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

Divine C. Nwafor[1,2,3] , Allison L. Brichacek[4,†], Chase H. Foster[5,†], Brandon P. Lucke-Wold[6], Ahsan Ali[1], Mark A. Colantonio[2], Candice M. Brown[1,4,3] and Rabia Qaiser[7]

[1]Department of Neuroscience, West Virginia University School of Medicine, Morgantown, WV, USA. [2]West Virginia University School of Medicine, Morgantown, WV, USA. [3]Rockefeller Neuroscience Institute, West Virginia University, Morgantown, WV, USA. [4]Department of Microbiology, Immunology, and Cell Biology, West Virginia University School of Medicine, Morgantown, WV, USA. [5]Department of Neurosurgery, George Washington University Hospital, Washington D.C., USA. [6]Department of Neurosurgery, University of Florida, Gainesville, FL, USA. [7]Department of Neurosurgery, Baylor Scott and White, Temple, TX, USA.

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

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.[1] Moreover, TBI is a crucial contributor to prolonged disability and dependence.[2] 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.[3] An appreciation for TBI in the adult civilian population is becoming more prominent.[4,5] 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.[6-8]

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.[9,10] An epidemiological study showed that 28% of children who visited the emergency department were reported to have been struck by or against an object.[11] 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.[11] 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.[12-17] 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.[10] 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

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.[27-30]

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.[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.[27,28,44,45] 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] Adaptions 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 follow.[63]

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.[8] 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.[72] Cerebrovascular dysfunction is a hallmark finding in many pediatric conditions and often predicts cognitive outcomes.[73] 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.[74] Likewise, Taylor *et al.* showed that vascular alterations in children increased the risk for cognitive impairment.[75] 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.[76] 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.[77] Beyond 10 years of age, CBFV declines and approximate adult values (~50 cm/s).[77-79] 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.[80] One explanation for the increased CBF values in children is the increased metabolic and energy demand needed by the developing brain.[73] Other vascular parameters such as cerebral vasoreactivity (CVR), which measures vascular responsiveness to vasodilation *via* changes in blood carbon dioxide (CO_2), have also been shown to be elevated in children compared to adults.[81,82] 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).[73] 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.[83] pTBI patients often reveal a significant reduction in CBF compared to aTBI patients.[73] 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.[81,84,85] 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).[60,86] 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.[87]

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.[88-91] The mechanisms that lead to CA impairment and subsequent decrease in CBF are unclear; however, several pathways have been implicated.[73] 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.[92,93] Similarly, a vasodilatory N-methyl-D-aspartate (NMDA) agonist (MK801) reduced pial vessel vasoconstriction and improved CBF following pTBI.[86] More recently, the c-Jun N-terminal kinase (JNK) intracellular signaling pathway has been implicated in pTBI-induced CA impairment.[73] 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.[91,94]

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.[95] 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.[96] 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.[85] 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, Badaut 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 7 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 neuroinflammation 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 mortality114. In children, higher water content, a softer skull, and weaker cervical 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.[73] 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.[131-135] 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.[136] 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.[137] Brain imaging has been explored in the prediction of post-TBI behavioral deficits.[138] 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.[139-142]

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.[143,144] 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.[145] 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.[146] 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.[147] 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.[148] Likewise, combining GCS and serum biomarker concentrations improves outcome prediction, including increased specificity and sensitivity.[149] 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.[150]

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.[151] This complex mutualistic ecosystem encompasses a diverse collection of microbial genomes that outnumber the cells in the human body.[152] Several studies have shown both intrinsic and extrinsic factors can shape the composition of the microbiome.[153-155] 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.[153,155] Other factors that shape the gut microbiome include sex, genetics, lifestyle, and medications.[153] 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).[156-160] Furthermore, recent studies have highlighted the role of the gut microbiome in maintaining healthy brain function.[161] 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.[162] Additionally, there is a growing body of preclinical and clinical evidence supporting microbiome dysbiosis in the pathogenesis and progression of aTBI.[163-167] 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.[166,168-171] 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[1]
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[2]
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[3]
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[4]
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[5]
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[6]
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[7]
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[8]
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[9]
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[10]

(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. 2007[11]
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 • However, S100B may not be a reliable prognostic marker because even high concentrations are associated with a full neurological recovery	Piazza et al. 2007[12]
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 • The correlation between S100B and NSE was weak, but significant • S100B and NSE cannot distinguish between symptomatic mild TBI and asymptomatic head contusion in children with minor head trauma	Geyer et al. 2009[13]
S100B	Serum	109 children with mild TBI	Elevated	• S100B was significantly elevated in patients with intracranial lesions identified on CT	Castellani et al. 2009[14]
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. 2009[15]
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. 2010[16]
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. 2010[17]
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. 2010[18]
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 • Serum GFAP correlated with functional outcome at 6 months, indicating its prognostic value • Hypothermia therapy had no effect on serum GFAP levels	Fraser et al. 2011[19]
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 • S100B was significantly higher in children with an abnormal cranial CT vs normal cranial CT scan • S100B appears to predict TBI severity	Babcock et al. 2012[20]

(Continued)

Table 1. 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[21]
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[22]
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[23]
S100B, NSE, GFAP, neurofilaments (NF-H), secretagogin, and Hsp70	Serum	63 children with TBI		• 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[24]
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[25]
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[26]
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 lesson assessed by CT	Mondello et al. 2016[27]

(Continued)

Table 1. 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[28]
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[29]
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[30]
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[31]
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 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[32]
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[33]

(Continued)

Table 1. Continued.

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), αII-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. 2000[37]
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. 2000[38]
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. 2004[39]
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. 2004[40]
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. 2005[41]
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. 2006[42]
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 was associated with TBI severity • Suggests that elevated hsp60 may reflect the severity of early mitochondrial stress or damage following TBI	Lai et al. 2006[43]
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. 2006[44]
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. 2007[45]
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. 2008[46]

(Continued)

Table 2. 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[47]
IL-1β, IL-6, NGF, BNDF, 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 • Higher concentrations of NGF and IL-6, and lower concentrations of IL-1β at 48 h were associated with better neurologic outcome • No significant changes were seen for BNDF nor GDNF	Chiaretti et al. 2008[48]
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[49]
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[50]
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 IL-6, MMP9, HGF, and fibrinogen were increased in infants with mild inflicted TBI	Berger et al. 2009[51]
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[52]
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[53]
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[54]
CD64 and CD11b PCT, CRP, glucose, TG, TC, HDL, and LDL	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[55]
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[56]

(Continued)

Table 2. 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	Foaud et al. 2014[57]
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[58]
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[59]
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[60]
Albumin and hemoglobin	Serum	213 children with moderate to severe TBI	Mixed	• Hypoalbuminemia, hyperglycemia 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[61]
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[62]
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[63]
Cardiac troponin (cTnI)	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 (>.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[64]

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-1β), intracellular adhesion molecule (ICAM), nerve growth factor (NGF), doublecortin (DCX), brain-derived neurotrophic factor (BNDF), 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 group 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 (cTnI).

organs, and may usher in post-TBI complications such as sepsis, which in turn increases BBB permeability, promotes neuro-inflammation, and worsens cognitive outcomes.[95] Furthermore, damage to the intestinal epithelium following TBI may lead to gastrointestinal ischemia, stress ulcers, and intestinal dysautonomia.[165,172] Moreover, intestinal damage has been shown to correlate with the severity of brain injury and may be associated with TBI-related morbidity.[166] Furthermore, some studies have demonstrated in models of aTBI that specific bacterial species such as *Lactobacillus acidophilus* and *Clostridium butyricum* may be beneficial for post-TBI recovery.[173,174]

The pediatric microbiome is functionally and compositionally different from the adult microbiome[152]; 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.[175-236]

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.

ORCID iDs

Divine C. Nwafor   https://orcid.org/0000-0001-7347-9524
Candice M. Brown   https://orcid.org/0000-0001-5845-0221

REFERENCES

1. Centers for Disease Control and Prevention. *Surveillance Report of Traumatic BrainInjury-Related Hospitalizations and Deaths by Age Group, Sex, and Mechanism of Injury—United States, 2016 and 2017*. Atlanta, GA: Centers for Disease Control and Prevention, U.S. Department of Health and Human Services; 2021.
2. Blennow K, Brody DL, Kochanek PM, et al. Traumatic brain injuries. *Nat Rev Dis Primers*. 2016;2:16084.
3. Lindquist LK, Love HC, Elbogen EB. Traumatic brain injury in Iraq and Afghanistan veterans: New results from a national random sample study. *J Neuropsychiatry Clin Neurosci*. 2017;29(3):254-259.
4. Frost RB, Farrer TJ, Primosch M, Hedges DW. Prevalence of traumatic brain injury in the general adult population: a meta-analysis. *Neuroepidemiology*. 2013;40(3):154-159.
5. Leo P, McCrea M. Epidemiology. In: Laskowitz D, Grant G, eds. *Translational Research in Traumatic Brain Injury*. Boca RatonFL; 2016.
6. Cook LG, Chapman SB, Elliott AC, Evenson NN, Vinton K. Cognitive gains from gist reasoning training in adolescents with chronic-stage traumatic brain injury. *Front Neurol*. 2014;5:87.
7. Schmidt AT, Hanten GR, Li X, Vasquez AC, et al. Decision making after pediatric traumatic brain injury: trajectory of recovery and relationship to age and gender. *Int J Dev Neurosci*. 2012;30(3):225-230.
8. Kochanek PM, Wallisch JS, Bayir H, Clark RSB. Pre-clinical models in pediatric traumatic brain injury-challenges and lessons learned. *Childs Nerv Syst*. 2017; 33(10):1693-1701.
9. Hyder AA, Wunderlich CA, Puvanachandra P, Gururaj G, Kobusingye OC. The impact of traumatic brain injuries: a global perspective. *Neuro Rehabilitation*. 2007; 22(5):341-353.
10. Dewan MC, Mummareddy N, Wellons JC 3rd, Bonfield CM. Epidemiology of global pediatric traumatic brain injury: Qualitative review. *World Neurosurg*. 2016;91:497-509.
11. Taylor CA, Bell JM, Breiding MJ, Xu L. Traumatic brain injury-related emergency department visits, hospitalizations, and deaths - United States, 2007 and 2013. *MMWR Surveill Summ*. 2017;66(9):1-16.
12. Russell KL, Kutchko KM, Fowler SC, Berman NE, Levant B. Sensorimotor behavioral tests for use in a juvenile rat model of traumatic brain injury: assessment of sex differences. *J Neurosci Methods*. 2011;199(2): 214-222.
13. Arnett AB, Peterson RL, Kirkwood MW, et al. Behavioral and cognitive predictors of educational outcomes in pediatric traumatic brain injury. *J Int Neuropsychol Soc*. 2013;19(8):881-889.
14. Cnossen MC, Winkler EA, Yue JK, et al. Development of a prediction model for post-concussive symptoms following mild traumatic brain injury: A TRACK-TBI pilot study. *J Neurotrauma*. 2017;34(16):2396-2409.
15. McMahon P, Hricik A, Yue JK, et al. Symptomatology and functional outcome in mild traumatic brain injury: results from the prospective TRACK-TBI study. *J Neurotrauma*. 2014;31(1):26-33.
16. Wilde EA, Merkley TL, Lindsey HM, et al. Developmental alterations in cortical organization and socialization in adolescents who sustained a traumatic brain injury in early childhood. *J Neurotrauma*. 2021;38(1):133-143.
17. Wade SL, Zhang N, Yeates KO, Stancin T, Taylor HG. Social environmental moderators of long-term functional outcomes of early childhood brain injury. *JAMA Pediatr*. 2016;170(4):343-349.
18. Tau GZ, Peterson BS. Normal development of brain circuits. *Neuropsychopharmacology*. 2010;35(1):147-168.
19. Giza CC, Kolb B, Harris NG, Asarnow RF, Prins ML. Hitting a moving target: Basic mechanisms of recovery from acquired developmental brain injury. *Dev Neurorehabil*. 2009;12(5):255-268.
20. Denny-Brown DE, Russell WR. Experimental concussion: (section of neurology). *Proc Roy Soc Med*. 1941;34(11):691-692.
21. Denny-Brown D. Brain trauma and concussion. *Arch Neurol*. 1961;5:1-3.
22. Johnson VE, Meaney DF, Cullen DK, Smith DH. Animal models of traumatic brain injury. *Handb Clin Neurol*. 2015;127:115-128.
23. Morganti-Kossmann MC, Yan E, Bye N. Animal models of traumatic brain injury: is there an optimal model to reproduce human brain injury in the laboratory? *Injury*. 2010;41(suppl 1):S10-S13.
24. Thompson FJ, Hou J, Bose PK. Closed-head TBI model of multiple morbidity. *Methods Mol Biol*. 2016;1462:521-536.
25. Cernak I. Animal models of head trauma. *NeuroRx*. 2005;2(3):410-422.
26. Morales DM, Marklund N, Lebold D, et al. Experimental models of traumatic brain injury: Do we really need to build a better mousetrap? *Neuroscience*. 2005; 136(4):971-989.
27. Adelson PD, Robichaud P, Hamilton RL, Kochanek PM. A model of diffuse traumatic brain injury in the immature rat. *J Neurosurg*. 1996;85(5): 877-884.
28. Huh JW, Widing AG, Raghupathi R. Midline brain injury in the immature rat induces sustained cognitive deficits, bihemispheric axonal injury and neurodegeneration. *Exp Neurol*. 2008;213(1):84-92.
29. Ommaya AK, Goldsmith W, Thibault L. Biomechanics and neuropathology of adult and paediatric head injury. *Br J Neurosurg*. 2002;16(3): 220-242.
30. Semple BD, Carlson J, Noble-Haeusslein LJ. Pediatric Rodent Models of Traumatic Brain Injury. *Methods Mol Biol*. 2016;1462:325-343.
31. Ross DT, Meaney DF, Sabol MK, Smith DH, Gennarelli TA. Distribution of forebrain diffuse axonal injury following inertial closed head injury in miniature swine. *Exp Neurol*. 1994;126(2):291-298.

32. Meaney DF, Smith DH, Shreiber DI, et al. Biomechanical analysis of experimental diffuse axonal injury. *J Neurotrauma*. 1995;12(4):689-694.

33. Long JB, Bentley TL, Wessner KA, Cerone C, Sweeney S, Bauman RA. Blast overpressure in rats: recreating a battlefield injury in the laboratory. *J Neurotrauma*. 2009;26(6):827-840.

34. Goldstein LE, Fisher AM, Tagge CA, et al. Chronic traumatic encephalopathy in blast-exposed military veterans and a blast neurotrauma mouse model. *Sci Transl Med*. 2012;4(134):134ra60.

35. Rafaels KA, Bass CR, Panzer MB, et al. Brain injury risk from primary blast. *The journal of trauma and acute care surgery*. 2012;73(4):895-901.

36. Rubovitch V, Ten-Bosch M, Zohar O, et al. A mouse model of blast-induced mild traumatic brain injury. *Exp Neurol*. 2011;232(2):280-289.

37. Celander H, Clemedson CJ, Ericsson UA, Hultman HI. The use of a compressed air operated shock tube for physiological blast research. *Acta Physiologica Scandinavica*. 1955;33(1):6-13.

38. Koliatsos VE, Cernak I, Xu L, et al. A mouse model of blast injury to brain: Initial pathological, neuropathological, and behavioral characterization. *J Neuropathol Exp Neurol*. 2011;70(5):399-416.

39. Friess SH, Ichord RN, Ralston, et al. Repeated traumatic brain injury affects composite cognitive function in piglets. *J Neurotrauma*. 2009;26(7):1111-1121.

40. Milwood Hargrave J, Pearce P, Mayhew ER, Bull A, Taylor S. Blast injuries in children: A mixed-methods narrative review. *BMJ Paediatr Open*. 2019;3(1):e000452.

41. Frost A, Boyle P, Autier P, et al. The effect of explosive remnants of war on global public health: a systematic mixed-studies review using narrative synthesis. *Lancet Public Health*. 2017;2(6):e286-e296.

42. Marmarou A, Foda MA, van den Brink W, Campbell J, Kita H, Demetriadou K. A new model of diffuse brain injury in rats. Part I: Pathophysiology and biomechanics. *J Neurosurg*. 1994;80(2):291-300.

43. Shitaka Y, Tran HT, Bennett RE, et al. Repetitive closed-skull traumatic brain injury in mice causes persistent multifocal axonal injury and microglial reactivity. *Journal of neuropathology and experimental neurology*. 2011;70(7):551-567.

44. Mychasiuk R, Farran A, Angoa-Perez M, Briggs D, Kuhn D, Esser MJ. A novel model of mild traumatic brain injury for juvenile rats. *J Vis Exp*. 2014;(94):51820.

45. Brody DL, Benetatos J, Bennett RE, Klemenhagen KC, Mac Donald CL. The pathophysiology of repetitive concussive traumatic brain injury in experimental models; new developments and open questions. *Mol Cell Neurosci*. 2015;66(Pt B): 91-98.

46. Lifshitz J, Rowe RK, Griffiths DR, et al. Clinical relevance of midline fluid percussion brain injury: Acute deficits, chronic morbidities and the utility of biomarkers. *Brain Inj*. 2016;30(11):1293-1301.

47. Sullivan HG, Martinez J, Becker DP, Miller JD, Griffith R, Wist AO. Fluid-percussion model of mechanical brain injury in the cat. *J Neurosurg*. 1976;45(5): 521-534.

48. Millen JE, Glauser FL, Fairman RP. A comparison of physiological responses to percussive brain trauma in dogs and sheep. *J Neurosurg*. 1985;62(4):587-591.

49. McIntosh TK, Noble L, Andrews B, Faden AI. Traumatic brain injury in the rat: Characterization of a midline fluid-percussion model. *Cent Nerv Syst Trauma*. 1987;4(2):119-134.

50. Dixon CE, Lyeth BG, Povlishock JT, et al. A fluid percussion model of experimental brain injury in the rat. *J Neurosurg*. 1987;67(1):110-119.

51. Hayes RL, Stalhammar D, Povlishock JT, et al. A new model of concussive brain injury in the cat produced by extradural fluid volume loading: II. Physiological and neuropathological observations. *Brain Inj*. 1987;1(1):93-112.

52. Van KC, Lyeth BG. Lateral (Parasagittal) fluid percussion model of traumatic brain injury. *Methods Mol Biol*. 2016;1462:231-251.

53. Yamaki T, Murakami N, Iwamoto Y, et al. A modified fluid percussion device. *J Neurotrauma*. 1994;11(5):613-622.

54. Kabadi SV, Hilton GD, Stoica BA, Zapple DN, Faden AI. Fluid-percussion-induced traumatic brain injury model in rats. *Nat Protoc*. 2010;5(9):1552-1563.

55. Alder J, Fujioka W, Lifshitz J, Crockett DP, Thakker-Varia S. Lateral fluid percussion: model of traumatic brain injury in mice. *J Vis Exp*. 2011;(54):3063.

56. Frey LC, Hellier J, Unkart C, et al. A novel apparatus for lateral fluid percussion injury in the rat. *J Neurosci Methods*. 2009;177(2):267-272.

57. Giza CC, Griesbach GS, Hovda DA. Experience-dependent behavioral plasticity is disturbed following traumatic injury to the immature brain. *Behav Brain Res*. 2005;157(1):11-22.

58. Rowe RK, Harrison JL, Ellis TW, Adelson PD, Lifshitz J. Midline (central) fluid percussion model of traumatic brain injury in pediatric and adolescent rats. *J Neurosurg Pediatr*. 2018;22(1):22-30.

59. Newell EA, Todd BP, Luo Z, Evans LP, Ferguson PJ, Bassuk AG. A mouse model for juvenile, lateral fluid percussion brain injury reveals sex-dependent differences in neuroinflammation and functional recovery. *J Neurotrauma*. 2020; 37(4):635-646.

60. Armstead WM, Kurth CD. Different cerebral hemodynamic responses following fluid percussion brain injury in the newborn and juvenile pig. *J Neurotrauma*. 1994; 11(5):487-497.

61. Armstead WM, Riley J, Vavilala MS. Dopamine prevents impairment of autoregulation after traumatic brain injury in the newborn pig through inhibition of Up-regulation of endothelin-1 and extracellular signal-regulated kinase mitogen-activated protein kinase. *Pediatr Crit Care Med*. 2013;14(2):e103-e111.

62. Curvello V, Hekierski H, Riley J, Vavilala M, Armstead WM. Sex and age differences in phenylephrine mechanisms and outcomes after piglet brain injury. *Pediatr Res*. 2017;82(1):108-113.

63. Lighthall JW, Dixon CE, Anderson TE. Experimental models of brain injury. *J Neurotrauma*. 1989;6(2):83-97.

64. Namjoshi DR, Good C, Cheng WH, et al. Towards clinical management of traumatic brain injury: a review of models and mechanisms from a biomechanical perspective. *Disease models & mechanisms*. 2013;6(6):1325-1338.

65. Laurer HL, Bareyre FM, Lee VM, et al. Mild head injury increasing the brain's vulnerability to a second concussive impact. *J Neurosurg*. 2001;95(5):859-870.

66. Hylin MJ, Orsi SA, Rozas NS, et al. Repeated mild closed head injury impairs short-term visuospatial memory and complex learning. *J Neurotrauma*. 2013;30(9):716-726.

67. Smith SL, Andrus PK, Gleason DD, Hall ED. Infant rat model of the shaken baby syndrome: preliminary characterization and evidence for the role of free radicals in cortical hemorrhaging and progressive neuronal degeneration. *J Neurotrauma*. 1998;15(9):693-705.

68. Bonnier C, Mesples B, Gressens P. Animal models of shaken baby syndrome: Revisiting the pathophysiology of this devastating injury. *Pediatr Rehabil*. 2004; 7(3):165-171.

69. Bonnier C, Mesplès B, Carpentier S, Henin D, Gressens P. Delayed white matter injury in a murine model of shaken baby syndrome. *Brain Pathol*. 2002;12(3):320-328.

70. Kawawata Y, Ehara A, Yamaguchi T, Seo Y, Shimoda K, Ueda S. Repeated mild shaking of neonates induces transient cerebral microhemorrhages and anxiety-related behavior in adult rats. *Neurosci Lett*. 2018;684:29-34.

71. Finnie JW, Blumbergs PC, Manavis J, et al. Neuropathological changes in a lamb model of non-accidental head injury (the shaken baby syndrome). *J Clin Neurosci*. 2012;19(8):1159-1164.

72. Kenney K, Amyot F, Haber M, et al. Cerebral vascular injury in traumatic brain injury. *Exp Neurol*. 2016;275(Pt 3):353-366.

73. Ichkova A, Rodriguez-Grande B, Bar C, Villega F, Konsman JP, Badaut J. Vascular impairment as a pathological mechanism underlying long-lasting cognitive dysfunction after pediatric traumatic brain injury. *Neurochem Int*. 2017;111: 93-102.

74. Bakker MJ, Hofmann J, Churches OF, Badcock NA, Kohler M, Keage HA. Cerebrovascular function and cognition in childhood: a systematic review of transcranial Doppler studies. *BMC Neurol*. 2014;14:43.

75. Taylor ZE, Spinrad TL, VanSchyndel SK, et al. Sociodemographic risk, parenting, and effortful control: Relations to salivary alpha-amylase and cortisol in early childhood. *Dev Psychobiol*. 2013;55(8):869-880.

76. Nation DA, Sweeney MD, Montagne A, et al. Blood-brain barrier breakdown is an early biomarker of human cognitive dysfunction. *Nat Med*. 2019;25(2):270-276.

77. Udomphorn Y, Armstead WM, Vavilala MS. Cerebral blood flow and autoregulation after pediatric traumatic brain injury. *Pediatr Neurol*. 2008;38(4): 225-234.

78. Soustiel JF, Shik V, Shreiber R, Tavor Y, Goldsher D. Basilar vasospasm diagnosis: Investigation of a modified "Lindegaard Index" based on imaging studies and blood velocity measurements of the basilar artery. *Stroke*. 2002;33(1):72-77.

79. Martin PJ, Evans DH, Naylor AR. Measurement of blood flow velocity in the basal cerebral circulation: advantages of transcranial color-coded sonography over conventional transcranial Doppler. *J Clin Ultrasound*. 1995;23(1):21-26.

80. Biagi L, Abbruzzese A, Bianchi MC, Alsop DC, Del Guerra A, Tosetti M. Age dependence of cerebral perfusion assessed by magnetic resonance continuous arterial spin labeling. *J Magn Reson Imaging*. 2007;25(4):696-702.

81. Philip S, Udomphorn Y, Kirkham FJ, Vavilala MS. Cerebrovascular pathophysiology in pediatric traumatic brain injury. *J Trauma*. 2009;67(2 suppl l): S128-S134.

82. Leung J, Kosinski PD, Croal PL, Kassner A. Developmental trajectories of cerebrovascular reactivity in healthy children and young adults assessed with magnetic resonance imaging. *J Physiol*. 2016;594(10):2681-2689.

83. Vavilala MS, Newell DW, Junger E, et al. Dynamic cerebral autoregulation in healthy adolescents. *Acta Anaesthesiol Scand*. 2002;46(4):393-397.

84. Adelson PD, Clyde B, Kochanek PM, Wisniewski SR, Marion DW, Yonas H. Cerebrovascular response in infants and young children following severe traumatic brain injury: a preliminary report. *Pediatr Neurosurg*. 1997;26(4):200-207.

85. Pop V, Badaut J. A neurovascular perspective for long-term changes after brain trauma. *Transl Stroke Res*. 2011;2(4):533-545.

86. Armstead WM. NMDA and age dependent cerebral hemodynamics after traumatic brain injury. *Exp Toxicol Pathol*. 2004;56(1-2):75-81.

87. Prins ML, Lee SM, Cheng CL, Becker DP, Hovda DA. Fluid percussion brain injury in the developing and adult rat: a comparative study of mortality, morphology, intracranial pressure and mean arterial blood pressure. *Brain Res Dev Brain Res*. 1996;95(2):272-282.

88. Freeman SS, Udomphorn Y, Armstead WM, Fisk DM, Vavilala MS. Young age as a risk factor for impaired cerebral autoregulation after moderate to severe pediatric traumatic brain injury. *Anesthesiology*. 2008;108(4):588-595.

89. Nagel C, Diedler J, Gerbig I, Heimberg E, Schuhmann MU, Hockel K. State of cerebrovascular autoregulation correlates with outcome in severe infant/pediatric traumatic brain injury. *Acta Neurochir Suppl*. 2016;122:239-244.

90. Vavilala MS, Muangman S, Tontisirin N, et al. Impaired cerebral autoregulation and 6-month outcome in children with severe traumatic brain injury: preliminary findings. *Dev Neurosci*. 2006;28(4-5):348-353.

91. Vavilala MS, Tontisirin N, Udomphorn Y, et al. Hemispheric differences in cerebral autoregulation in children with moderate and severe traumatic brain injury. *Neurocrit Care*. 2008;9(1):45-54.

92. Armstead WM, Kreipke CW. Endothelin-1 is upregulated after traumatic brain injury: a cross-species, cross-model analysis. *Neurol Res*. 2011;33(2):133-136.

93. Armstead WM, Raghupathi R. Endothelin and the neurovascular unit in pediatric traumatic brain injury. *Neurol Res*. 2011;33(2):127-132.

94. Vavilala MS, Lee LA, Boddu K, et al. Cerebral autoregulation in pediatric traumatic brain injury. *Pediatr Crit Care Med*. 2004;5(3):257-263.

95. Nwafor DC, Brichacek AL, Mohammad AS, et al. Targeting the blood-brain barrier to prevent sepsis-associated cognitive impairment. *J Cent Nerv Syst Dis*. 2019;11:1179573519840652.

96. Saunders NR, Liddelow SA, Dziegielewska KM. Barrier mechanisms in the developing brain. *Front Pharmacol*. 2012;3:46.

97. Muramatsu K, Fukuda A, Togari H, Wada Y, Nishino H. Vulnerability to cerebral hypoxic-ischemic insult in neonatal but not in adult rats is in parallel with disruption of the blood-brain barrier. *Stroke*. 1997;28(11):2281-2288. discussion 2288-2289.

98. Shlosberg D, Benifla M, Kaufer D, Friedman A. Blood-brain barrier breakdown as a therapeutic target in traumatic brain injury. *Nat Rev Neurol*. 2010;6(7):393-403.

99. Tomkins O, Shelef I, Kaizerman I, et al. Blood-brain barrier disruption in post-traumatic epilepsy. *J Neurol Neurosurg Psychiatry*. 2008;79(7):774-777.

100. Korn A, Golan H, Melamed I, Pascual-Marqui R, Friedman A. Focal cortical dysfunction and blood-brain barrier disruption in patients with Postconcussion syndrome. *J Clin Neurophysiol*. 2005;22(1):1-9.

101. Kirchhoff C, Stegmaier J, Bogner V, et al. Intrathecal and systemic concentration of NT-proBNP in patients with severe traumatic brain injury. *J Neurotrauma*. 2006;23(6):943-949.

102. Stahel PF, Morganti-Kossmann MC, Perez D, et al. Intrathecal levels of complement-derived soluble membrane attack complex (sC5b-9) correlate with blood-brain barrier dysfunction in patients with traumatic brain injury. *J Neurotrauma*. 2001;18(8):773-781.

103. Badaut J, Ajao DO, Sorensen DW, Fukuda AM, Pellerin L. Caveolin expression changes in the neurovascular unit after traumatic brain injury: signs of blood-brain barrier healing? *Neuroscience*. 2015;285:215-226.

104. Fukuda AM, Adami A, Pop V, et al. Posttraumatic reduction of edema with aquaporin-4 RNA interference improves acute and chronic functional recovery. *J Cereb Blood Flow Metab*. 2013;33(10):1621-1632.

105. Pop V, Sorensen DW, Kamper JE, et al. Early brain injury alters the blood-brain barrier phenotype in parallel with beta-amyloid and cognitive changes in adulthood. *J Cereb Blood Flow Metab*. 2013;33(2):205-214.

106. Abrahamson EE, Ikonomovic MD. Brain injury-induced dysfunction of the blood brain barrier as a risk for dementia. *Exp Neurol*. 2020;328:113257.

107. Storck SE, Hartz AMS, Bernard J, et al. The concerted amyloid-beta clearance of LRP1 and ABCB1/P-gp across the blood-brain barrier is linked by PICALM. *Brain Behav Immun*. 2018;73:21-33.

108. Cirrito JR, Deane R, Fagan AM, et al. P-glycoprotein deficiency at the blood-brain barrier increases amyloid-beta deposition in an Alzheimer disease mouse model. *J Clin Invest*. 2005;115(11):3285-3290.

109. Alasmari F, Ashby CR Jr., Hall FS, Sari Y, Tiwari AK. Modulation of the ATP-binding cassette B1 transporter by neuro-inflammatory cytokines: Role in the pathogenesis of Alzheimer's disease. *Front Pharmacol*. 2018;9:658.

110. Jullienne A, Roberts JM, Pop V, et al. Juvenile traumatic brain injury induces long-term perivascular matrix changes alongside amyloid-beta accumulation. *J Cereb Blood Flow Metab*. 2014;34(10):1637-1645.

111. Aldrich EF, Eisenberg HM, Saydjari C, et al. Diffuse brain swelling in severely head-injured children. A report from the NIH traumatic coma data bank. *J Neurosurg*. 1992;76(3):450-454.

112. Lang DA, Teasdale GM, Macpherson P, Lawrence A. Diffuse brain swelling after head injury: more often malignant in adults than children? *J Neurosurg*. 1994;80(4):675-680.

113. Fraunberger E, Esser MJ. Neuro-inflammation in pediatric traumatic brain injury-from mechanisms to inflammatory networks. *Brain Sci*. 2019;9(11):319.

114. Fukuda AM, Pop V, Spagnoli D, Ashwal S, Obenaus A, Badaut J. Delayed increase of astrocytic aquaporin 4 after juvenile traumatic brain injury: possible role in edema resolution? *Neuroscience*. 2012;222:366-378.

115. Kumar RG, Diamond ML, Boles JA, et al. Acute CSF interleukin-6 trajectories after TBI: Associations with neuroinflammation, polytrauma, and outcome. *Brain Behav Immun*. 2015;45:253-262.

116. Zetterberg H, Blennow K. Fluid biomarkers for mild traumatic brain injury and related conditions. *Nat Rev Neurol*. 2016;12(10):563-574.

117. Levin HS, Diaz-Arrastia RR. Diagnosis, prognosis, and clinical management of mild traumatic brain injury. *Lancet Neurol*. 2015;14(5):506-517.

118. Kochanek PM, Berger RP, Fink EL, et al. The potential for bio-mediators and biomarkers in pediatric traumatic brain injury and neurocritical care. *Front Neurol*. 2013;4:40.

119. Reuter-Rice K, Eads JK, Berndt SB, Bennett E. Chapter 6 state of the science of pediatric traumatic brain injury: biomarkers and gene association studies. *Annu Rev Nurs Res*. 2015;33(1):185-217.

120. Papa L, Ramia MM, Kelly JM, Burks SS, Pawlowicz A, Berger RP. Systematic review of clinical research on biomarkers for pediatric traumatic brain injury. *J Neurotrauma*. 2013;30(5):324-338.

121. Mondello S, Schmid K, Berger RP, et al. The challenge of mild traumatic brain injury: role of biochemical markers in diagnosis of brain damage: Serum markers for brain damage in mild TBI. *Med Res Rev*. 2014;34(3):503-531.

122. Marshall LF. Head injury: recent past, present, and future. *Neurosurgery*. 2000; 47(3):546-561.

123. Berger RP, Beers SR, Richichi R, Wiesman D, Adelson PD. Serum biomarker concentrations and outcome after pediatric traumatic brain injury. *J Neurotrauma*. 2007;24(12):1793-1801.

124. Iankova A. The glasgow coma scale clinical application in emergency departments: Andriana Iankova discusses the use of the glasgow coma scale assessment tool in adult patients with head injury whose conscious levels may be compromised by alcohol. *Emerg Nurse*. 2006;14(8):30-35.

125. Jennett B, Bond M. Assessment of outcome after severe brain damage a practical scale. *Lancet*. 1975;305(7905):480-484.

126. Shore PM, Berger RP, Varma S, et al. Cerebrospinal fluid biomarkers versus glasgow coma scale and glasgow outcome scale in pediatric traumatic brain injury: The role of young age and inflicted injury. *J Neurotrauma*. 2007;24(1):75-86.

127. Robertson CMT, Joffe AR, Moore AJ, Watt JM. Neurodevelopmental outcome of young pediatric intensive care survivors of serious brain injury. *Pediatr Crit Care Med*. 2002;3(4):345-350.

128. Ducrocq SC, Meyer PG, Orliaguet GA, et al. Epidemiology and early predictive factors of mortality and outcome in children with traumatic severe brain injury: experience of a French pediatric trauma center. *Pediatr Crit Care Med*. 2006;7(5):461-467.

129. Tsao JW, Hemphill JC, Johnston SC, Smith WS, Bonovich DC. Initial Glasgow Coma Scale score predicts outcome following thrombolysis for posterior circulation stroke. *Arch Neurol*. 2005;62(7):1126-1129.

130. Benesch CG, McDaniel KD, Cox C, Hamill RW. End-stage Alzheimer's disease: Glasgow coma scale and the neurologic examination. *Arch Neurol*. 1993;50:1309-1315.

131. Singh N, Singhal A. Challenges in minor TBI and indications for head CT in pediatric TBI—an update. *Child's Nerv Syst*. 2017;33(10):1677-1681.

132. Dennis EL, Babikian T, Giza CC, Thompson PM, Asarnow RF. Diffusion MRI in pediatric brain injury. *Child's Nerv Syst*. 2017;33(10):1683-1692.

133. Kirkham FJ. Indications for the performance of neuroimaging in children. *Handb Clin Neurol*. 2016;136:1275-1290.

134. Dodd AB, Epstein K, Ling JM, Mayer AR. Diffusion tensor imaging findings in semi-acute mild traumatic brain injury. *J Neurotrauma*. 2014;31(14):1235-1248.

135. Wintermark M, Sanelli PC, Anzai Y, et al. Imaging evidence and recommendations for traumatic brain injury: Conventional neuroimaging techniques. *J Am Coll Radiol*. 2015;12(2):e1-e14.

136. Dennis EL, Ellis MU, Marion SD, et al. Callosal function in pediatric traumatic brain injury linked to disrupted white matter integrity. *J Neurosci*. 2015;35(28):10202-10211.

137. Ware AL, Goodrich-Hunsaker NJ, Lebel C, et al. Post-acute cortical thickness in children with mild traumatic. *Brain Injury versus Orthopedic Injury*. 2020;37:1892-1901.

138. Johnson CP, Juranek J, Kramer LA, Prasad MR, Swank PR, Ewing-Cobbs L. Predicting behavioral deficits in pediatric traumatic brain injury through uncinate fasciculus integrity. *J Int Neuropsychol Soc*. 2011;17(04):663-673.

139. Ashwal S, Holshouser B, Tong K, et al. Proton spectroscopy detected myo-inositol in children with traumatic brain injury. *Pediatr Res*. 2004;56(4):630-638.

140. Ashwal S, Holshouser BA, Shu SK, et al. Predictive value of proton magnetic resonance spectroscopy in pediatric closed head injury. *Pediatr Neurol*. 2000;23(2):114-125.

141. Holshouser BA, Ashwal S, Luh GY, et al. Proton MR spectroscopy after acute central nervous system injury: Outcome prediction in neonates, infants, and children. *Radiology*. 1997;202(2):487-496.

142. Kreis R, Arcinue E, Ernst T, Shonk TK, Flores R, Ross BD. Hypoxic encephalopathy after near-drowning studied by quantitative 1H-magnetic resonance spectroscopy. *J Clin Invest*. 1996;97(5):1142-1154.

143. Brenner DJ. Computed tomography — An increasing source of radiation exposure. *N Engl J Med*. 2007;357:2277-2284.

144. Blackwell CD, Gorelick M, Holmes JF, Bandyopadhyay S, Kuppermann N. Pediatric head trauma: Changes in use of computed tomography in emergency departments in the united states over time. *Ann Emerg Med*. 2007;49(3): 320-324.

145. Raschle N, Zuk J, Ortiz-Mantilla S, et al. Pediatric neuroimaging in early childhood and infancy: challenges and practical guidelines: Raschle et al. *Ann N Y Acad Sci*. 2012;1252(1):43-50.

146. Wilkinson AA, Dennis M, Simic N, et al. Brain biomarkers and pre-injury cognition are associated with long-term cognitive outcome in children with traumatic brain injury. *BMC Pediatr*. 2017;17(1):173.

147. Korley FK, Diaz-Arrastia R, Wu AHB, et al. Circulating brain-derived neurotrophic factor has diagnostic and prognostic value in traumatic brain injury. *J Neurotrauma*. 2016;33(2):215-225.

148. Lo T-YM, Jones PA, Minns RA. Pediatric brain trauma outcome prediction using paired serum levels of inflammatory mediators and brain-specific proteins. *J Neurotrauma*. 2009;26(9):1479-1487.

149. Lo T-YM, Jones PA, Minns RA. Combining coma score and serum biomarker levels to predict unfavorable outcome following childhood brain trauma. *J Neurotrauma*. 2010;27(12):2139-2145.

150. Marzano LAS, Batista JPT, de Abreu Arruda M, et al. Traumatic brain injury biomarkers in pediatric patients: a systematic review. *Neurosurg Rev*. 2021.

151. Cong X, Xu W, Romisher R, et al. Gut microbiome and infant health: brain-gut-microbiota axis and host genetic factors. *Yale J Biol Med*. 2016;89(3):299-308.

152. Ihekweazu FD, Versalovic J. Development of the pediatric gut microbiome: Impact on health and disease. *Am J Med Sci*. 2018;356(5):413-423.

153. Shin A, Preidis GA, Shulman R, Kashyap PC. The gut microbiome in adult and pediatric functional gastrointestinal disorders. *Clin Gastroenterol Hepatol*. 2019; 17(2):256-274.

154. Yassour M, Vatanen T, Siljander H, et al. Natural history of the infant gut microbiome and impact of antibiotic treatment on bacterial strain diversity and stability. *Sci Transl Med*. 2016;8(343):343ra81.

155. Yatsunenko T, Rey FE, Manary MJ, et al. Human gut microbiome viewed across age and geography. *Nature*. 2012;486(7402):222-227.

156. Haikal C, Chen QQ, Li JY. Microbiome changes: an indicator of Parkinson's disease? *Transl Neurodegener*. 2019;8:38.

157. Sampson TR, Debelius JW, Thron T, et al. Gut microbiota regulate motor deficits and neuroinflammation in a model of parkinson's disease. *Cell*. 2016;167(6): 1469-1480. e1412.

158. Durgan DJ, Lee J, McCullough LD, Bryan RM Jr. Examining the role of the microbiota-gut-brain axis in stroke. *Stroke*. 2019;50(8):2270-2277.

159. Spychala MS, Venna VR, Jandzinski M, et al. Age-related changes in the gut microbiota influence systemic inflammation and stroke outcome. *Ann Neurol*. 2018;84(1):23-36.

160. Seo DO, Holtzman DM. Gut microbiota: From the forgotten organ to a potential key player in the pathology of Alzheimer's disease. *J Gerontol A Biol Sci Med Sci*. 2020;75(7):1232-1241.

161. Logsdon AF, Erickson MA, Rhea EM, Salameh TS, Banks WA. Gut reactions: How the blood-brain barrier connects the microbiome and the brain. *Exp Biol Med*. 2018;243(2):159-165.

162. Braniste V, Al-Asmakh M, Kowal C, et al. The gut microbiota influences blood-brain barrier permeability in mice. *Sci Transl Med*. 2014;6(263):263ra158.

163. Treangen TJ, Wagner J, Burns MP, Villapol S. Traumatic brain injury in mice induces acute bacterial dysbiosis within the fecal microbiome. *Front Immunol*. 2018;9:2757.

164. Nicholson SE, Watts LT, Burmeister DM, et al. Moderate traumatic brain injury alters the gastrointestinal microbiome in a time-dependent manner. *Shock*. 2019; 52(2):240-248.

165. Zhu CS, Grandhi R, Patterson TT, Nicholson SE. A review of traumatic brain injury and the gut microbiome: Insights into novel mechanisms of secondary brain injury and promising targets for neuroprotection. *Brain Sci*. 2018;8(6):113.

166. Ma EL, Smith AD, Desai N, et al. Bidirectional brain-gut interactions and chronic pathological changes after traumatic brain injury in mice. *Brain Behav Immun*. 2017;66:56-69.

167. Urban RJ, Pyles RB, Stewart CJ, et al. Altered fecal microbiome years after traumatic brain injury. *J Neurotrauma*. 2020;37(8):1037-1051.

168. Hang CH, Shi JX, Li JS, Wu W, Yin HX. Alterations of intestinal mucosa structure and barrier function following traumatic brain injury in rats. *World J Gastroenterol*. 2003;9(12):2776-2781.

169. Bansal V, Costantini T, Kroll L, et al. Traumatic brain injury and intestinal dysfunction: uncovering the neuro-enteric axis. *J Neurotrauma*. 2009;26(8): 1353-1359.

170. Katzenberger RJ, Ganetzky B, Wassarman DA. The gut reaction to traumatic brain injury. *Fly*. 2015;9(2):68-74.

171. Kharrazian D. Traumatic brain injury and the effect on the brain-gut axis. *Altern Ther Health Med*. 2015;21(suppl 3):28-32.

172. Olsen AB, Hetz RA, Xue H, et al. Effects of traumatic brain injury on intestinal contractility. *Neuro Gastroenterol Motil*. 2013;25(7):e463-593.

173. Li H, Sun J, Du J, et al. Clostridium butyricum exerts a neuroprotective effect in a mouse model of traumatic brain injury via the gut-brain axis. *Neuro Gastroenterol Motil*. 2018;30(5):e13260.

174. Ma Y, Liu T, Fu J, et al. Lactobacillus acidophilus exerts neuroprotective effects in mice with traumatic brain injury. *J Nutr*. 2019;149(9):1543-1552.

175. Fridriksson T, Kini N, Walsh-Kelly C, Hennes H. Serum neuron-specific enolase as a predictor of intracranial lesions in children with head trauma: A pilot study. *Acad Emerg Med*. 2000;7(7):816-820.

176. Berger RP, Pierce MC, Wisniewski SR, Adelson PD, Kochanek PM. Serum S100B concentrations are increased after closed head injury in children: a preliminary study. *J Neurotrauma*. 2002;19(11):1405-1409.

177. Akhtar JI, Spear RM, Senac MO, Peterson BM, Diaz SM. Detection of traumatic brain injury with magnetic resonance imaging and S-100B protein in children, despite normal computed tomography of the brain. *Pediatr Crit Care Med*. 2003; 4(3):322-326.

178. Spinella PC, Dominguez T, Drott HR, et al. S-100beta protein-serum levels in healthy children and its association with outcome in pediatric traumatic brain injury. *Crit Care Med*. 2003;31(3):939-945.

179. Bandyopadhyay S, Hennes H, Gorelick MH, Wells RG, Walsh-Kelly CM. Serum neuron-specific enolase as a predictor of short-term outcome in children with closed traumatic brain injury. *Acad Emerg Med*. 2005;12(8): 732-738.

180. Berger RP, Adelson PD, Pierce MC, Dulani T, Cassidy LD, Kochanek PM. Serum neuron-specific enolase, S100B, and myelin basic protein concentrations after inflicted and noninflicted traumatic brain injury in children. *J Neurosurg Pediatr*. 2005;103(1):61-68.

181. Berger RP, Kochanek PM. Urinary S100B concentrations are increased after brain injury in children: A preliminary study. *Pediatr Crit Care Med*. 2006;7(6):557-561.

182. Berger RP, Dulani T, Adelson PD, Leventhal JM, Richichi R, Kochanek PM. Identification of inflicted traumatic brain injury in well-appearing infants using serum and cerebrospinal markers: A possible screening tool. *Pediatrics*. 2006; 117(2):325-332.

183. Beers SR, Berger RP, Adelson PD. Neurocognitive outcome and serum biomarkers in inflicted versus non-inflicted traumatic brain injury in young children. *J Neurotrauma*. 2007;24(1):97-105.

184. Haqqani AS, Hutchison JS, Ward R, Stanimirovic DB. Protein biomarkers in serum of pediatric patients with severe traumatic brain injury identified by ICAT–LC-MS/MS. *J Neurotrauma*. 2007;24(1):54-74.

185. Piazza O, Storti MP, Cotena S, et al. S100B is not a reliable prognostic index in paediatric TBI. *Pediatr Neurosurg*. 2007;43(4):258-264.

186. Geyer C, Ulrich A, Gräfe G, Stach B, Till H. Diagnostic value of S100B and neuron-specific enolase in mild pediatric traumatic brain injury: Clinical article. *J Neurosurg Pediatr*. 2009;4(4):339-344.

187. Castellani C, Bimbashi P, Ruttenstock E, Sacherer P, Stojakovic T, Weinberg A-M. Neuroprotein s-100B: A useful parameter in paediatric patients with mild traumatic brain injury? *Acta Paediatr*. 2009;98(10):1607-1612.

188. Bechtel K, Frasure S, Marshall C, Dziura J, Simpson C. Relationship of serum S100B levels and intracranial injury in children with closed head trauma. *Pediatrics*. 2009;124(4):e697-e704.

189. Berger RP, Bazaco MC, Wagner AK, Kochanek PM, Fabio A. *Trajectory Analysis of Serum Biomarker Concentrations Facilitates Outcome Prediction after Pediatric Traumatic and Hypoxemic Brain Injury*. Developmental Neuroscience; 2010.

190. Su E, Bell MJ, Wisniewski SR, Adelson PD, et al. α-Synuclein levels are elevated in cerebrospinal fluid following traumatic brain injury in infants and children: The effect of therapeutic hypothermia. *Dev Neurosci*. 2010;32(5-6):385-395.

191. Hallen M, Karlsson M, Carlhed R, Hallgren T, Bergenheim MS. 100B in serum and urine after traumatic head injury in children. *J Trauma*. 2010;69(2):284-289.

192. Fraser DD, Close TE, Rose KL, et al. Severe traumatic brain injury in children elevates glial fibrillary acidic protein in cerebrospinal fluid and serum. *Pediatr Crit Care Med*. 2011;12(3):319-324.

193. Babcock L, Byczkowski T, Mookerjee S, Bazarian JJ. Ability of S100B to predict severity and cranial CT results in children with TBI. *Brain Injury*. 2012;26(11): 1372-1380.

194. Berger RP, Hayes RL, Richichi R, Beers SR, Wang KKW. Serum concentrations of ubiquitin C-terminal hydrolase-L1 and αII-spectrin breakdown product 145 kDa correlate with outcome after pediatric TBI. *J Neurotrauma*. 2012;29(1): 162-167.

195. Su E, Bell MJ, Kochanek PM, et al. Increased CSF concentrations of myelin basic protein after tbi in infants and children: absence of significant effect of therapeutic hypothermia. *Neurocritical Care*. 2012;17(3):401-407.

196. Bouvier D, Fournier M, Dauphin JB, et al. Serum S100B determination in the management of pediatric mild traumatic brain injury. *Clin Chem*. 2012;58(7): 1116-1122.

197. Žurek J, Fedora M. The usefulness of S100B, NSE, GFAP, NF-H, secretagogin and Hsp70 as a predictive biomarker of outcome in children with traumatic brain injury. *Acta Neurochir*. 2012;154(1):93-103.

198. Studer M, Goeggel Simonetti B, Heinks T, et al. Acute S100B in serum is associated with cognitive symptoms and memory performance 4 months after paediatric mild traumatic brain injury. *Brain Injury*. 2015;29(13-14):1667-1673.

199. Papa L, Zonfrillo MR, Ramirez J, et al. Performance of glial fibrillary acidic protein in detecting traumatic intracranial lesions on computed tomography in children and youth with mild head trauma. *Acad Emerg Med*. 2015;22(11):1274-1282.

200. Mondello S, Kobeissy F, Vestri A, Hayes RL, Kochanek PM, Berger RP. Serum concentrations of ubiquitin C-terminal hydrolase-L1 and glial fibrillary acidic protein after pediatric traumatic brain injury. *Sci Rep*. 2016;6(1):28203.

201. Rhine T, Babcock L, Zhang N, Leach J, Wade SL. Are UCH-L1 and GFAP promising biomarkers for children with mild traumatic brain injury? *Brain Inj*. 2016;30(10):1231-1238.

202. Papa L, Mittal MK, Ramirez J, et al. In children and youth with mild and moderate traumatic brain injury, glial fibrillary acidic protein out-performs S100β in detecting traumatic intracranial lesions on computed tomography. *J Neurotrauma*. 2016;33(1):58-64.

203. Papa L, Mittal MK, Ramirez J, et al. Neuronal biomarker ubiquitin C-terminal hydrolase detects traumatic intracranial lesions on computed tomography in children and youth with mild traumatic brain injury. *J Neurotrauma*. 2017;34(13): 2132-2140.

204. Metzger RR, Sheng X, Niedzwecki CM, et al. Temporal response profiles of serum ubiquitin C-terminal hydrolase-L1 and the 145-kDa alpha II-spectrin breakdown product after severe traumatic brain injury in children. *J Neurosurg Pediatr*. 2018; 22(4):369-374.

205. Park S-H, Hwang S-K. Prognostic value of serum levels of S100 calcium-binding protein b, neuron-specific enolase, and interleukin-6 in pediatric patients with traumatic brain injury. *World Neurosurgery*. 2018;118:e534-e542.

206. Park D-W, Park S-H, Hwang S-K. Serial measurement of S100B and NSE in pediatric traumatic brain injury. *Child's Nerv Syst*. 2019;35(2):343-348.

207. Stukas S, Higgins V, Frndova H, et al. Characterisation of serum total tau following paediatric traumatic brain injury: a case-control study. *Lancet Child Adolesc Health*. 2019;3(8):558-567.

208. Yeung C, Bhatia R, Bhattarai B, Sinha M. Role of salivary biomarkers in predicting significant traumatic brain injury: An exploratory study. *Pediatr Emerg Care*. 2020. Publish Ahead of Print.

209. Whalen MJ, Carlos TM, Kochanek PM, et al. Interleukin-8 is increased in cerebrospinal fluid of children with severe head injury. *Crit Care Med*. 2000;28(4):929-934.

210. Clark RSB, Kochanek PM, Adelson PD, et al. Increases in bcl-2 protein in cerebrospinal fluid and evidence for programmed cell death in infants and children after severe traumatic brain injury. *J Pediatr*. 2000;137(2):197-204.

211. Lai Y, Kochanek PM, Adelson PD, Janesko K, Ruppel RA, Clark RSB. Induction of the stress response after inflicted and non-inflicted traumatic brain injury in infants and children. *J Neurotrauma*. 2004;21(3):229-237.

212. Shore PM, Jackson EK, Wisniewski SR, Clark RSB, Adelson PD, Kochanek PM. Vascular endothelial growth factor is increased in cerebrospinal fluid after traumatic brain injury in infants and children. *Neurosurgery*. 2004;54(3):605-612. discussion 611-612.

213. Satchell MA, Lai Y, Kochanek PM, et al. Cytochrome c, a biomarker of apoptosis, is increased in cerebrospinal fluid from infants with inflicted brain injury from child abuse. *J Cerebr Blood Flow Metabol*. 2005;25(7):919-927.

214. Cousar JL, Lai Y, Marco CD, et al. Heme oxygenase 1 in cerebrospinal fluid from infants and children after severe traumatic brain injury. *Dev Neurosci*. 2006;28(4-5): 342-347.

215. Lai Y, Stange C, Wisniewski SR, et al. Mitochondrial heat shock protein 60 is increased in cerebrospinal fluid following pediatric traumatic brain injury. *Dev Neurosci*. 2006;28(4-5):336-341.

216. Briassoulis G, Mavrikiou M, Margeli A, et al. Circulating levels of KL-6 in acute respiratory distress syndrome sepsis or traumatic brain injury in critically ill children. *Pediatr Pulmonol*. 2006;41(8):790-795.

217. Briassoulis G, Papassotiriou I, Mavrikiou M, Lazaropoulou C, Margeli A. Longitudinal course and clinical significance of TGF-β1, sL- and sE-Selectins and sICAM-1 levels during severe acute stress in children. *Clin Biochem*. 2007;40(5-6): 299-304.

218. Chiaretti A, Antonelli A, Mastrangelo A, et al. Interleukin-6 and nerve growth factor upregulation correlates with improved outcome in children with severe traumatic brain injury. *J Neurotrauma*. 2008;25(3):225-234.

219. Chiaretti A, Antonelli A, Genovese O, et al. Nerve growth factor and doublecortin expression correlates with improved outcome in children with severe traumatic brain injury. *J Trauma*. 2008;65(1):80-85.

220. Chiaretti A, Antonelli A, Riccardi R, et al. Nerve growth factor expression correlates with severity and outcome of traumatic brain injury in children. *Eur J Paediatr Neurol*. 2008;12(3):195-204.

221. Chiaretti A, Barone G, Riccardi R, et al. NGF, DCX, and NSE upregulation correlates with severity and outcome of head trauma in children. *Neurology*. 2009; 72(7):609-616.

222. Sanchez de Toledo J, Adelson PD, Watson RS, et al. Relationship between increases in pancreatic enzymes and cerebral events in children after traumatic brain injury. *Neurocritical Care*. 2009;11(3):322-329.

223. Berger RP, Ta'asan S, Rand A, Lokshin A, Kochanek P. Multiplex assessment of serum biomarker concentrations in well-appearing children with inflicted traumatic brain injury. *Pediatr Res*. 2009;65(1):97-102.

224. Swanson CA, Burns JC, Peterson BM. Low plasma d-dimer concentration predicts the absence of traumatic brain injury in children. *J Trauma Inj Infect Crit Care*. 2010;68(5):1072-1077.

225. Chang TP, Nager AL. Pediatric traumatic brain injury: the utility of beta-natriuretic peptide. *J Trauma*. 2010;68(6):1401-1405.

226. Au AK, Aneja RK, Bell MJ, et al. Cerebrospinal fluid levels of high-mobility group box 1 and cytochrome C predict outcome after pediatric traumatic brain Injury. *J Neurotrauma*. 2012;29(11):2013-2021.

227. Fitrolaki D-M, Dimitriou H, Kalmanti M, Briassoulis G. CD64-Neutrophil expression and stress metabolic patterns in early sepsis and severe traumatic brain injury in children. *BMC Pediatr*. 2013;13(1).

228. Lin C, Wang N, Shen Z-P, Zhao Z-Y. Plasma copeptin concentration and outcome after pediatric traumatic brain injury. *Peptides*. 2013;42:43-47.

229. Foaud H, Labib JR, Metwally H, El-Twab K. Plasma D-dimer as a prognostic marker in ICU admitted egyptian children with traumatic brain injury. *J Clin Diagn Res*. 2014;8(9):PC01-PC05.

230. Walko TD, Bola RA, Hong JD, et al. Cerebrospinal fluid mitochondrial DNA: A novel DAMP in pediatric traumatic brain injury. *Shock*. 2014;41(6):499-503.

231. Au AK, Aneja RK, Bayır H, et al. Autophagy biomarkers beclin 1 and p62 are increased in cerebrospinal fluid after traumatic brain injury. *Neurocritical Care*. 2017;26(3):348-355.

232. Hicks SD, Johnson J, Carney MC, et al. Overlapping microRNA expression in saliva and cerebrospinal fluid accurately identifies pediatric traumatic brain injury. *J Neurotrauma*. 2018;35(1):64-72.

233. Luo H-C, Fu Y-Q, You C-Y, Liu C-J, Xu F. Comparison of admission serum albumin and hemoglobin as predictors of outcome in children with moderate to severe traumatic brain injury: A retrospective study. *Medicine*. 2019;98(44): e17806.

234. Gao N, Zhang-Brotzge X, Wali B, et al. Plasma osteopontin may predict neuroinflammation and the severity of pediatric traumatic brain injury. *J Cerebr Blood Flow Metab*. 2020;40(1):35-43.

235. Lele AV, Alunpipatthanachai B, Qiu Q, et al. Plasma levels, temporal trends and clinical associations between biomarkers of inflammation and vascular homeostasis after pediatric traumatic brain injury. *Dev Neurosci*. 2019;41(3-4):177-192.

236. Lele AV, Alunpipatthanachai B, Clark-Bell C, et al. Cardiac-cerebral-renal associations in pediatric traumatic brain injury: Preliminary findings. *J Clin Neurosci*. 2020;76:126-133.