The Utility of clinical and Blood-Based Biomarkers to Discriminate between Typical and Prolonged Pediatric mTBI Symptom Recovery

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THE UTILITY OF CLINICAL AND BLOOD-BASED BIOMARKERS TO DISCRIMINATE BETWEEN TYPICAL AND PROLONGED PEDIATRIC MTBI SYMPTOM RECOVERY

by

Morgan E. Nitta, M. S.

A Dissertation submitted to the Faculty of the Graduate School, Marquette University, in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy

Milwaukee, WI

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ABSTRACT

THE UTILITY OF CLINICAL AND BLOOD-BASED BIOMARKERS TO DISCRIMINATE BETWEEN TYPICAL AND PROLONGED PEDIATRIC MTBI SYMPTOM RECOVERY

Morgan E. Nitta, M.S.

Marquette University, 2022

Prolonged recovery following mild traumatic brain injury (mTBI) is poorly understood, particularly in pediatric samples, despite significant work to understand prolonged postconcussive symptoms (PPS). Emerging evidence in adult mTBI literature suggests blood-based biomarkers have diagnostic and prognostic value, but there is limited research examining this in pediatric mTBI. Further, while adult research documents that combining physiological biomarkers, emotional distress and symptom reports more optimally differentiates between mTBI and healthy controls, it is unknown if this finding will replicate in pediatric samples. This project examined foundational relationships between clinical, cognitive, inflammatory markers, and kynurenine pathway (KP) metabolites following mTBI in adolescents (N=104). Across the entire sample, higher report of emotional distress was related to lower levels of kynurenic acid (KynA), a putatively neuroprotective metabolite of the KP. Further, adolescents with prolonged recovery (PPS+) had lower KynA than those with typical mTBI recovery (PPS-) and healthy controls (HC). The HC group had higher KynA/QuinA ratios (a neuroprotective index) compared to both mTBI groups. Females, regardless of group, had lower KynA and lower KynA/3HK and KynA/QuinA than males. Finally, combinations of key variables discriminated HC and mTBI, as well as adolescents with and without PPS. Taken together, this research suggests a differential balance of KP metabolites in adolescent females following mTBI is strongly associated with emotional distress at a post-acute timepoint.
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Introduction

Traumatic brain injury (TBI) encompasses any trauma to the head by an external force resulting in direct injury to the brain. TBI classification is typically graded on a spectrum ranging from mild to severe. Outcomes following a severe TBI can include significant, and potentially longstanding, physical and cognitive impairments. While it was traditionally assumed that mild traumatic brain injuries (mTBI) resulted in only transient symptoms, there is increasing evidence that mTBI may have longstanding effects on cognitive, social, and psychological functioning (Hellewell et al., 2020; McInnes et al., 2017; Røe et al., 2009; Starkey et al., 2018), and it is especially concerning that many of these injuries are not evaluated by medical professionals (Cassidy et al., 2004; McCrea et al., 2004). It is also notable that mTBI is one of the most common neurological conditions in youth and adolescents (Brazinova et al., 2018; Polinder et al., 2018). It is estimated that 33 million children sustain a mTBI annually (Davis et al., 2017), resulting in nearly 400,000 hospital visits (Langlois et al., 2006). High prevalence and emerging evidence of poor recovery in children and adolescents illustrate that mTBI is a significant public health concern (Lumba-Brown et al., 2018).

Given the significant prevalence of pediatric mTBI, there have been efforts to comprehensively understand recovery processes and predict patient outcomes. One challenge which has emerged is the classification and diagnosis of mTBI. Numerous mTBI diagnostic criteria have been proposed (see Kirkwood et al., 2008 for review). One standard definition proposed by the World Health Organization (WHO) defines mTBI as an acute brain injury resulting from mechanical energy to the head from external forces including: (1) 1 or more of the following: confusion or disorientation, loss
of consciousness for 30 minutes or less, post-traumatic amnesia for less than 24 hours, and/or other transient neurological abnormalities such as focal signs, symptoms, or seizure; (2) Glasgow Coma Scale score of 13-15 after 30 minutes post-injury or later upon presentation for healthcare. (Carroll et al., 2004, p. 7)

While clearly stated, these criteria and the disparate application of definitions across clinics and research groups has resulted in heterogenous patient samples, which decreases the likelihood that outcome findings will generalize.

In response to different injury parameters resulting in a similar diagnosis, there have been many attempts to quantify mTBI severity (Coffeng et al., 2020; Mayer et al., 2017; Nelson et al., 2011; Ruff & Jurica, 1999). Historically, on the mild end of a spectrum, the term concussion is frequently used. Currently, concussion is often used to describe a sport-related head injury\(^1\) (Kirkwood et al., 2008). Concussion historically referred to an injury that could be managed independent of medical care, similar to a sprained wrist or minor cut. At the more extreme end of a severity spectrum, mild complicated TBI, which is most commonly observed in research populations recruited from emergency departments, describes injuries that have positive neuroimaging findings (e.g., skull fracture, subdural hematoma). Thus, the spectrum of severity in mTBI may be associated with overall symptom burden and can in part also explain outcome differences across studies.

Regardless of severity, neurobehavioral changes following a mTBI may include somatic, cognitive, and emotional/behavioral symptoms. Some of the most frequently reported symptoms include headache, fatigue, difficulty remembering, dizziness,

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\(^1\) The terms concussion and mTBI are used interchangeably throughout this paper, as diagnostic criteria and clinical guidelines significantly overlap (e.g., see Guskiewicz et al., 2007).
irritability, and sensitivity to light/noise (Blinman et al., 2009; Mittenberg et al., 1997; Ponsford et al., 1999). The expected duration of these postconcussive symptoms varies, though most youth and adolescents report symptom reprieve within a few weeks post-injury (Ledoux et al., 2019; McCrea et al., 2003). While most symptoms typically resolve, approximately 10-20% of youth experience symptom burden extending beyond a typical recovery window, lasting weeks to months after a brain injury (Babcock, Byczkowski, Wade, Ho, Mookerjee, et al., 2013; Ponsford et al., 1999; Yeates et al., 2009; Zemek et al., 2016).

Many researchers and clinicians believe that extended mTBI recovery reflects post-concussive syndrome (PCS). PCS encompasses nonspecific cognitive (e.g., difficulty concentrating and memory complaints), somatic (e.g., headaches and nausea), and emotional (e.g., emotional lability and irritability) symptoms that persist beyond the typical recovery window (Janusz et al., 2012). Formal diagnostic criteria for PCS were outlined in the Diagnostic Statistical Manual of Mental Disorders, Fourth Edition (American Psychological Association [APA], 1994; DSM-IV), describing a condition where postconcussive symptoms persist for 3 months following injury and are functionally limiting (APA, 1994). However, PCS is not included in the current Diagnostic Statistical Manual of Mental Disorders- 5th edition (DSM-5; APA, 2013). In contrast to DSM-5, the International Classification of Diseases, 10th Revision (ICD-10; World Health Organization, 2004) has outlined diagnostic criteria as well. Broadly, diagnostic criteria for PCS includes report of postconcussive symptoms (i.e., headache, fatigue, irritability, insomnia) which may be accompanied by feelings of depression or anxiety. Notably, ICD-10 criteria has not been validated in mTBI pediatric samples.
(Barlow, 2016) and is considered controversial by some given the nonspecific presentation of symptoms and ambiguous definition (e.g., see Iverson & Lange, 2003).

Despite debate regarding the existence of PCS, a small (but substantial) number of youths experience persistent postconcussive symptoms (PPS), thus increasing their risk for potentially long-term medical and psychological difficulties. Prolonged mTBI recovery in children and adolescents is associated with impaired cognitive function (Lambregts et al., 2018), depression (Ho et al., 2020; Peterson et al., 2013), school absences (Rozbacher et al., 2017b) and lower quality of life (Fineblit et al., 2016; Pieper & Garvan, 2014; Valovich McLeod et al., 2019). A comprehensive understanding as to why some youth experience prolonged symptom burden is clearly warranted.

There have been significant efforts to identify factors associated with prolonged recovery from pediatric mTBI. Some demographic factors appear relevant. For example, there is ample research suggesting that females experience postconcussive symptoms more frequently and for longer periods of time than males. Females report more symptoms of depression and anxiety following a concussion than males (Bunt et al., 2020; Covassin & Elbin, 2011; Fehr et al., 2019; Kontos et al., 2012). Further, those youth and adolescents who have sustained a previous concussion are at greater risk of experiencing higher symptom burden and prolonged symptom experiences with subsequent head injuries. Previous concussion history predicted longer symptom resolution, and those youth with a concussion within one year of their current injury had a symptom duration almost three times that of youth with no prior concussion (Eisenberg et al., 2013). Finally, adolescents (considered age range 13-18 years) tend to take a longer time to recover when compared to younger children (Davis et al., 2017).
These key demographic and concussion history variables are valuable in understanding pediatric mTBI recovery, but the identification and combination of more specific factors could significantly improve clinical intervention and management of PPS. Fortunately, there have been significant efforts to understand recovery within both clinical and neurophysiological disciplines. The following review of relevant clinical and physiological predictors of recovery in pediatric mTBI highlights the complex interplay of psychological and biological contributors to mTBI recovery. Indeed, the utility of integrating both clinical (i.e., symptom report scales, pre-injury psychological functioning, and cognitive measures) and neurophysiological factors (i.e., blood-based biomarkers of brain injury and inflammation) to understand mTBI recovery, while promising, has primarily been explored in adults.

**Clinical Measures**

Psychologists and physicians often utilize symptom report questionnaires to monitor and predict recovery from mTBI. Symptom rating scales ask patients (or caregivers) to report the presence and severity of common symptoms following mTBI. Common postconcussion symptom inventories include the Sport Concussion Assessment Tool (SCAT), which is currently in its 5th edition (Echemendia et al., 2017) or the Post-Concussion Scale (Lovell et al., 2006; Lovell & Collins, 1998). The total number of symptoms endorsed (regardless of severity) or a total symptom severity score (i.e., a sum score of all symptom severity ratings) are commonly used to track symptom recovery in clinical settings and outcome-based research. Nelson and colleagues (2016) highlighted how early symptom reporting (within 24 hours following injury) predicts symptom resolution. Additionally, athletes who reported higher symptom severity scores at one
week post-concussion were more likely to have a prolonged recovery (Nelson et al., 2016). Notably, there is some evidence to suggest that average symptom severity rather than the total symptom severity score may be a better predictor of pediatric mTBI recovery (Kowalczyk et al., 2020), suggesting the possibility that interpretation and utilization of symptom scales may be further refined.

When considering a total number of symptoms or total symptom severity score, it is important to note that the experience of specific symptoms varies over time after one sustains a mTBI. Somatic symptoms (e.g., headache and dizziness) peak immediately or in the subacute stage, whereas cognitive symptoms (e.g., memory difficulties) are often reported months after injury (Eisenberg et al., 2014; Ponsford et al., 1999; Yeates et al., 2009). Emotional symptoms also tend to appear later during the recovery process (Eisenberg et al., 2014) and for some tend to increase between 3 and 6 months following injury (Ewing-Cobbs et al., 2018). This symptom presentation timeline is notable, given that clinicians and researchers often use clinical measures to evaluate and define recovery. If cognitive and emotional symptoms are denied early in the recovery process but reported later, there is the potential for one to misattribute those difficulties to factors or issues unrelated to the injury.

A primary challenge in understanding recovery from mTBI in youth and adolescents is the nonspecific nature of the symptoms. For example, core features of attention-deficit/hyperactivity disorder (ADHD) such as distractibility or difficulty concentrating parallel symptoms reported by youth following mTBI. Some suggest that contributing factors such as hospitalization, pre-injury psychopathology, exposure to trauma, other noncranial injuries, and/or the use of medication introduces significant
variability to the broader recovery experience, endorsement of nonspecific symptoms, and possible prolonged symptom duration (Carroll et al., 2004). Further, even healthy (i.e., non-injured) youth often report experiencing postconcussive symptoms. One survey of non-injured children and adolescents reported that approximately 19% of boys and 28% of girls endorsed clinically significant levels of postconcussion symptoms (Iverson et al., 2015). Thus, given the common endorsement of symptoms across different clinical and healthy samples, it is quite clear that there are limitations associated with solely using symptom endorsement as a recovery metric.

Some researchers have posited that the nonspecific symptoms endorsed following concussion are related to general injury factors (Smith-Seemiller et al., 2003; Wood, 2004). However, evidence from studies comparing mTBI to other peripheral injuries suggests that prolonged postconcussive symptom experience is likely attributable to brain injury. For example, youth with mTBI continue to display PPS over time when compared to the recovery trajectory of youth who sustain orthopedic injuries (McNally et al., 2013; Taylor et al., 2010; Yeates et al., 2012). This suggests that while postconcussive symptoms are endorsed in non-head injury samples, prolonged postconcussive symptom experience is unique to mTBI. Additionally, it was identified that children with TBI were nearly 10 times more likely to have a diagnosis of depression when compared to an orthopedic control group six months following their injury (Luis & Mittenberg, 2002). Thus, the complex psychosocial experience of being injured cannot fully explain the experience of somatic, emotional/behavioral, and cognitive symptoms.

In addition to identifying group differences in mTBI symptom experience, researchers have employed prospective research designs. Longitudinal studies,
particularly those with prospective data collection can account for and control the probability of endorsing specific and nonspecific symptoms. Prospective studies of mTBI that utilize baseline testing (i.e., completion of symptom reports, neurocognitive tasks, and balance testing) can assist in clinical management and help clinicians conceptualize how preinjury symptom reporting may influence postinjury symptom experience. While not common practice, there is evidence that preinjury symptom report may predict recovery trajectories in pediatric mTBI. Ewing-Cobbs and colleagues (2018) reported a strong relationship between pre-injury psychological health and post-injury symptoms. That is, parent retrospective report of affective problems on a broad measure of emotional and behavioral function predicted elevated somatic, emotional, and fatigue symptom scores in a sample of youth and adolescents with mTBI assessed in an emergency department (ED; Ewing-Cobbs et al., 2018). It has also been documented that pre-injury depression and anxiety (Morgan et al., 2015; Yang et al., 2015; Yeates, 2010) predicts PPS. Notably, even a positive family history of mood disorders is associated with PPS following sport-related concussion (SRC; Morgan et al., 2015). Further, in a sample of children who experienced post-concussive symptoms between 4- and 26-weeks following their mTBI, children reported their pre-injury anxiety as higher than the general population (Peterson et al., 2015). Overall, there is significant evidence to suggest that the experience of emotional symptoms preinjury may impact post-injury symptom reporting, resolution, and recovery.

In addition to emotional functioning, somatization is increasingly recognized as a pre-injury factor which may influence post-injury symptom experience. Briefly, somatization is a poorly understood psychological construct which describes excessive
report of physical symptoms, which may or may not be explained by a medical condition. Within mTBI literature, higher report of pre-injury somatic symptoms predicted post-concussion symptoms in females with mTBI. Females with higher pre-injury somatization scores had persistent symptoms up to one month following their injury, while those with lower pre-injury somatization scores were more likely to have recovered (Root et al., 2016). In another study, higher report of pre-injury somatic symptoms was also associated with delayed symptom resolution in a sample of children and adolescents evaluated in the ED for mTBI (Grubenhoff et al., 2016). These pre-injury somatic symptoms were recalled retrospectively, and thus they may be subject to bias. However, in a prospective study of high school and collegiate athletes, Nelson and colleagues (2016) also reported that higher pre-injury somatization scores predicted a longer recovery from concussion. Further, adolescents presenting to a concussion clinic who reported more physical symptoms at initial visit were more likely to have an extended recovery (Wright et al., 2021).

Neuropsychological testing is also frequently used in TBI evaluations, though its utility in predicting extended recovery is debated. For example, poorer performance on cognitive testing in an ED was associated with greater mTBI symptom burden. Specifically, lower performance on reaction time measures and poorer cognitive flexibility predicted outcome, above and beyond sex and baseline symptom burden (Brooks et al., 2016). However, there is also overwhelming evidence to suggest mild decrements in performance on working memory, processing speed, and other cognitive tasks quickly resolve and return to a baseline level days after injury (Belanger & Vanderploeg, 2005; Frencham et al., 2005). Importantly, evidence from large,
prospective studies suggest that neuropsychological test performance does not predict recovery in ED samples (Hang et al., 2015; Nelson et al., 2017b; Rausa et al., 2018) and in high school and college athletes with SRC (McCrea et al., 2003; Nelson et al., 2016). Despite neurocognitive measures having low predictive power acutely following injury, some suggest that cognitive testing is most helpful when it is clear that an individual is experiencing PPS (McCrea et al., 2005). Clinically, neuropsychological assessment integrates report of postconcussive symptoms, mood, and cognitive functioning to help patients understand recovery via identification of strengths and challenges.

In summary, there are many challenges in using symptom report scales and cognitive instruments to quantify recovery. First, postconcussive symptoms are nonspecific in nature; they are associated with other psychological issues (e.g., ADHD and depression) and are often reported by healthy research participants. While uncommonly studied and subject to recall bias, preinjury disposition to experience symptoms may explain post injury report of symptoms, particularly affective and somatic symptoms following mTBI. Further, because cognitive changes following mTBI are minimal and spontaneously resolve, the use of neuropsychological measures acutely may not be useful alone in understanding recovery. In response to these limitations, many researchers have attempted to identify objective biomarkers markers of injury to further our understanding of prolonged recovery in pediatric mTBI.

**Neurophysiological changes**

It has been proposed that objective physiological markers and measures of mTBI injury might be considered in clinical contexts and improve recovery estimates (Dimou & Lagopoulos, 2013; Mayer et al., 2018). Physiological measures are broad and can include
assessment of acute injury characteristics such as loss of consciousness (LOC) or post-traumatic amnesia (PTA), various neuroimaging techniques, or quantification of neuronal injury in blood samples. Given that symptom reporting after mTBI is complicated by many factors, there is the potential that physiological markers of brain injury may supplement clinical conceptualization of recovery.

To begin, it seems plausible that more severe injuries would result in prolonged symptom duration. That is, youth who experience physical signs of injury such as extended LOC or PTA may represent the most “severe” mTBI and as a result experience an extended recovery. Supporting this assumption is evidence suggesting that physical signs and acute injury characteristics predict recovery trajectory. Yeates and colleagues (2009) conducted a longitudinal study of postconcussive symptoms in a pediatric ED and identified that LOC, PTA, altered mental status, and disorientation were strongly associated with PPS. Additionally, acute symptom burden and LOC were predictive of post-concussive symptom ratings up to one month following mTBI (Bernard et al., 2016). Nevertheless, the relationship between physical signs of mTBI and PPS is difficult to disentangle given that a much smaller proportion of mTBI with LOC is reported in SRC research (Guskiewicz et al., 2000). While LOC predicted longer symptom duration following mTBI in an outpatient sports medicine clinic (Fehr et al., 2019), LOC and amnesia were not predictive of prolonged symptom duration in a different study of participants with SRC (Meehan et al., 2013). Thus, differing outcomes may be associated with sample characteristics and how injuries are defined (e.g., definitions of mTBI inconsistently apply criteria of LOC duration or PTA).
LOC and PTA are observable physical markers of mTBI severity, and they are physical signs of rapid and acute neuronal dysfunction. However, many mTBI injuries are not observed (Powell et al., 2008), and thus the reliability of this severity indicator is poor. Further, mTBI rarely causes gross pathology detectable using computerized tomography (CT) scans or magnetic response imaging (MRI; Zetterberg et al., 2016), despite postconcussive cognitive, somatic, and emotional symptoms. However, the biomechanical insult to the brain results in a metabolic cascade (Giza & Hovda, 2014), which may include axonal injury, altered neurotransmission, neuroinflammation, excess glutamate release, and altered metabolism (Polinder et al., 2018). These metabolic changes can be quantified in cerebral spinal fluid (CSF) or blood and may contribute to clinical conceptualization of recovery above and beyond neuroimaging techniques. Thus, blood-based biomarkers provide an accessible, cost-effective measurement of neuronal dysfunction, and the use of these biomarkers to quantify the metabolic impact of injury on recovery is promising.

Blood-based biomarkers can be categorized into brain injury markers, markers of inflammation, or metabolic markers of injury, and research of fluid biomarkers has largely appeared in moderate to severe TBI outcome studies. An emerging literature suggests that elevations of these markers are observed in milder brain injuries, but few studies have examined blood-based biomarkers as they relate to symptom recovery in pediatric mTBI (Lugones et al., 2018; Mannix et al., 2020).

Brain injury specific markers (i.e., measure of axonal damage [tau, neurofilament light] or astroglial proteins [S100ß and glial fibrillary acid protein, GFAP] that cross the blood brain barrier) measured in blood serum or plasma following injury have been
associated with symptom outcome in the adult mTBI literature, though few studies report relationships in pediatric mTBI samples. For example, S100β is approved for use as a marker of necessity for CT scans in pediatric head injury (Bouvier et al., 2012; Weinberg & Castellani, 2010), but few studies report a significant relationship between elevated S100β and prolonged symptom duration (Babcock, Byczkowski, Wade, Ho, & Bazarian, 2013; Berger et al., 2002; Piazza et al., 2007). Further, GFAP and ubiquitin carboxy-terminal Hydrolase L1 (UCH-L1), when combined, have been approved by the Federal Drug Authority (FDA) for use to identify adult patients with low risk of intracranial injury (Bazarian et al., 2018). Literature remains in its nascent stages with regard to documenting relationships between brain injury specific markers, clinical symptoms, and outcome in the pediatric literature (e.g., see Mannix et al., 2020).

In addition to brain injury markers that can be quantified in blood serum or plasma, recent work has explored inflammation as a common mechanism underlying symptom presentation (Rathbone et al., 2015). Briefly, systemic inflammatory mediators, cytokines and chemokines, communicate with the central nervous system (CNS) to recruit neurotrophils and monocytes to repair injury (Patterson & Holahan, 2012). As such, peripheral circulating cytokines and chemokines may be markers of recovery processes occurring in the CNS. It is well-established that cytokine and chemokine production increases in the brain in rodent models of head injury, and these increases are associated with cognitive and behavioral changes (see Rathbone et al., 2015 for review). Further, increases in circulating cytokines have been reported in moderate to severe TBI (Maier et al., 2001) and more recently in adult mTBI (Kalabalikis et al., 1999; Morganti-Kossmann et al., 2019; Ritzel et al., 2018).
Beyond measured elevations, increased peripheral markers of postinjury inflammation also appear to be related to symptom duration and recovery in adult mTBI (Su et al., 2014). For example, C-reactive protein (CRP) measured in serum at hospital admission predicted persistent concussion symptoms and psychological distress at three-months post-injury mTBI in an adult sample. Further, subacute (i.e., within 6 hours of injury) elevations in interleukin (IL)-6 and IL1-receptor agonist (IL-1RA) were associated with longer symptom duration in high school and college football players (Nitta et al., 2019). Di Battista and colleagues (2019) also reported higher blood concentrations of chemokines in athletes with concussions compared to healthy controls within seven days of injury, and these elevations were positively correlated with the number of days required to achieve medical clearance. In another study, symptom severity within seven days of mTBI was positively correlated with inflammatory cytokines in male athletes, but an inverse relationship was found in females (Di Battista et al., 2020). This suggests possible sex differences in inflammatory response post-injury. These studies highlight innovative approaches to understanding typical and atypical recovery from mTBI, but replication and additional research is needed, particularly in pediatric samples.

The relationships between inflammatory markers and postconcussive symptoms is also of particular interest given parallel findings between the cytokines tumor necrosis factor (TNF)-a, IL-1β, IL-6, interferon-γ (IFNγ), and “sickness behavior” (Dantzer & Kelley, 2007; Di Battista et al., 2020). Sickness behavior, characterized by fatigue, malaise, decreased appetite, apathy, and lethargy, resembles depressive symptomatology. Irritability and/or depressed mood are commonly reported symptoms following mTBI,
suggesting a possible relationship between inflammation and postconcussive symptom experiences.

Finally, the inflammatory response to head injury also activates immunoregulatory networks like the kynurenine pathway (KP). KP metabolites are neuroactive and can be neuroprotective by modulating neuroplasticity or neurotoxic by effecting NMDA receptors and neurotransmission of glutamate (Savitz, 2019). This is significant given that excess glutamate release following mTBI is a primary component of the neurometabolic cascade following mTBI.

Briefly, the KP is activated by proinflammatory cytokines which increase the production of kynurenine (KYN) from tryptophan (TRP). KYN is metabolized into either neuroprotective kynurenic acid (KynA) or the neurotoxic metabolites 3-hydroxykynurenine (3HK) and quinolinic acid (QuinA). QuinA acts as an NMDA receptor agonist (Stone & Perkins, 1981), potentially having neurotoxic effects, whereas KynA promotes neuroprotection by acting as a competitive antagonist to ionotropic excitatory amino acid receptors (Foster et al., 1984; Kessler et al., 1989). Inflammatory response shows brain formation of QuinA is predominate over the formation of KynA (Heisler & O’Connor, 2015; Tutakhail et al., 2020), thus following injury, synthesis of more neurotoxic metabolites occurs. The ratios of neuroprotective and neurotoxic metabolites (e.g., KynA/QuinA and KynA/3HK) provide markers for competing physiological effects, and they can also be measured in blood serum or plasma samples.

An association between post concussive mood symptoms and KP metabolites is increasingly evident in mTBI. Atypical ratios of kynurenine metabolites have been associated with moderate or severe TBI (Morganti-Kossmann et al., 2019; Yan et al.,
2015) and more recently in SRC. Recent work conducted by Singh and colleagues (2016) reported a decreased ratio of KynA/QuinA in football players following SRC. Further, football athletes with a concussion history had significantly greater levels of neurotoxic QuinA compared to athletes without a concussion history (Meier, Drevets, et al., 2016). Additionally, athletes with longer recovery (quantified by return-to-play decisions) had higher QuinA and lower KynA/QuinA levels at 1-month post-injury. In both studies, a decreased KynA/QuinA ratio and increased level of QuinA was correlated with significant depressive symptoms. Finally, we reported higher KynA/3HK (i.e., greater neuroprotection) was associated with fewer depressive symptoms at a later timepoint in athletes with SRC (Meier, Nitta, et al., 2020). Thus, the immunological and metabolic response following mTBI is associated with frequently reported emotional sequelae, and measurement of these markers may highlight a potential mechanism and serve as a marker of risk and recovery of mTBI.

Notably, most of the work assessing inflammatory and kynurenine markers has been conducted to investigate adult male athletes’ recovery from SRC. Further, inflammatory markers and KP markers have known sex differences, and thus we do not know how that impacts an association with injury. There is a critical need to identify injury biomarkers to facilitate monitoring of pediatric mTBI recovery and to document potential relationships between those biomarkers and symptom report. While research suggests that children, adolescents, and adults experience some similar symptoms following mTBI, recovery and neuropathology associated with pediatric mTBI is different from adult mTBI (Field et al., 2003; Ommaya et al., 2002). Developing brains have differing degrees of myelination, elastic properties, and blood-brain barrier integrity.
(Prins & Hovda, 2003). Thus, it is unclear how these key differences might impact the probability of replicating blood-based biomarker findings derived in adult mTBI samples.

**Summary and Primary Aims**

There have been significant efforts to identify factors that assist clinicians and researchers in monitoring mTBI recovery. Currently, concussion symptom scales and neurocognitive testing are frequently utilized in management of mTBI. However, symptom reporting is subject to retrospective report bias, and the nonspecific nature of concussion symptoms makes it difficult to attribute symptoms solely to a brain injury. Further, neurocognitive functioning, as quantified by performance on neuropsychological measures, rapidly returns to a baseline level in a great majority of those who sustain mTBI (McCrea et al., 2003). Emerging evidence in adult mTBI literature suggests blood-based biomarkers have diagnostic and prognostic value, but there is limited research examining blood-based biomarkers in pediatric mTBI.

The integration and synthesis of clinical variables, injury characteristics, and biomarkers is an important next step in understanding what might contribute to the experience of persistent symptoms following pediatric mTBI. Highlighting the potential value of this work, a panel of blood-biomarkers improved injury classification of mTBI vs. healthy controls (AUC= 0.98) when combined with symptom severity scores in SRC (Meier et al., 2020). The utility of clinical measures and biological markers used in conjunction to quantify recovery is promising, but these efforts have largely utilized adult mTBI samples. Indeed, with increased focus on pediatric mTBI, there are direct calls for research to incorporate targeted, multidisciplinary integrated approaches (Polinder et al., 2018; Takagi et al., 2019). This research directly responds to this call by advancing
understanding of recovery in pediatric mTBI through the integration of clinical and neurophysiological measurement.

Broadly, this project explored and reported foundational relationships between psychological and physiological factors relevant to recovery from mTBI in a sample of adolescents. The primary aims for this project were as follows:

1. Explore and establish relationships between clinical variables and a panel of blood-based biomarkers in adolescent participants.

2. Evaluate differences in clinical variables and blood-based biomarkers (a) between healthy controls (HC), adolescents with mTBI who experience PPS, and adolescents with mTBI who experience no PPS, and (b) assess possible interactions of group and sex, prior history of concussion, and/or presence of acute injury characteristics (i.e., LOC and PTA).

3. Identify key variables, and possible combinations of variables, that discriminate between (a) healthy controls and mTBI and (b) no PPS and prolonged recovery from mTBI.

Method

Sample

A comprehensive dataset consisting of demographic variables, clinical measures and blood-based biomarkers obtained from healthy adolescents and adolescents who sustained a mTBI (at least one-month post-injury) was utilized. The final sample for this project combined clinical and physiological data from two independent research studies from the Brain Injury Research Program at the Medical College of Wisconsin.
The primary study (Study 1; *Neuroimaging and blood biomarkers of persistent post-concussive symptoms in adolescents*, PI: Timothy B. Meier, Ph.D.) was approved by the Children’s Hospital of Wisconsin Institutional Review Board. The sample size for this study was 43 adolescents with mTBI and 16 healthy controls (HC group). The HC group was recruited from the community. Adolescent participants (ages 14-18) with diagnosed mTBI were largely recruited from a sports medicine clinic (*n* = 4 recruited from the community via word of mouth), and visits were completed at least one-month post injury. Additional relevant data was obtained from a single timepoint within a prospective investigation of SRC primarily in high school and collegiate football athletes (Study 2; *Project Head to Head II*, PI: Michael McCrea, Ph.D.), and only athletes age 18 years or below were included. Study 2 was approved by the Medical College of Wisconsin Institutional Review Board. Data from 20 adolescents with mTBI and 25 healthy controls were used from this study. Study 2 is a prospective study with multiple visit timepoints, including a 45-day post injury timepoint. Only data from the participants’ 45-day visit were included to best capture clinical and physiological differences in prolonged symptom recovery from mTBI and to generally match the time point of Study 1.

In all data collection, adults and parents of minors provided written consent to participate, and minors provided written assent. Concussion diagnosis was based on criteria from the Zurich Consensus Statement of Concussion in sport (McCrory et al., 2013). Concussion is described as a brain injury caused by “a direct blow to the head, face, neck, or elsewhere on the body with an ‘impulsive’ force transmitted to the head” (McCrory et al., 2013, p. 3). In both research studies, patients completed a health and
demographic history form and clinical assessments, including concussion symptom scales, psychological rating scales, personality rating scales, and neurocognitive measures. Blood-based biomarkers were obtained from blood serum samples. Participants and healthy controls were excluded from the study if they reported a history of moderate to severe head injury, psychiatric condition, autoimmune disease, neurodevelopmental disorders (e.g., ADHD), neurological condition (e.g., epilepsy or migraines), endocrine disease, or cardiovascular disease. Participants and healthy controls were also excluded if they use neuroactive medications unrelated to PPS (e.g., antidepressants or anxiolytics).

All participants with mTBI were recruited at least 26 days status post-injury, with overlapping time ranges between samples. In the primary study (Study 1), approximately 47 adolescents with mTBI completed visits 32 median days since injury (interquartile range [IQR] 29-52 days). Participants from Study 2 completed visits 45 median days since injury (IQR 43-47 days).

**Key Variables of Interest**

**Clinical Instruments**

**Demographic and Health History.** Each participant responded to a demographic and health history questionnaire, which included data on age, sex, race, ethnicity, body mass index (BMI), grade point average (GPA), prior history of concussion, and medication use.

**Weschler Test of Adult Reading (WTAR; Wechsler, 2001).** The WTAR is a word-reading task that was used as a proxy marker of general intellectual ability. Word reading tasks are believed to be resistant to change following head injury, and thus
performance likely represents a reliable marker of pre-morbid intellectual (verbal) functioning. Normative data with standard scores (Std.S) with mean of 100 and standard deviation of 15 were reported.

**Sport Concussion Assessment Tool-3 (SCAT3, 2013).** The SCAT3 is a brief evaluation of subjective symptoms, cognitive impairment, and balance, and it asks about LOC, PTA, and retrograde amnesia (RTA). The SCAT3 consists of three primary components, including the Symptom Inventory, Sideline Assessment of Concussion (SAC), and Balance Error Scoring System (BESS). The Symptom Inventory asks participants to rate 22 symptoms on a 0 (*no issue*) to 6 (*severe*) Likert scale. The number of symptoms reported (i.e., symptom endorsed as $\geq 1$) and symptom severity (i.e., summation of *all* symptom ratings) are tallied producing an overall symptom severity score and total number of symptoms. The total number of symptoms reported on the SCAT3 Symptom Inventory was used to define groups. As such, only the raw total symptom severity score was used as a variable in Aim 1. Further, dichotomous yes/no items pertaining to the experiences of acute injury characteristics (LOC, PTA, and RGA) were used. The SCAT-3 demonstrated good discrimination and test-retest reliability in adolescent samples (Chin et al., 2016).

**Wechsler Adult Intelligence Scale-Fourth Edition (WAIS-IV; Wechsler, 2008)-Processing Speed Index (PSI).** The WAIS-IV contains two subtests, Coding and Symbol Search, which assess speed of information processing. When performance on these subtests is combined, an overall index of processing speed is derived$^2$. Processing

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$^2$ All participants were administered WAIS-IV processing speed subtests. A subset of participants under the age of 16 ($n = 33$) were administered WAIS-IV subtests, despite the tasks being normed with individuals age 16 and above. Scaled scores significantly differed between participants age $\geq 16$ and age $<16$ ($p < 0.001$; Cohen’s $d = 0.69$).
speed was reported as a standard score (Mean Index SS = 100; SD = 15) generated from the normative sample presented in the WAIS-IV manual.

**Brief Symptom Inventory-18 (BSI-18; Derogatis, 2000).** The Brief Symptom Inventory-18 (BSI-18) is a self-report measure of psychological distress. The BSI-18 was developed as a screening instrument for psychiatric disorders, and it contains a global severity index (BSI-GSI) and anxiety (BSI-A), depression (BSI-D), and somatization (BSI-S) subscales. Respondents are asked to assess symptoms on a scale of 0 (Not at all) to 4 (Extremely). Raw scores for the total scale and subscales were reported. The BSI-18 demonstrates good internal consistency, and it is considered an appropriate measure to evaluate mood and emotional functioning following mTBI (Lancaster et al., 2016).

**Minnesota Multiphasic Personality Inventory-Adolescent-Restructured Form (MMPI-A-RF; Archer et al., 2016).** The MMPI-A-RF is a broadband measure of adolescent psychopathology and personality traits. Patients were administered items contained on the Restructured Clinical (RC) Somatic Complaints (RC1) scale. Broadly, MMPI RC1 assesses malaise, gastrointestinal complaints, head pain complaints, neurological complaints, and cognitive complaints. Participant’s MMPI RC1 T-scores (Mean T = 50; SD = 10) are reported and were derived by utilizing normative data reported in the MMPI-A-RF manual.

**Blood-based Biomarkers**

Venous blood was collected using Red Top BD Vacutainer tubes, left to clot at room temperature for 30 minutes, centrifuged at 1,500 relative centrifugation force (RCF) for 15 minutes, and serum collected and stored at -80 °C.
Quinolinic acid (QuinA), kynurenic acid (KynA), 3-hydroxykynurenine (3HK), tryptophan (TRP), and kynurenine (KYN) concentrations were determined from serum blind to diagnosis using high-performance liquid chromatography with tandem mass spectrometry detection by Charles River Laboratories, Inc. according to their standard protocol. Serum concentrations of IL-6, IL-1RA, and CRP were measured in duplicate, blinded to concussion status, using a Meso Scale Discovery (MSD) QuickPlex SQ 120 instrument and MSD V-PLEX assays following manufacturer instructions.

The lower limit of detection (LLOD) and the upper limit of detection (ULOD) were assessed for inflammatory markers to ensure sensitivity of measurement. Biomarkers below the LLOD or above the ULOD were excluded from analyses (IL-6, n = 12). IL-6 was below the level of detection for 12 participants, and thus, not included in analyses including IL-6. When cleaning the inflammatory markers data, samples with a coefficient of variation (CV) between duplicates >25% were also excluded (IL-6, n = 5), and these overlapped with IL-6 samples that were below the level of detection. All blood markers were natural log transformed to approximate the normal distribution, and they reported in log pg/mL.

**Inflammatory Markers.** Based on SRC inflammatory markers research (Nitta et al., 2019), inflammatory markers of C-Reactive Protein (CRP), Interleukin-6 (IL-6), and IL-1 receptor antagonist (IL-1RA) were investigated. The natural log transformed values were utilized.

**Kynurenine Pathway (KP) Metabolites.** The primary KP metabolites of interest include neuroprotective Kynurenic acid (KynA) and neurotoxic Quinolinic acid (QuinA),
3-hydroxykynurenine (3HK). Their associated ratios (KynA/3HK, KynA/QuinA) were also derived and the natural log transformed values were utilized.

**Data Analytic Plan**

Statistical analyses were conducted with IBM SPSS Statistics version 27 (IBM, 2020). Data and blood samples were cleaned to ensure sensitive detection, and participants with incomplete clinical and physiological data were excluded. T-tests and Chi-square analyses were conducted to evaluate if participants obtained from Study 1 or 2 differed in meaningful and unanticipated ways on key variables (e.g., age, time since injury, ratio of male to female participants, injury characteristics, history of prior concussion).

Participants were separated into three groups: (1) a heathy control (HC) group consisting of noninjured adolescents, (2) adolescents with mTBI with typical recovery defined by less than 3 reported symptoms on the SCAT 3 Total Symptoms at time of visit (PPS-), and (3) those with prolonged recovery at time of visit (PPS+). Prolonged recovery was defined as the presence of 3 or more symptoms endorsed on the SCAT-3 total symptom report and self-report of current functioning <90% back to a pre-injury baseline level of functioning.

Analyses and hypotheses are presented by Aim. A two-tailed alpha of 0.05 was considered statistically significant for all analyses. Post hoc tests were conducted using Bonferroni correction based on number of comparisons. Adjustment for multiple comparisons were used within key subsets of variables (i.e., adjustment for clinical measures, inflammatory markers, and KP metabolites).

**Primary Aim 1**
Pearson’s $r$ correlations were generated to assess the associations between key clinical variables (i.e., SCAT-3 symptom severity, BSI-GSI and subscales, MMPI RC1 T-score, and PSI) and blood biomarkers (i.e., inflammatory markers, and kynurenine metabolites). Given the relatively small group sizes, correlations across variables are reported for the full sample including individuals who sustained an mTBI and healthy controls.

Given that symptoms following mTBI are nonspecific, it was hypothesized that clinical measures of mood and postconcussive symptoms would be significantly, positively correlated. It was also predicted that SCAT-3 symptom severity scores and BSI-18 scores would be significantly, positively correlated, given overlapping item content. It was also expected that these scales would exhibit a moderate positive correlation with MMPI RC1, as somatization scales are often correlated with internalizing symptoms (Markon, 2010; Simms et al., 2012). Further, there is some debate as to whether a somatoform construct is uniquely different from an internalizing construct (Kotov et al., 2017). It was also hypothesized that neurocognitive measures were likely to be related to measures assessing mood such that lower scores on PSI were be negatively correlated with emotional and somatic symptom reports (Weschler, 2006).

Research which highlights relationships between elevated inflammatory markers and “sickness behavior” (Dantzer & Kelley, 2007; Di Battista et al., 2020) suggests that positive relationships between IL-1RA, IL-6, and CRP and BSI-18 subscales are likely to emerge. Further, given that the KP is related to mood symptoms broadly (Savitz, 2019) and within athletes with concussions (Meier, Nitta, et al., 2020; Singh et al., 2016), these relationships were also hypothesized to be present.
**Primary Aim 2**

To address Primary Aim 2, univariate analyses were conducted to identify differences between groups on clinical variables and biomarkers. Continuous variables were assessed using a one-way analysis of variance (ANOVA) when comparing mean values in the HC, PPS+, and PPS- groups. Univariate analyses were conducted including (a) report of emotional symptoms (BSI-GSI, BSI-Dep, BSI-Anx, BSI-Som, MMPI RC1), (b) neurocognitive performance (PSI), (c) inflammatory markers (IL-6, IL-1RA, and CRP), and (d) KP metabolites and ratios (KynA, 3HK, QuinA, KynA/3HK, KynA/QuinA). Assessing variables within their respective domains decreases the number of comparisons made, and thus reduces the error rate. Exploratory analyses also included evaluating differences between HC and mTBI group across blood-based biomarkers given limited documentation of how these measures function in pediatric samples.

Finally, 2-way ANOVAs assessed for interactions between group (HC, PPS+, and PPS-) and the dichotomous variables of sex (male vs. female\(^3\)) and the presence of acute injury characteristics (+LOC/PTA vs. -LOC/PTA) across clinical and biomarkers. Additionally, a 2-way ANOVA assessed for interactions between mTBI groups (PPS+ and PPS-) and history of prior concussion (prior history of concussion vs. no prior concussion history) across clinical and biomarkers.

Given that severity of mTBI is related to PPS (Yeates et al., 2009), it was anticipated that there would be an interaction between the PPS+ group and presence of acute injury characteristics when predicting postconcussive, emotional, and somatic

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\(^3\) It should be noted that sex and gender are considered binary in this research, despite the spectrum of physiological, societal, cultural, and historical variance across symptom presentation. This variable is presented with the understanding that there is societal and cultural nuances to further explore.
symptom reporting. Further, females are more likely to experience PPS+ than males following an mTBI (Kontos et al., 2012), so it was predicted that a significant interaction would emerge between the PPS+ group and female sex when predicting postconcussive emotional and somatic symptom reporting.

Finally, there is limited research to warrant anticipated findings regarding differences of inflammatory markers or kynurenine metabolites between groups. However, some preliminary thoughts are offered. First, KP metabolites are related to sickness behavior and psychiatric conditions like major depressive disorder (Savitz, 2019), so a difference between the PPS+ and PPS- group may emerge where PPS+ has lower neuroprotective KynA and lower KynA/QuinA and KynA/3HK ratios. Further, there may be an interaction of sex and group across inflammatory markers given Di Battista and colleagues’ (2020) recent findings that male athletes with concussion showed a positive relationship with inflammatory markers and an inverse relationship in females.

**Primary Aim 3**

Binary logistic regression was conducted to examine whether potential predictors discriminated between HC and all adolescents with mTBI. Binary logistic regression was also conducted to examine whether potential predictors discriminated between PPS+ and PPS- groups. Predictors entered into the logistic regression were selected based on Aim 1 and Aim 2 findings. Independent variables identified in Aim 2 that differentiated groups with a statistical probability of $p < 0.2$ were considered as potential predictors and placed into a logistic regression model (Meehan et al., 2016). Simultaneously, associations between variables reported in Aim 1 were considered to identify multicollinearity between variables. For example, given strong, positive correlations across clinical
measures, BSI-GSI was entered into the model as a predictor rather than individual subscales. Additionally, only kynurenine pathway ratios (i.e., KynA/QuinA and KynA/3HK) were entered as predictors as these are considered indices which capture the overall balance of the kynurenine pathway. Finally, the predictive probabilities from the logistic regression models was used to calculate area under the receiver operating characteristic curves (AUCs) to determine if combined clinical and biomarker variables improve group discrimination (i.e., HC vs. mTBI (all) and PPS- vs. PPS+).

In the adult mTBI literature, a combination of SCAT-3 symptom severity scores and blood-based biomarkers improved discrimination between non-injured controls and SRC (Meier, Huber, et al., 2020). It was hypothesized that a similar finding would emerge within this adolescent mTBI sample when discriminating between injured and non-injured adolescents utilizing other symptom report measures (e.g., BSI-GSI). Further, if warranted, including other clinical variables as predictors (i.e., MMPI RC1 and PSI) may also contribute to improved discrimination between groups. Finally, the discriminability of inflammatory and kynurenine markers was exploratory in nature.
Results

Sample Characteristics

Data from 104 adolescents were included (adolescents sustained mTBI \( n = 63 \); healthy controls \( n = 41 \)). With regard to study differences, 59 adolescents were included from Study 1 and 45 adolescents were included from Study 2. As expected, Study 2 participants were significantly older than Study 1 participants, and all females were recruited from the Study 1 (see Table 1).

Table 1.

<table>
<thead>
<tr>
<th>Differences between Study 1 and Study 2 participants</th>
<th>Study 1</th>
<th>Study 2</th>
<th>Statistics</th>
</tr>
</thead>
<tbody>
<tr>
<td>n = 59</td>
<td>15.58(1.00)</td>
<td>17.0 0(1.15)</td>
<td>( F(1, 102)= 45.34, p &lt; .001 )</td>
</tr>
<tr>
<td>n = 45</td>
<td>55.93%</td>
<td>0%</td>
<td>( X^2(1)= 36.87, p &lt; .001 )</td>
</tr>
<tr>
<td>Age</td>
<td>40.68%</td>
<td>44.44%</td>
<td>( X^2(1)= 0.15, p = .70 )</td>
</tr>
<tr>
<td>Sex (%female)</td>
<td>40.68%</td>
<td>44.44%</td>
<td>( X^2(1)= 0.15, p = .70 )</td>
</tr>
<tr>
<td>History of Prior-Concussions (% yes)</td>
<td>38.10%</td>
<td>20.00%</td>
<td>( F(1, 61)= 0.41, p = .52 )</td>
</tr>
<tr>
<td>Number of days since injury</td>
<td>38.10%</td>
<td>20.00%</td>
<td>( FET= 0.25 )</td>
</tr>
</tbody>
</table>

Note: M and SD represent mean and standard deviation, respectively. Loss of Consciousness (LOC); Posttraumatic Amnesia (PTA); Retrograde amnesia (RGA)

The mTBI group was further stratified into PPS+ (\( n = 22 \)) and PPS- (\( n = 41 \)) groups. Table 2 presents full sample descriptive statistics and demographics. There were age differences between the PPS+, PPS-, and HC groups, where the PPS+ group was significantly younger than the HC and PPS- groups (\( p < .05 \)). Females were more likely to be in the PPS+ group (\( p <0.001 \)), and PPS+ and PPS- group were more likely to report
a prior history of concussion ($p = .01$). Groups did not differ on demographic variables of race, ethnicity, body mass index (BMI), number of days since injury, injury characteristics, as well as estimates of intellectual function (WTAR). See Table 2 for a complete summary of sample demographics by group.
Table 2.
Sample Characteristics Across Sample of Healthy Control (HC) and mTBI with PPS- and PPS+ Subgroups

<table>
<thead>
<tr>
<th></th>
<th>HC</th>
<th>mTBI</th>
<th>PPS-</th>
<th>PPS+</th>
<th>Statistics*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n = 41)</td>
<td>(n = 63)</td>
<td>(n = 41)</td>
<td>(n = 22)</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>16.53(1.25)</td>
<td>15.96(1.26)</td>
<td>16.22(1.37)</td>
<td>15.50(0.86)</td>
<td>F(2,101)=6.60, p = .002</td>
</tr>
<tr>
<td>Sex (% female)</td>
<td>22.0</td>
<td>38.1</td>
<td>24.4</td>
<td>63.6</td>
<td>X^2 (2) = 13.17, p &lt;0.001</td>
</tr>
<tr>
<td>Race (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>American Indian/Alaskan Native</td>
<td>0.0</td>
<td>1.6</td>
<td>2.4</td>
<td>0</td>
<td>X^2 (6) = 7.95, p = .24</td>
</tr>
<tr>
<td>Black/African American</td>
<td>14.6</td>
<td>19.0</td>
<td>24.4</td>
<td>9.1</td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>85.4</td>
<td>77.8</td>
<td>73.2</td>
<td>86.4</td>
<td></td>
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<tr>
<td>Not Reported Ethnicity</td>
<td>0</td>
<td>1.6</td>
<td>0</td>
<td>4.5</td>
<td></td>
</tr>
<tr>
<td>Hispanic or Latino (%)</td>
<td>4.9</td>
<td>6.3</td>
<td>0.0</td>
<td>18.2</td>
<td>X^2 (6) =12.13, p = .06</td>
</tr>
<tr>
<td>Not Hispanic or Latino</td>
<td>92.7</td>
<td>88.9</td>
<td>92.7</td>
<td>81.8</td>
<td></td>
</tr>
<tr>
<td>Unknown/Not Reported</td>
<td>2.4</td>
<td>4.8</td>
<td>7.3</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>History of Prior Concussions (% yes)</td>
<td>24.4</td>
<td>54.0</td>
<td>53.7</td>
<td>54.5</td>
<td>X^2 (2) =8.91, p = .01</td>
</tr>
<tr>
<td>BMI</td>
<td>25.25(5.51)</td>
<td>24.61(4.60)</td>
<td>24.98(4.96)</td>
<td>23.91(3.86)</td>
<td>F(2,101)= 0.54, p = .59</td>
</tr>
<tr>
<td>Number of days since injury**</td>
<td>--</td>
<td>49.90(39.48)</td>
<td>54.17(45.50)</td>
<td>41.95(23.59)</td>
<td>F(1, 61) = 1.38, p = .24</td>
</tr>
<tr>
<td>LOC/PTA/RG A** (% yes)</td>
<td>--</td>
<td>31.7</td>
<td>34.31</td>
<td>27.3</td>
<td>X^2 (1) = 0.03, p = .86</td>
</tr>
<tr>
<td>Clinical Variables</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>SCAT-3 Sx Sev</td>
<td>2.00(3.97)</td>
<td>11.68(17.51)</td>
<td>1.98(3.10)</td>
<td>29.77(18.97)</td>
<td>F(2,101)= 78.87, p &lt;.001</td>
</tr>
<tr>
<td>WTAR</td>
<td>104.27(10.53)</td>
<td>100.03(15.75)</td>
<td>101.12(14.26)</td>
<td>98.00(14.62)</td>
<td>F(2,101) = 1.73, p = .18</td>
</tr>
</tbody>
</table>

Note: M and SD represent mean and standard deviation, respectively. Loss of Consciousness (LOC); Posttraumatic Amnesia (PTA); Retrograde amnesia (RGA); Sport Concussion Assessment Tool-3 Symptom Severity (SCAT-3 Sx Sev); Weschler Test of Adult Reading (WTAR) Standard Score. *Statistical comparisons are made between HC, PPS+, and PPS- groups. **Statistical comparison made between PPS+ and PPS- groups.
Aim 1.

Pearson’s $r$ correlations (Table 3) quantified the direction and magnitude of relationships between key clinical variables (i.e., SCAT-3 symptom severity, BSI-GSI and subscales, MMPI RC1 T-score, and PSI), and blood biomarkers (i.e., inflammatory markers and KP metabolites and ratios). Parametric correlations were reported given that PSI and MMPI RC1 are standardized scores, and inflammatory markers are reported after linear transformation. Notably, nonparametric correlations were assessed in exploratory analyses as well given that raw sum scores are reported for SCAT3 and BSI-18 scales, and no notable differences in relationships were observed.

Clinical Measures.

SCAT3 Symptom Severity, BSI-GSI, BSI-Dep, BSI-Anx, BSI-Som, and MMPI RC1 were significantly positively correlated ($r$’s > .46). SCAT3 Symptom Severity ($r = -.27$), BSI-GSI ($r = -.21$), BSI-SOM ($r = -.27$) and MMPI RC1 ($r = -.23$) were negatively correlated with PSI (i.e., higher severity of symptoms correlated with slower processing speed). PSI was not significantly correlated with BSI-Anx or BSI-Dep.

Inflammatory Markers.

Inflammatory markers CRP, IL-6, and IL-1RA were significantly correlated ($r$’s > .40). CRP, IL-6, and IL-1RA were not significantly correlated with clinical measures ($r$ ranged -.12 to .12), with the exception of IL-1RA being positively correlated with PSI ($r = .29$). IL-6 and IL-1RA were significantly positively correlated with QuinA ($r = .35$ and $r = .27$, respectively). IL-6 was negatively correlated with KynA/QuinA ($r = -.22$).

KP Metabolites.
Within kynurenine pathway metabolites, KynA was significantly positively correlated 3HK \((r = .61)\), QuinA \((r = .42)\), KynA/3HK \((r = .59)\), KynA/QuinA \((r = .67)\). 3HK was positively correlated with QuinA \((r = .58)\) and negatively correlated with KynA/3HK \((r = -.29)\). KynA/3HK and KynA/QuinA were significantly positively correlated \((r = .66)\).

When considering relationships between kynurenine pathway metabolites and clinical measures, KynA was significantly negatively correlated with SCAT3 symptom severity \((r = -.35)\), BSI-GSI \((r = -.37)\), BSI-Dep \((r = -.38)\), BSI-Anx \((r = -.27)\), BSI-Som \((r = -.38)\), and MMPI RC1 \((r = -.24)\) and significantly positively correlated with PSI \((r = .32)\).

3HK was negatively correlated with SCAT3 symptom severity \((r = -.23)\), BSI-GSI \((r = -.26)\), BSI-Dep \((r = -.27)\), BSI-Som \((r = -.30)\), and MMPI RC1 \((r = -.21)\). 3HK was not significantly correlated with BSI-Anx. 3HK was positively correlated with PSI \((r = .31)\).

QuinA was significantly negatively correlated with SCAT3 symptom severity \((r = -.20)\), BSI-GSI \((r = -.24)\), BSI-Dep \((r = -.22)\), and BSI-Som \((- .27)\). QuinA was not significantly correlated with BSI-Anx or MMPI RC1.
Table 3

**Parametric Correlations of Key Clinical Variables, Inflammatory Markers, and Kynurenine Pathway Metabolites**

<table>
<thead>
<tr>
<th>Variable</th>
<th>1</th>
<th>2</th>
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<th>12</th>
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<th>15</th>
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</thead>
<tbody>
<tr>
<td>1. SCAT3 Symptom Severity</td>
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<td>2. BSI-18 Global Severity Index</td>
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<tr>
<td>3. BSI-18 Depression Raw Score</td>
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<td>.93**</td>
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<td>4. BSI-18 Anxiety Raw Score</td>
<td>.81**</td>
<td>.95**</td>
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<td>5. BSI-18 Somatization Raw Score</td>
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<td>7. PSI</td>
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<td>-.16</td>
<td>-.27**</td>
<td>-.23*</td>
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<td>8. IL-6</td>
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<td>-.04</td>
<td>-.11</td>
<td>-.01</td>
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<td>9. IL-1RA</td>
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<td>-.07</td>
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<td>-.08</td>
<td>-.12</td>
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<td>11. KynA</td>
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<td>-.37**</td>
<td>-.38**</td>
<td>-.27**</td>
<td>-.38**</td>
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<td>12. 3HK</td>
<td>-.23*</td>
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<td>-.27**</td>
<td>-.16</td>
<td>-.30**</td>
<td>-.21*</td>
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<td>.12</td>
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<td>.61**</td>
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<td>13. QuinA</td>
<td>-.20*</td>
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<td>-.22*</td>
<td>-.17</td>
<td>-.27**</td>
<td>-.16</td>
<td>.19</td>
<td>.35**</td>
<td>.27**</td>
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<td>.42**</td>
<td>.58**</td>
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<tr>
<td>14. KynA/3HK</td>
<td>-.19</td>
<td>-.18</td>
<td>-.18</td>
<td>-.16</td>
<td>-.15</td>
<td>-.08</td>
<td>.07</td>
<td>-.05</td>
<td>.01</td>
<td>-.04</td>
<td>.58**</td>
<td>-.29**</td>
<td>-.09</td>
<td></td>
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</tr>
<tr>
<td>15. KynA/QuinA</td>
<td>-.19</td>
<td>-.18</td>
<td>-.21*</td>
<td>-.13</td>
<td>-.16</td>
<td>-.13</td>
<td>.16</td>
<td>-.22*</td>
<td>-.18</td>
<td>-.20*</td>
<td>.67**</td>
<td>.14</td>
<td>-.40**</td>
<td>.66**</td>
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</table>

*Note.* Sport Concussion Assessment Tool-3 Symptom Severity (SCAT-3 Sx Sev), Brief Symptom Inventory-18 (BSI-18), Minnesota Multiphasic Personality Inventory-RC1 (MMPI RC1), WAIS-IV Processing Speed Index (PSI), C-Reactive Protein (CRP); interleukin (IL)-6, IL1-receptor agonist (IL-1RA), Kynurenic Acid (KynA); 3 Hydroxykynurenine (3HK); Quinolinic Acid (QuinA).

* p <.05, ** p <.01, *** p <.001
Aim 2.

Univariate analyses identified differences between HC, PPS+, and PPS- groups across clinical measures (i.e., BSI-GSI, BSI-Dep, BSI-Anx, BSI-Som, MMPI RC1, PSI), inflammatory markers (i.e., CRP, IL-6, and IL-1RA), and KP metabolites and ratios (i.e., KynA, 3HK, QuinA, KynA/3HK, KynA/QuinA). Additionally, 2-way ANOVAs assessed for interactions between group and the dichotomous variables of sex (male vs. female), history of prior concussion (history of prior concussion vs. no prior concussion history), and the presence of acute injury characteristics (+LOC/PTA vs. -LOC/PTA) in separate models. Supplementary analyses were conducted to assess HC and mTBI group differences on inflammatory markers and KP metabolites given limited documentation of how these biomarkers function in a pediatric population (see Supplementary Table 1).

Group Differences (PPS+ vs PPS- vs HC)

Table 4 reports clinical measure group differences. The PPS+ reported higher raw scores on the BSI-GSI, as well as the BSI-Dep, BSI-Anx, and BSI-Som subscales compared to the PPS- and HC groups (p’s <.001, $\eta^2_{partial}$ range 0.27-0.51). The PPS+ group had greater MMPI RC1 t-scores compared to PPS- and HC groups (p <.001, $\eta^2_{partial}$ = 0.28). The PPS+ had lower scores than the PPS- and HC groups on measures of processing speed (p <.001, $\eta^2_{partial}$ = 0.15).
Table 4.

*Group Differences between PPS+, PPS-, and HC across Clinical Measures*

<table>
<thead>
<tr>
<th></th>
<th>PPS+</th>
<th>PPS-</th>
<th>HC</th>
<th>p-value</th>
<th>η² partial</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n = 22)</td>
<td>(n = 41)</td>
<td>(n = 41)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BSI-GSI</td>
<td>13.55(11.43)</td>
<td>1.34(2.58)</td>
<td>1.29(2.49)</td>
<td>$F(2,101)= 40.19, p &lt; .001$</td>
<td>0.44</td>
</tr>
<tr>
<td>BSI-Dep</td>
<td>4.18(4.61)</td>
<td>0.37(1.18)</td>
<td>0.56(1.38)</td>
<td>$F(2,101)= 20.99, p &lt; .001$</td>
<td>0.29</td>
</tr>
<tr>
<td>BSI-Anx</td>
<td>4.05(4.11)</td>
<td>0.39(0.86)</td>
<td>0.32(0.72)</td>
<td>$F(2,101)= 29.40, p &lt; .001$</td>
<td>0.37</td>
</tr>
<tr>
<td>BSI-Som</td>
<td>5.32(3.64)</td>
<td>0.59(1.16)</td>
<td>0.41(1.16)</td>
<td>$F(2,101)= 52.67, p &lt; .001$</td>
<td>0.51</td>
</tr>
<tr>
<td>MMPI RC1</td>
<td>56.54(11.48)</td>
<td>44.46(7.79)</td>
<td>41.68(8.92)</td>
<td>$F(2,101)= 19.93, p &lt; .001$</td>
<td>0.28</td>
</tr>
<tr>
<td>PSI</td>
<td>95.81(13.72)</td>
<td>107.93(15.27)</td>
<td>112.34(15.34)</td>
<td>$F(2,101)= 8.80, p &lt; .001$</td>
<td>0.15</td>
</tr>
</tbody>
</table>

*Note:* Brief Symptom Inventory-18 Global Severity Index (BSI-GSI), BSI-18 Depression Subscale (BSI-Dep), BSI-18 Anxiety Subscale (BSI-Anx), BSI-18 Somatization Subscale (BSI-Som), Minnesota Multiphasic Personality Inventory-RC1 (MMPI RC1), WAIS-IV Processing Speed Index (PSI).

Table 5 presents comparison of blood-based biomarkers between the three established groups. CRP, IL-6 and IL-1RA did not significantly differ between groups ($p$’s > .05). See Figure 1 for distribution of inflammatory markers across groups.
Table 5.

Group Differences between PPS+, PPS-, and HC across Inflammatory Markers and Kynurenic Pathway (KP) Metabolites

<table>
<thead>
<tr>
<th></th>
<th>PPS+ (n = 23)</th>
<th>PPS- (n = 41)</th>
<th>HC (n = 41)</th>
<th>p-value</th>
<th>η² partial</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>m(sd)</td>
<td>m(sd)</td>
<td>m(sd)</td>
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<tr>
<td><strong>Inflammatory Markers</strong></td>
<td></td>
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</tr>
<tr>
<td>CRP</td>
<td>13.36(1.62)</td>
<td>13.40(1.64)</td>
<td>13.33(1.39)</td>
<td>F(2, 102)= 0.02, p = .98</td>
<td>--</td>
</tr>
<tr>
<td>IL-6</td>
<td>-0.80(0.63)</td>
<td>-0.73(0.74)</td>
<td>-0.92(0.59)</td>
<td>F(2, 90)= 0.77, p = .47</td>
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</tr>
<tr>
<td>IL-1RA</td>
<td>5.46(0.45)</td>
<td>5.43(0.44)</td>
<td>5.54(0.57)</td>
<td>F(2, 132)= 0.25, p = .78</td>
<td>--</td>
</tr>
<tr>
<td><strong>KP Metabolites</strong></td>
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</tr>
<tr>
<td>KynA</td>
<td>3.39(0.39)</td>
<td>3.65(0.29)</td>
<td>3.82(0.38)</td>
<td>F(2, 101)= 10.50, p &lt;.001</td>
<td>0.17</td>
</tr>
<tr>
<td>3HK</td>
<td>3.23(0.25)</td>
<td>3.38(0.27)</td>
<td>3.45(0.38)</td>
<td>F(2, 101)= 3.40, p = .04</td>
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</tr>
<tr>
<td>QuinA</td>
<td>5.74(0.31)</td>
<td>5.87(0.31)</td>
<td>5.84(0.31)</td>
<td>F(2, 101)= 1.25, p = .29</td>
<td>--</td>
</tr>
<tr>
<td>KynA/3HK</td>
<td>0.16(0.33)</td>
<td>0.27(0.28)</td>
<td>0.37(0.33)</td>
<td>F(2, 101)= 3.21, p = .04</td>
<td>--</td>
</tr>
<tr>
<td>KynA/QuinA</td>
<td>-2.35(0.40)</td>
<td>-2.22(0.38)</td>
<td>-2.03(0.31)</td>
<td>F(2, 101)= 6.55 p = .002</td>
<td>0.12</td>
</tr>
</tbody>
</table>

Note. All markers are log transformed to approximate the normal distribution and reported in pg/mL; C-Reactive Protein (CRP); interleukin (IL)-6, IL1-receptor agonist (IL-1RA), Kynurenic Acid (KynA); 3-Hydroxykynurenine (3HK); Quinolinic Acid (QuinA).

KynA was significantly lower in PPS+ compared to PPS- and HC (p < .001, η² partial = 0.17). KynA/QuinA ratio was significantly higher in the HC group compared to PPS+ and PPS- groups (p = .003, η² partial = 0.08). See Figure 2 for distribution of KP metabolites across groups.

Exploratory analyses comparing a combined mTBI group with HC (see Supplementary Table 1) identified similar differences. CRP, IL-6 and IL-1RA did not significantly differ between adolescents with mTBI and healthy controls (p’s >0.05). KynA was significantly lower in adolescents with mTBI than HC (p = 0.001, η² partial = 0.11). KynA/3HK ratio and KynA/QuinA ratio was significantly lower in adolescents with mTBI than HC group (p = 0.03, η² partial = 0.04 and p = 0.001, η² partial = 0.10).
**Interaction of Sex.** Two-way ANOVAs were used to compare differences of clinical measures, inflammatory markers, and KP metabolites, and between PPS+, PPS-, and HC groups, sex, and potential interactions with the appropriate Bonferroni correction for multiple comparisons.

There was a significant interaction of group and sex for BSI-GSI symptoms ($p = .02$, partial $\eta^2=0.08$), where females in the PCS+ group reported significantly more symptoms than males from the PCS- and HC groups. Overall, females reported more significant symptoms than males ($p < .001$, $\eta^2_{partial} = 0.28$).

There were no significant differences of group or sex across all three inflammatory markers.

There was a significant effect of group on KynA ($p = .01$, $\eta^2_{partial}=0.08$). Post-hoc analyses showed decreased KynA in PPS+ group compared to HC’s and PPS-. There was also a significant effect of sex for KynA, KynA/3HK ratio, and KynA/Quin ratio (see Table 6). Females had significantly lower KynA ($p <.001$, $\eta^2_{partial} = 0.22$). Further, females had lower KynA/3HK ratios ($p = .003$, $\eta^2_{partial} = 0.09$) and KynA/QuinA ratios ($p <0.001$, $\eta^2_{partial} = 0.12$). There were no significant interactions between group and sex (see Table 6).
Table 6.

Two-way General Linear Model (Group x Sex) across Clinical Measures, Inflammatory Markers, and Kynurenine Pathway (KP) Metabolites

<table>
<thead>
<tr>
<th>Measure</th>
<th>Group</th>
<th>p</th>
<th>η²</th>
<th>Sex</th>
<th>p</th>
<th>η²</th>
<th>Group by Sex</th>
<th>p</th>
<th>η²</th>
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<tr>
<td></td>
<td>F(df)</td>
<td></td>
<td></td>
<td>F(df)</td>
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<tr>
<td><strong>Clinical Measures</strong></td>
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<tr>
<td>BSI-GSI</td>
<td>29.36 (2, 98)</td>
<td>&lt;.001</td>
<td>0.37</td>
<td>14.20 (1, 98)</td>
<td>&lt;.001</td>
<td>0.13</td>
<td>4.15 (2, 98)</td>
<td>.02</td>
<td>0.08</td>
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<tr>
<td>MMPI RC1</td>
<td>16.94 (2, 98)</td>
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<td>0.26</td>
<td>0.19 (1, 98)</td>
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<td>--</td>
<td>0.15 (2, 98)</td>
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<tr>
<td>PSI</td>
<td>7.83 (2, 98)</td>
<td>.001</td>
<td>0.14</td>
<td>0.54 (1, 98)</td>
<td>.82</td>
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<td>1.95 (2, 98)</td>
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<tr>
<td>CRP</td>
<td>0.10 (2, 98)</td>
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<td>0.08 (1, 98)</td>
<td>.77</td>
<td>--</td>
<td>0.42 (2, 98)</td>
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<tr>
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<td>0.10 (1, 87)</td>
<td>.75</td>
<td>--</td>
<td>2.20 (2, 87)</td>
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<tr>
<td>IL-1RA</td>
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<td>.80</td>
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<td>0.87 (1, 98)</td>
<td>.35</td>
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<td>0.21 (2, 98)</td>
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<td><strong>KP Metabolites</strong></td>
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<tr>
<td>KynA</td>
<td>4.50 (2, 98)</td>
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<td>27.81 (1, 98)</td>
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<td>0.22</td>
<td>0.60 (1, 98)</td>
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<tr>
<td>3HK</td>
<td>1.16 (2, 98)</td>
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<td>--</td>
<td>5.55 (1, 98)</td>
<td>.02</td>
<td>--</td>
<td>0.23 (1, 98)</td>
<td>.80</td>
<td>--</td>
</tr>
<tr>
<td>QuinA</td>
<td>0.77 (2, 98)</td>
<td>.46</td>
<td>--</td>
<td>1.88 (1, 98)</td>
<td>.17</td>
<td>--</td>
<td>1.07 (1, 98)</td>
<td>.25</td>
<td>--</td>
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<tr>
<td>KynA/3HK</td>
<td>1.51 (2, 98)</td>
<td>.27</td>
<td>--</td>
<td>9.54 (1, 98)</td>
<td>.003</td>
<td>0.09</td>
<td>1.51 (1, 98)</td>
<td>.23</td>
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</tr>
<tr>
<td>KynA/QuinA</td>
<td>4.05 (2, 98)</td>
<td>.02</td>
<td>--</td>
<td>13.00 (1, 98)</td>
<td>&lt;.001</td>
<td>0.12</td>
<td>0.31 (1, 98)</td>
<td>.73</td>
<td>--</td>
</tr>
</tbody>
</table>

*Note. Brief Symptom Inventory-18 Global Severity Index (BSI-GSI), Minnesota Multiphasic Personality Inventory-RC1 (MMPI RC1), WAIS-IV Processing Speed Index (PSI), C-Reactive Protein (CRP); interleukin (IL)-6, IL1-receptor agonist (IL-1RA), Kynurenic Acid (KynA); 3-Hydroxykynurenine (3HK); Quinolinic Acid (QuinA).*

**Interaction of History of Prior Concussion.** Two-way ANOVAs were used to compare differences across clinical measures, kynurenine markers, and inflammatory markers between PPS+, PPS-, and HC groups, history of prior concussion, and potential interactions.

There was a significant interaction of group and history of prior concussion for BSI-GSI symptoms ($p = .005$, partial $\eta^2=0.10$), where participants who reported at least
one prior concussion in the PCS+ group reported significantly more symptoms on the BSI. There was a significant effect of prior concussion ($p = .01$, partial $\eta^2=0.07$), where participants who reported history of concussion reported more symptoms, though this effect is driven by PCS+ group.

CRP, IL-6, and IL-1RA did not differ across groups or between participants with or without a history of concussion. Further, there were no significant interactions of group and history of prior concussion across three inflammatory markers.

There was a significant effect of group on KynA ($p < .001$, partial $\eta^2=0.19$), where PPS+ has significantly lower KynA than the PPS- and HC groups. The HC group had a significantly higher KynA/QuinA ratio compared to both PPS+ and PPS- groups ($p = .002$, partial $\eta^2=0.12$). There were no significant effects of history of prior concussion. There were no significant interactions between group and history of prior concussion (see Table 7).
Table 7.

Two-way General Linear Model (Group x History of Prior Concussion) across Clinical Measures, Inflammatory Markers, and Kynurenine Pathway (KP) Metabolites

<table>
<thead>
<tr>
<th>Measure</th>
<th>Group</th>
<th>History of Prior Concussion</th>
<th>Group by Concussion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F(df)</td>
<td>p</td>
<td>$\eta^2$ partial</td>
</tr>
<tr>
<td><strong>Clinical Measures</strong></td>
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<td></td>
</tr>
<tr>
<td>BSI-GSI</td>
<td>41.01 (2, 98)</td>
<td>&lt;.001</td>
<td>0.46</td>
</tr>
<tr>
<td>MMPI RC1</td>
<td>16.63 (2, 98)</td>
<td>&lt;.001</td>
<td>0.25</td>
</tr>
<tr>
<td>PSI</td>
<td>9.38 (2, 98)</td>
<td>&lt;.001</td>
<td>0.16</td>
</tr>
<tr>
<td><strong>Inflammatory Markers</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CRP</td>
<td>0.001 (2, 98)</td>
<td>.99</td>
<td>--</td>
</tr>
<tr>
<td>IL-6</td>
<td>0.59 (2, 87)</td>
<td>.55</td>
<td>--</td>
</tr>
<tr>
<td>IL-1RA</td>
<td>0.40 (2, 98)</td>
<td>.97</td>
<td>--</td>
</tr>
<tr>
<td><strong>KP Metabolites</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>KynA</td>
<td>11.69 (2, 98)</td>
<td>&lt;.001</td>
<td>0.19</td>
</tr>
<tr>
<td>3HK</td>
<td>3.77 (2, 98)</td>
<td>.03</td>
<td>--</td>
</tr>
<tr>
<td>QuinA</td>
<td>1.23 (2, 98)</td>
<td>.30</td>
<td>--</td>
</tr>
<tr>
<td>KynA/3HK</td>
<td>3.49 (2, 98)</td>
<td>.03</td>
<td>--</td>
</tr>
<tr>
<td>KynA/QuinA</td>
<td>6.53 (2, 98)</td>
<td>.002</td>
<td>0.12</td>
</tr>
</tbody>
</table>

Note. Brief Symptom Inventory-18 Global Severity Index (BSI-GSI), Minnesota Multiphasic Personality Inventory-RC1 (MMPI RC1), WAIS-IV Processing Speed Index (PSI), C-Reactive Protein (CRP); interleukin (IL)-6, IL1-receptor agonist (IL-1RA), Kynurenic Acid (KynA); 3-Hydroxykynurenine (3HK); Quinolinic Acid (QuinA).

**Group by Injury Characteristics.** Additional two-way ANOVAs evaluated whether there were PPS+ and PPS- group differences between clinical measures, inflammatory markers, KP metabolites, and injury characteristics (PTA, LOC, RGA), and potential interactions (see Table 8). Participants in the HC group were excluded from these analyses because they did not sustain a mTBI.

PCS+ had higher BSI-GSI raw scores and t-scores on MMPI RC1 compared to the PCS- group ($p$’s <.001). There was no difference between the PCS+ and PCS- groups on measures of processing speed.
CRP, IL-6, and IL-1RA did not differ across groups or between injured adolescents with or without the presence of acute injury characteristics.

There was a significant effect of group ($p = .01$, partial $\eta^2=0.11$) on KynA. KynA and QuinA were significantly different between presence of injury characteristics but did not survive post-hoc corrections. There were no significant interactions between group and injury characteristics (see Table 8).
### Table 8.

Two-way General Linear Model (Group x Injury Characteristics) across Clinical Measures, Inflammatory Markers, and Kynurenine Pathway (KP) Metabolites

<table>
<thead>
<tr>
<th>Measure</th>
<th>Group (PPS+, PPS-)</th>
<th>Injury Characteristics</th>
<th>Group by Injury Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F(df)</td>
<td>p</td>
<td>η² partial</td>
</tr>
<tr>
<td>Clinical Measures</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BSI-GSI</td>
<td>39.93 (1, 58)</td>
<td>&lt;.001</td>
<td>0.41</td>
</tr>
<tr>
<td>MMPI RC1</td>
<td>22.64 (1, 58)</td>
<td>&lt;.001</td>
<td>0.28</td>
</tr>
<tr>
<td>PSI</td>
<td>4.81(1, 58)</td>
<td>.03</td>
<td>--</td>
</tr>
<tr>
<td>Inflammatory Markers</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CRP</td>
<td>0.03(1, 58)</td>
<td>.86</td>
<td>--</td>
</tr>
<tr>
<td>IL-6</td>
<td>0.11 (1, 52)</td>
<td>.74</td>
<td>--</td>
</tr>
<tr>
<td>IL-1RA</td>
<td>0.49(1, 58)</td>
<td>.49</td>
<td>--</td>
</tr>
<tr>
<td>KP Metabolites</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>KynA</td>
<td>6.97 (1, 58)</td>
<td>.01</td>
<td>0.11</td>
</tr>
<tr>
<td>3HK</td>
<td>2.01(1, 58)</td>
<td>.16</td>
<td>--</td>
</tr>
<tr>
<td>QuinA</td>
<td>1.00 (1, 58)</td>
<td>.32</td>
<td>--</td>
</tr>
<tr>
<td>KynA/3HK</td>
<td>2.47(1, 58)</td>
<td>.12</td>
<td>--</td>
</tr>
<tr>
<td>KynA/Quin A</td>
<td>1.92 (1, 58)</td>
<td>.17</td>
<td>--</td>
</tr>
</tbody>
</table>

*Note. Brief Symptom Inventory-18 Global Severity Index (BSI-GSI), Minnesota Multiphasic Personality Inventory-RC1 (MMPI RC1), WAIS-IV Processing Speed Index (PSI), C-Reactive Protein (CRP); interleukin (IL)-6, IL1-receptor agonist (IL-1RA), Kynurenic Acid (KynA); 3-Hydroxykynurenine (3HK); Quinolinic Acid (QuinA).
Aim 3.

Discrimination of adolescents with mTBI (mTBI) from healthy controls (HC)

Results of the binary logistic regression indicated that, overall, BSI-GSI, PSI, and MMPI RC1 were predictive of group ($R^2 = 0.17$ (Cox-Snell), $0.23$ (Nagelkerke), $X^2(3) = 19.32, p < .001$), but individually clinical variables were not significant predictors ($p$’s > .05). KynA/3HK and KynA/QuinA were predictive of group ($R^2 = 0.10$ (Cox-Snell), $0.14$ (Nagelkerke), $X^2(2) = 11.34, p = .003$), and KynA/QuinA was a significant predictor in the model ($b = 2.03$, Wald $X^2(1) = 6.00, p = .01$). When both clinical and kynurenine metabolites are included simultaneously, KynA/QuinA was the only significant predictor in the model ($b = 1.77$, Wald $X^2(1) = 4.15, p = .04$). The combined model correctly predicted $77.8\%$ of mTBI and $56.1\%$ of HC giving an overall percentage correct prediction rate of $69.2\%$. See Table 9 for full results.
Table 9.

*Binary logistic regression models (Clinical, KP ratios, Clinical + KP ratios) predicting mTBI vs. HC*

<table>
<thead>
<tr>
<th>mTBI vs. HC</th>
<th>95% CI for Odds Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>b(se)</td>
</tr>
<tr>
<td>Clinical</td>
<td></td>
</tr>
<tr>
<td>BSI-GSI</td>
<td>-0.01(.07)</td>
</tr>
<tr>
<td>PSI</td>
<td>0.03(.02)</td>
</tr>
<tr>
<td>MMPI RC1</td>
<td>-0.05(.03)</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>KP ratios</td>
<td></td>
</tr>
<tr>
<td>KynA/3HK</td>
<td>0.03(.90)</td>
</tr>
<tr>
<td>KynA/QuinA</td>
<td>2.03(.83)**</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Clinical + KP ratios</td>
<td></td>
</tr>
<tr>
<td>BSI-GSI</td>
<td>-0.07(.06)</td>
</tr>
<tr>
<td>PSI</td>
<td>0.02(.02)</td>
</tr>
<tr>
<td>MMPI RC1</td>
<td>-0.05(.03)</td>
</tr>
<tr>
<td>KynA/3HK</td>
<td>0.12(.97)</td>
</tr>
<tr>
<td>KynA/QuinA</td>
<td>1.78(.87)*</td>
</tr>
</tbody>
</table>

R²= 0.17 (Cox-Snell), 0.23 (Nagelkerke). Model X²(3)=19.17, p < 0.001.

R²= 0.10 (Cox-Snell), 0.14 (Nagelkerke). Model X²(2)=11.34, p < 0.001.

R²= 0.23 (Cox-Snell), 0.31 (Nagelkerke). Model X²(5)=26.56, p < 0.001.

*Note. Brief Symptom Inventory-18 Global Severity Index (BSI-GSI), Minnesota Multiphasic Personality Inventory-RC1 (MMPI RC1), WAIS-IV Processing Speed Index (PSI), C-Reactive Protein (CRP); interleukin (IL)-6, IL1-receptor agonist (IL-1RA), Kynurenic Acid (KynA); 3-Hydroxykynurenine (3HK); Quinolinic Acid (QuinA).*

*p < 0.05, **p < 0.01, ***p < 0.001

Table 10 presents ROC AUC values corresponding with prediction models that include clinical measures only, KP ratios only, and a combination of clinical measures and KP ratios. AUC values ranged from 0.671 to 0.765 for discriminating mTBI from HC groups (HC selected as the predictive state).
Table 10.

Area Under the Receiver Operating Curve (AUC) of predicted probabilities for mTBI vs. HC

<table>
<thead>
<tr>
<th>Predictors</th>
<th>AUC [95% CI]</th>
<th>Asymptotic Sig</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. BSI-GSI + PSI + MMPI RC1</td>
<td>0.743 [0.648, 0.840]</td>
<td>0.000</td>
</tr>
<tr>
<td>2. KynA/3HK + KynA/QuinA</td>
<td>0.671 [0.564, 0.779]</td>
<td>0.003</td>
</tr>
<tr>
<td>3. BSI-GSI + MMPI RC1 + PSI + KynA/3HK + KynA/QuinA</td>
<td>0.765 [0.674, 0.855]</td>
<td>0.000</td>
</tr>
</tbody>
</table>

Note. Brief Symptom Inventory-18 Global Severity Index (BSI-GSI), WAIS-IV Processing Speed Index (PSI), Minnesota Multiphasic Personality Inventory-RC1 (MMPI RC1), Kynurenic Acid (KynA); 3-Hydroxykynurenine (3HK); Quinolinic Acid (QuinA).

Discrimination of PPS+ from PPS-

Results of the binary logistic regression indicated that, overall, BSI-GSI, PSI, and MMPI RC1 were predictive of PPS group ($R^2$= 0.03 (Cox-Snell), 0.05 (Nagelkerke), $X^2$(2)=2.10, p <.001). BSI-GSI was a significant predictor in the model ($b$ = 0.41, Wald $X^2$(1) = 9.88, $p$ = .002). PSI was also a significant predictor ($b$ = -0.24, Wald $X^2$(1) = 5.44, $p$ = .02). KynA/3HK and KynA/QuinA was not predictive of PPS group ($R^2$= 0.03 (Cox-Snell), 0.05 (Nagelkerke), $X^2$(2)=2.10, $p$=.35). When simultaneously including clinical and KP metabolites, BSI-GSI and PSI were the only significant predictors of PPS group in the model ($b$ = 0.40, Wald $X^2$(1) = 8.61, $p$ =.003, and $b$ = -2.40, Wald $X^2$(1) = 5.49, $p$ = .02). This combined model correctly predicted 92.7% of PCS- and 81.8% of PCS+ giving an overall percentage correct prediction rate of 88.9%. See Table 11 for full results.
### Table 11.

*Binary Logistic Regression models (Clinical, KP ratios, Clinical + KP ratios) predicting PPS+ vs. PPS-.*

<table>
<thead>
<tr>
<th>PPS+ vs PPS-</th>
<th>b(se)</th>
<th>Lower</th>
<th>Odds Ratio</th>
<th>Upper</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Clinical</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BSI-GSI</td>
<td>0.40(.13)**</td>
<td>1.16</td>
<td>1.50</td>
<td>1.92</td>
</tr>
<tr>
<td>PSI</td>
<td>-0.09(.04)*</td>
<td>0.85</td>
<td>0.92</td>
<td>0.99</td>
</tr>
<tr>
<td>MMPI RC1</td>
<td>0.04(.05)</td>
<td>0.94</td>
<td>1.05</td>
<td>1.16</td>
</tr>
</tbody>
</table>

R² = 0.53 (Cox-Snell), 0.73 (Nagelkerke). Model X²(3) = 47.50, p < .001

<table>
<thead>
<tr>
<th><strong>KP ratios</strong></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>KynA/3HK</td>
<td>-0.92(1.23)</td>
<td>0.04</td>
<td>0.40</td>
<td>4.45</td>
</tr>
<tr>
<td>KynA/QuinA</td>
<td>-0.38(0.93)</td>
<td>0.11</td>
<td>0.69</td>
<td>4.24</td>
</tr>
</tbody>
</table>

R² = 0.03 (Cox-Snell), 0.05 (Nagelkerke). Model X²(2) = 2.10, p = .35

<table>
<thead>
<tr>
<th><strong>Clinical +KP ratios</strong></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>BSI-GSI</td>
<td>0.40(.14)**</td>
<td>1.14</td>
<td>1.5</td>
<td>1.96</td>
</tr>
<tr>
<td>PSI</td>
<td>-0.24(.04)*</td>
<td>0.65</td>
<td>0.79</td>
<td>0.96</td>
</tr>
<tr>
<td>MMPI RC1</td>
<td>0.04(1.96)</td>
<td>0.94</td>
<td>1.05</td>
<td>1.16</td>
</tr>
<tr>
<td>KynA/3HK</td>
<td>-0.42(1.60)</td>
<td>0.01</td>
<td>0.66</td>
<td>30.5</td>
</tr>
<tr>
<td>KynA/QuinA</td>
<td>0.32(5.73)</td>
<td>0.06</td>
<td>1.38</td>
<td>31.76</td>
</tr>
</tbody>
</table>

R² = 0.53 (Cox-Snell), 0.73 (Nagelkerke). Model X²(5) = 26.67, p < .001

*Note. Brief Symptom Inventory-18 Global Severity Index (BSI-GSI), BSI-18 Depression Subscale (BSI-Dep), BSI-18 Anxiety Subscale (BSI-Anx), BSI-18 Somatization Subscale (BSI-Som), Minnesota Multiphasic Personality Inventory-RC1 (MMPI RC1), WAIS-IV Processing Speed Index (PSI), C-Reactive Protein (CRP); interleukin (IL)-6, IL1-receptor agonist (IL-1RA), Kynurenic Acid (KynA); 3-Hydroxykynurenine (3HK); Quinolinic Acid (QuinA).*

*p < .05, **p < .01, ***p < .001

AUC ROC values for the significant symptom measures (BSI-GSI, PSI, and MMPI RC1), significant kynurenine ratios (KynA/3HK and KynA/QuinA), and combination of biomarker and clinical measures are presented in Table 12. AUC values ranged from 0.618 to 0.945 for discriminating PPS+ from PPS-.
Table 12.

Area Under the Receiver Operating Curve (AUC) of predicted probabilities for PCS- vs. PCS+

<table>
<thead>
<tr>
<th>Predictors</th>
<th>AUC [95% CI]</th>
<th>Asymptotic Sig</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. BSI-GSI + PSI + MMPI RC1</td>
<td>0.945 [0.886, 1.00]</td>
<td>0.000</td>
</tr>
<tr>
<td>2. KynA/3HK + KynA/QuinA</td>
<td>0.618 [0.467, 0.768]</td>
<td>0.126</td>
</tr>
<tr>
<td>3. BSI-GSI + MMPI RC1 + PSI + KynA/3HK, KynA/QuinA</td>
<td>0.945 [0.887, 1.00]</td>
<td>0.000</td>
</tr>
</tbody>
</table>

*Note. Brief Symptom Inventory-18 Global Severity Index (BSI-GSI), WAIS-IV Processing Speed Index (PSI), Minnesota Multiphasic Personality Inventory-RC1 (MMPI RC1), Kynurenic Acid (KynA); 3 Hydroxykynurenine (3HK); Quinolinic Acid (QuinA).*

Discussion

A significant number of children and adolescents experience prolonged symptoms after sustaining an mTBI, which can have long-lasting negative effects (Ho et al., 2020; Peterson et al., 2013; Rozbacher et al., 2017a; Wilmoth et al., 2019). While most postconcussive symptoms typically resolve, recovery trajectory can vary from weeks to months after mTBI (Babcock, Byczkowski, Wade, Ho, Mookerjee, et al., 2013; Ponsford et al., 1999; Yeates et al., 2009; Zemek et al., 2016). Given the relatively high prevalence rate and potential negative impact, there have been substantial efforts to better understand clinical outcomes and physiological processes underlying pediatric mTBI.

Research has identified several risk factors of prolonged recovery in pediatric mTBI. It is well-documented that adolescence (Davis et al., 2017), female sex (Bunt et al., 2020; Covassin & Elbin, 2011; Fehr et al., 2019; Kontos et al., 2012), pre-injury report of depression, anxiety, and somatic symptoms (Ewing-Cobbs et al., 2018; Grubenhoff et al., 2016; Morgan et al., 2015; Root et al., 2016; Yang et al., 2015; Yeates, 2010), and prior history of concussion (Eisenberg et al., 2013) increase the likelihood of
an extended recovery. In addition to these risk factors, emerging evidence from the adult mTBI literature suggests that physiological markers of injury, including peripheral inflammatory markers and kynurenine pathway (KP) metabolites, may also predict prolonged recovery (Meier, Nitta, et al., 2020; Nitta et al., 2019). It is currently unknown if physiological markers of injury function similarly in pediatric mTBI and are predictive of outcome.

A primary objective of this research study was to elucidate relationships between clinical and physiological measures in a combined ample of adolescents with mTBI approximately 50 days post injury and healthy controls. Further, we reported both clinical and physiological differences between healthy controls and adolescents with typical and prolonged symptom duration post mTBI. Variables of sex, history of prior concussion, and acute injury characteristics were also considered to examine potential interactions between identified risk factors and mTBI recovery. Finally, clinical and physiological measures were independently and simultaneously considered as predictors of mTBI and prolonged symptom experience. Elaboration and exploration of each empirical question is continued in detail below.

**Aim 1. Associations between clinical and physiological measures**

Aim 1 documented the direction and strength of relationships between clinical measures, inflammatory markers, and KP metabolites in a combined clinical and nonclinical pediatric sample. These associations have been explored in adult samples, but it is unclear whether established relationships would be observed in a pediatric sample. While research suggests that symptom presentation in adult and pediatric samples do not significantly vary (Howell et al., 2019; McCrory et al., 2004), there is evidence that
physiological markers of interest are differentially expressed across the lifespan (Bjelosevic et al., 2017; Ignjatovic et al., 2011; Mayer et al., 2018). Thus, a critical first step in understanding relationships between clinical and blood-based biomarkers in the context of recovery from pediatric mTBI requires documenting associations between cognitive, emotional, and somatic symptom reporting with serum biomarkers in a pediatric sample.

Consistent with our hypothesis, symptom report measures were strongly (positively) correlated. Specifically, the SCAT3 Symptom Severity Score, BSI-GSI and subscales, and MMPI RC1 demonstrated strong, positive correlations amongst one another. These findings make clear that the primary variance of self-report measures utilized in mTBI research (e.g., SCAT3 and BSI-18) often reflect emotional distress and postconcussive symptoms (Nelson et al., 2018; Thomas, 2012). Further, symptom report appears to be broad and general, and there is debate if somatic symptoms and internalizing symptoms are distinct constructs or represent an underlying internalizing factor (Kotov et al., 2017).

Processing speed was quantified with performance-based neuropsychological measures and exhibited discrepant relationships with symptom checklists, inflammatory markers, and KP metabolites. First, processing speed exhibited meaningful associations with depression, anxiety, and somatic scales where lower PSI was correlated with higher report of symptoms. These associations reflect small-medium to medium effects though variable statistical significance. While PSI was not statistically significantly correlated with depression and anxiety symptoms, a similar magnitude of associations with somatic symptoms was observed and was statistically significant (i.e., -0.21 to -0.17). It is
interesting to note that one study reported observing significant negative correlation between PSI and BSI-Dep and BSI-Som in a population of adults 6 months after mTBI, but no relationship between PSI and BSI-Anx (Nelson et al., 2017a). Although Nelson and colleagues’ sample size was considerably larger \( n = 415 \), the magnitude of their significant correlations are only slightly smaller compared to the current study. Current findings of nonsignificant correlations between PSI and BSI-Dep and BSI-Anx may be related a relatively small sample size and/or a restricted range of symptom reporting (i.e., low raw scores). Nevertheless, we observed expected relationships between somatic symptoms measured by the BSI-18 and processing speed, and small associations between processing speed and report of anxiety and depressive symptoms. It seems clear that lower scores on processing speed measures can be associated with report of emotional distress.

Relationships between processing speed, inflammation, and KP metabolites have not been documented in a pediatric sample. Here, we observed that higher levels of IL-1RA and neuroprotective KynA were positively correlated with processing speed. These initial findings are novel and add to a small literature disentangling physiological correlates of cognitive tasks. Few studies report relationships between KP metabolites and cognitive measures. Most findings are reported in psychiatric samples, where there are differences in associations between KP and verbal memory (Platzer et al., 2017), processing speed, and visual learning (Zhou et al., 2019). This research is in its nascent stages, and the relationships between KP metabolites and neurocognitive outcomes are limited to mood disorders. However, given the established emotional distress component
of mTBI, an underlying pathophysiology describing associations between neurocognitive performance and distress in pediatric mTBI should be explored.

When considering inflammatory markers approximately 50 days post mTBI, we hypothesized a potential relationship between inflammatory markers and clinical symptoms given previously reported associations between inflammatory cytokines, “sickness behavior,” and depression (Dantzer & Kelley, 2007; Di Battista et al., 2020; Howren et al., 2009). However, the data from the present study evidenced no significant relationship between inflammatory markers CRP, IL-6, IL-1RA and SCAT3 Symptom Severity, BSI-18 and subscales, or MMPI RC1 at this post-acute timepoint. With a larger sample size of adolescents experiencing prolonged symptom recovery, we might observe significant relationships between inflammation and symptoms of emotional distress given emerging evidence of inflammatory processes and mood disorders specifically in pediatric populations (Gabbay et al., 2009; Henje Blom et al., 2012; Lee et al., 2020).

In contrast to inflammatory markers reported, KP metabolites were strongly correlated with report of mood symptoms, and there is a robust literature highlighting the kynurenine pathway as a potential mechanism of depressive symptoms and mood disorders. In this sample, lower circulating neuroprotective KynA was associated with increased report of depression, anxiety, and somatic symptom reporting. This is expected given that KynA is neuroprotective and acts as a competitive antagonist to ionotrophic excitatory amino acid receptors (Foster et al., 1984; Kessler et al., 1989), decreasing excitotoxicity. However, along the neurotoxic arm of the KP, the relationships of metabolites 3HK and QuinA with symptom report was unexpected. In previous literature, increased 3HK and QuinA have been associated with mood (Savitz, 2019), and more
specifically, symptoms of depression (Öztürk et al., 2021). Higher 3HK and QuinA serum levels were related to fewer postconcussive symptoms, as well as fewer reported depressive, anxiety, and somatic symptoms. This is surprising given that we would expect a positive relationship between neurotoxic 3HK and QuinA and increased report of symptoms.

Finally, the ratios of KynA to 3HK and QuinA have been used as putative indices of neuroprotection in mTBI (Meier, Savitz, et al., 2016), where lower KynA/3HK and KynA/QuinA ratios have been associated with greater emotional distress. The KynA/QuinA ratio was negatively correlated with depression symptoms consistent with the literature suggesting that lower neuroprotective ratio of KynA/QuinA is associated with low mood, depression, and bipolar disorder (Arnone et al., 2018; Meier et al., 2018; Savitz, 2019). The relationships of KP metabolites and clinical measures reported here add to a very limited literature on the function of this pathway in adolescents. Overall, KP metabolite relationships performed in both expected and unexpected ways, and additional research documenting KP metabolites and psychological distress and symptoms of mood, anxiety, and somatization is warranted, particularly in pediatric samples.

Overall, Aim 1 highlights the nuanced relationships between blood-based biomarkers, cognitive functioning, and symptom reporting in pediatric samples. Inflammatory markers of CRP, IL-6, and IL-1RA and KP ratio of KynA/3HK were not significantly correlated with symptom report in this adolescent sample, potentially highlighting the differential expression of these markers in a pediatric population. When assessing the performance of KynA alone, reported relationships of increased KynA and
increased report of depression, anxiety, and somatic symptom reporting is consistent with reported literature. Without inflammatory activation, the neuroprotective KynA branch of the KP is preferred, and thus the expected KynA and unexpected 3HK and QuinA performance across this broad adolescent sample likely represents baseline metabolite levels without inflammatory activation. One might anticipate significant relationships between neurotoxic and KP ratios and symptom reporting would emerge in a sample consisting of only adolescent mTBI patients.

The associations between clinical measures, inflammatory markers, and KP metabolites should be considered tentative in light of limited sample size and a mix of clinical and non-clinical participants. Many associations demonstrated similar magnitudes though some failed to reach clinical significance. Briefly, exploratory analyses of these relationships solely in sample of adolescents with mTBI identified patterns similar correlations. Replication in ideally diverse samples (i.e., healthy adolescents, adolescents with mood disorders) is needed to further explicate physiological relationships with symptom reporting in pediatric populations.

**Aim 2. Clinical and physiological differences in typical and atypical symptom recovery post mTBI**

**Clinical Differences**

Diverse emotional, cognitive, and somatic symptoms are commonly reported following mTBI. There is evidence suggesting that somatic symptoms are most commonly reported in an acute stage following injury (Howell et al., 2019) while emotional and cognitive symptoms tend to be endorsed later in recovery (Eisenberg et al., 2014; Ewing-Cobbs et al., 2018; Ponsford et al., 1999). Overall, when considering the BSI-GSI, adolescents experiencing a prolonged recovery reported significantly more
symptoms than both healthy controls and adolescents who recovered from mTBI, which is consistent with a broad literature. With respect to BSI-18 subscales, adolescents experiencing prolonged recovery from mTBI reported more symptoms of depression and anxiety. This finding aligns with a robust literature documenting that prolonged symptom recovery in pediatric mTBI frequently includes the experience of emotional symptoms (e.g., see Sheldrake et al., 2022).

Report of somatic symptoms were also significantly different across groups with very large effect sizes. Across both the BSI-18 somatic symptoms subscale and MMPI RC1, adolescents with prolonged recovery reported more physical symptoms (e.g., nausea, chest pain, weakness) compared to healthy controls and typically recovered adolescents. Notably, despite higher levels of somatic symptom reporting in the prolonged recovery group, mean MMPI RC1 T-scores and BSI-18 subscales remained well below the clinical cut-offs (T>64) across groups. Upon further inquiry, only seven participants in the entire sample obtained MMPI RC1 T-scores above this cut-off, five from the prolonged recovery group, one typically recovered adolescent, and one healthy control. This suggests that while somatic symptoms are frequently reported in the acute stage of recovery from mTBI, adolescents with prolonged recovery continue to report a greater number of somatic symptoms, though not to a degree conveying clinical significance. Additionally, a limited number of participants reported BSI-18 symptoms above the clinical cut-off across subscales, including the BSI-Som subscale. The role of somatic symptom reporting in understanding mTBI recovery is promising and underappreciated, especially given that acute endorsement of somatic symptoms has been documented to predict prolonged mTBI recovery (Nelson et al., 2016).
With respect to neurocognitive outcomes, participants experiencing a prolonged mTBI recovery had slower processing speed. Typically, a mTBI does not have lasting effects on cognitive functioning, and while some injured adolescents may experience acute difficulties with speed of information processing, clinically cognitive recovery is quick with limited functional impact (Sarmiento et al., 2020; Sicard et al., 2021). Thus, lower performance on measures of processing speed in the prolonged recovery group is somewhat unexpected. On the other hand, there is some evidence that individuals with prolonged recovery from mTBI may experience cognitive difficulties up to one-month post-injury (Mayer et al., 2012). It is likely that lower processing speed scores in the prolonged symptom group is associated with higher reported symptom burden and distress, as some report mood and postconcussive symptoms are associated with poorer performance on cognitive measures (Barker-Collo et al., 2015).

In addition to considering broad group differences, we also assessed group differences in light of documented risk factors for prolonged recovery from mTBI (i.e., sex, history of prior concussion, acute injury characteristics). First, results of this study are consistent with a replicated finding that females are more likely to experience prolonged symptoms after mTBI (Covassin & Elbin, 2011; Iverson et al., 2017). Females with prolonged recovery from mTBI reported more psychological symptoms than females in the healthy control and typical recovery groups. Overall, this interaction is driven by female sex, where females overall reported more symptoms on the BSI-18 than males. Females, regardless of injury status, are more likely to endorse symptoms than males on the BSI-18 but no differences were observed for PSI or MMPI RC1. Further, results of this study are consistent with a broad literature documenting that adolescents
who sustain multiple concussions are at risk for experiencing extended symptom duration with subsequent head injuries (Eisenberg et al., 2013; Ponsford et al., 1999). Not only did participants with prolonged recovery report more BSI symptoms, participants with prolonged recovery from their current mTBI who previously sustained a concussion reported more symptoms on the BSI-18.

Finally, while acute injury characteristics such as loss of consciousness (LOC), posttraumatic amnesia (PTA), and retrograde amnesia (RGA) are hypothesized to be risk factors for prolonged recovery (McCrorry et al., 2013; Nelson et al., 2013), no significant interactions between adolescents with mTBI and the presence of acute injury characteristics across clinical symptom report, somatic symptoms or processing speed were identified. These findings are consistent with research that suggests LOC does not predict recovery time or cognitive symptoms (Erlanger et al., 2003; McCrea et al., 2013). However, others have identified that LOC, PTA, and RGA (Bernard et al., 2016; Cantu, 2001; Fried et al., 2022) may predict symptom duration and cognitive impairment (Yeates et al., 2009). Notably, this investigation considered acute injury characteristics simultaneously given that LOC, PTA, and RGA rarely occur in concussion, and null findings may be related to combining the three dichotomous conditions (i.e., the predictive value of one factor is nullified by the presence of factors that are not predictive). Thus, these injury defining features considered simultaneously may not predict post-acute outcomes. Taken together, there continues to be conflicting findings regarding whether LOC, PTA, and RGA are risk factors for prolonged mTBI recovery.

Overall, differences in symptom reporting between groups is consistent with and adds to the existing pediatric mTBI literature. Adolescents who have an extended
symptom recovery (as defined by persistent postconcussive symptoms and not being 100% back to baseline) continue to report emotional and somatic symptoms approximately 50 days post injury. Further, this study replicates previous findings that female sex and a concussion history increase risk for prolonged symptoms, though there was no increased risk for prolonged symptom duration if mTBI was associated with LOC, RGA, and PTA.

These findings emphasize the importance of broad clinical expertise in understanding relationships between mTBI, cognitive functioning, and emotional distress. When emotional distress and cognitive difficulties persist beyond 4-6 weeks, there is a unique role for neuropsychologists to facilitate identification and management of symptoms. Sarmiento and colleagues (2020) highlight the value of neuropsychological assessment to identify and screen for other potential contributors to prolonged symptom recovery, including depression, anxiety, learning difficulties, or ADHD. Broadly understanding a patient’s challenges facilitates more meaningful treatment recommendations, improving the probability of positive outcomes. Further, given that diagnostic criteria for mTBI and predictors of recovery rely significantly on symptom report, a nuanced understanding of this recovery process in females is warranted (Valera et al., 2021), especially given the increased likelihood that females will endorse more postconcussive symptoms at baseline. As pathophysiological correlates of mood, anxiety, and somatic symptom reporting are further refined, a more comprehensive understanding of symptom reporting in pediatric mTBI will emerge.

_Inflammatory markers_
Emerging research has identified differences in physiological responses following brain injury as measured in fluid blood and serum biomarkers. Mechanical forces in mTBI disrupts cellular processes and leads to axonal injury, altered neurotransmission, neuroinflammation, excess glutamate release and altered metabolism (Giza & Hovda, 2014; Polinder et al., 2018). Critically, few investigations have considered relationships between blood-based biomarkers and prolonged symptom duration in adolescent mTBI.

Prospective studies of inflammatory markers and mTBI document acute elevations as predictive of prolonged symptom duration. For example, in a sample of high school and football athletes with SRC, elevated IL-6 measured at 6 hours post injury was associated with longer symptom recovery (Nitta et al., 2019). In an adult mTBI study, elevated CRP predicted prolonged concussion symptoms (Su et al., 2014). We extended research on peripheral inflammatory markers to a post-acute timepoint in an adolescent mTBI sample and reported no significant group differences across studied inflammatory markers of IL-6, IL-1RA, and CRP.

When considering risk factors, inflammatory markers were not significantly different across sex, despite some studies reporting differences in inflammatory cytokines between male and female with mTBI (Di Battista et al., 2020). Notably, the sex difference observed by Di Battista and colleagues was observed in the acute stage post mTBI, which again highlights the temporal nature of inflammatory markers. When considering sex alone, adult studies have also reported higher levels of CRP in females compared to males (Hutchinson et al., 2000), and these differences extend to pediatric samples (Lambert et al., 2004). In contrast to these findings, we reported no significant difference between males and females across all inflammatory markers. Further, CRP,
IL-6, and IL-1RA did not differ significantly based on prior concussion history or the presence of acute injury characteristics at this timepoint post mTBI.

In summary, this research did not identify a unique inflammatory response in adolescents approximately 50 days after mTBI. Null findings lend support to the hypothesis that increased or higher levels of peripheral inflammatory markers are restricted to the acute (within 24-hours post-injury) time period (Nitta et al., 2019; Parkin et al., 2019). This finding contributes to an emerging literature and understanding of the role of inflammatory processes occurring post injury and relationships with symptoms in pediatric sample.

**Kynurenine Pathway Metabolites**

While elevations in inflammatory markers appear most related to symptoms in the acute stages of recovery from mTBI, disruption of neurometabolic processes are likely to extend for weeks or months (Giza & Hovda, 2001). The inflammatory response following traumatic brain injury activates the immunoregulatory kynurenine pathway (KP). Downstream metabolites of this pathway have both neuroprotective and neurotoxic effects on the brain, and the ratios of neuroprotective KynA and neurotoxic 3HK and QuinA represent the overall balance of the pathway. Measurements of KP metabolites and their associated ratios have been associated with outcomes in adult mTBI literature, though it is unclear if these findings might replicate in pediatric mTBI samples.

In a study of college football players, concussed athletes had decreased KynA/QuinA ratios at one day, one week and one month post-injury (Singh et al., 2016). We similarly found that adolescents with mTBI (regardless of symptom duration) had increased levels of KynA and lower KynA/QuinA compared to healthy controls. After
delineating mTBI group based on symptom duration, healthy controls had higher KynA/QuinA ratios relative to both prolonged and typically recovered mTBI groups. Further, adolescents with prolonged recovery had lower neuroprotective KynA when compared to those with typical recovery and healthy controls. These findings parallel previous work which reported athletes with SRC who had longer recovery (quantified by return-to-play decisions) had higher QuinA and lower KynA/QuinA levels at one month post-injury (Meier, Savitz, et al., 2016). Notably, given the relatively limited sample size, there may have been insufficient power to detect additional differences. For example, while 3HK and KynA/3HK group differences were consistent with hypotheses, they were not statistically different after applying a correction for multiple comparison.

With respect to the risk of prolonged symptoms in the context of a history of prior concussion, KP metabolite performance in this investigation differed from previous findings. In contrast to the current findings, several studies reported relationships between KP metabolites and a history of prior concussions, suggesting a potential primed immune response from previous head injury. Specifically, football players with a mTBI who reported a prior history of concussion had lower neurotoxic KynA/QuinA compared to athletes without a prior concussion history (Meier et al., 2020b) and greater levels of neurotoxic QuinA compared to athletes with no concussion history (Meier et al., 2016). In this investigation, there was no effect of prior concussion on KP metabolites or ratios, and KP metabolites did not differ between participants with a history of concussion and those with no previous concussions. KP metabolites differences were also not reported based on acute injury characteristics.
Perhaps our most notable finding relates to sex, KP metabolites, and clinical measures. Meier and colleagues (2018) reported that in a sample of females with and without depression, females had lower KynA/3HK and KynA/QuinA ratios compared to males. When considering the additional risk factor of sex in this investigation, females also had decreased KynA compared to males across the groups with a very strong effect, likely driving additional findings of lower KynA/3HK and KynA/QuinA ratios in females compared to males. Despite identified risk of prolonged symptom recovery in females, there was no significant interactions between group and sex across KP metabolites. We hypothesized that females with prolonged recovery from mTBI would have lower KynA/QuinA and KynA/3HK than females with typical recovery and females in the healthy control group. However, we suspect that sex differences and KP metabolites may be potentially due to a methodological confound whereas females were highly represented in the PPS+ group.

Given the distinct sex differences identified, it is important to consider the relationship between the immune system and sex hormones. Indeed, previous work has included variables that may impact KP metabolite expression such as menstrual phase, oral contraceptive use, and hormones (Meier et al., 2018). The use of oral contraceptives has been reported to lower KP ratios, driven by lower KynA. Further, hormonal changes associated with puberty in females confer additional risk for poorer outcomes from moderate to severe TBI (Morrison et al., 2004). Additionally, there is also a relationship between hormonal changes and symptom reporting in mTBI. In a study of sex differences following mTBI, female symptom report scores surpassed males beginning around the onset of puberty (Bazarian et al., 2010). In another independent study of women
presenting to the ED following mTBI, females in the luteal phase of their menstrual cycle (i.e., higher progesterone) had significantly lower quality of life and more postconcussive symptoms one month after injury (Wunderle et al., 2014). Taken together, the effects of group and KP metabolites may be driven by female sex rather than prolonged symptom duration, and there are clearly additional variables to consider in future research explicating relationships between mTBI, female sex, and symptom duration.

Further, symptom report cannot be solely attributed to physiological differences. Consideration of psychosocial factors as they relate to gender-based symptom reporting is also important to consider. In a scoping review of sex differences in traumatic brain injury, Valera and colleagues (2021) highlight how psychosocial factors may contribute to sex and gender differences in symptom reporting, including differences in the perception of somatic symptoms, differences in symptom labeling, as well as differences in socialization and willingness to disclose discomfort. Understanding symptom recovery in mTBI is certainly multifactorial in nature. Nevertheless, the interplay between inflammation, neuroimmune pathways, sex, psychological factors highlights the need for a biopsychosocial approach to conceptualizing mTBI recovery and management.

**Aim 3. Integrating clinical and blood-based biomarkers as predictors**

Finally, this research evaluated whether a combination of clinical and physiological measures better classified adolescents who (1) did or did not sustain an mTBI and (2) do and do not report prolonged postconcussive symptoms. The integration and synthesis of clinical variables and blood-based biomarkers is critical in understanding factors that contribute to the experience of persistent symptoms following pediatric mTBI. Indeed, there are direct calls for research to incorporate targeted, multidisciplinary
integrated approaches to understand prolonged symptom recovery (Polinder et al., 2018; Takagi et al., 2019).

**Discrimination between mTBI and HC**

Overall, with respect to clinical measures, BSI-GSI, PSI, and MMPI RC1 simultaneously discriminated between healthy controls and injured adolescents, though no individual measure was significantly predictive on its own. A model including KP ratios of KynA/3HK and KynA/QuinA significantly predicted group membership, and a higher KynA/QuinA ratio was associated with increased likelihood of being in the non-injured control group. When clinical variables and KP ratios were combined in a predictive model, KynA/QuinA continued to be a significant predictor of group membership, and the combined model was improved when compared to clinical measures and KP ratios alone. KynA/QuinA as significant predictor in the combined model suggests that there are immunological differences between healthy non-injured adolescents and adolescents with mTBI at this post-acute timepoint.

Combinations of clinical and cognitive measures have been investigated to more aptly diagnose mTBI in the subacute state (Garcia et al., 2018). Garcia and colleagues (2018) identified that combining a cognitive screener and balance measure effectively identified acute concussion (AUC = 0.73). The ability of these measures to identify concussion at an acute timepoint is similar to the predictive ability of combined symptom report and PSI at a post-acute timepoint (AUC = 0.74) in this study.

To our knowledge, only one study has simultaneously combined clinical and physiological measures in a prediction model. In a sample of high school and college football athletes, we previously reported that a combination of symptom severity score
and blood-based biomarkers in mTBI effectively differentiated those who did and did not sustain a concussion. A combination of the SCAT3 symptom severity score, brain injury markers, and inflammatory markers improved AUC classification of SRC and football controls from .95 to .99, and SRC and non-football athletes from .94 to .98 (Meier, Huber, et al., 2020). AUC values in the current investigation did not reach similarly high AUC levels for the combined model (AUC= 0.76) but classification was improved from models including only clinical or KP ratios (AUC=0.73 and AUC=0.67, respectively). Direct comparison between studies is limited as blood-based biomarkers varied between studies (i.e., brain injury specific markers versus inflammatory markers and KP metabolites) as well as the time point post injury that blood was collected. Further, discrimination between groups is likely more pronounced and detectable in the acute phase and group differences likely diminish over time, thus lower AUC values in this study are expected. However, nevertheless, it is notable that we identified KP ratios significantly discriminate healthy controls from adolescents with mTBI providing valuable insight into biomechanisms of mTBI recovery.

**Discrimination between PPS+ and PPS-**

While differences identified between healthy controls and mTBI adds to our understanding of mTBI, differentiation within an injured group is likely of greater clinical significance. Understanding the complex interplay between clinical and physiological variables may better help in the identification of postconcussive syndrome (PCS), and thus, management and intervention for the small, but meaningful, subset of pediatric patients with prolonged mTBI recovery. In contrast to discrimination between individuals who sustained a concussion and healthy non-injured controls, both BSI-GSI
and PSI were significant predictors of PPS+ in the clinical measure prediction model, but neither KynA/3HK or KynA/QuinA ratios were significant predictors in the physiological prediction model. This pattern remained in the combined prediction model as BSI-GSI and PSI continued to be significant predictors of group membership. Overall, KP ratios discrimination was fair (AUC=0.62) but had a limited contribution overall in discriminating between prolonged and typical recovery.

These findings highlight a few different important issues. First, PPS+ and PPS- groups were stratified based on symptom report, and thus, it is not surprising that BSI-GSI strongly predicted prolonged versus typical recovery. As is the case with much of the research pertaining to clinical conditions, symptoms will always be very strong discriminators when symptom checklists are used as a diagnostic tool. Further, we highlighted in Aim 1 strong correlations across measures of mood, anxiety, and somatization as measured by the BSI-18. Thus, general symptom reporting is strongly associated with a prolonged recovery from mTBI, and not surprisingly, a very strong discriminator.

Despite the predictive ability of BSI-GSI in this investigation, there are potentially drawbacks to solely relying on symptom reporting to identify PCS. Utilizing symptom report measures in different ways to identify PPS in pediatric mTBI has the potential to result in high rates of misclassification (Mayer et al., 2020). Thus, the standard practice of using symptom report alone to identify prolonged recovery from mTBI may fail to identify adolescents who would benefit from intervention. Further, there is significant risk for attributing prolonged emotional distress solely to a concussion. High report of emotional symptoms or somatic symptoms could be explained
by psychosocial factors, depression, anxiety, and/or ADHD. Clinically, if there is a question of whether postconcussive symptom report is related to previous mTBI or other factors or conditions, this research supports using additional symptom measures and cognitive screening to identify possible etiology.

We hypothesized that combining physiological and clinical measures may improve classification. However, in this model KP ratios did not significantly predict or add to prediction of persistent postconcussive symptoms above and beyond clinical measures. Nevertheless, findings expand understanding of physiological mechanisms underlying symptom report post mTBI. Given identified relationships between KP metabolites and psychological distress, KP metabolite differences between groups could moderate the relationship between prolonged recovery and distress reported post mTBI. While symptoms measures will always be more predictive than physiological measures, their additive nature has the potential to improve specificity of classification or provide physiological endpoints for clinical trials. To our knowledge this is the first study to combine KP metabolites and clinical measures to discriminate typical and prolonged symptom recovery, and additional work is needed to better explicate the relationship of physiological measures and clinical symptom report at both acute and post-acute recovery timepoints.

Limitations

As with all empirical research, there are some notable limitations to this work that should be kept in mind when interpreting findings. First, this sample was comprised of participants recruited from two different research studies. Study 1 participants were largely recruited from a sports medicine clinic while Study 2 participants were recruited...
to participate in a prospective research study. This poses a potential sampling bias where it is possible that those participants recruited from the medical clinic were more likely to experience an atypical recovery and have increased symptom reporting. In fact, no adolescent with mTBI from Study 2 was assigned to the PPS+ group based on symptom reporting in the post-acute phase. Notably, because Study 2 only included male participants, there was also a decreased likelihood of a Study 2 participant experiencing prolonged symptoms. Finally, Study 1 participants were more likely to be younger, thus there was increased likelihood that the PPS+ group would be comprised of younger, female adolescents. However, it is important to note that being younger and female are well-established risk factors for extended symptom recovery (Covassin & Elbin, 2011; Fehr et al., 2019; Kontos et al., 2012). Despite a potential sampling bias, this is the first study to report KP metabolites in a pediatric mTBI sample.

Additionally, there are some noteworthy methodological differences between the two studies that should be acknowledged. First, Study 1 participants were instructed to fast for 24 hours prior to their visit, while Study 2 participants did not. Fasting can affect tryptophan (TRP) levels, which is the upstream amino acid from which the KP metabolites are synthesized. To ensure that differences in KP metabolites reported here cannot be attributed to TRP differences between Study methods, we completed sensitivity analyses to ensure identified differences were not related solely to methodological differences between studies. Indeed there were significant differences in TRP levels between Study 1 and Study 2, but when we covaried TRP, there continued to be significant group differences for KynA and KynA/3HK.
Finally, it should be noted that some clinical measures were designed and normed for adult populations. Specifically, the lowest age norms for the WTAR and PSI measures is 16 years. Approximately one third of the sample was below the age of 16, and thus, WTAR and PSI scores were calculated using adult norms. Despite PPS+ being statistically significantly younger, WTAR scores did not differ across groups. However, given the unique PSI findings in this study, a comparison of adult (WAIS-IV) and pediatric (WISC-5; Wechsler, 2014) Wechsler processing speed tasks and normative data was conducted. Notably, raw scores on the processing speed subtests used to generate the Processing Speed Index score were broadly equivalent between the WAIS-IV and WISC-V. Regardless, the finding that adolescents with extended recovery from mTBI have slower processing speed than those adolescents who recovered from mTBI should be interpreted cautiously.

**Clinical Implications**

This research adds substantially to a growing literature documenting relationships between clinical and physiological measures in pediatric mTBI. A significant proportion of the pediatric mTBI literature focuses on symptom reporting and disruption of physiological systems in the acute window of recovery to identify diagnostic and prognostic tools. We investigated these relationships at a later timepoint in mTBI recovery to broaden conversations regarding underlying pathophysiological recovery and its relationship with symptom duration and presentation.

Broadly, this study again identifies female sex as a risk factor for prolonged symptom experience following mTBI and further documents relationships between mTBI, female sex, and emotional distress. Further, this study extends results from the
adult mTBI literature to the pediatric mTBI literature. Females have lower neuroprotective KynA, thus sustaining an mTBI may have an additive effect of decreased neuroprotection for excitotoxicity. This finding suggests an underlying pathophysiological relationship and mechanism for emotional distress. Interestingly, in a rat model of concussion, treatment with KynA diminished the efflux of potassium (Katayama et al., 1990), thus helping to maintain extracellular homeostatic conditions. Clinical trials for a KynA analogue, 4-chlorokynurenine or AV-101, a selective antagonist acting on NMDA receptors, are currently being conducted for use in major depressive disorder and other KP therapeutic targets are being explored (e.g., see Meier & Savitz, 2022). As research continues to disentangle the role of the KP in mTBI and symptom duration, there is potential for medication modulation of the KP to address depression symptoms or development of mood disorders post mTBI.

This clinical translational pediatric mTBI study is relevant to and can improve current clinical practice. There are certainly risks associated with the mismanagement of mTBI in pediatric populations. Pediatric patients with prolonged symptom experience following a mTBI are more likely to utilize and seek medical consultation from a variety of providers (Jimenez et al., 2017), placing significant burden on the health system. First, clinicians should be aware of risk factors for prolonged recovery, including those outlined in this study (i.e., female sex, early adolescence). Early identification of risk factors may increase the likelihood that clinicians provide early interventions which can range from providing brief psychoeducation regarding recovery to mental health intervention focused on mTBI recovery and specific symptoms reported. Finally, patients, families, and clinicians may benefit from the knowledge that there is a clear
pathophysiological mechanism underlying extended symptom duration. For patients seeking an explanation for their distress, it may be helpful to integrate biological factors into mTBI education. Though the clinical intervention provided at this time may remain the same, it may be validating for patients to learn that there is an underlying pathophysiological mechanism to their recovery experiences.

**Future Directions**

Relationships between blood-based biomarkers, measures of general emotional distress, depression, anxiety, and somatic symptoms, and processing speed were documented. Future research might include quantifying emotional symptoms using symptom measures specific for different types of internalizing symptoms. For example, quantifying a broader range of depressive symptoms may better capture the different ways depressive symptoms are expressed (e.g., more physical or cognitive depression symptoms). Thus, different types of mood symptoms may be differentially associated with KP metabolites given research on “sickness behavior” (Dantzer & Kelley, 2007). Further, this study only considered the long term impact of mTBI on processing speed. While processing speed exhibited unexpected relationships with biomarkers, future studies may consider including other cognitive tests commonly found to be affected by mTBI in the acute phase of recovery. Attention, concentration, and working memory are cognitive processes often impacted by mTBI in the acute phase of recovery, and processing speed is a key component in completing cognitive tasks which require these skills. Inclusion of neurocognitive measures that evaluate a broader range of cognitive constructs is warranted. Finally, while this investigation included data from a single timepoint following mTBI, prospective studies of pediatric mTBI which integrate clinical
measures and blood-based biomarkers is warranted to further disentangle the physiological underpinnings of symptom presentation across the timeline of recovery.

There is clearly a strong relationship between female sex and prolonged symptom experience following mTBI. While there are numerous studies that include male participants, there is a dearth of research documenting physiological recovery in females. It is critically important to include female adolescents in mTBI research studies, as there appears to be a significant risk for prolonged symptom duration and physiological differences in recovery. Further, some research suggests that girls’ soccer had the highest rates of SRC compared to boys and other sports (Covassin & Elbin, 2011; Lincoln et al., 2011). These data suggest differential expression of metabolites in females. Further, there is some research suggesting that the hormone and differences in menstrual cycles may impact symptom experience. This study focused solely on the inflammatory makers and kynurenine pathway metabolites, but future research would benefit from the inclusion of other physiological variables, including pubertal state, phase of menstrual cycle, or estrogen or progesterone levels. Continued research of pediatric mTBI in females will elucidate the relationship between psychosocial factors and physiological functioning to confer risk for prolonged recovery.

Conclusion

There is an unavoidable potential that many children and adolescents may sustain an mTBI during their lifetime as they participate and benefit from the many joys of sports, play, and childhood. For the small but substantial number of pediatric patients who experience prolonged symptom recovery, results of this investigation contribute to a growing literature working to develop improved monitoring of mTBI recovery as well as
improved therapeutic interventions. Key relationships between symptom presentation and potential underlying physiological mechanisms were reported, and results suggest that symptoms and physiological variables function differently at this post-acute timepoint and across a key demographic variable of sex. Continued research to understand associations between pathophysiological processes and mTBI symptom presentation across recovery phases will expand our understanding of treatment targets, whether that be targeted mental health and behavioral therapy, the provision of psychoeducation, pharmaceutical intervention, or a potential unique combination.
BIBLIOGRAPHY


Supplementary Table 1.

Differences between mTBI and HC across Blood-based Biomarkers

<table>
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<tr>
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<th>mTBI</th>
<th>HC</th>
<th>p-value</th>
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<tr>
<td></td>
<td>(n = 63)</td>
<td>(n = 41)</td>
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<td></td>
<td>m(sd)</td>
<td>m(sd)</td>
<td>F(1, 102)=</td>
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<td></td>
<td></td>
<td></td>
<td>p =</td>
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<tr>
<td>CRP</td>
<td>13.38(1.62)</td>
<td>13.33(1.39)</td>
<td>0.03, p = .86</td>
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<td></td>
<td>n = 63</td>
<td>n = 41</td>
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<tr>
<td>IL-6</td>
<td>-0.75(0.70)</td>
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<td>1.41, p = .24</td>
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<td></td>
<td>n = 57</td>
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<td>IL-1RA</td>
<td>5.44(0.44)</td>
<td>5.54(0.57)</td>
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<td>n = 41</td>
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<td>3.82(0.38)</td>
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Note. All markers are log transformed to approximate the normal distribution and reported in pg/mL; C-Reactive Protein (CRP); interleukin (IL)-6, IL1-receptor agonist (IL-1RA), Kynurenic Acid (KynA); 3-Hydroxykynurenine (3HK); Quinolinic Acid (QuinA).
Figure 1. Box plot distributions of inflammatory markers across PPS+, PPS-, and HC groups. Natural log-transformed levels of biomarkers are shown in pg/ml. CRP = c-reactive protein; IL-1RA = interleukin 1 receptor antagonist; IL-6 = interleukin 6.
Figure 2. Box plot distributions of kynurenine pathway metabolites across PPS+, PPS-, and HC groups. Natural log-transformed levels of biomarkers are shown in pg/ml. KynA = kynurenic acid, 3HK = 3-Hydroxykynurenine, QuinA = quinolinic acid.