Journal of Neurotrauma

DOI: 10.1089/neu.2018.6083

Title: Interleukin-8 Predicts Fatigue at 12 Months Post-injury in Children with Traumatic Brain Injury.

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Abstract

Despite many children experiencing fatigue after childhood brain injury, little is known about the predictors of this complaint. To date, traditional indices of traumatic brain injury (TBI) severity have not reliably predicted persisting fatigue (up to 3 years post-injury). This study aimed to establish if persisting fatigue is predicted by serum biomarker concentrations in child TBI. We examined if acute serum biomarker expression would improve prediction models of 12-month fatigue based on injury severity. Blood samples were collected from 87 children (1 – 17 years at injury) sustaining mild to severe TBI (GCS range 3-15; mean 12.43; classified as mild TBI (n=50, 57%) vs moderate/severe TBI n=37, 43%), and presenting to the Emergency Departments (ED) and Pediatric Intensive Care Units (PICU) at one of three tertiary pediatric hospitals (Royal Children's Hospital (RCH); Hospital for Sick Children (HSC), Toronto St Justine Children's Hospital (SJH), Montreal). Six serum biomarker concentrations were measured within 24 hours of injury [interleukin-6 (IL-6), interleukin-8 (IL-8), soluble vascular cell adhesion molecule (SVCAM), S100 calcium binding protein B (S100B), neuron specific enolase (NSE), and soluble neural cell adhesion molecule (sNCAM)]. Fatigue at 12 months post-injury was measured using the Pediatric Quality of Life Inventory Multidimensional Fatigue Scale (parent report), classified as present/absent using previously derived cut-points. At 12 months post-injury, 22% of participants experienced fatigue. A model including interleukin-8 (IL-8) was the best serum biomarker for estimating the probability of children experiencing fatigue at 12 months post-injury. IL-8 also significantly improved predictive models of fatigue based on severity.

Keywords: Fatigue, serum biomarkers, Glasgow Coma Scale (GCS), traumatic brain injury (TBI), children

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Introduction

Traumatic brain injury (TBI) is a common cause of childhood disability, with reported incidence between 47 and 280 per 100,000.[1] Fatigue is one of the most frequently reported symptoms following TBI[2-7] and has been associated with poor quality of life.[8] Fatigue is defined as an overwhelming sense of tiredness or lack of energy, associated with impaired physical and/or cognitive functioning.[9] After childhood brain injury, fatigue interferes with academic achievement, social/emotional wellbeing, physical activity,[10] and is associated with reduced social participation.[11]

Fatigue in pediatric TBI

Fatigue is multi-faceted, with biological and psychological contributions, yet, its relationship with brain injury severity remains unclear.[12, 13] In a recent systematic review of fatigue after child acquired brain injury[10] rates of fatigue in mild TBI were high (approximately 60%) soon after injury, although this resolved at follow-up.[14, 15] Fatigue persists at 13 years after injury in more severe TBI.[16] Studies have primarily included mild TBI,[14, 15, 17] although one study included moderate/severe TBI.[16] Our group has published new data examining fatigue in children with mild-to-severe TBI, although associations between fatigue and TBI severity were inconsistent which may have been related to variation in sample size and time follow-up. In a sub-study of 35 adolescents, TBI severity (mild vs moderate/severe) was not associated with fatigue at 6 weeks post-injury.[18] In a subsequent follow-up, including a larger group of children and adolescents (2 to -<18 years, n=109), moderate/severe TBI was associated with worse fatigue 12 months post-injury, relative to mild TBI but there was considerable overlap between the groups (adolescent samples).[19] Studies in adults with TBI have also shown overlap in the severity of symptoms of fatigue across injury severity groups [4, 6, 20, 21] A high prevalence of fatigue is seen across the spectrum of TBI severities, from around 45% within one week post-injury to 37% one year post-injury.[12]

Biomarkers

Serum biomarkers provide an objective measure of the impact of injury on the brain, reflecting injury to the neuro-vascular unit.[22] Emerging research indicates that protein

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biomarkers are useful to predict clinical outcomes from childhood TBI, particularly following mild injuries.[23-28] Several biomarkers have been extensively studied following severe childhood TBI; S100 calcium binding protein B protein (S100β), glial fibrillary acidic protein (GFAP), neuron specific enolase (NSE), Tau, neurofilament light protein (NFL) and amyloid protein, brain-derived neurotrophic factor.[29, 30] Our research team have previously shown, using a proteomic approach, a massive inflammatory response, and release of putative brain-specific proteins across the blood-brain barrier into the blood in children with severe TBI.[31] These biomarkers were then associated with attention problems in long term follow up.[32] In a more recent study our group used panel of nine relatively brain-specific as and inflammatory serum protein biomarkers. In this study we determined that increased concentrations of serum NSE and decreased expression of soluble neuron cell adhesion molecule (sNCAM) was associated with long-term cognitive/behavioural outcomes (attention, executive function and memory).[33] The current study therefore builds on this earlier work, in selected specific proteins sensitive to injury to the cells of the neurovascular unit is as well as marking neuro-inflammation. To our knowledge, research examining serum biomarkers in association with fatigue is limited. Given the high incidence of fatigue after TBI, further research is necessary.

This study extends our earlier work documenting significant fatigue after childhood TBI, and explores the potential prognostic value of biomarkers to predict fatigue 12 months post-injury. Study objectives were to determine: (1) which combination(s) of biomarkers most strongly predict fatigue 12-months post-injury; and (2) whether adding biomarker information to injury severity results in better prediction accuracy than injury severity alone. We hypothesized that: (1) one or more biomarkers would significantly improve models predictive of fatigue at 12-months post-injury; and (2) serum biomarkers combined with TBI severity would increase prediction accuracy relative to TBI severity alone.

Materials and methods

Design

Details of the Biomarker and Quality of Life in Children with Traumatic Brain Injury (BTBI) study have been previously described.[19] In brief, BTBI was a multi-site, international

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prospective observational study of children with mild, moderate, and severe TBI presenting to one of three pediatric hospitals (1 Australian, 2 Canadian), assessed from injury to 12-months post-injury. TBI was defined as a history of head injury presenting within 24 hours of event, with non- trivial mechanism and signs and symptoms of head trauma exceeding scalp abrasions and lacerations. Non-trivial mechanism is established according to features of history and physical examination, and included motor vehicle accidents, bicycle related, falls (above ground level), sports and other injuries. It was distinguished from trivial injury mechanisms (ground level falls, walking into stationary objects) with no signs or symptoms of head trauma other than scalp abrasions or lacerations).[21] Mild TBI was defined as Glasgow Coma Scale (GCS) score of 13-15 (lowest hospital-recorded in first 24 hours).[34] Radiological evidence of intracranial injury was not included in criteria as not all participants had CT scans.

Participants

The BTBI study was approved by the research ethics boards at each participating institution. Participants enrolled into the study were recruited on admission to the Emergency Departments (ED), hospital wards or Pediatric Intensive Care Units (PICUs) of The Royal Children's Hospital (RCH), Melbourne, Australia, Central Hospitalier Universitaire (CHU), Sainte-Justine, Montreal, Canada and The Hospital for Sick Children (HSC) Toronto, Canada (August 2012 - June 2014). Frontline clinical staff (PICU or ED treating physicians or primary nurses) and trained research personnel at each site identified potentially eligible participants through screening of daily admissions. We followed international guidelines for the conduct of medical research and medical record access for screening was conducted in the same manner at each site. Study site research personnel were trained for all study procedures including screening, consent and data collection by the BTBI study Research Coordinator. Inclusion criteria were: child age between birth - 17 years, presentation within 24 hours of a TBI sustained via non-trivial mechanism, minimum of 4 hours length of stay in the ED or admission to hospital, and bloods sampled for biomarker measurements. Children were excluded if they had a history of previous TBI requiring hospital admission, diagnosis of birth trauma, moderate to severe neurodevelopmental disorder, or parents not fluent in English or French. Blood samples could be biobanked

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prior to consent if blood was drawn for clinical indications. Following informed consent blood could be drawn for biobanking and research purposes if clinical blood tests were not ordered. Once it was deemed clinically appropriate by frontline clinical staff, the principal site investigator or trained research personnel met the family to explain the study and obtain informed consent. Assent was obtained for mature minors and recorded in accordance with ethics boards at all participating hospitals.

Procedure

Demographic and clinical information was extracted from the participants' electronic medical records: child age, sex, mechanism of injury, hospital recorded GCS (lowest and highest in first 24 hours), [35] pediatric trauma score, and interventions. Head CT was ordered at the discretion of the treating physician, as clinically indicated, and scans were interpreted and reported on by radiologists blind to the study protocol. Abnormal CT were recorded if any of the following were present: intracranial hemorrhage (epidural, subdural, or subarachnoid), diffuse axonal injury (DAI), compression or trapping of a lateral ventricle, partial or complete effacement of the basal cisterns, midline shift, cerebral edema, or contusion. The severity of the TBI was categorized using GCS within 24 hours as: mild TBI (GCS 13-15); moderate TBI (GCS 9-12); severe TBI (GCS <9).[35] Although the common data element recommendation is for highest hospital recorded GCS,[36] this can lack sensitivity in the mild TBI group. Further, in our earlier work from our group more broadly,[37, 38] child functional and quality of life outcomes have been predicted by injury severity was classified based on the child's lowest GCS within 24 hours. Whether highest or lowest GCS was used to categorize TBI severity groups was determined post-hoc, based on planned comparison between the two regression models. Due to small numbers of moderate/severe injuries, these groups were collapsed for analysis. A structured interview[39] and parent-report measures were completed at the time of injury in person and again 12-months post-injury via telephone, administered by a trained psychologist researcher. Demographic and clinical data were entered into a web-based case report form. Parent-report measures were entered via Teleform into a database. All data were electronically transferred to a secure central database at SickKids Hospital, Toronto.

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The Pediatric Quality of Life Inventory Multidimensional Fatigue Scale (PedsQL, parent form)[40, 41] was completed 12-months post-injury. General, sleep/rest and cognitive fatigue symptoms were rated on a 5-point Likert scale (0=never a problem; to 4=almost always a problem). Items were reverse scored and transformed to a 0-100 point scale. Higher scores indicated better quality of life (i.e., fewer fatigue symptoms). Data were compared to published normative data (parent ratings, n=259; age 2-19 years).[42] Total fatigue scores were dichotomized using previously derived cut-points into those with (scores 2 SD below normative data)/ and without (scores within 2 SD of normative data) 'fatigue'.[42-44]

Blood sample collection and laboratory analyses.

Clinical blood samples (arterial or venous blood taken from wherever access was available) were collected from participants within 24 hours post-injury, in serum separator tubes (BD, Franklin Lakes, New Jersey, United States), allowed to clot and were centrifuged (at 5,000 rpm for 10 minutes). Serum was separated, and stored at – 80 ° C as soon as possible following collection. Serum samples from participating sites were shipped on dry ice and stored at – 80 ° C at the biobanking facility at the Hospital for Sick Children, Toronto. Serum samples were divided into 100 µL aliquots using one rapid thaw freeze cycle and again stored at – 80 ° C. Based on our previous work,[32] we measured a combination of six inflammatory and brain-specific serum protein biomarkers which were associated with outcomes following childhood TBI. Biomarkers were measured using multiplex immunoassays on a Luminex 200 analyser using xPonent 3.1.971.0 software (Luminex Corporation, Austin, TX, USA) by the Analytical Facility for Bioactive Molecules (The Hospital for Sick Children, Toronto) as follows: IL-6 and IL-8 -MILLIPLEX MAP Human Cytokine/Chemokine Magnetic Bead Panel – Immunology Multiple Assay; sVCAM-1 and sNCAM - MILLIPLEX MAP Human Neurodegenerative Disease Magnetic Bead Panel 3 – Neuroscience Multiplex Assay (Millipore-EMD, Billerica, MD, USA); NSE – MILLIPLEX MAP Human Cancer/Metastasis Biomarker Magnetic Bead Panel – Cancer Multiplex Assay (Millipore-EMD, Billerica, MD). S100B was measured using a commercially available Human S100B enzyme linked immunosorbent assay (ELISA kit (Millipore). All measurements were conducted in accordance with manufacturers' instructions. The first available sample from each patient within 24 hours of injury was used for analysis.

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Statistical analysis.

Group based analyses (participants in the analysed sample versus lost to follow-up and mild versus moderate/severe TBI) for demographic and clinical data were conducted using Chi-square and t-tests. Biomarker concentrations were graphed using scatterplots (against GCS for each of the mild and moderate/severe groups) and boxplots (for the fatigued/not fatigued groups).

The optimal biomarker prediction model was built using bivariate, then multivariate, logistical regression models to identify the parsimonious model from all available combinations of biomarkers. Biomarkers that did not significantly discriminate group membership (with/without fatigue) or were less parsimonious were excluded. The most parsimonious model was determined using likelihood ratio tests, and was retained for the purpose of this investigation.

Two logistical regression models were tested to assess the predictive value of combining severity (mild versus moderate/severe TBI) categorized from GCS with the selected biomarkers. The model combining TBI severity (mild versus moderate/severe) and biomarker was tested against the TBI severity alone model using likelihood ratio tests. Child age and sex were entered into both models as covariates. TBI severity classification based on the common data element recommended[36] highest versus lowest hospital recorded GCS in 24 hours post-injury, as described by others,[45, 46] were compared. Model quality was compared post-hoc using the Akaike information criterion (AIC) and Bayesian information criterion (BIC). The highest quality model (lowest AIC/BIC values) was retained. All statistical models were performed in Stata, version 15.[47]

Results

Demographics

Of 159 children initially enrolled, 87 had sufficient data for inclusion in analysis (1 child was excluded due to extreme biomarker expressions identified for IL-6 and IL-8, Figure 1). The identified IL-8 and IL-6 outlier did not have any clinical, physiological, or other apparent differences to explain their outlying serum expression. Post-hoc analysis revealed that the

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results of the prediction models did not differ with or without this participant data, and so therefore they were removed from analyses.

[Figure 1 here]

Participants in the analyzed sample did not differ significantly from those excluded from analysis, for demographic or pre-injury variables (Table 1). Most participants were boys (67.8%), with a median age of 11.0 (mean=9.8 years, SD=5.0, range 11 months – 16 years). The majority of injuries were sustained via falls (33.3%), sport (21.8%) or motor vehicle accidents (23.0%), with a few injuries being sustained via bicycle accidents (8.1%) or other injuries (13.8%).

[Table 1 here]

TBI severity was categorized as: mild TBI (GCS 13-15, n=50); moderate TBI (GCS 9-12, n=8); severe TBI GCS <9 (n=29); collapsed into mild (n=50, 57.5%) versus moderate/severe (n=37, 42.5%). [35] A total of 55 children had clinical CT scans performed. Table 2 demonstrates demographic and injury details for those with mild versus moderate/severe injuries. As shown, when compared to children with moderate/severe TBI, children with mild TBI were older at injury (and follow-up). An analysis using injury severity measures showed that those with mild TBI had higher GCS and PTS scores, and were most likely to have sustained injuries through falls (42.0%), compared to those with moderate/severe TBI were most likely to have sustained injury through motor vehicle accidents (43.2%). Those with moderate/severe TBI were more likely to have exhibited abnormal clinical brain CT, and also exhibited a longer time from injury to blood draw (mean 14.1 hrs, SD 10.1 hrs) relative to those with mild TBI (mean 8.42 hrs, SD 6.8 hrs, p=.002). Concentrations for the 6 serum biomarkers are displayed against lowest hospital GCS within 24 hours post-injury for the mild and moderate/severe groups respectively (Figure 2).

[Table 2 here]

[Figure 2 here]

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Fatigue prediction

Fatigue was reported in 19 (21.8%) children at 12-months post-injury (mild TBI 14.3%; moderate/severe TBI 31.6%, p=.05). Those with/without fatigue differed in their lowest GCS score within 24 hours post-injury, with the fatigued group experiencing lower initial GCS than the non-fatigued group (Table 3). The fatigue group was also more likely to have a greater proportion of females, late questionnaire return, and higher rates of tertiary educated parents. Biomarker expression is displayed in boxplots for those with and without fatigue at 12 months in Figure 3. Bivariate associations between biomarkers and fatigue are reported in Supplementary Table 1. The most parsimonious biomarker model consisted of only one predictor (IL-8): (OR 1.04; 95% CI 1.01, 1.08, p=0.007) (positive log likelihood ratio test (p=0.001). With one unit increase in IL-8, the odds of fatigue at 12 months increased by 4%.

[Figure 3 here]

The injury severity model using lowest hospital recorded GCS to categorize TBI severity (within 24 hours post-injury) was identified as the highest quality model (AIC = 87.9, BIC = 97.8, versus highest GCS; AIC = 90.1, BIC = 99.9) so was retained. Table 4 presents the logistical regression analysis for fatigue at 12-months post-injury, predicted by TBI severity (based on lowest GCS) alone, with age and sex as covariates. Logistical regression analysis indicated TBI severity was a significant predictor of fatigue at 12-months post-injury. Moderate/severe TBI was associated with a 367.7% increased likelihood of fatigue 12-months post-injury. Sex was a significant predictor of fatigue; and females were 411.6% more likely to demonstrate fatigue 12-months post-injury. Child age at injury was not significantly predictive of fatigue.

[Table 4 here]

The final, combined logistical regression model included severity and biomarker expressions predicted fatigue at 12 months post-injury. In this model, biomarker expression of IL-8 (24 hours post-injury) was the only significant predictor of fatigue at 12 months post-injury. Every increase of one unit (pg/ml) of IL-8 resulted in a 4% increased likelihood of experiencing fatigue at 12 months post-injury, even after controlling for

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confounders of age and sex. Age and sex were non-significant in the combined model. Likelihood ratio test results showed that adding the biomarker IL-8 to the severity model resulted in a statistically significant improvement in model fit (p = 0.01) for predicting post-injury fatigue at 12 months.

Further post-hoc analysis indicated that those with other injuries demonstrated significantly elevated concentrations of Il8 (mean = 29.88, SD = 34.09) relative to those with no other concomitant injuries (mean = 13.13, SD = 13.43, p=.001). Those with intracranial lesions demonstrated significantly elevated concentrations of Il8 (mean = 24.49, SD = 29.26), relative to those without intracranial lesions (mean = 13.32, SD = 14.73, p=.02). In order to account for potential influence of concomitant and intracranial injuries on the relationship between IL-8 and fatigue, the final model was repeated to include other organ injury and intracranial lesions. IL-8 remained a significant predictor of 12-month fatigue (Table 5).

[Table 5 here]

Discussion

From a panel of six inflammatory and brain proteins we demonstrated that early measurement of IL-8 has prognostic value following TBI in children. To date, the blood based inflammatory response following TBI and its relation to fatigue has not been well established. In this study we demonstrated a novel application of biomarkers to predict fatigue 12-months post-injury. The current study extends our earlier work examining the relationship of injury-related and other factors contributing to post-injury fatigue.[18, 19, 48]

Prognostic value of serum biomarkers

Of the biomarkers tested, IL-8 was shown to be the strongest single predictor of persistent fatigue in our population. Interleukin-8 is a potent chemoattractant for neutrophils, is a member of the CXC chemokine family (CXCL8), a group of signalling molecules that act as biological markers of the inflammatory response to injury, and is secreted by glial cells, macrophages, and endothelial cells.[49-51] It has been described as a "help me" cry of neurons and astrocytes in response to central nervous system injury.[52] The early release of

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IL-8 from astrocytes in response to other cytokines soon after brain injury has been demonstrated, indicating its role in mediating secondary brain damage. [53-55]

Our findings are consistent with prior research documenting that IL-8, released into the blood within24 hours following injury, provided a prognostic marker for outcome from child TBI. Lo and colleagues demonstrated that serum IL-8 concentrations measured within 24 hours post-injury independently predicted unfavorable outcome (Glasgow Outcome Scale, GOS).[56] Further, IL-8 along with another inflammatory marker (L-selectin) had a higher prognostic value for unfavorable outcome relative to putative brain specific (glial or neuronal) proteins.[56] These authors suggested that serum inflammatory markers might be more sensitive to the neuro-inflammation burden compared to the relatively brain-specific proteins or, alternatively, neuro-inflammation might directly effect outcomes more than had been previously suspected. Although we cannot differentiate between these hypotheses, our data provides broad support for the sensitivity of IL-8 for negative sequela from child TBI. The prognostic value of paired severity (based on GCS) and IL-8 data in our study confirms prior findings,[57] in which eight serum biomarkers (S100b, NSE, IL-6, IL-8, IL-10, L-selectin, SICAM, and endothelin) and GCS were tested for best prognostic model of unfavorable outcome from pediatric TBI. Pairing severity with Il-8 serum expression significantly increased prediction of unfavorable outcome, relative to severity alone. Interleukin-8 is not exclusive to the brain, however, and is a non-specific marker of inflammation. Therefore, elevated IL-8 concentrations may result from inflammation in multiple organs as a result of the TBI, although additional, post-hoc analysis indicated that IL-8 remained predictive of fatigue at 12 months post-injury even after controlling for other organ injuries.

Initial, bivariate models indicated some preliminary evidence for the role of sNCAM predicting fatigue outcome; decreased expression of sNCAM was associated with greater chance of fatigue at 12 months. Other members of our group recently demonstrated that lowest measured sNCAM was associated with greater difficulties with attention, hyperactivity/impulsivity and executive functioning on behavioral questionnaires at 3 year follow-up.[33] Attentional vigilance has been implicated as a contributor to post-TBI fatigue

in adults.[58] Therefore, exploration of the association between sNCAM, attention, and fatigue may be a target for future studies.

We hypothesized that biomarkers would increase prediction of 12-month fatigue over individual biomarkers. It has been demonstrated combining serum expression of two or more biomarkers (inflammatory mediators and relative brain-specific proteins), rather than an isolated biomarker, is likely to better explain TBI outcomes.[25, 32] Our findings failed to support this, with IL-8 alone being the strongest predictor of fatigue at 12 months after child TBI. Further validation studies are required given the exploratory nature of the current study.

TBI severity

Fatigue at 12-months post-injury was predicted by injury severity alone (derived from lowest GCS score), where biomarkers were not included. Specifically, moderate/severe TBI was associated with increased risk of fatigue at 12 months post-injury. We examined lowest hospital and highest hospital recorded GCS in order to classify severity groups, and found that the lowest hospital recorded GCS within 24 hours post-injury was more significant in our predictive models. However, not all children had clinical CT requested, such that presence/absence of CT abnormalities cannot be consistently used to ascertain injury severity. We previously demonstrated that moderate/severe TBI was associated with a worse 12-month fatigue compared to mild TBI.[19] The current data represents a sub-sample of this previously analysed cohort (those with complete biomarker and fatigue data), so results should be interpreted with caution. Our current findings contribute to previous fatigue studies of one or two injury severity categories (either mild or moderate/severe).[14-17] A more precise and non-categorical measure of injury severity would allow for potentially more sensitive analysis of the impact of TBI severity on fatigue.

Female sex

There was evidence of the influence of female sex in association with increased risk of significant fatigue, although the significance of female sex was no longer evident when acute biomarkers were included in our model. In the general population incidence rates of fatigue are reported to vary, and female to-male ratios in the order of 2:1 to 3:1 have been

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reported.[59] In the context of mild TBI, females compared with males appear to report more symptoms, including fatigue, both before and after sustaining an injury, [60] Female sex has been identified as a risk for persisting post-concussion symptoms (of which fatigue is one of the most common complaints).[61, 62] Therefore, sex-related differences in fatigue warrant further research.[12]

Study limitations

The current study was limited in scope for several reasons. Firstly, we did not consider recognised secondary factors that may contribute to post-TBI fatigue (e.g., psychological distress, pain, depression).[12] Secondary factors include physical disability, psychological, biochemical, endocrine and sleep disturbance thought likely to contribute to fatigue.[12] Secondly the timing of biomarker analysis is an important factor. We examined biomarker concentrations in a single sample taken within 24 hours of the brain. However, variation between the initial and peak blood concentration of biomarker expression has been reported in previous studies irrespective of mechanism of injury,[63] suggesting the time of the biomarker sample is an important variable to account for in TBI studies.[64] Variability in the time of the blood sample and difference in the sample time between TBI severity groups may have impacted on the predictive performance of biomarkers,[65] potentially reducing the sensitivity of biomarkers to detect associations with fatigue. However, it has been suggested that Il-8 has a variable and less reliable peak following brain trauma, such that there may be individual variability in the timing of this peak,[66] and the sensitivity of early samples to outcome. Future studies could aim to better standardise the time when samples are drawn relative to the time of injury, and potentially explore intra-individual peaks in Il-8. Furthermore, with respect to its clinical implications, this study was exploratory and prognostic thresholds for fatigue remain unclear. Thirdly, pre-determined cut points from published normative data were used to classify those with/without fatigue which are collapsed across all participants. Future research would benefit from using age and sex matched controls, and this would allow more specific cut-off thresholds for serum concentrations. Additional limitations result from the sample size and heterogeneity. While attrition analyses indicated that the retained sample was representative of the available pool of children who suffered TBI for key child and TBI factors (child age, sex, TBI

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severity), the small retained sample is a limitation. Further, although this study is significant as it is the first study to demonstrate the potential use of serum biomarkers to predict fatigue outcome in child TBI, the sample included a broad range of children's age and TBI severity. We controlled for specific child and TBI severity factors in analysis to address these co-variables. In the future larger studies would allow for a better understanding of the influence of other potentially confounding factors associated with serum biomarker expression or fatigue outcome, and would allow for further subgroup analysis within the TBI severity groups. In addition, due to the scope of the study and sample size, it was not appropriate to consider other potentially confounding factors resulting in fatigue. Future studies with a larger sample size could include a multivariate analysis of key additional factors affecting persistent fatigue (mood, pain, medication). Finally, we relied on parent ratings of child fatigue, to allow for valid assessment of younger children included in the sample. Although psychometrically robust, this fails to capture subjective fatigue from the child's perspective.[18, 43, 67-72] Future evaluation of child fatigue ratings should next be examined.

In conclusion, our study demonstrates that IL-8 provides a potentially useful biomarker for post-TBI fatigue in child brain injury. IL-8 was associated with parent-rated child fatigue 12-moths post-injury. Future studies extend present findings and establish prognostic thresholds in IL-8 for predicting fatigue in order to help risk stratify patients for follow-up and intervention.

Acknowledgements

The following members of the Biomarker and Quality of Life in Children with Traumatic Brain Injury group, by site, including non-author contributors:
Site: Hospital for SickKids: Dr. Kathy Boutis, Ms Helena Frndova, Dr. Anne-Marie Guerguerian, Sumaira Hussain, Dr. Jamie Hutchison, Ms Judith Van Huyse, Dr. Martin Post, Ms Amy Wilkinson.
Site: Royal Children's Hospital (RCH): Dr. Vicki Anderson, Dr. Franz Babl, Dr. Warwick Butt, Dr. Alison Crichton, Carmel Delzoppo, Dr Mardee Greenham,

Site: Hospital Saint-Justine: Dr Miriam Beauchamp, Cindy Beaudoin, Mariana Dumitrascu, Dr. Catherine Farrell, Charlotte Gagner, Dr. Jocelyn Gravel,

In addition:

We thank the participants of the Pediatric Research Academic Initiative at HSC Emergency (PRAISE) for identifying and participating in enrolment of study participants at the Toronto site.

We would like to acknowledge the contribution of the Canadian Critical Care Translational Biology Group and the Canadian Critical Care Trials Group who contributed to the work of the Canadian sites.

We thank Hayley Craig-Barnes (SickKids) for her expert technological laboratory work in biobank management and biomarker laboratory assays.

We thank Brenton Ward (RCH) for assisting in recruitment of participants at RCH.

We thank the parents and children for participating in this study.

Funding Source

All phases of this study were supported in part by a jointly awarded grant from The Ontario Neurotrauma Foundation, Toronto, Canada and The Victorian Neurotrauma Initiative, Melbourne, Australia and the Victorian Government's Infrastructure Support Program, Melbourne, Australia to VA, MHB and JSH. AC was supported by Moving Ahead CRE Seed Grant; a National Health and Medical Research Council Postgraduate Scholarship, and a Neurosciences Victoria Brain and Mind Scholarship, Melbourne, Australia. FEB and EO were supported in part by a Centre of Research Excellence Grant, National Health and Medical Research Council, Canberra, Australia. FEB was supported in part by a grant from the Royal Children's Hospital Foundation, Melbourne, Australia, a Melbourne Campus Clinician Scientist Fellowship, Melbourne, Australia and an NHMRC Practitioner Fellowship, Canberra, Australia.

Author Disclosure Statement

No competing financial interests exist.

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Table 1. Comparison of participants included and excluded from analysed sample.

	Lost to follow up	Completed follow-up	*p value*
	N (%)= 71 (45.0)	*N (%)=87 (55.0)*	
Demographics			
Age at Injury (years), *M (SD)*	8.7 (5.2)	9.8 (5.0)	0.91
Months to follow-up (12m), *M (SD)*	12.8 (0.8)	12.9[a] (1.3)	0.60
Age at 12m follow-up, *M (SD)*	8.8 (5.9)	10.8[a] (5.0)	0.95
Male, *n (%)*	40 (67.8)	68[b] (78.2)	0.16
Ethnicity (Caucasian), *n (%)*	33 (61.1)	56 (73.7)	0.13
Level of parental education (Tertiary), *n (%*	22 (43.1)	32[c] (45.7)	0. 78
Severity			
Lowest Glasgow Coma Scale, *M (SD)*	11.3 (5.0)	10.8 (4.9)	0.53
Distribution of Lowest Glasgow Coma Scale			0.93
<8, *n (%)*	14 (21.5)	17 (20.2)	
8-12, *n (%)*	5 (7.7)	7 (8.3)	
13-14, *n (%)*	3 (4.6)	6 (7.1)	
15, *n (%)*	43 (66.2)	54 (64.3)	
Highest Glasgow Coma Scale, *M (SD)*	12.4 (4.3)	12.8 (3.9)	0.55
Severity classification[d]			0.26
Mild TBI, *n (%)*	47 (66.2)	50 (57.5)	
Moderate/severe TBI, *n (%)*	24 (33.8)	37 (42.5)	
Pediatric Trauma Score , *M (SD)*	8.3 (3.3)	8.6 (2.7)	0.77
Neurosurgery, *n (%)*	8 (11.3)	10 (11.5)	0.96
Mechanism of injury			0.68
Motor Vehicle Accident, *n (%)*	13 (18.3)	20 (23.0)	
Bicycle, *n (%)*	7 (10.0)	7 (8.1)	

Fall, *n (%)*	30 (42.3)	29 (33.3)	
Sport, *n (%)*	15 (21.1)	19 (21.8)	
Other, *n (%)*	6 (8.5)	12 (13.8)	
Pre-injury function			
ABAS GAC Composite, *M (SD)*	103.0 (16.1)	100 (16.5)	0.16
Psychological Difficulties[d], *n (%)*	9 (17.3)	10 (12.7)	0.46
PIFOS Fatigue disability, *n (%)*	1 (1.6)	1 (1.2)	0.84

TBI, Traumatic Brain Injury

[a] Missing 2 obs ; [b] Missing 11 obs; [c]missing 17 obs; [d] lowest hospital recorded GCS was used to classify severity as this was found to be most predictive of fatigue outcomes and retained in the final analysis; [e]measured on Brief Infant Toddler Social Emotional Assessment and Strengths and Difficulties Questionnaire.

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Table 2. Comparison of participants with mild and moderate/severe TBI severity.

	TBI severity		
	Mild	Moderate/Severe	*p value*
	N (%)= 50 (57.5)	*N (%)=37 (43.5)*	
Demographics			
Age at Injury (years), *M (SD)*	11.0 (4.4)	8.2 (5.4)	0.01
Months to follow-up (12m), *M (SD)*	12.8 (1.2)	13.0 (1.3)	0.67
Age at 12m follow-up, *M (SD)*	12.1 (4.4)	9.2 (5.3)	0.01
Biomarker sample, hours from injury, M (SD)	8.4 (6.8)	14.1 (10.1)	0.002
Distribution of biomarker samples, hours from injury			<.001
0-4hr, *n (%)*	0 (0.0)	4 (12.5)	
4-8hrs, *n (%)*	0 (0.0)	8 (25.0)	
8-12hrs, *n (%)*	0 (0.0)	3 (9.4)	
12-16hrs, *n (%)*	39 (79.6)	2 (6.3)	
16-20hrs, *n (%)*	5 (10.2)	2 (9.4)	
>20 hrs, *n (%)*	5 (10.2)	12 (37.5)	
Male, *n (%)*	41 (82.0)	27 (73.0)	0.31
Ethnicity (Caucasian), *n (%)*	32 (76.2)	10 (29.4)	0.52
Level of parental education (Tertiary), *n (%)*	21 (48.8)	11 (40.7)	0.51
Severity			
Lowest Glasgow Coma Scale, *M (SD)*	14.6 (0.6)	5.7 (3.1)	<.001
Distribution of Lowest Glasgow Coma Scale			<.001
≤8, *n (%)*	0 (0.0)	29 (76.3)	
9-12, *n (%)*	0 (0.0)	9 (23.7)	
13-14, *n (%)*	19 (38.8)	0 (0.0)	

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15, *n (%)*	30 (61.2)	0 (0.0)	
Highest Glasgow Coma Scale, *M (SD)*	15.0 (0.2)	9.0 (84.8)	<.001
Pediatric Trauma Score , *M (SD)*	10.3 (1.0)	6.5 (2.6)	<.001
Neurosurgery, *n (%)*	2 (4.0)	8 (21.6)	0.01
CT findings			
Clinical CT completed	23 (46.9)	32 (84.2)	<.001
Abnormal brain CT, *n (%)*	6 (26.0)	24 (75.0)	<.001
Mechanism of injury			0.001
Motor Vehicle Accident, *n (%)*	4 (8.0)	16 (43.2)	
Bicycle, *n (%)*	3 (6.0)	4 (10.8)	
Fall, *n (%)*	21 (42.0)	8 (21.6)	
Sport, *n (%)*	15 (30.0)	4 (10.8)	
Other, *n (%)*	7 (14.0)	5 (13.5)	
Pre-injury function			
ABAS GAC Composite, *M (SD)*	99.4 (17.5)	101.1 (12.3)	0.66
Psychological Difficulties[a], *n (%)*	8 (18.2)	2 (5.7)	0.10
PIFOS Fatigue disability, *n (%)*	1 (2.2)	0 (0.0)	0.36
Other injury[b]			
Spine fracture	0 (0.0)	1 (50.0)	N/A
Spinal cord injury	0 (0.0)	0 (0.0)	N/A
Thoracic injury	0 (0.0)	5 (13.2)	<0.05
Cardiovascular injury	0 (0.0)	0 (0.0)	N/A
Abdominal injury	0 (0.0)	1 (2.6)	0.25
Genital-urinal injury	0 (0.0)	1 (2.6)	0.25
Major fracture	8 (16.3)	11 (29.0)	0.16
Peripheral injury	0 (0.0)	0 (0.0)	N/A

TBI, Traumatic Brain Injury; M, Mean; SD, Standard Deviation; CT, Computed tomography; ABAS GAC, Adaptive Behavior Assessment System General Adaptive Composite; PIFOS, Pediatric Injury Functional Outcome Scale

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[a]measured on Brief Infant Toddler Social Emotional Assessment and Strengths and Difficulties Questionnaire.

[b]Proportion of those with other injuries. Spinal fracture or cord injury is proportional to the total number of participants with spinal injuries (n=2).

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Table 3. Comparison of participants with/without fatigue, for the entire sample and those with mild and moderate/severe TBI severity.

	Entire Sample			TBI Severity					
				Mild TBI			Moderate/Severe TBI		
	Fatigue status			Fatigue status			Fatigue status		
	No Fatigue *N (%)=19 (19.5)*	Fatigue *N (%) = 68 (78.2)*	*p-value*	Fatigue *N (%) = 7 (14.0)*	No Fatigue *N (%) = 43 (86.0)*	*p-value*	Fatigue *N (%) = 7 (14.0)*	No Fatigue *N (%) = 43 (86.0)*	*p-value*
Demographics									
Age at Injury (years), *M (SD)*	10.5 (5.1)	9.6 (5.0)	0.49	11.9 (4.3)	10.8 (4.5)	0.56	9.7 (5.5)	7.7 (5.2)	0.28
Months to follow-up (12m), *M (SD)*	13.3 (1.3)	12.8 (1.2)	0.09	13.4 (1.1)	12.8 (1.3)	0.20	13.3 (1.4)	12.8 (1.2)	0.28
Age at 12m follow-up, *M (SD)*	10.63 (5.0)	11.63 (5.0)	0.47	13.0 (4.4)	11.9 (4.5)	0.53	10.7 (5.44)	8.7 (5.2)	0.29
Biomarker sample, hours from injury, M (SD)	11.9 (8.5)	10.6 (9.0)	0.58	7.8 (8.4)	8.5 (6.6)	0.79	14.3 (7.9)	14.0 (11.1)	0.94
Distribution of biomarker samples, hours from injury			0.49			0.61			0.61
0-4hr, *n (%)*	2 (11.8)	2 (3.1)		0 (0.0)	0 (0.0)		2 (9.1)	2 (20.0)	
4-8hrs, *n (%)*	1 (5.9)	7 (10.9)		0 (0.0)	0 (0.0)		7 (21.8)	1 (10.0)	

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8-12hrs, *n (%)*	1 (5.9)	2 (3.1)		0 (0.0)	0 (0.0)		2 (9.1)	1 (10.0)	
12-16hrs, *n (%)*	6 (35.3)	35 (54.7)		6 (85.7)	33 (78.6)		2 (9.1)	0 (0.0)	
16-20hrs, *n (%)*	2 (11.8)	6 (94)		0 (0.0)	5 (11.9)		1 (4.6)	2 (20.0)	
>20 hrs, *n (%)*	5 (29.4)	12 (18.8)		1 (14.3)	4 (9.52)		8 (36.4)	4 (40.0)	
Male, *n (%)*	11 (57.9)	57 (83.8)	0.02	4 (57.1)	36 (85.7)	0.07	7 (58.3)	21 (80.8)	0.15
Ethnicity (Caucasian), *n (%)*	12 (75.0)	44 (73.3)	0.10	4 (66.7)	27 (77.1)	0.17	2 (20.0)	8 (32.0)	0.48
Level of parental education (Tertiary), *n (%)*	2 (20.0)	30 (50.0)	0.08	2 (50.0)	18 (47.4)	0.92	0 (0.0)	12 (54.6)	0.02
Severity									
Lowest Glasgow Coma Scale, *M (SD)*	8.7 (5.2)	11.3 (4.7)	0.04	14.6 (0.5)	14.6 (0.6)	1.00	5.25 (3.05)	6.0 (3.4)	0.50
Distribution of Lowest Glasgow Coma Scale			0.22			0.81			0.49
≤8, *n (%)*	4 (21.1)	26 (38.2)		N/A	N/A		7 (26.9)	2 (16.7)	
9-12, *n (%)*	3 (15.8)	16 (23.5)		N/A	N/A		19 (73.1)	10 (83.3)	
13-14, *n (%)*	2 (10.5)	7 (10.3)		3 (42.9)	16 (38.1)		N/A	N/A	
15, *n (%)*	10 (52.6)	19 (27.9)		4 (57.1)	26 (61.9)		N/A	N/A	
Highest Glasgow Coma Scale, *M (SD)*	11 (5.0)	12.8 (4.0)	0.10	15.0 (0.0)	15.0 (0.21)	0.57	8.6 (5.0)	9.2 (4.8)	0.77
Pediatric	8.2 (3.3)	8.7 (2.5)	0.47	11.2 (0.4)	10.2 (1.0)	0.02	6.4 (2.9)	6.5	0.89

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Trauma Score , *M (SD)*								(2.5)	
Neurosurgery, *n (%)*	2 (10.5)	8 (11.8)	0.88	0 (0.0)	2 (4.8)	0.56	2 (16.7)	6 (23.1)	0.26
CT findings									
Abnormal brain CT, *n (%)*	9 (75.0)	21 (48.0)	0.18	1 (14.3)	5 (11.9)	0.86	8 (66.7)	16 (61.5)	0.26
Mechanism of injury			0.48			0.42			0.61
Motor Vehicle Accident, *n (%)*	14 (20.6)	6 (31.6)		0 (0.0)	4 (9.5)		10 (38.5)	10 (38.5)	
Bicycle, *n (%)*	5 (7.4)	2 (10.5)		0 (0.0)	3 (7.1)		2 (7.7)	2 (16.7)	
Fall, *n (%)*	26 (38.2)	3 (15.8)		2 (28.6)	19 (45.2)		7 (26.9)	1 (8.3)	
Sport, *n (%)*	14 (20.6)	5 (26.3)		4 (57.1)	10 (23.8)		4 (15.4)	1 (8.3)	
Other, *n (%)*	9 (13.2)	3 (15.8)		1 (14.3)	6 (14.3)		3 (11.5)	2 (16.7)	
Pre-injury function									
ABAS GAC Composite, *M (SD)*	100.8 (16.0)	97.4 (18.9)	0.47	86.67 (17.9)	101.0 (16.9)	0.06	103.9 (17.2)	100.5 (14.68)	0.57
Psychological Difficulties[a], *n (%)*	2 (12.5)	8 (12.7)	0.98	2 (33.3)	6 (16.22)	0.32	2 (7.7)	0 (0.0)	0.37
PIFOS Fatigue disability, *n (%)*	0 (0.0)	1 (1.6)	0.58	0 (0.0)	1 (2.6)	0.67	0 (0.0)	0 (0.0)	N/A
Other injury[b]									
Spine fracture	0 (0.0)	1 (100.0)	0.16	0 (0.0)	0 (0.0)	N/A	0 (0.0)	1 (100.0)	0.20
Spinal cord	0 (0.0)	0 (0.0)	N/A	0 (0.0)	0 (0.0)	N/A	0 (0.0)	0 (0.0)	N/A

injury									
Thoracic injury	3 (15.8)	2 (2.9)	0.03	0 (0.0)	0 (0.0)	N/A	3 (25.0)	2 (7.7)	0.14
Cardiovascular injury	0 (0.0)	0 (0.0)	N/A	0 (0.0)	0 (0.0)	N/A	0 (0.0)	0 (0.0)	N/A
Abdominal injury	1 (5.3)	0 (0.0)	0.57	0 (0.0)	0 (0.0)	N/A	1 (8.3))	0 (0.0)	0.14
Genital-urinal injury	1 (5.3)	0 (0.0)	0.57	0 (0.0)	0 (0.0)	N/A	1 (8.3)	0 (0.0)	0.14
Major fracture	2 (10.5)	17 (25.0)	0.18	0 (0.0)	8 (19.1)	0.21	2 (16.7)	9 (34.6)	0.25
Peripheral injury	0 (0.0)	0 (0.0)	N/A	0 (0.0)	0 (0.0)	N/A	0 (0.0)	0 (0.0)	N/A

TBI, Traumatic Brain Injury; M, Mean; SD, Standard Deviation; CT, Computed tomography; ABAS GAC, Adaptive Behavior Assessment System General Adaptive Composite; PIFOS, Pediatric Injury Functional Outcome Scale

[a]measured on Brief Infant Toddler Social Emotional Assessment and Strengths and Difficulties Questionnaire.

[b]Proportion of those with other injuries. Spinal fracture or cord injury is proportional to the total number of participants with spinal injuries (n=2).

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Table 4. Factors associated with abnormal 12m fatigue. Summary of biological (severity alone versus severity and biomarkers combined) variables entered into logistic regression to predict fatigue (n=87).

	TBI Severity Alone Model		TBI Severity and Biomarker Model[a]	
	OR (95% CI)	p *value*	OR (95% CI)	p *value*
TBI Injury severity (GCS classified)	3.677 (1.148 – 11.782)	0.028	2.583 (0.751 – 8.812)	0.132
Age at injury	1.102 (0.977 – 1.242)	0.114	1.139 (0.995 – 1.303)	0.059
Sex	4.116 (1.253 – 13.524)	0.020	3.092 (0.854 – 11.196)	0.086
Biomarker (IL8) pg/ml			1.044 (1.005 – 1.084)	0.027

TBI, Traumatic Brain Injury; GCS, Glasgow Coma Scale; OR, odds ratio; CI, confidence interval

[a]Likelihood ratio test results showed that adding biomarker IL8 in Model 2 resulted in a statistically significant improvement in model fit (p = 0.0145).

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Table 5. Factors associated with abnormal 12m fatigue. Summary of biological variables (severity alone, versus severity and biomarkers combined, with presence/absence of organ and intracranial injuries) entered into logistic regression to predict fatigue (n=87).

	TBI Severity Alone Model		TBI Severity and Biomarker Model[a]	
	OR (95% CI)	p *value*	OR (95% CI)	p *value*
TBI Injury severity (GCS classified)	3.114 (0.761 - 12.732)	0.114	2.198 (0.467 - 10.357)	0.319
Age at injury	1.090 (0.963 - 1.235)	0.174	1.138 (0.988 - 1.311)	0.073
Sex	4.771 (1.401 - 16.250)	0.012	3.166 (0.806 - 12.440)	0.099
Presence of other organ injuries	0.415 (0.098 - 1.769)	0.235	0.189 (0.032 - 1.111)	0.065
Presence of intracranial injuries	1.600 (0.380 - 6.745)	0.522	1.699 (0.355 - 8.123)	0.507
Biomarker (IL8) pg/ml			1.055 (1.013 – 1.098)	0.009

TBI, Traumatic Brain Injury; GCS, Glasgow Coma Scale; OR, odds ratio; CI, confidence interval

[a]Likelihood ratio test results showed that adding biomarker IL8 resulted in a statistically significant improvement in model fit (p = 0.0031).

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Figure legends

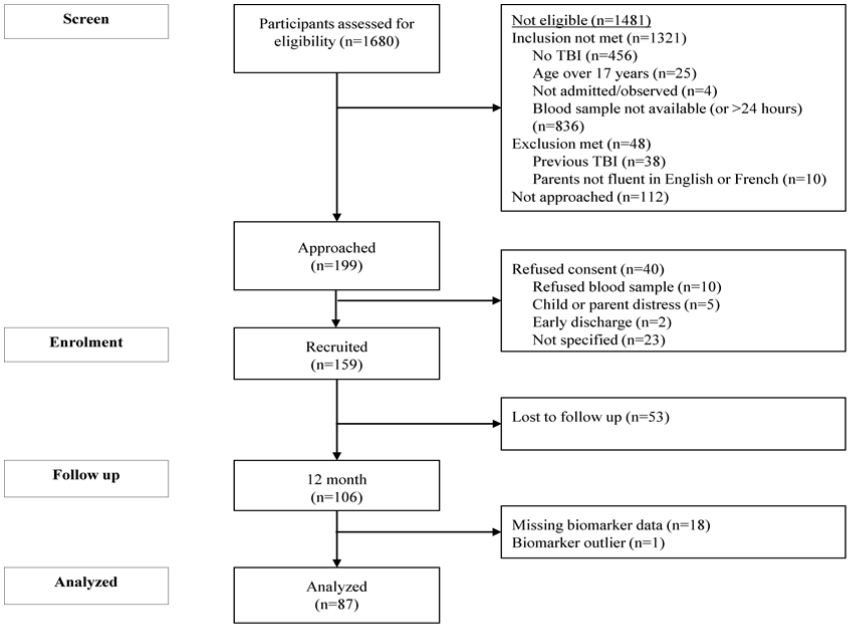

Figure 1. Study flow diagram of participants.

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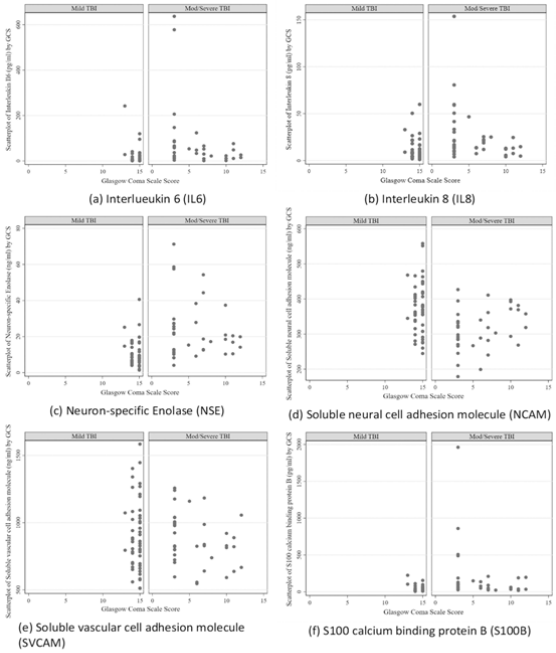

Figure 2. Scatterplot of serum biomarkers by Glasgow Coma Scale score (lowest on admission), by TBI severity group.

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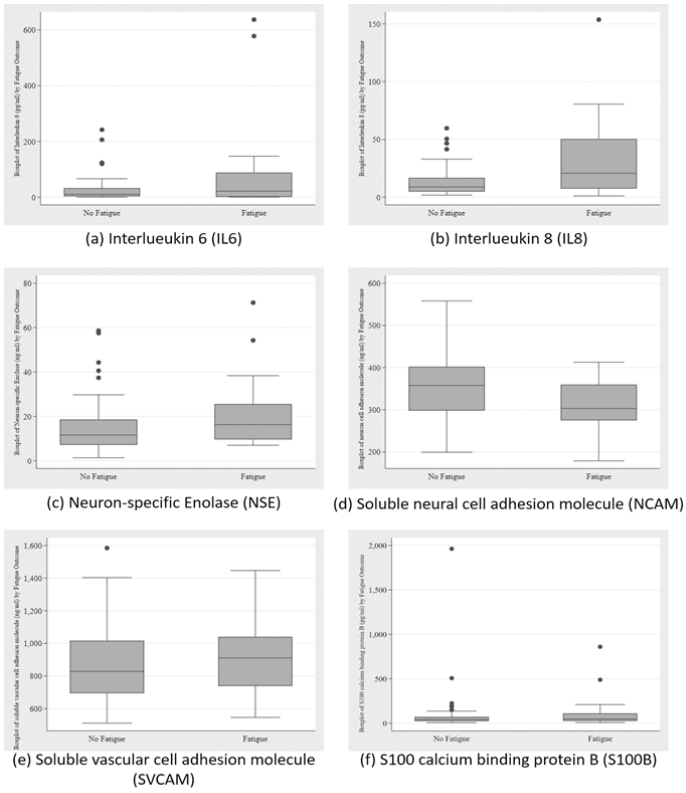

Figure 3. Boxplot of serum biomarker expression by fatigue outcome at 12 months post-injury.

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Supplementary Table 1. Bivariate logistical regression of biomarker expression against fatigue at 12 months.

Biomarker	Odds Ratio	95% CI	p
Il-6	1.007	1.000, 1.015	0.056
Il8	1.043	1.011, 1.076	0.007
NSE	1.031	0.995, 1.067	0.090
Ncam	0.991	0.983, 0.999	0.029
SVCAM	1.001	0.999, 1.003	0.480
S100B	1.001	0.999, 1.002	0.581

IL6, Interlueukin 6 (IL6); IL8, Interleukin 8; NSE, Neuron-specific Enolasse; NCAM, Neuron cell adhesion molecule, SVCAM, Soluble vascular cell adhesion molecule; S100B, S100 calcium binding protein B

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S100B – Human S100B ELISA by Millipore (EZHS100B-33K)

From the Millipore Protocol Booklet:

The limit of sensitivity of this assay is 2.7 pg/mL S100B (50 μL sample size).

1) The approximate range of this assay is 2.7 pg/mL to 2000 pg/mL S100B 50 μL sample size). Any result greater than 2000 pg/mL in a 50 μL sample should be diluted using Assay Buffer and repeated until the results fall within range.
2) **Sensitivity**: The lowest level of S100B that can be detected by this assay is 2.7 pg/mL using a 50 μL sample size, as derived from Statistical Ligand Immunoassay Analysis of multiple assays (n = 12) calculating the mean plus 2 standard deviations of the minimal detectable concentrations.
3) **Specificity**: The antibody pair used in this assay measures Human S100B and has no cross reactivity with S100A1, S100A6 and S100A13.
4) **Precision**: The assay variations of EMD Millipore S100B ELISA kits were studied on two samples at two levels on the S100B standard curve. The mean intra-assay variation was calculated from results of twenty-four determinations of the indicated samples. The mean inter-assay variations of each sample were calculated from results of six separate assays with duplicate samples in each assay

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Intra-Assay Variation

Sample Number	Mean S100B Levels (pg/mL)	Intra-Assay %CV
1	26.9	4.8
2	278.5	2.9

Inter-Assay Variation

Sample Number	Mean S100B Levels (pg/mL)	Intra-Assay %CV
1	28.5	4.4
2	280	1.9

NCAM and sVCAM-1 – Human Neurodegenerative Disease Magnetic Bead Panel 3 by Millipore (HNDG3MAG-36K)

The range of this assay is 61 pg/mL to 250,000 pg/mL sVCAM-1 and 24 pg/mL to 100,000 pg/mL NCAM.

1) **Sensitivity**: Minimum Detectable Concentration (MinDC) is calculated using the MILLIPLEX Analyst 5.1. It measures the true limits of detection for an assay by mathematically determining what the empirical MinDC would be if an infinite number of standard concentrations were run for the assay under the same conditions. MinSC+2SD = Minimum Detectable Concentration + 2 standard deviations.

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Analyte	Overnight Protocol (n=6 assays)	
	MinDC (pg/mL)	MinDC+2SD (pg/mL)
NCAM	4.81	13.48
sVCAM-1	6.44	12.24

Specificity: There was no or negligible cross-reactivity between the antibodies for an analyte and any of the other analytes in this panel.

2) **Precision**: Intra-assay precision is generated from the mean of the %CVs from 16 reportable results across two different concentrations of analytes in a single assay. Inter-assay precision is generated from the mean of the %CVs across two different concentrations of analytes across 10 different assays.

Analyte	Overnight Protocol	
	MinDC (pg/mL)	MinDC+2SD (pg/mL)
NCAM	3.5	4.9
sVCAM-1	2.8	7.3

IL6 and IL8 – Human Cytokine/Chemokine Magnetic Bead Panel by Millipore (HCYTOMAG-60K)

The range of this assay is 3.2 pg/mL to 10,000 pg/mL for both IL6 and IL8

1) **Sensitivity**: Minimum Detectable Concentration (MinDC) is calculated using MILLIPLEX® Analyst 5.1. It measures the true limits of detection for an assay by mathematically determining what the empirical MinDC would be if an infinite number of standard concentrations were run for the assay under the same conditions. MinSC+2SD = Minimum Detectable Concentration + 2 standard deviations.

Analyte	MinDC (pg/mL)	MinDC+2SD (pg/mL)
IL6	0.9	1.3
IL8	0.4	0.7

Specificity: There was no or negligible cross-reactivity between the antibodies and any of the other analytes in this panel.

2) **Precision**: Intra-assay precision is generated from the mean of the % CV's from sixteen reportable results across two different concentration of cytokines in a single assay. Inter-assay precision is generated from the mean of the % CV's from four reportable results across two different concentrations of cytokines across six different experiments.

Analyte	Intra-Assay %CV	Inter-Assay %CV
IL6	2.0	18.3
IL8	1.9	3.5

NSE – Human Cancer Metastasis Biomarker Bead Panel by Millipore (HCMBMAG-22K)

The range of this assay is 0.036 ng/mL to 150 ng/mL.

1) **Sensitivity**: Minimum Detectable Concentration (MinDC) is calculated using MILLIPLEX® Analyst 5.1. It measures the true limits of detection for an assay by mathematically determining what the empirical MinDC would be if an infinite number of standard concentrations were run for the assay under the same conditions. MinSC+2SD = Minimum Detectable Concentration + 2 standard deviations.

Analyte	MinDC+2SD (ng/mL)
NSE	0.011

Specificity: There was no or negligible cross-reactivity between the antibodies for an analyte and any of the other analytes in this panel.

2) **Precision**: Intra-assay precision is generated from the mean of the %CV's from 8 reportable results across two different concentrations of analytes in a single assay. Inter-assay precision is generated from the mean of the %CV's across two different concentrations of analytes across 5 different assays.

Analyte	Intra-Assay %CV	Inter-Assay %CV
NSE	9	15

Journal of Neurotrauma
Interleukin-8 Predicts Fatigue at 12 Months Post-injury in Children with Traumatic Brain Injury. (DOI: 10.1089/neu.2018.6083)
This paper has been peer-reviewed and accepted for publication, but has yet to undergo copyediting and proof correction. The final published version may differ from this proof.