Pediatric Traumatic Brain Injury: An Update on Preclinical Models, Clinical Biomarkers, and the Implications of Cerebrovascular Dysfunction

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ABSTRACT

Traumatic brain injury (TBI) is a leading cause of pediatric morbidity and mortality. Recent studies suggest that children and adolescents have worse post-TBI outcomes and take longer to recover than adults. However, the pathophysiology and progression of TBI in the pediatric population are studied to a far lesser extent compared to the adult population. Common causes of TBI in children are falls, sports/recreation-related injuries, non-accidental trauma, and motor vehicle-related injuries. A fundamental understanding of TBI pathophysiology is crucial in preventing long-term brain injury sequelae. Animal models of TBI have played an essential role in addressing the knowledge gaps relating to pTBI pathophysiology. Moreover, a better understanding of clinical biomarkers is crucial to diagnose pTBI and accurately predict long-term outcomes. This review examines the current preclinical models of pTBI, the implications of pTBI on the brain’s vasculature, and clinical pTBI biomarkers. Finally, we conclude the review by speculating on the emerging role of the gut-brain axis in pTBI pathophysiology.

KEYWORDS: pediatric traumatic brain injury, traumatic brain injury, gut-brain axis, biomarkers, vascular dysfunction, research models

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Introduction

Traumatic brain injury (TBI) is a significant public health issue and a leading cause of mortality globally. The CDC 2017 surveillance report estimates that TBI-related hospitalization was 224,000 in the United States. Moreover, TBI is a crucial contributor to prolonged disability and dependence. Epidemiological studies and biomedical research have focused on understanding the pathophysiology of TBI in the military setting because military service members are at an increased risk of blast-related injuries from explosives or blunt force to the head during warfare. An appreciation for TBI in the adult civilian population is becoming more prominent. However, TBI pathophysiology and progression in the pediatric civilian population is studied to a lesser extent than the adult population despite some studies showing that children have worse post-TBI outcomes and take longer to recover.

Young children (0–4 years old) and adolescents (15–19 years old) are at an increased risk of developing TBI, predominantly resulting from falls or motor vehicle accidents. An epidemiological study showed that 28% of children who visited the emergency department were reported to have been struck by or against an object. Likewise, the CDC revealed that over 812,200 children (age 17 or younger) were treated in the United States for a concussion or TBI in 2014. Importantly, children that survive TBI-related events have an elevated risk of developing psychological, social, sensorimotor, and cognitive impairments in later childhood and into adulthood. When juxtaposed to an increased risk of death post-TBI, these findings highlight the significant economic and public health burden of pediatric traumatic brain injury (pTBI) on society. Thus, it becomes paramount that adequate funding and research be directed towards understanding the pathophysiology of TBI in the pediatric population since no therapies currently exist that further permission provided the original work is attributed as specified on the SAGE and Open Access pages.
effectively mitigate the consequential effects of pTBI long-term. 8

Although TBI studies in adults - humans, pigs, and rodents have provided a functional understanding of TBI pathophysiology, several nuances exist and should be considered before extrapolating results from adult TBI (aTBI) to pTBI. For instance, many aspects of the central nervous system (CNS) in the pediatric population, e.g., as myelination and synapse formation, are in continual development, and brain injury in children could severely impact these brain maturation processes with lasting neurological consequences.18,19 In this review, we examine: 1) current preclinical models of TBI and their use in pTBI, 2) the implications of pTBI on the brain’s vasculature, and 3) clinical pTBI biomarkers. For simplicity, we will refer to the pediatric population in this review as the neonatal period to adolescence. We will conclude the review by briefly discussing an emerging role in pTBI pathogenesis’s gut-brain axis.

Research Models of TBI and Their Application in the Pediatric Population

The development of an experimental animal research model that recapitulates the mechanisms of TBI has been a persistent challenge for researchers.20,21 The multi-planar and heterogeneous physical forces involved, coupled with concussive, rotational, sheering, and ischemic intracerebral injuries that result, have proven challenging to recreate TBI in a rigorous and reproducible manner.22-24 For the past several decades, the modalities that have been developed for use in animal subjects generally focus on imparting a single component of these multifactorial injurious mechanisms and then studying the consequential brain injury pattern. Several such paradigms for studying TBI in animals have been described in the literature.22,25,26 These models have proven paramount in advancing our understanding of the complex pathophysiology that underlies TBI. However, studies on the applicability of these models to human head injuries, especially those involving the pediatric brain, are sparse.25-30

TBI models can be broadly grouped into 2 major classes: penetrating vs non-penetrating injury. Once differentiated into the above classes, the models have then been designed to simulate either focal or diffuse injury through specific modifications. The models vary in their ability to produce mild to severe injury. Some models are more amenable to adjusting severity gradients than others. Ease of implementation is also an important consideration when choosing the appropriate model due to the high throughput of animals often needed for TBI experiments. This section explores some of the more commonly used models in TBI research and discusses their use in pTBI research.

Closed Head Animal Models of TBI

“Closed head” animal models of TBI refer to the generation of brain injury by applying an impulse, i.e., force, through an intact skull with or without skin incision and outer table exposure. The major variations of this overarching category include impact and non-impact modalities. In general, non-impact impulse models impart an inertial force to the head and cause angular brain momentum and resultant diffuse injury, while fixed impact models result in more focal TBI.24 However, modifications can be made to each model to simulate the desired predominant injury pattern. Each of these will be described in turn below.

Non-Impact Models. In non-impact impulsive loading, angular momentum is imparted to the brain by inducing rapid head movement and slower deceleration without external loading. These models, therefore, recapitulate diffuse axonal or shear injury caused by the stretching and compression of anisotropic tissue.31,32 Interest in this specific modality has experienced renewed vigor, given that a vast component of modern human TBI is secondary to the primary impact from sporting collisions, motor vehicle accidents, and wartime blast injuries.

Non-impact blast impulse models have a conspicuous translation to the injuries experienced by soldiers in modern warfare. Replicating those conditions is usually accomplished by using a piston actuator to compress air or gas through an expansion chamber several feet in length instead of using a formal solid weight. 33-35 Alternatively, some studies expose experimental animals to actual detonated explosives to replicate the desired wartime conditions with greater fidelity.36 In models employing compressed air or gas via a shock tube, the imparted force can be titrated to achieve different severities of TBI, ranging from mild to fatal.31,33,35,37,38 To the best of our knowledge, there are no non-impact blast impulse models that have been validated in pediatric rodent animal models, and there is only one study that employed a non-impact model in young porcine subjects. The authors showed that 3- to 5-day old piglets receiving repetitive rapid axial rotation with a pneumatic actuator exhibited worsened composite cognitive function and increased mortality compared to single injury and sham piglets.39 Given that 1 in 6 children live in a conflict zone frequently exposed to explosives, the absence of non-impact blast impulse models for pediatric populations is a critical barrier to progress pTBI research on a global scale.40,41

Impact Models. Impact-based TBI research models use a variable weight generally conducted through a tube propelled by gravity or a pneumatic or electromagnetic actuator to transmit a mechanical load directly onto the skull.42,43 The resultant injury (focal vs diffuse) is a function of both the mass of mechanical load used and applied to the skull, both of which can be easily modified. These models include the classic “weight-drop” model described by Marmarou et al., whereby a brass load is dropped through a two-meter Plexiglass tube and onto a murine skull.42 Several closed head impact models recapitulate TBI in the pediatric animal subjects.37,28,44,45 The modifications to standard models for application to young subjects are minor, and mainly consist of altering the impulse amplitude or manner of fixation to the apparatus. The weight-drop impact model is less commonly used in pTBI research today than the controlled
cortical impact model (discussed in the open head animal models of TBI section), which affords high precision and reproducibility.8

Open Head Animal Models of TBI

Similar to closed head analogs, “open head” animal models of TBI, in which a craniectomy is performed so that impulses may be applied directly to the exposed dura, can be broadly divided into 2 schemas: fluid percussion and direct cortical impact. These models sacrifice the significant head movement of other models in exchange for in situ extradural impulses. The open head models can induce a focal cortical contusion of moderate to severe grade (direction cortical impact) or diffuse brain/axonal injury (fluid percussion).24,46

Fluid Percussion Models. The fluid percussion model has evolved since its inception by Denny-Brown and Russell in the 1940s. The original design relies on applying an impulse to the skull vertex alone after preparatory craniectomy. Historically, this model was developed in feline specimens, but researchers adopted the model for applications in other mammals.47,48 Some of the earliest adaptations to murine subjects were described in 1987 by McIntosh et al. and Dixon et al., who further modified the original design to allow lateral and midline testing.49,50 The Stalhammar apparatus was later developed, marking one of the seminal points in the model's history.51 In the Stalhammar method, a weighted pendulum is released from a known height to strike a piston attached to a fluid reservoir – often isotonic saline.51,52 The mechanical impulse is transmitted through the fluid to cause a localized deformation of the exposed brain.

Although these models have remained relatively unchanged since their inception, researchers have made refinements to allow for more precise digital manipulation of experimental parameters like pulse pressure, pulse velocity, and load duration.53-56 One crucial improvement was to perform the craniectomy and fluid percussion at sites other than the “standard” parasagittal location. In addition, the induction of apnea following percussion is a well-documented complication of this model and should be closely monitored to prevent mortality, particularly in pTBI models.52,54 Adoptions of the lateral and midline fluid percussion models to the pediatric rat have also been described.57,58 More recently, a study by Newell et al. was the first to demonstrate the feasibility of the lateral fluid percussion model in juvenile mice.59 Fluid percussion models have also been utilized in newborn and young piglets to assess hemodynamic responses post-TBI.60-62

Cortical Impact Models. Controlled cortical impact (CCI) models share some of the general concepts of the weight drop, impact accelerator, and fluid percussion models. For this reason, they are commonly referred to as rigid percussion models.25 In the most common paradigm for this model, a limited circular craniectomy is performed on an anesthetized subject where the head is securely affixed to the experimental apparatus by stereotactic pinning to prevent cranial motion. A direct extradural impulse is applied by a rigid piston that is driven electromechanically, pneumatically, or electromagnetically.63,64 Thus, the velocity and depth of the impulse can be scaled to affect both the severity of resultant brain deformation and the local contusion and axonal damage that follows.65

While initially developed for studying aTBI, CCI is the most commonly used TBI model to study pTBI progression and pathogenesis. For pediatric applications, postnatal day (PND) 17-35 in rats and PND 21 in mice are frequently used in CCI studies.8 There are, however, limitations to this model. The generated TBI is often too severe, confounding postoperative assessments and post-mortem analyses. To address this limitation, researchers have created closed head cortical impact models that allow for repetitive concussive impulses to the same subject.65,66 This refined model simulates a repetitive injury that mimics recurrent mild TBI; this novel feature will enable researchers to study the impact of mild, repetitive injuries that commonly occur in student-athletes.22,24

Models of Inflicted TBI

Shaken baby syndrome, also known as abusive head trauma or inflicted TBI, is often overlooked in the pTBI literature. As a result, very few models mimic the effects of shaken baby syndrome in babies less than one-year-old. Although models of shaken baby syndrome are utilized by a much smaller number of research groups, their studies can recapitulate the widespread cortical hemorrhage pattern suffered by human infant patients. At least 3 rodent models have been reported in the literature. Smith et al. were the first to describe such a model in 1998.67 Their protocol involved exposing anesthetized PND 6 rat pups to one daily shaking episode for 3 consecutive days via a mechanical tabletop shaker set to 200 cycles per minute followed by euthanasia on PND 9. Bonnier and colleagues described another experimental model differing from the previous approach in that anesthetized PND 8 were exposed to shaking at 900 cycles per minute for 15 seconds on a tabletop shaker.68,69 Two decades later, Kawamata et al. described a refined apparatus and design consisting of lucent, fenestrated, and cushioned plastic tubes. PND 3 and PND7 mice were shaken by the same common tabletop shaker at 250 cycles per minute in single-minute bouts 5 times daily. The authors conclude that the model may help study the effects of cerebral microhemorrhages on behavioral outcomes in early development.70

Research data on models of shaken baby syndrome in larger mammals is limited. Finnie and colleagues have published a few studies on their immature ovine model of inflicted TBI.8,71 This model involves manually grasping the axilla of anesthetized lamb subjects and vigorously shaking them in ten, 30-second bouts over a half hour. The development of a large animal model of pediatric inflicted TBI was galvanized by the hypothesis that the large volume gyrencephalic brains
and relatively weak cervical muscles of ovine animals better replicated the forces and injuries experienced by human babies. Simulating abuse in anesthetized juvenile animals is controversial and presents a barrier to developing additional inflicted TBI models. The reconciliation of these ethical concerns through the development of novel models should be a focus of future research.

Cerebrovascular Dysfunction Following TBI in Children
A major consequence following TBI is the damage to the brain’s vasculature. Cerebrovascular damage in animal and human TBI studies has been described in the context of hypoperfusion, hemorrhage, ischemia, edema, and blood flow abnormalities. Cerebrovascular dysfunction is a hallmark finding in many pediatric conditions and often predicts cognitive outcomes. For example, a systematic review by Bakker et al. found that decreased blood flow velocities in premature infants and children with sickle cell disease were associated with poor cognitive performance. Likewise, Taylor et al. showed that vascular alterations in children increased the risk for cognitive impairment. Moreover, recent studies suggesting the role of cerebrovascular dysfunction in neurodegenerative diseases further support the importance of vascular integrity in maintaining brain function and health. This section briefly discusses the functional and structural vascular alterations evident following pTBI.

Cerebral Blood Flow Dysfunction Following pTBI
Transcranial doppler (TCD) ultrasound and magnetic resonance imaging arterial spin labeling (MRI ASL) has been used to measure cerebral blood flow (CBF) in children. TCD studies showed that newborns exhibit low (~24 cm/s) cerebral blood flow velocity (CBFV); however, CBFV then rapidly rises (~95 cm/s) and peaks at 6-9 years of age. Beyond 10 years of age, CBFV declines and approximate adult values (~50 cm/s). Furthermore, MRI ASL studies by Biagi et al. showed that CBF was highest in children 4-12 years of age and rapidly declined in adult subjects. One explanation for the increased CBF values in children is the increased metabolic and energy demand needed by the developing brain. Other vascular parameters such as cerebral vasoreactivity (CVR), which measures vascular responsiveness to vasodilation via changes in blood carbon dioxide (CO2), have also been shown to be elevated in children compared to adults. These findings make it apparent that cerebral hemodynamics in normal physiology differs significantly in early childhood compared to adults.

Cerebral autoregulation (CA) is crucial for maintaining a steady-state CBF under a precise range of cerebral perfusion pressure (CPP). In normal physiology, Vavilala et al. showed that the cerebral autoregulatory index (i.e., how fast blood flow velocity returns to baseline after a transient decrease in mean arterial pressure) is lower in adolescents than in adults. pTBI patients often reveal a significant reduction in CBF compared to aTBI patients. It remains unclear whether the lower autoregulatory index in the pediatric population may be responsible for the more significant CBF alterations in pTBI compared to aTBI patients. Nonetheless, it is widely known that a drastic decrease in CBF puts the developing brain at risk for ischemia and neuronal death. Animal models of pTBI in piglets have revealed CA impairment couples a reduction in CBF and greater constrictions in pial vessels. Interestingly, CA impairment was more prominent in newborn TBI piglets (1-5 days old) compared to juvenile piglets (3-4 weeks old). The findings from the study mentioned above were corroborated by the results in another study demonstrating worsened and prolonged hypotension in PND 17 and PND 28 rats compared to adult rats following TBI.

Furthermore, human clinical studies in children have shown that CA impairment following TBI is a significant predictor of poor outcomes. More importantly, young age appears to be a risk factor for CA impairment. The mechanisms that lead to CA impairment and subsequent decrease in CBF are unclear; however, several pathways have been implicated. For example, endothelin-1 (ET-1) has been shown to increase in pTBI animal models, and treatment with ET-1 antagonist, BQ-123, mitigates CA impairment by attenuating CBF decline and pial artery vasoconstriction. Similarly, a vasodilatory N-methyl-D-aspartate (NMDA) agonist (MK801) reduced pial vessel vasoconstriction and improved CBF following pTBI. More recently, the c-Jun N-terminal kinase (JNK) intracellular signaling pathway has been implicated in pTBI-induced CA impairment. These therapeutic targets that mitigate CA impairment are especially clinically relevant in the pediatric population since clinical studies have shown that CA impairment is present in about 17% of mild pTBI patients and 42% of moderate-severe pTBI patients.

Blood-Brain Barrier Dysfunction Following pTBI
The brain’s vasculature’s primary structural unit is the blood-brain barrier (BBB). The BBB regulates a stringent transport of molecules and cells between the periphery and the brain parenchyma. This unique structure is composed of endothelial cells held together tightly by junctional proteins, astrocyte endfeet processes, surrounding pericytes, and a basal lamina. Transporter and protein composition at the level of the BBB changes with brain maturity. For example, the immature BBB relies heavily on the inward transport of glucose and amino acids compared to the adult BBB. Likewise, P-glycoprotein (P-gp) efflux transporter expression at the BBB has been shown to be increased at PND 7 compared to PND 28 in rats. Moreover, Muramatsu et al. demonstrated that PND 7 rats had increased immunoglobulin G (IgG) compared to PND 21 rats following 24 h post-hypoxia-ischemia insult. Thus, an indication that the
BBB is more responsive to hypoxia-ischemia insults in younger rats (PND 7) compared to older rats (PND 2).97

Following TBI, the BBB becomes compromised. BBB perturbation post-TBI is evidenced by increased BBB permeability and loss of junctional proteins.98 While most TBI patients tend to show acute BBB breakdown (days to weeks), some studies have shown that the breakdown of the BBB post-TBI may last for years.99-102 Models of pTBI, like aTBI, have revealed increased extravasation of dyes or IgG into the brain parenchyma.103 Additionally, Badau et al. showed increased BBB permeability to IgG coupled to the loss of junctional protein claudin 5 in PND 17 rats at day 3 post-pTBI. As measured via caveolin-1, cellular transcytosis was increased at days one and seven following pTBI initiation in the same study.104

Interestingly, claudin 5 levels have been shown to significantly increase at day 7 and up to 60 days post-pTBI compared to controls.103,105

Amyloid-beta accumulation is implicated in TBI pathogenesis and is thought to be mediated by BBB dysfunction at the transporter level.106 Several studies have demonstrated that the P-gp efflux transporter is crucial for clearing amyloid-beta.107,108 Failure to remove amyloid-beta from the brain promotes inflammation and neurodegeneration, which in turn impairs normal brain function.109 Jullienne et al. and Pop et al. showed that P-gp transporter expression is decreased in PND 17 rats following pTBI. Consequently, the decrease in P-gp expression was coupled to increased brain amyloid-beta accumulation.105,110 Increased expression of perlecan and fibronectin perivascular matrix proteins post-pTBI is also thought to mediate the accumulation of brain amyloid-beta.110

A complication of BBB damage seen earlier and more frequently in children than in adults is the accumulation of fluid in the brain (edema).111,112 Edema in pTBI patients is often associated with poorer outcomes and increased mortality.114 In children, higher water content, a softer skull, and weaker cerebral support are thought to be responsible for the increased risk of diffuse edema seen in children compared to adults.113 Increased expression of water channel aquaporins (AQP4) present on the end-feet processes of astrocytes is thought to mediate the vasogenic edema formation seen post-pTBI.114 This finding is supported by a study showing that inhibition of AQP4 via small-interfering RNA (siRNA) in PND 17 pTBI rats reduced edema and improved cognitive outcomes compared to controls.104 Conversely, the increased expression of AQP4 in PND 17 rats is also thought to play a role in edema resolution; however, it appears that this benefit is present 3 days post-pTBI.114

Breakdown of the BBB post-TBI is often associated with neuroinflammation, neuronal death, and long-term neurological deficits. Recent insights have generated new interest in studying the role of vascular integrity as a target for developing therapeutics that may be used to manage TBI acutely and long-term. For additional insights, readers are referred to this excellent review on vascular impairment post-pTBI.73

**Neuroimaging and Assessment of Clinical Biomarkers Following TBI in Children**

Despite many proposed TBI therapies with encouraging early phase trials, none have made it through phase III clinical trials.115 Discovering biomarkers is an important part of understanding the pathophysiology of any disease and identifying new ideas for therapies. While many biomarkers have been investigated and reviewed for the diagnosis, prognosis, and treatment of aTBI, and a few have focused on the pediatric population, no standard biomarkers have been widely adopted in the field.116-121 In general, TBI diagnosis is determined by the severity of primary cerebral lesions and secondary brain damage. Secondary brain damage can result from several biochemical and molecular mechanisms, including reactive oxygen species (ROS) production, lipid peroxidation, excessive glutamate release, and neuroinflammation.122 The current standard for the assessment of TBI severity in both aTBI and pTBI is the Glasgow Coma Score (GCS), which classifies TBI as mild (13-15), moderate (9-12), or severe (≤8).123 Scoring is based on subsectional scales of the eye, verbal, and motor responses, with a score of 0 being no response (deep coma or death) and the highest score being “normal” ability to respond to the task, i.e., fully awake and alert). Iankova describes the clinical application of the GCS, including changes in the scale and the inconsistencies with scoring, as healthcare professionals are prone to subjective interpretation or discrepancies in the GCS assessment techniques.124 Likewise, the Glasgow Outcome Scale (GOS) defines functional/neurological outcome and is typically scored as: 1 = death; 2 = persistent vegetative state; 3 = severe disability; 4 = moderate disability; and 5 = good recovery.125 Although GOS is a global and nonspecific clinical score in infants and children with TBI, it is most widely used to assess late neurologic outcomes in this subset of patients.126-128 However, GCS and GOS are not specific to TBI, and these scales can be used to assess the severity of brain-related injuries, including stroke and Alzheimer’s Disease.129,130

Several reviews have described the use of brain imaging techniques following TBI in pediatric populations.131-133 Popular imaging techniques include computed tomography (CT) and MRI. TBI can result in physical changes of the brain structure, including axonal and white matter (WM) injury. The corpus callosum is a common area of injury in TBI, and its location permits the assessment of its structural integrity and function via imaging. Corpus callosum white matter integrity is measured using fractional anisotropy (FA). At the same time, the corpus callosum function can be assessed using interhemispheric transfer time (IHTT),
which measures the time it takes for information to cross cerebral hemispheres. Dennis et al. have shown that callosal function is associated with disrupted white matter integrity in pTBI. A recent study that examined post-TBI (mild TBI) cortical thickness, which varied by brain sub-region, compared to controls with orthopedic injury highlights the complexity of using neuroimaging techniques to predict TBI outcomes. Brain imaging has been explored in the prediction of post-TBI behavioral deficits. In addition, several metabolites detected during brain imaging have been examined for their applicability as biomarkers of TBI, including N-acetyl aspartate, creatine, choline, lactate, and myoinositol.

Although CT scans are becoming increasingly common in head injury cases, physicians must consider the risk of unnecessary exposure to radiation, which is especially dangerous for children. Although MRI provides a better resolution of brain structure and function compared to CT, this technique has its own challenges in the pediatric population, including lack of proper child-sized equipment, increased movement-related artifacts, and the use of sedatives. The goals of brain imaging are 2-fold and include detecting injuries that may require surgical or therapeutic intervention, as well as determining the prognosis of rehabilitative therapy or other long-term treatment plans. However, brain imaging techniques can be expensive, risky, and provide minimal information for targeting patient treatment or an assessment of patient prognosis. A biological fluid biomarker or panel of markers can provide a potentially faster, less expensive, and less stressful option to identify targeted therapeutic options for TBI patients. For the purposes of this review, we will briefly discuss some of these biomarkers that have been explored in TBI-defined pediatric clinical samples.

Some of the most commonly measured brain-specific biomarkers include neuron-specific enolase (NSE), S100 calcium-binding protein B (S100B), myelin basic protein (MBP), glial fibrillary acidic protein (GFAP), and ubiquitin carboxyl-terminal hydrolase L1 (UCH-L1). The outcomes of pediatric clinical studies using these markers are listed in Table 1. Many nonspecific markers for inflammation, damage, degeneration, and changes in metabolism have also been explored, and these are listed in Table 2. Since TBI can result in long-term cognitive changes or deficits, it is also important to explore brain biomarkers that may help to predict these differences. Wilkinson et al. found that the combination of high levels of NSE and low soluble neuron cell adhesion molecule (sNCAM) may be used to predict children at risk of attention and functioning issues following TBI. Brain-derived neurotrophic factor (BDNF), a protein with many roles in the maintenance and regeneration of neurons, is decreased following brain injury and is associated with abnormalities seen on a head CT and 6-month recovery prognosis. Lo, Jones, and Minns found that the prognostic pairing of inflammatory mediators and brain-specific proteins yields better unfavorable outcome predictive values in pTBI than using individual markers. Likewise, combining GCS and serum biomarker concentrations improves outcome prediction, including increased specificity and sensitivity. A combination of markers will likely be required to best diagnose or determine outcome prediction measurement for pTBI. For an in-depth discussion on the relevance of pTBI biomarkers, the reader is referred to this excellent review.

**A Putative Role for the Gut-Brain Axis (GBA) in pTBI Pathophysiology**

The gut microbiome plays an important role in pediatric health and disease, with strong evidence suggesting an influential part of the microbiome in maintaining brain function, homeostasis, and stress responses through the brain-gut axis. This complex mutualistic ecosystem encompasses a diverse collection of microbial genomes that outnumber the cells in the human body. Several studies have shown both intrinsic and extrinsic factors can shape the composition of the microbiome. For instance, children have a higher degree of interpersonal variation in their microbiome composition, especially within the first 3 years of life compared to adults. Additionally, the host’s geographical location also tends to have a significant effect on gut microbiome composition, with pronounced differences seen between individuals from different geographical and cultural backgrounds. Other factors that shape the gut microbiome include sex, genetics, lifestyle, and medications. These factors should be considered when examining the bi-directional underpinnings of the gut-brain axis in maintaining overall health.

Disruption of the microbiome has been implicated in many neurodegenerative diseases, including Parkinson’s disease (PD), Alzheimer’s disease (AD), and multiple sclerosis (MS). Furthermore, recent studies have highlighted the role of the gut microbiome in maintaining healthy brain function. For example, Braniste et al. showed that germ-free mice, beginning with intrauterine life, are prone to increased BBB permeability compared to pathogen-free mice with normal gut flora. However, the re-introduction of microbes to these germ-free mice decreased BBB permeability and improved cognitive outcomes. Additionally, there is a growing body of preclinical and clinical evidence supporting microbiome dysbiosis in the pathogenesis and progression of aTBI. However, studies investigating the relationship between gut microbiome dysfunction and TBI progression in the pediatric population remain scarce.

The intestinal epithelium is a mechanical barrier that prevents commensal bacteria and pathogenic microbe translocation from the gut lumen into the bloodstream. However, following TBI, the permeability of the intestinal epithelium increases, providing a mechanism for bacterial translocation into systemic circulation. The translocated microbes are then propelled through blood vessels, ultimately gain access to other...
Table 1. Potential brain-specific biomarkers in human pediatric Traumatic brain injury.

| BIOMARKER | SAMPLE TYPE | POPULATION | CHANGE AFTER TBI | MAIN OUTCOMES | REF |
|-----------|-------------|------------|------------------|---------------|-----|
| NSE       | Serum       | 22 children with intracranial lesion on CT and 28 without | Elevated     | • NSE was not sensitive or specific enough to predict intracranial lesion | Fridriksson et al. 2000 |
| S100B     | Serum       | 45 children, aged 0 to 13, with mild (n = 27), moderate (n = 6), or severe TBI (n = 12) and 16 controls | Elevated     | • S100B concentrations were increased in almost half of patients, and was only detectable after 12 hrs in patients with severe closed head injury | Berger et al. 2002 |
| S100B     | Blood       | 17 children, aged 5 to 18 years, with mild TBI | Elevated     | • S100B concentrations were statistically increased in patients with head and other bodily injuries vs those with only head injury • However, there was no significant difference in S100B concentrations in patients with positive vs negative MRI | Akhtar et al. 2003 |
| S100B     | Serum       | 136 healthy children total, 27 children with TBI | Elevated     | • S100B is increased after TBI and its elevation appears to be correlated with poor outcome | Spinella et al. 2003 |
| NSE       | Serum       | 86 children with closed TBI | Elevated     | • NSE concentrations significantly higher in patients with poor outcome compared to good outcome • NSE was a poor predictor of abnormal CT, however, NSE may predict global, short-term physical disability in children with closed TBI | Bandyopadhyay et al. 2005 |
| NSE, S100B, and MBP | Serum | 100 children with mild TBI (56 noninflicted, 44 inflicted) and 64 controls | Elevated     | • NSE and S100B concentrations were significantly increased in TBI patients vs controls • MBP concentrations were significantly elevated in TBI patients with intracranial hemorrhage vs without intracranial hemorrhage | Berger et al. 2005 |
| S100B     | Serum and urine | 15 children with traumatic or hypoxemic brain injury and 14 healthy controls | Elevated     | • S100B concentrations are higher in serum and urine of brain injured vs healthy children • S100B levels peak earlier in serum than urine, indicating urine may be helpful in later TBI diagnosis | Berger et al. 2006 |
| NSE, S100B, and MBP | Serum or CSF | 14 children with inflicted TBI | —            | • S100B was not sensitive nor specific for inflicted TBI, while NSE and MBP warrant further studies | Berger et al. 2006 |
| NSE, S100B, and MBP | Serum | Children with inflicted vs noninflicted TBI (n = 15 per group) | Elevated     | • Functional and cognitive tests showed significant between-group differences, with inflicted TBI being more severe • There were statistically different time to peak comparisons for each biomarker | Beers et al. 2007 |
| NSE, S100B, and MBP | Serum | 152 children <13 years with acute TBI | Elevated     | • Higher concentrations for all biomarkers were associated with worse outcome at all timepoints, and highest correlations were seen in the peak concentrations • Initial and peak NSE, and initial MBP, concentrations correlate with outcome in children ≤4 | Berger et al. 2007 |

(Continued)
Table 1. Continued.

| BIOMARKER | SAMPLE TYPE | POPULATION | CHANGE AFTER TBI | MAIN OUTCOMES | REF |
|-----------|-------------|------------|------------------|---------------|-----|
| S100B     | Serum       | Six children, aged 1 to 17 years, with severe TBI | Elevated | • S100B concentrations upregulated in 5 of 6 patients compared to a control reference of pooled healthy human serum | Haqqani et al. 2007¹¹ |
| S100B     | Serum       | Children, age 1 to 15 years, with mild (n = 9), moderate (n = 2), or severe (n = 4) TBI | Elevated | • Children with severe TBI had the highest S100B concentrations<br>• However, S100B may not be a reliable prognostic marker because even high concentrations are associated with a full neurological recovery | Piazza et al. 2007¹² |
| S100B and NSE | Serum | Children aged 6 months to 15 years, 53 with contusion and 95 with mild TBI | Elevated | • No significant differences in mean S100B or NSE levels between children with mild TBI compared to contusions<br>• The correlation between S100B and NSE was weak, but significant<br>• S100B and NSE cannot distinguish between symptomatic mild TBI and asymptomatic head contusion in children with minor head trauma | Geyer et al. 2009¹³ |
| S100B     | Serum       | 109 children with mild TBI | Elevated | • S100B was significantly elevated in patients with intracranial lesions identified on CT | Castellani et al. 2009¹⁴ |
| S100B     | Serum       | 152 children with head trauma (24 with intracranial injury and 128 without) | Elevated | • Mean S100B concentrations were significantly higher in children with intracranial injury, however the overall ability of S100B to detect intracranial injury was poor | Bechtel et al. 2009¹⁵ |
| S100B, NSE, and MBP | Serum | 72 children with TBI and 28 children with hypoxic ischemic encephalopathy | — | • Trajectory analysis of S100B, NSE, and MBP was able to predict poor outcome in patients with high probability | Berger et al. 2010¹⁶ |
| α-Synuclein | CSF | 47 infants and children with severe TBI and 9 control patients | Elevated | • α-synuclein concentrations were increased in TBI patients compared to controls, and levels were higher in patients treated with normothermia vs hypothermia | Su et al. 2010¹⁷ |
| S100B     | Serum and urine | 105 children with either no CT taken or negative CT; 6 children with a positive TBI CT | Elevated | • Serum S100B levels were higher in TBI vs control patients, but no difference was seen in urine | Hallen et al. 2010¹⁸ |
| GFAP      | Serum and CSF | 27 children with severe TBI | Elevated | • Peak GFAP concentration occurred on day 1 post-TBI and was higher in CFS than serum<br>• Serum GFAP correlated with functional outcome at 6 months, indicating its prognostic value<br>• Hypothermia therapy had no effect on serum GFAP levels | Fraser et al. 2011¹⁹ |
| S100B     | Serum       | Children <19 years, who presented within 6 hours of moderate to severe TBI | Elevated | • Mean S100B levels were higher in children with moderate to severe TBI compared to mild TBI<br>• S100B was significantly higher in children with an abnormal cranial CT vs normal cranial CT scan<br>• S100B appears to predict TBI severity | Babcock et al. 2012²⁰ |

(Continued)
| BIOMARKER | SAMPLE TYPE | POPULATION | CHANGE AFTER TBI | MAIN OUTCOMES | REF |
|-----------|-------------|------------|------------------|---------------|-----|
| UCH-L1 and SBDP145 | Serum | 39 children with TBI and 10 healthy controls | Elevated | • Significantly increased UCH-L1 concentration in children with moderate and severe, but not mild, TBI, while no differences in SBDP145 concentrations between any groups  
• UCH-L1 and SBDP145 had a significant negative correlation with GCS and the correlation was stronger than that of NSE, S100B, and MBP, however no biomarkers correlated with the presence of clinical symptoms or abnormalities on head CT | Berger et al. 2012 |
| MBP | CSF | 27 children with severe TBI and 57 controls | Elevated | • Mean MBP concentrations were increased in TBI patients ≥1 year compared to <1 year  
• MBP concentrations are increased up to 5 days after TBI, but not affected by therapeutic hypothermia | Su et al. 2012 |
| S100B | Serum | 446 children, aged <16 years, with mild TBI | Elevated | • Increased S100B levels, taken in the first 3 hours of TBI management, correlate with CT and have the potential to reduce the need for CT  
• Median S100B concentrations correlated with TBI severity | Bouvier et al. 2012 |
| S100B, NSE, GFAP, neurofilaments (NF-H), secretagogin, and Hsp70 | Serum | 63 children with TBI | Elevated | • Elevated S100B, GFAP, and NSE levels in patients with worse outcome or death  
• NF-H grew faster in TBI patients with worse outcome or death | Zurek and Fedora 2012 |
| S100B | Serum | 36 children, aged 6 to 16, with mild TBI and 27 control children with orthopedic injuries | Elevated | • Groups exhibited similar levels of elevation following injury  
• TBI-specific S100B differences were associated with children who had post-concussive symptoms (PCS), including verbal memory performance  
• This shows that S100B is not specific for TBI, but is associated with cognitive PCS | Studer et al. 2015 |
| GFAP | Serum | 197 children with TBI and 60 trauma controls | Elevated | • Significantly increased GFAP concentrations measured within 6 hrs of TBI correlated with intracranial lesion on CT and TBI severity | Papa et al. 2015 |
| UCH-L1 and GFAP | Serum | 45 children aged <15 with TBI and 40 healthy control children | Elevated | • There were higher GFAP, UCH-L1, and S100B, but not MBP concentrations in children with TBI compared to controls, and these negatively correlated with GSC scores on admission  
• Increased concentrations of GFAP and UCH-L1 correlated with TBI severity  
• UCH-L1 and GFAP predicted poor outcome better than S100B and MBP  
• UCH-L1 has potential to detect acute intracranial lesion assessed by CT | Mondello et al. 2016 |

(Continued)
| BIOMARKER | SAMPLE TYPE | POPULATION | CHANGE AFTER TBI | MAIN OUTCOMES | REF |
|-----------|-------------|------------|------------------|---------------|-----|
| GFAP and UCH-L1 | Serum | 25 children with mild TBI and 20 children with orthopedic injury, aged 11 to 16 years | Elevated | • GFAP was significantly higher in acute TBI compared to orthopedic injury, while there was no difference in UCH-L1 • GFAP and UCH-L1 were not predictive of post-concussive symptoms over one month post-injury | Rhine et al. 2016 |
| GFAP and S100B | Serum | 114 children with mild TBI and 41 controls without head trauma | Mixed | • There was significantly increased GFAP, but not S100B, concentrations in TBI patients vs controls • GFAP, but not S100B, was able to predict intracranial lesion on head CT | Papa et al. 2016 |
| NSE and sNCAM | Serum | 23 children with TBI | Mixed | • Higher concentrations of NSE and lower levels of sNCAM were associated with abnormal behavior in children and may predict long-term attention-related problems after TBI | Wilkinson et al. 2017 |
| UCH-L1 | Serum | 196 children, aged 2 weeks to 21 years, with mild/moderate TBI and 60 trauma controls | Elevated | • There were statistically increased levels of UCH-L1 in patients with intracranial lesions compared to trauma controls, or trauma with or without TBI symptoms • Levels of UCH-L1 increased with severity of CT lesions • UCH-L1 may be useful in predicting traumatic intracranial lesions on CT | Papa et al. 2017 |
| UCH-L1 and SBDP145 | Serum and CSF | 19 children, aged 24 weeks to 15.7 years, with severe TBI and 17-20 noninjured controls | Elevated | • Serum UCH-L1 levels were increased in TBI patients compared to controls (median 361 vs 147 pg/mL; \( P < .001 \)), peaking at 12 hours after injury then falling back to control levels by 120 hours • CSF UCH-L1 levels correlated with serum levels, though they were overall higher compared to serum levels (median 3372 in TBI vs 525 pg/mL in controls) • Serum SBDP145 levels were also increased in TBI patients compared to controls (172 vs 69 pg/mL; \( P < .001 \)), peaking at 48 hours post-injury and remaining elevated at 120 hours | Metzger et al. 2018 |
| S100B and NSE (and IL-6) | Serum | 15 pediatric patients with TBI | Elevated | • Median serum concentrations at admission (S100B = 178 pg/mL, NSE = 16 pg/mL, and IL-6 = 15 pg/mL) were elevated compared to one week post-injury • S100B and NSE levels both at admission and 1 week post-admission were higher in the poor GCS group compared to the good GCS group • Elevated S100B and NSE at 1 week post-TBI correlated with unfavorable outcome (poor GOS) at 6 months post-injury | Park and Hwang 2018 |
| BIOMARKER           | SAMPLE TYPE | POPULATION                                                                 | CHANGE AFTER TBI                                                                 | MAIN OUTCOMES                                                                                           | REF                      |
|---------------------|-------------|-----------------------------------------------------------------------------|--------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------|--------------------------|
| S100B and NSE       | Serum       | 10 pediatric TBI patients (n = 5 favorable outcome, n = 5 unfavorable outcome) | Elevated                                                                      | • Median serum S100B levels were elevated at 1 day post-TBI compared to one week later (134 vs 41 pg/mL)  
• Median NSE levels were also elevated one day vs one week post-TBI (15 vs 5 pg/mL)  
• S100B levels 1 week post-TBI were elevated in the unfavorable group compared to the favorable outcome group | Park, Park, and Hwang 2019[34]                                                   |
| Tau                 | Serum       | 158 pediatric TBI and 416 control participants                             | Elevated                                                                      | • Serum tau levels were negatively associated with GCS the first day post-TBI  
• Median tau values for patients with GCS 3-8 was 8.48 pg/mL, GCS 9-12, 7.08 pg/mL, and GCS 13-15 was 2.86 pg/mL  
• Tau levels not strongly associated with CT findings in mild TBI patients | Stukas et al. 2019[35]                                                             |
| GFAP, S100B, and NSE| Saliva      | 24 children with acute, isolated TBI (n = 14 significant brain injury, n = 10 non-significant brain injury) and 50 controls (n = 25 with musculoskeletal injury, n = 25 with no injury) | Mixed                                                                          | • S100B levels were elevated in TBI patients compared to controls with musculoskeletal injury (P = .02), but not uninjured controls  
• Salivary levels of GFAP and NSE were not significantly different between TBI patients and controls | Yeung, Bhatia, Bhattarai, and Sinha 2020[36]                                           |

Key: neuron-specific enolase (NSE), S100 calcium-binding protein B (S100B), myelin basic protein (MBP), cerebrospinal fluid (CSF), glial fibrillary acidic protein (GFAP), ubiquitin carboxyl-terminal hydrolase L1 (UCH-L1), α-2-spectrin breakdown product 145 kDa (SBDP145), soluble neuron cell adhesion molecule (sNCAM)
Table 2. Nonspecific biomarkers potentially predictive of human pediatric traumatic brain injury.

| BIOMARKER                          | SAMPLE TYPE | POPULATION                              | CHANGE AFTER TBI                                                                 | MAIN OUTCOMES                                                                                                                      | REF          |
|------------------------------------|-------------|-----------------------------------------|--------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------|--------------|
| IL-8                               | CSF         | 27 children with severe TBI and 24 controls | Elevated                                                                        | IL-8 concentrations were significantly increased in TBI patients compared to controls, increased IL-8 was associated with poor outcome | Whalen et al. 200037 |
| B-cell lymphoma 2 (Bcl-2)          | CSF         | 23 children with severe TBI and 19 controls | Elevated                                                                        | Bcl-2 levels were increased in TBI patients compared to controls, survival was better for patients with increased Bcl-2, indicating it may have a protective role in TBI | Clark et al. 200038 |
| Heat shock protein-70 (hsp70)      | CSF         | 20 infants and children with TBI        | Elevated                                                                        | Hsp70 concentrations were increased in TBI patients compared to controls, indicating an endogenous stress response, hsp70 levels were positively associated with inflicted vs accidental TBI | Lai et al. 200439 |
| VEGF                               | CSF         | 14 infants and children with severe TBI and 5 noninjured controls | Elevated                                                                        | Mean VEGF was increased in children with TBI compared to controls, as well as a trending increase in adenosine, peak VEGF concentrations occurred at 22.4 h post-TBI, indicating a rapid vascular regenerative response | Shore et al. 200440 |
| Cytochrome c, Fas, caspase-1, IL-1β, caspase-3 | CSF         | 67 infants and children with TBI and 19 controls | Elevated                                                                        | Increased cytochrome c concentrations correlated with inflicted TBI, but not GCS or survival, however, increased Fas and caspase-1 did not discriminate between inflicted and accidental TBI, there were no differences in caspase-3 concentrations after TBI, and only differences in IL-1β at later timepoints | Satchell et al. 200541 |
| Heme oxygenase 1 (HO-1)            | CSF         | 48 infants and children with TBI and 7 control patients | Elevated                                                                        | HO-1 concentrations increased in TBI vs control patients, increased HO-1 was associated with increased injury severity and poor neurological outcome post-TBI | Cousar et al. 200642 |
| Heat shock protein-60 (hsp60)      | CSF         | 34 infants and children with severe TBI and 7 control patients | Elevated                                                                        | Peak hsp60 levels were increased in TBI patients compared to controls, and associated with TBI severity, suggests that elevated hsp60 may reflect the severity of early mitochondrial stress or damage following TBI | Lai et al. 200643 |
| KL-6 and CRP                        | Plasma      | Children with severe TBI, sepsis, ARDS, or cancer (n = 9 per group) | -                                                                               | ARDS patients had higher early KL-6 concentrations compared to all other groups, while TBI patients had the lowest KL-6 concentrations, KL-6 was highest in non-surviving ARDS patients | Briassoulis et al. 200644 |
| TGF-1β, ICAM, L- and E-selectins    | Plasma      | Patients with sepsis, TBI, or ARDS vs ventilated controls with chronic illness (n = 10 per group) | Mixed                                                                           | TBI patients had the highest concentrations of soluble ICAM, biomarker concentrations were similar in surviving vs nonsurviving TBI patients | Briassoulis et al. 200745 |
| IL-6 and NGF                        | CSF         | 29 children with severe TBI and 31 matched controls | Elevated                                                                        | Early (2 h) NGF, but not IL-6, concentrations correlated with head injury severity (GCS), upregulation of IL-6 and NGF after injury was associated with better neurologic outcome at 6 months and may reflect a mechanism of neuroprotection | Chiaretti et al. 200846 |

(Continued)
| BIOMARKER                | SAMPLE TYPE | POPULATION                        | CHANGE AFTER TBI | MAIN OUTCOMES                                                                 | REF          |
|-------------------------|-------------|-----------------------------------|------------------|-------------------------------------------------------------------------------|--------------|
| NGF and DCX             | CSF         | 12 children with severe TBI and 12 matched controls | Elevated         | • NGF was higher in children who had good outcomes, and DCX correlated with NGF, indicating these markers have a neuroprotective role | Chiaretti et al. 2008 | 87 |
| IL-1β, IL-6, NGF, BDNF, and GDNF | CSF         | 27 children with severe TBI and 21 matched controls | Mixed            | • NGF and IL-1β correlated with injury severity at 2 h post-TBI                | Chiaretti et al. 2008 | 88 |
| NGF, DCX, BDNF, GDNF, and NSE | CSF         | 32 children with severe TBI and 32 matched controls | Mixed            | • Early (2 h) concentrations of NGF, DCX, and NSE, but not BDNF or GDNF, correlated with injury severity  
• Increased NGF and DCX, and decreased NSE were associated with better neurological outcome | Chiaretti et al. 2009 | 89 |
| Lipase and amylase      | Serum       | 51 children with severe TBI       | Elevated         | • Early increases in pancreatic enzymes lipase and amylase after TBI suggests an interaction between the brain and GI system | Sanchez et al. 2009 | 90 |
| VCAM, ICAM, IL-12, eotaxin, TNFR2, IL-6, MMP9, HGF, and fibrinogen | Serum       | 16 infants with mild inflicted TBI and 20 control infants | Mixed            | • VCAM, ICAM, IL-12, eotaxin, and TNFR2 were significantly decreased, and IL-6, MMP9, HGF, and fibrinogen were increased in infants with mild inflicted TBI | Berger et al. 2009 | 91 |
| D-dimer, MMP9, and S100B | Plasma      | 64 children with head trauma identified on CT | Elevated         | • D-dimer, but not MMP9 or S100B, was significantly associated with TBI identified by head CT  
• Low D-dimer levels indicate absence of significant brain injury | Swanson et al. 2010 | 92 |
| Beta-natriuretic peptide (BNP) | Serum      | 95 children with head injury: Bleed positive (n = 21) vs bleed negative (n = 74) | No change        | • BNP concentrations were similar in the positive vs negative bleed groups  
• BNP levels did not correlate with injury severity, making it a poor prediction marker for TBI outcome | Chang and Nager 2010 | 93 |
| HMGB1 and cytochrome c  | CSF         | 37 children with severe TBI and 12 controls | Elevated         | • Elevated levels of HMGB1 and cytochrome c were associated with poor outcome post-TBI, suggesting that apoptosis may play an important role in TBI patients | Au et al. 2012 | 94 |
| CD64 and CD11b          | Whole blood and plasma | 15 children with severe TBI compared to 30 children with sepsis and 15 controls | Elevated         | • There was significantly decreased levels of TC, LDL, and HDL in septic, and moderate changes in TBI patients, compared to controls  
• Neutrophil CD64 expression was increased in septic patients compared to TBI and controls, while no differences in CD11b were seen between any groups  
• Patients with TBI show patterns of increased glucose combined with a moderate decrease in cholesterol-lipoprotein | Fitrolaki et al. 2013 | 95 |
| Copeptin                | Plasma      | 126 children with acute severe TBI and 126 controls | Elevated         | • Plasma copeptin levels increased in TBI children compared to controls  
• Patients with 6-month unfavorable outcome or mortality also had increased glucose and C-reactive protein, a lower GCS score, CT classification of 5 or 6, unreactive pupils, and traumatic subarachnoid hemorrhage on initial CT scan  
• Copeptin predicts 6 month unfavorable outcome and mortality | Lin et al. 2013 | 96 |

(Continued)
| BIOMARKER                  | SAMPLE TYPE | POPULATION                                      | CHANGE AFTER TBI | MAIN OUTCOMES                                                                                                                                                                                                 | REF |
|----------------------------|-------------|-------------------------------------------------|------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----|
| D-dimer                    | Plasma      | 46 children, >16 years, with TBI and 20 healthy controls | Elevated         | • Significant increase in D-dimer concentrations associated with TBI on head CT  
• High D-dimer concentrations suggest brain injury and poor prognosis                                                                                                                                   | Foud et al. 2014 |
| mtDNA and HMGB1            | CSF         | 42 children with severe TBI and 13 control patients | Elevated         | • Mean mtDNA concentrations were increased in TBI compared to control patients  
• Mean mtDNA was higher in patients who later died or had severe disability  
• There was a significant correlation between mtDNA and HMGB1 concentrations, signifying that DAMPs are increased after TBI | Walko et al. 2014 |
| Beclin 1 and p62           | CSF         | 30 children with severe TBI and 30 control patients | Elevated         | • Mean and peak levels of both markers were increased up to 7 days, suggesting increased autophagy after TBI  
• Peak p62 levels were higher in patients with poor outcome                                                                                                                                     | Au et al. 2017 |
| miRNAs                    | CSF or saliva | Children with mild TBI (n = 60), severe TBI (n = 8), or controls | Mixed            | • Six miRNAs had similar changes in both CSF and saliva (four were downregulated: miR-182-5p, miR-221-3p, miR-26b-5p, miR-320c, and 2 were upregulated: miR-29c-3p, miR-30e-5p)  
• Changes in miR-320c were directly correlated with attention difficulty                                                                                                      | Hicks et al. 2018 |
| Albumin and hemoglobin    | Serum       | 213 children with moderate to severe TBI         | Mixed            | • Hypoaalbuminemia, hyperglycaemia upon admission, and a GSC <8 were independent risk factors for mortality  
• Strong correlation between admission serum albumin and hemoglobin levels and GCS, and these may predict mortality                                                                 | Luo et al. 2019 |
| Osteopontin (OPN)         | Plasma      | 66 children, aged 3-9 years, with TBI (n = 11 mild TBI, n = 5 moderate TBI, n = 50 severe TBI) | Elevated         | • Plasma OPN levels correlated with severe TBI, according to GCS, and intracranial lesions  
• OPN levels increased up to 72 h post-TBI and correlated with mortality                                                                                                                        | Gao et al. 2020 |
| IL-6, angiopoietin-2 (AP-2), endothelin-1 (ET-1), endocan-2 (EC-2) | Plasma | 28 children with TBI (n = 14 mild TBI, n = 3 moderate TBI, n = 11 severe TBI) | Mixed            | • Inverse relationship between GCS and AP-2, GCS and IL-6, and injury severity score (ISS) and ET-1  
• Direct relationship detected between GCS and ET-1 and ISS and AP-2                                                                                                                             | Lele et al. 2019 |
| Cardiac troponin (cTnl)    | Plasma      | Children with mild (n = 14), moderate (n = 3), or severe (n = 11) TBI | Elevated         | • 14/28 patients had at least one sample with elevated (>0.4 ng/mL) cTnl within 1-10 days in the hospital  
• The average admission GCS was 12 in patients with elevated cTnl and 9 in normal levels of cTnl                                                                                     | Lele et al. 2020 |

Key: interleukin (IL), cerebrospinal fluid (CSF), vascular endothelial growth factor (VEGF), C-reactive protein (CRP), acute respiratory distress syndrome (ARDS), transforming growth factor-beta1 (TGF-β1), intercellular adhesion molecule (ICAM), nerve growth factor (NGF), doublecortin (DCX), brain-derived neurotrophic factor (BDNF), glial-derived neurotrophic factor (GDNF), neuron-specific enolase (NSE), gastrointestinal (GI), vascular cellular adhesion molecule (VCAM), tumor necrosis factor receptor 2 (TNFR2), matrix metalloproteinase-9 (MMP9), hepatocyte growth factor (HGF), high-mobility box 1 (HMGB1), procalcitonin (PCT), triglycerides (TG), total cholesterol (TC), high-density-lipoproteins (HDL), low-density-lipoproteins (LDL), mitochondrial DNA (mtDNA), micro-ribonucleic acids (miRNAs), osteopontin (OPN), angiopoietin-2 (AP-2), endothelin-1 (ET-1), endocan-2 (EC-2), cardiac troponin (cTnl)
organisms, and may usher in post-TBI complications such as sepsis, which in turn increases BBB permeability, promotes neuro-inflammation, and worsens cognitive outcomes. Furthermore, damage to the intestinal epithelium following TBI may lead to gastrointestinal ischemia, stress ulcers, and intestinal dysautonomia. Moreover, intestinal damage has been shown to correlate with the severity of brain injury and may be associated with TBI-related morbidity. Furthermore, we emphasize the importance of elucidating the role of the microbiome in pTBI. Together, this review addresses the current gaps in the pathophysiology of TBI in the pediatric population. Furthermore, we emphasize the importance of elucidating the role of the microbiome in pTBI and TBI in preclinical and clinical TBI research.

The pediatric microbiome is functionally and compositionally different from the adult microbiome yet, to our knowledge, there are no preclinical studies that have examined the gut-brain axis in pTBI despite numerous aTBI studies demonstrating a promising role for gut dysbiosis in disease progression. Thus, preclinical and clinical studies that will examine microbiome dysbiosis in pTBI are urgently needed and could provide insights into the development of therapies that mitigate pTBI associated long-term neurological sequelae in children.

Conclusion

Traumatic brain injury poses a significant economic and public health crisis. Despite the considerable advances in studying TBI in the adult population, fewer studies have examined the pathophysiology and progression of TBI in the pediatric population. Given the considerable differences in CNS development and cerebrovascular function between children and adults, as highlighted in this review, newer studies must investigate pTBI pathophysiology separate from aTBI. Furthermore, the diagnostic and prognostic value of neuroimaging and clinical biomarkers in pTBI needs to be explored. Ideally, optimal biomarkers should be easily accessible, minimally invasive, and rely on objective measures. Equally important is the emerging role of the microbiome in TBI pathophysiology. While some studies have examined the role of the microbiome in aTBI, to our knowledge, no studies have elucidated a role for the microbiome in pTBI. Taken together, this review addresses the current gaps in the pathophysiology of TBI in the pediatric population. Furthermore, we emphasize the importance of distinguishing between aTBI and pTBI in preclinical and clinical TBI research.

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