Characterisation of serum total tau following paediatric traumatic brain injury: a case-control study

Sophie Stukas, Victoria Higgins, Helena Frndova, Jasmine Gill, Evyatar Hubara, Anne-Marie Guerguerian, Kathy Boutis, Miriam Beauchamp, Catherine Farrell, Franz E Babl, Carmel Delzoppo, Mardee Greenham, Amy A Wilkinson, Alison Crichton, Vicki Anderson, Khosrow Adeli, Jamie Hutchison, Cheryl Wellington, on behalf of the Serum Biomarkers and Quality of Life in Children with Traumatic Brain Injury investigators, the National Biobank and Database for Patients with Traumatic Brain Injury investigators, the Canadian Critical Care Translation Biology Group, and the Canadian Traumatic Brain Injury Research Consortium

Summary

Background Traumatic brain injury (TBI) is a major health problem in children. Blood-based biomarkers interpreted by use of normative values might improve the accuracy of diagnosis. Ultrasensitive assays can quantify serum concentrations of the neuronal microtubule-associated protein tau, which is increased in adult brains following TBI. We aimed to determine if serum total tau correlates with TBI diagnosis, severity, and radiological findings on CT scans in children younger than 18 years.

Methods In this case-control study, we included venous blood samples from healthy control children in the Canadian Laboratory Initiative on Pediatric Reference Intervals (CALIPER) biobank. For TBI cases, we recruited children (aged 0–17 years) who presented to the emergency department within 24 h of a TBI in three tertiary-care paediatric hospitals (Toronto, Vancouver, and Melbourne). Children were eligible if they required hospital observation for a minimum of 4 h or admission to the intensive care unit, and were excluded if they had had hospital treatment for a previous TBI, had birth trauma, or their parents could not speak English or French and therefore could not readily give consent. All available control samples were used and a case-control match was therefore not done. Venous and arterial blood samples were collected from patients with TBI within 28 h of injury (day 1). We used an ultrasensitive single-molecule immunoassay to measure serum total tau in blood samples. We first generated reference intervals of serum total tau from the control group, and used these normative data to interpret injury-associated changes in serum total tau in children with TBI. Concentrations of serum tau were measured in all CALIPER participants and patients with TBI, and no participants were excluded before analysis.

Findings We included samples from 416 control participants from the CALIPER cohort. Median total tau concentrations did not differ between sexes (p=0·12), but three significant reference intervals based on age groups were identified (1–3 years [0·88–19·2 pg/mL], 4–15 years [0·93–5·31 pg/mL], and 16–19 years [0·79–4·20 pg/mL]). Blood samples were obtained from 158 patients with TBI recruited between April 30, 2011, and June 28, 2013. Serum total tau on day 1 of TBI was negatively associated with Glasgow Coma Scale (GCS) score (r_s=–0·42, 95% CI –0·55 to –0·28, p<0·0001). Median total tau was 2·86 pg/mL (IQR 1·52–4·83) in patients with GCS score 13–15 points (n=114), 7·08 pg/mL (3·75–41·1) in those with GCS score 9–12 points (n=13), and 8·48 pg/mL (2·53–70·6) in those with GCS score 3–8 points (n=31). Notably, participants who had GCS scores of 15 points had median total tau concentrations (2·57 pg/mL [1·50–4·61]) indistinguishable from those of control participants (2·46 pg/mL [1·77–3·42]), whereas those with GCS score 13–14 points had elevated total tau (6·41 pg/mL [2·97–42·5]). Serum total tau was not strongly associated with CT findings in patients with mild TBI.

Interpretation Serum total tau might help to differentiate between patients with mild TBI (GCS 13–14 *vs* GCS 15), but larger studies are needed to validate these results before this biomarker can be used for diagnosis and prognosis.

Funding Canadian Institutes of Health Research, Ontario Neurotrauma Foundation, and Victoria Neurotrauma Foundation

Introduction

Traumatic brain injury (TBI) is the leading cause of death and acquired disability in children. In high-income countries, an estimated 691 in 100 000 children are treated in emergency departments because of TBI annually, 74 in 100 000 require hospital admission, and nine in 100 000 die.[1] Diagnosis and management of TBI in children can be challenging as their physiology, injury mechanism, clinical symptoms and signs, and risk of long-term sequelae and delayed recovery vary on the basis of age and developmental stage.[2,3] Although CT scans can provide rapid diagnosis of intracranial injuries, there is concern about exposure to radiation, the need for sedation in very young children, and overuse of the technique.[4]

Lancet Child Adolesc Health 2019

Published Online
June 20, 2019
http://dx.doi.org/10.1016/S2352-4642(19)30194-4

See Online/Comment
http://dx.doi.org/10.1016/S2352-4642(19)30200-7

Djavad Mowafaghian Centre for Brain Health and Department of Pathology and Laboratory Medicine, University of British Columbia, Vancouver, BC, Canada (S Stukas PhD, J Gill BMLSc, Prof C Wellington PhD); **Canadian Laboratory Initiative on Pediatric Reference Intervals Program, Department of Pediatric Laboratory Medicine and Department of Laboratory Medicine and Pathobiology, Faculty of Medicine** (V Higgins PhD, Prof K Adeli PhD) **and Institute of Medical Science and Interdepartmental Division of Critical Care** (A-M Guerguerian MD, Prof J Hutchison MD), **University of Toronto, Toronto, ON, Canada; Department of Critical Care Medicine** (H Frndova MEng, E Hubara MD, A-M Guerguerian, Prof J Hutchison), **Program in Neuroscience and Mental Health** (A-M Guerguerian, A A Wilkinson PhD, Prof J Hutchison), **and Division of Paediatric Emergency Medicine, Department of Paediatrics** (K Boutis MD), **Hospital for Sick Children, Toronto, ON, Canada; Department of Psychology, University of Montreal, Montreal, QC, Canada** (M Beauchamp PhD); **Research Centre** (M Beauchamp) **and Division of Paediatric Intensive Care, Department of Paediatrics** (C Farrell MD), **Sainte-Justine Hospital, Montreal, QC, Canada; Murdoch Children's Research Institute, Melbourne, VIC,**

Australia (F E Babl MD, C Delzoppo BHealthSc, M Greenham PhD, A Crichton PhD, Prof V Anderson PhD); **Department of Pediatrics** (F E Babl) **and School of Psychological Sciences** (Prof V Anderson), **University of Melbourne, Melbourne, VIC, Australia; Royal Children's Hospital, Melbourne, VIC, Australia** (F E Babl, C Delzoppo, Prof V Anderson); **Krembil Research Institute, Toronto Western Hospital, Toronto, ON, Canada** (A A Wilkinson); **and Department of Pediatrics, Monash University, Melbourne, VIC, Australia** (A Crichton)

Correspondence to: Prof Cheryl Wellington, Djavad Mowafaghian Centre for Brain Health, Department of Pathology and Laboratory Medicine, University of British Columbia, Vancouver, BC V6T 1Z3, Canada
wcheryl@mail.ubc.ca

Research in context

Evidence before this study

Children have a high incidence of traumatic brain injury (TBI) caused by falls, vehicle crashes, sport participation, and abuse. In preparation for this study, we searched for blood biomarker studies in paediatric TBI in National Centre for Biotechnology Information (USA) databases from Jan 1, 1990, to Sept 30, 2018, using the search terms "traumatic brain injury", "children OR pediatric", "blood OR serum OR plasma", and "biomarker". Studies were included if participants were aged between 0 and 19 years and one or more blood proteins or metabolites were measured. Studies were excluded if participants were older than 19 years, blood biomarkers were not measured, data were derived from animal models, or if they were not primary publications. Both the number of control participants (if present) and patients with TBI, and type of control group (ie, healthy or orthopaedic injury) were used to ascertain study quality. 165 publications were found, of which 64 remained after applying the exclusion criteria above. 51 studies focused solely on paediatric TBI, and 13 studies compared patients with TBI to control participants (two studies included control children with orthopaedic injury and 11 studies included healthy children as controls). 22 studies included 50 participants or fewer, 12 studies had between 51–100 participants, and 13 studies had more than 100 participants. However, only one study had more than 100 participants in each of the TBI and control groups. Therefore, there are few studies in children that compare a well powered TBI cohort with an equally well powered control group, and valid normative data for paediatric TBI blood biomarkers are not available.

Added value of this study

Our study used a rigorous approach to establish normative data for paediatric serum total tau concentrations that meet the guidelines of the US Clinical and Laboratory Standards Institute. We studied a well powered cohort of healthy children aged 1 to less than 19 years to guide interpretation of changes in serum total tau concentrations in children after TBI. Serum total tau varies by age but not by sex in children. After TBI, serum total tau concentrations were elevated in all patients with a Glasgow Coma Scale (GCS) score below 15 points, could discriminate between patients with mild TBI (GCS 13–14 *vs* GCS 15), and peaked on the first day of injury, except in patients with severe TBI, in whom it remained elevated for at least 7 days.

Implications of all the available evidence

Serum total tau could help to differentiate patients with various degrees of mild TBI, as its concentrations in patients with GCS scores of 15 points subjects were indistinguishable from those of control participants, whereas concentrations in patients with GCS scores of 13–14 were elevated. Serum total tau at day 1 was not useful for predicting positive radiological findings on CT scans. As serum total tau concentrations vary widely in children younger than 4 years, its diagnostic value for TBI might be weaker in infants and toddlers compared with school-aged children and adolescents.

Blood biomarkers can potentially assist in clinical prediction and decision making. Biomarkers indicative of neuronal (γ-enolase, also known as neuron-specific enolase [NSE], and ubiquitin carboxy-terminal hydrolase isozyme L1 [UCH-L1]), axonal (neurofilament light polypeptide [NF-L] and microtubule-associated protein tau), and astroglial (glial fibrillary acidic protein [GFAP] and the calcium-binding protein S100-B [S100B]) damage in TBI have been studied.[5,6] However, studies of TBI biomarkers in children lag behind studies in adults in both size and scope.[7,8] A particular challenge is the rapid development and maturation of the CNS during childhood and adolescence, which might affect baseline and post-injury biomarker concentrations.[3,9]

Serum total tau is increased after TBI in adults and might be associated with post-concussion symptoms.[10–15] However, there are no large-scale studies of serum total tau after paediatric TBI, nor have paediatric normative concentrations for serum total tau been established to guide post-TBI interpretation. We therefore aimed to establish valid reference intervals for a research assay of serum total tau for children aged 1–18 years, using healthy participants from the Canadian Laboratory Initiative on Pediatric Reference Intervals (CALIPER) cohort, to guide the interpretation of laboratory test results.[9,16] We used these reference intervals as a proxy of tau concentrations before injury to assess the diagnostic potential of serum total tau in a cohort of patients with TBI. We characterised the response of serum total tau on day 1 of injury in relation to TBI severity and describe its temporal profile in the first week after injury.

Methods

Study design and participants

In this case-control study, serum samples from healthy control participants were drawn from CALIPER, a Canada-wide, multicentre collaborative project coordinated by the Hospital for Sick Children (Toronto, ON, Canada),[9] as well as from the Hospital for Sick Children's core laboratory. CALIPER was initiated in 2008 and recruited healthy male and female children aged 1–18 years. For our study, we excluded CALIPER participants from the neonatal or paediatric intensive care units, those undergoing therapeutic drug monitoring and testing, and those who had any reported abnormal laboratory test results. Participants with a history of chronic illness or metabolic disease, acute illness (including mild TBI and concussion) within the past month, or prescribed medication over the past month

were excluded. Additional control samples were selected from specific outpatient clinics (eg, dentistry, fracture, or day surgery clinics) at the Hospital for Sick Children that see metabolically healthy children for the purpose of the patient visit, and were included in the pool of samples analysed by the hospital's core laboratory. Any blood tests ordered for these patients were reviewed to ensure the results were within the reference intervals for healthy individuals. These samples have been used for previously published CALIPER studies (appendix).[1,2]

TBI cases were obtained from the Serum Biomarkers and Quality of Life in Children with Traumatic Brain Injury (BTBI) study. Children with TBI were identified through screening of daily hospital admissions, and enrolled after informed or deferred consent by a parent or guardian was obtained on admission at one of three tertiary-care paediatric hospitals: the Hospital for Sick Children, Sainte-Justine Hospital (Montreal, QC, Canada), and the Royal Children's Hospital (Melbourne, VIC, Australia) using the Pediatric Research Academic Initiative in SickKids Emergency (PRAISE) training programme. Participants were followed up for 12 months after injury. Eligibility criteria were age from 0 to 17 years at the time of injury, presentation to the emergency department requiring observation for a minimum of 4 h, or admission to the intensive care unit, within 24 h of a clinically diagnosed TBI of any severity, and consent for blood sampling. Participants were excluded if they had a previous TBI that had required hospital admission, birth trauma (ie, injuries sustained during labour or delivery), or whose parents or guardians were not fluent in English or French.

The study protocol was approved by the ethics review board at each institution. The use of CALIPER and BTBI samples was approved by the University of British Columbia Clinical Research Ethics Board.[9] Research was completed in accordance with the Helsinki Declaration and in compliance with the standards of the Canadian and Australian National Research Councils.

Procedures

Information on patient demographics, Glasgow Coma Scale (GCS) score for consciousness, injury mechanisms, and physiological parameters were collected from children with TBI into a case-report form using standardised procedures. The maximum GCS score on hospital admission was used to classify TBI severity as per the US National Institutes of Health's Common Data Element recommendations: mild 13–15 points, moderate 9–12 points, and severe 3–8 points. CT of the head was ordered at the discretion of the treating physician. CT scans were interpreted by board-certified radiologists masked to study protocol. Radiology reports were uploaded to a central database, in which a second board-certified radiologist reviewed and coded the data. The main radiological findings of interest were presence of intracranial lesions, including intracranial haemorrhage (epidural, subdural, or subarachnoid), diffuse axonal injury, compression or trapping of a lateral ventricle, partial or complete effacement of the basal cisterns, midline shift, cerebral oedema, or contusion.

We analysed the time courses of total tau in children with mild, moderate, and severe TBI who had had repeat serum sampling to determine if tau concentrations followed different trajectories. In the subset of children with severe TBI in this sampling group, we also determined if these trajectories were different between those with suspected secondary brain injury and those without. To do this, we analysed their correlation with physiological and treatment intensity variables that are associated with secondary brain injury, including hypotension, hypoxia, seizures, high intracranial pressure, and intensity variables for intracranial pressure therapy, according to their relative risk of adverse effects as published by the Brain Trauma Foundation Severe TBI Management Guidelines.[17] Predefined threshold values used for the physiological variables are listed in the appendix.

See Online for appendix

Clinical blood samples (both venous and arterial) for quantification of serum total tau were collected from patients with TBI within 28 h of injury (day 1). Additional serum samples collected on days 2, 3, and sometimes 7 were also available for some participants depending on their time of discharge from hospital after injury. If blood was drawn for clinical reasons unrelated to the study, deferred consent was allowed for the storage of up to three blood samples. When consent was not obtained after the blood draw, samples were discarded. Venous blood samples were drawn for CALIPER participants. For all samples, blood was collected into serum separator tubes, allowed to clot for 30 min, centrifuged (4000–5000 rpm, 8–10 min), separated, and stored at –80°C within 5 h of collection.

Total tau concentration in serum was measured by single-molecule array (Simoa) research immunoassay on an HD-1 analyser (Quanterix; Lexington, USA) following the manufacturer's protocol (see appendix for details on quality control, lot use, and lot harmonisation).

Statistical analysis

The number of participants needed was determined using the US Clinical and Laboratory Standards Institute guidelines for establishing reference intervals, which require between 40 and 120 participants (appendix).[16] No participants were excluded for the primary analysis, but outliers were removed from the normative dataset when generating the reference intervals of serum total tau (appendix). The intervals represent the central 95% of analyte concentrations with 90% confidence within a normative population, and were established in accordance with guideline EP28-A3c (appendix). When establishing the intervals, we used the Tukey test to identify outliers for normally distributed data and the adjusted Tukey test for skewed data.

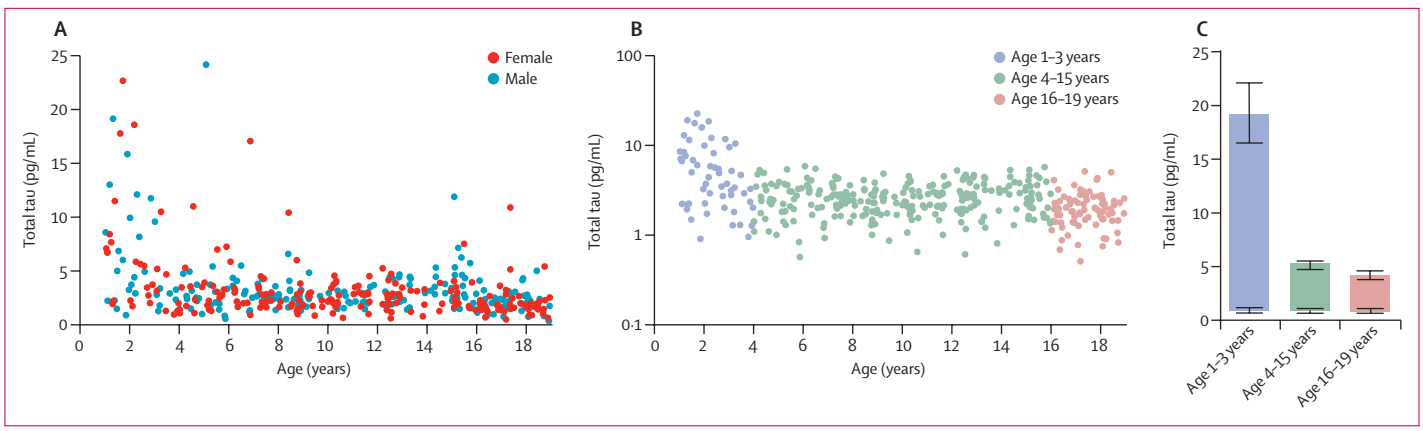

Figure 1: **Generation of reference intervals for serum total tau concentrations from control samples**
(A) Serum total tau concentrations in healthy children from the Canadian Laboratory Initiative on Paediatric Reference Intervals (CALIPER) cohort. (B) Three age partitions for serum total tau concentrations were generated and outliers removed. Outliers were removed using the Tukey test twice for normally distributed data and the adjusted Tukey test twice for skewed data. No partitions were required for sex. (C) Upper and lower limits (97·5th and 2·5th percentiles) and their respective 90% CIs for each reference interval of serum total tau were defined (appendix).

Descriptive statistics including means and SDs or medians and IQRs and frequency were used to describe continuous and categorical variables. We tested the association between serum total tau and demographic and injury characteristics using the Spearman's rank correlation test, Mann-Whitney test (two groups), and Kruskal-Wallis test (three or more groups) for continuous variables, or Fisher's exact test for categorical variables. Diagnostic accuracies were calculated using receiver operating characteristic (ROC) curves and the associated area under the curve (AUC). Where appropriate, ROC curves were built using logistic regression models. Serial tau samples were analysed using the Wilcoxon matched-pairs signed rank test or Friedman's test with Dunn's multiple comparison test.

All statistical tests were two-sided and a p value of less than 0·05 was considered significant. p values from analyses of secondary or subgroups comparisons are reported after correcting for multiple comparisons to limit type I errors. For comparisons of three or more groups of non-normally distributed data, we corrected for multiple comparisons using Dunn's test, following the group-wise Kruskal-Wallis test. No multiple comparisons were used for the area under the ROC (AUROC) analysis, when calculating reference intervals, and when comparing samples from CALIPER and outpatient participants.

Statistical analyses were done using Prism (version 7.03, GraphPad), SPSS (version 24), and R (version 3.5.1).

Role of the funding source

The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication. All authors approved the final version of the manuscript before submission.

Results

Participants were recruited between April 30, 2011, and June 28, 2013. Of 10 125 healthy children in the CALIPER cohort, 8628 blood samples were examined for eligibility and 4718 were confirmed to be eligible for analysis. Of these, 339 samples that were evenly distributed across the study age range and between sexes were randomly selected and included and analysed in this study. However, because we only had serum samples from one child younger than age 3 years and 23 children aged 3–5 years, additional residual samples from 77 children (39 boys and 38 girls) of these ages were collected from the Hospital for Sick Children's core laboratory (appendix). As no significant difference was detected in serum total tau concentrations in outpatients aged from 3 to 5 years versus those from the CALIPER cohort (appendix), the outpatients were merged with the CALIPER participants to form the control group (n=416, median age 10·4 years [IQR 6·2–15·40]), and 210 (51%) samples from boys and 206 (49%) samples from girls were used to establish reference intervals for serum total tau.

Median total tau in serum samples from all 416 participants in the control group was 2·54 pg/mL (IQR 1·78–3·56 pg/mL, range 0·36–24·2 pg/mL; figure 1). The Harris and Boyd method[8] for partitioning biochemical reference data into subgroups was used to test any significant differences in age and sex. Although total tau concentrations did not significantly differ between sexes (median for boys 2·67 pg/mL, IQR 1·96–3·67, and girls 2·31 pg/mL, 1·70–3·51; p=0·12), three age partitions were required (ie, 1–3 years, 4–15 years, and 16–19 years), each containing more than the required 40 participants per interval. The youngest age category represented the widest reference interval (0·88–19·2 pg/mL). Reference intervals were narrower for the middle (0·93–5·31 pg/mL) and oldest (0·79–4·2 pg/mL) age groups (figure 1, appendix).

 Published online June 20, 2019 http://dx.doi.org/10.1016/S2352-4642(19)30194-4

We screened 1680 children with TBI across our three sites. Of them, 199 met the eligibility criteria and were approached for consent. Consent was refused in 40 patients, and one patient who was enrolled did not have blood drawn for the study. Total tau was therefore measured in serum samples from day 1 from 158 paediatric patients with TBI (figure 2, table). Participants were divided into three groups on the basis of the severity of their TBI; the median age for those with mild TBI was 11·5 years (IQR 6·6–14·3), 5·0 years (2·2–14·3) for those with moderate TBI, and 5·0 years (2·2–14·3) for those with severe TBI. Within the TBI cohort, there was no effect of sex on serum total tau (median for boys 3·43 pg/mL IQR 1·57–6·30, and girls 3·04 pg/mL 1·70–14·2; p=0·91), age, or exact time of sample collection within the first 28 h (appendix). Serum total tau at day 1 in the TBI cohort was negatively associated with GCS score (r_s=–0·42, 95% CI –0·55 to –0·28, p<0·0001).

12 outlier patients were then excluded from the 4–15 years age group and five from the 16–19 years age group. After exclusion, there were significant differences in total tau concentrations between control CALIPER patients (n=399; median total tau 2·46 pg/mL, IQR 1·77–3·42, p<0·0001 for group-wise differences in GCS scores) and each of the three TBI groups stratified by GCS score: median total tau was 2·86 pg/mL (IQR 1·52–4·83) in patients with a GCS score of 13–15 points (n=114), 7·08 pg/mL (3·75–41·1) in those with a GCS score of 9–12 points (n=13), and 8·48 pg/mL (2·53–70·6) in those with a GCS score of 3–8 points (n=31; figure 3, appendix). Serum total tau was higher than the defined reference interval in 21 (18%) patients with GCS scores 13–15 points, 7 (54%) with 9–12 points, and 17 (55%) with 3–8 points.

As serum total tau concentrations were widely distributed within the mild TBI group and the spectrum of mild TBI is itself broad and poorly defined by GCS, we subdivided this group into those with GCS scores of 15 points (n=105) and those with 13–14 points (n=9). This division showed that the total tau concentrations in control participants were indistinguishable from patients with GCS scores of 15 points (median 2·57 pg/mL, IQR 1·50–4·61; figure 3). It also identified significant differences between patients in the GCS 13–14 group (6·41 pg/mL, 2·97–42·5, p=0·014) and either the control group or the GCS 15 group, and showed that serum total tau in 17 (16%) of patients in the GCS 15 group and four (44%) of those in the GCS 13–14 group was above the defined reference interval (figure 3, appendix).

The diagnostic accuracy of serum total tau for TBI was assessed using the AUROC adjusted bivariately for age (<4 years *vs* ≥4 years; figure 3, appendix). Compared with control participants, the AUC was 0·499 (95% CI 0·428–0·571, p=0·98) for those in the GCS 15 group, 0·787 (0·57–1·00, p=0·0036) for the GCS 13–14 group, 0·89 (0·790–0·999, p<0·0001) for the GCS 9–12 group, and 0·73 (0·60–0·869, p<0·0001) for the GCS 3–8 group. These data suggest that an injury threshold of a GCS score below 15 points is associated with increased serum total tau concentrations.

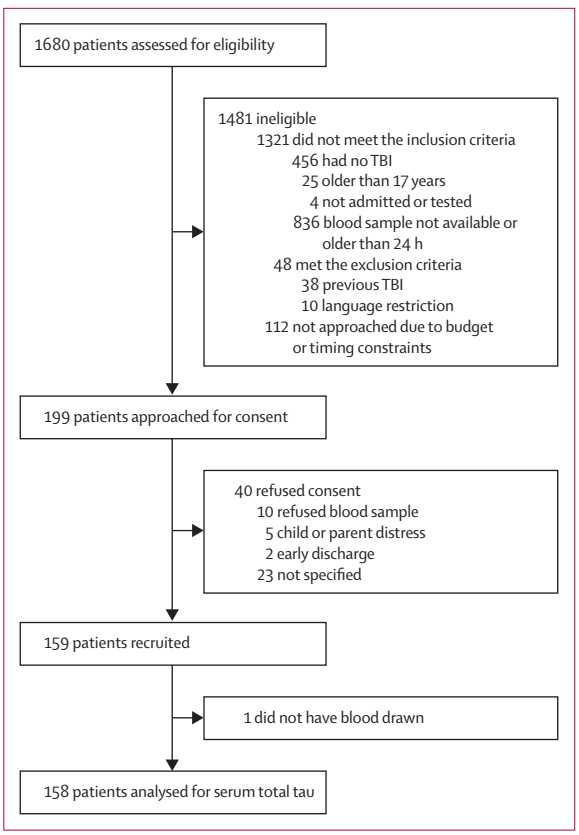

Figure 2: **Profile of patients with TBI recruited and screened through the Serum Biomarkers and Quality of Life in Children with TBI programme**
TBI=traumatic brain injury.

We next compared serum total tau concentrations in 61 patients in the GCS 13–15 group who had head CT scans to determine whether total tau was predictive of intracranial lesions (appendix). Median serum total tau was 4·48 pg/mL (IQR 2·35–12·2) in patients with abnormal CT findings and 2·89 pg/mL (1·55–4·36) in those with normal CT findings, with an AUROC of 0·649 (95% CI 0·49–0·81, p=0·061; figure 4).

As the change in biomarker concentrations over time might be more informative than a single measurement at one timepoint, we quantified serum total tau in a subset of 36 patients with TBI who had additional samples drawn on days 2, 3, and sometimes 7 after injury (table, appendix). In those with mild (n=6) and moderate (n=10) TBI, serum total tau concentrations consistently decreased over the first 2 days, with median percent changes of –53% (IQR –84% to –28%) in those with mild TBI and –70% (–78% to –19%) in those with moderate TBI (figure 5). By contrast, patients with severe TBI (n=20) showed a range of trajectories; total tau declined rapidly in four (20%), remained stable and within the

	Day 1 (n=158)	Multi-timepoint sample group (n=36)
Median age, years	10·6 (5·0–14·1)	8·1 (3·5–13·5)
Sex		
Male	117 (74%)	24 (67%)
Female	41 (26%)	12 (33%)
Median weight, kg	35 (18·0–59·8)	30 (15·5–60·0)
GCS score		
3–8	31 (20%)	20 (56%)
9–12	13 (8%)	10 (28%)
13–15	114 (72%)	6 (17%)
Mechanism of injury*		
Motor vehicle accident	35 (22%)	13 (36%)
Bicycle	14 (9%)	5 (14%)
Fall	59 (37%)	8 (22%)
Sport	34 (22%)	5 (14%)
Other	18 (11%)	6 (17%)
Neurosurgery†	17 (11%)	8 (22%)
Total Pediatric Trauma Score	10 (7–11)	6 (3–8)
Associated major trauma		
Head	142 (90%)	34 (94%)
Spine	6 (4%)	4 (11%)
Thorax	13 (8%)	4 (11%)
Cardiovascular	1 (1%)	1 (3%)
Abdomen	7 (4%)	2 (6%)
Genitourinary	4 (3%)	3 (8%)
Pelvic or long bone fracture	41 (26%)	17 (47%)
Other limb injury	2 (1%)	2 (6%)
Admission CT findings		
Participants with scan and complete data set‡	97 (61%)	30 (83%)
Epidural haemorrhage	7 (77%)	2 (7%)
Subdural haemorrhage	33 (34%)	16 (53%)
Subarachnoid haemorrhage	18 (19%)	7 (23%)
Diffuse axonal injury	3 (3%)	2 (7%)
Lateral ventricle	10 (10%)	6 (20%)
Basal cistern	7 (7%)	5 (17%)
Grey or white matter difference	11 (11%)	5 (17%)
Midline shift	12 (12%)	6 (20%)
Oedema	16 (16%)	9 (30%)
Contusion	25 (26%)	10 (33%)
Skull or facial fracture	45 (46%)	18 (60%)
Serum sample information		
Median day 1 post-TBI collection time, h	6·8 (3·8–17)	10·3 (5·8–16·7)
Multipoint sample draws		
Median number of samples per participant (range)	NA	2 (2–4)
Day 2 post-TBI	..	32 (89%)
Day 3 post-TBI	..	19 (53%)
Day 7 post-TBI	..	6 (17%)

Data are number (%) or median (IQR), unless otherwise indicated. TBI=traumatic brain injury. GCS=Glasgow Coma Scale. NA=not applicable. *Values may not add up to 100% because of rounding. †Neurosurgery includes placement of extraventricular cerebrospinal fluid drain or placement of intraparenchymal pressure monitor, craniectomy, and craniotomy and evacuation. ‡A total of nine participants at day 1 and three participants with more than one sample had CT scans performed but incomplete or missing forms.

Table: **Demographics and injury characteristics of participants with TBI**

reference interval range in ten (50%), or was above the reference interval and occasionally rising in six (30%) of patients. We then determined whether the trajectory of serum total tau in children with severe TBI was associated with secondary brain injury including intracranial hypertension, hypoxia, and hypotension. Patients with severe TBI were divided into those who had consistently increased total tau (n=6) and those with low or rapidly decreasing total tau (n=14; appendix). The only significant association was with increased hypoxia in patients with increased total tau (four [67%]) compared with those in the group with low or decreasing tau (two [14%], p=0·038; appendix).

Discussion

To our knowledge, this is the first large-scale study to report on serum total tau concentrations in both healthy children and children with TBI. We show that serum total tau decreases with age, with three significant age partitions in healthy children: from 1 to less than 4 years, 4 to less than 16 years, and from 16 to less than 19 years. Serum total tau was significantly increased following TBI in paediatric patients with a GCS score below 15 points, suggesting that the release of tau from the CNS into the blood is increased, or its clearance is impaired, following an injury threshold.

Generating valid normative data is a crucial step in the analytical and clinical validation of biomarkers. Patients in control groups for TBI studies are often drawn from pools of patients with orthopaedic trauma, routine outpatients, or healthy family members, and in the case of sports-related studies, pre-season baseline assessments for concussion-related endpoints. Additionally, the relatively small sample sizes (20–50 participants) of these cohorts might not represent true population norms. Representative sampling is especially important in paediatrics to account for growth and development. One well recognised example of an age-dependent biomarker is S100B; serum or plasma concentrations of S100B negatively correlate with age in children but not in adults, with the highest concentrations detected in the first 1–2 years of life.[18–21] A similar association has also been described for UCH-L1 in children.[21] In this study, we show that serum total tau is also inversely correlated with age, with the most marked changes occurring in the first 4 years of life, potentially because of synaptic pruning or axonal development that occurs in early childhood. Whether there are differences in the phosphorylation or truncation of developmental versus neurodegenerative total tau needs to be investigated. Although modelling can be used to correct for age-dependent effects, the high and variable nature of these blood biomarkers in healthy infants and toddlers, together with the additional challenge of accurately measuring severity of injury in this age group, might hinder their clinical usefulness for TBI.

The advent of ultrasensitive techniques such as Simoa and rolling circle amplification enhanced immunoassay

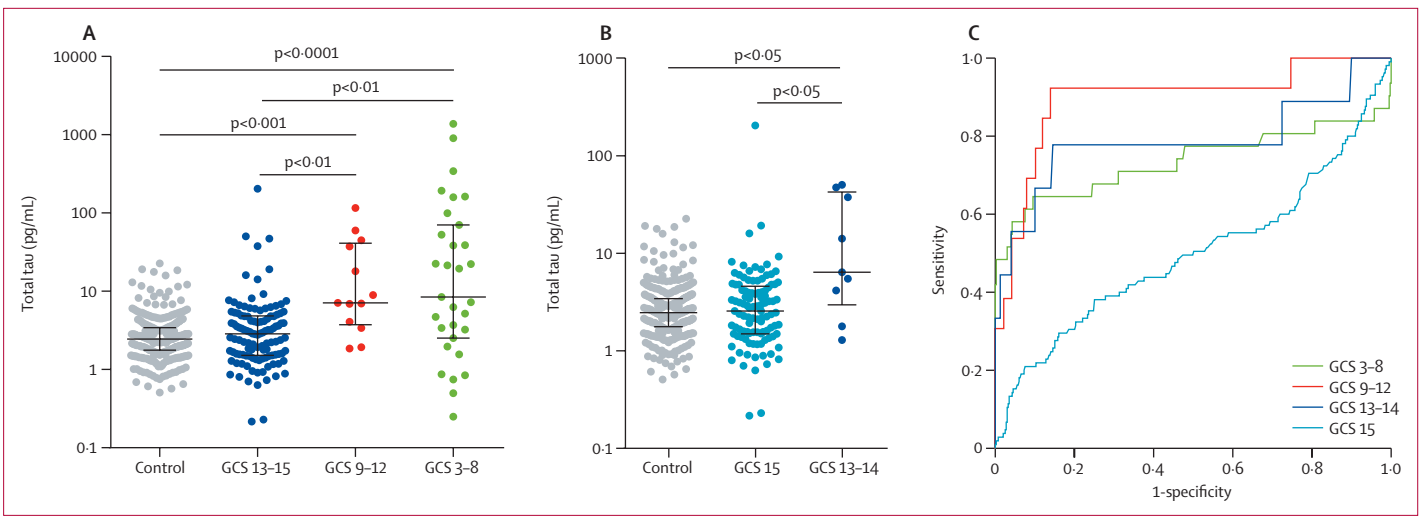

Figure 3: **Serum total tau concentrations in control participants and patients with acute TBI**
(A) Serum total tau concentrations from CALIPER participants and patients with TBI divided by injury severity on the basis of GCS scores. (B) Differences in concentrations of serum total tau between subgroups of mild TBI (GCS 15 and GCS 13–14) and control. The median and the first and third quartiles (error bars) are shown. (C) Multivariate receiver operating characteristic curves for serum tau concentrations in subgroups by TBI severity compared with control participants generated from logistic regression models corrected for age (<4 years *vs* ≥4 years; appendix). Significance was calculated with the Kruskal-Wallis test and Dunn's multiple comparison test, which does not report specific p values. Comparisons that are not illustrated were not significant as reported by Dunn's test. CALIPER=Canadian Laboratory Initiative on Paediatric Reference Intervals. GCS=Glasgow Coma Scale. TBI=traumatic brain injury.

using multi-arrayed fiberoptics (a-EIMAF) has facilitated many studies on total tau from blood as a biomarker of neurological injury and disease.[22,23] With the exception of one small study using conventional ELISA,[24] our study represents the first major investigation of serum total tau in children with and without TBI. We show that serum total tau at day 1 after injury is significantly increased in patients with a GCS score lower than 15; however, as 28 (53%) of these individuals ranked above the reference interval for their age group, there was significant overlap between control and TBI groups. Although our results are consistent with findings for TBI in adults,[12,14,15] the magnitude of the total tau response might differ between adults and children. For example, the TRACK-TBI study[15] of 196 adults with TBI (82% had mild TBI) detected a 15–25% increase of plasma total tau compared with 20 healthy control participants during the acute phase of injury,[15] whereas we report that median serum total tau at day 1 was three times higher in patients with GCS scores lower than 15 than in control participants (an increase of 15 times in the 75th percentile). Guzel and colleagues[24] reported an increase of 2–2·5 times in serum total tau at day 1 after injury in patients with GCS 14–15 over controls.[24] It is probable that study results will depend on the specific assay platform used, as the antigen standards, antibodies, and techniques will vary; this caveat is highlighted by the difference in absolute total tau concentrations and magnitude of response following injury in studies that have used a-EIMAF[15,25] versus Simoa.[10–13]

Blood biomarkers are entering into clinical use for adult patients with TBI, with the introduction of S100B to the Scandinavian Guidelines[26] in 2013 and the 2018 US Federal Drug Administration's approval of GFAP and UCH-L1 on the basis of the ALERT-TBI study[27] for reducing the unnecessary use of CT head scans.[5] For children, three clinical decision rules derived from large multicentre studies in the USA and Canada and the UK have been developed to identify which patients with mild TBI should have a head CT.[4] Although elements of the Canadian Assessment of Tomography for Childhood Head Injury are in the Canadian Paediatric Society's position statement, in Australia, from where most of the CT data in our study are derived, no clinical decision

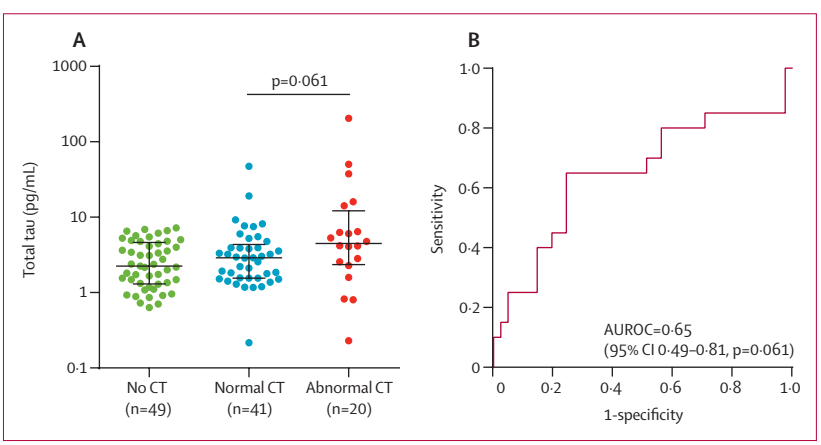

Figure 4: **Serum total tau concentrations in patients with mild TBI with normal and abnormal CT scans**
(A) Serum total tau in patients with mild TBI (GCS 13–15) with normal CT scans, abnormal CT scans (ie, presence of intracranial lesions), and those for whom a CT scan was not done (excluding four patients who had missing or incomplete scan data). Median, first, and third quartiles are shown. Statistical significance is based on the Mann-Whitney test. (B) ROC curve showing the ability of serum tau concentrations to distinguish between normal and abnormal CT scans among patients with mild TBI. Additional data for these groups of patients are shown in the appendix. GCS=Glasgow Coma Scale. ROC=receiver operating characteristic. TBI=traumatic brain injury. AUROC=area under the receiver operating characteristic curve.

For the **Canadian Assessment of Tomography for Childhood Head Injury** see https://www.mdcalc.com/catch-canadian-assessment-tomography-childhood-head-injury-rule

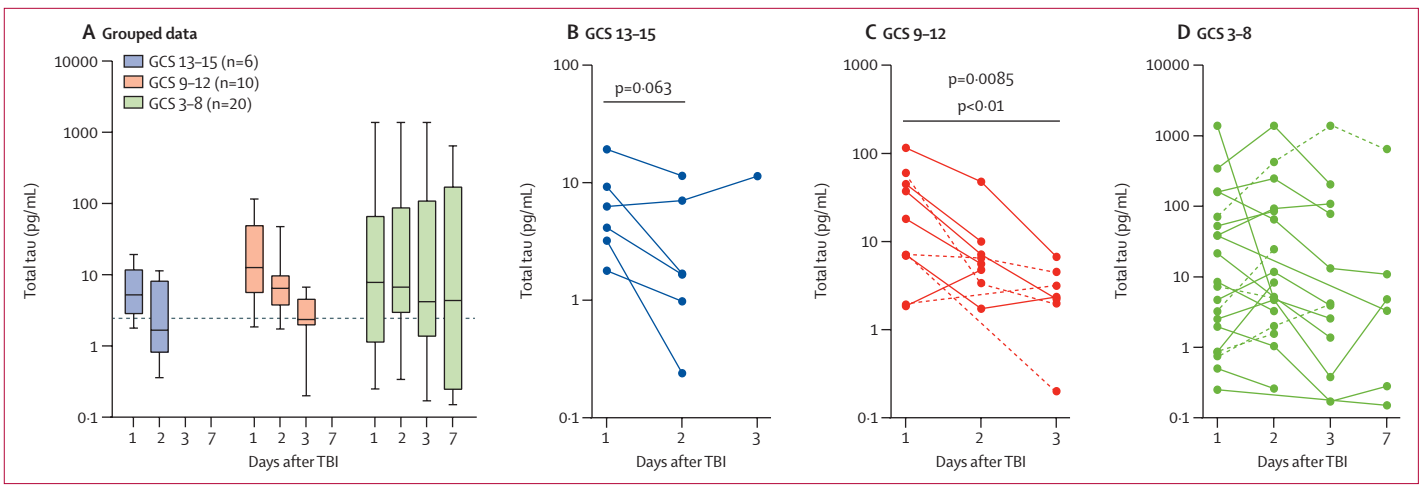

Figure 5: **Trajectory of serum total tau in the first week after TBI, by injury severity**
(A) Concentration of serum total tau after TBI in 36 patients. Dashed black line represents control median concentrations of serum total tau. Bars represents median, first, and third quartiles, with whiskers at the minimum and maximum values. (B–D) Linked samples showing individual trajectories over time. Dashed lines denote participants aged under 4 years. (B) Six samples from patients with GCS scores 13–15 at days 1 and 2 were analysed using a Wilcoxon matched-pair signed rank test. (C) Five of ten GCS 9–12 patients and (D) 11 of 20 GCS 3–8 patients with samples from days 1, 2, and 3 were analysed using a Friedman's test and Dunn's multiple comparison test to estimate a trajectory for serum total tau concentrations. GCS=Glasgow Coma Scale. TBI=traumatic brain injury.

rules predominate.[4] Consistent with published studies in adults,[14,15,25] serum total tau did not show any positive predictive value for the presence of intracranial lesions on CT scans. However, the use of CT was not within the screening or inclusion criteria and thus there is a potential for selection or ascertainment bias with respect to CT imaging. Whether serum biomarkers can add value to the current clinical decision rules to reduce CT use remains an important question.

Finally, we evaluated the pattern of serum total tau over the first week after TBI in a subset of patients, as biomarker trajectory might be more informative than single-point data.[6] Peak serum total tau occurred on day 1 after injury in patients with mild and moderate TBI, with a rapid decline by day 2, following a similar pattern to data reported for S100B, NSE, GFAP, and UCH-L1.[6] For patients with severe TBI, however, serum total tau did not follow a uniform trajectory. Concentrations of total tau steadily increased over time in only six (30%) of 20 patients with severe TBI who had undergone repeated sampling, which was consistent with a previous report[12] of sustained elevations in plasma total tau in adult patients with TBI followed up for up to 90 days. Future studies with larger cohorts of patients with severe TBI will be required to determine if total tau concentrations or differences in trajectory are associated with clinical management, physiological parameters, at-risk genotypes, or are predictive of outcome.

Strengths of our study include well powered case and control groups, and the ability to accurately measure total tau in 630 of 631 serum specimens using the Quanterix Advantage total tau singleplex ultrasensitive assay.

Our study has several limitations. Control and TBI samples were collected through independent studies with no control samples from participants younger than 1 year. Outpatient control samples from young children were used to supplement CALIPER samples, so that only 339 (82%) of 416 control samples were collected from healthy children in the community. The outpatient control samples were not specifically excluded for orthopaedic injury, which might have affected total tau concentrations. It will be important for future neurotrauma studies to exclude orthopaedic injury cases from CALIPER. Additionally, CALIPER does not specifically collect data on exposure to acute illness (including mild TBI and concussion); it is therefore possible that some CALIPER control participants might have had mild TBI that did not require hospital admission or assessment in the emergency department, or for which medical attention was not sought within the month preceding blood collection. The ethnicity of CALIPER participants is representative of the Canadian population but not specifically addressed in our study. The ethnicity of the patients who provided the case samples is also not available; however, 60 (38%) of 158 case samples are from patients of Canadian origin and therefore are expected to correspond to the ethnic distribution of CALIPER participants. The remaining 92 (62%) case samples are from Australian patients with unknown correlation with the CALIPER ethnicity distribution.

The case sample size is significantly smaller for patients with moderate and severe TBI, reflecting the natural incidence of this injury type. Control and TBI samples were assayed with separate lots of reagents in a research-use-only assay, which is not yet available for clinical use, although the variation in assay performance was less than 20% and there was significant overlap between the readings for control participants and patients with GCS scores of 15. This limitation can be addressed by using a single assay lot wherever possible, as well as validated reference materials and increased sample sizes to bridge assay lots for consistency, endeavours that were beyond

the scope of our study. This study is restricted to a single biomarker, serum total tau, in children with TBI compared with valid normative data from healthy children, with insufficient granularity within the first 28 h of injury and data beyond day 1 after injury. Future studies using a similarly rigorous approach and larger cohorts are needed to validate our results, particularly for total tau trajectories during and beyond the first day of injury, and to identify additional biomarkers for diagnosis and prognosis. Finally, future studies could consider plasma rather than serum samples, as total tau concentrations are higher in adult plasma compared with serum.[28]

Contributors

AMG, KB, MB, CF, FB, CD, MG, VA, and JH were responsible for the design of the case study, its conduct (participant accrual, screening, enrolment, sample collection), and coordination or were the site principal investigator. KA and VH were responsible for the design of the CALIPER study and its conduct. SS, KA, JH, and CW conceived and designed the tau biomarker study. SS, EH, KB, CD, MG, AW, AC, VH, KA, JH were responsible for data collection; SS, VH, JG, and JH were responsible for data analysis; and SS, AMG, KB, KA, JH and CW were responsible for data interpretation. SS, JH, and CW wrote the initial draft of the manuscript.

Declaration of interests

We declare no competing interests.

Data sharing

Consent forms, case report forms, and the procedures manual for the BTBI study are available on request. The BTBI database containing de-identified data is planned to be housed in the Brain-CODE bioinformatics platform (Ontario Brain Institute, Ontario, ON, Canada) after publication of primary papers from the BTBI study, where it will be available to other researchers upon receipt and approval of database sharing protocols with complete consensus by the BTBI Steering Committee.

Acknowledgments

We thank Douglas Fraser and Francois Lauzier for critically reviewing the manuscript on behalf of the Canadian Critical Care Translation Biology Group and Canadian Traumatic Brain Injury Research Consortium (CTRC). The Canadian Traumatic Brain Injury Research Consortium programme is supported by a project grant (353989) to KA from the Canadian Institutes of Health Research (CIHR); VH is supported by a Doctoral Award from CIHR. The CTRC (JH, CW) is supported by an operating grant from the CIHR (TBI-144225) and Ontario Neurotrauma Foundation (ONF) (2015-ABI-CTRC-1009). BTBI was supported by a project grant from ONF (2006-ABI-COMOR-44) and a joint grant from the ONF and the Victoria Neurotrauma Foundation (2010-VNIDCP08–817).

References

1 Thurman DJ. The epidemiology of traumatic brain injury in children and youths: a review of research since 1990. *J Child Neurol* 2016; **31:** 20–27.

2 Keenan HT, Presson AP, Clark AE, Cox CS, Ewing-Cobbs L. Longitudinal developmental outcomes after traumatic brain injury in young children: are infants more vulnerable than toddlers? *J Neurotrauma* 2019; **36:** 282–92.

3 Anderson V, Spencer-Smith M, Wood A. Do children really recover better? Neurobehavioural plasticity after early brain insult. *Brain* 2011; **134:** 2197–221.

4 Babl FE, Borland ML, Phillips N, et al. Accuracy of PECARN, CATCH, and CHALICE head injury decision rules in children: a prospective cohort study. *Lancet* 2017; **389:** 2393–402.

5 Mondello S, Sorinola A, Czeiter E, et al. Blood-based protein biomarkers for the management of traumatic brain injuries in adults presenting to emergency departments with mild brain injury: a living systematic review and meta-analysis. *J Neurotrauma* 2018; published online July 2. DOI:10.1089/neu.2017.5182.

6 Thelin EP, Zeiler FA, Ercole A, et al. Serial sampling of serum protein biomarkers for monitoring human traumatic brain injury dynamics: a systematic review. *Front Neurol* 2017; **8:** 300.

7 Papa L, Ramia MM, Kelly JM, Burks SS, Pawlowicz A, Berger RP. Systematic review of clinical research on biomarkers for pediatric traumatic brain injury. *J Neurotrauma* 2013; **30:** 324–38.

8 Lugones M, Parkin G, Bjelosevic S, et al. Blood biomarkers in paediatric mild traumatic brain injury: a systematic review. *Neurosci Biobehav Rev* 2018; **87:** 206–17.

9 Adeli K, Higgins V, Trajcevski K, White-Al Habeeb N. The Canadian laboratory initiative on pediatric reference intervals: a CALIPER white paper. *Crit Rev Clin Lab Sci* 2017; **54:** 358–413.

10 Shahim P, Tegner Y, Wilson DH, et al. Blood biomarkers for brain injury in concussed professional ice hockey players. *JAMA Neurol* 2014; **71:** 684–92.

11 Olivera A, Lejbman N, Jeromin A, et al. Peripheral total tau in military personnel who sustain traumatic brain injuries during deployment. *JAMA Neurol* 2015; **72:** 1109–16.

12 Bogoslovsky T, Wilson D, Chen Y, et al. Increases of plasma levels of glial fibrillary acidic protein, tau, and amyloid beta up to 90 days after traumatic brain injury. *J Neurotrauma* 2017; **34:** 66–73.

13 Gill J, Merchant-Borna K, Jeromin A, Livingston W, Bazarian J. Acute plasma tau relates to prolonged return to play after concussion. *Neurology* 2017; **88:** 595–602.

14 Gill J, Latour L, Diaz-Arrastia R, et al. Glial fibrillary acidic protein elevations relate to neuroimaging abnormalities after mild TBI. *Neurology* 2018; **91:** e1385–89.

15 Rubenstein R, Chang B, Yue JK, et al. Comparing plasma phospho tau, total tau, and phospho tau-total tau ratio as acute and chronic traumatic brain injury biomarkers. *JAMA Neurol* 2017; **74:** 1063–72.

16 Henny J, Vassault A, Boursier G, et al. Recommendation for the review of biological reference intervals in medical laboratories. *Clin Chem Lab Med* 2016; **54:** 1893–900.

17 Brain Trauma F, American Association of Neurological S, Congress of Neurological S, et al. Guidelines for the management of severe traumatic brain injury. Introduction. *J Neurotrauma* 2007; **24**(suppl 1)**:** S1–2.

18 Portela LV, Tort AB, Schaf DV, et al. The serum S100B concentration is age dependent. *Clin Chem* 2002; **48:** 950–52.

19 Spinella PC, Dominguez T, Drott HR, et al. S-100beta protein-serum levels in healthy children and its association with outcome in pediatric traumatic brain injury. *Crit Care Med* 2003; **31:** 939–45.

20 Gazzolo D, Michetti F, Bruschettini M, et al. Pediatric concentrations of S100B protein in blood: age- and sex-related changes. *Clin Chem* 2003; **49:** 967–70.

21 Mondello S, Kobeissy F, Vestri A, Hayes RL, Kochanek PM, Berger RP. Serum concentrations of ubiquitin c-terminal hydrolase-l1 and glial fibrillary acidic protein after pediatric traumatic brain injury. *Sci Rep* 2016; **6:** 28203.

22 Bogoslovsky T, Gill J, Jeromin A, Davis C, Diaz-Arrastia R. Fluid biomarkers of traumatic brain injury and intended context of use. *Diagnostics* 2016; **6:** e37.

23 Zetterberg H, Blennow K. From cerebrospinal fluid to blood: the third wave of fluid biomarkers for Alzheimer's disease. *J Alzheimer Dis* 2018; **64**(suppl1)**:** S271–79.

24 Guzel A, Karasalihoglu S, Aylanc H, Temizoz O, Hicdonmez T. Validity of serum tau protein levels in pediatric patients with minor head trauma. *Am J Emerg Med* 2010; **28:** 399–403.

25 Gardner RC, Rubenstein R, Wang KKW, et al. Age-related differences in diagnostic accuracy of plasma glial fibrillary acidic protein and tau for identifying acute intracranial trauma on computed tomography: a TRACK-TBI study. *J Neurotrauma* 2018; **35:** 2341–50.

26 Unden J, Ingebrigtsen T, Romner B, Scandinavian Neurotrauma C. Scandinavian guidelines for initial management of minimal, mild and moderate head injuries in adults: an evidence and consensus-based update. *BMC Med* 2013; **11:** 50.

27 Bazarian JJ, Biberthaler P, Welch RD, et al. Serum GFAP and UCH-L1 for prediction of absence of intracranial injuries on head CT (ALERT-TBI): a multicentre observational study. *Lancet Neurol* 2018; **17:** 782–89.

28 Keshavan A, Heslegrave A, Zetterberg H, Schott JM. Stability of blood-based biomarkers of Alzheimer's disease over multiple freeze-thaw cycles. *Alzheimer Dement* 2018; **10:** 448–51.