Review

Inflammatory Biomarkers in mTBI

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Inflammatory Biomarkers in mTBI

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Abstract: Mild TBI (mTBI) accounts for most TBI cases, the leading cause of morbidity and mortality worldwide. Despite their high incidence, mTBI pathophysiology remains largely unknown. Recent studies have shown that the inflammatory response is activated early after mTBI and can persist for several weeks or months. However, limited evidence on the utility of inflammatory biomarkers as predictors of clinical outcomes in mTBI was previously provided. Thus, this study aims to provide an overview of the current knowledge on the role of inflammation in the pathogenesis of mTBI and the potential of some inflammatory biomolecules as biomarkers of mTBI. Thus, it was shown that the increase of IL-6, TNF-α, and IL-1β plasma levels could be implicated in the development of the early post-concussion symptoms. On the other hand, the persistence of the increased plasmatic concentrations of IL-10 and IL-8 for as long as six months following the brain injury event could suggest chronic inflammation leading to neuroinflammation and late or persistent symptoms. In this context, our findings showed that the inflammatory biomarkers could be relevant in diagnosing or predicting recovery or long-term outcomes of mTBI.

Keywords: mild traumatic brain injury; post-concussion syndrome; neuroinflammation; pro-inflammatory cytokines; diagnosis; outcomes; recovery

1. Introduction

Traumatic brain injury (TBI) is a leading cause of morbidity and mortality worldwide. Mild TBI (mTBI), also known as concussion, accounts for most TBI cases and is characterized by a transient alteration of consciousness or cognitive function. Despite the high incidence of mTBI, its pathophysiology remains largely unknown. In recent years, there has been a growing interest in the role of inflammation in the pathogenesis of mTBI [1]. The prevalence of concussion varies across different populations and settings. In the general population, the estimated prevalence of concussion ranges from 1.6% to 3.8% [2] with higher values in specific high-risk populations, such as athletes and military personnel [3,4]. The prevalence of concussions in high school athletes has been estimated to be as high as 11.2% [5].

The inflammatory response is a complex process that involves the activation of various cell types, including microglia and astrocytes, and the release of a variety of pro-inflammatory and anti-inflammatory mediators. Studies have shown that the inflammatory response is activated early after mTBI and can persist for several weeks or months. Several inflammatory biomarkers have been proposed as potential markers of mTBI, including interleukin-1 (IL-1), interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), and C-reactive protein (CRP). Recent studies showed that these molecules could be found in increased concentrations in serum, cerebrospinal fluid (CSF), and saliva of the individuals that have undergone mTBI, in a severity and outcome dependent manner [6–8]. While inflammation is characterized by the activation of immune cells and the release of inflammatory mediators, such as cytokines and chemokines [9], in the context of concussion, it has

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been proposed as a possible contributor to the pathophysiology of the injury and persistence of symptoms in some individuals [10].

Several studies have investigated the levels of inflammatory biomarkers in individuals with concussions and reported controverted results. While some studies reported increased levels of cytokines (IL-6 and TNF-α) in the serum or CSF [7,8], others did not observe significant differences in concussed individuals, as compared to age and sex-matched controls [11,12]. In this context, limited evidence on the utility of inflammatory biomarkers as predictors of clinical outcomes in concussion was provided. Several studies suggested that higher levels of inflammatory biomarkers may be associated with worse clinical outcomes, such as prolonged recovery or persistent symptoms [8,13], whereas other studies failed to confirm this association [11,12].

Thus, this study aims to provide an overview of the current knowledge on the role of inflammation in the pathogenesis of mTBI and the potential of some inflammatory biomolecules as biomarkers of mTBI. While summarizing the studies investigating inflammatory biomarkers in mTBI in diagnosis, prognosis, therapy, we will also discuss the limitations of the current literature and future directions for research in this field.

2. Materials and Methods

The aim of this meta-analysis was to examine the evidence on the association between inflammatory biomarkers and mTBI, as well as the potential utility of inflammatory biomarkers as diagnosis and prognosis tools in mTBI.

2.1. Search strategy

A systematic literature search was conducted using the PubMed, Embase and Cochrane Library databases. The search was limited to articles published in English from January 2000 to December 2021. The search terms used were "mild traumatic brain injury" OR "concussion" AND "inflammatory biomarkers" OR "cytokines" OR "chemokines" OR "CRP" OR "IL-1" OR "IL-6" OR "TNF-alpha". The reference lists of identified studies were also searched for additional relevant studies.

2.2. Selection criteria

Inclusion criteria for this meta-analysis were: (1) observational or interventional studies that examined the association between inflammatory biomarkers and mTBI or concussion; (2) studies that measured inflammatory biomarkers in serum or plasma; (3) studies that were written in English.

The exclusion criteria were considered: (1) case reports, case series, or reviews; (2) studies that did not measure inflammatory biomarkers in serum or plasma; and (3) studies that were not written in English or that were not available in full text.

2.3. Data extraction and quality assessment

Two reviewers independently screened the abstracts and full-text articles for eligibility and extracted data from eligible studies. Any discrepancies were resolved by mutual consensus. The following data were extracted from each study: first author, year of publication, study design, sample size, inflammatory biomarkers measured, and main findings.

The quality of the included studies was assessed using the Newcastle-Ottawa Scale (NOS) for observational studies and the Cochrane Risk of Bias tool for randomized controlled trials (RCTs).

2.4. Data synthesis and analysis

Descriptive statistics were used to summarize the characteristics of the included studies. The effect sizes of the associations between inflammatory biomarkers and mTBI or concussion were calculated using standardized mean differences (SMDs) and 95% confidence intervals (CIs). Heterogeneity among the studies was assessed using the I2 statistic. Subgroup analyses were conducted based on the type of inflammatory biomarker (cytokine, chemokine, or other) and the study design (observational or RCT).

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Statistical analysis was conducted using R software (version 3.6.2). A random-effects model was used to calculate the overall effect sizes due to the expected heterogeneity among the studies. The level of statistical significance was set at $p < 0.05$.

Publication bias was assessed using Egger's test and funnel plots. Sensitivity analyses were conducted by excluding studies with a high risk of bias and using a fixed-effects model.

3. Results

3.1. Overview of the studies included in the present meta-analysis

The initial search provided 92 studies. After excluding duplicates, 61 studies were included in the initial screening procedure. 40 publications were excluded because they did not fulfil the inclusion criteria, and 21 studies were included in the final screening procedure. Eight studies were finally included in the meta-analysis (Figure 1). There were no significant concerns about publication bias and the quality of the studies (Figures 2A,B).

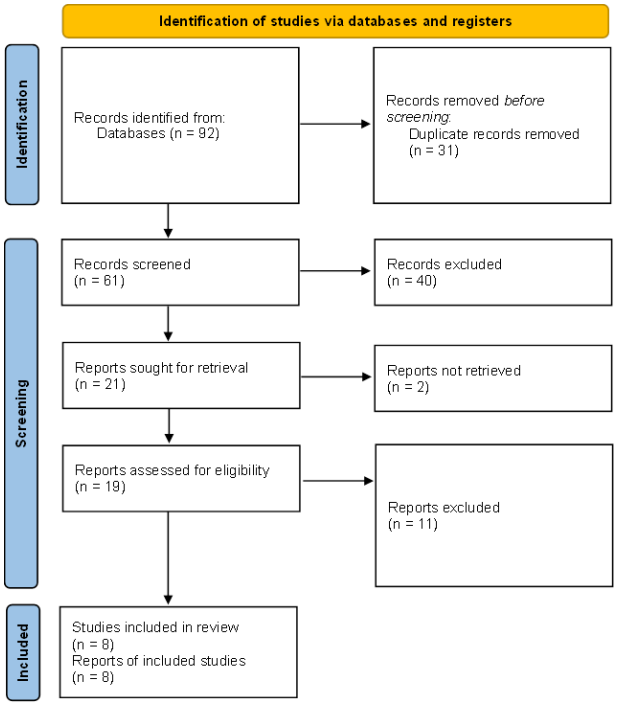

Figure 1. PRISMA 2020 flow diagram for new systematic reviews which included searches of databases and registers only; according to the guidelines provided by [14].

Parkin et al. [15] analyzed changes in blood protein expression after pediatric mTBI using a multiplex immunoassay. The study compared patients with normal recovery (n = 9) with those with persisting symptoms (n = 9) and measured the blood protein expression of apolipoprotein, enolase 2, GFAP, IL-1β, IL-6, IL-8, IL-10, S100B, tau, and TNF-α at admission, one to four days, two weeks, and three months post-injury. The results of the study showed that there were significant differences in IL-6 ($p < 0.001$) and tau ($p = 0.048$) protein expression across time post-injury irrespective of clinical outcome. Additionally, IL-8 protein expression significantly differed across time post-injury in children with persisting symptoms ($p = 0.041$). The study also identified an increase in TNF-α expression at one to four days post-injury in children with persisting symptoms, as compared with normal recovery ($p = 0.031$) [15].

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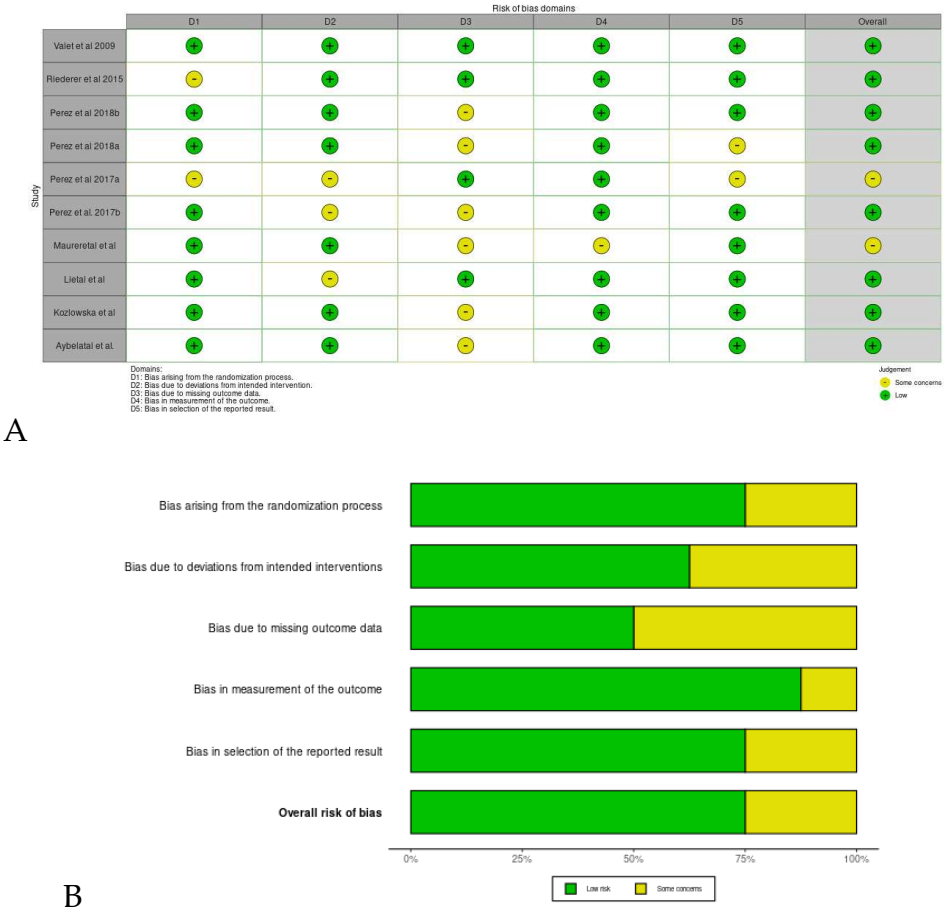

Figure 2. The analysis of publication bias (A) and the quality of the studies (B).

Rodney et al. [16] investigated the relationship between repetitive TBIs and inflammation in military personnel and veterans. The study included a cohort of 106 participants with a history of TBI, grouped into those with repetitive TBIs (more than 3), those with 1-2 TBIs, and controls with no TBIs. The primary outcomes were the association between serum levels of inflammatory-related proteins (TNF-α, IL-6, and IL-10), TBI history, and PTSD symptoms. They found that IL-6 mean concentration was significantly higher in the repetitive TBI group compared to those with 1-2 TBI or no TBI history. In addition, for participants with a history of TBI, PTSD symptom severity, intrusion and avoidance were significant predictors of higher IL-6 and IL-10 concentrations, respectively. They concluded that repetitive TBIs concurrent with high PTSD symptoms in military personnel and veterans are associated with chronic inflammation, particularly elevated concentrations of IL-6 [16].

Thompson et al. [17] compared plasma inflammatory biomarkers concentrations up to 6 months in young and older adults with and without mTBI. Their study included a prospective cohort of younger (21 - 54 years) and older (55 + years) adults diagnosed with mTBI and age/sex-matched non-injured controls (n = 313). They used multiplex assays to quantify concentrations of selected plasma inflammatory markers at Day 0, Month 1, and 6. The main measures were the concentrations of plasma inflammatory markers. This study showed persistent ageing-related differences between control groups in concentrations of four cytokines for up to 6 months. At Day 0, IL-6, IL-8, and fractalkine were higher in the older TBI group compared to the older control and the younger TBI groups, while IL-10 was higher in the older TBI compared to controls. At Month 1, significantly higher concentrations of IL-8, fractalkine, and TNF-α were seen. At six months post-injury, significantly higher concentrations of IL-6 and IL-8 were seen, while a lower concentration of IL-7 was found in older versus younger TBI groups. They concluded that the neuroinflammatory signature accompanying mTBI in older adults differs from that of younger adults. They also

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suggested that the differences seen are notable for their roles in neutrophil attraction (IL-8), neuronal-microglial-immune cell interactions (fractalkine), and chronic inflammation (IL-6) [17].

Powell et al. [18] investigated the effects of mTBI history, lifetime mTBI incidence, and recency on blood biomarker concentrations of axonal protein neurofilament light (NfL), glycolytic enzyme neuron-specific enolase (NSE), astrocyte-expressed S100 calcium-binding protein B (S100B), and neurotrophic cytokine interleukin-6 (IL-6) in healthy, active-duty Special Operations Forces combat soldiers. They found that soldiers with mTBI history had higher NSE concentrations than those without, and lifetime mTBI incidence had significant main effects on NSE and S100B concentrations. Additionally, mTBI recency had a significant main effect on NfL concentration [18].

Sun et al. [19] investigated the relationship between the changes in inflammation-related cytokine levels and cognitive consequences in individuals with mTBI. Their study included two cohorts of individuals with mTBI and matched healthy controls that were followed up at 1- and 3-months post-injury. Cognitive assessments and a 9-plex panel of serum cytokines (IL-1β, IL-4, IL-6, IL-8, IL-10, IL-12, chemokine (C-C motif) ligand 2 CCL2, IFN-γ, and TNF-α) were measured. The results showed that the levels of IL-1β, IL-6, and CCL2 were acutely elevated in mTBI patients, as compared to controls. The increased levels of CCL2 were associated with more severe PCS, while elevated IL-1β was associated with worse working memory in the acute phase. Acute high CCL2 levels predicted higher information processing speed at three months post-injury [19].

Ryan et al. [20] examined immune activation and the alterations in cytokine levels following TBI in children and healthy age-matched controls. The study enrolled 208 children, including those with severe TBI (initial GCS ≤ 8), mild TBI (GCS 14/15), and healthy controls. They found that all children with TBI had increased IL-6 at baseline. The mTBI group had significantly increased IFN-γ versus controls, while in severe TBI, IFN-γ was decreased compared to controls at baseline. At baseline, IL-8, IL-10, IL-17A, and TNF-α were decreased in mTBI compared to controls, which persisted at two weeks post-mTBI. They also found that cytokines increased in severe TBI, especially in response to endotoxin, while suppressed responses were found in mild TBI coupled with persistent immune dysfunction post-injury [20].

Zhao et al. [21] investigated the sex differences in CBF and serum inflammatory cytokines levels in patients with acute stage mTBI. The results showed that while both male and female patients had increased levels of IL-1β and IL-6, female patients demonstrated overexpression of IL-8 and low expression of IL-4. Regarding CBF levels, male patients showed increases in three brain regions, whereas female patients showed decreases in three brain regions. Furthermore, the study found correlations between inflammation levels and CBF in certain brain regions. The findings suggested that females may be more sensitive to inflammatory and CBF changes, and more likely to experience cognitive impairment post-mTBI [21].

Devoto et al. [22] investigated the levels of inflammatory biomarkers (TNF-α, IL-6, and IL-10) in active-duty personnel with and without a history of TBI and assessed the impact of comorbid PTSD on inflammation levels. The study found that IL-6 and TNF-α concentrations were higher in the TBI group than in the control group and that within the TBI group, those with high PTSD had higher levels of IL-6 and TNF-α compared to those with low PTSD. No significant differences were found in IL-10 or the IL-6/IL-10 ratios between those with low and high PTSD. Exploratory factor analysis identified four symptom profiles in the TBI group, with the third component most relating to PTSD and depression symptoms and high inflammation. The study highlighted the association between TBI, PTSD, and inflammation in a military sample, emphasizing the need for interventions to mitigate the associated risks [22].

Vedantam et al. [23] also investigated the plasma levels of inflammatory cytokines and their associations with neuropsychological outcomes in patients with mTBI. The study recruited patients with mTBI and orthopedic injury controls without mTBI from emergency rooms at three trauma centers. Plasma cytokines levels were measured at enrollment and at six months after injury, and neuropsychological tests were performed at one week, one month, three months, and 6 months after the injury. The results showed that levels of IL-2 and IL-6 within 24 hours post-injury were significantly higher for mTBI patients than controls. Increased plasma IL-2 concentrations at 24 hours

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were associated with more severe 1-week post-concussive symptoms. On the other hand, increased plasma IL-10 levels at six months were associated with greater depression and more severe PTSD symptoms. The study concludes that plasma cytokines levels were significantly associated with early and late PCS, PTSD, and depression scores after mTBI, highlighting the potential role of inflammation in the pathophysiology of post-traumatic symptoms after mTBI [23].

Table 1. Summarized description of the studies that were selected for the meta-analysis.

Study	Experimental design	Evaluated biomarkers	Main findings
[15]	pediatric mTBI patients: • normal recovery (n = 9), • persistent symptoms (n = 9).	• TNF-α, • IL-1β, • IL-6, • IL-8, • IL-10, • S100B, • Tau, • Enolase 2, • GFAP.	• significant differences in IL-6 and tau expression following mTBI; • significant differences in IL-8 expression in children with persisting symptoms; • increased TNF-α expression in children with persisting symptoms, as compared with normal recovery.
[16]	106 participants with history of TBI: • repetitive TBI (n = 44), • 1 - 2 TBIs (n = 33), • no TBIs (n = 29).	• TNF-α, • IL-6, • IL-10.	• IL-6 significantly higher in the repetitive TBI group, as compared to the other groups; • higher IL-6 and IL-10 concentrations correlated with PTSD symptoms.
[17]	313 participants: • Young mTBI patients (n = 96); • Older mTBI patients (n = 75); • age/sex-matched non-injured controls (n_y = 80, n_o = 62).	• IL-6, • IL-7, • IL-8, • IL-10, • TNF-α, • Fractalkine.	• increased IL-6, IL-8, and fractalkine in older mTBI patients, as compared to older controls and younger mTBI patients (acute); • increased IL-10 in the older mTBI patients, as compared to their controls (acute); • increased TNF-α in mTBI patients (1-month post-injury); • increased and persistent IL-6 and IL-8 in mTBI patients (6 months post-injury); • decreased IL-7 levels in older mTBI patients, as compared to the younger ones.
[18]	104 active-duty combat soldiers grouped by • mTBI history, • lifetime mTBI incidence.	• NfL, • NSE, • S100B, • IL-6.	• increased NSE concentrations in soldiers with mTBI history, as compared to those without mTBI history; • significant main effects on NSE and S100B concentrations of lifetime mTBI incidence; • significant main effect on NfL concentration of mTBI recurrence.
[19]	• mTBI patients (n = 52, n = 43), • matched healthy control (n = 54).	• IL-1β, • IL-4, • IL-6, • IL-8, • IL-10, • IL-12, • CCL2, • IFN-γ, • TNF-α.	• IL-1β, IL-6, and CCL2 significantly increased in mTBI patients, as compared to controls; • CCL2 increase in direct correlation with PCS severity; • IL-1β increase in inverse correlation with working memory performance.
[20]	208 children • severe TBI (initial GCS ≤ 8) (n = 6), • mTBI (GCS 14/15) (n = 104), • healthy controls (n = 98).	• IL-6 • IL-8, • IL-10, • IL-17A, • TNF-α,	• increased IL-6 in mTBI patients, as compared to controls; • decreased IL-8, IL-10, IL-17A, and TNF-α in mTBI, as compared to controls; • significantly increased IFN-γ in mTBI patients, as compared to controls; • significantly decreased IFN-γ in severe TBI patients, as compared to controls.

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Study	Experimental design	Evaluated biomarkers	Main findings
		• IFN-γ.	
[21]	41 patients with acute stage mTBI: • 17 females, • 24 males; 23 sex-, age-, and education-matched healthy participants: • 13 females, • 10 males.	• IL-1β, • IL-4, • IL-6, • IL-8.	• increased levels of IL-1β and IL-6 after mTBI; • overexpression of IL-8 and low expression of IL-4 in females that had undergone mTBI.
[22]	63 active-duty personnel with and without a history of TBI, 20 age/sex matched controls.	• TNF-α, • IL-6, • IL-10.	• Increased IL-6 and TNF-α in the TBI patients, as compared to controls group; • positive correlation between IL-6 and TNF-α and PTSD severity.
[23]	104 patients with mTBI, 53 orthopedic injury controls	• IL-2, • IL-6, • IL-10.	• increased plasma IL-2 and IL-6 levels in mTBI patients (after 24 h from the injury), as compared to controls; • significant correlation between plasma IL-2 levels (24 h) and 1-week PCS severity; • significant correlation between plasma IL-10 (6 months) and PTSD symptoms severity.

3.2. Meta-Analysis

3.2.1. Interferon-γ

Six studies were included in the meta-analysis on IFN-γ levels in mTBI and PCS, with 546 patients. The statistical analysis showed that the sample was heterogeneous with an I2 of 95.3%, and Q of 107.36, 5 degrees of freedom and a p-value < 0.0001. A random effects model showed an SMD of 24.3, but with no statistical significance (p = 0.21) (Figure 3A). The influence analysis showed that omitting each of the studies and recalculating the SMD was associated with a non-significant result, indicating that IFN-γ levels do not differ significantly between the groups of the study (Figure 3B).

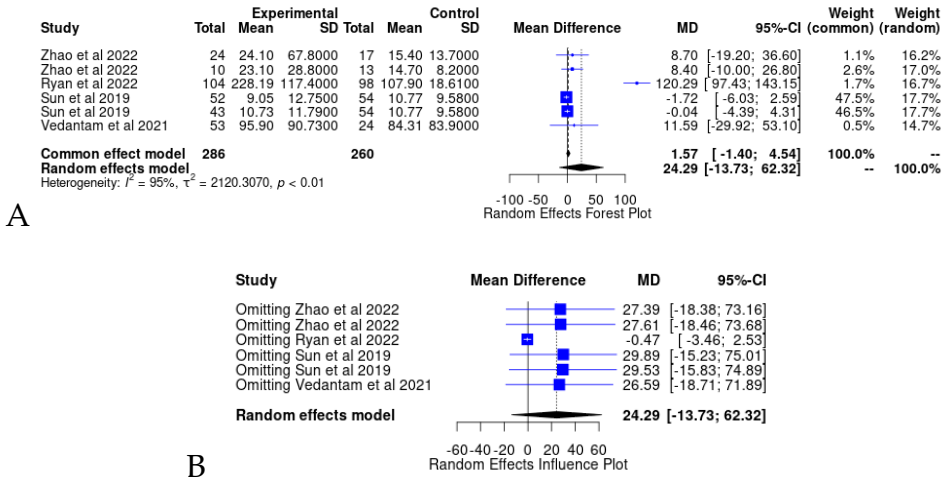

Figure 3. Statistical analysis of the studies included in the meta-analysis on IFN-γ levels in mTBI and PCS (Forest plot – A, effect influence plot – B).

3.2.2. TNF-α

Data from nine studies with 687 participants were combined to analyze TNF-α levels. The heterogeneity of the data was high (I2 = 99.6%, Q = 1926.30, d.f. = 7, p < 0.000001). We used a random

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effects model with an SMD of 1.08 and a p-value of 0.65. This confirmed that there was no statistically significant difference in the levels of mTBI and control patients (Figure 4).

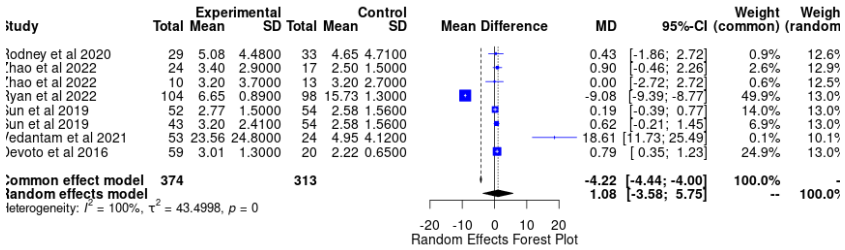


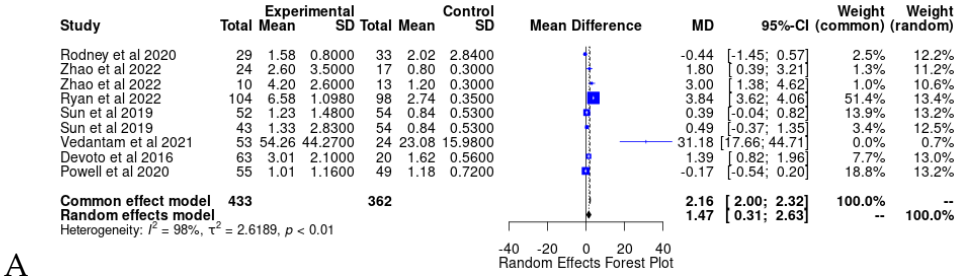
Figure 4. Statistical analysis of the studies included in the meta-analysis on TNF-α levels in mTBI and PCS (Forest plot).

3.2.3. IL-2

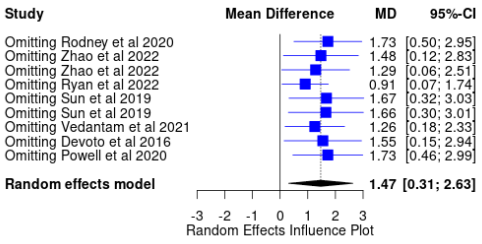
Only one study investigated the difference of IL-2 levels between normal controls and patients with mTBI. Vedantam et al. [23] reported an increase up to 6.93 (s.d. 4.65) in mTBI patients, as compared to the levels of 4.88 (s.d. 3.33) in normal controls.

3.2.4. IL-6

For IL-6 we analyzed data from 9 studies and 795 participants. I2 was 98.4% (Q = 506.71, d.f. = 8, p < 0.0001). The random effects model analysis showed an SMD of 4.47, with a p-value of 0.013 (Figure 5A). Influence analysis showed that the result was robust and remained significant after omitting each of the studies and recalculating the SMD (Figure 5B).



A



B

Figure 5. Statistical analysis of the studies included in the meta-analysis on IL-6 levels in mTBI and PCS (Forest plot – A, effect influence plot – B).

3.2.5. IL-1β

Six studies were included in the analysis of IL-1β levels, with 421 participants. The I2 was 92.7% (Q = 68.27, d.f. = 5, p < 0.0001). The random effects model showed an SMD of 2.7 (p = 0.0072) (Figure 6A). The sensitivity analysis confirmed the robustness of the results, which remained significant after omitting each of the studies (Figure 6B).

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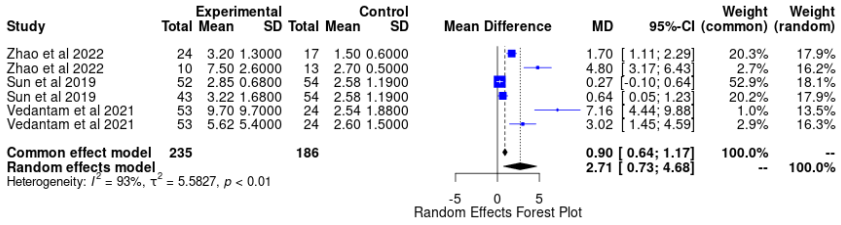
A



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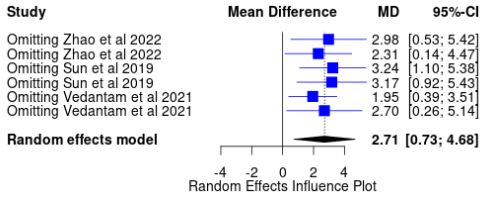
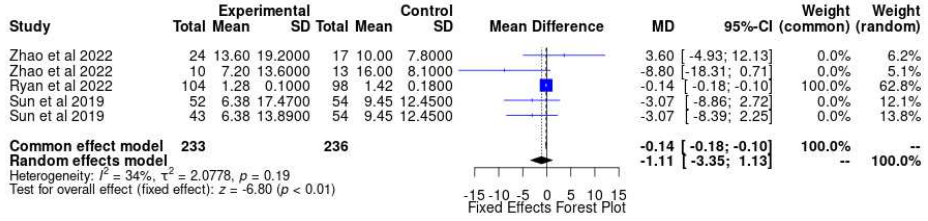


Figure 6. Statistical analysis of the studies included in the meta-analysis on IL-1β levels in mTBI and PCS (Forest plot – A, effect influence plot – B).

3.2.6. IL-4

Data from 5 studies were included in the analysis of IL-4, with 469 participants. The heterogeneity of the sample was low, with an I2 of 34.1% (Q = 6.07, d.f. = 4, p = 0.19). We used a fixed effects model, which showed an SMD of 0.14 (p < 0.0001) (Figure 7A). Sensitivity analysis showed that omitting the study of Ryan et al. [20] changed the SMD significantly, and this study severely influences the final results. This makes the result less robust (Figure 7B).

A



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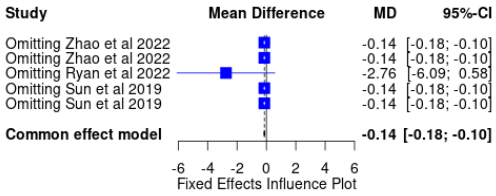

Figure 7. Statistical analysis of the studies included in the meta-analysis on IL-4 levels in mTBI and PCS (Forest plot – A, effect influence plot – B)

3.2.7. IL-8

For IL-8, we analyzed the data from 5 studies with 469 participants. The I2 was 99.9% (Q = 2764,16, d.f. = 4, p < 0.000001), and the random effects model we used showed no significant difference between the groups of the study (SMD = 6.96, p =0.45) (Figure 8A).

3.2.8. IL-10

Interleukin 10 levels were analyzed in 7 studies with 608 participants. The heterogeneity of the data was high (I2 = 94.2%, Q = 103,51, d.f. = 6, p < 0.0001). The random effects model showed no significant difference between the groups of the study (SMD = 0.16, p = 0.53) (Figure 8B).

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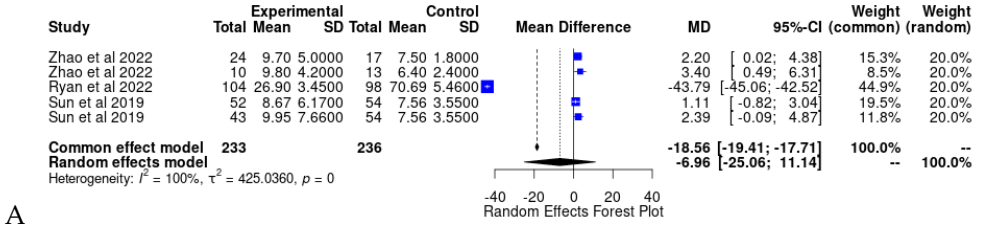

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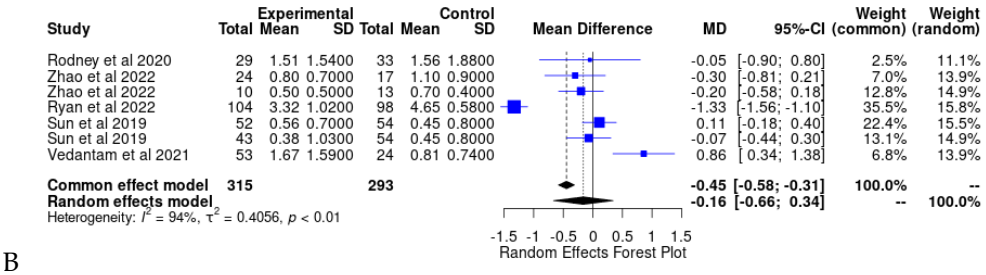


B

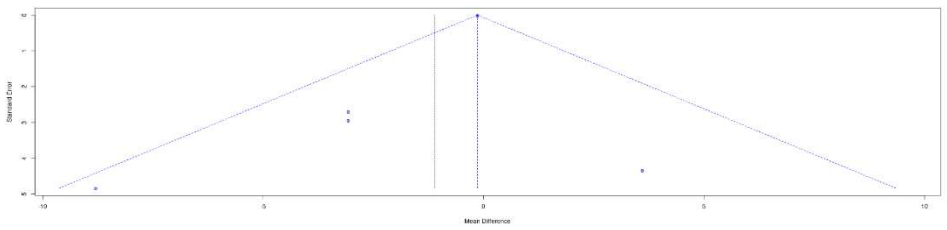
Figure 8. Statistical analysis of the studies included in the meta-analysis (Forest plots) on IL-8 (A) and IL-10 (B) levels in mTBI and PCS

3.3. Persistent PCS and inflammatory biomarkers

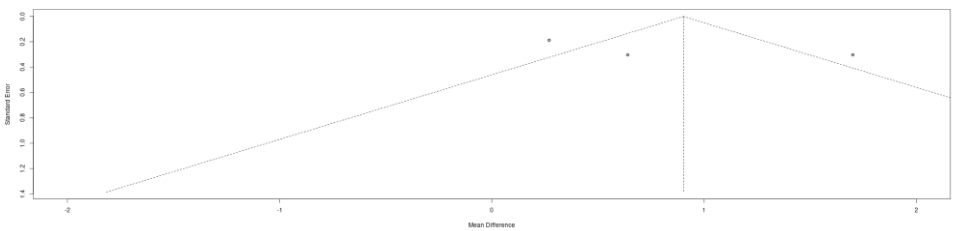
Only two studies investigated the levels of inflammatory biomarkers in persistent PCs. Ventadam et al. [23] measured this in patients with mTBI and controls without injury. They reported that IL-6 was reduced from 54.26 (s.d. 44.27) to 31.15 (s.d 28.6), IL-2 was increased from 6.93 (s.d. 4.65) to 11.33 (s.d. 6.26), IL-10 was increased from 1.67 (s.d. 1.59) to 9.75 (s.d. 0.86), IL-1b was reduced from 9.7 (s.d.) to 5.62 (s.d. 5.4), TNF-α was reduced from 23.56 (s.d. 24.8) to 10.18 (s.d. 9.53), and IFN-γ was reduced from 95.9 (s.d. 90.73) to 33.1 (s.d. 33.2). Powell et al. [18], on the other hand, reported an increase of IL-6 levels from 1.01 (s.d. 1.16) to 1.08 (s.d. 1.14) at one year after a mTBI. Elevated plasma IL-2 levels at 24 hours were also associated with more severe PCS 1 week after the injury. Six months after the injury, elevated plasma IL-10 levels were associated with greater depression scores and more severe PTSD symptoms [18].

3.4. Publication Bias

Visual inspection of funnel plots and Egger's tests showed no publication bias for any of the biomarkers which exhibited significant differences between mTBI and control individuals (Figure 9A – IL-1β, 9B – IL-4, 9C – IL-6).



A



B

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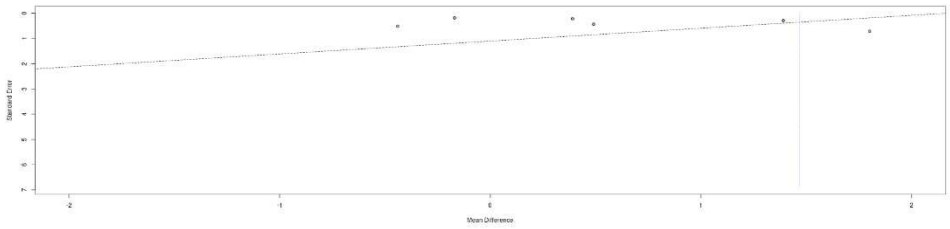

C

Figure 9. Egger test for (A) – IL-1β, (B) – IL-4, (C) – IL-6.

4. Discussion

The role of inflammation in mTBI and PCS has recently been investigated by many studies. In the present meta-analysis, we investigated the role of inflammatory biomarkers in diagnosing mTBI and the prognosis of PCS: IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IFN-γ, and TNF-α.

IL-2 is a cytokine that plays a crucial role in the immune system by promoting the proliferation and activation of T cells and other immune cells being produced by various cells, including T cells, B cells, and natural killer cells, in response to antigen stimulation. IL-2 acts on cells that express the high-affinity IL-2 receptor (IL-2R) to promote cell growth, differentiation, and effector function. IL-2 has been implicated in a variety of physiological and pathological processes, including immune responses, autoimmunity, transplantation, cancer, and infectious diseases [24]. Dysregulated IL-2 function can lead to various immunological disorders, such as immunodeficiency, autoimmune diseases, or chronic inflammation. Recent research data have also suggested a potential role of IL-2 in the pathophysiology of TBI and its associated symptoms. Although data from only one study only were available, the significant difference in IL-2 serum levels between controls and mTBI patients and a significant increase at six months post-injury suggested that it is equally involved in acute inflammatory response, contributing to the development of early symptoms and long-term recovery. Thus, IL-2 could be an important inflammatory biomarker of TBI diagnosis and PCS prognosis.

IL-1β is a pro-inflammatory cytokine primarily produced by activated macrophages and monocytes in response to infection, injury, or stress. IL-1β is involved in various physiological and pathological processes, including inflammation, fever, tissue repair, and immunity, acting on various target cells, including endothelial cells, fibroblasts, and immune cells, by binding to the IL-1 receptor (IL-1R) and activating intracellular signalling pathways. IL-1β promotes the production of other proinflammatory cytokines, such as TNF-α and IL-6, and induces the expression of adhesion molecules and chemokines, which attract immune cells to the site of injury or infection [25]. Changes in IL-1β functions have been implicated in various diseases, including rheumatoid arthritis, type 2 diabetes, and Alzheimer's disease. IL-1β has also been implicated in the pathophysiology of TBI and its associated symptoms [25]. The recent studies included in this meta-analysis suggested that the significantly increased IL-1β levels reported in mTBI patients associated with the acute phase of inflammatory response could indicate its role in the pathophysiology of mTBI, thus being a good candidate as a diagnosis biomarker. Furthermore, it was shown that IL-1b levels decreased six months after the injury suggesting a good potential in prognosis and outcome prediction.

IL-10 is an anti-inflammatory cytokine produced by T cells, B cells, macrophages, and dendritic cells. IL-10 plays a key role in regulating the immune response by inhibiting the production of proinflammatory cytokines, such as TNF-α and IL-1β, and promoting the differentiation and activation of regulatory T cells. IL-10 is involved in a wide range of physiological and pathological processes, including autoimmune diseases, allergies, infections, and cancer. Differences of IL-10 expression or function have been implicated in various immunological disorders, such as immunodeficiency, chronic inflammation, or cancer progression [26]. The potential role of IL-10 in the pathophysiology of traumatic brain injury (TBI) and its associated symptoms has been investigated by several studies. However, the present meta-analysis failed to show a significant difference in the expression of IL-10 in patients with an mTBI compared to control individuals, and the role of this cytokine as a diagnostic or prognostic biomarker in mTBI seems to be insignificant.

IL-4 is a cytokine primarily produced by T helper 2 cells (Th2), mast cells, and basophils. It regulates the immune response by promoting the differentiation and activation of Th2 cells, inducing the production of IgE antibodies, and suppressing the activity of Th1 cells and macrophages. IL-4 is involved in several physiological and pathological processes, including allergy, asthma, autoimmunity, and infectious diseases. Dysregulated IL-4 expression or function has been implicated in various immunological disorders, such as allergic diseases, immunodeficiency, and autoimmune diseases. Although the levels of IL-4 were reduced in mTBI patients, and the result was statistically significant, sensitivity analysis failed to confirm the robustness of the result and further studies are needed to confirm the role of this cytokine as a diagnostic or prognostic biomarker in mTBI [27].

Interleukins 6 and 8 are also proinflammatory cytokines that are primarily produced by immune cells, including macrophages, monocytes, and T cells, in response to infection, inflammation, or injury [28,29]. IL-6 acts on various target cells, including hepatocytes, lymphocytes, and endothelial cells, by binding to the IL-6 receptor (IL-6R) and activating intracellular signaling pathways. IL-6 promotes the production of acute-phase proteins, such as CRP, and the differentiation and activation of immune cells, such as Th17 cells and B cells. Various inflammatory or immune-mediated diseases, such as rheumatoid arthritis, multiple sclerosis, and cancer, were previously characterized by impaired IL-6 activity [28]. In the present meta-analysis, we found that the levels of IL-6 were significantly different between mTBI patients and control individuals, a result that was also confirmed by the sensitivity analysis and indicating the potential role of this cytokine as a diagnostic and prognostic biomarker in mTBI. The levels of IL-8, however, were not significantly different. On the other hand, no clear information about the possible use of IL-8 was observed when we meta-analyzed the previous reports. Despite this, it is already known that the dysregulations of IL-8 expression or functions were seen in chronic obstructive pulmonary disease, cystic fibrosis, and sepsis [30]. The recruitment and activation of neutrophils and other immune cells to the site of infection or injury, as well as endothelial cells and leukocytes activation, are concurring in the intracellular signaling pathways to which IL-8 participates. Kossmann et al [31] discussed the possible implication of IL-8 in TBI when they observed that it is released into the CSF following a traumatic event resulting in brain injury. In this way, the non-specific participation of IL-8 to the TBI pathophysiology could be associated with blood-brain barrier dysfunction and nerve recovery [31]. Notwithstanding, Whalen et al [32] previously described IL-8 as a potential target in anti-inflammatory therapy and suggested that it participates in the main process undergoing the acute inflammatory component of TBI.

TNF-α and IFN-γ are proinflammatory cytokines primarily produced by activated immune cells, including macrophages, T cells, and natural killer cells, in response to infection, inflammation, or injury. TNF-α acts on various target cells, including endothelial cells, macrophages, and immune cells, by binding to the TNF receptor (TNFR) and activating intracellular signaling pathways. TNF-α promotes the production of other proinflammatory cytokines, such as IL-1β and IL-6, and induces the expression of adhesion molecules and chemokines, which attract immune cells to the site of injury or infection. TNF-α activities impairments have been reported in various inflammatory or immune-mediated diseases, such as rheumatoid arthritis, inflammatory bowel disease, and cancer [33,34]. The present study showed no significant difference between mTBI patients and controls, and therefore we conclude that blood TNF-a has no value as a diagnostic biomarker for mTBI.

Being a cytokine involved in regulating the immune response by promoting the differentiation and activation of Th1 cells, natural killer cells, and macrophages, it was showed that IFN-γ inhibits the proliferation and activation of Th2 cells and regulatory T cells. Dysregulated IFN-γ functions have been implicated in various immunological disorders, such as autoimmune diseases, chronic infections, and cancer [35]. There was statistical significance in the serum levels of IFN-γ between mTBI and individuals without a history of mTBI. However, the data regarding IFN-γ is rather scarce, thus further analysis could shed more light onto this cytokine potential as a diagnosis or prognosis biomarker.

The data on the difference of the serum inflammatory biomarkers at 6- and 12-months post-injury were limited, and no clear conclusions can be made.

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Overall, these findings suggest that inflammatory biomarkers may serve as potential diagnostic and therapeutic targets for mTBI. Further research is needed to understand better the role of these biomarkers in the pathophysiology of mTBI and to explore their potential as diagnostic or therapeutic tools. Additionally, identifying specific cytokine profiles associated with different mTBI symptom clusters may improve diagnosis and treatment efficacy.

5. Conclusions

Despite the increased incidence of mTBI, there is scarce evidence about its pathophysiology. The implication of inflammation in mTBI was previously shown. However, the inflammatory biomarkers used in diagnosis, prognosis, and therapy need further documentation. This study provides reasonable evidence based on the meta-analysis of several studies presenting the variations of inflammatory cytokines in mTBI. Thus, it was showed that the increase of IL-6, TNF-α, and IL-1β plasma levels could be implicated in the development of the early post-concussion symptoms. On the other hand, the persistence of the increased plasmatic concentrations of IL-10 and IL-8 for as long as six months following the brain injury event could suggest chronic inflammation leading to neuroinflammation and late or persistent symptoms. In this context, our findings showed that inflammatory biomarkers could be relevant in diagnosing or predicting recovery or long-term outcomes of mTBI.

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Conflicts of Interest: The authors declare no conflict of interest.

Abbreviation list: TBI – traumatic brain injury; mTBI – mild traumatic brain injury; PCS – post-concussion syndrome; CSD – cerebrospinal fluid; IL-1 – interleukin-1; IL-1β – interleukin-1β; IL-4 – interleukin-4; IL-6 – interleukin-6; IL-8 – interleukin-8; IL-10 – interleukin-10; IL-12 – interleukin-12; TNF-α – tumor necrosis factor-alpha; CRP – C-reactive protein; SMD – standardized mean difference; NOS – Newcastle-Ottawa Scale; RCT – randomized controlled trials; CI –confidence intervals; PTSD – post-traumatic stress disorder; NfL – neurofilament light chain protein; NSE – neuron-specific enolase, S100B – S100 calcium-binding protein B; CCL2 – chemokine (C-C motif) ligand 2; IFN-γ – interferon gamma; GCS – Glasgow Coma Scale; CBF – cerebral blood flow; GFAP – glial fibrillary acidic proteins; Th – T helper cells;

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