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Study protocol for investigating the clinical performance of an automated blood test for glial fibrillary acidic protein and ubiquitin carboxy-terminal hydrolase L1 blood concentrations in elderly patients with mild traumatic BRAIN Injury and reference values (BRAINI-2 Elderly European study): a prospective multicentre observational study

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ABSTRACT

Introduction Two blood brain-derived biomarkers, glial fibrillar acidic protein (GFAP) and ubiquitin carboxy-terminal hydrolase L1 (UCH-L1), can rule out intracranial lesions in patients with mild traumatic brain injury (mTBI) when assessed within the first 12 hours. Most elderly patients were excluded from previous studies due to comorbidities. Biomarker use in elderly population could be affected by increased basal levels. This study will assess the performance of an automated test for measuring serum GFAP and UCH-L1 in elderly patients to predict the absence of intracranial lesions on head CT scans after mTBI, and determine both biomarkers reference values in a non-TBI elderly population.

Methods and analysis This is a prospective multicentre observational study on elderly patients (≥65 years) that will be performed in Spain, France and Germany. Two patient groups will be included in two independent substudies. (1) A cohort of 2370 elderly patients (1185<80 years and 1185≥80 years; BRAINI2-ELDERLY DIAGNOSTIC AND PROGNOSTIC STUDY) with mTBI and a brain CT scan that will undergo blood sampling within 12 hours after mTBI. The primary outcome measure is the diagnostic performance of GFAP and UCH-L1 measured using an automated assay for discriminating between patients with positive and negative findings on brain CT scans. Secondary outcome measures include the performance of both biomarkers in predicting early (1 week) and midterm (3 months) neurological status and quality of life after trauma. (2) A cohort of 480 elderly reference participants (BRAINI2-ELDERLY REFERENCE STUDY) in whom reference values for GFAP and UCHL1 will be determined.

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ This is a large multicentre prospective study to validate the value of serum biomarkers, glial fibrillar acidic protein and ubiquitin carboxy-terminal hydrolase L1, concentrations for predicting brain CT findings after mild traumatic brain injury (mTBI) in the elderly population most commonly affected by mild TBI.
⇒ Comorbidities will be considered but will not prevent patient inclusion, making the sample similar to real-world data.
⇒ Comorbidities' effect on the concentrations of these blood biomarkers will be assessed.
⇒ Reference values for these biomarkers will also be obtained in a large group of non-TBI patients, helping fine-tune the test's value.
⇒ Variability in the indications of CT scans can influence their positivity rate across countries and centres, thereby influencing the study's statistical power.

Ethics and dissemination Ethical approval was obtained from the Institutional Review Boards of Hospital 12 de Octubre in Spain (Re#22/027) and Southeast VI (Clermont Ferrand Hospital) (Re# 22.01782.000095) in France. The study's results will be presented at scientific meetings and published in peer-review publications.

Trial registration number NCT05425251.

INTRODUCTION

Traumatic brain injury (TBI) poses a significant health burden.[1] The world's population is becoming older as life expectancy increases. This shift in the population's age has resulted in a change in TBI's demography, increasing the age range of TBI sufferers and making falls the most frequent cause of TBI.[1–4] Mild TBI (mTBI), as defined by the Glasgow Coma Scale (GCS) score of 13–15, is the most frequent form of TBI and poses the most significant healthcare burden caused by TBI.[5 6] Mild TBI is one of the most frequent traumatic emergencies in the elderly population (≥65 years), and as the population ages, the risk of suffering mTBI and the number of patients affected by mTBI increases.[7 8] Falls are more frequent as age increases because elderly patients have increased muscle weakness, impaired equilibrium, visual decline, comorbidities and concomitant medication use. Moreover, they are more prone to intracranial and systemic complications after mTBI owing to different factors, such as more frequent use of antithrombotic treatment, comorbidities and increased brain frailty(ie, a combination of progressive accumulation of health deficits, combined with failing of repair processes that determines a brain with less reserve to cope with further injury). Therefore, this highly vulnerable population is responsible for most emergency mTBI consultations in industrialised countries.[8]

The initial management of mTBI in the emergency department (ED) relies on performing non-contrast brain CT if the patient meets specific conditions, including clinical decision rules (CDRs). The prevalence of abnormalities detected using CT in different studies is approximately 10%, and <1% of patients will require a neurosurgical procedure. CDRs, such as the New Orleans Criteria,[9] the Canadian Head CT rule,[10] or national guidelines, such as the French guidelines, have been designed to help physicians select individual risk factors or their combinations to guide the performance of CT after mTBI. These percentages could be higher in the elderly because they develop haemorrhagic lesions more frequently. Consequently, most CDRs consider age >60 or 65 years to be a risk factor for brain injury after mTBI requiring an indication for a CT scan.[9–11] Furthermore, a higher incidence of intracranial pathology in the elderly population and the difficulties in assessing their condition due to cognitive decline, even with the use of the GCS, encourage CT scanning. While CDRs have been developed and used for several decades, variability among physicians regarding CT indications still exists.[11 12] While increased age is a risk factor for the prescription of CT after mTBI for different CDRs, there is no consensus on the use of this factor by ED physicians, as found in a recent European survey.[11] The variability in the management of mTBI among different hospitals within a country and in Europe implies that CDRs are not correctly followed by physicians attending these patients due to the complexity of these rules or the difficulty in actually applying them in certain conditions such as intoxicated patients, hearing loss or speech difficulties. In addition, CDRs can be ignored by physicians because of the fear of missing brain injury or pressure from patients or relatives. Consequently, approximately 40% of the CT scans obtained in the emergency room (ER) do not follow these CDRs.[12] Therefore, the number of CTs performed for mTBI is very high, with important consequences to patients, such as increased health expenditure and radiation exposure. This is also a specific and important problem for elderly patients who frequently experience recurrent falls and sometimes receive one CT for each fall. In addition, elderly patients using anticoagulants or antithrombotic drugs are more frequently hospitalised due to the insecurity of physicians regarding possible deterioration. This results in more possibly unneeded CT scans and hospitalisations for these patients.

Therefore, CDRs for the performance of CT after mTBI could be more robust after including a more objective parameter that could be assessed easily and in a very short timeframe. Blood concentrations of brain-damage biomarkers could play a major role in increasing the objectivity of CDRs, the safety of managing mTBI in the ED, or even including prognostic information on the probability of deterioration or poor outcomes in elderly patients. The use of a blood assay to rule out the presence of traumatic lesions has been proposed previously. Serum protein S-100B has long been included in the Scandinavian Guidelines for the initial management of mTBI, and its utility in assessing patients at intermediate risk has been demonstrated.[13–15] However, the use of S-100B has not gained generalised acceptance for the screening of mTBI.[11] Combining serum levels of two brain-specific proteins, ubiquitin carboxy-terminal hydrolase L1 (UCH-L1) and glial fibrillar acidic protein (GFAP), can predict the absence of clinically relevant lesions on CT scans in patients with mTBI and reduce unnecessary CT scanning.[16] GFAP and UCH-L1 were approved by the US Food and Drug Administration (FDA) for clinical use in adult patients with mTBI to help determine the need for a CT scan within 12 hours after injury. FDA approval was obtained in 2018 for measurement in serum using a lab-based immunoassay (Banyan Brain Trauma Indicator (Banyan Brain trauma Insert)) and in 2021 for measurement in plasma using a portable hand-held device (Abbott i-STAT Alinity Traumatic Brain Injury plasma assay). Both tests confirmed a good correlation between them when tested in the same patient cohort (ALERT-TBI).[17 18] However, different biomarker (GFAP, UCH-L1, and S100B)-based tests could lose specificity in elderly populations because many biomarker levels increase with age.[19–21]

Furthermore, patients with neurological comorbidities were excluded from these studies,[16 22]specifically those suffering from mild cognitive impairment and neurological or psychiatric conditions. A consequence of patient exclusion for pre-existing conditions is that the median age of recruitment in clinical studies is much lower than that of the actual population seen in the ED. However, age-related changes and neurological comorbidities could

increase the basal levels of brain-specific biomarkers, thereby decreasing the predictive capability of these tests.[23 24] Therefore, the application of these biomarkers in elderly patients could be controversial without specific information on their blood levels in patients with or without mTBI.

Traditionally, little attention has been paid to patients with mTBI beyond its management in the ER. Furthermore, most patients with mTBI are not followed up after hospital discharge in a structured way. No further follow-up is deemed necessary if there are no findings on the CT scan. However, a large proportion of these patients, estimated between 5% and 43%, suffer from different symptoms that reduce their quality of life, diminish their capacity to return to work or study, and make them incapable of returning to their normal life.[6 25] As stated previously, the elderly might have an increased risk of complications and poorer outcomes, increasing the burden on patients' relatives, caregivers and society in terms of increased dependency on others; however, whether they fare worse than younger patients after mTBI remains unclear.[26]

A test that can apply to elderly patients with cut-off values specifically designed for them, including those most vulnerable due to neurological comorbidities, is required. No specific study has been performed on the prognosis of mTBI in the elderly in terms of quality of life and independence. In addition, there is no information regarding the prognostic value of brain-specific biomarkers in the elderly population. Automated assays assessing serum concentrations of GFAP and UCH-L1 have been developed on the VIDAS platform (bioMérieux, Marcy l'Etoile, France), and their clinical evaluation is currently in progress, including in the BRAINI study.[27] The BRAINI2-Elderly study will attempt to refine this test for all elderly patients. This study generally aims to assess the predictive performance of the biomarkers GFAP and UCH-L1 in improving the clinical management of mTBI in the elderly population. The primary aim is to evaluate the performance of two biomarkers, GFAP and UCH-L1, in ruling out intracranial lesions on a head CT scan after mTBI in the elderly population. This study secondarily aims to assess the performance of GFAP and UCH-L1 in early and midterm prognoses and to provide reference values for GFAP and UCH-L1 using a non-TBI elderly population.

METHODS AND ANALYSIS

Study design

The BRAINI2-Elderly study is a multicentre observational study in which prospective data are collected from Spain, France and Germany. It consists of two independent substudies: (1) a cohort study of elderly patients with mTBI (BRAINI2-ELDERLY DIAGNOSTIC AND PROGNOSTIC STUDY) and (2) a cross-sectional study of elderly reference participants (BRAINI2-ELDERLY REFERENCE STUDY).

Study setting

Braini2-Elderly is part of the BRAINI2 Project, cofunded by the European Union European Institute of Technology BRAINI2-EIT Health. The BRAINI2-Elderly includes six centres in Spain within University Hospitals (four in Madrid, Spain, including SERMAS Hospital 12 de Octubre, Hospital Gregorio Marañón, Hospital Infanta Cristina and Hospital de La Princesa, 1 in Barcelona, Spain, ICS Hospital Vall d'Hebron and 1 in Mallorca, Hospital Son Espasses), 5 in France within University Hospitals (Grenoble, Clermont-Ferrand, Lyon Edouard Herriot, Lyon-sud and Nantes), and one in Germany (Klinikum rechts der Isar, Munich). The centre selection was based on experience in TBI management and documented patient recruitment capacity.

Study population

For the BRAINI2-ELDERLY DIAGNOSTIC and PROGNOSTIC STUDY, patients will be included if they meet the following criteria: age ≥65 years, assessed for mTBI with a GCS score of 13–15 in the recruiting centres, need for brain CT scan as part of clinical care, and performance of blood sampling within 12 hours after injury and within 6 hours after brain CT.

Patients will be excluded if they have at least one of the following criteria: age <65 years, GCS score of 2–12 on admission, unknown time of injury, time since injury exceeding 12 hours, primary admission for a non-traumatic neurological disorder (such as stroke or spontaneous intracranial haematoma), penetrating head injury, brain tumour, mechanical ventilation from the trauma scene or prehospital management, venipuncture not feasible, no realisation of brain CT scan, under judiciary control, participation in an interventional drug study, and neurosurgical operation 1 month before the study.

All inclusion and exclusion criteria will be verified, and patients will be included after obtaining written informed consent. Next-of-kin consent will be possible, according to local and national requirements, if the patient is not in the condition of providing consent.

Study outcomes

The BRAINI2-ELDERLY DIAGNOSTIC and PROGNOSTIC STUDY primarily aims to evaluate the performance of the two biomarkers, GFAP and UCH-L1, alone and in combination, in ruling out intracranial lesions on CT scans after mTBI in the elderly population, independent of their comorbidities. The primary outcome measure is the performance of these two biomarker assays in terms of sensitivity, specificity, positive and negative predictive values (NPV), likelihood ratios and their corresponding lower limit of the 95% CI with respect to brain CT scan findings (positive vs negative).

Secondary outcomes will be early (1 week±3 days) and midterm clinical recovery (3 months±1 week) of mTBI patients. Neuroworsening will be evaluated at both time points and considered present if patients develop one of the following criteria: (A) a decrease in GCS score of >2

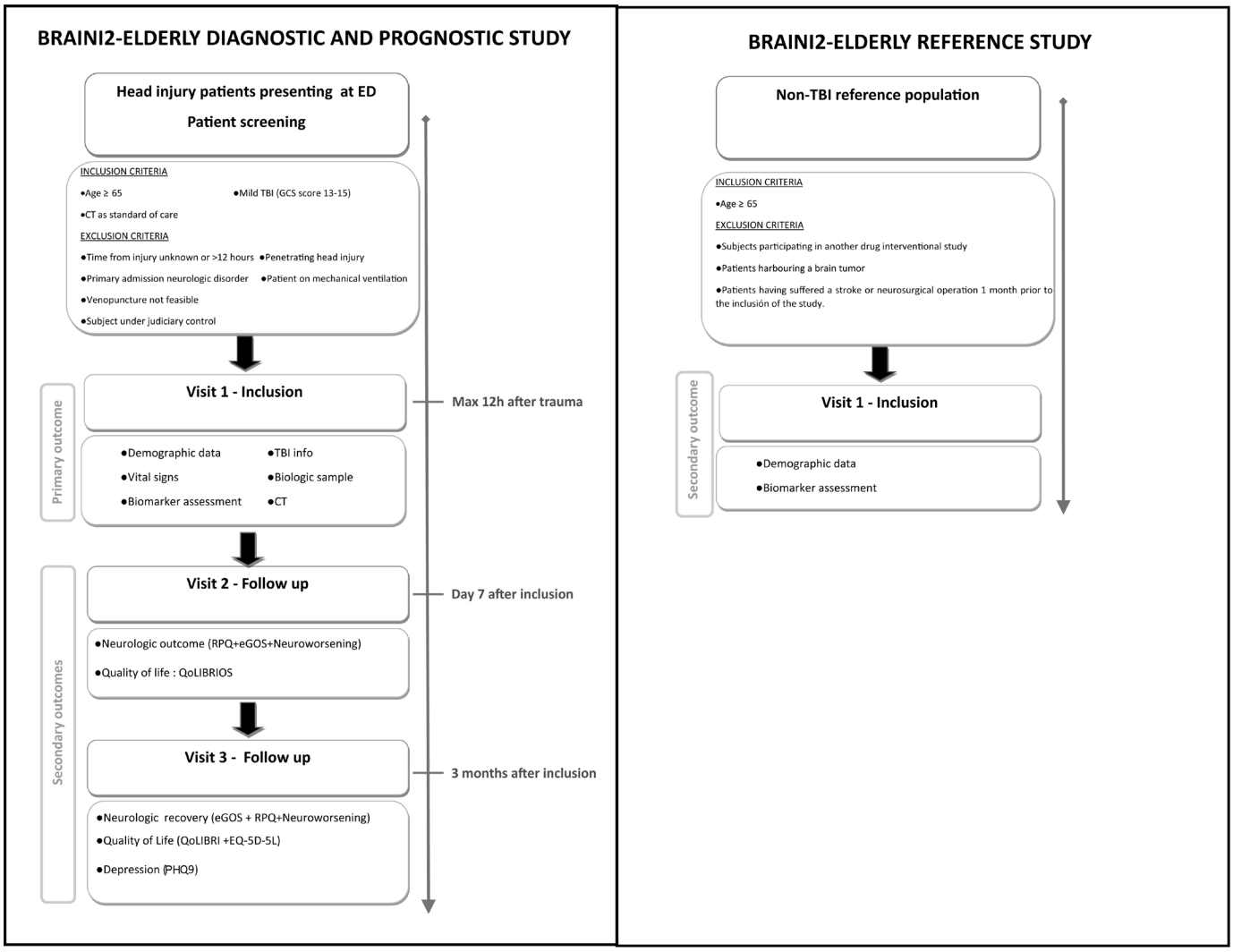

Figure 1 Study design and flow of the BRAINI2-Elderly study. ED, emergency department; eGOS, extended Glasgow Outcome Scale; EQ-5D, European Quality of Life-5 Dimensions; GCS, Glasgow Coma Scale; QOLIBRI, Quality of Life after Brain Injury; PHQ9, Patient Health Questionnaire; RPQ, Rivermead Post-concussion Symptoms Questionnaire; TBI, traumatic brain injury.

points from the initial GCS score in the absence of sedatives, (B) a deterioration in neurological status sufficient to warrant any intervention, including mechanical ventilation, sedation, osmotherapy, corticosteroids, neurosurgical intervention, or admission to the intensive care unit, and (C) admission to a hospital ward exclusively based on the deterioration of neurological condition due to TBI. Patient status and well-being will be evaluated at 1 week using the Glasgow Outcome Scale-Extended (GOSE),[28 29] the Rivermead Post-concussion Symptoms Questionnaire,[30] and the simplified version of the Quality of Life after Brain Injury (QOLIBRI-OS) questionnaire.[31] Three months after TBI, patient status and well-being will be assessed using the GOSE, the 5-level European Quality of Life-5 Dimensions version,[32] and the QOLIBRI-OS and screened for depression symptoms using the Patient Health Questionnaire (PHQ-9).[33]

The study design and flow are shown in figure 1.

In the BRAINI2-ELDERLY REFERENCE STUDY, the study outcome is the distribution of the reference values for GFAP and UCH-L1 in the non-TBI elderly population. The normal ranges of these biomarkers will be determined overall and based on age, comorbidities and other demographic and clinical data.

Data collection and monitoring

All study data will be collected and stored in a secure web-based electronic case report form (eCRF). Clinical research associates or qualified staff members under the centre's principal investigator's supervision will enter the data at each participating centre. The study database will be created using eCRF.

Data collection will comprise demographic and baseline information at admission, including the presence or absence of comorbidities, including neurological and psychiatric comorbidities, the frailty of the patients as measured using the Clinical Frailty Scale, concomitant

medications, particularly antithrombotic medications, the cause and mechanism of the injury, clinical symptoms and signs of mTBI, and the presence and extent of associated systemic trauma. The reason for the responsible physician prescribing a brain CT scan and the interpretation of CT findings by the local radiologist will also be collected. In addition, common analytical blood data will be recorded. The outcome assessment will be performed by trained central outcome assessors during a structured telephone interview at 1 week and 3 months and stored in the eCRF. Anonymised DICOM CT images will be transferred to a centralised platform (secure web central database France Life Imaging (FLI-IAM) Platform (Digital and Ethics, France) to be evaluated by central CT readers. DICOM CT images will be uploaded, and only the patient code will be associated with the CT because all DICOM images will be anonymised. Staff blinded to the results of the blood biomarkers will capture all data, particularly functional outcome assessments and CT scan analysis.

Each centre's principal investigator will ensure the completeness and accuracy of the information recorded in the eCRF and the transfer of the CTs to the centralised platform. In addition, data checks during the study period will be scheduled every 3 months during the duration of the study to identify queries and resolve them promptly.

Data analysis CT scans

Patients with mild TBI will be eligible for inclusion if they undergo head CT scanning within 12 hours after the injury as part of their clinical care. CTs will be uploaded to a secure web central database (FLI-IAM Platform) that complies with the European rules of General Data Protection Regulation. Two independent central readers will classify CT findings as positive or negative. In cases of disagreement, final adjudication will be performed by a third independent neuroradiologist. Because CT status (positive or negative) is the outcome of the study's primary aim, the criteria for CT status are one of the most important aspects of the study. Items that will classify CT as positive or not are listed in table 1. All acute haemorrhagic lesions and findings associated with diffuse axonal injury or brain swelling will be classified as CT-positive. The presence of depressed skull fractures will also be classified as CT-positive, whereas linear skull or cranial base fractures will be recorded but not classified as CT-positive. Subdural hypodense hygromas, calcifications, ischaemia, facial fractures or scalp injuries will not be classified as CT-positive. However, all findings are recorded by each central reader and classified using the Common Data Elements definition, and this information is stored in the eCRF.

Data analysis: biomarkers

A 10 mL blood sample will be obtained from each participant in the study within 12 hours after the reported time of injury to determine biomarkers using best practices in phlebotomy to prevent sample haemolysis and ensure the simple is fully processed within 2 hours after blood sampling. Blood samples will be collected using two (5 mL) gel separator tubes for serum and allowed to clot for 30 min at room temperature (18°C–25°C). The samples will then be centrifuged at 2000 G×g for 10 min. Within 1 hour maximum of centrifugation, the serum will be processed, aliquoted (5×1 mL) in bar-coded cryovials, and stored in a freezer at −80°C until shipment on dry ice to bioMérieux biobank (Marcy l'Étoile, France). All measurement procedures will be independently conducted, and the conductors will be blinded to the clinical data. The samples will be batch analysed with a clinical-use automated VIDAS3 platform using the VIDAS TBI (GFAP, UCH-L1) kit (bioMérieux) for quantitative measurement of GFAP and UCH-L1 in human serum.

Table 1 Items used for qualifying CTs as positive or negative

Items that will classify CT as positive	Items that will not classify CT as positive
Epidural haematoma	Subdural hygroma
Acute or subacute subdural haematoma	Chronic subdural haematoma (no high intensity lesion inside the haematoma)
Indeterminate extra-axial haemorrhage	All parenchymal or extraparenchymal calcifications
Subarachnoid haemorrhage	Brain tumours
Intraventricular haemorrhage	Linear non-depressed fracture
Depressed cranial fracture	Cranial base fracture
Brain contusion (including high intensity/mixed lesions and hypodense lesions)	Pneumoencephalus
Intraparenchymal traumatic haematoma	Hypodense chronic ischaemic lesions
Brain swelling	Facial fractures
Petechial haemorrhage	Scalp injury
Gliding contusions	
Signs of traumatic axonal injury (hyperdense lesions in brainstem or corpus callosum)	
Brain swelling or oedema	
Compressed cisterns	

The development status of the test when drafting this manuscript is clinical trials readiness 7.

Serum aliquots not used for the study's main purpose will be stored within the bioMérieux biocollection (CODECOH DC-2008-50; Marcy l'Étoile, France) for future use. These samples may be used under the conditions described in the BRAINI2 consortium agreement between partners and in compliance with signed informed consent.

Sample size and inclusion plan

The study's primary endpoint is the diagnostic performance of the VIDAS TBI (GFAP, UCH-L1) assay in predicting whether the tested patient will be positive or negative for brain injury visible on head CT scan in the elderly population (BRAINI2-ELDERLY DIAGNOSTIC and PROGNOSTIC STUDY).

Two different age groups are considered in the elderly population: 65–79 years and ≥80 years. Ideally, at least 120 patients with positive CT findings will be included in each group. To calculate the sample size for this study, we will focus on measuring the NPV of GFAP and UCH-L1, alone or in combination, to rule out intracranial abnormalities on CT in mTBI patients (GCS 13–15). The goal is to safely rule out the need for CT without missing many patients with intracranial abnormalities. Assuming the prevalence of CTs showing intracranial abnormalities in this population is 11%, with a targeted NPV≥98.8%, sensitivity ≥96.7%, and specificity ≥34.2%, and adding a 10% lost to follow-up, a required target sample of 120 CT-positive mTBI patients and a sample size of 1185 patients (1077+10%) should be included using strata. The overall number of mTBI patients to be included is 2370 (1185<80 years and 1185≥80 years). As inclusion of patients is a dynamic process the steering committee will increase the number of centres contributing to the study in the participating countries if there are delays in the recruitment process. Also, If CT positivity, confirmed by central reviewers, is significantly different than in our initial assumption (11% CT positivity), the sample size might be adjusted accordingly, in order to maintain recruitment of at least 120 CT positive in each patient group. The targeted sample size will be adjusted following study midterm interim analysis.

To obtain similar-sized groups from each age group, 65–79 and ≥80 years, each centre will be allocated to include a certain number of participants from each group. It is known from the experience of the BRAINI study that the number of patients suffering from TBI and the probability of undergoing CT as a standard of care increases with age. Therefore, the probability of inclusion will increase with age, and the oldest group in the study can be overrepresented. Therefore, every 6 months, inclusions from each centre will be analysed based on age. Adjustments to the inclusion allocation will be made if deemed necessary. Therefore, the target numbers of included patients according to age group will be established for each centre following the study inclusion plan.

Regarding the BRAINI2-ELDERLY/REFERENCE group, there is a need to recruit two patient groups (with and without comorbidities) of a similar size. The standard approach recommended by the Clinical Laboratory Standards Institute (CLSI) EP28-A3c guidelines (CLSI. EP28-A3C, Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline-Third Edition, Clinical and Laboratory, Standards Institute, Wayne, Pennsylvania, USA, 2008) to establish reference values is to collect and analyse a minimum of 120 samples from healthy participants from the local population. This has the advantage of allowing 90% confidence limits to be computed non-parametrically for each reference limit. Therefore, 480 non-TBI patients will be included in the study, with 120 non-TBI patients in each of the 4 groups (65–79 years with comorbidities; 65–79 years without comorbidities; ≥80 years with comorbidities and ≥80 without comorbidities).

Statistical considerations

The BRAINI study's results will be used as a reference for the statistical analysis of the different outcomes in the BRAINI2 study. Initially, the predictive performance of the BRAINI predefined cut-off values for the two biomarkers will be validated in the BRAINI2 subsample of patients without comorbidities. Next, diagnostic accuracy indicators (sensitivity, specificity, predictive values and likelihood ratios) will be estimated. Validation will be performed in a bivariate (age and biomarkers) and multivariate context. The predictive performance of the biomarkers will then be reassessed in the entire BRAINI2 cohort. For secondary outcomes (prognosis), the best-performing predictive model developed in the BRAINI will be validated in the BRAINI2 subsample without comorbidities. Then, the predictive performance of the biomarkers will be reassessed in the entire BRAINI2 cohort. The STARD, REMARK and TRIPOD guidelines will be implemented.

Finally, the distribution of reference values for GFAP and UCHL-1 in the non-TBI population (BRAINI2-ELDERLY-Reference) will be described based on age and comorbidities. In addition, the CLSI-EP28-A3C guidelines for 'Defining, Establishing and Verifying Reference Intervals in the Clinical Laboratory' will be implemented (CLSI https://clsi.org/).

Patients and public community involvement

Patient associations are partners of the BRAINI2 consortium and are part of the governing structure of the study (general assembly). They are involved in this research's design and conduction as they participate in the choice of secondary outcome measures. In addition, they contribute to the assessment protocol, with some questions evaluating cognitive impairment after TBI. Patient association representatives and

selected patients were present during the initial visits to some clinical centres.

Ethics and dissemination

Ethics approval was obtained from each recruitment centre: (1) the Comité Ético de Investigación Clínica of Hospital 12 de Octubre, Madrid, Spain, on 22 February 2022 (Ref #22/027), and the study started recruitment on 1 March and (2) Southeast VI of Clermont Ferrand Hospital, France, on 5 September 2022 (Re# 22.01782.000095), and the study started recruitment on 5 December 2022. In Germany, the study has been authorised on 10 May 2023 by the competent authority, the national 'Bundesinstitut für Arzneimittel und Medizinprodukte' (BfArM), as meeting the expected legal requirements of a performance study in the scope of the regulation (EU) 2017/746 on in vitro diagnostic regulation.

This study's results will be presented at national and international meetings, including meetings of patient associations, and published in peer-reviewed journals. There will be no notifications of individual results to the patients. All active collaborating investigators, research coordinators and institutions will receive credit from the main publications of the study under the name of BRAINI2 investigators.

DISCUSSION

This study's results will need to be discussed considering the different large clinical studies already published (ALERT-TBI, CENTER-TBI, TRACK-TBI) and after a full analysis of the BRAINI study.[16 17 20 22 27 34–37] Both ALERT-TBI and CENTER-TBI studies have shown the ability of both GFAP and UCH-L1 to rule out the presence of lesions on CT. The CENTER-TBI study did not show an absolute advantage of combining both biomarkers (GFAP and UCH-L1).[22] However, this study used samples within 24 hours after mTBI and a research-use-only assay with a poor agreement between replicates of biomarker assessment. The ALERT-TBI cohort evaluated only a small proportion of patients aged >65 years (25%); in both studies, patients with neurological comorbidities were excluded.[16] In the European BRAINI study,[27] the proportion of patients >65 years represents a large proportion of the cohort (55%, unpublished observation). Therefore, the BRAINI study will be able to determine if previous predefined cut-offs for GFAP and UCH-L1 in the general adult population are valid for this subset of the population and define alternate thresholds, thereby improving the specificity of the test for this population. The VIDAS3 platform will measure GFAP and UCH-L1 within 12 hours post-TBI. This study will be able to externally validate this cut-off in elderly and very elderly patients (>80 years) and demonstrate whether GFAP and UCH-L1 can be used effectively in mTBI patients with neurological comorbidities using the same analytical platform and procedures. Regarding prognosis, both TRACK-TBI and CENTER-TBI have demonstrated the role of both biomarkers in improving functional outcome prediction in the general mTBI population.[36 38] In this study, GFAP and UCH-L1 will be assessed for their ability to predict mid-term neurological outcomes and quality of life in the elderly.

However, this study has some limitations. Variability in mTBI management and CT prescriptions between centres and countries is expected. However, this may have influenced the CT-positive prevalence across centres and affected the study's statistical power. The reason for performing cranial CT will be recorded to understand this variability. Variability between central readers can also arise when assessing the presence of intracranial lesions. A precise definition of the lesions will be used to consider CT-positivity and has been included in the protocol. Final adjudication will be performed by a third central reader. A sensitivity analysis will be performed to estimate the potential effect of disagreement among the neuroradiology reviewers.

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Contributors All the authors initiated the study design, wrote the study protocol and drafted the manuscript. AL is the first author because he has contributed to the study concept, design of the work, literature review, analysis, interpretation of data, drafting the work, final approval of the version to be published and agreement to be accountable for all aspects of the work. J-FP, PB, MAP, OM, VP, DV, VS, AL and JdlC: contributed to study concept, drafting of the work, critical revision, final approval of the version to be published and agreement to be accountable for all aspects of the work. All participants were part of the steering committee of the study. All authors attested to significantly contributing to the study's protocol refinement of the study protocol and approved the final manuscript. All members of BRAINI2 contributed to the design and application of the protocol.

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Competing interests OM and VP are employees of bioMérieux.

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