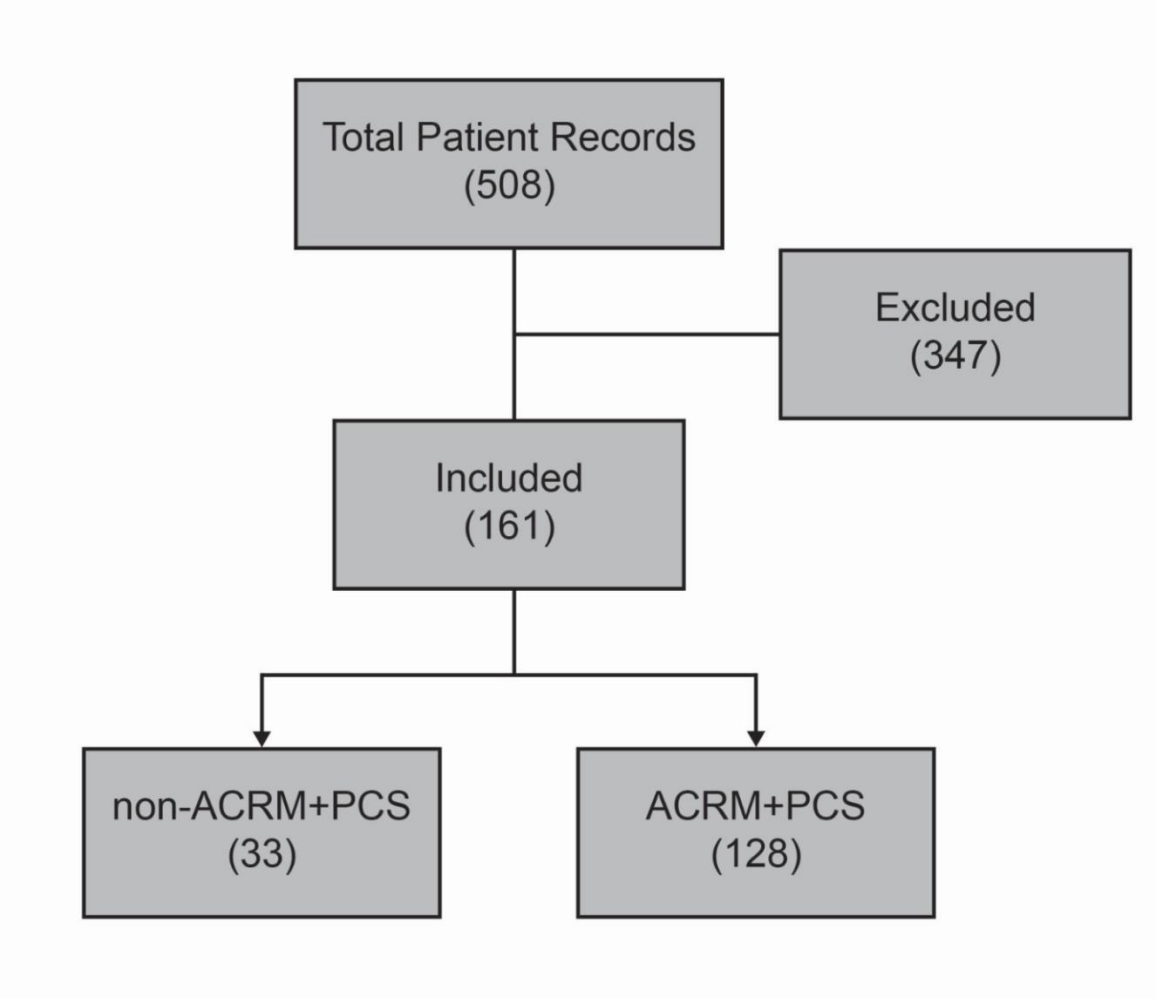

Figure 1. Flow Chart of Patient Selection Process

The most common reasons for exclusion were age at the time of injury (38.0%), insufficient information (34.6%), date of clinical assessment (7.5%), positive CT or MRI scan (5.2%), and no head or body injury (4.6%).

Table 1. Reasons for Exclusion

Reason	No. (%)
Age at the time of injury	132 (38.0)
Insufficient Info	120 (34.6)
Date of Clinical Assessment	26 (7.5)
Positive CT or MRI Scan	18 (5.2)
No head or body injury	16 (4.6)
Less than 3 Symptom Categories	12 (3.4)
Severe Brain Injury	7 (2.0)
Positive Neurofocal Sign unrelated to head injury	5 (1.4)
Clinical Assessment < 30 days from time of injury	4 (1.1)
Repeats	4 (1.1)
Follow up patient	2 (0.6)
Recovered at time of Study	1 (0.3)
	347

2.4.2. Demographic Characteristics

The ACRM+PCSx and non-ACRM+PCSx head trauma groups had no significant difference in age, sex, history of substance abuse, and the duration of delay between the injury and first clinical assessment (Table 2). The differences in education, pre-existing psychiatric conditions, systemic injuries, previous concussions, and the duration of delay between the injury and primary treatment were not assessed due to a large amount of missing data (>20% of any group). The most common mechanism of injury for both groups was motor vehicle accidents. Sports injuries and blunt trauma were more common in the non-ACRM group, whereas falls were more common in the ACRM group.

2. Patient Demographics

Characteristics		**No. (%)**		
		Non-ACRM+PCSx (n=33)	**ACRM+PCSx (n=128)**	**P Value**
Age at time of injury				
	Mean (SD)	37.8 (10.8)	38.0 (11.8)	0.990[a]
Sex				
	Male	9 (27.3)	52 (40.6)	0.159[b]
	Female	24 (72.7)	76 (59.4)	
Highest Education Attained				
	High School	0 (0)	7 (5.5)	
	College	2 (6.1)	8 (6.3)	
	University	6 (18.2)	29 (22.7)	
	Unknown	25 (75.8)	84 (65.6)	
Previous Psychiatric Condition				
	No	1 (3.0)	2 (1.6)	
	Yes	7 (21.2)	39 (30.5)	
	Unknown	25 (75.8)	87 (68.0)	
History of Substance Abuse				
	No	25 (75.8)	83 (64.8)	0.351[b]
	Yes	7 (21.2)	36 (28.1)	
	Unknown	1 (3.0)	9 (7.0)	
Previous Concussions				
	0	0 (0.0)	1 (0.8)	
	1	7 (21.2)	15 (11.7)	
	2	2 (6.1)	4 (3.1)	
	3+	7 (21.2)	23 (18.0)	
	Unknown	17 (51.5)	85 (66.4)	
Duration of Delay Between Injury and Primary Treatment (Days)				
	0-1	13 (39.4)	96 (75.0)	
	2-7	2 (6.1)	16 (12.5)	
	8-14	2 (6.1)	0 (0.0)	
	15-20	0 (0.0)	0 (0.0)	
	>21	0 (0.0)	0 (0.0)	
	Unknown	16 (48.5)	16 (12.5)	
Duration of Delay Between Injury and First Clinical Assessment (Days)				
	28-49	3 (9.1)	13 (10.2)	
	50-99	5 (15.2)	23 (18.0)	0.320[c]
	100-149	6 (18.2)	18 (14.1)	
	150-199	7 (21.2)	12 (9.4)	

	>200	11 (33.3)	60 (46.9)	
	Unknown	1 (3.0)	2 (1.6)	
Systemic Injuries				
	No	9 (27.3)	21 (16.4)	0.252[b]
	Yes	19 (57.6)	77 (60.2)	
	Unknown	5 (15.2)	30 (23.4)	
Mechanism of injury				
	MVA	12 (36.4)	63 (49.2)	0.013[*c]
	Sports	6 (18.2)	9 (7.0)	
	Bike	0 (0.0)	4 (3.1)	
	Falls	2 (6.1)	29 (22.7)	
	Blunt Force	10 (30.3)	15 (11.7)	
	Assault	2 (6.1)	7 (5.5)	
	Other	0 (0)	1 (0.8)	
	Unknown	1 (3.0)	0 (0.0)	
	Blast	0 (0.0)	0 (0.0)	
ACRM Criteria				
	Head Impact or Acceleration or Deceleration injury to head or body			
	No	0 (0.0)	(0.0)	
	Yes	33 (100.0)	128 (100.0)	
	Post Traumatic Amnesia			
	No	33 (100.0)	8 (6.3)	
	Yes	0 (0.0)	65 (50.8)	
	Unknown	0 (0.0)	55 (43.0)	
	Loss of Consciousness			
	No	33 (100.0)	30 (23.4)	
	Yes	0 (0.0)	33 (25.8)	
	Unknown	0 (0.0)	65 (50.8)	
	Altered Mental Status/Focal Neurological Signs			
	No	33 (100.0)	30 (23.4)	
	Yes	0 (0.0)	33 (25.8)	
	Unknown	0 (0.0)	65 (50.8)	

Abbreviations: MVA, Motor Vehicle Accident.
[a]Whitney-Mann test.
[b]Pearson's Chi-Square Test
[c]Fisher-Freeman-Halton Exact Test was used due to an expected frequency <5
* $p < 0.05$

2.4.3. Symptomatic Differences

Headache and poor memory were the most common symptoms in both the ACRM+PCSx and non-ACRM+PCSx head trauma groups. There were no statistically significant differences between the symptomatology of the two groups (Table 3 and Figure 2). Appendix C shows the differences between the original 37 symptoms captured.

Table 3: Symptom Comparison between the ACRM+PCSx and Non-ACRM+PCSx groups.

	No. (%)		
Symptoms	**Non-ACRM+PCSx (n =33)**	**ACRM+PCSx (n=128)**	**P-value**
Physical			
Headache	33 (100.0)	121 (94.5)	0.346[a]
Dizziness	23 (69.7)	101 (78.9)	0.262
Nausea/vomiting	13 (39.4)	49 (38.3)	0.907
Sleep Disturbance	19 (57.6)	68 (53.1)	0.647
Fatigue	14 (42.2)	53 (41.4)	0.916
Blurred Vision	7 (21.2)	32 (25.0)	0.651
Sensitivity to light	24 (72.7)	71 (55.5)	0.072
Double Vision	4 (12.1)	8 (6.3)	0.269[a]
Noise Sensitivity	17 (51.5)	47 (36.7)	0.121
Psychological			
Irritable/ Easily Angered	11 (33.3)	55 (43.0)	0.316
Feeling Depressed	6 (18.2)	41 (32.0)	0.119
Restlessness	8 (24.2)	32 (25.0)	0.928
Cognitive			
Poor memory	24 (72.7)	101 (78.9)	0.447
Poor concentration	20 (60.6)	77 (60.2)	0.962
Taking Longer to Think	1 (3.03)	18 (14.1)	0.127[a]

P-values determined by χ^2 test for association.
[a] Fisher's Exact Test

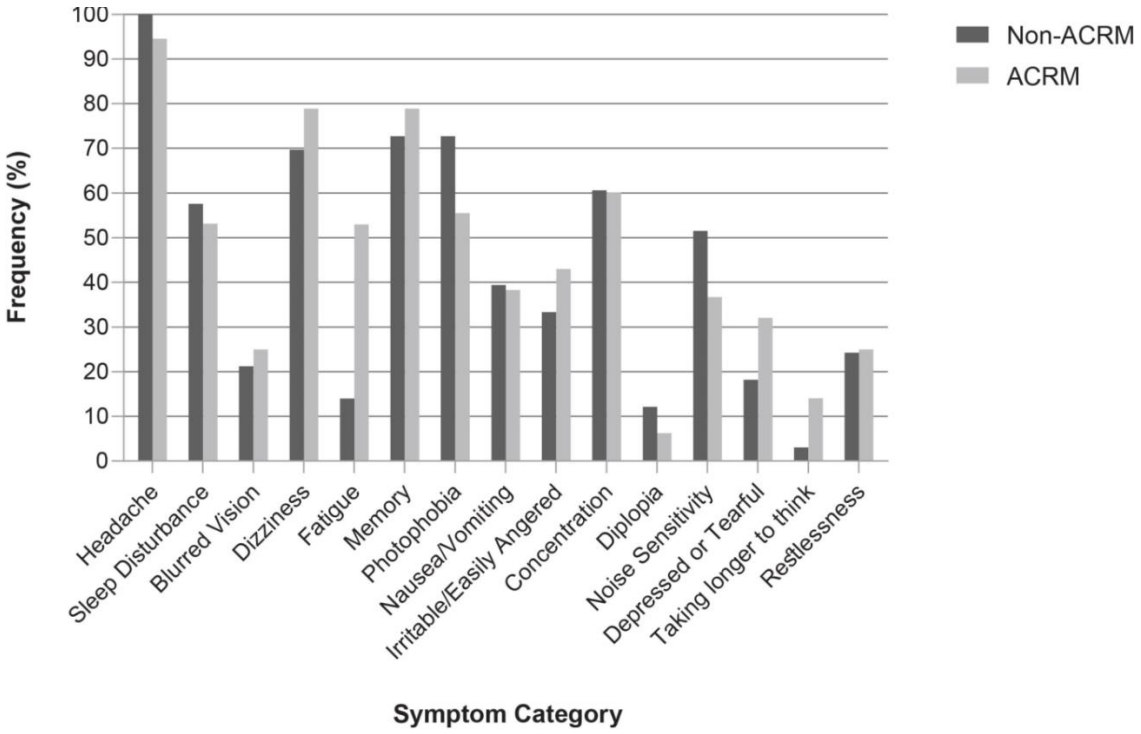

Figure 2. Difference in symptomatology between the ACRM+PCSx and Non-ACRM+PCSx groups.

2.5. Discussion

This study shows that a significant proportion (20.5%) of patients with chronic post-concussive symptoms referred to a concussion clinic did not sustain a mTBI that met the ACRM (1993) criteria using retrospective data. Korley et al.conducted a prospective study recruiting ER patients who had sustained a head injury but did not meet the ACRM (1993) criteria [13]. This group, which they labeled Head Injury but BRainInjury Debatable (HIBRID), is comparable to our non-ACRM+PCSx group. Korley et al. reported a high incidence of post-concussive symptoms in HIBRID patients (32.7%) at 1-month post-

injury[13]. Our study supports these findings and affirms that these post-concussive symptoms can persist in the chronic stages. All patients included in our study were assessed at more than 28 days after injury, with the majority assessed at more than 150 days post injury.

For specific symptomatology, Korley et al. reported that the HIBRID group had a lower incidence of moderate/severe post-concussion symptoms compared to the ACRM groups[13]. Italso had lower incidence of moderate/severe depression compared to the ACRM group,but more than trauma and healthy controlgroups[13]. These results contrastour findings which suggest that there are no significant differences in the symptomatology of the ACRM and non-ACRM head trauma groups. It is possible that the symptomatic differences observed during the acute stages by Korley et al. could have resolved with time. Hence, they were not evident in our study, which included participants at more chronic stages. As a prospective study, Korley et al. were able to use the RPQ to look at the symptom severity and not just the presence or absence of symptoms.

One explanation for the similar symptoms seen in both the ACRM and non-ACRM groups is that they both may have suffered a brain injury.It is possible that the non-ACRM population had sustained a low threshold head trauma, resulting in milder brain injuries. Initially, these milder injuries did not elicit any instantaneous symptoms andthus were not captured by the ACRM (1993) and other current diagnostic criteria[13,14]. Nonetheless, these injuries can produce post-concussive symptoms that are essentially

indistinguishable from those seen after a mTBI that meets the ACRM (1993) criteria. In our study, both groups are similar in their demographic variables except for the mechanism of injury. However, without further details it is difficult to ascertain whether differences in the mechanism of injury indicate a difference in the severity of head injury. Considering this, a need to incorporate more sensitive testing that could detect milder brain injuries, such as advanced imaging techniques or biomarker quantification, becomes evident[14–16]. Early detection and labeling of these sub-threshold mTBIs would help us to provide appropriate care and mitigate long-term consequences.

Another explanation for persisting post-concussion symptoms in both groups is that there may be a common underlying pathophysiology[17]. Neuro-inflammation is a secondary injury mechanism associated with symptoms seen after TBI[18–20]. It is also associated with many post-concussion-like symptoms seen in systemic infections and musculoskeletal trauma, such as whiplash and surgery[21–23]. The neuro-inflammation hypothesis posits that whenever a trauma occurs, irrespective of the cause, the body launches an immune response that triggers neuroinflammation. This neuroinflammation, through altered brain function, maybe responsible for triggering various post-concussion symptoms [17,20]. This could explain the presence of persistent post-concussive-like symptoms in both the non-ACRM and ACRM participants. Despite missing data, a high prevalence of comorbid systemic injuries (ACRM: 60.2%, non-ACRM: 57.6%) was observed in both groups. These systemic injuries maybe responsible for similar symptomology seen in both groups. While neuroinflammation has been seen objectively

in mTBI patients with persistent post-concussion symptoms using single photon-emission computed tomography (SPECT)[24], this has not been investigated in the non-ACRM head injury groups suffering from post-concussion symptoms. This suggests a need to investigate the role of neuroinflammation in post-concussion symptomatology in the non-brain injured population[18].

Development of post-concussive symptoms following trauma also depends on the presence of various pre- and post-injury risk factors besides the head injury itself. Female sex, pain, previous anxiety and affective disorder, acute post-traumatic stress, higher IQ, history of drug or alcohol abuse, previous head trauma, genetics, personality, and environmental factors are associated with the persistent post-concussive symptoms after trauma[4,25–31]. In our study, we were unable to explore these factors in depth due to missing information. However, consistent with the finding that persistent post-concussion symptoms are more common in women, most patients referred for post-concussion symptoms in our study were female (62.1%, n=100). Despite missing data, there was also a high prevalence of previous concussions in both the ACRM (26.08%) and non-ACRM groups (48.4%), which makes both groups potentially more prone to developing similar post-concussion symptoms.To better understand the role of non-ACRM head injuries in causing post-concussion symptoms, future studies may consider comparing patients with no previous history of head trauma.

Considering the role of various injury and non-injury-related factors in shaping the clinical outcomes following trauma, the concept of a single underlying cause seems

inadequate[32,33]. There is a growing acceptance of the "Biopsychosocial Model," which stipulates that the complex interactions between biological, psychological and social factors and various unique injury-related and non-injury-related factors are responsible for persistence of post-concussion symptoms[31,33–35]. Kenzie et al. 2017 and 2018 proposed a model in which four groups of factors interact with each other to affect mTBI injury and recovery[36,37]. These include cellular (e.g., axonal injury, neuroinflammation), network (intrinsic connectivity networks), experiential (e.g., psychological, and cognitive symptoms) and social factors (healthcare access and available social supports). These factors are further influenced by injury biomechanics, injury context and personal characteristics (e.g., genetics, age, sex)[36,37]. Currently, these models provide the most plausible explanation for why the ACRM and other non-ACRMhead injured groups may have similar clinical presentations. Any trauma that triggers one of these scales, regardless of the cause, may give rise to post-concussion symptoms. Similarly, Iverson (2019) proposed the idea of network connectivity[32]. This theory implies that post-concussion symptoms occur together, not because they are all due to a common underlying pathology, but because they are strongly interrelated, each triggering and worsening the next.

2.6. Conclusions

This study found that a significant proportion of head trauma patients present with chronic post-concussion symptoms despite not meeting the ACRM(1993) criteria for

mTBI (20.5%). There is no difference in the chronic symptomatology between the patients who meet the ACRM (1993) criteria and those who do not. Future studies should focus on exploring these populations using emerging techniques that can better detect and evaluate the extent of head injury. They may also consider exploring the role of other factors such as previous mTBIs, psychiatric factors and neuroinflammation in the head trauma population that does not reach the threshold for mTBI.

2.7. Limitations

The conclusions drawn by the study are impacted by certain limitations. In a retrospective chart review, data is prone to omissions, inconsistencies, and the possibility of recall bias during consultations. Moreover, there is also the problem of incomplete or missing data in patient charts. This prevented us from exploring the role that previous mTBI and psychiatric history may play in influencing post-concussion symptoms, as well as how symptoms at the time of injury relate to those present before the head trauma.

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SUPPLEMENTARY MATERIAL:

Appendix A: Data Collection Form

ID_____ IF EXCLUDED, WHY _____ REVIEWER _____

(0=No, 1= Yes. 2=Unknown)

Post-Concussion Syndrome Retrospective Chart Review

If information is not available, please note that it is unavailable on the line.

Demographics & History			
Sex: Male □Female□ NA □ 0=male 1=female		**Age at time of injury:** _____ (years)	
Learning Disabilities: 0=no 1=yes 2=n/i	YES □NO □ _____ (what)	**Highest Attained Education Level:** HS (1) College(2) Uni(3) Other(4) N/A(5)	_____ _____
Previous psychiatric conditions: 0=no 1=yes 2=n/i	YES □NO □ _____ (what)	**Prior Sleep Disorder?** 0=no 1=yes 2=n/a	YES □ NO □ _____ (what)
History of substance abuse: 0=no 1=yes 2=n/a	YES □ NO □ _____ (what)	**Drugs/alcohol intake at time of concussion:** 0=no 1=yes 2=n/i	YES □ NO □ _____ (what)
Neuropsychiatric Assessment? 0=no 1=yes 2=n/i	YES □ NO □ _____	**Previous TBI/concussion? How many?** 0=no/unknown # =1+	YES □ NO □ _____ (How many)

Concussion Related Information			
Date of Injury:	__ __ / __ __ / __ __ __ __ (DD / MMM / YYYY)	**Date of Clinical Assessment (@R)**	__ __ / __ __ / __ __ __ __ (DD / MMM / YYYY)
Duration of delay between injury and primary treatment (days): _____ □ 0-1 (1) □ 2-7 (2) □ 8-14 (3) □ 8-14 (4) □ 15-20 (5) □ >21 (6) □ N/A (7) (Check box)		**Duration of delay between injury and first clinical assessment (days) _____** □ 0-49 (1) □ 50-99 (2) □ 100-149 (3) □ 150-199 (4) □ >200 (5) □ N/A (6) (Check box)	

Head impact? YES □ NO □ UNKNOWN □ 0=no 1=yes 2=unknown	**Post-traumatic amnesia?** YES □ NO □ UNKNOWN □ 0=no 1=yes 2=unknown	**Loss of Consciousness at time of concussion?** YES □ NO □ UNKNOWN □ 0=no 1=yes 2=unknown	**Immediate onset of symptoms?** YES □ NO □ UNKNOWN □ What? _____
Medications? _____ _____ _____ _____ _____		**Category?** **Lipid Regulator** (1) □ **Antidepressants** (2)□ **Narcotics** (3) □ **B-blockers** (4) □ **Ace inhibitors** (5) □ **Migraine meds** (6) □	**Category?** **Diuretics** (7) □ **Anti-convulsant**(8) □ **Hypnotics/sedative**(9)□ **Analgesics** (10)□ **Other** (11)□ .

Post-Concussion Syndrome Retrospective Chart Review

If information is not available, please note that it is unavailable on the line

Mechanism of Injury			
MVA (1) □ _____ (details)	**Sports Inj (2)** □ _____ (details)	**Bike Accident (3)** □ _____ (details)	**Falls (4)** □ _____ (details)
Blast (5) □ _____ (details)	**Blunt Force (6)** □ _____ (details)	**Assault (7)** □ _____ (details)	**Other (8)** □ _____ (details)
Other systemic injuries (What): YES (1) □ NO (2) □ _____ _____			

Symptoms After Injury			
Anxiety or Restless □	**Fatigue/Easily Tired** □	**Mood Changes** □	**Slowed Thinking** □
Back Pain □	**Feel in a Fog** □	**Nausea** □	**Slurred Speech** □
Balance Problems □	**General Body Pain** □	**Neck Pain** □	**Smell Problems** □
Blurred Vision □	**Headache** □	**Noise in Ear/Tinnitus** □	**Stuttering** □
Confusion □	**Hypersomnia** □	**Numbness/Tingling** □	**Trouble With Words** □

Depression/Sadness □	**Impaired [ ]**□	**Personality Change** □	**Vertigo/Spinning** □
Trouble falling asleep □	**Impaired Memory** □	**PTSD** □	**Vomiting** □
Disorientation □	**Insomnia** □	**Pressure in Head** □	**OTHER** □
Dizziness □	**Irritable/Easy Angered**□	**Sensitivity to Light** □	_____
Double Vision □	**Light Headedness** □	**Sensitivity to Noise** □	_____

Office Use
Meets ACRM Criteria □ 0=no 1=yes 2=unknown

Appendix B: Categorization of Symptomatology according to RPQ

Headache o Headache o Head Pressure	**Sleep Disturbance** o Insomnia o Hypersomnia o Trouble falling asleep.	**Irritable/Easily Angered/ Frustrated** o Irritable/Easily Angered/ Frustrated	**Blurred Vision** o Blurred Vision
Dizziness o Dizziness o Light-headed o Vertigo/Spinning o Balance Problems	**Fatigue** o Fatigue	**Memory** o Impaired Memory o Trouble with Words	**Photophobia** o Light sensitivity
Nausea/Vomiting o Nausea o Vomiting	**Restlessness** o Anxiety or Restlessness	**Concentration** o Impaired Concentration o Feel in a fog	**Diplopia** o Double Vision
Noise Sensitivity o Noise Sensitivity	**Depressed or Tearful** o Depressed	**Taking longer to think** o Slowed Thinking	

Other Symptoms (Not Included in RPQ Analysis)

Speech Problems

- Slurred Speech
- Stuttering

Cognitive Changes

- Confusion
- Disorientation

Pain

- Back Pain
- Neck Pain
- General Body Pain

Mood Changes

- Personality Changes
- PTSD (diagnosis)

Tinnitus/ Ringing in ears

-

Smell Problems

-

Numbness/Tingling

Appendix C: Raw Symptom Scores

	No. (%)	
Raw Symptoms	**Non-ACRM+PCS (n =40)**	**ACRM+PCS (n=130)**
Anxiety/restlessness	10 (25.0)	32 (24.6)
Back pain	9 (22.5)	32 (24.6)
Balance	13 (32.5)	38 (29.2)
Blurred vision	9 (22.5)	33 (25.4)
Confusion	2 (5.0)	10 (7.7)
Depression/Sadness	8 (20.0)	41 (31.5)

Trouble falling asleep	7 (17.5)	31 (23.8)
Disorientation	0 (0.0)	4 (3.1)
Dizziness	16 (40.0)	76 (58.5)
Double vision	3 (7.5)	8 (6.2)
Fatigue/Easily Tired	18 (45.0)	53 (40.8)
Feel in a Fog	5 (12.5)	8 (6.2)
General Body Pain	1 (2.5)	2 (1.5)
Headache	38 (95.0)	119 (91.5)
Hypersomnia	2 (5.0)	7 (5.4)
Impaired Concentration	21 (52.5)	72 (55.4)
Impaired Memory	27 (67.5)	93 (71.5)
Insomnia	22 (55.0)	59 (45.4)
Irritable/ Easily Angered	13 (32.5)	54 (41.5)
Lightheaded	5 (12.5)	13 (10.0)
Mood Changes	9 (22.5)	32 (24.6)
Nausea	16 (40.0)	47 (36.2)
Neck pain	23 (57.5)	58 (44.6)
Noise in Ear/Tinnitus	9 (22.5)	27 (20.8)
Numbness /Tingling	8 (20.0)	35 (26.9)
Personality Changes	1 (2.5)	1 (0.8)
PTSD	6 (15.0)	17 (13.1)
Pressure in Head	12 (30.0)	49 (37.7)
Sensitivity to Light	26 (65.0)	71 (54.6)
Sensitivity to Noise	18 (45.0)	35.4 (130)
Slowed Thinking	4 (10.0)	18 (13.8)
Slurred Speech	0 (0.0)	2 (1.5)
Smell Problems	0 (0.0)	3 (2.3)
Stuttering	2 (5.0)	5 (3.8)
Trouble With Words	9 (22.5)	37 (28.5)
Vertigo/Spinning	8 (20.0)	23 (17.7)
Vomiting	1 (2.5)	10 (7.7)

Post-Publication Update:

Following the publication of our study in May 2023, the ACRM updated the criteria for mTBI diagnosis after 30 years[1,2]. This new criterion is more inclusive than the previous criteria and acknowledges the short comings of the ACRM (1993) criteria. ACRM (2023) not only takes immediate signsbut also various post-concussive symptoms into account to make an mTBI diagnosis, provided they occur within a certain timeframe and in combination with certain clinical exam and laboratory findings. In addition, if an individual sustains a head injury and presents with at least two of the specified post-concussive symptoms in the absence of any immediate signs, or clinical exam and or laboratory findings,that person would be labelled as "suspected mTBI". This has changed the interpretation of our study significantly.

According to the ACRM 2023 criteria, our "non-ACRM+PCSx" population and Korley et al (2018) "HiBRID" population is equivalent to "Suspected mTBI" population. It basically means that an mTBI could not be ruled out in this population, hence explaining similar presentation in both these populations. We had mentioned in our study that similar symptomology could be due to shortcomings of the ACRM (1993) criteria, and the new updated criteria supports our views. However, in our opinion, ACRM (1993) is still has some shortcomings e.g., a head injured patient presenting with only one symptom, such as vomiting, would not be considered having sustained an mTBI according to ACRM

(2023)in the absence of any other signs, clinical exam or laboratory or imaging findings. Thus, a need for an objective marker of mTBI diagnosis in inevitable.

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Chapter 3

Inflammatory Cytokines Associated with Mild Traumatic Brain Injury and Clinical Outcome: A Systematic Review and Meta-Analysis

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3.1. ABSTRACT

Mild traumatic brain injuries (mTBIs) trigger a neuroinflammatory response, that leads to perturbations in the inflammatory cytokine levels, resulting in a distinctive profile. A systematic review and meta-analysis were conducted to synthesize the data related to the levels of inflammatory cytokines in patients with mTBI. The electronic databases EMBASE, MEDLINE and PUBMED were searched from January 2014 to December 12, 2021. A total of 5138 articles were screened using a systematic approach based on the PRISMA and R-AMSTAR guidelines. Of these articles, 174 were selected for full-text review and 26 were included in the final analysis. The results of this study demonstrate that within 24 hours, patients with mTBI have significantly higher levels of Interleukin-6 (IL-6), Interleukin-1 Receptor Antagonist (IL-1RA), and Interferon- γ(IFN-γ) in blood, compared to healthy controls in majority of the included studies. Similarly, one week following the injury, patients with mTBI have higher circulatory levels of Monocyte Chemoattractant Protein-1/C-C Motif Chemokine Ligand 2 (MCP-1/CCL2), compared to healthy controls in majority of the included studies. The results of the meta-analysis also confirmed these findings by demonstrating significantly elevated blood levels of IL-6, MCP-1/CCL2, and Interleukin-1 beta (IL-1β) in the population with mTBI compared to healthy controls ($p<0.0001$), particularly in the acute stages (<7 days). Furthermore, it was found that IL-6, Tumor Necrosis Factor-alpha (TNF-α), IL-1RA, IL-10, and MCP-1/CCL2 were associated with poor clinical outcomes following an mTBI. Finally, this research highlights the lack of consensus in the methodology of mTBI studies that

measure inflammatory cytokines in blood and provides direction for future mTBI research.

KEYWORDS: MTBI, Neuroinflammation, Cytokines, Mild Traumatic Brain Injury

3.2. INTRODUCTION

Most traumatic brain injuries are classified as mild traumatic brain injuries (mTBI) or concussions. mTBI induces a variety of symptoms including headaches and other physical, cognitive, and emotional symptoms, commonly referred to as post-concussion symptoms. These symptoms often resolve spontaneously within a few days to months. However, up to 56% of individuals with mTBI either develop prolonged symptoms or do not recover [1–4]. Persistent post-concussive symptoms are usually associated with increased healthcare costs, disability, and reduced quality of life [5–9].

Interestingly, post-concussion like symptoms appear to be nonspecific to mTBIs, as they are also seen in individuals who have sustained other body injuries [10–14]. Following a head injury, a cascade of acute neurochemical, metabolic, and cellular changes are triggered within the brain [15]. Neuroinflammation is a secondary consequence of mTBI that appears to be one of many factors associated with post-concussion symptoms [16,17]. It is observed that post-concussion like symptoms areassociated with inflammatory cytokines independent of head injuries [18]. For example, headache, one of the most common post-concussion symptoms, is associated with elevated levels of Tumor Necrosis Factor-alpha (TNF-α), Interleukin-1 beta (IL-1β) and Interleukin-10 (IL-10)

[19,20]. Similarly, depression is associated with elevated C-Reactive Protein (CRP) and Interleukin-6 (IL-6) levels, while anxiety is associated with an increase in CRP, TNF-α, and Interferon- γ(IFN-γ) levels[21–23]. The same holds true for chronic subjective dizziness which is associated with elevated TNF-α and IFN-γ levels [24]. Furthermore, IL-1βis associated with benign paroxysmal positional vertigo (BPPV) [25]. This indicates that inflammation is associated with various post-concussion like symptoms, irrespective of the triggering cause.

To establish an association between the inflammatory cytokines and mTBI-related symptoms, the best approach one can take is to measure cytokine levels intracranially.Since mTBI is a minor injury with no signs of obvious trauma on routine imaging, it is not feasible to undertake a lumbar puncture to measure intracranial cytokine levels. To overcome this issue, many studies attempting to study inflammation in mTBI measure cytokine levels peripherally in blood. However, as mentioned earlier, inflammation is not exclusive to mTBI, so while measuring cytokine levels peripherally is convenient, it can present issues in differentiating the source of inflammation. Hence, it is important to characterize the inflammatory cytokine profile that is unique to patients with mTBI, both in blood and CSF.

A systematic review and meta-analysis were conducted to examine and analyze the evidence presented from clinical studies linking mTBI with various inflammatory cytokines, both in blood and CSF. Our primary aim is to compare the inflammatory

cytokine levels between populations with mTBI and healthy control (HC) groups. The secondary aim is to compare the inflammatory cytokine levels between the population with mTBI and trauma control (TC) groups. Finally, the last aim is to explore the associations between post-mTBI inflammatory cytokine levels and clinical outcomes and prognosis. This research would help us identify the inflammatory cytokine profile exclusive to mTBI, that sets it apart from healthy controls as well as trauma controls. In addition, this research would help us identify the inflammatory cytokines that have the most potential to be used as prognostic mTBI biomarkers.

3.3. Methodology

3.3.1. Search Strategy

A systematic screening approach in fulfillment with Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) and the Revised Assessment of Multiple Systematic Reviews (R-AMSTAR) guidelines was implemented[26]. Potentially eligible studies were identified by systematically searching the databases PUBMED, EMBASE and MEDLINE. The searches were limited to the literature published from January 2014 to December 12th, 2021. Studies published prior to January 2014 were discussed in our previous study [18]. The search strategy was developed using combinations of the following MeSH terms: (“mild traumatic brain injur*” OR “concussion”) AND (“neuroinflammat*” OR “cytokine*”). A secondary manual search, using Google Scholar, was also conducted to ensure that all relevant articles were captured.

3.3.2. Study Screening

Citations were uploaded into Covidence for title, abstract, and full-text screening as well as duplicate removal. Two independent reviewers conducted the study screening in duplicate, from title to full-text screening stages (SM, OA). Disagreements regarding article inclusion were settled by mutual consensus after discussing the disputed articles together. Any further discrepancies were discussed with other team members and eventually resolved by the principal investigator (TG, MPR).

3.3.3. Selection Criteria

Only studies providing information on inflammatory cytokines in the CSF, blood, plasma, or serum of the patients with mTBI were considered for review. The research question and inclusion/exclusion criteria were established a priori. Inclusion criteria were defined as: (1) concussions or mild traumatic brain injuries; (2) neuroinflammation; (3) inflammatory cytokines; (4) articles published in English. Exclusion criteria were defined as: (1) complicated mild and more severe forms of traumatic brain injuries with Glasgow Coma Scale (GCS) < 13; (2) no blood or CSF cytokines; (3) no comparison healthy or trauma controls or baseline (pre-mTBI) groups; (4) review articles or abstracts or letter to editors; (5) cadaver/non-human studies; and (6)articles that included TBIs but did not make distinctions between various types of TBIs. Reference lists of related studies were also searched for additional reports.

3.3.4. Data Extraction

Two independent reviewers (SM, MM) abstracted the relevant data from the included articles on an Excel sheet. The characteristics extracted from each study included the author, publication year, study design, sample size, mTBI setting, mTBI diagnostic criteria, control type, and patient demographics (e.g., age, sex, etc.). Details about the number of previous mTBIs, time since last mTBI, and GCS data were also recorded. Furthermore, data regarding methods of cytokine measurement, type of biospecimen analyzed, the time interval between injury and cytokine measurement, cytokine levels (both mTBI and control groups), along with any relevant p-values, were recorded. In addition, any acute or chronic functional outcomes associated with a particular cytokine were noted. This included the presence of persistent symptoms, reduced or lack of return to normal activities (work, school, sports), abnormal neurocognitive function, and Glasgow Outcome Scores (GOSE).

To conduct the meta-analysis, the mean cytokine concentrations, and their standard deviations (SD) for case and control groups were extracted at each follow-up visit. The timing of cytokine concentration measurement varied from <24 hours to >1 month. Other descriptive statistics such as medians and measures of variance (e.g., 95% confidence intervals [CI] and range) were also extracted. Efforts were made to contact the authors of studies that presented their data as either medians only, or in a log-transformed format only, to ensure that all possible mean values were available to conduct a thorough

meta-analysis. Data from studies that had overlapping populations was extracted but a distinction was made in the analysis.

3.3.5. Reporting Quality

Risk of bias and study quality were evaluated using the Newcastle-Ottawa Quality Assessment Scale (NOS) [27]. A score of 0 or 1 was given for each category/criterion on the NOS scale, where the maximum possible score of 8/8 could be achieved (maximum score of 1 for each category). The total scores were categorized according to the methodological quality of each study. Potential confounding factors (including type of biospecimen, assay type, time since mTBI, type of mTBI population and control for confounding inflammatory variables etc.) were also considered for a more detailed bias and quality analysis of studies.

3.3.6. Data Analysis

Review Manager (RevMan) Version 5.4 (The Cochrane Collaboration, 2020) was used for the meta-analysis. Meta-analyses were conducted whenever the mean values of an inflammatory cytokine were available in at least three or more studies, with a minimum of 30 participants in each study. For the studies that did not report SD, it was calculated from SEM (Standard error of mean) and CI 95% (Confidence Interval 95%) using the following formulas:

$$\boldsymbol{SEM} = \frac{\boldsymbol{Upper\ limit\ of\ CI}\ 95\% - \boldsymbol{Lower\ limit\ of CI}\ 95\%}{\mathbf{3.92}}$$

$$\boldsymbol{SD} = \boldsymbol{SEM} \times \sqrt{\boldsymbol{n}}(\boldsymbol{number\ of\ participants}$$

Due to a limited number of studies that qualified for the meta-analysis, only four analyses were conducted comparing meanTNF-α, IL-6, IL-1β and MCP-1/CCL2 levels in serum/plasma/blood between the patients with mTBI and healthy control groups. A high level of heterogeneity was expected due to the utilization of different assay methods (i.e., Multiplex and ELISA), time of cytokine measurement, control for inflammatory variables, and different blood fractions and dilutions used. To account for this heterogeneity, a random effects model and inverse variance approach were utilized to estimate the pooled standardized (Std.) mean differences, their corresponding 95% confidence interval, and p-values. The Std. mean difference is used when the included studies measure the same outcome in different ways. It standardizes the differences in the measurement of the same outcome before pooling the means. It does not however remove the heterogeneity among the study population. Random effects models are preferable if significant heterogeneity is expected as this model accounts for both within-study variability and between-study variability. Heterogeneity was tested using Cochrane's Q test with the p-value set at 0.1 for significance and quantified using the I^2 statistic (I^2>40% as low, 40–60% as moderate, and >60% as substantial heterogeneity). The sources of heterogeneity were evaluated and the risk of bias across studies, publication bias, and selective reporting were assessed. Sensitivity analyses were conducted by

excluding the studies in which mean and standard deviation were estimated (from reported standard deviations and range values) to assess the consistency of the estimated mean differences. If multiple studies conducted by the same group had overlapping populations, only the most recent study with the largest mTBI population size was included in the meta-analysis. If cytokines were measured at different time points, the time-point with the largest population size was used for the total cytokine analysis. For acute and chronic cytokine analysis, the most time-appropriate data consistent with the timings of the other studies was used.

3.4. Results

3.4.1 Study Characteristics:

A total of 5138 studies were yielded across the three databases Embase, 139 from Medline, 662 articles from PubMed, and 7 from other sources. After removing duplicates, a systematic screening process was conducted as shown in Figure 1, yielding a total of 26 articles that met the selection criteria (Figure 1). Out of 26 studies, 25 compared blood cytokine levels between patients with mTBI and healthy controls and 3 studies compared the levels between patients with mTBI and trauma controls (some studies had both control types). Only one study compared cytokine level differences in the CSF. This CSF data was not included in the qualitative or quantitative analysis. The characteristics of all the included studies are described in Table 1.

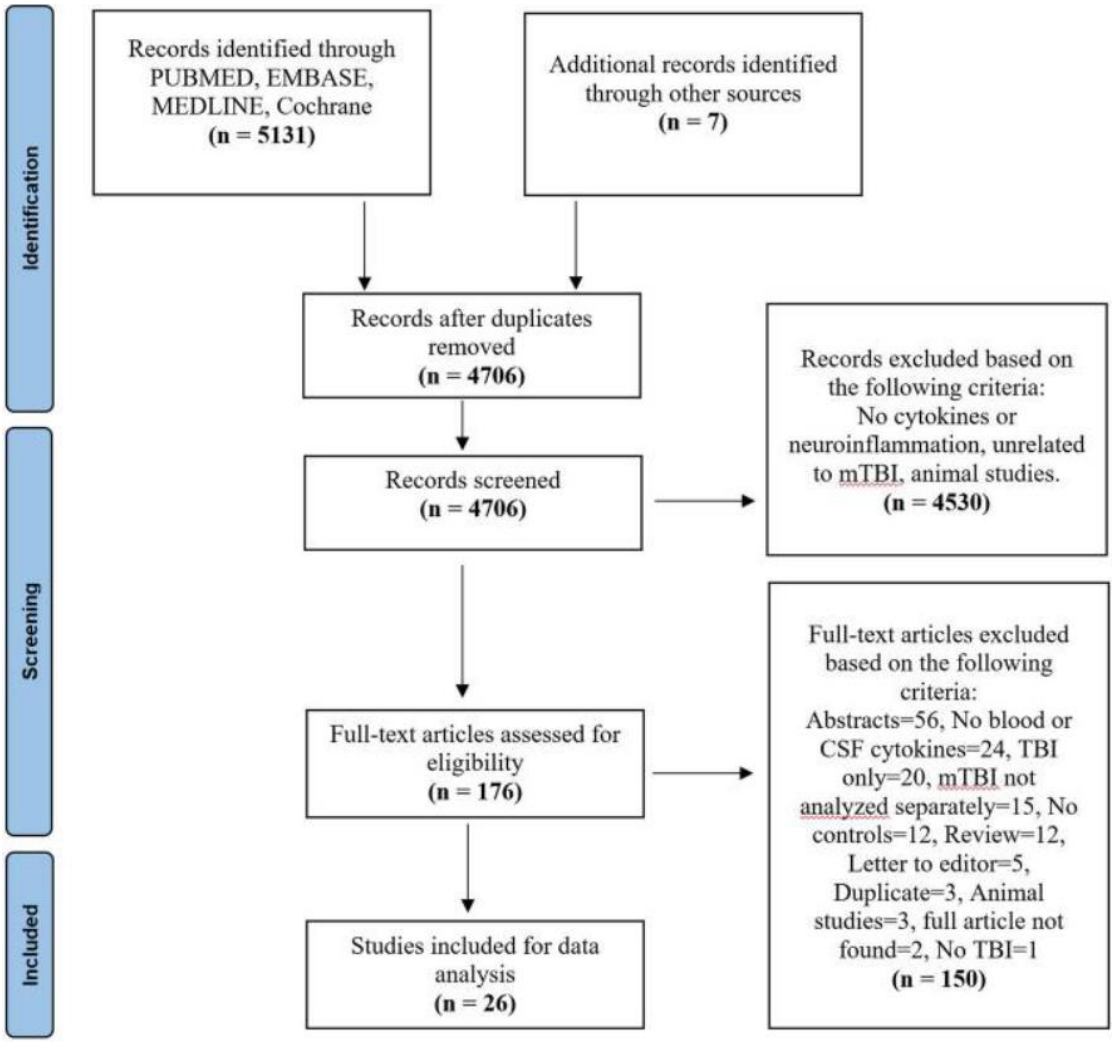


Figure 1. Prisma Flow Chart

Table 1. Study Characteristics

Author Year	Population	Biomarkers Tested	Biomarker Assessment	Specimen Used	Sig. Data	Time (Acute/Chronic)	mTBI Dx/ Setting	Variable Control	Prognosis/Outcome
Shan 2016	mTBI=55 TC=17 HC=44	TNF-α, IL-1β, CXCL1, CXCL8, and CCL2	ELISA (R&D Systems, USA)	Plasma	None of the biomarkers selected have a sig. difference between the groups.	Acute mTBI (1-8) hours mTBI (9-24) hours OI < 24 hours	Zurich 2012/ General Trauma	Yes	NR
Meier 2020	mTBI=106 HC=134	IL-6, IL-1RA, and CRP	Multiplex (Meso Scale Diagnostics, USA)	Serum	IL-6, IL-1RA, and CRP are sig. elevated at < 6 hrs. in mTBI groups compared to healthy (p=0.001) IL-1RA is sig. elevated at 24-48 hrs. in mTBI groups compared to healthy (p<0.05).	Acute Pre-injury baseline Within 6 hours 24 to 48 hours	CDC/ SRC	Partial	Elevated IL-1RA (p=0.03) and IL-6 (p=0.08) ass. with symptom duration. .
Nitta 2019	mTBI=40 HC=43	IL-6, IL-1β, IL-1RA, IL-10, TNF-α, CRP, and IFN-γ	Multiplex (Meso Scale Diagnostics, USA)	Serum	IL-1RA and IL-6 levels at 6 hr. visit are sig. higher in athletes with mTBI (p < 0.001).	Acute Baseline (6 hrs.) 24–48 hrs. Day 8, 15 and 45	CDC/ SRC	Partial	IL-6 levels at 6 hours ass. with the duration of symptoms (p = 0.031).
Feng 2018	mTBI=16 HC=11	TNF-α	ELISA (Biotech Co., China)	Plasma CSF	Plasma TNF-α is sig. higher in mTBI compared to controls (p=0.009) Day 3 plasma TNF-α is sig. higher than day 1, 5, and 7 (P<0.05) CSF TNF-α levels higher (non-sig) in mTBI patients at Day 3	Acute Days 1,3,5 and 7	GCS/ General Trauma	Yes	NR
Goetzl 2019	mTBI=32 HC=21	IL-6	ELISA (R&D Systems, USA).	Plasma NDE levels	IL-6 sig. increased in both acute (P<0.0001) and chronic (P<0.1) mTBI compared to controls.	Acute: 7 days Chronic: 3-12 months	NCAA/ SRC	No	NR

Di Battista 2020	mTBI=41 HC=55	IL-6	Ella™ (Protein Simple, Biotechne, USA).	Plasma	No sig. results (differences).	Acute <7 days	Berlin 2016/ SRC	No	No sig. correlation between IL-6 and either symptom burden or days to medical clearance (p>0.05).
Tylicka 2020	mTBI=29 HC=13	IL-8, IL-11	ELISA (R&D Systems, UK).	Plasma	IL-8 sig. higher in mTBI (p=0.033)	Acute 2-6 hours	GCS/ General Trauma	Yes	NR
Rusiecki 2020	mTBI=90 HC=50	IL-1α, IL1β, IL4, IL-6, IL8, IL-10, TNFα, MCP-1, IL-13, IL-17, TNF-β	Multiplex (Ray Biotech, USA)	Serum	Controls' cytokine levels are greater than cases' for IL-6 (p = 0.02), IL-8 (p = 0.01) and IL-1β (p = 0.05)	Chronic 281.8 days (mean)	DoD-VA criteria/ Military	Partial	Decreased IL-8 levels ass. with PTSD (p=0.01)
Begum 2020	mTBI=23 HC=12	92 cytokines	Multiplex (Olink Biosciences, Sweden)	Serum	IL-7 levels sig. increased in mTBI (P < 0.05) MCP-1 was sig. reduced in mTBI at > 1 week (P=0.03) CXCL1 was sig. increased in mTBI at < 1 week (P=0.02)	Acute: 2–5 days Chronic: 15–75 days	ACRM/ SRC	Partial	Reduced MCP-1 levels relate to an increase in the number(r = 0.455, p = 0.013) and severity of symptoms (r = −0.378, p = 0.043).
Vedanta-m 2020	mTBI=53 TC=12	IL-1β, IL-2, IL-4, IL-6, IL-10, IL12p70, IL-17a, IFNγ, TNFα	Luminex Magpix (Luminex, USA)	Plasma	Sig. elevated IL-2 (p=0.014) and IL-6 (p=0.01) levels in mTBI within 24 hours post-injury. Sig. elevation in IL-6 (p=0.044) at 6 months post-injury in mTBI.	Acute: < 24 hrs. Chronic: 6 months	ACRM/ General Trauma	No	At 24 hrs, elevated IL-2 (p=0.001) and lower IL-6 (p = 0.035) and IL-17-a levels (p = 0.007) ass. with severe PCS at 1 week (p = 0.001. At 6 months, elevated IL-10 ass. with depression (p=0.004) and PTSD (p=0.001).
O'Brien 2020	mTBI=58 HC=47	IL-1β and IL-18	Simoa (Quanterix, MA)	Serum	No sig. results (differences).	Acute:Baseline, 2, 6 and 13 days	NR/ SRC	Partial	NR

Sun 2019	mTBI=95 HC=54	CCL2, IL-1β, IL-4, IL-6, IL-8, IL-10, IL-12, IFN-γ, TNF-α	Multiplex (Luminex, USA)	Serum	CCL2, IL-1β, and IL-6 levels (acute) higher in mTBI at all time points compared to HC ($p < 0.001$), except IL-1β at 3 months time-point.	Acute and Chronic Cohort 1: Within 7 days post injury, 1 month, 3 months Cohort 2: Within 7 days post injury	WHO/ General Trauma	Yes	Elevated CCL2 level ass. with more severe PCS ($p < 0.001$) and predicted information processing speed at 3 months ($p=0.009$). IL-1β is negatively ass. with working memory in acute phase ($p < 0.001$) and positively in chronic phase ($p=0.015$).
Di Battista 2019	mTBI=42 TC =30 HC=102	IFN-γ, IL-8, TNF-α, MCP-1, MCP-4, MCP-1β, MIP-1α.	Multiplex (Meso Scale Diagnostics, USA)	Plasma Supernatant	Patients with mTBI have higher levels of MCP-4 ($p < 0.001$) and MIP-1β ($p = 0.001$) compared to HC.	Acute 2-7 days	Berlin 2016/ SRC	No	MCP-1 ($p = 0.007$) and MCP-4 ($p < 0.001$) positively correlate with days to recovery in mTBI patients.
Guedes 2020	mTBI=150 HC=45	TNF-α, IL-6, IL-10,	Simoa (Quanterix, Lexington, MA)	Plasma	No sig. differences in the plasma or exosomal concentrations of any biomarker.	Chronic 6.83 -9.53 years (median)	DoD–VA/ Military	No	PCS severity correlate with plasma TNF-α ($r = −0.2328$, $p = 0.02$). PTSD correlated weakly with plasma TNF-α ($r = −0.2267$, $p = 0.0255$). A marginally sig. correlation between PTSD and exosomal IL-6 ($r = 0.1893$, $p = 0.08$).
Gill 2018	mTBI=42 HC=22	TNFα, IL-6, IL-10	Simoa (Quanterix, Lexington, MA)	NDEs from blood	mTBI has elevated concentrations of IL-10 ($p< 0.05$).	Chronic >3 months	WARC AT/ Military	Yes	Exosomal IL-10 levels are related to PTSD symptoms ($B=0.8$, $t=2.60$, $p < 0.01$). IL-10 regression model ($p < 0.01$) shows PTSD sig. related ($p < 0.01$) and depression ($p = 0.063$) and PCS severity

									do not relate to exosomal IL-10 (p = 0.26).
Thompson 2020	mTBI=171 HC=122	IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12, IFN-γ and TNF-α	Multiplex (Bio-Rad, USA)	Plasma	Within 24 hours of injury, concentrations of IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12, IFN-γ, and TNF-α were sig. elevated in mTBI. At 1 month, TNF-α, Il-7 and IL-8 levels were sig. elevated in mTBI. At 6 months, TNF-α, IL-7, IL-8 and IL-12 were sig. elevated in mTBI. These comparisons are for ages 21-54.	Acute: <24 hrs. Chronic: 1 and 6 months.	CDC/ General Trauma	Yes	NR
Edwards 2020	mTBI=45 HC=49	IL-6, IL-10, and TNF-α	Simoa (Quanterix, Lexington, MA)	Serum	At < 8 hrs. IL-6 levels in mTBI are greater than HC ($p < 0.001$). No sig. differences at the second time point.	Acute < 8 hrs and 24 hrs later	DoD–VA/ Military	Yes	NR
Kanefs-ky 2019	mTBI=61 HC=82	TNF-α, IL-6 and IL-10	Simoa (Quanterix, Lexington, MA)	Plasma	IL-6 elevated in the mTBI w LOC group compared to both the mTBI w/out LOC and control groups ($p < 0.001$ for both comparisons).	NR	WARC AT/ Military	Yes	Increased TNF-α in mTBI ass. with severe PTSD ($r = 0.36$, $p = 0.005$). mTBIs with LOC are ass. with elevated IL-6 levels and pain, compared to mTBI without LOC and HC.
Brahma-jothi 2020	mTBI=5 HC=5	TNF-α, IL-6,	ELISA (R&D Systems, USA)	Plasma	TNF-α and IL6 sig. elevated in chronic stages, but not acute ($p < 0.0001$).	Acute: Baseline Chronic: 1-2 yrs.	NR/ Military	No	NR
Powell 2020	mTBI=55 HC=49	IL-6	ELISA (ALPCO Diagnostics, USA)	Venous Blood	No sig. results (differences).	Chronic 1+ yrs.	Self reported/ Military	No	NR
Bai 2020	mTBI=112 HC=72	IL-6, CCL2, IL-1B	Multiplex (Luminex, USA)	Serum	IL-1β, IL-6, and CCL2 acutely elevated in mTBI relative to HC (all for $p < .001$).	Acute <7 days	WHO/ General Trauma	Partial	NR

Brett 2020	mTBI=73 HC=128	IL-6, IL-1RA, and CRP	Multiplex (Meso Scale Diagnostics, USA)	Serum	No sig. results (differences).	NR	DoD-VA/ SRC	Partial	Sig. interaction between prior mTBI and IL-1RA levels on the ImPACTMemory Composite, p = 0.044. At low levels of IL-1RA, athletes with multiple mTBI had worse memory performance than those without prior mTBI (p = 0.014). Higher IL-1RA levels sig. ass. with more symptoms (elevated BSI-GSI scores, p=0.046) and worse memory (p=0.017).
Chaban 2020	mTBI=207 HC=207	IFN-g, IL-8, IL-9, TNF-α, IL-1RA, MCP-1	Multi-plex (Bio-Rad, USA)	Plasma	IFN-g, IL-8, IL-17A, IL-9, MCP-1 and TNF-α were sig. higher in mTBI than HC at all time points.	Acute: <72 hrs. and 2 weeks. Chronic: 3 and 12 months.	WHO/ General Trauma	Yes	NR
Di Battista 2018	mTBI=16 HC=27	MCP-1, MCP-4	Multiplex (Meso Scale Diagnostics, USA)	Venous blood	MCP-1 and MCP-4 were elevated in acute mTBI.	Acute <7 days	NR/ SRC	Partial	NR
Ryan 2021	mTBI=104 HC=98	IL-2, IL-4, IL-6, IL-8, IL-10, IL-17A, IFN-γ TNF-α	Multiplex (Meso Scale Diagnostics, USA)	Peripheral blood plasma	IL-6 and IL-1RA asig. elevated in mTBI (p < 0.005). IL-8, IL-10, IL-17A, TNF-α sig. reduced (p-value < 0.0001) in mTBI.	Acute: Less than 24 hours	GCS 14-15/ SRC	Yes	NR
Meier 2021	mTBI=23 HC=47	IL-6, IL-10, IL-1β, IL-	Multiplex (Meso Scale	Serum	Serum IL-6 and IL-1RA levels sig. elevated in mTBI relative to baseline	Acute Pre-injury	CDC/ SRC	Partial	

1RA and TNF-α	Diagnostics, USA)		levels ($p<0.05$).	baseline Within 6 hours

A total of 3248 participants were included across all studies, where 1746 of these patients had at least one mTBI. There were 1502 controls, 1431 of which were healthy controls, and 71 were trauma controls. The mean sample size of patients with at least one mTBI was 67.15 ± 50.11 whereas, the mean sample size for controls was 57.24 ± 39.02. The mean age of patients with at least one mTBI was 27.37 years, and the mean age of control patients was 27.14 years. 74.5% of mTBI patients were male. Sport-related injuries were the most common source of mTBI in the majority of included studies (42%), followed by general trauma (31%), and military-related injuries (27%). The diagnostic criteria used for mTBI diagnosis was heterogenous.

3.4.2. Study Quality

Most studies in this review have a level of evidence of IV (N = 14; 53.8%). There was substantial agreement between the two reviewers at the title/abstract screening stage (κ = 0.80 [95%CI, 0.70 to 0.90]) and the full-text screening stage (κ = 0.79 [95%CI, 0.60 to 0.90]. The mean NOS score for the included studies was 6.38 ± 1.19, which indicates a fair quality of evidence for non-randomized studies. The areas of best performance based on the NOS checklist were the case definition (N = 25; 96%) and the definition of controls (N = 25; 96%). The area of worst performance was the non-response rate (the number of patients that were lost to follow-up), which was not provided in any of the included studies.

3.4.3. mTBI vs. Healthy Controls: Qualitative Review of Blood Inflammatory Cytokines

The most common blood inflammatory cytokines assessed in the included studies were IL-6, TNF-α, IL-10, IL-1β, Interleukin-8 (IL-8), IFN-γ, Interleukin-1 Receptor Antagonist (IL-1RA), Interleukin 4 (IL-4), and MCP-1/CCL2 (Figure 6). It should be noted that MCP-1 is also referred to as CCL-2 and both these terms are used interchangeably. Most of the included studies extracted peripheral inflammatory cytokine specimens from plasma (46%, n = 12). The remaining studies extracted inflammatory cytokines from either serum (38 %, n = 10) or whole blood (15 %, n = 4). About 42% of the studies (n = 11), assessed cytokine levels within 24 hours of injury. However, most studies (53.8%, n = 14) assessed cytokine levels 30 days or later following a mTBI. The systematic review found elevated levels of IL-6 (time points: <24 hrs., 1-7 days and≥30 days), TNF-α (≥30 days), IL-1β(1-7 days), IL-8 (time points: <24 hrs. and ≥30 days), IFN-γ (<24 hrs.), IL-1RA (<24 hrs.), and MCP-1/CCL2 (time points: 1-7 days and≥30 days) in patients with mTBI, compared with healthy controls, where any significant findings were replicated in at least two studies. The evidence was particularly strong for IL-6, IFN-γ, IL-1RA levels (at <24 hrs.) and MCP-1/CCL2 (between 1-7 days), where ≥ 60 % of the studies found significant elevated levels in patients with mTBI compared to healthy controls at these time points.

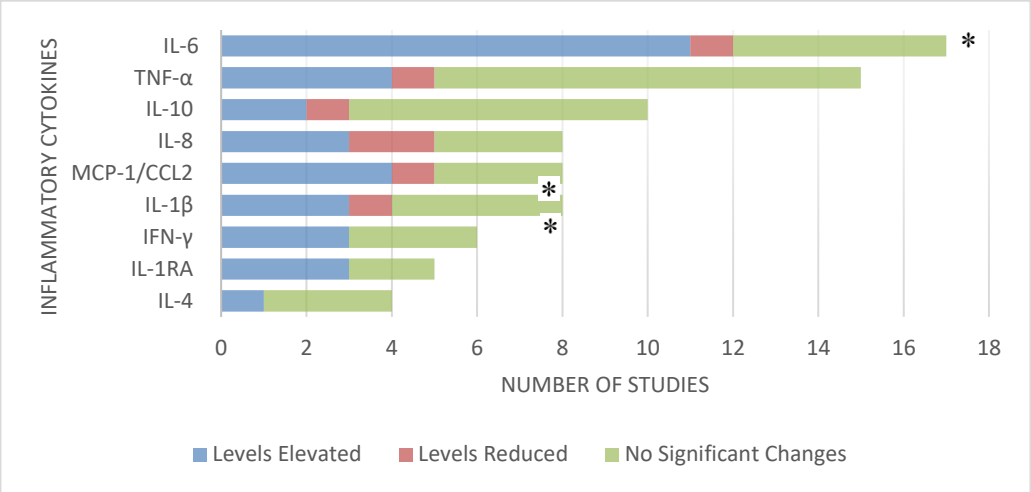


Figure 6. Summary of Results for mTBI vs. Healthy Controls* *Meta-analysis demonstrated acutely elevated levels of the respective cytokines in patients with mTBI.*

There are four groups of research articles that had subject overlap, where the participant pools were utilized multiple times by researchers in the same group. These groups are labeled as Groups A, B, C, and D. Group A published four of the included studies [28–31], Group B published two studies [32,33], Group C published four studies [34–37], and finally Group D published three articles [38–40]. For this review, clear distinctions were made if two or more studies had overlapping populations at a certain time point. This is to avoid any potential impact on the statistical analysis and the results of this review, caused by falsely giving undue weight to a certain study population.

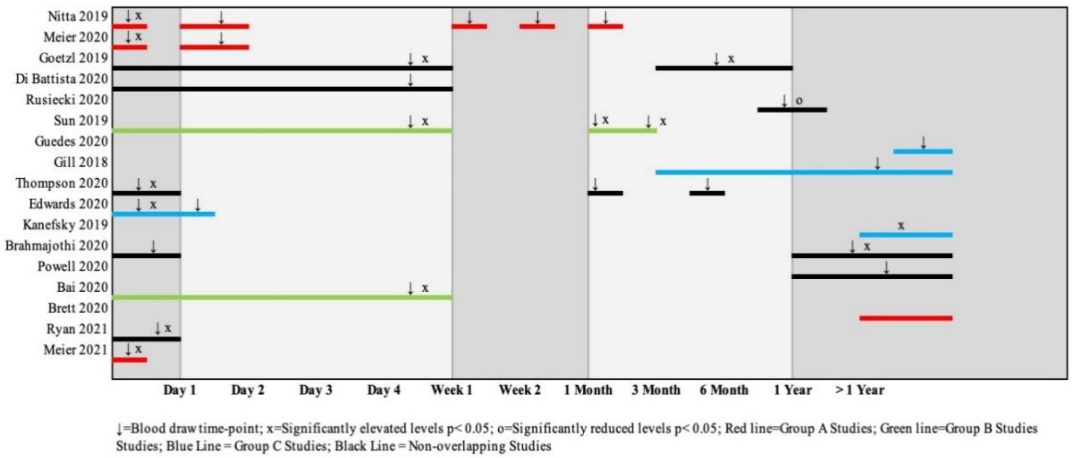
IL-6:

Blood IL-6 levels were assessed in 65% of the included studies (n = 17/26) [28–37,40–46] (Supplementary Figure 1). Out of these, most of the studies (65%, n = 11/17)

showed significantly elevated IL-6 levels in patients with mTBI at a minimum of one time-point compared to healthy controls [28,29,31–33,36,37,42,44–46]. On the other hand, one study showed significantly reduced IL-6 levels in the mTBI population compared to healthy controls (5.8%, n=1/17) [41]. The remaining studies showed no significant differences between the two populations at any time point (29.4%, n=5/17) [30,34,35,40,43]. It should be noted that there was a subject overlap to some extent between the four studies conducted by Group A [28–31], two by Group B [32,33] and three by Group C [34–36].

Within 24 hours, six out of seven studies measuring IL-6 showed elevated levels in the mTBI population [28,29,31,37,42,44]. Out of these six studies, three studies were conducted by group A [28,29,31]. The one study that did not find any significant differences between the two populations at this time point, had a very small sample size (n=5) for each group [46].

Out of the 16 studies comparing IL-6 levels, only 35.2% of the studies completely and 35.2% of the studies partially controlled for the confounding inflammatory variables. The remaining studies (29.6%) did not control for any confounding inflammatory variables. Confounding inflammatory variables include factors such as infections, auto-immune diseases, anti-inflammatory drug intake and other conditions that affect cytokine levels.

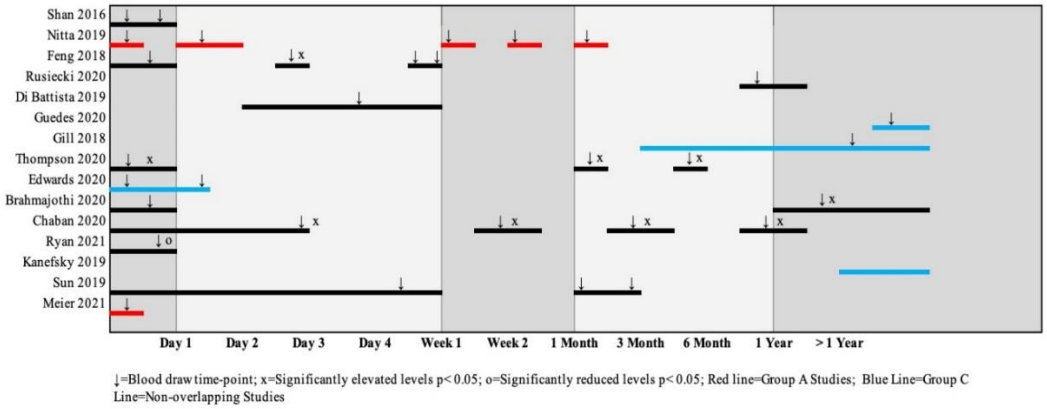


Supplementary Figure 1: Sampling points for IL-6

TNF-α

Our review identified 15 studies comparing circulating TNF-α levels between the patients with mTBI and healthy controls [29,31,33–37,39,41,42,44,46–49](Supplementary Figure 2). Out of these, four studies (25.6%; n = 4/15) showed significantly elevated TNF-α levels, and one found significantly reduced levels in patients with mTBI at a minimum of one time-point, compared to healthy controls [42,44,46,48,49]. Although all these studies looked at blood cytokines, one looked at CSF and found elevated TNF-alpha associated with mTBI [48].The remaining 10 studies showed no significant differences in the TNF-α levels between cases and controls [29,31,33–35,37,39,41,42,47].

Out of all studies comparing TNF-α levels, 60% (n = 9) of the studies completely and 20% (n=3) partially controlled for confounding inflammatory variables. The remaining studies did not control for any confounding inflammatory variable. There was a subject overlap to some extent between the two studies conducted by Group A [29,31] and three studies by Group C [34–36].

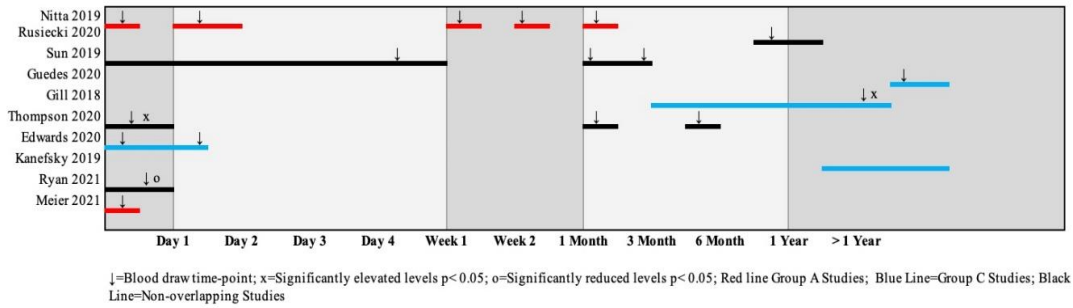


Supplementary Figure 2: Sampling points for TNF-α

IL-10:

Ten studies assessed IL-10 levels in blood following an mTBI [29,31,33–37,41,42,44](Supplementary Figure 3). This review identified that two out of these ten studies [34,42] found significantly elevated levels; whereas one study found significantly reduced levels [44] in mTBI patients at a minimum of one time-point, compared to healthy controls. The remaining studies found no significant differences between the two populations.

Out of all studies comparing IL-10 levels, 60% (n = 6/10) of the studies completely and 30% (n=3/10) partially controlled for confounding inflammatory variables. The remaining one study did not control for any confounding inflammatory variables. There was a subject overlap to some extent between the two studies conducted by Group A [29,31] and three by Group C [34–36].

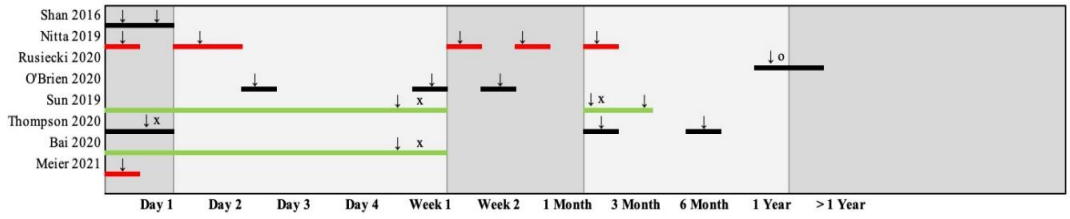


Supplementary Figure 3: Sampling points for IL-10

IL-1β:

A total of eight studies compared IL-1β levels in blood between mTBI patients and healthy controls [29,31–33,41,42,47,50](Supplementary Figure 4). Three out of eight studies showed significantly elevated IL-1β levels in patients with mTBI compared to healthy controls at a minimum of one time point [31–33,42,50]. On the other hand, one study showed significantly reduced IL-1β levels in patients with mTBI compared to healthy controls [41]. The remaining four studies, however, found no significant IL-1β level differences in blood between the cases and controls [29,47].

Out of the eight studies comparing IL-1β levels, 37.5 % (n = 3) of the studies completely and 62.5 % (n=5) partially controlled for the confounding inflammatory conditions. There was a subject overlap to some extent between the two studies conducted by Group A [29,31] and the two by Group B [32,33].



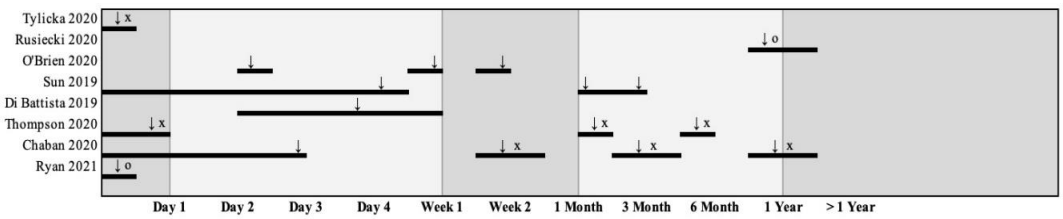
↓=Blood draw time-point; x=Significantly elevated levels p< 0.05; o=Significantly reduced levels p< 0.05; Red line=Group A Studies; Green Line=Group B Studies; Black Line=Non-overlapping Studies

Supplementary Figure 4: Sampling points for IL-1β

IL-8:

Eight of the included studies assessed IL-8 levels in blood following an mTBI [33,39,41,42,44,49–51](Supplementary Figure 5). Three of the included studies showed significantly elevated IL-8 levels in patients with mTBI, compared to healthy controls [42,49,51]. Furthermore, two studies showed a significant reduction in IL-8 levels in the mTBI population when compared to healthy controls [41,52]. The remaining studies showed no significant differences in IL-8 levels between the cases and controls.

Out of the eight studies measuring IL-8 levels in blood, 62.5 % (n=5) completely and 37.5 % (n = 3) partially controlled for the confounding inflammatory conditions.



↓=Blood draw time-point; x=Significantly elevated levels p< 0.05; o=Significantly reduced levels p< 0.05

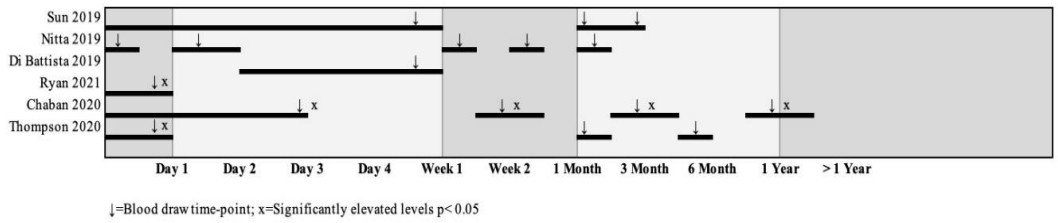
Supplementary Figure 5: Sampling points for IL-8

IFN-γ:

Six of the included studies assessed IFN-γ levels in blood following an mTBI [29,33,39,42,44,49](Supplementary Figure 6). Out of these, three studies showed significantly elevated IFN-γ levels in patients with mTBI, when compared to healthy controls at a minimum of one time point[42,44,49]; whereas the remaining three studies showed no significant differences between the two populations [29,33,39].

Within 24 hours, two out of three studies measuring blood IFN-γ levels showed elevated levels in mTBI population, but one study did not find any significant differences between the two populations during this period [29,42,52].

Out of the six studies comparing IFN-γ levels, 67 % (n = 4) of the studies completelycontrolled and 33 % (n = 2) partially controlled for the confounding inflammatory variables.



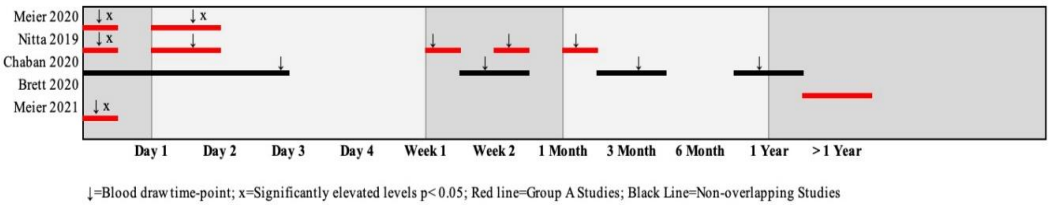
Supplementary Figure 6: Sampling points for IFN-γ

IL-1RA:

Blood IL-1RA levels were assessed in five of the included studies [28–31,49](Supplementary Figure 7). Out of these, three studies showed significantly elevated IL-1RA levels in patients with mTBI when compared to healthy controls at

less than 24 hrs[28,29,31]. The two remaining studies showed no such differences at any time point [30,49].

Of the five studies assessing IL-1RA levels, 20 % (n = 1) of the studies completely controlled for confounding inflammatory variables, whereas 80% (n=4) partially controlled for them. It should be noted that there was a subject overlap to some extent between the four studies conducted by Group A [28–31].

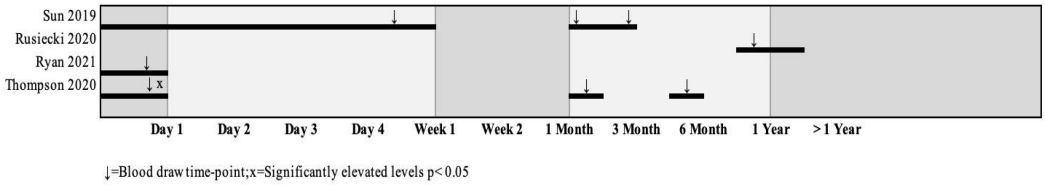


Supplementary Figure 7: Sampling points for IL-1RA

IL-4:

Circulating IL-4 levels were assessed in four of the identified studies [33,41,42,44](Supplementary Figure 8). Out of these, one study showed significantly elevated IL-4 levels in patients with mTBI, compared to healthy controls at a minimum of one-time point[42]. The remaining three studies, however, did not report any significant differences [33,41,44].

Out of the four studies comparing IL-4 levels, 75 % (n = 3) of the studies completely and 25 % (n=1) of them partially controlled for the confounding inflammatory conditions.



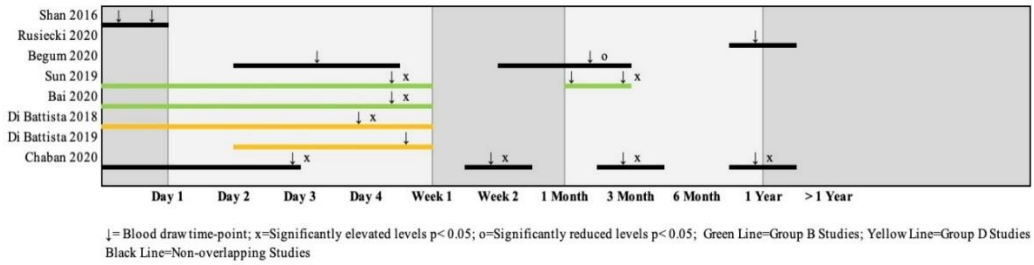
Supplementary Figure 8: Sampling points for IL-4

MCP-1/CCL2

Circulating MCP-1/CCL2 levels were assessed in eight of the identified studies [32,33,38,39,41,47,49,53](Supplementary Figure 9). Out of these, four studies reported significantly elevated MCP-1/CCL2 levels in the mTBI population when compared to healthy controls [32,33,38,49]; whereas one study reported a significant reduction in MCP-1/CCL2 levels in the mTBI population [53]. The remaining studies showed no significant differences in MCP-1/CCL2 levels between the two populations.

Within one week, four out of six studies measuring MCP-1/CCL2 showed elevated levels in the mTBI population [32,33,38,49] but the remaining two studies found no significant differences between the two populations at this time point [39,53].

Out of the eight studies comparing MCP-1/CCL2 levels, 37.5 % (n = 3) completely and 50% (n=4) partially controlled for the confounding inflammatory conditions. The remaining studies did not control for any confounding inflammatory variables. There was a subject overlap to some extent between the two studies conducted by Group D [38,39] and the two by Group B [32,33].



Supplementary Figure 9: Sampling points for MCP-1/CCL2

3.4.4. mTBI vs. Healthy Controls: Meta-Analysis of blood Inflammatory Cytokines

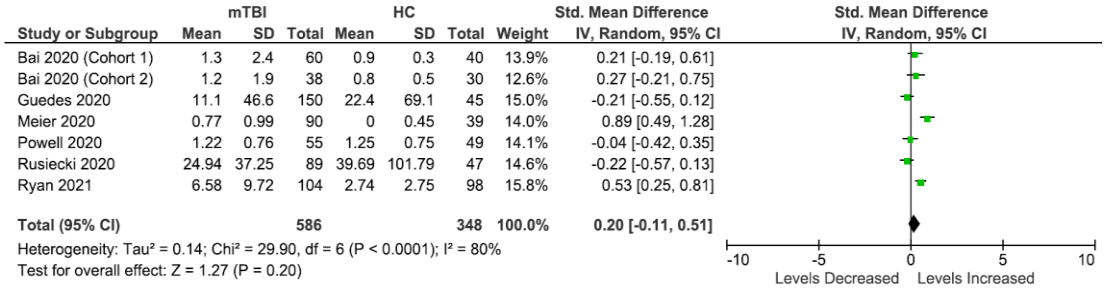
Eleven studies (12 cohorts) involving 987 participants with mTBI were utilized to conduct meta-analyses on IL-6, TNF-α, IL-1β, and MCP-1/CCL2 levels in blood. The results show significantly higher circulating levels of IL-6, IL-1β and MCP-1/CCL2 in the mTBI population in the acute stages (within 1 week), compared to healthy controls. No differences were observed for any inflammatory cytokine in the chronic stages.

IL-6

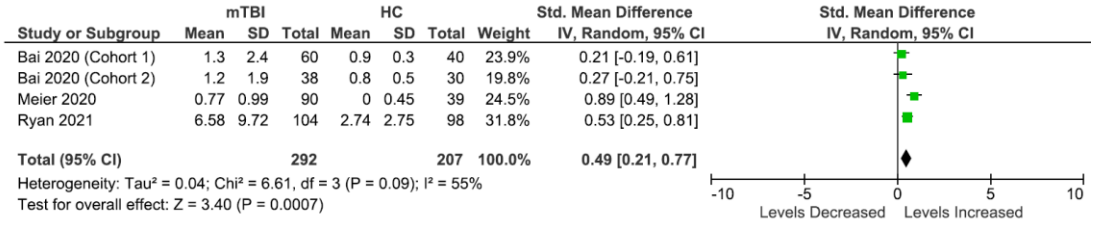
Six studies (7 cohorts) were included in the IL-6 analysis, involving 586 participants with mTBI and 348 healthy controls (Figure 2). The analysis shows no significant differences in the levels of IL-6 in blood between the two populations (SMD: 0.2 [95% CI: -0.11, 0.51] pg/ml, p=0.20 I^2=80%) (Figure 2A). The large heterogeneity is partly due to inconsistent results from the included studies due to differences in the timings of assessment, the fraction of blood specimen analyzed, techniques of biomarker assessment, and inflammatory confounding variables.

Further sub-analysis based on timing i.e., acute and chronic stages showed significantly elevated circulating IL-6 levels in mTBI population compared to healthy population in the acute stages (less than 7 days) (SMD: 0.49 [0.21, 0.77] pg/ml, p=0.0007 I^2=55%) (Figure 2B). However, no significant differences were observed between the two populations (SMD: -0.17 [95% CI: -0.37 to 0.04) pg/ml, p=0.11) in the chronic stages (more than 6 months) (Figure 2C).

(A)



(B)



(C)

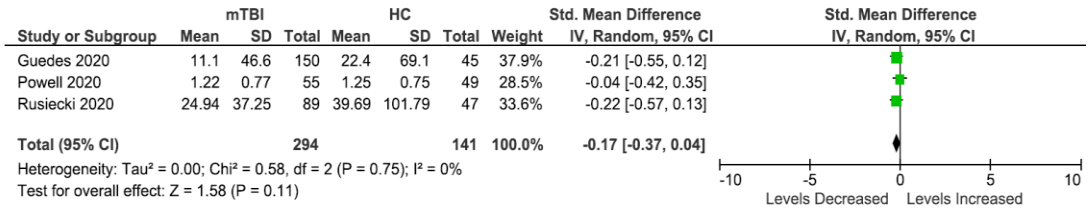


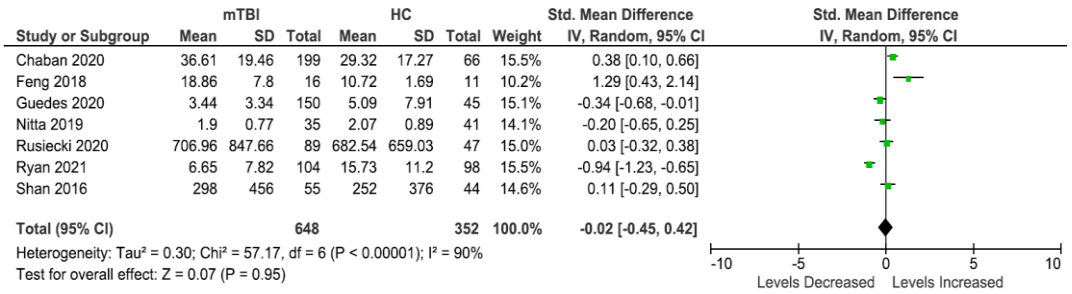
Figure 2. IL-6 Meta-analysis: (A) All studies; (B) Acute IL-6 meta-analysis; (C) Chronic IL-6 Meta-analysis.

TNF-α

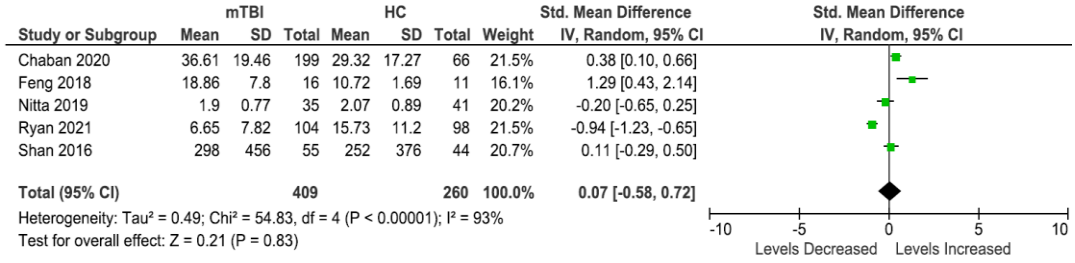
Seven studies were included in the TNF-α meta-analysis, involving 648 participants with mTBI and 352 healthy controls (Figure 3). The analysis shows no significant differences in the levels of TNF-α in blood between the cases and controls (SMD: -0.02 [95% CI: -0.45, 0.42] pg/ml, p=0.95, I^2=90%) (Figure 3A).

Further sub-analyses also showed no differences between the two populations at both acute and chronic stages (Figures 3B and 3C), and heterogeneity remained high.

(A)



(B)



(C)

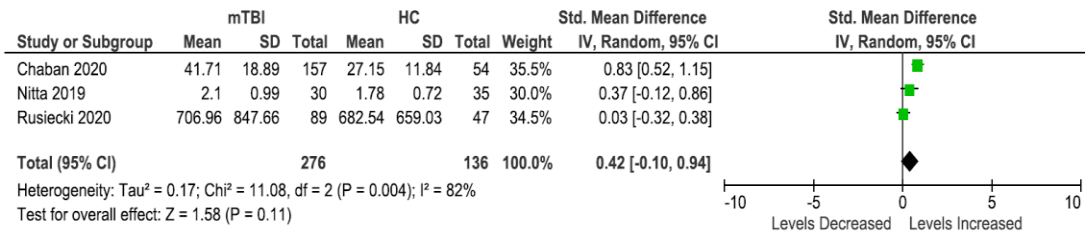


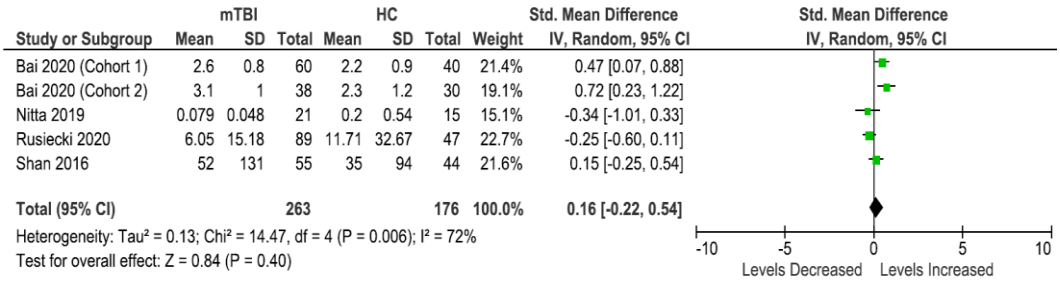
Figure 3. TNF-α Meta-analysis: (A) All studies; (B) Acute TNF-α meta-analysis; (C) Chronic TNF-α meta-analysis.

IL-1β:

Four studies (five cohorts) were included in the IL-1β analysis, involving 263 participants with mTBI and 176 healthy controls (Figure 4). The analysis showed no significant difference in the levels of IL-1β in blood between the cases and controls (SMD: 0.16 [95% CI: -0.22, 0.54] pg/ml) (p=0.40, I^2=72%) (Figure 4A).

Further sub-analyses showed significantly elevated blood IL-1β levels in the mTBI population in the acute stages (< 7 days) and brought down the heterogeneity (SMD: 0.42 [95% CI: 0.10, 0.74] pg/ml) (p=0.01, I^2=40%) (Figure 4B). A meta-analysis on chronic levels could not be conducted due to an insufficient number of studies.

(A)



(B)

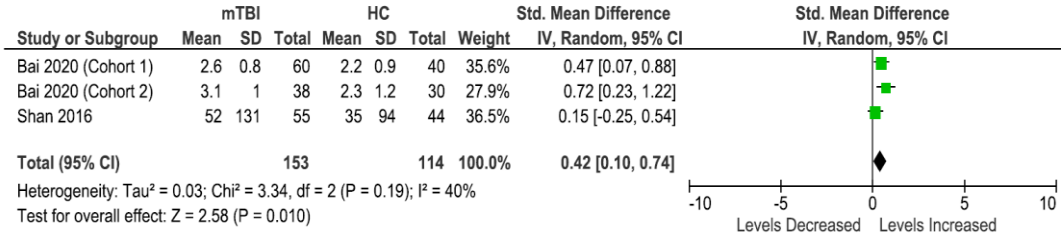


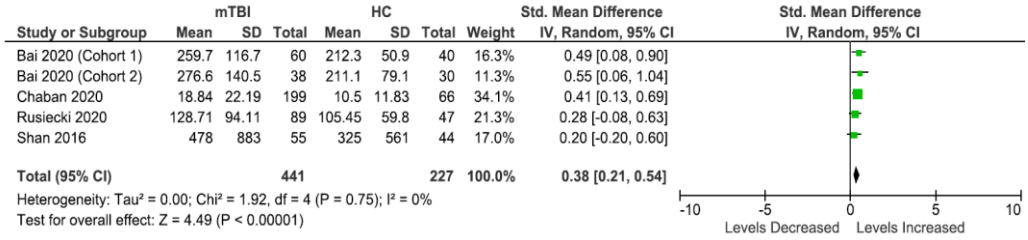
Figure 4. IL-1β Meta-analysis: (A) All Studies; (B) Acute IL-1β Meta-analysis.

MCP-1/CCL2:

Four studies (five cohorts) were included in MCP-1/CCL2 analysis, involving 441 patients with mTBI and 227 healthy control subjects (Figure 5). Patients with mTBI had significantly elevated concentrations of MCP-1/CCL2 (SMD: 0.38 [95% CI: 0.21, 0.54] pg/ml) (p=0.00001, I^2=0%) (Figure 5A) in blood compared to healthy controls.

Further sub-analysis based on timings demonstrated that blood MCP-1/CCL2 levels are particularly elevated in the acute stages in patients with mTBI compared to healthy controls (within 7 days) (SMD: 0.40 [95% CI: 0.22, 0.59] pg/ml) (p=0.0001, I^2=0%) (Figure 5B). Sub-meta-analysis on chronic levels was not possible due to insufficient data.

(A)



(B)

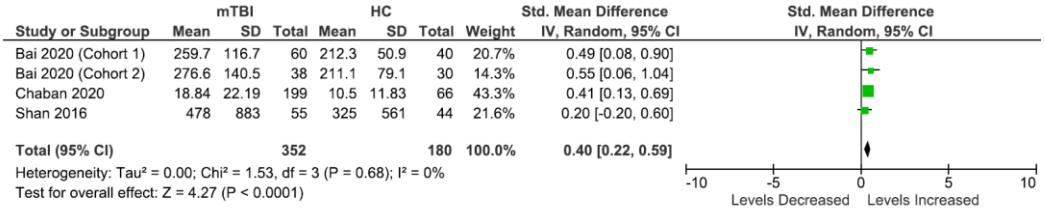


Figure 5. MCP-1/CCL2 Meta-analysis: (A) All studies; (B) Acute MCP-1/CCL2 Meta-analysis.

3.4.5. mTBI vs Trauma Controls:

Only three studies compared inflammatory cytokine levels in blood between the patients with mTBI and trauma controls [38,47,54].

Shan (2016) found no significant differences in the TNF-α, IL-1β, and the chemokines (CXCL1, CXCL8, and MCP-1/CCL2) levels in acute stages (less than 24 hours) between the two groups [47]. Vedantam (2021) observed significantly elevated IL-2 and IL-6 levels in patients with mTBI in the acute stages (<24 hrs.); however, in the chronic stages only IL-6 levels remained elevated [54]. Di Battista (2019) found that athletes with sport-related concussion had higher levels of the chemokines' monocyte chemoattractant protein-4 (MCP-4) ($p<0.001$) and macrophage inflammatory protein-1β (MIP-1β) ($p = 0.001$) compared to healthy athletes (within one week of injury). At medical clearance, there were no significant biomarker contributions towards the class separation between the athletes with SRC vs. healthy athletes [39].

3.4.6. Prognosis

The relationship between inflammatory cytokines in blood and mTBI prognosis was analyzed in 13 studies (Table 1).

For this analysis, the population was considered to have poor functional outcomes if they had any of the following conditions:

- Persistent symptoms (including emotional/psychological)
- Reduced or lack of return to normal activities (work, school, sports)
- Abnormal neurocognitive tests/functioning
- Low GOSE scores (<8)

IL-6:

Some studies showed that elevated IL-6 levels in blood at 6 hours post-mTBI are significantly associated with the duration of symptoms ($p = 0.031$) [28,29,31]. On the contrary, Di Battista (2020) showed that there is no significant correlation between IL-6 levels and either symptom burden or days to medical clearance [40].

Guedes et al (2020) found a mild correlation between elevated IL-6 levels in blood and PTSD in the chronic stages [35].

MCP-1/CCL2:

Acutely elevated MCP-1/CCL2 levels in blood are associated with greater PCS severity and are positively associated with information processing speed at 3 months post-injury [33]. Similarly, acutely elevated MCP-1/CCL2 levels in blood (within 1 week) are positively correlated with days to recovery in athletes with sport-related mTBI [39]. On the other hand, Begum (2020) reports that reduced serum MCP-1/CCL2 levels in blood are associated with an increase in the number ($r = 0.455$, $p = 0.013$) and severity of symptoms ($r = -0.378$, $p = 0.043$) [53].

TNF-α:

Plasma TNF-α levels correlate with persistent PCS and PTSD symptoms [35,36]. Within the mTBI groups, increased circulating TNF-α concentrations is associated with greater PTSD symptoms ($r = 0.36$, $p = 0.005$) [36].

IL-1RA:

Acutely elevated circulating IL-1RA levels (within 6 hours of mTBI) appear to be significantly associated with greater symptom duration ($p = 0.03$) [28]. In addition, there is a significant interaction between prior concussions and levels of IL-1RA on the ImPACT Memory Composite scores ($p = 0.044$)[30].At low levels, athletes with multiple mTBIs show worse memory performance than those without prior mTBIs ($p = 0.014$). Overall, elevated levels are associated with greater symptoms (higher BSI-GSI scores, $\chi2(1) = 3.98$, $p = 0.046$) and worse memory (ImPACT Speed Composite scores, $\chi2(1) = 5.67$, $p = 0.017$) [30].

IL-10:

At 3 months post-mTBI, elevated circulating IL-10 levels are found to be related to PTSD symptoms ($B=0.8$, $t=2.60$, $p < 0.01$)[34].At the six-month mark, elevated plasma IL-10 levels are associated with greater depression scores ($p=0.004$) and more severe PTSD symptoms ($p=0.001$) [54].

3.5. Discussion

This study reports significantly higher blood concentrations of IL-6, CCL-2/MCP1 and IL-1β in subjects with mTBI, compared to healthy controls, particularly in the acute stages. While both positive and negative results have been reported for the individual studies, this report strengthens the assertion that mTBI is accompanied by a peripheral inflammatory response [15,17,18,55–57].

Despite extensive knowledge about the protracted recovery and long-term consequences of mTBI, challenges associated with its diagnosis, prognosis, and

management remain unresolved. This could be partly attributed to a lack of understanding of mTBI pathophysiology. mTBI appears to be a multifaceted problem, with various biological and non-biological factors at play that determine the clinical outcome [58–60]. Neuroinflammation constitutes one of the many secondary pathologies associated with mTBI and represents only a single piece of an intricate puzzle [61]. Understanding this neuroinflammation would unravel one of many unknowns of mTBI. To achieve this objective, we have conducted a systematic review and meta-analysis to consolidate and analyze the data on the inflammatory cytokines associated with mTBI. As a result, we are able to identify a few circulating inflammatory cytokines associated with mTBI. The results of the systematic review show significantly elevated levels of IL-6, IFN-γ, IL-1RA (within 24 hours) and MCP-1/CCL2 (between 1-7 days) in blood in patients with mTBI, compared to healthy controls. These results are further supported by the results of the meta-analysis which demonstrate significantly elevated blood levels of IL-6, IL-1β and MCP-1/CCL2 levels in mTBI during the acute stages (within a week). Taken together, these results show a strong association between elevated IL-6, IL-1β, and MCP-1/CCL-2 levels in blood and mTBI during the acute stages. However, due to inherent heterogeneity associated with cytokine-related data, these findings should be interpreted with caution.

IL-6 is a non-specific indicator of inflammation. It is one of the most frequently measured cytokines in mTBI studies. Our review shows that circulating IL-6 levels are consistently higher in patients with mTBI, compared to healthy controls, in most of the studies (65%, n = 11/17), especially during the acute stages. Interestingly, blood IL-6 levels also seem to be elevated in individuals with mTBI,

when compared to those with trauma controls, particularly during the acute phase [54]. Apart from IL-6, the peripheral inflammatory cytokine profile associated with mTBI appears to be quite different than the one associated with bodily trauma controls [39]. However, due to limited availability of data, no meaningful inferences can be drawn on the circulating inflammatory cytokine level differences between the patients with mTBI and trauma controls without further research. This review also shows that acutely elevated IL-6 levels in blood are consistently associated with poor prognosis, particularly in terms of duration of symptoms [28,29,31]. However, one study found no significant correlation between IL-6 levels in blood and either symptom burden or days to medical clearance[40]. This discrepancy can be attributed to differences in the timings of cytokine level measurements, as Di Battista (2020) measured IL-6 levels in the late acute stages compared to others. Chronically, IL-6 appears to be associated with PTSD [35]. Overall,

these findings indicate that circulating IL-6, while not highly specific, is a strong indicator of mTBI in early acute stages and could be used to predict clinical outcomes.

MCP-1/CCL2 belongs to the chemokine family of cytokines and is also a non-specific marker of inflammation. This review uncovers a strong association between elevated blood MCP-1/CCL2 levels and mTBI. This association is particularly strong within the first week following an mTBI, as 66% of the studies measuring blood MCP-1/CCL-2 show elevated levels in patients with mTBI, compared to healthy controls. This finding is further supported by the results of the meta-analysis. Beyond one week, MCP-1/CCL2 levels remain elevated, extending into the chronic stages; however, the evidence is more robust within one week of the mTBI. With regards to

prognosis, the evidence is quite conflicting as some studies indicate associations between elevated levels of MCP-1/CCL-2 levels and greater symptom severity, days to recovery, and information processing speed [33,39]. On the other hand, Begum (2020) reports that reduced serum MCP-1/CCL2 levels are associated with an increase in the number ($r = 0.455$, $p = 0.013$) and severity of symptoms ($r = -0.378$, $p = 0.043$) [53]. In addition, due to limited data available, no meaningful inferences can be drawn on MCP-1/CCL-2 level differences between the patients with mTBI and trauma controls without further research. Overall, we can infer that MCP-1/CCL2, just like IL-6, is an indicator of acute mTBI and could be used to predict clinical outcomes.

This meta-analysis also shows significantly elevated IL-1β levels in the acute stages (within a week) of mTBI, compared to healthy controls. TNF-α is the second most common cytokine explored in mTBI studies. This review, however, is unable to detect any significant differences in TNF-α levels between the patients with mTBI and healthy controls. In addition, despite the evidence of elevated IL-1RA, IL-8, and IFN-γ levels in patients with mTBI, particularly within 24 hours, we were not able to conduct a meta-analysis due to a limited number of studies.

While this review suggests that TNF-α [35,36,62], IL-1RA [28,30] and IL-10 [34,54], in addition to IL-6 [29,31,35,36]and MCP-1/CCL2 [33,39]have the potential to predict the outcome of mTBI, the data in this thesis is too limited to draw concrete conclusions about these associations.

Neuroinflammation plays both a protective and detrimental role in mTBI [15,17,63]. While it usually offers neuroprotection early on after an mTBI, persistent neuroinflammation appears to be associated with poorer outcomes [63]. As a result, further research is necessary to study the levels of inflammatory cytokines in the

chronic stages and their association with persistent symptoms and recovery. Identifying these chronic cytokines may not only be beneficial in monitoring the prognosis of mTBI but may also aid in developing and monitoring targeted treatment strategies for persistent post-concussive symptoms. Although it appears promising, the inherent non-specific nature of these cytokines makes them an unsuitable candidate for the suggested use when employed alone. Recently, many specific markers of neuronal injury, such as UCHL1, GFAP and S100B have gained much popularity as specific markers of brain injury. Future research may consider utilizing these cytokines in combination with neuronal injury markers to assess prognosis and monitor treatment efficacy, as suggested by others [64]. Additionally, future studies may benefit from measuring cytokines and conducting clinical assessment longitudinally at multiple time points to fully understand the relationship between the biomarker recovery trajectory and mTBI recovery trajectory [65].

This study highlights the significant heterogeneity in blood-based inflammatory cytokine data related to mTBI. We acknowledge this limitation and recommend that future studies adopt standardized cytokine analysis methods to minimize data heterogeneity and associated outliers that can result in a 100-fold change across studies. This heterogeneity not only jeopardizes data reliability, accuracy, and reproducibility but also hinders progress in the field. MacDonald et al (2021) recognized and elaborated on these limitations and proposed potential solutions to mitigate them. Incorporating these strategies in future research will help address this issue[64].

3.5.1. Limitations

Our findings must be interpreted with caution.First, this review shows that there is considerable heterogeneity in the data, leading to difficulties in pooling and analyzing the data to formulate a meaningful conclusion. Heterogeneity was caused by many reasons, some of which include differences in the time elapsed between the initial mTBI and blood sample collection, cytokine analysis technique, blood fraction used for analysis, confounding variable control, mTBI diagnostic criteria, data reporting and functional outcomes measured. Hence, there is a need for a standardized approach in acquiring and reporting data to allow for comparisons.

Secondly, the results of this review show that about 76% of mTBI patients were male. This is because most studies are conducted in the military (27%) and sports populations (42%), which happen to be male-dominant settings. Since females are more at-risk for poor recovery and develop persistent symptoms more frequently compared to males [66–68], we could not assess prognosis accurately based on this data. Future studies, especially those assessing prognosis in mTBI patients, may want to incorporate more female participants in their studies.

Thirdly, the cytokine alterations observed in the mTBI population do not necessarily reflect a pathophysiology associated with head injury alone. There are other variables that should be considered while measuring cytokine levels as they are known to cause considerable fluctuations. These include time of blood collection, sex differences, other injuries (orthopedic, whiplash, muscle strains etc) at the time of mTBI, acute and chronic illnesses, co-existing psychiatric conditions, and medication intake amongst others. While we attempted to take some of these limitations into

account, the results should still be interpreted with caution due to the factors mentioned above.

Lastly, most studies reported their results using medians or log-transformed means. Although this way of reporting leads to more consistent results, a thorough meta-analysis cannot be conducted using medians as we have found in this review. Future studies should follow a more standardized methodology so that the data reported using means and SD is not as heterogeneous and is more consistent to allow for a more thorough analysis.

3.5.2. Strengths

This systematic review and meta-analysis have several strengths. An exhaustive effort was made to capture the data by searching a variety of databases and acquiring the missing data by directly contacting the authors, extracting data from graphs and tables, or usingstandardized estimation methods for calculating mean (SD). Although the latter two strategies may not be accurate, they provide estimates close to real values and are frequently employed in meta-analyses.

3.5.3. Conclusions

Overall, we found substantial evidence of increased inflammatory cytokine levels in patients with mTBI. The evidence was particularly strong for IL-6, IL-1β, and MCP-1/CCL2. The results of this study were however limited by low study numbers as well as methodological heterogeneity between the studies.

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Chapter 4

Association Between Mild Traumatic Brain Injury-Induced Inflammatory Cytokines and Emotional Symptom Traits: A Systematic Review

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4.1. Abstract

Both mild traumatic brain injuries (mTBI) and systemic injuries trigger a transient neuroinflammatory response that result in similar clinical outcome. The ensuing physical, cognitive, and emotional symptoms fail to subside in approximately 15–20% of the mTBI population. Emotional impairments, particularly depression, anxiety, and post-traumatic stress disorder (PTSD), are commonly associated with poor recovery following mTBI. These emotional impairments also have a significant neuroinflammatory component. We hypothesized that the inflammatory cytokines seen in mTBI patients with emotional symptoms would coincide with those commonly seen in patients with emotional symptoms without mTBI. A systematic review was conducted to identify the most common neuroinflammatory cytokines in the mTBI population with psychological symptoms (depression, anxiety, PTSD). Electronic databases EMBASE, MEDLINE, Cochrane Central Register of Controlled Trials (CENTRAL), PUBMED, and PSYCINFO were searched from data inception to August 31, 2021. A systematic screening approach was employed from screening to data analysis. A total of 994 articles were screened, 108 were selected for full article review, and 8 were selected for data analysis.The included studies consisted of 875 patients of which 81.3% were male. The mean sample size of patients with at least one mTBI was 73.8 ± 70.3 (range, 9-213), with a mean age of 33.9 ± 4.8 years. The most common cytokines associated with poor psychological outcomes involving PTSD and/or depression in the chronic mTBI population were IL-6, TNFα, IL-10, and CRP.

Keywords: concussion; mild traumatic brain injury; inflammatory cytokines; neuroinflammation; depression; PTSD

4.2. Introduction

Mild traumatic brain injuries (mTBI) account for 80–90% of all traumatic brain injuries (TBI) [1,2]. Following mTBI, people suffer from many physical, cognitive, and psychological/emotional symptoms, collectively called post-concussive symptoms. A minority of patients, about 15–20%, recover slowly or not at all [3–5]. Almost all of these have depressive or anxiety symptoms [6].

mTBI triggers a cascade of biomolecular changes in the brain acutely [7]. These lead to behavioral changes, amongst other symptoms, following mTBI. Interestingly, it was observed that patients who sustain systemic injuries not involving the brain display the same characteristic symptoms as those who suffer mTBI [8.] This raised the possibility that humoral mechanisms triggered by the systemic injury produced the same effect on the brain as a concussive injury to the brain itself [9].

Both systemic injuries and neurotrauma cause inflammatory changes, and presumably the systemic inflammatory cytokines likely affect the brain in the same way as the well-described inflammatory changes that follow a brain injury [9]. These changes include activation of immune cells and increased systemic concentrations of circulating inflammatory cytokines. Immediately following a mTBI, neuroinflammation seems to play a role in neuroprotection; however, continued neuroinflammation can be detrimental and could be responsible for persistent symptoms [10,11]. We have identified that each

symptom could be induced by altered levels of inflammatory cytokines [9]. For example, headaches are associated with elevated Tumor Necrosis Factor-α (TNF-α), Interleukin-10 (IL-10), Interleukin-1β (IL-1β), Interleukin-6 (IL-6), and Interferon-α (IFN-α) levels [9,12,13]. Cognitive impairments are associated with upregulated IL-1β, IL-6, and TNF-α levels [9,14.]Fatigue and sleep disturbances are also associated with cytokines TNF-α, IL-6, and IL-1β [9,15,16]. Similarly, our previous review has demonstrated that depression and anxiety are associated with elevated TNF-α, IL-6, IFN-α, and C-Reactive Protein (CRP) levels [9]. Overall, the most common systemic cytokines associated with mTBI include IL-6 [17–23], TNF-α[20,23–25], IL-10 [20,26], IL-1β [19,20], Interleukin-8 (IL-8) [20,25], Interferon Gamma (IFN-γ) [20,25], Interleukin-1RA (IL-1RA) [17,21], Interleukin-4 (IL-4) [20], and C–C motif chemokine ligand 2 (CCL2) [19,27].

Like mTBI patients, systemic inflammation seems to be associated with psychological conditions such as depression, anxiety, and post–traumatic stress disorder (PTSD) in the non–concussed population [28–30]. This led us to question whether the same inflammatory mediators were active in mTBI patients and played a role in the genesis of emotional impairments, particularly depression, anxiety, and PTSD, that are hallmarks of persistent post-concussion symptoms (PPCS) [6].

To help us understand the relation between inflammation and the emotional symptoms seen after mTBI, we conducted a systematic review to identify the most common neuroinflammatory cytokines associated with poor emotional recovery in the mTBI population. This study focuses on mTBI/concussions as they are the most common among all TBI subtypes [1,2]. Additionally, emotional symptoms are more predominant in

this TBI subgroup [6]. We hypothesize that the inflammatory cytokines seen in mTBI patients with emotional symptoms would coincide with those commonly seen in patients with emotional symptoms without mTBI.

4.3. Methods

4.3.1. Search Strategy

Three separate searches were conducted across five databases (PUBMED, EMBASE, MEDLINE, Cochrane Central Register of Controlled Trials (CENTRAL), and PSYCINFO) for the literature on mTBI. All three searches were identical except for the outcomes in question (depression, anxiety, and PTSD). The searches only included literature from data inception to August 31st, 2021. The search terms included "mild traumatic brain injury", "neuroinflammation", "concussion", and similar phrases (Appendix Table I, II, and III). A manual search using Google Scholar was conducted to capture any articles that may have been missed. The inclusion and exclusion criteria and the research question were established as a priori. Inclusion criteria were: (1) mTBI; (2) neuroinflammation or at least one blood or cerebrospinal fluid (CSF) cytokine identified for the population of interest; (3) emotional symptomatology (anxiety, depression, or PTSD); (4) human studies; (5) English language. Exclusion criteria were: (1) moderate and severe TBI (Glascow Coma Scale (GCS) < 13); (2) no inflammatory markers; (3) no emotional symptomatology; (4) review articles; (5) cadaver/non-human studies. This study focused on mTBI and excluded any studies that only included moderate or severe

TBIs (Traumatic Brain Injury) or did not distinguish between various types of TBIs. The full search strategy is provided as Supplementary tables S1, S2, and S3.

4.3.2. Study Screening

In fulfilment of the Revised Assessment of Multiple Systematic Reviews (R-AMSTAR) and the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) guidelines, a systematic screening approach was implemented [31,32]. Two independent reviewers conducted the study screening in duplicate, from title to the full-text screening stage. Any discrepancies were discussed between the reviewers and were resolved thereafter. The same systematic approach was also used to screen the references of the included studies to capture any additional relevant papers (Figure 1).

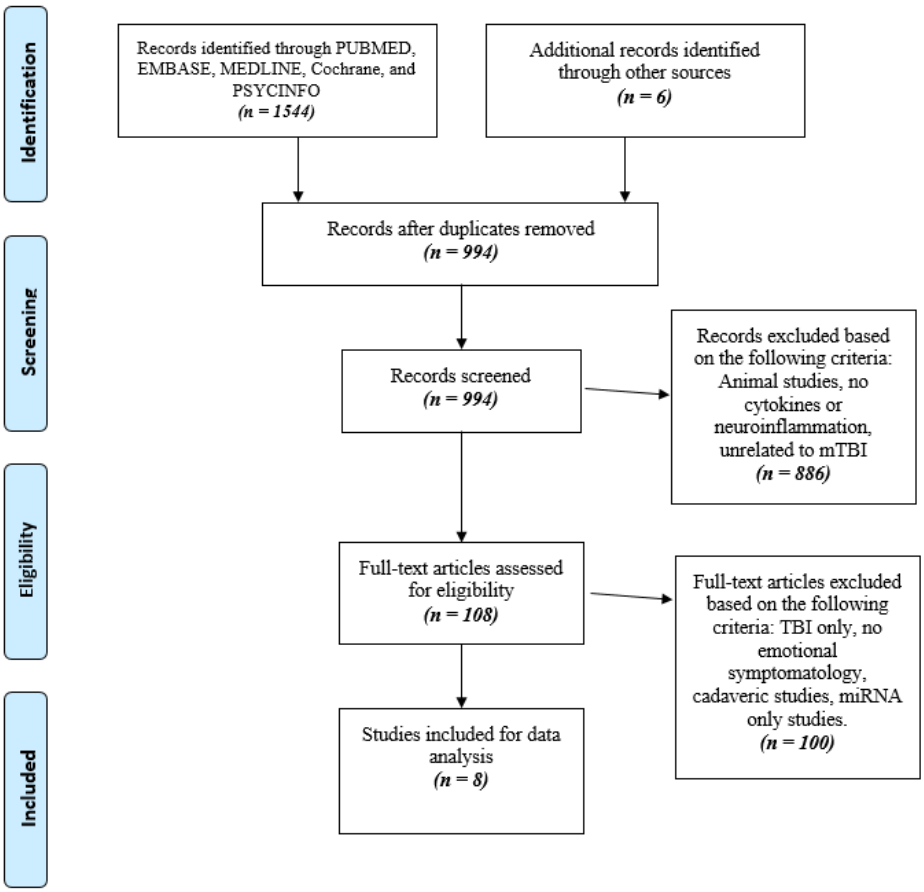

Figure 1. Prisma Flow Chart

4.3.3. Data Abstraction

Two independent reviewers abstracted the relevant data from the articles included in this review. The data included the author, year of publication, sample size, study design, acute (< one month) and chronic (> one month) mTBI, and patient demographics (e.g., age, gender, etc.) for each study. Data regarding cytokine levels, time of cytokine analysis, biospecimen analyzed, and screening tools for anxiety/PTSD/depression were also recorded. The reviewers documented the data onto a shared spreadsheet.

4.3.4. Quality Assessment

The methodological index for non-randomized studies (MINORS) was used to evaluate the quality of the studies included in this review [33]. Both reviewers recorded a score from 0 to 2 for each of the 12 categories on the MINORS checklist. For comparative studies, a maximum score of 24 could be achieved, whereas a maximum score of 16 was possible for non-comparative studies. For each of the included non-comparative and comparative studies, a total score of 13–16 or 19–24 was considered excellent quality, 9–12 or 13–18 was considered fair quality, and 0–8 or 0–12 was considered poor quality, respectively [33]. The levels of evidence for all included studies were also assessed.

4.3.5. Statistical Analysis

Descriptive statistics including mean, range, standard deviation, and 95% confidence interval (CI) were presented where appropriate. A kappa (κ) statistic was used at each screening stage to assess the level of agreement between the reviewers. Near perfect agreement was categorized as any κ value between 0.81 to 0.99. Furthermore, a κ of 0.61 to 0.80 was considered significant agreement, a κ of 0.41 to 0.60 was moderate agreement, a κ of 0.21 to 0.40 was fair agreement and a κ value of 0.20 or less was considered slight agreement.

4.4. Results

4.4.1. Study Characteristics

A combined total of 994 papers were yielded across the five databases for the three searches. The specific searches for depression, anxiety, and PTSD yielded 576, 216, and 202 papers, respectively. A systematic screening process was followed as shown in Figure 1, yielding eight papers that met the inclusion criteria after excluding duplicates. Of the included studies, seven were case control studies (87.5%), one was a cohort study (12.5%), and two (25.0%) were conference abstracts. The main study characteristics and outcomes are described on Table 1.

Author and Year	Population	mTBI Setting	Cytokine (s)	Biospecimen	Outcome of interest	Acute/ Chronic (Average Time)
Bellgowan et al. (2012)	*n* = 9 (Cases = 9, Controls = 0)	Sports	IL-1β	Blood - venous plasma	IL-1β levels were significantly elevated at 24–48 h compared to either 1 week or 1-month post-concussion. Depression and anxiety ratings significantly higher at 24–48 h and 1 week, but not 1 month after concussion.	Acute (24–hours, 1 week, 4 weeks)
Ghai et al. (2020)	*n* = 69 (Cases = 27, Non-mTBI Veterans (DC) = 11, Community Controls (CC) = 31)	Military	CRP	Blood - venous plasma	Elevated CRP levels in chronic mTBI patients compared to CC and DC controls. Chronic mTBI group had significantly greater comorbid PTSD and depressive symptoms, compared to CC and DC.	Chronic (4.6 years)
Gill et al. (2018)	*n* = 64 (Cases = 42, Controls = 22)	Military	IL-6, IL-10, TNFα	Blood exosomal plasma	Chronic mTBI patients had elevated exosomal IL-10 levels compared to controls. PTSD was significantly related, and depression tended to be related to exosomal IL-10.	Chronic (3–36 months)
Guedes et al. (2020)	*n* = 195 (Cases = 150, Controls = 45)	Military	IL-6, TNF-α, IL-10	Blood venous plasma	mTBI group had increased PTSD and depression symptoms compared to controls. Plasma TNF-α (p = 0.02) and exosomal IL-6 (p = 0.08) levels correlated with PTSD.	Chronic (NR)

Kanefsky et al. (2019)	*n* = 143 mTBI+LOC (n = 25), mTBI without LOC (n = 36), Controls = 82)	Military	TNF-a, IL-6, IL-10	Blood venous plasma	Both mTBI groups (+/- LOC) reported significantly greater depression and PTSD symptoms compared to controls. IL-6 was elevated in the mTBI with LOC group compared to both the mTBI w/out LOC and control groups. Within the mTBI groups, increased TNF-α concentrations were associated with greater PTSD symptoms (r = 0.36, p = 0.005).	Chronic (NR)
Peskind et al. (2015)	*n* = 105 (Cases = 35, Non-mTBI Veterans (DC) = 16 Community Controls (CC) = 55	Military	IL-7, IL-6	CSF	mTBI veterans had greater PTSD and depression and elevated CSF IL-7 levels compared to the DC. CSF IL-6 levels did not differ between mTBI Veterans and DC but was significantly higher in both Veteran groups than in CCs.	Chronic (NR)
Su et al. (2014)	*n* = 213 (Cases = 213, Controls = 0)	Trauma	CRP	Blood venous plasma	CRP levels were significantly correlated with PCS, persistent psychological problems and persistent cognitive impairments.	Chronic (1-3 months)
Vedantam et al. (2021)	*n* = 77 (Cases = 53, OI Controls = 24)	Trauma	IL-1β, IL-2, IL-4, IL-6, IL-10, IL-17, IFN-γ, TNFα, IL-12p70	Blood exosomal plasma	Within 24 h, IL-2 and IL-6 levels were significantly elevated in the mTBI population vs. OI controls. At 6 months post-injury, mTBI group had elevated IL-6 (p = 0.044) levels vs. OI controls. Elevated IL-10 levels at 6-month post-mTBI was significantly associated with severe PTSD (p = 0.004) symptoms and worse mood (p = 0.001).	Acute (24 h) / Chronic (6 months)

Table 1. Study Characteristics and Outcomes.

Abbreviations: mTBI, mild traumatic brain injury; LOC, Loss of Consciousness; IL, Interleukin; DC, Deployed Controls (non-mTBI Veterans); CC, Community Controls; CRP, C-Reactive Protein; PTSD, Post-Traumatic Stress Disorder; TNF, Tumor Necrosis Factor; CSF, Cerebrospinal Fluid; PCS, Post-Concussive Symptoms; OI, Orthopedic Injury; IFN, Interferon; NR, Not Reported. Definitions: Acute: Refers to a mTBI < less than 4 weeks. Chronic: Refers to a mTBI > 4 weeks.

4.4.2. Study Quality

All the studies included in this review have a level of evidence of IV (n = 8; 100%). There was considerable agreement between the two reviewers at the title/abstract screening stage (κ = 0.84 [95%CI, 0.70 to 0.90]) and the full-text screening stage (κ = 0.75 [95% CI, 0.60 to 0.90]. The mean MINORS score for non-comparative and comparative studies were 12.0 ± 1.4 and 20.1 ± 1.8 respectively, which indicates fair quality of evidence for non-randomized studies. The areas of best performance based on the MINORS checklist were "endpoints appropriate for aim" (n = 8; 100%) and "inclusion of consecutive patients" (n = 8; 100%). The area of worst performance was "unbiased assessment of endpoints", which was not found in any of the included studies.

4.4.3. Patient Characteristics

A total of 875 patients were included across all the studies in this review. The mean sample size of patients with at least one mTBI was 73.8 ± 70.3 (range, 9–213). Furthermore, the mean sample size for non-mTBI/healthy controls was 47.7 ± 24.4 (range, 22–82). The mean age of the study groups (at least one mTBI) was 33.9 ± 4.8 years, while the mean age of the control groups was 31.3 ± 6.3 years. In addition, 81.3% (442/544) of the participants were male; two studies did not specify the sex distribution of their population [34,35]. Of the included studies, two studies did not specify age, and two did not include a healthy control population (Table 1).

4.4.4. Outcomes

4.4.4.1. Depression

All the included papers in this review had a study population (n = 875) that had symptoms of depression (Table 1). The screening tools that were utilized to diagnose depression in the study populations for each paper include the nine-item Patient Health Questionnaire Depression Scale (PHQ-9) (n = 2), the Quick Inventory of Depressive Symptomatology (QIDS) (n = 2), the Center for Epidemiologic Studies Depression Scale (CES-D) (n = 1), and the Beck Depression Inventory (BDI) (n = 1). One paper did not specify the screening tools used to diagnose depression [34]. The biomarkers assessed in the studies involving depression include IL-6 (n = 5), IL-10 (n = 4), TNF-α (n = 4), IL-1β (n = 2), CRP (n = 2), and IL-7 (n = 1). The study populations across all papers involving those diagnosed with depression were either military personnel (74.6%; n = 653/875), athletes (1.0%, n = 9/875), or admitted at the hospital (24.3%, n = 213/875).

4.4.4.2. Post-traumatic stress disorder (PTSD):

Six of the included papers in this review involved a study population (n = 653) that had symptoms of PTSD (Table 1). PTSD was assessed through the PTSD Checklist Military Version (PCL-M) (n = 4) as well as the Post-traumatic Stress Checklist—Civilian Form (PCL-C) (n = 1). One study did not specify the screening tools utilized to assess PTSD [34]. The biomarkers assessed in the studies involving PTSD include IL-6 (n = 5), IL-10 (n = 4), TNF-α (n = 4), IL-1β (n = 1), CRP (n = 1), and IL-7 (n = 1). The study populations across all papers involving those diagnosed with PTSD were all veteran military personnel (n = 653).

4.4.4.3. Anxiety

Two of the included papers in this review had a study population (*n* = 222) that had symptoms of anxiety (Table 1). The Beck Anxiety Inventory (BAI) was utilized as a screening tool for anxiety in one of the studies [36]. One study did not specify the screening tools utilized to assess anxiety [35]. The relevant biomarkers assessed in the anxiety study population were CRP and IL-1β. The study populations across all papers involving those diagnosed with anxiety were all athletes (4.1%, *n* = 9) or admitted to the hospital (95.9%, *n* = 213).

4.4.4.4. Cytokines

The most notable biomarkers assessed in the eight studies included in this systematic review were IL-6 (66.7%; *n* = 584), TNF-α (54.7%; *n* = 479), IL-10 (54.7%; *n* = 479), CRP (32.2%; *n* = 282), IL-7 (12.0%; *n* = 105), and IL-1β (9.8%; *n* = 86). Four studies assessed a biomarker that was chosen to be excluded due to a small sample size or lack of clinical significance (e.g., IL-4 and IL-17a) (Table 1). Seven studies used blood samples to measure the levels of biomarkers, while one study analyzed CSF (Table 1). All eight studies assessed cytokine levels in chronic mTBI, and two assessed cytokine levels in acute mTBI.

a) IL-6

Five of the eight included studies assessed IL-6 levels within the mTBI population Table 1) [26,34,37–39].

Kanefsky et al. (2019) demonstrated increased prevalence of adverse emotional outcomes (PTSD $p< 0.001$; depression $p = 0.001$) in mTBI patients, in comparison to non-mTBI controls. It was also observed that chronic mTBI patients with loss of consciousness (LOC) had significantly elevated IL-6 levels compared to those without LOC and non-mTBI controls.This study controlled for factors affecting both inflammatory cytokine levels and psychiatric conditions, rendering results more reliable [38].

Peskind et al. (2015) and Guedes et al. (2020), did not detect any variations in plasma IL-6 levels between mTBI and non-mTBI military veterans. However, both studies noted increased PTSD and depression prevalence among mTBI individuals compared to non-mTBI individuals. Neither of the two studies controlled for inflammatory conditions [34,37]. Guedes et al. (2020) discovered a mild correlation between exosomal IL-6 levels and PTSD ($p = 0.08$), despite controlling for confounding psychiatric variables [37]. Interestingly, Peskind et al. (2015), noted that CSF IL-6 levels were upregulated in military veterans (both mTBI and non-mTBI) in comparison to community controls, indicating prevalence of increased inflammation among military personnel [34].

Gill et al. (2018) did not find discrepancies in IL-6 levels within neuronal derived exosomes between mTBI and non-mTBI military personnel, despite controlling for inflammatory conditions [26].

Vedantam et al. (2021) found significantly upregulated plasma IL-6 levels within mTBI patients when compared to orthopedic injury (OI) controls, both at 24 h ($p = 0.01$)

and six months post-injury (p = 0.044) [39]. However, the regression model did not demonstrate any statistically significant association between elevated IL-6 levels and emotional outcomes across mTBI population. This study controlled for confounding psychiatric conditions.

b) TNF-α

In this systematic review, four of the eight included studies assessed TNF-α levels in mTBI populations [26,37–39]. However, none of these studies found any significant variations in systemic TNF-α levels between mTBI and non-mTBI groups. Two of these four studies controlled for both confounding inflammatory and psychiatric variables [26,3].

Guedes et al. (2020) together with Kanefsky et al. (2021) found statistically significant findings regarding TNF-α levels and adverse psychological outcome(s) within the mTBI population [37,38]. Guedes et al. (2020) found that chronically elevated plasma TNF-α levels correlated weakly with PTSD (r = −0.2267, p = 0.0255), after controlling for confounding psychiatric variables [37]. These findings were supported by Kanefsky et al. (2020), who found that there is a link between chronic mTBI and PTSD symptoms through TNF-α after controlling for factors affecting both inflammatory cytokine levels and psychiatric conditions [38].

c) IL-10

In this review, four of the eight studies assessed IL-10 levels within mTBI populations [26,37–39]. Out of these, two studies found a statistically significant relationship between IL-10 levels and emotional symptoms [26,39].

Gill et al. (2018) found elevated IL-10 levels in patients with at least one mTBI event, in comparison to healthy controls. There was also a significant relationship between upregulated IL-10 levels and PTSD (B = 0.8, t = 2.60, p< 0.01), and a weak relationship between IL-10 levels and depression (B = 0.421, t = 1.41, p = 0.063) within mTBI populations. This study controlled for factors affecting both inflammatory cytokine levels and psychiatric conditions [26].

These findings were supported by Vedantam et al. (2021), who showed that elevated IL-10 levels at six months post-injury were significantly correlated with depression (p = 0.001) and with more severe PTSD symptoms (p = 0.004) [39]. However, this study was unable to find significant variations in plasma IL-10 levels between mTBI patients and orthopedic injury controls, both at 24 h and six months post-injury (p> 0.05). The lack of discrepancies in IL-10 levels between the two populations, however, could be attributed to the ability of both mTBI and OI to cause inflammation, as well as the absence of controls for confounding inflammatory variables.

Guedes et al. (2020) and Kanefsky et al. (2021), on the other hand, did not find any significant link between IL-10 levels with mTBI and emotional symptoms [37,38].

d) CRP

Two of the eight studies that this study reviewed assessed CRP levels in mTBI populations [36,40].

Ghai et al. (2020) found significantly elevated CRP levels in chronic mTBI veterans in comparison to controls (non-mTBI veterans and community controls) [40]. MTBI veterans also had significantly greater co-morbid PTSD and depressive symptoms, in comparison to controls. This study did not control for confounding inflammatory conditions, though datasets were controlled for psychiatric variables.

Su et al. (2014) also found that elevated baseline CRP levels were associated with an upregulated risk of persistent symptoms (2.72; 95% CI: 1.61–4.59), persistent psychological issues (1.54; 95% CI: 1.06–2.22), and persistent cognitive impairments (1.69; 95% CI: 1.14–2.51) within the mTBI population. However, no non-mTBI controls were included in this study [36].

e) IL-1β

In this review, two of the eight included studies assessed IL-1β levels in mTBI populations (Table 1) [35,39]. Bellgowan et al. (2012) revealed that IL-1β levels were significantly elevated at 24–48 h post-mTBI, in comparison to either one week ($p< 0.01$) or one-month post-mTBI ($p< 0.05$) [35]. There was no variation in IL-1β levels between one week and one-month post-mTBI. It was also observed that both depressive and anxiety ratings were significantly upregulated at 24–48 h and one week ($t[7] = –3.59$; $p<$

0.01; $t[7] = -2.51$; $p < 0.05$; respectively), though not at one month post-mTBI. Since there were no non-mTBI controls used in this study, no conclusion can be placed regarding how IL-1β levels in mTBI differ from those of controls.

Vedantam et al. (2021) found no significant differences between IL-1β levels, both at 24 h and at six months post-mTBI, in comparison to orthopedic controls. The relationship between IL-1β levels and emotional symptoms was not made clear in this study [35,39].

f) IL-7

Peskind et al. (2015) assessed CSF IL-7 in mTBI populations. In comparison to deployed control veterans, mTBI veterans had greater post-concussive symptoms, combat exposure, PTSD, depression, sleep disturbance, and alcohol use. CSF IL-7 was elevated in mTBI veterans, in comparison to deployed and community controls (15.762.7 [SEM], 8.563.2, and 8.364.4 pg/mL ($p < 0.03$) [34].

4.5. Discussion

This study found evidence supporting the association between upregulated cytokine levels (IL-6, TNF-α, IL-10, CRP, and IL-1β) and adverse psychological outcomes in mTBI patients.

Previously, our group reviewed evidence that individuals with systemic injuries developed near-identical symptoms to mTBI patients [9]. Since mTBIs are associated with cerebral inflammatory responses [41] that are responsible for post-mTBI symptoms, we

hypothesized that circulating inflammatory mediators similarly affected the brain following systemic injury. According to our previous review, IL-6, IFN-α, and TNF-α and CRP were associated with depression and anxiety. Furthermore, irritability was found to be associated with TNF-α and IL-1β[9]. The results of this current review align with our previously proposed hypothesis.

IL-6 acts as a regulator of inflammatory procedures by inducing either a pro- or anti-inflammatory response [11]. IL-6 levels are typically upregulated shortly after a mTBI event [21,22] can remain elevated for weeks or months following such an injury [18,23]. Acutely elevated IL-6 levels are associated with greater symptom duration and severity in mTBI [17,21,38]. One study, however, does not support such an association [42]. This discrepancy could be attributed to population differences, as Di Battista et al., (2020) assessed cytokine levels in both males and females whereas others did so in a predominantly male population. Elevated IL-6 levels are also seen in psychological conditions, even in the absence of head trauma. For example, PTSD patients have elevated IL-6 (42% higher, $p = 0.02$) levels that are significantly related to condition severity [43]. Similarly, significantly upregulated IL-6 levels are observed in depressed and anxious populations in comparison to controls [44-47]. In this review, only two of the five studies assessing IL-6 levels found upregulated levels in mTBI patient populations compared to controls [38-39]. However, it is evident that most of the included studies assessing IL-6 did not control for the confounding inflammatory conditions or treatments that could affect IL-6 levels. Furthermore, three out of these five studies showed that mTBI populations have increased depression and PTSD symptoms in comparison to non-mTBI populations, out

of which only two controlled for confounding psychiatric conditions [34,37,38]. One study did not show any statistically significant relationship between elevated IL-6 levels and emotional outcome in mTBI population [39]. Overall, based on the available literature, elevated IL-6 levels are associated with PTSD in the chronic mTBI population. However, more research is needed to further explore this association.

TNF-α is a pro-inflammatory cytokine associated with the neuroinflammatory responses following an mTBI event [11]. TNF-α levels are elevated in both acute [20,25] and chronic [20,23,25] inflammation, following mTBI. In children, acutely elevated TNF-α proteomic expression at 1–4 days post-mTBI (p = 0.031) is associated with persisting symptoms [48]. Elevated TNF-α levels are associated with poor psychological health even in the absence of an mTBI event [49]. PTSD and depression are consistently seen to be associated with elevated TNF-α levels, in comparison to healthy controls [50-55]. Increased serum TNF-α levels positively correlate with increased anxiety and/or depression [56].In this systematic review, we were unable to find any evidence of significant variations in systemic TNF-α levels between mTBI and non-mTBI groups having emotional symptoms. However, we found that elevated TNF-α levels within mTBI population is associated with adverse psychological outcome(s) [37,38]. In conclusion, elevated TNF-α levels are associated with PTSD in chronic stages especially in the male-predominant mTBI population.

IL-10 is a prominent anti-inflammatory cytokine detected in both acute and chronic stages of mTBI [20,37]. In the absence of a head injury, the relation between IL-10 and psychological outcomes, PTSD, and depression is inconclusive [57,58]. For example,

one study demonstrated reduced serum IL-10 levels in depressed patients in comparison to non-depressed patients [59], though other studies identified upregulation in serum IL-10 level within depressed patients [59-61]. Similarly, studies have found both significantly elevated and depressed IL-10 levels in PTSD patients in comparison to control groups [62–64]. Overall, this review supports the finding that elevated IL-10 levels seen in chronic mTBI patients are associated with PTSD and depression. This could be seen as a compensatory mechanism for increased inflammation observed following a mTBI [26].

CRP is a non-specific marker of systemic inflammation. Following a mTBI event, CRP levels increase for several days before gradually declining, potentially over weeks [65]. Elevated levels of serum CRP, specifically within the first 24 h of a mTBI event, are associated with greater injury severity [36]. High-sensitivity serum CRP levels within two weeks of an mTBI event are prognostic biomarkers for potential disability six months later [65,66]. Elevated CRP levels are also seen to be associated with psychological impairments, even in the absence of a mTBI event [67]. Patients with PTSD exhibit significantly upregulated CRP levels in comparison to those who did not meet the clinical criteria for PTSD [68,69]. Similarly, patients with depression and anxiety have upregulated CRP levels in comparison to controls [67,70,71]. Overall from this review, it is evident that elevated CRP levels in the mTBI patient population are positively associated with depression and PTSD. Additional research is required to further explore this correlation.

IL-1βlevels are acutely elevated following a mTBI [19,20]. Elevated IL-1βlevels are frequently associated with emotional symptoms even without mTBI [72,73]. Based on the

current evidence, acutely elevated IL-1βlevels are associated with adverse emotional outcomes in mTBI patients. However, more studies are needed to further support this.

4.6. Limitations

The limitations of this systematic review arise from the quality of the evidence available regarding this topic, as all the included studies had level IV evidence. The study design, comparative groups, biomarkers investigated, and variations in psychological assessments contribute to heterogeneity. Moreover, incomplete documentation of data (for example, specific levels of biomarkers, psychological scores, and certain outcomes) for the population of interest limited our ability to ascertain the influence of several biomarkers on specific psychological outcomes.

Medications, autoimmune disorders, and other comorbidities (e.g., metabolic syndrome, type II diabetes) affecting the immune system are some of the factors that can impact the levels of cytokines within the investigated patient populations. Although some studies included in this review accounted for these factors through their exclusion criteria, most of the studies did not.

A very large percentage of the study population, across all included studies, were male. Future studies should aim to include more female participants so that the samples can be more representative of the mTBI population.

Furthermore, there are variations between the included studies regarding the time of cytokine assessment post-injury. Most studies included assessed participants in the chronic stages (from one month and up to multiple years), and it is not clear what

elevations of cytokines in the acute stage of mTBI are associated with increased emotional symptoms. The specific times of sample collection post-injury should be reported in future studies. In addition, the scales used to assess the psychological outcomes should be more standardized in order to reduce data heterogeneity.

Despite these limitations, the datasets from this study do support an association between emotional symptoms and neuroinflammation in the mTBI population.

4.7. Conclusions

There is a positive correlation between elevated IL-6 and TNF-α levels and PTSD in chronic mTBI patient population. Similarly, elevated IL-10 and CRP levels are associated with PTSD and depression in chronic mTBI population. However, it is challenging to ascertain a clear relationship between individual inflammatory cytokines and specific psychological outcomes in mTBI population, due to the heterogeneity in biospecimens analyzed, time of cytokine assessment post-injury, outcome assessments, comparative groups, lack of female participants, and control for confounding factors. Additionally, more standardized protocols for cytokine and psychological outcome analysis should be utilized in future mTBI studies to allow for a clearer understanding of the relationships studied. Measurement of these cytokines can prove useful as biomarkers to identify patients at risk of emotional symptoms following a mTBI event, prompting early rehabilitation and hence expediting patient recovery.

4.8. Clinical Significance

Chronically elevated IL-6 and TNFα levels following mTBI could be used to identify patients at risk of developing PTSD. Similarly, chronically elevated IL-10 and CRP levels could help identify mTBI patients at risk of developing depression and PTSD. Early detection of patient populations at risk of poor emotional outcome(s) would help clinicians plan early rehabilitation to mitigate losses.

Supplementary Table S1: Search Strategy for Depression

EMBASE	**MEDLINE**	**PUBMED**	**COCHRANE**	**PSYCHNFO**
Strategy: 1. exp inflammation/ or inflammat*.mp. 2. exp cytokine/ or cytokine*.mp. 3. exp neuroinflammation/ or neuroinflammat*.mp. 4. 1 or 2 or 3 5. mtbi.mp. 6. exp brain concussion/ or exp concussion/ or concussion.mp. 7. exp mild traumatic brain injury/ or mild traumatic brain injur*.mp. 8. 5 or 6 or 7 9. exp depression/ or depression.mp. or exp long term depression/ or exp major depression/ 10. 4 and 8 and 9 11. limit 11 to (human and english language).	**Strategy**: 1. exp inflammation/ or inflammat*.mp. 2. exp cytokine/ or cytokine*.mp. 3. exp neuroinflammation/ or neuroinflammat*.mp. 4. 1 or 2 or 3 5. mtbi.mp. 6. exp brain concussion/ or exp concussion/ or concussion.mp. 7. exp mild traumatic brain injury/ or mild traumatic brain injur*.mp. 8. 5 or 6 or 7 9. exp depression/ or depression.mp. or exp long term depression/ or exp major depression/ 10. 4 and 8 and 9 11. limit 11 to (human and english language).	**Strategy**: ((depression) AND ((mtbi) OR (concussion) OR (brain concussion)OR (mild traumatic brain injur*)) AND ((inflammat*) OR (cytokine*) OR (neuroinflammat*))	**Strategy:** ((depression) AND ((mtbi) OR (concussion) OR (brain concussion)OR (mild traumatic brain injur*)) AND ((inflammat*) OR (cytokine*) OR (neuroinflammat*))	**Strategy:** 1. exp inflammation/ or inflammat*.mp. 2. exp cytokine/ or cytokine*.mp. 3. exp neuroinflammation/ or neuroinflammat*.mp. 4. 1 or 2 or 3 5. mtbi.mp. 6. exp brain concussion/ or exp concussion/ or concussion.mp. 7. exp mild traumatic brain injury/ or mild traumatic brain injur*.mp. 8. 5 or 6 or 7 9. exp depression/ or depression.mp. or exp long term depression/ or exp major depression/ 10. 4 and 8 and 9 11. limit 11 to (human and english language).

Supplementary Table S2: Search Strategy for PTSD

EMBASE	**MEDLINE**	**PUBMED**	**COCHRANE**	**PSYCHNFO**
Strategy: 12. exp inflammation/ or inflammat*.mp. 13. exp cytokine/ or cytokine*.mp. 14. exp neuroinflammation/ or neuroinflammat*.mp. 15. 1 or 2 or 3 16. mtbi.mp. 17. exp brain concussion/ or exp concussion/ or concussion.mp. 18. exp mild traumatic brain injury/ or mild traumatic brain injur*.mp. 19. 5 or 6 or 7 20. ptsd.mp. or exp posttraumatic stress disorder 21. 4 and 8 and 9 22. limit 11 to (human and english language).	**Strategy**: 12. exp inflammation/ or inflammat*.mp. 13. exp cytokine/ or cytokine*.mp. 14. exp neuroinflammation/ or neuroinflammat*.mp. 15. 1 or 2 or 3 16. mtbi.mp. 17. exp brain concussion/ or exp concussion/ or concussion.mp. 18. exp mild traumatic brain injury/ or mild traumatic brain injur*.mp. 19. 5 or 6 or 7 20. ptsd.mp. or exp posttraumatic stress disorder 21. 4 and 8 and 9 22. limit 11 to (human and english language). 23.	**Strategy**: (((PTSD) OR (posttraumatic stress disorder)) AND ((mtbi) OR (concussion) OR (brain concussion)OR (mild traumatic brain injur*)) AND ((inflammat*) OR (cytokine*) OR (neuroinflammat*))	**Strategy:** (((PTSD) OR (posttraumatic stress disorder)) AND ((mtbi) OR (concussion) OR (brain concussion)OR (mild traumatic brain injur*)) AND ((inflammat*) OR (cytokine*) OR (neuroinflammat*))	**Strategy:** 12. exp inflammation/ or inflammat*.mp. 13. exp cytokine/ or cytokine*.mp. 14. exp neuroinflammation/ or neuroinflammat*.mp. 15. 1 or 2 or 3 16. mtbi.mp. 17. exp brain concussion/ or exp concussion/ or concussion.mp. 18. exp mild traumatic brain injury/ or mild traumatic brain injur*.mp. 19. 5 or 6 or 7 20. ptsd.mp. or exp posttraumatic stress disorder 21. 4 and 8 and 9 22. limit 11 to (human and english language). **23.**

Supplementary Table S3: Search Strategy for Anxiety

EMBASE	MEDLINE	PUBMED	COCHRANE	PSYCHNFO
Strategy: 23. exp inflammation/ or inflammat*.mp. 24. exp cytokine/ or cytokine*.mp. 25. exp neuroinflammation/ or neuroinflammat*.mp. 26. 1 or 2 or 3 27. mtbi.mp. 28. exp brain concussion/ or exp concussion/ or concussion.mp. 29. exp mild traumatic brain injury/ or mild traumatic brain injur*.mp. 30. 5 or 6 or 7 31. anxiety.mp. or exp anxiety disorder/ or exp anxiety/ 32. 4 and 8 and 9 33. limit 11 to (human and english language).	**Strategy**: 24. exp inflammation/ or inflammat*.mp. 25. exp cytokine/ or cytokine*.mp. 26. exp neuroinflammation/ or neuroinflammat*.mp. 27. 1 or 2 or 3 28. mtbi.mp. 29. exp brain concussion/ or exp concussion/ or concussion.mp. 30. exp mild traumatic brain injury/ or mild traumatic brain injur*.mp. 31. 5 or 6 or 7 32. anxiety.mp. or exp anxiety disorder/ or exp anxiety/ 33. 4 and 8 and 9 34. limit 11 to (human and english language).	**Strategy**: (((anxiety) OR (anxiety disorder)) AND ((mtbi) OR (concussion) OR (brain concussion)OR (mild traumatic brain injur*)) AND ((inflammat*) OR (cytokine*) OR (neuroinflammat*))	**Strategy:** (((anxiety) OR (anxiety disorder)) AND ((mtbi) OR (concussion) OR (brain concussion)OR (mild traumatic brain injur*)) AND ((inflammat*) OR (cytokine*) OR (neuroinflammat*))	**Strategy:** 24. exp inflammation/ or inflammat*.mp. 25. exp cytokine/ or cytokine*.mp. 26. exp neuroinflammation/ or neuroinflammat*.mp. 27. 1 or 2 or 3 28. mtbi.mp. 29. exp brain concussion/ or exp concussion/ or concussion.mp. 30. exp mild traumatic brain injury/ or mild traumatic brain injur*.mp. 31. 5 or 6 or 7 32. anxiety.mp. or exp anxiety disorder/ or exp anxiety/ 33. 4 and 8 and 9 34. limit 11 to (human and english language).

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Chapter 5

5.1. Summary of Main Findings

mTBIs are referred to as “silent epidemic” because of their high incidence but frequent underdiagnosis [1]. In the last two decades, awareness about concussions has increased dramatically in the general population. It is particularly due to the combined efforts of scientists and mass media geared towards public awareness as well as the long-term effects of concussion observed in athletes. The understanding of the pathophysiology underlying mTBI is slowly evolving. However, it appears that apart from the type and severity of head injury, mTBI outcome relies on a multitude of non-injury factors, unrelated to head trauma.

Previously, we had explored the cause for non-specificity of post-concussive symptoms, based on overlapping pathophysiology seen inhead trauma and many other conditions [2]. In this thesis, our primary goal was to identify the features (clinical characteristics and pathological biomarkers) exclusive to mTBI. This goal was accomplished through multiple studies that not only expanded our current knowledge of mTBI but laid the groundwork for our future studies, particularly for developing a treatment for mTBI.

In Chapter 2, we conducted a retrospective chart review to determine the percentage of head trauma patients presenting with post-concussive symptoms despite not meeting the ACRM (1993) criteria for mTBI diagnosis. The results showed that 20.5% of

head trauma patients, presenting with chronic post-concussive symptoms, do not meet this criterion. Although subthreshold brain injuries are common in the military and sports community, this phenomenon is understudied in the general population Therefore, we conducted a comprehensive retrospective chart review to characterize this head trauma population. We compared the symptomatology between the head trauma patients that meet the threshold for mTBI diagnosis set by the ACRM (1993), to those who do not. However, no symptom-specific differences were found between the two head trauma populations.

Following the publication of our study in May 2023 [3], the ACRM updated their criteria for mTBI diagnosis after 30 years [4]. This new criterion is more inclusive than the previous criteria (1993). The ACRM (2023) criteria not only takes into account the immediate signs of mTBI, but also various other post-concussive symptoms to make an mTBI diagnosis, provided they occur within a certain timeframe and in combination with certain clinical exam and laboratory findings. In addition, if an individual sustains a head injury and presents with at least two of the specified post-concussive symptoms in the absence of any immediate signs, clinical exam, laboratory or imaging findings, that person would be labelled as "suspected mTBI". This has changed the interpretation of our study significantly. According to the ACRM (2023) criteria, our head trauma population that presents with chronic post-concussive symptoms, despite not meeting the ACRM (1993) criteria for mTBI diagnosis, is equivalent to the "Suspected mTBI" population, under ACRM (2023) criteria. This means that an mTBI could not be ruled out in this population, hence explaining the similar presentation in both populations. We had

mentioned in our study that similar symptomology could be due to shortcomings of the ACRM (1993) criteria, and the new updated criteria supports our views[3].

When no symptom-specific differences were found between the two head trauma populations, we further explored an mTBIrelated secondary pathophysiology. We conducted a systematic review and a meta-analysis to compile and analyze the data on inflammatory cytokine levels in populations with mTBI (Chapter 3). We compared the cytokines levels between the populations with and without an mTBI (both healthy and trauma). The results of the systemic review showed significantly elevated IL-6, IL-1RA, IFN-γ levels (at <24 hrs.) and MCP-1/CCL2 levels (within a week) in patientswith mTBI compared to healthy controls. Meta-analysis further supported these findings by demonstrating significantly elevated levels of IL-6, MCP-1/CCL2, and IL-1β levels in patients with mTBI compared to the healthy controls ($p<0.0001$) in acute stages (<7 days). Due to limited number of studies, the results of comparison between the concussed and trauma populations were inconclusive. This study also showed that elevated blood levels of IL-6, TNF-α, IL-1RA, IL-10, and MCP-1/CCL2 were associated with poor prognosis following an mTBI.This research particularly highlighted the lack of consensus between various mTBI studies, showing differences with respect to the time elapsed between the injury and biomarker assessment, type of biospecimen used, method of analysis, outcomes measured, and confounding factors control.

One of the largest factors associated with protracted recovery following mTBI are emotional impairments. Emotional impairments have a significant neuroinflammatory component, similar to the one seen in mTBI. To explore the common pathology, we

conducted a detailed systematic review to identify the inflammatory cytokines commonly associated with psychological symptoms in populations with mTBI (Chapter 4). The results showed that IL-6, TNF-alpha, IL-10, and CRP are commonly associated with poor psychological outcomes (PTSD and/or depression) in populations with mTBI especially in the chronic stages of the illness.

5.2. Clinical Implications:

The implications of the foundational work in this thesis span across various aspects of mTBI symptomatology and pathophysiology. In general, this body of work highlights the link between neuroinflammation and post-concussive symptoms. The significance and clinical implications of this work have been discussed in detail in each specific chapter.

The first chapter of this thesis highlights how there is a significant proportion of head trauma patients with post-concussive symptomatologythat are not captured by the current subjective criteria of mTBI diagnosis. Due to missed diagnosis, such patients do not receive appropriate and timely care, hence resulting in poor clinical outcome and increased burden on the healthcare system and society. Brain injury cannot be ruled out in this population, so clinically this population should be treated or managed in the same way as one would manage the population with diagnosed mTBI. Early rehabilitation in this population would likely mitigate the long-term consequences of mTBI.

Additionally, similar clinical presentation of head trauma patients who meet the mTBI diagnostic criteriaand those who do not, undermine the fact that the brain injury

and its severity alone do not determine the clinical outcome. It is rather a complex interaction of various injury and non-injury factors that shape the prognosis. Clinically, it means that all the known risk factors should be considered while predicting the outcome of patients with head injuries. Extensive research has been done to identify various risk factors that lead to poor recovery. It will be helpful to translate this knowledge into the clinic by identifying patients prone to prolonged recovery. Sage et al (2021) has proposed a scoring system to estimate an individual's risk of poor recovery and to manage patients. It would be useful to incorporate a scoring system such as this one in an acute care setting, particularly in the ER or urgent care clinics, particularly in the ER or urgent care clinics, to identify the vulnerable population and to manage them adequately to mitigate the long-term consequences of mTBI [5].

Our research has also identified several cytokines in the blood that are frequently associated with mTBI, particularly in the acute stages. We have also found a several cytokines in the blood that are consistently associated with adverse clinical outcomes in mTBI. Clinically, these cytokines could be utilized in combination with other established neuronal injury markers to identify tpatients at risk of poor recovery, monitor prognosis and treatment efficacy, and to identify patients in need of specialized care.

Lastly, we explored a potential link between mTBI pathophysiology (neuroinflammation) and poor recovery (emotional impairments). This study shows that chronically elevated IL-6 and TNF-alpha levels are associated with PTSD whereas chronically elevated IL-10 and CRP levels are associated with depression and PTSD in mTBI patients. Clinically, this information can be useful to identify mTBI patients at risk

for PTSD and depression. Early identification of such patients could help clinicians start targeted rehabilitation therapies earlier, helping prevent long-term consequences related to the emotional impairments which are associated with mTBI.

5.3. Future Directions

The knowledge obtained from this thesis provides directions for future studies that have been discussed in detail in each specific chapter.

The similarities between the head trauma group that does not reach the threshold for mTBI diagnosis according to the current standard diagnostic criteria and the mTBI group make it quite evident that the subthreshold mTBI group must be further studied. As such, all current mTBI diagnostic criteria need to be revisited and updated to capture this population. As mentioned earlier, the ACRM has recently updated the criteria for mTBI diagnosis. One of the major changes is incorporation of objective testing, including biomarker assessment, for mTBI diagnosis. This is an important development in the field of mTBI diagnostics that will increase the specificity of mTBI diagnosis, a problem that has long been overlooked. Despite many developments, a standardized biomarker for mTBI diagnosis has not yet been found. Future research needs to focus on exploring standardized objective markers for concussion diagnosis. These not only include blood-based biomarkers alone but also many radiological markers.

This thesis also highlights the lack of homogeneity among various mTBI studies, particularly with regards to cytokine data reporting. There are vast differences with respect to the time elapsed between the injury and biomarker assessment, type of

biospecimen used, assay technique, method of analysis, outcomes measured, and confounding factors control. Future studies should adopt a standardized approach for data reporting. This would make it easier to pool and analyze data to make meaningful inferences.

This work also draws attention to the fact that although post-concussive symptoms are observed predominantly in females, most of the current mTBI research data is obtained from males. This is because majority of mTBI studies are performed in male-predominant sports and military populations. Future studies should also explore general population and include more female participants to avoid this bias.

Additionally, a quantifiable system or protocol should be incorporated in an acute clinical setting to identify the population at risk of poor recovery after a head trauma. Once identified, tailored treatment options should be made available to this population early on to prevent persistent symptoms. Apart from symptomatic treatment, other therapies that target chronic inflammation should be explored and implemented.

5.4. Concluding Statement

The pathophysiology of mTBI is extremely complex and the journey to fully understanding all the aspects of its pathophysiology is not yet complete. However, with each research development, we become closer to understanding the pathophysiology of mTBI. We hope that this work on mild traumatic brain injury and associated neuroinflammation provides a valuable contribution to the growing body of literature.

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