STUDY PROTOCOL

General information	
Full study title and acronym	• **The adding value of undetectable protein S100 B in ruling out intracranial injuries following mild head injury** • Version 1.0 17 dec 2018 • PS100B study
Name and contact details of principal investigator	• Andrea Fabbri • Emergency Department AUSL Romagna - Forlì, Italy
Study duration	• The estimated study start will be april 2020, with last subject visited april 2021 (study duration: 12 months)
Study sites	The study will be in the area of Emergency Department and involves 7 centers: 1. Emergency Dept AUSL Romagna – Forlì (Coordinating center). Principal Investigator: Dr. Andrea Fabbri. 2. Emergency Dept Hospital Martini – Turin. Investigator: Dr. Fabio De Iaco. 3. Emergency Dept, AOU di Padova – Dr Vito Cianci. 4. Emergency Dept AUSL Romagna – Cesena. Investigator: Dr. Alessandro Valentino 5. Emergency Dept AUSL Romagna – Rimini. Investigator: Dr.ssa Tiziana Perin 6. Emergency Dept AUSL Romagna – Ravenna Investigator: Dr. Andrea Rossi 7. Emergency Dept. ASL Roma 2 - Roma – Dr. Francesco Rocco Pugliese
1. Introduction, study rationale and study objective(s)	
Introduction and rationale	Traumatic brain injury (TBI) is a common cause of death and disability, primarily in the young but increasingly among the elderly. The injury panorama stretches from the severely injured, unconscious patients in need of neuro-intensive care in the more common mildly injured patients, sometimes without any visual lesions. Many survivors, even from seemingly mild injuries, may suffer from

	permanent disabilities and be in need of long term rehabilitation with costs for society. TBI is a complex disease with changing clinical symptoms over time; it is heterogenic in nature and may contain a plethora of different hemorrhagic and non hemorrhagic injures, both inside and outside the brain parenchyma. Most (up to 95%) of head injuries are classified as mild head injury (MHI), defined as Glasgow Coma Scale (GCS) 14-15 and a set of clinical variables. Previous studies reporte only 5% to 10% of positive CT scan and a set of clinical variables and risk factors were included in the predictive model with good sensitivity and high specificity. There is concern in asintomatic subjects, where the likelihood of intracranial lesion is particularly low also in the presence of risk factors and or symptoms, since in these cases the sensitivity is low and the negative predictive value (NPV) very high, i.e. high number of cases with negative head CT scans. Due to the considerable resource use and high number of unnecessary CT scans, recent efforts have been concentrated on optimizing CT use after MHI. Due to the high socioeconomic cost of missing cases of intracranial complication, CT rates remain high. The methods are often limited, and better surrogate markers of brain injury have been sought to help the treating clinician. In many fields of medicine, biological markers ("biomarkers") of injury have been introduced. A biomarker is defined as "A characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes or pharmacologic responses to a therapeutic intervention. While a number of potential markers of brain tissue fate exist, the most studied protein biomarker of brain injury is S100B. The first human TBI study of S100B's value as a serum biomarker of brain injury assessment was published by Ingebrigten and coworkers in 1995, although increased S100B levels in cerebrospinal fluid (CSF) foolowing various neurological disorders had been previously described in patients by Sindic et al. in 1982. Later on, the protein S100B, a 21-kDa calcium-binding glial-specific protein mainly expressed by astrocytes, has received a special attention as a possible biomarker for brain damage after minor head injury, especially for cerebral edema and brain contusion. The half-life of S100B has been shown to be in the range of 60 to 120 min in patients with TBI and 90 min. Protein S100B has a predictive negative value (NPV) that reaches up to 99% for intracerebral

	hemorrhage (ICH) and 100% for neurosurgical injuries. Adding S100B protein blood level to current recommendations could therefore reduce the need for CT examination and save costs. A set of clinical variables have just defined to predict intracranial lesions with accuracy wich accounts over 85%. The protein S100B has been introduced in the Scandinavian guidelines for initial management of minimal, mild and moderate head injuries in adults.
Study objective(s)	To assess the reliability of S100B as a negative predictive tool for ICH after mild head injury in reducing the number of negative and unnecessary CT scan.
2. Investigational material	
Investigational material and comparator product(s)	Venous blood samples must be obtained from each patient within 6 hours after injury. We chose a 6-hour cut-off because of the short half-life of the S100B protein, ranging between 25 and 120 minutes. Serum S100B levels must be determined by eletrochemiluminescence immunoassay on the Roche cobas e602 instrument (Roche Diagnostics, Meylan, France). The analytical range is between 0.005 g/L and 39 g/L. The cut-off was set as 0.105 g/L as specified by Roche Diagnostics.
3. Study population	
Recruitment, enrollment period, and sample size	• Approximately 4000 subjects will be recruited over a planned recruitment period of 12 months.
Inclusion criteria	• Age ≥ 18 years • Informed consent
Exclusion criteria	• Refusal of informed consent • Serum sampling for S100 B time interval from injury >6 hours. • Any symptom after head injury e.g. diffuse headache, vomiting, clinical signs of skull base fracture, focal neurological deficit, post-traumatic seizure. • Unknown time of the trauma and missing informed consent.
4. Study design and study procedure	
Study design	• This is a observational, prospective, multicenter, national study in patient with mild head injury admitted to the Emergency Department.

Study procedure	The treating emergency physicians completed standardized data form prior to cranial CT. Items in the questionnaire included age, gender, antithrombotic medication, mechanism of injury, LOC, amnesia, alcohol or drug intoxication, GCS 15. Antithrombotic medication included antiplatelet therapy, P2Y12 inhibitors and anticoagulant therapy [vitamin K antagonists (VKA) or new oral anticoagulants (NOA)].
5. Statistics	
Primary and secondary endpoint(s)	To assess the reliability of S100B, a negative predictive value (NPV) >99.7% for rule out of ICH in subjects following mild head injury,.
Statistical methods	Mean value, standard deviation (SD), median, inter-quartile range (IQR) and frequencies will be used to describe data distribution. A multivariable logistic regression with forward stepwise selection with a *P* value lower than 0.05 for removal of variables will be performed. The odds ratio (OR) and 95% confidence intervals (95% CI) will be also calculated. Proportions will be performed to compare by means of Fisher's exact test. Sensitivity and specificity will be evaluated, together with the negative predictive value (NPV). We'll test the associations between each risk factor and the primary outcome measure using chi-square tests for nominal variables, the Mann–Whitney U test for ordinal variables, and the unpaired 2-tailed *t*-test for continuous variables by using SPSS software, version 17.0 (SPSS Inc., Chicago, Illinois). The operating characteristics by calculating the area under the receiver operating characteristic (ROC) curve for variables selected by the multivariable logistic regression analysis will be calculated.
Sample size, level of significance, and power	In sample size calculation , we estimated that 4,000 patients would analbe us to stimate a NPV 99.7% with 95%CI 99.5% 99.9% and that 92% power for an alfa of 0.05 to test the null hypothesis that the NPV was less than 99.5%.
6. Safety assessment	
	Biochemical analysis of S100 B was performed from routine blood

	samples included in the current clinical pathway of head injury, which is operating in any Emergency department.
Follow up of ongoing AEs and SAEs	30 day telephone call follow up will be obtained by Glasgow Outcome Scale.
7. References	
Literature references	• Unden et al. BMC Medicine 2013, 11:50. Scandinavian guideline for initial management of minimal, mild and moderate head injuries in adults: an evidence and consensus-based update. • Eric Peter Thelin et al. Acta Neurochir (2017) 159: 209-225. A review of the clinical utility of serum S100B protein levels in the assessmentof traumatic brain injury. • A. David et al. Diagnostic and Interventional Imaging (2017) 98, 551- 556. Evaluation of S100B blood level as a biomarker to avoid computed tomography in patients with mild head trauma under antithrombotic medication • Cassidy JD, Carroll LJ, Peloso PM, Borg J, von Holst H, Holm L, Kraus J, Coronado VG: Incidence, risk factors and prevention of mild traumatic brain injury: results of the WHO Collaborating Centre Task Force on Mild Traumatic Brain Injury. J Rehabil Med. 2004, 43 (Suppl): 28-60. • Ingebrigtsen T, Romner B, Kock-Jensen C: Scandinavian guidelines for initial management of minimal, mild, and moderate head injuries. The Scandinavian Neurotrauma Committee. J Trauma. 2000, 48: 760-766. 10.1097/00005373-200004000-00029. • Haydel MJ, Preston CA, Mills TJ, Luber S, Blaudeau E, DeBlieux PM: Indications for computed tomography in patients with minor head injury. N Engl J Med. 2000, 343: 100-105. 10.1056/NEJM200007133430204. • Stein SC, Fabbri A, Servadei F, Glick HA: A critical comparison of clinical decision instruments for computed tomographic scanning in mild closed traumatic brain injury in adolescents and adults. Ann Emerg Med. 2009, 53: 180-188. 10.1016/j.annemergmed.2008.01.002. • Stein SC, Spettell C: The Head Injury Severity Scale (HISS): a practical classification of closed-head injury. Brain Inj. 1995, 9: 437-444. 10.3109/02699059509008203. • Zongo D, Ribéreau-Gayon R, Masson F, Laborey M, Contrand B, Salmi LR, Montaudon D, Beaudeux JL, Meurin A, Dousset V, Loiseau H, Lagarde E: S100-B protein as a screening tool for the early assessment of minor head injury. Ann Emerg Med. 2012, 59: 209-218. 10.1016/j.annemergmed.2011.07.027.

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