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Performance of glial fibrillary acidic protein (GFAP) and ubiquitin carboxy-terminal hydrolase L1 (UCH-L1) biomarkers in predicting CT scan results and neurological outcomes in children with traumatic brain injury (BRAINI-2 paediatric study): protocol of a European prospective multicentre study

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ABSTRACT

Introduction In light of the burden of traumatic brain injury (TBI) in children and the excessive number of unnecessary CT scans still being performed, new strategies are needed to limit their use while minimising the risk of delayed diagnosis of intracranial lesions (ICLs). Identifying children at higher risk of poor outcomes would enable them to be better monitored. The use of the blood-based brain biomarkers glial fibrillar acidic protein (GFAP) and ubiquitin carboxy-terminal hydrolase-L1 (UCH-L1) could help clinicians in this decision. The overall aim of this study is to provide new knowledge regarding GFAP and UCH-L1 in order to improve TBI management in the paediatric population.

Methods and analysis We will conduct a European, prospective, multicentre study, the BRAINI-2 paediatric study, in 20 centres in France, Spain and Switzerland with an inclusion period of 30 months for a total of 2880 children and adolescents included. To assess the performance of GFAP and UCH-L1 used separately and in combination to predict ICLs on CT scans (primary objective), 630 children less than 18 years of age with mild TBI, defined by a Glasgow Coma Scale score of 13–15 and with a CT scan will be recruited. To evaluate the potential of GFAP and UCH-L1 in predicting the prognosis after TBI (secondary objective), a further 1720 children with mild TBI but no CT scan as well as 130 children with moderate or severe TBI will be recruited. Finally, to establish age-specific reference values for GFAP and UCH-L1 (secondary objective), we will include 400 children and adolescents with no history of TBI.

Ethics and dissemination This study has received ethics approval in all participating countries. Results from our study will be disseminated in international peer-reviewed journals. All procedures were developed in order to assure data protection and confidentiality.

Trial registration number NCT05413499.

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ The multicentre design of the study with broad prospective recruitment in several European countries and the wide spectrum of clinical presentations of the patients included will ensure the generalisability of our findings.
⇒ The precision of the primary outcome measurement will be ensured through evaluation of CT images by two independent neuroradiological experts.
⇒ The follow-up of the included children will enable confirmation of the absence of intracranial lesions, which may have been detected postdischarge from the emergency department.
⇒ As performing a CT scan for patient management is not mandatory as an inclusion criterion, not all children included will ultimately be considered in the main analysis, as the primary outcome measure will be the CT scan result.

INTRODUCTION

Background and rationale

Mild traumatic brain injury (mTBI) in children is a frequent reason for paediatric emergency department (ED) visits[1] and a rare cause of acute complications: of the children who undergo a CT scan in the ED, between

5% and 10% exhibit intracranial lesions (ICLs) on the CT scan and less than 1% require neurosurgical intervention.[2 3] Although ICL remains a serious complication requiring rapid diagnosis, physicians should not routinely order a head CT scan, as this would unnecessarily expose a large number of children to ionising radiation associated with an increased risk of cancer.[4 5] Moreover, in light of the high prevalence of mTBI,[6] the ED would become overcrowded and CT overused. To assist physicians in their decision-making, several clinical decision rules have been proposed in recent years[7–9] with the aim of identifying children at higher or lower risk of ICL in order to better target CT scan indications. However, the rate of CT scans performed has remained high, up to 35%, and has not decreased with application of the clinical decision rules.[10–12] Aside from the presence of ICLs in the acute period, other complications such as headaches, dizziness, asthenia, memory, concentration and sleep disorders can occur after an mTBI. These postconcussion symptoms (PCS) are still present in approximately 30% of children more than a month after injury,[13 14] with a possible impact on their quality of life.[15] Although some risk factors for prolonged PCS have been identified, such as being an adolescent[13 16] or a girl,[16] a history of concussion[13] or reporting more acute concussion symptoms,[17 18] no single factor can predict the recovery or neurological outcome.[19] Thus, knowing when to scan or not and when to closely monitor a child after an mTBI remains a challenge for clinicians. Despite the incorporation of anamnestic and clinical parameters into conventional clinical decision rules, their efficacy in achieving this objective is insufficient.[10–12 19] Developing new strategies to reduce CT utilisation while minimising the risk of delayed ICL diagnosis, and to identify children at higher risk of poor outcomes or PCS is imperative.

One of the most promising approaches in this context is the utilisation of blood-based brain biomarkers. Several biomarkers have been identified in recent years, such as calcium channel binding protein S100 subunit beta (S100B), glial fibrillar acidic protein (GFAP) and ubiquitin carboxy-terminal hydrolase-L1 (UCH-L1),[20 21] which have blood concentration kinetics compatible with the time required to manage paediatric mTBI patients in ED. The latter two have the advantage over S100B that they can be measured over a longer time frame after the TBI and are not influenced by skin pigmentation and multiple traumas.[22 23] GFAP is a cytoskeletal protein belonging to the class of intermediate filaments mostly specific to astrocytes, and UCH-L1 is a proteolytically stable and abundant protein found almost exclusively in the cytoplasm of neurons and involved in the ubiquitinylation and deubiquitinylation of certain proteins destined to be degraded by the proteasome.[24] In mTBI, both GFAP and UCH-L1 are detectable within 1 hour of injury. GFAP peaks at 20 hours after injury and slowly declines over 72 hours. UCH-L1 increases rapidly, peaks at 8 hours after injury and declines rapidly over 24 hours. In adults, previous studies have shown the values of these biomarkers as predictors of ICL, with significantly higher concentrations in patients with ICL on CT scans compared with those without.[25 26] A recent study in 1959 adults with mild and moderate TBI showed that the combination of these two biomarkers in a single test had high sensitivity (97.6%) and negative predictive value (99.6%) for detection of ICL.[27]

To date, there is little data on these two biomarkers in the paediatric population and on their relevance to TBI management in children. Elevated serum concentrations of GFAP and UCH-L1, assessed at a median time of 4.7 hours (range: 0.5–20.6 hours) postinjury, were observed in 45 children following TBI of any severity, in comparison to measurements in 40 healthy matched controls.[28] An increasing gradient in the concentration of these biomarkers was also demonstrated along the severity continuum from mild to severe TBI.[28] In cohorts of children and young adults with mild to moderate TBI, Papa *et al* found an area under the curve for ICL detection on CT of 0.85 (95% CI 0.72 to 0.98) for GFAP (n=92, including eight children with ICL) and 0.83 (95% CI 0.73 to 0.93) for UCH-L1 (n=151, including 17 children with ICL).[29 30] With a cut-off point set to maximise sensitivity at 100%, the specificity was 36% for GFAP and 47% for UCH-L1.[29 30] The performance of a test combining the two biomarkers has not been evaluated to date in children.

Several studies have shown that GFAP and UCH-L1 can predict poor outcomes as assessed by the Glasgow Outcome Scale (GOS) score after TBI.[28 31 32] Regarding the association between GFAP and UCH-L1 and the presence of PCS after an mTBI, the results of the studies to date differ. Rhine *et al* found that neither GFAP nor UCH-L1 were predictive of PCS in 25 children over the 1 month postinjury period.[33] In contrast, Mannix *et al* found a correlation in 13 children and young adults between GFAP concentrations and the Rivermead PCS scoring at 1 month.[34] However, many unknowns remain, and the scope of the results of these studies may be limited by their small numbers.

In addition, no physiological reference values have been established to date for children in regards to UCH-L1, and only one recent study has proposed a paediatric reference interval for GFAP, in a cohort of Danish children.[35] As with other brain biomarkers,[36] it has been shown that age can influence their concentrations and that GFAP levels after severe trauma were higher in a paediatric cohort than in adults.[31]

The overall aim of the BRAINI-2 paediatric study is to provide new knowledge regarding GFAP and UCH-L1 in children and adolescents in order to improve TBI management in the paediatric population.

Objectives

The primary objective is to assess the performance of GFAP and UCH-L1 used separately and in combination as markers to detect the presence or absence of ICL on CT scans in a population of children with mTBI.

The secondary objectives are (1) to evaluate the potential of GFAP and UCH-L1 for early prediction of prognosis after TBI of any severity, including early clinical deterioration within 72 hours after TBI, as well as neurological outcome, occurrence of PCS and health-related quality of life (HRQoL) at 1 month and 3 months after TBI and (2) to establish age-specific reference values for serum GFAP and UCH-L1 concentrations in a non-TBI paediatric population.

METHODS AND ANALYSIS

The study protocol was drafted in accordance with the 'Standard Protocol Items: Recommendations for Interventional Trials' recommendations and checklist (see online supplemental appendix 1).[37] The BRAINI-2 paediatric study is part of a European Institute of Innovation and Technology (EIT Health) project entitled BRAINI-2, with bioMérieux as the lead partner, that aims to evaluate the VIDAS TBI assay, which is an automated assay for the measurement of serum GFAP and UCH-L1, in two vulnerable populations with distinct physiological specificities: older adults and children. A prospective multicentre study, the BRAINI study (NCT04032509), is currently underway to evaluate the performance of GFAP and UCH-L1 in predicting ICLs in a general adult population after mTBI.[38]

Study design and settings

We will conduct a European, prospective, non-controlled, multicentre study in 20 participating centres: 15 centres in France (the University Hospitals of Nantes, Clermont-Ferrand, Rennes, Brest, Lille, Montpellier, Limoges, Grenoble, Saint-Etienne, Louis Mourier-Colombes, Trousseau-Paris, Robert Debré-Paris, the General Hospitals of Lorient, Saint-Nazaire and La Roche-sur-Yon), four university hospitals in Spain (three in Madrid, including SERMAS 12 de Octubre, La Paz, Niño Jesus University Hospitals and one in Barcelona, the Hospital Vall d'Hebron), and one university hospital in Switzerland (the Children's Hospital, Cantonal Hospital of Lucerne). The recruitment commenced on 2 August 2022, with a projected inclusion period of 30 months, thus estimating an end date of January 2025 for the enrolment of a total of 2880 children and adolescents.

Study population

All patients included will be children or adolescents aged from birth to under 18 years, with a parental affiliation to an appropriate health insurance system, and for whom oral or written informed consent will be obtained from one of the parents of the child or the holder of parental responsibility, and/or from the child or adolescent. In order to achieve the different objectives, children with mild, moderate and severe TBI, as well as those without TBI, will be considered for inclusion based on predefined criteria (table 1). The anticipated enrolment comprises a total of 2880 children, categorised as follows: (1) 2480 children and adolescents with TBI, encompassing 630 cases of mTBI with CT scan, pertinent to both the primary and secondary objectives concerning prognosis, 1720 cases of concussion without CT scan indication and 130 cases of moderate or severe TBI, pertinent to the secondary prognosis objective and (2) 400 children and adolescents with no TBI history, relevant to the secondary objective on establishing reference values. The non-TBI group will be further divided into three age subgroups (under 2 years, 2–9 years and 10 years and above) to consider potential age-related variations in biomarker physiological levels.

Outcome measures

Primary outcome

The primary outcome is the presence of ICL on CT scans (ie, CT-scan positive), defined by one or more of the descriptions[7] detailed in table 2. The decision to perform a cranial CT scan will be guided by national or local guidelines or the attending physician's discretion. Typically, all participating centres adhere to the clinical decision rules outlined by the Pediatric Emergency Care Applied Network (PECARN)[7] to identify children at risk of ICL warranting a CT scan. However, it is noteworthy that two French centres (Nantes and Rennes) use a modified PECARN rule, which includes the S100B protein assay.[21] The CT images will be independently reviewed by two neuroradiologists blinded to the initial interpretation of the CT scan by a local radiologist, the results of biomarker assays, and the outcome of the child. In case of disagreement, a third radiologist will be asked to provide a final interpretation of the CT scan. In case multiple CT scans are performed, the one closest to the blood sampling will be retained. The interrater agreement will be assessed by Cohen's Kappa coefficient[39] and interpreted according to the scale proposed by Landis and Koch.[40]

Secondary outcomes

To assess the predictive capacity of GFAP and UCH-L1 for TBI prognosis, the study will evaluate outcomes such as early clinical deterioration, neurological outcome, occurrence of PCS and HRQoL.

Early clinical deterioration is defined by the occurrence of death from TBI, neurosurgical intervention, intubation for TBI or hospital admission of two nights or more associated with ICL on CT scans for persistent neurological symptoms such as persistent alteration in mental status, recurrent emesis due to TBI, persistent severe headache, or ongoing seizure management,[7] within 72 hours after TBI. This outcome will be assessed on day 3 postinjury in all children with TBI.

Neurological outcome will be assessed using the paediatric version of the Glasgow Outcome Scale-Extended (GOS-E Peds)[41] at 1 month and 3 months in all children with TBI. The GOS-E Peds measures the functional outcomes following TBI in children and adolescents aged

Table 1 Inclusion and non-inclusion criteria

Inclusion criteria	Non-inclusion criteria
For the mTBI group	For all of the TBI groups
► GCS score of 13–15 on admission ► Admission within 24 hours of the injury ► Indication for head CT scan according to national or local guidelines or the in-charge physician AND/OR diagnosis of concussion consistent with the fourth Zurich consensus statement[51] and defined by the presence of≥1 symptom on the validated Acute Concussion Evaluation (ACE) questionnaire[45] ► Ability to follow-up by telephone, mail, or email	► Time of injury unknown or exceeding 24 hours ► Blood sampling not possible within 24 hours after the injury ► Pre-existing neurological disorders affecting the assessment of neurological outcome, seizure disorder/epilepsy, brain tumour, history of neurosurgery, stroke, or encephalopathy ► Penetrating brain injury with skull fracture ► No clear primary mechanism of trauma ► Venipuncture not feasible ► Pregnancy ► Intoxication ► No possibility for transferring CT scan images to the centralised platform ► Participation in another interventional research study
For the moderate or severe TBI group	
► GCS score of 3–12 on admission ► Admission within 24 hours of the injury ► Indication for head CT scan according to national or local guidelines or the in-charge physician ► Ability to follow-up by telephone, mail or email	
For the non-TBI group	
► Admission for any reason other than TBI ► Indication of blood sampling for their routine management ► GCS score of 15 ► Without a chronic pathology	► Pre-existing neurological disorders, seizure disorder/ epilepsy, brain tumour, history or indication of neurosurgery, stroke or encephalopathy ► History of TBI ► Orthopaedic trauma or surgery within the past month ► Suspected meningitis or encephalitis ► Venipuncture not feasible ► Pregnancy ► Intoxication ► Participation in another interventional research study

GCS, Glasgow Coma Scale; mTBI, mild traumatic brain injury; TBI, traumatic brain injury.

Table 2 Items used for determining CT scan positive or not

CT scan positive	Not CT scan positive
Intracranial haemorrhage (epidural/ acute or subacute subdural/ extra-axial/ subarachnoid/ intraventricular/ petechial/ intraparenchymal haemorrhage)	Parenchymal or extraparenchymal calcifications
Brain contusion	Pneumocephalus
Cerebral oedema	Chronic subdural haematoma
Traumatic infarction	Brain tumour
Diffuse axonal injury	Linear non-depressed fracture
Shearing injury	Facial fracture
Sigmoid sinus thrombosis	Hypodense chronic ischaemic lesion
Midline shift of intracranial contents	Scalp injury
Signs of brain herniation	Basilar skull fracture
Diastasis of the skull	Normal CT
Depressed skull fracture	

from birth to 18 years, on a scale of eight levels ranging from 1 (indicating death) to 8 (representing the highest level of good recovery).

The occurrence of PCS will be assessed using the Rivermead Post-Concussion Symptoms Questionnaire (RPQ)[42] at 1 month and 3 months in children aged 5 years or older with mTBI. The RPQ consists of a 16-item symptom inventory checklist measuring postinjury symptoms across physical, cognitive and emotional domains. PCS is defined as an increase from the perceived preconcussion baseline of three or more concussion symptoms.[13 15]

HRQoL will be assessed using the Paediatric Quality of Life Inventory (PedsQL) Generic Core Scales[43 44] at 1 month and 3 months in all children with TBI. The age-appropriate PedsQL questionnaires comprise 23 items assessing multidimensional HRQoL scores for children and adolescents aged from 5 to 18 years. Additionally, a proxy measure available for parents will be used for all children aged 1 month and over.

For the establishment of age-specific reference values, the endpoints will be the serum GFAP and UCH-L1 concentrations measured in three age groups (under 2 years, 2–9 years and 10 years and above) in the non-TBI population.

Biomarkers

The Banyan Brain Trauma Indicator (BTI) is a manual immunoassay measuring the GFAP and UCH-L1 biomarker serum concentrations that obtained FDA clearance in February 2018. However, the Banyan BTI, which is a 4-hour manual assay, presents limitations for use in ED and has not been validated in paediatrics. BioMérieux obtained the CE Mark in September 2023 for the VIDAS TBI (GFAP, UCH-L1), which is an automated, quantitative, enzyme-linked immunofluorescent assay for measurement of serum GFAP and UCH-L1 in adult patients with mTBI that provides result within 1 hour of blood sampling. A 2.5 mL blood sample will be collected from each child under 10 years of age or 4 mL from each child 10 years of age or over (within 24 hours of head injury for the children with TBI). The whole blood samples will be collected in serum separator tubes and fully processed to serum within 2 hours after blood sampling. The blood samples will be centrifuged at 2000×g for 15 min at 18–25°C between 30 min and 90 min after the blood sampling. The serum will be separated and aliquoted immediately or within 30 min of the centrifugation step. The blood samples collected for the study will be processed and stored on site at −80°C, before being sent to bioMérieux for analysis by VIDAS TBI assay.

Study timing and data collected

After checking that the child is eligible to participate in the study according to the inclusion and exclusion criteria specific to each study group, the inclusion visit will involve collecting data on demographic characteristics, health history, vital signs, age-adapted GCS score, presence of extra-cranial injury and specifically for the children with TBI: the circumstances of the TBI, the general and neurological examination, the data from the Acute Concussion Evaluation questionnaire[45] for the diagnosis of concussion, the initial management of the child and the CT scan findings according to the local radiologist, if applicable (figure 1). The blood samples will be processed as outlined above and then sent to bioMérieux for biomarker testing using the VIDAS TBI test. The biomarker results will not be available while the child is still hospitalised. Therefore, no patient management decision will be made based on the results of the GFAP and UCH-L1 assays.

STUDY PERIOD	INCLUSION	FOLLOW-UP*		
TIMEPOINT	Day 0	Day 3	Month 1	Month 3
ENROLMENT				
Eligibility screen	X			
Informed consent	X			
Inclusion in one of the three population groups	X			
EXAMINATIONS				
Clinical examination	X			
Blood sampling	X			
Head CT scan#	X	X		
ASSESSMENTS				
Serum GFAP and UCH-L1 concentrations	X			
Presence of intracranial lesion on CT scan#	X			
Early clinical deterioration		X		
Neurological outcome			X	X
Post-concussion symptoms			X	X
Health-related quality of life			X	X

Figure 1 Study schedule of enrolment, examinations and assessments. #A CT scan will be performed in some patients with mTBI during the initial management and/or early monitoring, between day 0 and day 3, and in all of those with moderate or severe TBI. *The follow-up will only involve children with TBI (not children from the non-TBI control population) and the search for post-concussion symptoms will only concern children with mTBI. GFAP, glial fibrillar acidic protein; mTBI, mild TBI; TBI, traumatic brain injury; UCH-L1, ubiquitin carboxy-terminal hydrolase-L1.

Routine follow-up care after hospital discharge varies across participating sites. Each centre provides families with instructions for home monitoring. Additionally, some centres (Clermont-Ferrand, Brest, Nantes, Rennes and Lucerne) systematically refer children experiencing persistent PCS to a concussion clinic or arrange specialist consultations with a neuropaediatrician or neuropsychologist. As part of the study, enrolled children will undergo follow-up at 1 and 3 months post-TBI, where their medical records will be reviewed at each participating centre. Follow-up will involve telephone, mail or email interviews (figure 1). Data from various questionnaires, including the GOS-E Peds,[41] RPQ[42] and PedsQL,[43 44] will be collected by research assistants using an electronic case report form (eCRF) to assess neurological outcome, occurrence of PCS and HRQoL, respectively. In addition, details of the management (inpatient services, length of stay) and the occurrence of early clinical deterioration, neuroimaging after the initial emergency management, medical consultation, hospital readmission or TBI complications after hospital discharge will also be collected.

Statistical analysis

Performance of the biomarkers to detect ICL on CT scans

We will assess the performance of GFAP and UCH-L1 to detect ICL on CT scans separately and in combination in the VIDAS TBI assay in terms of sensitivity, specificity and positive and negative predictive values. First, cut-off points for each biomarker separately and in combination will be derived from receiver operating characteristic (ROC) curves. In order not to bias the cut-off determination on the ROC curves, a strategy of under-sampling and/or oversampling will be used to obtain balanced groups between children with a negative CT scan (ie, without ICL) and children with a positive CT scan (ie, with ICL). Selection of the best-performing combination of GFAP and UCH-L1 cut-off points will be performed to maximise specificity with a high sensitivity (≥98%), which will ensure correct identification of almost all children with ICL. Models such as decision tree or logistic regression could also be evaluated to combine the two biomarkers. The model with the associated cut-off points that will reach the best performances will be selected using cross-validation and bootstrap validation techniques, which limit overoptimistic estimates of performance.

The performance of GFAP and UCHL-1 will be also calculated using cut-off points based on the 95th percentile of the age-specific reference values for each biomarker. The final interpretation of the VIDAS TBI assay will consider the results for the two biomarkers: the VIDAS TBI assay will be defined as positive when the result of at least one biomarker is positive, that is, above the 95th percentile, and as negative when the results of both biomarkers will be negative, that is, below the 95th percentile.

Potential of biomarkers to predict prognosis after TBI

The association (or correlation) of GFAP and UCH-L1 concentrations with early clinical deterioration (as a binary variable), neurological outcome (GOS-E Peds score as a continuous and/or binary variable), presence of PCS (RPQ score as a continuous and/or a binary variable) and HRQoL (PedsQL score as a continuous variable) will be studied. We will perform bivariate analyses using linear or logistic regression models, depending on the outcome being studied, with GFAP and/or UCH-L1 concentrations as explanatory variables. The interaction between the two concentrations will be also analysed.

Establishment of age-specific reference values

Age-specific reference values of GFAP and UCH-L1 will be established from a non-TBI paediatric population divided in the three age groups. We will use specific statistical methods recommended by the Clinical Laboratory Standards Institute (CLSI) for determining reference values and reference intervals for quantitative clinical laboratory tests.[46]

Sample size justification

In order to establish cut-off points applicable to the entire paediatric age range and evaluate biomarker performance, a sample size of 90 children with positive CT scans, indicating ICL, is deemed sufficient to construct the best predictive model combining both biomarkers, ensuring maximum specificity with a sensitivity of at least 98%. Considering an anticipated prevalence of ICL of approximately 15% among children undergoing CT scans following mTBI, and aiming to maintain the margin of error for estimates within 3% with a type 1 error of 5%, we project a recruitment target of 600 children. Factoring in a potential loss to follow-up or secondary exclusions of up to 5% of participants, we estimate that a minimum of 630 children with mTBI and CT scans must be recruited.

With an expected loss to follow-up rate at 3 months of 15%, we project having data from 1997 children with mTBI available for analysis of the association between GFAP and UCH-L1 concentrations and the presence of PCS at 3 months. Considering an estimated percentage of positive biomarker results of approximately 10% in patients without PCS and a prevalence of PCS at 3 months of 10%,[14 19] we will be able to detect a minimum OR ≥2 with a risk α of 5% for a power of 90%.

To establish reference values, the standard approach recommended by the CLSI EP28-A3c guideline is to collect and analyse a minimum of 120 samples from healthy subjects from the local population.[46] This has the advantage of also allowing 90% confidence limits to be computed non-parametrically for each reference limit. Consequently, the target recruitment for each age group is between 130 and 135 with a 10% rate of observations potentially deleted from the reference set, for a total of 400 children for the non-TBI group.

Patient and public involvement

Patients were not involved in the design, or conduct, or reporting or dissemination plans of this research.

ETHICS AND DISSEMINATION

Ethics approval and consent to participate

The protocol (V.N°1.0–10 May 2022) was approved by an ethics committee in France on 23 June 2022 (N° SI 22.01960.000103); in Spain on 2 August 2022, for Hospital Vall d'Hebron (N° PR(AG)271/2022); on 23 August 2022, for Hospital 12 de Octubre (N° CEIm: 22/382); on 25 October 2022, for Hospital Niño Jesus (N° R-0073/22); on 23 March 2023, for Hospital La Paz (N° 2023.280); in Switzerland on 21 February 2023 (N° 2022–01302). Parents and the child or adolescent will receive verbal information and a written document describing the study (see online supplemental appendix 2). Informed consent will be obtained from one of the parents of each child or the holder of parental responsibility, and from each child or adolescent from the age of 6 years. The consent is written in Spain and Switzerland, and oral in France, in accordance with national regulations. All the consent forms have been approved by the corresponding ethics committees in that way.

Data collection, storage, monitoring and confidentiality

Data collection for each child/adolescent participating in the research will be by means of an eCRF. Each person responsible for completion of the eCRF (investigator, research staff) will have a 'user' account with specific computer rights linked to their role (eg, the right to enter or modify data or to lock, monitor or sign a page of the eCRF). The data will be compiled directly from the eCRF into the database hosted on a dedicated server with controlled access (account/password) according to the user's role. Any addition, modification or deletion of data will be recorded in a non-editable electronic file (the audit trail). The anonymised images from the CT scans will be uploaded by the investigators and stored for the duration of the study on a secure web central database. All investigators will archive all of the study data for at least 15 years after the completion of the study. All investigators will commit to keeping the identities of the individuals who participate in the study confidential by assigning them a code. This code will be used for all of the eCRF and all the attached documents (reports of CT scans, laboratory tests, etc). It will be the only information that will allow a connection with the participant to be made retrospectively. Monitoring will be carried out by the sponsor, the Nantes University Hospital. A clinical research associate will regularly check the quality of the data reported in the eCRF. Every 2 months, inclusions from each centre will be analysed. Adjustments and contingency plans may be proposed if necessary, particularly in the event of under or overinclusion, in order to obtain a sample in line with the composition and number of children pre-established for the different groups.

Dissemination

For widespread dissemination, the results of this study will be presented at national and international congresses and published in peer-reviewed journals.

DISCUSSION

To date, very little data are available on the GFAP and UCH-L1 biomarkers in paediatric patients, particularly those with TBI. This gap in knowledge may be filled by the BRAINI-2 paediatric study, the results of which can be expected to have a major impact on the management of children with TBI. The number of children exposed unnecessarily to ionising radiation and, therefore, the risk of certain cancers, such as leukaemia and brain tumours, associated with CT irradiation would be reduced.[4 5] The sedation sometimes required for young children prior to brain imaging would be avoided. Additionally, avoiding unnecessary CT scans would reduce overcrowding in the ED and allow radiologists to dedicate more time to patients who need their expertise the most. Identifying children at risk of early clinical deterioration would enable close hospital monitoring to be implemented, while those at risk of developing PCS would benefit from specific information, parental guidance and even specialist follow-up in order to limit the onset, intensity, and duration of symptoms.[47 48]

From a methodological point of view, this study has a number of strengths. The quality of the measurement of our primary outcome will be ensured by the reading of the CT images by two independent neuroradiological experts. The 3-month follow-up of the children included will allow verification of the absence of ICL, which would have been diagnosed after the ED discharge. The generalisability of our results will be supported by the multicentre design of the study, with broad recruitment planned in several European countries, and by the wide spectrum of clinical presentations of the patients with TBI included.

However, this study has some limitations. The indication for a CT scan may vary from centre to centre, depending on local or national recommendations. As performing a CT scan for patient management is not mandatory as an inclusion criterion, not all children included will ultimately be considered in the main analysis, as the primary outcome measure will be the CT scan result. However, for all children, even those without a CT scan, early clinical deterioration will be sought. For children with TBI, we will not be able to assess the association between the values of the biomarkers and their long-term prognosis, such as the occurrence of prolonged PCS, as the follow-up will stop 3 months after the head trauma. Nevertheless, this study will enable one of the largest international cohorts to date of children with concussion to be monitored prospectively. Finally, to establish the age-specific reference values, the children without TBI will be divided into three pre-established age groups. It is possible that the variations in physiological biomarker values according to age do not correspond absolutely with these predefined age groups,

particularly in the case of high variability in the first years of life.[49] The number of children included may be insufficient in one of the newly identified age groups. Using continuous models in our statistical analyses to establish continuous rather than discrete age group reference values could resolve this limitation.[50]

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