Serum biomarkers in severe paediatric traumatic brain injury—a narrative review

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Abstract: Severe traumatic brain injury continues to present complex management and prediction challenges for the clinician. While there is some evidence that better systems of care can improve outcome, multiple multi-centre randomised controlled trials of specific therapies have consistently failed to show benefit. In addition, clinicians are challenged in attempting to accurately predict which children will recover well and which children will have severe and persisting neurocognitive deficits. Traumatic brain injury is vastly heterogeneous and so it is not surprising that one therapy or approach, when applied to a mixed cohort of children in a clinical trial setting, has yielded disappointing results. Children with severe traumatic brain injury have vastly different brain injury pathologies of widely varying severity, in any number of anatomical locations at what may be disparate stages of brain development. This heterogeneity may also explain why clinicians are unable to accurately predict outcome. Biomarkers are objective molecular signatures of injury that are released following traumatic brain injury and may represent a way of unifying the heterogeneity of traumatic brain injury into a single biosignature. Biomarkers hold promise to diagnose brain injury severity, guide intervention selection for clinical trials, or provide vital prognostic information so that early intervention and rehabilitation can be planned much earlier in the course of a child's recovery. Serum S100B and serum NSE levels show promise as a diagnostic tool with biomarker levels significantly higher in children with severe TBI including children with inflicted and non-inflicted head injury. Serum S100B and serum NSE also show promise as a predictor of neurodevelopmental outcome. The role of biomarkers in traumatic brain injury is an evolving field with the potential for clinical application within the next few years.

Keywords: Biomarkers; brain injuries; Glasgow outcome score; intensive care unit; pediatrics

Introduction

Traumatic brain injury is the commonest cause of death and disability amongst children greater than one year of age (1) and continues to present complex management and prediction challenges for the clinician. Traumatic brain injury is commonly classified into mild [Glasgow Coma Score (GCS) 13–15], moderate (GCS 9–12) or severe (GCS 3–8) based on the GCS. TBI should not be viewed as a single pathophysiological event, but a cascade that involves two separate injury phases, primary and secondary (2). Primary brain injury occurs at the time of the initial impact and results directly from forces generated causing injury to the physical structures of the brain. The physical structures of the brain include neurons, neural stem cells, glial cells and blood vessels. A typical neuron consists of a cell body, axon and dendrites and is generated by neural stem cells during childhood. Glial cells are non-neuronal cells that

maintain homeostasis, form myelin and provide support and protection for neurons. In the central nervous system, glial cells include oligodendrocytes, astrocytes, ependymal cells and microglia.

The physical structures of the brain are closely protected and shielded from the systemic compartment by two separate barriers, the blood-brain barrier and the blood-cerebrospinal barrier. Therefore, under normal circumstances, proteins originating from neurons and glial cells are rarely detectable in blood. If there is disruption to either or both of these barriers following trauma, proteins may extravasate across the disrupted barriers and result in leakage into the blood or CSF following impact.

Secondary brain injury is the result of a pathophysiologic cascade of events that occurs in the seconds, minutes, and days to weeks following the primary brain injury (3). There are two distinct types of secondary brain injury. The first is the endogenous cellular, biochemical or molecular cascades that are released in the secondary injury (or repair) response and are associated with (I) ischaemia and energy failure; (II) excitotoxicity; (III) inflammation; (IV) direct tissue disruption; and (V) axonal injury (3).

The second, occurs in parallel and results from secondary insults in critically ill children in the field, emergency room or paediatric intensive care unit. These secondary insults produce adverse consequences on a central nervous system that is at increased vulnerability after TBI. The primary goal of neurointensive care is the prevention of factors that promote this form of secondary brain injury such as hypoxia, hypotension, intracranial hypertension, hypercarbia, hyperglycaemia or hypoglycaemia, electrolyte abnormalities, seizures and hyperthermia (4). Children with severe TBI still present complex management and outcome prediction challenges for the paediatric intensivist, despite decades of investigation.

Current management & why TBI trials fail

Current principles for the management of children with severe TBI are contained in "Guidelines for the Management of Pediatric Severe Traumatic Brain Injury" published by the Brain Trauma Foundation (5). These evidence-based guidelines review all of the key goals and therapies. They were first published in 2003 (6) and subsequently updated in 2012 (7) and 2019 (5). The level of evidence informing these guidelines remains low. Of the 22 recommendations, there are none based on high quality evidence, only 3 based on moderate quality evidence (of

which two are therapies to be avoided) and the remaining 19 are based on low quality evidence. The three largest randomised controlled trials for children with severe TBI were all performed to test the utility of early, prophylactic therapeutic hypothermia. All three failed to demonstrate a positive impact on the primary outcome (8-10).

In comparison to other fields of medicine, clinical trials in severe TBI pose complicated methodological challenges. Severe TBI is not one single disease entity, against which, one therapy or approach can be easily tested. This vastly heterogeneous environment will vary on a case by case basis that is dependent upon competing risk factors or co-morbidities, a complex spectrum of brain injury pathologies, anatomical location, stage of brain development and maturity, widely varying severity of injury, and complicating factors such as extra-cranial injuries (causing additional hypoxia and hypotension) sustained during the traumatic event. It is, therefore, not surprising that one therapy or approach, when applied to a mixed cohort of children in a clinical trial setting, has yielded disappointing results.

A new path for brain injury research whereby children can be reliably stratified and selected for clinical trials, or provide vital prognostic information so that timely intervention and rehabilitation can be planned, is urgently required.

Biomarkers

A biomarker is 'a defined characteristic that is measured as an indicator of normal biological processes, pathogenic processes or responses to an exposure or intervention' (11). Thus, biomarkers are objective molecular signatures of markers of injury that are released into the bloodstream following traumatic brain injury and may represent a way of unifying the heterogeneity of TBI into a single biosignature.

In TBI, biomarkers can be used in one of two different ways. Firstly, biomarkers can be diagnostic. The FDA defines a diagnostic biomarker as "a biomarker that detects or confirms the presence of a disease or condition of interest, or identifies an individual with a subtype of disease (12)". In TBI, diagnostic biomarkers would have the potential to (I) diagnose children with TBI, including inflicted TBI, where a history of trauma may not be forthcoming; (II) discriminate between mild, moderate and severe TBI (III); reduce the need for ongoing neuroimaging; (IV) guide intervention selection and timing for clinical trials and ultimately (V) match therapy to pathophysiological patterns or biosignatures. Secondly, biomarkers can be prognostic.

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The FDA defines a prognostic biomarker as "a biomarker that is used to identify the likelihood of a clinical event, disease recurrence or disease progression in patients with a disease or medical condition of interest (12). In severe TBI, prognostic biomarkers would have the potential to (I) identify children at risk of neurocognitive deficits so that early intervention and rehabilitation can be planned much earlier in the course of a child's recovery or (II) identify children with a high likelihood of dying so that families can be counselled and prepared for a devastating outcome.

Brain biomarkers have attracted increasing attention with more than 90 different biomarkers studied in paediatric patients with TBI to date (13). Papa *et al*. (13) state that the ideal biomarker for traumatic brain injury would (I) have a high sensitivity and specificity for brain injury; (II) help stratify patients by severity of injury; (III) have a rapid appearance in accessible biological fluid; (IV) provide information about injury mechanisms; (V) have well-defined biokinetic properties; (VI) monitor progress of disease and response to treatment and (VII) predict functional outcomes. Biomarkers in traumatic brain injury may be sampled from blood, cerebrospinal fluid, brain interstitial fluid collected during brain microdialysis and most recently, saliva. Preliminary studies have shown that there is a correlation between salivary and serum S100B (14,15). The greatest contribution to the peripheral signal most likely comes from those brain cell biomarkers derived from the brain interstitial fluid and cerebrospinal fluid. Clinical studies have appropriately targeted serum biomarkers which are readily available in all children. For example, cerebrospinal fluid may be accessible in those children with an extra ventricular drain. However, less than 10% of children will have an extra ventricular drain inserted (13). Cerebral microdialysis and salivary biomarkers are still largely research tools. For the purposes of this review, we will focus on serum biomarkers that have received the most attention in paediatric clinical studies of severe TBI.

Types of biomarkers

There are many ways to classify biomarkers—the approach taken in this article is to classify brain injury biomarkers based on the primary and secondary brain injury mechanisms.

Primary brain injury: biomarkers of structural damage

Biomarkers are released in response to damage to neurons and glial cells during primary brain injury. The processes covered by these biomarkers thus far includes dendritic, neuronal cell body and axonal injury, demyelination and astroglial injury and responses.

S100B

S100 calcium binding protein B, or S100B, is a protein of the S100 family and is a low molecular weight (9–13 kDa), calcium binding protein. It is the most investigated brain injury biomarker in children to date (13). S100B is localised predominantly in astrocytic glial cells of the central nervous system and is specifically found in the cytoplasm and nucleus and thus will be present in brain interstitial fluid and cerebrospinal fluid. Small amounts of S100B are also found in peripheral locations including fat, skin, skeletal muscle, bone marrow (16) and abdominal organs (17). The biological half life is 2 hours and the elimination half-life is 30 minutes (18). There is a sharp rise in S100B levels within minutes after injury and levels decline rapidly over the next few hours and usually normalise within 24 hours (18).

Diagnostic Uses: There are 10 studies that have examined the diagnostic utility of S100B (19-28) in paediatric TBI (*Table 1*). S100B levels are significantly higher in children with TBI compared with healthy matched controls (19), higher in severe TBI compared with mild TBI (23) and higher in children with a lower presenting GCS (28). All but one study (24) found that S100B levels discriminated TBI severity.

Two studies examined S100B levels in children with inflicted and non-inflicted TBI (21,22). S100B levels were higher when the two brain injury groups were combined, but S100B levels could not discriminate between inflicted TBI (iTIB) and non-inflicted TBI (nTBI). Both studies showed that there was a delayed peak in S100B levels in infants with iTBI.

S100B levels are significantly higher in children with an abnormal CT scan (25-27) but the predictive power of S100B for an abnormal CT scan is variable with AUC of 0.67 (95% CI: 0.55–0.80) (25), 0.71 (95% CI: 0.58–0.81) (27) and 0.93 (95% CI: 0.873–0.987) (26). An MRI study showed that S100B levels could not discriminate between children with normal and abnormal scans (20).

Prognostic uses: There are 8 studies that have examined the predictive utility of S100B (24,28-34) and all found associations with various outcome measures (*Table 1*). A high S100B level at admission was associated with poor outcomes at hospital discharge (34). Outcome at 6 months post-injury, assessed using the Glasgow Outcome Score,

Table 1 S100B

Author	Year published	Study	Number of children	Biofluid & timing	Main findings
Park *et al.* (28)	2019	Prospective, observational study	15 children with TBI (9 in poor group GCS <9 and 6 in good group GCS 10–15)	Serum S100B (& NSE) within 6 hours of admission and one week after trauma	Diagnostic: high S100B level at admission & 1 week associated with poor GCS group
				Outcome assessed with GOS at 6 months post injury	Prognostic: higher S100B levels at 1 week levels associated with unfavourable outcome (GOS) (P=0.009)
Meshcheryakov *et al.* (29)	2018	Retrospective study	169 children with severe TBI (65 children had NSE samples)	Serum S100B on days 1–3, 6–8, 14–15 and 20–23	Diagnostic: not assessed
					Prognostic: higher S100B levels in children who died compared to those that survived (P=0.038); did not discriminate amongst survivors with a good or poor outcome (GOS)
Wilkinson *et al.* (30)	2016	Prospective, observational study	58 children with mild/moderate/severe TBI	Serum S100B (& NSE) taken daily until arterial line removed	Diagnostic: not assessed
					Prognostic: highest serum S100B level predictive of inattention at 1 year post injury (AUC 0.7); paired baseline level of inattention with trough serum level of S100B (AUC 0.94; P=0.036)
Babcock *et al.* (27)	2012	Secondary analysis of a prospective cohort	Of 679 children in TBI registry with mild/moderate/severe TBI	Serum S100B at admission and CT head	Diagnostic: S100B levels higher in children with abnormal CT (P=0.003) The AUC for predicting an abnormal CT scan was 0.71 (95% CI: 0.58–0.81)
			155 had a serum S100B level and 109 had both S100B and CT head performed		Prognostic: not assessed
Žurek *et al.* (31)	2012	Prospective, observational study	63 children with mild/moderate/severe TBI	Serum S100B, (& NSE, GFAP) at admission and every 24 hours for 6 days	Diagnostic: not assessed
				Outcome measured with GOS at 6 months post injury	Prognostic: Day 0 levels predicted worse outcome or death (P<0.001)
Hallén *et al.* (26)	2010	Prospective, observational study	111 children with mild/moderate/severe TBI (group 1 = 105 with no CT indicated or normal; group 2 = 6 with abnormal CT head	Serum S100B at admission and 6 hours later	Diagnostic: S100B levels higher in group 2 compared with group 1 at admission (P<0.001) and 6 hours after injury (P=0.016)
					At a cut-off value of 0.195 ug/L, AUC 0.93 with 100% sensitivity and 88% specificity for abnormal CT
					Prognostic: not assessed

Table 1 (*continued*)

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Table 1 (*continued*)

Author	Year published	Study	Number of children	Biofluid & timing	Main findings
Bechtel *et al.* (25)	2009	Prospective observational study	152 children with mild/moderate/severe TBI including 24 with ICI and 128 without ICI on CT head	Serum S100B within 6 hours after injury	Diagnostic: Serum S100B levels were greater in children with ICI compared with those that did not (P<0.001) AUC to detect ICH was 0.67 (95% CI: 0.55–0.8)
					Prognostic: not assessed
Lo *et al.* (24)	2009	Prospective, observational study	28 children with severe TBI (GCS <8) & non severe TBI (GCS >8)	Serum S100B (& 7 other biomarkers) at 24 h post injury	Diagnostic: Not elevated in children with severe TBI; did not discriminate between diffuse and focal brain injury
				Outcome assessed with GOS at 6 months after injury	Prognostic: High S100B levels on day 1 predicted worse outcome (P=0.015)
					Paired with NSE/L-selectin/IL-6 had an AUC 0.97/0.98/0.98 and 100% sensitivity for all and specificity 92%/96%/96%
Piazza *et al.* (23)	2007	Prospective, observational study	15 children with mild/moderate/severe TBI	Serum S100B at admission at after 48 hours	Diagnostic: Higher S100B levels in all children with TBI; levels higher in severe TBI compared with mild TBI
				Outcome assessed at 6 months post injury with GOS	Predictive: could not be assessed as all patients had a good outcome
Beers *et al.* (32)	2007	Prospective, cross-sectional study	15 children with nTBI, 15 children with iTBI (mild/moderate/severe)	Serum S100B (NSE (&MBP) at admission, 12–24 hours after injury and daily for 5 days in severe TBI	Diagnostic: not assessed
				Outcome assessed with neurocognitive testing (GOS/VABS/IQ) at 6 months post injury	Predictive: Time to peak S100B level significantly correlated with VABS score and IQ
Berger *et al.* (33)	2007	Prospective, case-control study	152 children with mild/moderate/severe TBI	Serum S100B (NSE & MBP) at arrival, 12–24 hours after injury and daily for 5 days in severe TBI	Diagnostic: not assessed
				Outcome assessed with GOS score at varying time points in first 12 months after injury	Predictive: Peak S100B level associated with worse outcome at all time points; Initial S100B level associated with worse outcome at all time points except for at 7–12 months post injury
Berger *et al.* (22)	2006	Prospective, case-control study	14 infants (<1 year) with inflicted TBI; 74 healthy control	Serum S100B (NSE & MBP) at admission	Diagnostic: S100B not sensitive or specific for children with iTBI
					Prognostic: not assessed

Table 1 (*continued*)

Table 1 (*continued*)

Author	Year published	Study	Number of children	Biofluid & timing	Main findings
Berger *et al.* (21)	2005	Prospective, case-control study	56 children with nTBI, 44 with iTBI and 64 controls	Serum S100B (NSE & MBP) at admission, 12–24 hours after injury and daily for 5 days in severe TBI	Diagnostic: Initial median serum S100B significantly higher in children with both forms of TBI compared with controls
					No difference in initial or peak S100B levels between children with nTBI and iTBI; delayed peak in S100B in iTBI compared with controls
					Ability of initial S100B level to diagnose TBI showed AUC 0.82 with 77% sensitivity and 72% specificity with a cut-off level of 0.017 ng/mL
					Prognostic: not assessed
Spinella *et al.* (34)	2003	Prospective, cohort study	27 children with mild/moderate/ severe TBI and 136 controls	S100B within 12 hours of injury & PCPC at hospital discharge	Diagnostic: not assessed
					Predictive: S100B levels predicted good versus poor outcome at hospital discharge & 6 months post injury
					A S100B level >2.0 ug/L predicted poor outcome at 6 months with sensitivity 86% and specificity 95%. The ROC AUC was 0.94 (+0.05)
Akhtar *et al.* (20)	2003	Prospective cohort study	17 children with mild/moderate/ severe TBI with normal CT head	Serum S100B at 6 hours and 12 hours post injury and brain MRI within 96 hours following injury	Diagnostic: There were no differences in mean S100B level between children with an abnormal MRI (n=7) and normal MRI (n=10)
					The mean S100B level was higher at both time points in children with head and body trauma compared with head alone (P=0.018 at t1 and P=0.025 at t2)
					Prognostic: not assessed
Berger *et al.* (19)	2002	Prospective, observational study with controls	45 children with mild (n=27); moderate (n=6) or severe (n=12) TBI including 2 with iTBI; 16 controls	Serum S100B collected on arrival to hospital and every 12 hours for 5 days	Diagnostic: Higher mean S100b levels in patients with TBI compared with healthy controls (P=0.008).
					S100B levels did not discriminate between degrees of brain injury severity, abnormal CT scan or clinical features of vomiting, loss of consciousness or post-traumatic seizures
					Prognostic: not assessed

Scientific literature (total papers = 16; diagnostic papers =10 and prognostic papers =8).

is associated with S100B levels at admission (24,31,33,34) and at one week following injury (28). Time to peak S100B levels was associated with neurocognitive outcome (32). Two other studies found that admission levels were associated with poor outcome at 12 months post-injury (30,33) and one found that admission S100B levels could discriminate between those that survived and those that died (29).

Limitations: The use of S100B is limited by its lack of specificity as extra-cerebral sources produce increases in levels of S100B in the setting of haemorrhagic shock, circulatory arrest or during cardiopulmonary bypass (35). The short half-life of S100B is also a limiting factor in acute TBI. Finally, S100B is not a useful marker in children less than 2 years of age due to high normative values in that age group (35-37).

Summary: S100B levels are significantly higher in children with TBI including children with inflicted and non-inflicted injury and there is limited evidence that higher levels are associated with increasing severity of TBI. S100B levels are not able to discriminate between inflicted and non-inflicted TBI but there is a longer time to peak S100B levels in infants with inflicted injury. This may reflect severity of the primary injury or increased secondary injury due to a delay in seeking medical attention. S100B levels are poor predictors of abnormal neuroimaging (CT and MRI). S100B levels shows promise as a predictor of outcome (GOS) at 6 months and 12 months after injury.

Neuron specific enolase

Enolases are glycolytic enzymes comprising three different subunits (α, β and γ). Neuron-specific enolase (NSE) is a 78 kDa dimeric, γ-isoenzyme that is located in the cytoplasm of central and peripheral neurons and neuroendocrine cells (38,39). NSE can be detected in the blood within 6 hours after injury (36) and has a serum half-life of 24 hours (28,36).

Diagnostic Uses: There are 5 studies that have examined the diagnostic utility of serum NSE (21,22,24,28,40) (*Table 2*). Three studies showed diagnostic potential with stratification of TBI injury (24,28,40). Serum NSE levels at admission and one week after injury were significantly higher in children with GCS <9 (poor group) compared with children with a GCS 10–15 (good group) (28). Similarly, children with a GCS <12 (poor group) had significantly higher mean levels of NSE when compared with children with a GCS >12 (good group) although higher levels were not associated with abnormalities on CT scan (40). One study found that NSE levels were two times higher in children with diffuse injury on CT scan compared to children with focal injuries (P=0.02) (24).

There are 2 studies that assessed NSE levels in children with inflicted versus non-inflicted TBI (21,22). Initial NSE levels were significantly higher in children when the two brain injury groups were combined and predicted TBI with an AUC 0.85 with 71% sensitivity and 64% specificity by using an NSE concentration cut-off 11.36 ng/mL (21). However initial and peak NSE levels could not discriminate between iTBI and nTBI although the peak NSE level was significantly delayed in infants with iTBI (22). The second, smaller study used the same NSE concentration cut-off to achieve a sensitivity of 77% and specificity of 66% (21).

Prognostic uses: There are 9 studies that have examined the predictive value of NSE for outcome (24,28-33,41,42) (*Table 2*). NSE levels taken within 24 hours of arrival to hospital were predictive of worse outcome as measured on the GOS at hospital discharge in one study (42), at 6 months in 4 studies (24,28,31,32) and at varying time points in the first year in another study (33). NSE levels taken within 24 hours of injury were predictive of attention related difficulties at 1 year following injury (30) and attention related and executive functioning problems at a median time of 3 years following injury (41). One study predicted children that survived compared to those that died but could not discriminate good or worse outcomes amongst survivors (29).

Limitations: The use of NSE is limited by the abundance of NSE expressed in red blood cells and platelets making the process of haemolysis a significant source of cross-contamination when measured in trauma (43). Another limitation is the slow elimination from plasma leading to difficulties in distinguishing between primary and secondary insults to the brain.

Conclusion: Serum NSE levels are significantly higher in children with TBI including children with inflicted and non-inflicted injury and there is evidence that higher levels are associated with increasing severity of TBI. There is mixed evidence that NSE levels are associated with abnormal CT findings. The longer time to peak NSE levels in infants with inflicted injury may reflect severity of the primary injury or increased secondary injury due to a delay in seeking medical care. NSE shows promise as a predictor of outcome (GOS) at hospital discharge, 6 months and 12 months following injury with prediction of short- and long-term attention-related and executive functioning problems.

Glial fibrillary acidic protein (GFAP)

GFAP is an astrocyte specific cytoskeleton protein and is a well established marker of glial damage in severe TBI (43).

Table 2 Neuron specific enolase

Author	Year published	Study	Number of Children	Biofluid & Timing	Main Findings
Park et al. (28)	2019	Prospective observational study	15 children with TBI (9 in poor group GCS <9 and 6 in good group GCS 10-15)	Serum NSE (& S100B) at admission and one week after trauma	Diagnostic: high level at admission & 1 week associated with poor GCS group (GCS <9)
					Prognostic: levels at one week after injury associated with unfavourable outcome (GOS) levels (P=0.009)
Meshcheryakov et al. (29)	2018	Retrospective study	169 children with severe TBI (43 children had NSE samples)	Serum NSE on days 1–3, 6–8, 14–15 and 20–23	Diagnostic: not assessed
					Prognostic: higher NSE levels in children who died compared to those that survived (P=0.038); did not discriminate amongst survivors with a good or poor outcome (GOS); did not state time NSE level taken
Wilkinson et al. (41)	2017	Prospective, observational study	23 children with mild/moderate/ severe TBI who completed follow up (85 children in original sample)	Serum NSE (& S100B & neuron cell adhesion molecular) at time of injury and daily for 2 weeks	Diagnostic: not assessed
					Prognostic: Higher levels of NSE associated with higher scores on the inattention, hyperactivity/impulsivity and executive functioning scales of Connors-3 and working memory; higher levels of NSE only associated with higher scores on the inhibit scale of the BRI
Wilkinson et al. (30)	2016	Prospective, observational study	58 children with mild/moderate/ severe TBI	Serum NSE (& S100B)	Diagnostic: not assessed
					Prognostic: highest serum NSE level predictive of inattention at 1 year post injury (AUC 0.72); paired high baseline levels of inattention and NSE level was the best predictor of inattention at 1 year
Žurek et al. (31)	2012	Prospective, observational study	63 children with mild/moderate/ severe TBI	Serum NSE (& S100B, GFAP) at admission and every 24 hours for 6 days	Diagnostic: not assessed
				Outcome measured with GOS at 6 months post injury	Prognostic: Higher NSE levels predicted worse outcomes (GOS) or death (P=0.02)
Lo et al. (24)	2009	Prospective, observational study	28 children with severe TBI (GCS <8) & non severe TBI (GCS >8)	Serum NSE (& 7 other biomarkers) at 24 h post injury	Diagnostic: median NSE levels two times higher in those with diffuse injury compared with focal injury (P=0.01)
					Prognostic: High NSE levels on Day 1 predicted worse outcome at 6 months (P=0.03)

Table 2 (*continued*)

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Table 2 (*continued*)

Author	Year published	Study	Number of Children	Biofluid & Timing	Main Findings
Beers et al. (32)	2007	Prospective, cross-sectional study	15 children with nTBI, 15 children with iTBI mild/ moderate/severe)	Serum NSE (& S100B & MBP) at admission, 12–24 hours after injury and daily for 5 days in severe TBI	Diagnostic: not assessed
				Outcome assessed with neurocognitive (GOS/VABS/IQ) testing at 6 months post injury	Predictive: initial, time to peak & peak NSE were associated with 6-month GOS & IQ; time to peak NSE higher in iTBI vs. nTBI; time to peak NSE and peak NSE were correlated with the VABS Composite score
Berger et al. (33)	2007	Prospective, case-control study	152 children with mild/ moderate/severe TBI	Serum NSE (& S100B & MBP) at arrival, 12–24 hours after injury and daily for 5 days in severe TBI	Diagnostic: not assessed
				Outcome assessed with GOS score at varying time points in first 12 months after injury	Predictive: Peak NSE level associated with worse outcome at all time points; Initial NSE level associated with worse outcomes in first 6 months after injury; initial & peak NSE levels more strongly correlated with outcome in children <4 years
Berger et al. (22)	2005	Prospective, case-control study	14 infants (<1 year) with inflicted TBI; 74 healthy control	Serum NSE (and S100B & MBP) at admission	Diagnostic: 76% sensitive & 66% specific for diagnosing iTBI
					Prognostic: not assessed
Berger et al. (21)	2005	Prospective, case-control study	56 children with nTBI, 44 with iTBI and 64 controls	Serum NSE (and S100B & MBP) at admission, 12–24 hours after injury and daily for 5 days in severe TBI	Diagnostic: Initial median serum NSE significantly higher in children with both forms of TBI compared with controls
					No difference in initial or peak NSE levels between children with nTBI and iTBI; delayed peak in S100B in iTBI compared with controls
					Ability of initial NSE level to diagnose TBI showed AUC 0.85 with 71% sensitivity and 64% specificity with a cut-off NSE level of 11.36 ng/mL

Table 2 (*continued*)

Table 2 (*continued*)

Author	Year published	Study	Number of Children	Biofluid & Timing	Main Findings
Bandyopadhyay et al. (42)	2005	Retrospective analysis of a prospective cohort	86 children with TBI (10 with GCS <13)	Serum NSE at arrival to ED and within 24 hours of injury	Diagnostic: not assessed Prognostic: Higher mean NSE levels in children with poor outcome (GOS) at hospital discharge; AUC 0.83 for discriminating good vs. poor outcome; at a cut-off value of 21.2, NSE was 86% sensitive and 74% specific in predicting poor outcome
Fridriksson et al. (40)	2000	Prospective, observational study	50 children with blunt head trauma; 22 with an abnormal CT head	Serum NSE at arrival to hospital	Diagnostic: Mean NSE levels were significantly higher when GCS <12 (36.6 ng/ml vs. 18.4 ng/mL); mean levels were not different between those with abnormal CT findings compared with those with a normal CT head Predictive: not assessed

Scientific literature (total papers =12; diagnostic papers = 5; prognostic papers =9).

GFAP biomarker levels are elevated within 3–34 hours in serum/plasma following severe TBI (16).

Diagnostic Uses: There are 3 studies that have examined the diagnostic utility of GFAP (31,44,45) (*Table 3*). In one case-control study, serum GFAP levels were significantly higher in children with TBI compared to healthy controls (44). Serum GFAP concentrations measured on arrival could discriminate between mild and severe TBI in one study (44) but were unable to discriminate TBI severity in two studies (31,45). Serum GFAP on arrival also failed to correlate with physiological variables, highest intracranial pressure or with indices of injury measured on CT head (45).

Prognostic uses: There are 3 studies that have examined the prognostic utility of GFAP (31,44,45) (*Table 3*). Serum GFAP concentrations measured on arrival were associated with worse outcomes at 6 months post injury (31,44,45).

Summary: The evidence supporting the use of serum GFAP as a diagnostic tool is limited but shows promise in the prediction of outcomes at 6 months after injury.

Myelin basic protein

Myelin basic protein is an oligodendrocyte protein and is a critical constituent of the insulating myelin sheath covering axons. Myelin basic protein maintains the correct structure of myelin, interacting with the lipids in the myelin membrane (46). Though also present in the peripheral nervous system, MBP exists predominantly in the central nervous system. The process of myelination is age-dependent and progresses from infancy into adolescence and degrees of myelinated axonal injury may thus differ in relation to patient age (47). Serum MBP concentrations peak 48 to 72 hours after injury and remain increased for up to 2 weeks (48).

Diagnostic Uses: Two studies have examined the diagnostic utility of MBP (21,22) (*Table 4*). Initial serum MBP levels were not different between inflicted and non-inflicted TBI (22) and were not able to discriminate between inflicted and non-inflicted TBI or controls (21). One study showed that peak MBP levels were higher in children with an intracranial haemorrhage on CT scan compared with children without intracranial haemorrhage and controls when these two groups were analysed together (21). There was a delay in peak MBP levels in children with iTBI when compared to children with nTBI (21)

Prognostic uses: There are 2 studies that have examined the prognostic utility of MBP (32,33) (*Table 4*). One found that a high peak MBP level was predictive of worse neurocognitive outcome (32) at 6 months post injury and

Table 3 Glial fibrillary acidic protein

Author	Year published	Study	Number of children	Biofluid & timing	Main findings
Mondello *et al*. (44)	2016	Case-Control	45 cases/40 healthy controls	Serum GFAP (and UCH-L1) on arrival to hospital	Diagnostic: increasing severity of TBI & the possible identification of brain injury not detected on CT
			Mild/moderate/ severe TBI		Predictive: predict outcome at 6 months (GOS)
Žurek *et al*. (31)	2012	Prospective, observational	63 children mild/ moderate/severe TBI	Blood GFAP levels at admission and daily for 6 days.	Diagnostic: levels were not diagnostic of brain injury severity
				Outcome measured with GOS at 6 months post injury	Prognostic: higher GFAP levels predicted worse outcomes (GOS) or death (P=0.002)
Fraser *et al*. (45)	2011		27 children with severe TBI	Serum GFAP at admission and daily until arterial line removed.	Diagnostic: did not correlate with clinical symptoms, physiological variables or high ICP or abnormal CT findings
				Outcome measured at 6 months post injury with PCPC	Predictive: high levels on Day 1 correlated with 6 month PCPC

Scientific literature (total papers = 3; diagnostic papers =3; prognostic papers =3).

Table 4 Myelin basic protein

Author	Year published	Study	Number of children	Biofluid & timing	Main findings
Berger *et al*. (33)	2007	Prospective, observational	152 children with mild/moderate/ severe TBI	Serum MBP (& S100B & NSE) at arrival, 12–24 hours after injury and daily for 5 days in severe TBI;	Diagnostic: not assessed
				outcome assessed with GOS score at varying time points in first 12 months after injury	Predictive: Peak MBP level associated with worse outcome at all time points; Initial MBP level associated with worse outcome in first 3 months after injury; peak MBP levels more strongly correlated with outcome than initial MBP levels in children <4 years
Beers *et al*. (32)	2007	Prospective, cross-sectional study	15 children with nTBI, 15 children with iTBI (mild /moderate/severe)	Serum MBP (& S100B & NSE) at admission, 12–24 hours after injury and daily for 5 days in severe TBI	Diagnostic: not assessed
				Outcome assessed with neurocognitive testing (GOS/ VABS/IQ) at 6 months post injury	Predictive: peak MBP significantly correlated with all 3 outcome measures; time to peak MBP levels ignificantly associated with IQ
Berger *et al*. (21)	2005	Prospective, case-control study	56 children with nTBI, 44 with iTBI and 64 controls	Serum MBP (and S100B & NSE) at admission, 12–24 hours after injury and daily for 5 days in severe TBI	Diagnostic: No difference in initial MBP between both forms of TBI and controls
					Peak MBP levels higher in children with ICH (0.33 *vs*. 0.17; P=0.005; AUC 0.69 with sensitivity (44%) and specificity (96%); no difference between children with iTBI compared with nTBI
					Prognostic: not assessed

Table 4 (*continued*)

Table 4 (*continued*)

Author	Year published	Study	Number of children	Biofluid & timing	Main findings
Berger *et al*. (22)	2005	Prospective, case-control study	14 infants (<1 year) with inflicted TBI; 74 healthy control	Serum MBP (and S100B & NSE) at admission	Diagnostic: 36% sensitive & 100% specific for diagnosing iTBI
					Prognostic: not assessed

Scientific literature (total papers =4; diagnostic papers =2 and prognostic papers =2).

the other at varying time points in the next 12 months after injury (33).

Summary: Interest in MBP has lessened in comparison to S100B, NSE and GFAP in large part due to an observed lack of clinical sensitivity but may have potential for predicting 6 month outcomes.

Ubiquitin C-terminal Hydrolase-L1 (UCH-L1)

UCH-L1 is an E2 ubiquitin-conjugating enzyme expressed in neurons where it functions to add and remove ubiquitin to proteins intended for degradation (49). It is found almost exclusively in the cytoplasm of neurons (50).

Diagnostic Uses: There are 3 studies that have examined the diagnostic utility of serum UCH-L1 (37,44,51) (*Table 5*). One showed that serum levels of UCH-L1 were significantly higher in children with TBI when compared to controls (51) and two found that UCH-L1 could discriminate between severe and moderate TBI (44,51). However one study found that UCH-L1 could not discriminate between mild TBI and controls (37). Serum UCH-L1 levels was not associated with either clinical symptoms or abnormal CT findings.

Prognostic uses: There are 2 studies that have examined the prognostic utility of serum UCH-L1 (37,44) (*Table 5*). One showed an elevated UCH-L1 at admission was associated with worse outcomes at 6 months (44) and one at three different time points within one year from injury on the GOS (37).

Summary: Serum UCH-L1 is one of the newest proposed biomarkers for TBI and there is currently limited data available. Serum UCH-L1 shows promise as both a diagnostic and prognostic marker of TBI in children.

α-II-Spectrin

α-II Spectrin is a cytoskeletal protein primarily found in neuronal axons and dendrites. This protein is broken down into spectrin breakdown products (SBDP) of differing molecular weights (120, 145, 150 kDa). SBDP145, formed by calpain-mediated cleavage of spectrin, is a marker of necrotic cell death process activation.

Diagnostic Uses: There are 2 studies that have examined the diagnostic utility of SBDP145 (37,51) (*Table 6*). These studies showed that the level at arrival and within 12 hours after injury was significantly higher when compared with controls but could not discriminate between brain injury severity. The level was also not associated with clinical symptoms or an abnormal CT scan (37).

Prognostic uses: One study examined the predictive utility of SBDP145 (37) (*Table 6*). This study showed that SBDP145 on arrival to hospital was associated with worse outcomes at 3 different time points within one year from injury.

Summary: The breakdown products of α-II Spectrin are newly proposed biomarkers for TBI and as a result there are limited data available. These markers show promise as both a diagnostic and prognostic marker of TBI in children.

Tau protein

The tau proteins are a group of six highly soluble protein isoforms that have roles primarily in maintaining the stability of microtubules in axons (52). Although tau is present in dendrites at low levels, it is active primarily in the distal portions of axons. Tau proteins are abundant in the neurons of the central nervous system and they are also expressed in very low levels in CNS astrocytes and oligodendrocytes. An elevated serum tau level is indicative of axonal injury (52).

There is one study in paediatric TBI that investigated the diagnostic utility of serum tau protein (*Table 7*) (52). This group measured serum total tau in 416 healthy children to generate age-related normative values. The investigators used these normative data to interpret serum total tau in 158 children with TBI. The study found that on Day 1 of TBI, median serum concentrations of total tau were three times higher in children with any reduction in GCS compared with normal GCS controls. This finding

Table 5 Ubiquitin C-Terminal Hydrolase-L1

Author	Year published	Study	Number of children	Biofluid & timing	Main findings
Metzger *et al.* (51)	2018	Prospective, observational with control samples	19 children with severe TBI matched to 4 controls	Serum UCH-L1 (and SBDP-145) at 12, 24, 72, 96 and 120 hours	Diagnostic: increased levels over controls; peak at 12 hours and baseline levels at 120 hours
					Predictive: not assessed
Mondello *et al.* (44)	2016	Case-control study	45 cases/40 healthy controls	Serum UCH-L1 (and GFAP) on arrival to hospital	Diagnostic: increasing severity of TBI & possibly brain injury undetected on CT & intracranial haemorrhage on CT
			Mild/moderate/ severe TBI	Outcome measured at 6 months (GOS)	Predictive: predict outcome at 6 months (GOS)
Berger *et al.* (37)	2012	Prospective, observational	39 cases with mild/ moderate/severe TBI and 10 controls	Serum UCH-L1 on arrival to hospital	Diagnostic: increased levels in moderate and severe TBI but not mild compared to controls
				Outcome measured as GOS at hospital discharge, 3–4 months and 6–8 months after discharge	Not diagnostic of clinical symptoms or abnormal CT head
					Predictive: predicted worse outcomes

Scientific literature (total papers =3; diagnostic papers =3; prognostic papers =2).

Table 6 Alpha II-spectrin

Author	Year published	Study	Number of Children	Biofluid	Main Findings
Metzger *et al.* (51)	2018	Prospective, observational	19 children with severe TBI matched to 4 controls	Serum SBDP-145 (and UCH-L1) at 12, 24, 72, 96 and 120 hours	Diagnostic: increased levels over controls; peak at 48 hours and remaining high at 120 hours
					Predictive: not assessed
Berger *et al.* (37)	2012	Prospective, observational with controls	39 cases with mild/moderate/ severe TBI and 10 controls	Serum SBDP-145 on arrival to hospital	Diagnostic: increased levels in TBI overall but did not discriminate between mild/moderate/severe
				Outcome measured as GOS at hospital discharge, 3-4 months and 6-8 months after discharge	Not diagnostic of clinical symptoms or abnormal CT head.
					Predictive: predicted worse outcomes.

Scientific literature (total papers =2; diagnostic papers =2; prognostic paper =1)

Table 7 Total Tau Protein

Author	Year published	Study	Number of children	Biofluid	Main findings
Stukas *et al.* (52)	2019	Case control study)	158 children with mild/ moderate/severe TBI and 416 healthy control samples	Serum total tau within 28 hours of injury)	Diagnostic: increased levels over controls but did not discriminate between mild/moderate/ severe TBI); Predictive: not assessed

Scientific literature (total papers =1; diagnostic papers =1; prognostic papers =0).

Table 8 Secondary brain injury

Author	Year published	Study	Number of children	Biofluid & timing	Main findings
IL-6					
Park et al. (53)	2018	Prospective observational study	15 children with TBI (9 in poor group GCS <9 and 6 in good group GCS 10–15)	IL-6 within 6 hours of admission and one week after trauma	Diagnostic: Both IL-6 levels did not discriminate between severity of brain injury
					Prognostic:IL-6 levels 1 week later were higher in the unfavourable outcome group but this was not significant (P=0.061).
sNCAM					
Wilkinson et al. (41)	2017	Prospective observational study	23 children with mild/ moderate/severe TBI who completed follow up (85 children in original sample)	Neuron cell adhesion molecular) at time of injury and daily for 2 weeks	Diagnostic: not assessed
					Prognostic: Lower levels of sNCAM were associated with higher scores on inattention, hyperactivity/ impulsivity and executive functioning scales and working memory
sNCAM, sE-Selectin, soluble intercellular adhesion molecule, soluble vascular cell adhesion molecule, IL6, IL-8					
Wilkinson et al. (30)	2016	Prospective observational study	58 children with mild/ moderate/severe TBI	Serum sNCAM, sE-Selectin, soluble intercellular adhesion molecule, soluble vascular cell adhesion molecule, IL6, IL-8 once daily in first week after injury	Diagnostic: not assessed
					Prognostic: the highest serum level of sE-selectin (AUC =0.71) and trough serum levels of sNCAM (AUC =0.75) were the highest predictors of inattention at 1 year post-injury
Heat shock proteins (Hsp)					
Žurek et al. (31)	2012	Prospective, observational study	63 children with mild/ moderate/severe TBI	Hsp at admission and every 24 hours for 6 days	Diagnostic: not assessed
				Outcome measured with GOS at 6 months post injury	Prognostic: Hsp not related to outcome
IL-6, IL-8, IL-10, L-selectin. SICAM, endothelin					
Lo et al. (24)	2009	Prospective, observational study	28 children with severe TBI (GCS <8) & non severe TBI (GCS >8)	Biomarker levels at 24 h post injury	Diagnostic: Only IL-6 (P=0.02) and SICAM (P=0.01) were significantly higher in children with severe injury than those with non-severe injury
					Prognostic: High L-selectin, IL-6 and IL-8 levels predicted worse outcome at 6 months

supported total tau as a marker of injury, however, there was a poor association with injury severity and abnormalities on CT scan images.

Secondary brain injury: biomarkers of cellular, biochemical & molecular cascades

Biomarkers reflecting aspects of the cellular, biochemical, or molecular cascades in the secondary brain injury response have been investigated to a lesser extent in children. The secondary injury biomarkers that have been investigated to date include IL-6, IL-8, 1L-10, L-selectin, sE-selectin, endothelin, soluble neuron cell adhesion molecule (sNCAM), soluble intercellular adhesion molecule (SICAM), soluble vascular cell adhesion molecule and heat shock proteins (*Table 8*). The most studied of these secondary injury biomarkers is IL-6 and the results are inconsistent with respect to both diagnostic and prognostic utility (53).

Primary & secondary brain injury: using paired or multiple biomarkers

Serum levels of most acute TBI biomarkers return to baseline levels within days to a week following TBI. However, the subacute and chronic effects of TBI can persist for months following the initial injury event. Thus, it may be appropriate to consider a continuum of TBI biomarkers that may be released at different time points following the initial brain injury event. Given the failure to find a single biomarker that satisfies all the criteria of an "ideal" biomarker in severe TBI, some investigators have examined combinations of biomarkers.

A prospective observational study was conducted in 28 children who had an arterial blood sample taken exactly at 24 hours post-injury (24). Serum concentrations of 8 different biomarkers–S100b, NSE, IL-6, IL-8, IL-10, soluble intracellular adhesion molecule (SICAM), L-selectin and endothelin–were quantified. Global outcome was assessed at 6 months post-injury using the Glasgow Outcome Score. None of the 8 biomarkers assessed individually achieved an AUC of more than 0.95 for predicting unfavourable outcome, but 5 of the 20 biomarker pairs assessed, had a high degree of outcome predictability. The primary brain injury biomarker pair that achieved the highest AUC was the combination of S100B with NSE. An S100B >0.04 ng/mL and NSE ≥10 mg/mL achieved an AUC 0.97 with 100% sensitivity and 92% specificity for the prediction of unfavourable outcome.

In that same study (24), two combinations using S100B and either L-selectin or IL-6 achieved an AUC of 0.98 and their specificity and sensitivity for unfavourable outcome prediction was 96% and 100% respectively. Their conclusion was that prognostic pairs combining serum levels of two biomarkers (brain proteins and inflammatory mediators) offer better outcome predictive values for unfavourable outcome after childhood brain trauma than may be achieved using individual marker levels.

A study by Wilkinson *et al*. (30) showed that the best predictors of inattention at 1 year following injury in 58 children with varying degrees of TBI were a combination of secondary brain injury biomarkers. In the two-variable ROC model, combinations of baseline levels of inattention with four biomarkers individually improved the model. The AUCs for combinations of baseline levels of inattention with trough serum levels of sNCAM and with highest serum levels of sE-selectin, S100b and NSE were 0.86, 0.87, 0.90 and 0.94, respectively.

Future directions

This review highlights the obstacles impeding the interpretation and generalisability of biomarker data. These obstacles include a wide variability in the patient cohort, clinical characteristics, study methodology and diagnostic and prognostic variables used as outcome measures. Nonetheless, serum S100B and serum NSE levels show promise as a diagnostic tool with biomarker levels significantly higher in children with severe TBI including children with inflicted and non-inflicted head injury. Serum S100B and serum NSE also show promise as a predictor of neurodevelopmental outcome.

In order to realise the full potential of biospecimens in paediatric traumatic brain injury, standardisation and adoption of best practice guidelines are needed to ensure the quality and consistency of specimens. A workgroup was formed in 2012 to specifically address this gap. The aim of the Pediatric TBI CDE Biospecimens and Biomarkers Workgroup was to provide recommendations for best practice guidelines to standardise the quality and accessibility of biospecimens for paediatric brain injury research (54). Consensus recommendations were developed and represent expert opinion on this subject. It is the hope that future investigators will be able to obtain biospecimens in a consistent way that will ease data interpretation on biomarker potential in clinical practice.

Blood biomarkers are entering clinical use for adult

patients with TBI, with the introduction of S100B to the 2013 Scandinavian Guidelines (55) and the 2018 US Federal Drug Administration's approval of GFAP and UCH-L1 for reducing the unnecessary use of CT head scans to help detect concussion in adults, the ALERT-TBI study (56). The Brain Trauma Indicator test measures two biomarkers: GFAP and UCH-L1 taken within 12 hours of injury and results returned to the clinician within 3–4 hours to aid decision making (57). The introduction of a blood biomarker into paediatric practice is not currently on the horizon, given the lack of current evidence for diagnostic and prognostic utility.

Conclusions

To date, no biomarker with sufficient sensitivity and specificity has been validated as a clinical tool in paediatric patients with severe TBI. Serum S100B and serum NSE levels show promise as a diagnostic tool with biomarker levels significantly higher in children with severe TBI including children with inflicted and non-inflicted head injury. Serum S100B and serum NSE also show promise as a predictor of neurodevelopmental outcome.

Further research is needed with standardisation of methodology and outcome variables with potential utility in the combination of biomarkers that measure and assess both primary and secondary brain injury.

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Footnote

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