Annals of Trauma & Acute Care

Short Communication
Published: 17 Jan, 2018

S100B and GFAP in Traumatic Brain Injury: A Pilot Study

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Abstract

Traumatic Brain Injury (TBI) is a leading cause of death and disability worldwide. There is great interest in the use of potential biomarkers of TBI for diagnosis, triage, prognostication and drug development. S100B and Glial Fibrillary Acid Protein (GFAP) are the most studied and candidate biomarkers of TBI. However, conflicting results hamper their routine application in clinical practice. In this study, serum samples of a small cohort of TBI patients at different severity and different time points from the injury were analysed by measuring the levels of S100B and GFAP.

Keywords: Traumatic brain injury; Biomarkers; Diagnosis; S100B; GFAP

Introduction

Traumatic brain injury (TBI) is a leading cause of mortality and morbidity in both civilian life and the battle field worldwide [1]. TBI is caused by a deformation of the brain resulting in a cascade of pathological events and death of brain cells. Survivors of TBI frequently experience long-term disabling changes in cognition, sensor motor function and personality. The rapidly evolving nature of TBI, makes challenging to recognize and treat those patients who are still capable of responding to therapy before irreversible damages occur, whilst avoiding the use of unwarranted medical or surgical interventions. For this reason in the last decades, extensive research was carried out on the use of biomarkers in biological fluids and reflecting brain damage [2]. Different proteins were mainly studied in serum/plasma or Cerebro Spinal Fluid (CSF), and among the most studied, there are S100B and the Glial Fibrillary Protein (GFAP) [3,4,5]. However, many studies have failed to show reproducible results, therefore their clinical applicability is still a matter of debate. The first candidate biomarker, S100B is a brain-enriched member of the S-100 family of low molecular weight (10.5 kDa) calcium-binding proteins. S100B is mainly present in mature, perivascular astrocytes and very low levels were found in CSF and serum (0.05 ng/ml) [6]. For this reason, S100B was initially considered a marker of astrocytic damage in TBI. However, as S100B does not cross the intact Blood-Brain Barrier (BBB), its release was later considered more reflective of BBB disruption rather than the severity of the brain tissue damage [7]. In addition, other studies showed the presence of this protein in Extra-Cranial (EC) trauma patients [7], and this because of its expression in melanocytes and in various non-nervous tissues including fat, skin and skeletal muscle [8,9]. The second most studied biomarker, GFAP, is a monomeric filament protein expressed by astocytes. This protein is a brain-specific protein and its level was found normal in poly trauma patients who did not have brain injury [10]. Also in this case, the protein does not cross the BBB, unless seriously compromised. However its release after TBI was associated with elevated ICP, reduced mean arterial pressure, poor Glasgow Outcome Scale (GOS) and mortality [11]. In this pilot study, the levels of S100B and GFAP were measured in serum of 5 mild TBI+EC (mTBI+EC), 5severeTBI+EC (sTBI+EC), and compared with serum level of EC injury only patients, with the aim to shad the light on the role of GFAP and S100B as potential biomarkers of TBI and on their use in the clinical management.

Materials and Methods

Study participants

Study participants were recruited from the Surgical Reconstruction and Microbiology Research Centre (SRMRC) at Queen Elizabeth Hospital of Birmingham (UK) as part of Golden Hour study (Ethics Ref. 13/WA/0399). Written informed consent was received from participants or valid proxy (family or a professional not directly involved in the study) prior to inclusion in the study. Serum samples from a total of 15 patients: 5 mTBI+EC, 5 sTBI+EC and 5 EC were taken at different time points (T0-1h, T4-12h, T48-72h) in each patient. EC injury patients had radiographically-

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Received Date: *19 Dec 2017*
Accepted Date: *08 Jan 2018*
Published Date: *17 Jan 2018*

Citation:
Di Pietro V, Belli A. S100B and GFAP in Traumatic Brain Injury: A Pilot Study. Ann Trauma Acute Care. 2018; 2(1): 1006.

Table 1: Clinical characteristic of the study subjects.

Study group	Number of samples	Age	Gender	Mechanism of injury[a] (%)		CT[b] (%)	GCS	Outcome (GOSe score)
		Mean ± SD (Range)	M/F	A	RTC	+/-	Median ± SD (Range)	Median ± SD (Range)
EC	5	35.6 14.5 (18-52)	3/2	0	100	NA	NA	NA
mTBI+EC	5	47.2 20.2 (23-72)	4/1	0	100	80/20	141.1 (13-15)	62.14 (1-7)
sTBI+EC	5	56 18.6 (31-78)	3/2	30	70	60/40	40.7 (3-8)	32.59 (1-8)

[a] Mechanism of injury: A, assault; F, RTC, road traffic accident;
[b] Computed tomography findings: -, no visual pathology; + visual pathology (swelling, contusion, mass lesion).
NA: Not Applicable

Table 2: S100B and GFAP concentrations measured by ELISA at different time points in blood of TBI patients.

	mTBI+EC T0	mTBI+EC T4-12h	mTBI+EC T48-72h	sTBI+EC T0	sTBI+EC T4-12h	sTBI+EC T48-72h	EC T0	EC T4-12h	EC T48-72h
S100B pg/ml	ND	ND	ND	834	ND	ND	15	ND	ND
	ND	ND	ND	832	376	ND	820	ND	ND
	ND	ND	ND	798	310	ND	798	ND	45
	ND	ND	ND	826	ND	ND	254	310	ND
	ND	ND	ND	306	ND	90	ND	ND	ND
GFAP ng/ml	ND	ND	ND	5.052	ND	ND	5.052	ND	ND
	ND	ND	ND	5.652	1.368	ND	5.652	1.368	ND
	ND	ND	ND	8.773	5.538	ND	8.773	5.538	ND
	ND	ND	ND	ND	0.041	ND	ND	0.041	ND
	ND	ND	ND	ND	ND	ND	ND	ND	ND

mTBI+EC= mild traumatic brain injury + extra-cranial injury
sTBI+EC= severe traumatic brain injury + extra-cranial injury
EC= extra-cranial injury
ND= not detectable

confirmed orthopaedic fractures, no head trauma, no infection, no history of neurological or psychiatric disorders and no alcohol or drug dependency. Mild TBI with EC included those with a non-penetrating head trauma and Glasgow Coma Scale (GCS) score ≥ 13. Severe TBI with EC included patients with GCS ≤ 8. Clinical, demographic and imaging parameters were collected for each patient. Some of these data are presented in Table 1.

Measurement of S100B and GFAP in serum patients

Release of S100B and GFAP was measured in serum patients by using immuno enzymatic ELISA kits (Abnova, Neihu District, Taipei City, Taiwan) according to the manufacturer instructions. Briefly, 100 µL of standards with known concentration and an equal amount of serum samples were incubated in microplate wells pre-coated with antibody specific for S100B or GFAP. After incubation, biotinylation, conjugation with streptavidin-horseradish peroxidase plates were incubated for 30 min at 37 ˚C with 3,3′,5,5′-tetramethylbenzidine. The reaction was stopped by addition of 50 µL of acidic solution and absorbance of the resulting yellow product was measured spectrophotometrically at 450 nm (Molecular Devices, Sunnyvale, CA, USA). Using this protocol, the standard curves were linear and ranged from 0.050 to 4 ng/ml for S100B and from 0.25 to 25 ng/ml for GFAP.

Results

The concentrations of S100B and GFAP are shown in Table 2. S100B was detected in 5 of 5 sTBI+EC patients at T0-1hour (within 1h from injury); in 2 of 5 patients at T4-12hour and in 1 patient only at T48-72 hours. S100B was also measured in 4 of 5 EC patients at T0-1 hour; in 1 of 5 at T4-12 hour and 2 of 5 at T48-72 hour. No signal was detected in mTBI+EC group. GFAP levels were detected in 3 of 5 sTBI+EC patients within 1h from injury. At T4-12h, the GFAP levels were still detectable in 3 of 5 sTBI+EC patients but not at T48-72 hour. In addition, GFAP protein was not detected in mTBI+EC patients or EC patients at any of the time points.

Discussion

For many years S100B was considered one of the most promising biomarker of TBI. Although many studies showed the limitation of its clinical applicability [12], S100 Bisstill included in an algorithm in Scandinavian guidelines to triage patients with mild TBI for CT imaging after head trauma [13]. In our pilot study, despite the small cohort of patients, we were not able to detect any S100B signal in mTBI+EC group, which includes an 80% of positive CT scan. In addition, S100B was measured in both sTBI+EC and EC groups, confirming a not specific role in the diagnosis of brain damage. Unlike S100B, GFAP resulted very specific for brain damage. As this marker was not found in multi trauma patients, an increase in circulating GFAP can be entirely attributable to cell damages occurring to the brain tissue. In mTBI+EC group, GFAP did not show any release, supporting previous findings on GFAP, which identify this biomarker as a predictor of mortality rather than a biomarker of mTBI [11,14]. One of the limitations of this study is the limit of detection of the technique used. ELISA test might be not sensitive enough to precisely determine low levels or traces of these 2 proteins in serum. However, our findings align with those of other researchers and support the concept that new class of molecules must be studied to accurately diagnose and monitor the different severity of TBI [15,16,17,18]. Nevertheless, these proteins can still have their clinical utility in determining much more severe forms of TBI or in predicting mortality. Certainly, their use in combination with other biomarkers can increase their diagnostic value in sensitivity and specificity [19].

Acknowledgements

This study was funded by the National Institute for Health Research (NIHR). The views expressed are those of the author(s) and not necessarily those of the NHS, the NIHR or the Department of Health.

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