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Interest of blood biomarkers to predict lesions in medical imaging in the context of mild traumatic brain injury

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ABSTRACT (210 words)

Mild traumatic brain injury (mTBI) is one of the common causes of emergency department visits around the world. Up to 90% of injuries are classified as mTBI. Cranial computed tomography (CCT) is a standard diagnostic tool for adults with mTBI. Alternatively, children can be admitted for inpatient observation with CCT scans performed only on those with clinical deterioration. The use of blood biomarkers is a supplementary tool for identifying patients at risk of intracerebral lesions who may need imaging. This review provides a contemporary clinical and laboratory framework for blood biomarker testing in mTBI management. The S100B protein is used routinely in the management of mTBI in Europe together with clinical guidelines. Due to its excellent negative predictive value, S100B protein is an alternative choice to CCT scanning for mTBI management under considered, consensual and pragmatic use. In this review, we propose points to help clinicians and clinical pathologists use serum S100B protein in the clinical routine. A review of the literature on the different biomarkers (GFAP, UCH-L1, NF [H or L], tau, H-FABP, SNTF, NSE, miRNAs, MBP, β trace protein) is also conducted. Some of these other blood biomarkers, used alone (GFAP, UCH-L1) or in combination (GFAP + H-FABP \pm S100B \pm IL10) can improve the specificity of S100B.

KEYWORDS: mTBI, biomarkers, S100B, GFAP, UCH-L1, H-FABP, SNTF, NFL, NHL, MBP, NSE

HIGHLIGHTS

- The S100B protein can be used in the clinical routine in the management of mTBI.
- S100B protein serum levels, in combination with a clinical algorithm, could reduce the need for computer tomography scans by one-third.
- Sampling should take place within 3 hours of trauma for S100B assay.
- For S100B and probably other biomarkers, specific cut-off levels and paediatric reference ranges had to be defined.
- GFAP in combination with H-FABP could improve the specificity of S100B.

1. INTRODUCTION

A mild traumatic brain injury (mTBI) is one of the common causes for emergency unit visits around the world, with an estimated incidence of 100–300 per 100,000 habitants [1]. Traumatic brain injuries (TBIs) are classified as severe, moderate or mild based on a Glasgow Coma Scale (GCS) score of 3–8, 9–12 or 13–15, respectively [2]. Up to 90% of the injuries are established as mTBI [1]. Cranial computed tomography (CCT) is often part of the classical management of adults with mTBI. In children, three recent epidemiological studies have recently shown that radiation exposure from CCT scans increases the incidence of cancers [3–5]. In order to reduce this exposure, a clinical observation (at hospital) may also be indicated, with a prescribed CCT scan only in case of clinical worsening. However, this strategy is more expensive than performing the CCT scan in the first place [6]. According to published studies in adults showing that 85–99% (93–100% for children) of mTBI have no intracerebral lesions, most CCT scans or clinical observations could be avoided [7,8]. That's why, clinical decision rules were established to help clinicians for the identification of patients with a low risk of intracerebral lesions [9,10]. In adults, mTBIs are clustered into three groups according to clinical and anamnestic criteria defined by the Canadian CT Head Rules, namely, mTBI with a low, medium or high risk of complications [9]. In children, the Pediatric Emergency Care Applied Research Network (PECARN) validated a similar clinical decision rule [11]. Patients with a low risk of complications can follow the monitoring process at home, decreasing the number of CCT scans to 50% for adults [12] and 10% for children [10] for all three risk categories. However, in the medium-risk category, most CCT scans remain negative.

In this context, the use of blood biomarkers is a supplementary tool to identify patients at risk of intracerebral lesions who may need imaging. This review provides a contemporary

clinical and laboratory framework for biomarker testing in mTBI management. In this review, we propose points to help clinicians and clinical pathologists use serum S100B protein in the clinical routine. A review of the literature on the different biomarkers (GFAP, UCH-L1, NF [H or L], tau, H-FABP, SNTF, NSE, miRNAs, MBP, β trace protein) is also conducted.

2. METHODS

The search strategy was developed and conducted with the affiliated Medical Library after input from clinical investigators on the research team. Studies were found through a search on the following electronic databases: Medline, Embase, the Cochrane Central Register of Controlled Trials (CENTRAL), Web of Science, Scopus and Google Scholar. Appropriate combinations of controlled vocabulary and key words were used with adjustments made to account for differences in indexing across databases: trauma, mTBI, mild traumatic brain injury, biomarker, biomarkers, S100B, S-100B, S100, S100b, GFAP, UCH-L1, UCHL1, NFH, NFL, Tau, H-FABP, SNTF, SBDPs, NSE, MBP, miRNAs, B Trace protein, BTP, adult, child, pediatric. The search was augmented by scanning the reference lists of identified relevant studies. All titles and abstracts pertinent to our study were retrieved and searched for full texts. The literature search and data analysis were performed from inception to March 15, 2019.

In this review, we propose points to help clinicians and clinical pathologists use serum S100B protein in the clinical routine. A review of the literature on the different biomarkers (GFAP, UCH-L1, NF [H or L], tau, H-FABP, SNTF, NSE, miRNAs, MBP, β trace protein) is also conducted. For biomarkers probably usable in the short term in clinical routine, we abstracted, in a table form, information related to study design, sample size, recruitment, patient characteristics (age, sex ratio, GCS score), laboratory aspects of biomarker (type of assay, concentrations, reference ranges, sampling information, time between mTBI and blood

sampling), comparison of CT-scan versus biomarker blood values (numbers of true positive, false positive, false negative, true negative, sensitivities, specificities, AUC, cutoff) and eventual clinical evolution (CE). Only English-language data were used for extraction.

3. BIOMARKERS OF INTEREST

Several molecules released from brain tissue after injury can serve as biomarkers of neurological complications. The cellular origins of these markers are diverse (astrocytes, neurons, ependymocytes), and they can be released into the blood or cerebrospinal fluid (CSF) (Table 1) [13]. Biomarkers show a temporal profile and kinetics associated with distinct pathobiological processes, such as necrosis, apoptosis, demyelination or neuro-regeneration [14]. In the context of mTBI, and thus, in this review, we focus on blood biomarkers.

2.1 Biomarker usable in clinical routine: S100B

The S100B protein is part of the family of S100 proteins and superfamily of calciproteins also called intracellular calcium-binding proteins. Characteristics include a low molecular weight (21 kDa) and a dimeric structure ($\beta\beta$ or $\alpha\beta$ chains)[15]. The β subunit characterizing the S100B protein, is mostly synthesized by astrocytes, but also by Schwann cells [16]. Other cells like histiocytes, adipocytes, chondrocytes and melanocytes explain its extracerebral synthesis [17]. The α subunit is, conversely, ubiquitously produced. The most important localization of the S100B protein is the cytosolic compartment. Several studies have established both intracellular (cell growth, cytoskeletal maintenance, energy metabolism, signal transduction) and extracellular functions of the protein (activation of different neuronal

1 and glial membrane receptors, such as the RAGE receptor and bFGF receptor 1), related to
2 protein–protein interactions [15,18]. As a result of cerebral lesions, the rapid release of S100B
3 from damaged glial cells into the bloodstream explains that the protein is detectable 1 hour
4 after trauma. With a short half-life between 30 and 100 minutes, S100B is then quickly
5 eliminated by the kidneys [19–21]. S100B can be measured in routine clinical practice by two
6 automated assays (Liaison XL[®] (DiaSorin) and Cobas[®] (Roche Diagnostics)) showing good
7 analytical performance [22]. Recently, bioMérieux also developed a prototype assay for
8 serum S100B measurement on a Vidas[®] 3 analyzer [23]. The hemolysis doesn't interfere with
9 the S100B assay due to the absence of intra erythrocytic concentration (as opposed to NSE).
10 However, leukocytes, and especially, lymphocytes contain a significant concentration of
11 S100B [22]. As melanocytes can release S100B protein under physiological conditions, dark-
12 skinned people have significantly higher S100B serum concentrations [24]. Moreover, the
13 S100B cellular exportation is synchronised with that of melanin. For several sports like
14 swimming, running, boxing, football, hockey or basketball, a minor increase of S100B serum
15 concentration has been constantly described [25]. In some contact sports, such as rugby, this
16 rise may be most significant [26].

17 For mTBI in adults, the prospective multicenter study published by Biberthaler *et al.*
18 [27] was the first to demonstrate the usefulness of serum S100B measurement (with a
19 decision making threshold at 0.1 µg/L), which was later confirmed by Undén and Romner's
20 [28] meta-analysis. In this context, Scandinavian guidelines were proposed for the
21 management of mTBI [29], and validated by interventional studies like those of Calgagnile *et*
22 *al.* [30], Undén *et al.* [31] and Allouchery *et al.* [32] (involving 512, 430 and 1,449 patients
23 with S100B assays, respectively).

24 All these studies showed a reduction of about one third in the number of CCT scans
25 performed, with sensitivities and negative predictive values close to 100%. Furthermore, the

relative CCT reduction is influenced by the age of the patient [32]. Non-optimal specificity of S100B may be explained by the S100B extracerebral synthesis in patients with acute fractures [33], muscle contusions [17] or other types of polytrauma [34]. The use of S100B is optimal in isolated mTBI. To reduce false negatives, serum samples must be obtained at 3 hours post-trauma compared to 6 hours [35], in conjunction with the short half-life of the S100B protein. A decision algorithm including S100B measurement for the management of mTBI patients is proposed Allouchery *et al.* [32]. This algorithm is given as an example and is similar to the Scandinavian recommendations [29]. Serum S100B haven't been cleared by the US Food and Drug Administration as an aid in the diagnosis of mTBI and this biomarker is now widely used in clinical practice in European countries.

For mTBI in children, a meta-analysis recently demonstrated the value of serum S100B as a biomarker in the management of pediatric mTBI, although this isn't a large multicenter study [36]. Blood collection should take place within 3 hours of trauma. Cut-off levels should be based on pediatric reference ranges [22,37,38] because S100B serum concentrations are higher at the beginning of life (Figure 1).

2.2 Biomarkers probably usable in the short term in clinical routine: GFAP, UCH-L1, NF (H or L), Tau and H-FABP

Glial fibrillary acid protein (GFAP) is a cytoskeletal protein belonging to the class of intermediate filaments specific for astrocytes [39]. The monomer, containing 322 amino acids (50 kDa) [40], has a tendency to polymerize and depolymerize dynamically in the cell compartment [41]. Ubiquitin carboxy-terminal hydrolase L1 (UCH-L1) is a 24-kDa protein essential to the ubiquitinylation and de-ubiquitinylation of unneeded or damaged proteins recognized by the proteasome [42]. UCH-L1 is considered as a promising biomarker in the

management of head trauma [44], due to a specific neuronal expression [43]. Neurofilaments are described as heteropolymeric components of the neuron cytoskeleton. Their structure consists in the association of a 68-kDa light neurofilament (NFL) subunit backbone with either 160-kDa medium (NFM) or 200-kDa heavy (NFH) subunit side arms [45]. As a result of TBI, the influx of intracellular calcium leads to a cascade of events causing the activation of calcineurin, a calcium-dependent phosphatase dephosphorylating neurofilament side arms, probably participating in axonal injury [46]. Tau is an intracellular microtubule-associated protein, mostly present in axons. This protein both participates in assembling axonal microtubule bundles and anterograde axoplasmic transport [47]. Because of its preferential localization in the axons, tau lesions could be related to axonal disruption [48]. During brain injury, a cleaved form of tau called c-tau is detected both in CSF and serum. In this context, c-tau is currently proposed as a potential biomarker of TBI.

The blood measurements of GFAP, UCH-L1, NF (H or L) and tau are not currently available in clinical routine practice. Indeed, in the major studies on this subject, the assays were carried out using enzyme-linked immunosorbent assay (ELISA) techniques [49–54]. Quanterix[®], an *in vitro* diagnostic company working on biomarkers in different fields including neurology, recently developed a fourplex assay using digital ELISA for the blood determination of GFAP, UCH-L1, NFL and total tau [55]. This innovative technology has a lower limit of detection adapted to their measurement in serum. In one study, the average coefficient of variation obtained for UCH-L1 assay in all the tested samples was high (29%), and one-third of them didn't meet the quality control requirements [56]. Given these points and the complex structure of GFAP, an *in vitro* diagnostic kit will require the verification of preanalytical and analytical performance and the determination of decision thresholds for routine clinical practice. Serum GFAP and UCHL-1 have recently been cleared by the US Food and Drug Administration as an aid in the diagnosis of mTBI [57]. In this study, serum

1 samples were analysed for UCH-L1 and GFAP concentration using chemiluminescent
2 enzyme-linked immunosorbent assays but the references of the reagents or of the analyzer are
3 not clearly cited. On their website, Banyan biomarkers[®] announces a collaboration with *in*
4 *vitro* diagnostic companies such as Abbott or bioMerieux. These companies are probably
5 working on the development of GFAP or UCH-L1 assay kits, which will be released leading
6 to the realization of studies based on interventional (and not only observational) impacts of
7 these biomarkers on large cohort of patients as already published for S100B.

8 Table 2 shows the main characteristics of the studies about the significance of
9 biomarkers for predicting lesions in medical imaging in the context of mTBI in adults. The
10 studies are heterogenous in terms of the sampling time, kit used and number of patients.
11 GFAP permits distinguishing patients with lesions on CCT scans from the first hour after
12 trauma (area under the curve (AUC) 0.86 [0.79–0.93]) and patients requiring a neurosurgical
13 intervention (AUC 0.94 [0.88–1]). GFAP is detectable for a long period of time (peak serum
14 concentration at the 20th hour after trauma). In the same study, UCH-L1 could also be used
15 from the first hour after trauma to identify patients with lesions on CCT scan (AUC 0.77
16 [0.68–0.85]) and patients requiring a neurosurgical intervention (AUC 0.92 [0.83–1]), with
17 results peaking earlier than with GFAP (before 8 hours post-trauma) [49]. Because GFAP is
18 detected for a long time, the assay can be performed within 48 hours after the trauma and
19 GFAP could be a better biomarker, than tau or NFL, to predict lesions on CCT scan [56]. In
20 the same cohort, GFAP and UCH-L1 presented similar sensitivity (97 and 100%,
21 respectively) and specificity values (18 and 21%, respectively) for detecting lesions on CCT
22 scan (Table 2) [50,51]. Because of the known neurospecificity of GFAP and UCH-L1, it's
23 unexpected that specificity results are less effective than those of the S100B protein. That's
24 why, highly interesting track is that of the combination of biomarkers. For example, the
25 combination of GFAP with heart fatty acid binding protein (H-FABP), an intracellular

vascular and brain fatty-acid transporter, could increase the specificity to 45.9% for a 100% sensitivity (Table 2) [52]. In the same study, the specificity could be improved to 55.9% (95% confidence interval: 46.8–64.9) thanks to the association of four biomarkers (GFAP, H-FABP, S100B and interleukin-10). On the other hand, tau doesn't seem relevant to detect lesions on CCT scans [53] or predict outcomes [58]. For GFAP, concentration levels are correlated with the outcome of patients after mTBI [49,59,60]. For mTBI in children, GFAP may be a promising diagnostic tool [61], whereas UCH-L1 appears more promising at mTBI diagnosis [62]. As the number of studies is limited, further approaches will be needed.

2.3 Biomarkers with insufficient evidence concept proofs for use in clinical routine

2.3.1 SNTF

The α II-spectrin N-terminal fragment (SNTF) or α II-spectrin breakdown product (SBDP) is part of the family of spectrin degradation products located in the cytoplasm of neurons. Spectrin is a major component of the cytoskeleton bound to the cell membrane of neurons. STNF-145 and STNF-150 are products of the degradation of spectrin obtained after calpain' cleavage, whereas STNF-120 is a degradation product produced by caspase 3 [63]. As a result of brain injury the accumulation of these fragments in damaged axons, produces an increase of the serum concentrations of STNF-145 and STNF-150 correlated with the outcome of children after TBI [44]. Recently, a study of concussions in hockey players showed that serum SNTF protein could be useful for diagnosing and predicting a concussion in athletes and providing decision support back to the game [64]. A validated SNTF assay isn't available yet in routine clinical practice.

2.3.2 Neuron-specific Enolase (NSE)

NSE, one of the five isozymes of the glycolytic enzyme enolase located in central and peripheral neuronal cell bodies, is known to rise after cell injury [65]. The availability of an automated assay for NSE determination and its strong neuronal selectivity represent two key points. However, its slow plasma elimination (half-life: about 48 hours) [66] and its high intra-erythrocytic and intraplatelet concentrations limit the use of NSE as a biomarker of brain trauma. Especially since it's a major source of error in the determination of the plasma level release of cerebral NSE (intra- or extra-vascular hemolysis, platelet activation) [67]. Therefore, this biomarker isn't used in the management of mTBI [68,69].

2.3.3 MBP

In oligodendroglial synthesis, myelin basic protein (MBP) is released into the bloodstream after head injury [70]. Its rapid degradation by proteases temporarily limits the analytical developments [71].

2.3.4 miRNAs

MicroRNAs (miRNAs) are small RNA molecules that are usually 20–24 nucleotides in length and are encoded from highly conserved DNA regions in the human genome but not translated into proteins [72]. In the last few years, molecular and cellular studies have highlighted the important role of miRNAs as biomarkers in the pathogenesis and progression of TBI. The serum concentrations of various specific microRNAs provide useful information in the diagnosis, severity and prognosis of TBI [73,74]. They could also be interesting for the outcome. Promising miRNAs, such as miR-21, miR-16, let-7i, miR-335 have been identified as suitable candidate biomarkers for TBI able to differentiate mild from severe TBI [75–77]. Recently, salivary detection of miRNAs was suggested for the diagnosis of mTBI [78] and more particularly in contact sports like rugby [79, 80] or mixed martial arts [81]. Salivary

miRNA levels may identify the duration and character of concussion symptoms such as memory difficulty, headaches or fatigue [82]. Select miRNAs were associated in serum with baseline concussion assessments at the beginning of the season and with neurocognitive changes from pre to post-season in collegiate football players [83].

2.3.5 *β Trace protein*

β Trace protein (BTP) is known to be mostly synthesized in the spinal leptomeninges and pachymeninx [84,85]. It would have a role closely related to prostaglandin metabolism as prostaglandin D synthase [86] and act as a carrier protein for retinoids [87]. BTP is located in high amounts in CSF, and it presents the highest CSF/serum ratio of all the CSF-specific proteins [88]. BTP, mainly used for the diagnosis of CSF leakage, can be measured in the serum by nephelometry using one of the two analyzers available in the routine : BNII[®] or BNProSpec[®] analyzers using the N Latex β -TP assay (Siemens Healthcare Diagnostics, Germany) [89]. Blood measurement of BTP has never been tested in the mTBI context; since we conducted the research in the Clermont-Ferrand teaching hospital, BTP assay with Siemens technology for the first 100 adults with mTBI also presented a blood assay of S100B (up to 3 hours post-trauma). We then studied the β trace's specificity and sensibility to eliminate intracranial injury. BTP measurement was not found to be a highly significant indicator for the correct stratification of patients with mTBI into the groups of presence or absence of lesions in CCT scans (area under the receiver operating characteristic [ROC] curve 0.51 [95% CI, 23–80%]; $p = 0.92$; data not published).

4. CONCLUSION

1 The S100B protein is used routinely in the management of mTBI in Europe together
2 with clinical guidelines. Because of its excellent negative predictive value, S100B is an
3 alternative of choice to CCT scanning for mTBI management, on the condition of a
4 considered, consensual and pragmatic use. This biomarker is mainly used in European
5 countries. Other biomarkers, used alone (GFAP, UCH-L1) or in combination (GFAP + H-
6 FABP ± S100B ± IL10 or GFAP + UCH-L1) need to be further evaluated together with
7 S100B to determine which combination of biomarkers is most accurate.

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FIGURE AND TABLE LEGENDS

Table 1. Biomarkers of mild traumatic brain injury

ELISA: enzyme-linked immunosorbent assay; ECLIA: electrochemiluminescence immunoassay; IFMA: immunofluorometric assay; IRMA: immunoradiometric assay; LIA: chemiluminescence immunoassay. Ella (Protein simple®) does immunoassays in a microfluidic Simple Plex cartridge.

Figure 1. Quantile regression analysis of serum S100B concentrations in healthy children using the DiaSorin® method (from Bouvier D *et al.*, 2015 [22]).

Serum S100B concentrations were determined in a cohort of 409 healthy children aged 0–16 years.

Table 2. Characteristics of Studies on the Interest of Biomarkers in Predicting Lesions in Medical Imaging

AUC: area under the curve, BDP: breakdown product, CI: confidence interval, CT: computed tomography, GCS: Glasgow Coma Scale, ECLIA: chemiluminescent enzyme-linked

1 immunosorbent assays; ELISA: enzyme-linked immunosorbent assay, GFAP: glial fibrillary
2 acid protein, H-FABP: heart fatty acid binding protein, MRI: magnetic resonance imaging,
3 NFH: heavy neurofilament subunit, NFL: light neurofilament subunit, SE: sensitivity, SD:
4 standard deviation, SP: specificity, UCH-L1: ubiquitin carboxy-terminal hydrolase L1.

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