Molecular and Cellular Neuroscience xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Molecular and Cellular Neuroscience

journal homepage: www.elsevier.com/locate/ymcne

Fluid markers of traumatic brain injury

Henrik Zetterberg [a,b,*], Kaj Blennow [a]

[a] Clinical Neurochemistry Laboratory, Institute of Neuroscience and Physiology, the Sahlgrenska Academy at the University of Gothenburg, Mölndal, Sweden
[b] UCL Institute of Neurology, Queen Square, London, UK

ARTICLE INFO

Article history:
Received 1 December 2014
Revised 21 January 2015
Accepted 2 February 2015
Available online xxxx

ABSTRACT

Traumatic brain injury (TBI) occurs when an external force traumatically injures the brain. Whereas severe TBI can be diagnosed using a combination of clinical signs and standard neuroimaging techniques, mild TBI (also called concussion) is more difficult to detect. This is where fluid markers of injury to different cell types and subcellular compartments in the central nervous system come into play. These markers are often proteins, peptides or other molecules with selective or high expression in the brain, which can be measured in the cerebrospinal fluid or blood as they leak out or get secreted in response to the injury. Here, we review the literature on fluid markers of neuronal, axonal and astroglial injury to diagnose mild TBI and to predict clinical outcome in patients with head trauma. We also discuss chronic traumatic encephalopathy, a progressive neurodegenerative disease in individuals with a history of multiple mild TBIs in a biomarker context. This article is part of a Special Issue entitled 'Traumatic Brain Injury'.

Contents

1. Introduction

Moderate to severe traumatic brain injury (TBI), in which there are intracranial bleedings and/or mass lesions in the brain parenchyma after a blow to the head, is easily diagnosed by clinical examination and standard neuroimaging techniques. However, mild TBI or concussion (defined as a head trauma resulting in brief loss of consciousness and/or alteration of mental state; the two terms are used interchangeably) is much harder to objectively detect and presents an everyday challenge in emergency care units globally. Concussion causes no gross pathology, such as hemorrhage, and no abnormalities on conventional computed tomography scans of the brain (McCrory et al., 2009), but rather rapid-onset neuronal dysfunction that resolves in a spontaneous manner over a few days to a few weeks. The dysfunction is at least partly caused by direct damage to axons and other structures in the central nervous system (CNS). Approximately 15% of concussion patients suffer persisting cognitive dysfunction (Roe et al., 2009; Williams et al., 2010) and diffuse axonal injury (DAI) appears to be the most important underlying pathology in such cases (Kirov et al., 2013). Further,

* Corresponding author at: Institute of Neuroscience and Physiology, Department of Psychiatry and Neurochemistry, The Sahlgrenska Academy at the University of Gothenburg, S-431 80 Mölndal, Sweden.
E-mail address: henrik.zetterberg@gu.se (H. Zetterberg).

http://dx.doi.org/10.1016/j.mcn.2015.02.003
1044-7431/

it has been shown that repetitive concussions increase the risk of chronic traumatic encephalopathy (CTE), a condition described in boxers and other sports athletes, as well as in military veterans, characterized by chronic and sometimes progressive neurological and/or psychiatric symptoms following repetitive brain injury (Stein et al., 2014). Fluid markers of neuronal, axonal and astroglial damage would be valuable to diagnose concussion in patients with head trauma, to predict short- and long-term clinical outcome and to tell when the brain has recovered from the TBI.

In this review, we start by discussing the different fluids that may be used as samples to determine markers of TBI. We thereafter discuss technical aspects of biomarker analyses. We then review the literature published so far on candidate biomarkers for mild TBI. Finally, we discuss the special case of CTE and emphasize the need of biomarker development for this disease entity. To obtain the data, we searched PubMed for English language articles on mild TBI and CTE using the keyword "traumatic brain injury" together with other keywords including: "concussion", "chronic traumatic encephalopathy", "biomarkers", "CSF", "blood", "saliva", "urine", "tears", "tau", "neurofilament light", and several other keywords relevant to every section. We largely selected publications in the past 5 years, but did not exclude important older publications. Selection criteria also included a judgment on the novelty of studies and their relevance for the well-informed researcher/clinician.

2. Marker matrices

2.1. Cerebrospinal fluid

Cerebrospinal fluid (CSF) is a clear fluid that surrounds the brain and provides mechanical support. It also carries nutrients and signaling molecules to neurons and helps in disposing metabolites that are further cleared into the blood via arachnoid villi in the intracranial dural sinuses and at the cranial and spinal nerve root sheaths. The total CSF volume is around 150 mL and the production and clearance rates are around 20 mL per hour. CSF is easily sampled through a lumbar puncture. Standard operating procedures for CSF sampling and handling have been established and the procedure can be done in outpatients (Blennow et al., 2010). Lumbar puncture is safe with post-lumbar puncture headache being the only significant side-effect that affects 2–20% of the patients (Zetterberg et al., 2010). The main advantage of CSF as a matrix in which to measure markers of CNS injury is that it communicates freely with the brain interstitial fluid that bathes the neurons. Biochemical changes in the brain are thus reflected in the CSF, which may be regarded as an accessible, although not perfect, sample of the brain interstitial fluid. Further, CSF has low protease activity and most molecules do not change upon sampling provided the sample is not contaminated by blood. The main disadvantage is that lumbar puncture may be regarded as invasive. Finally, although CSF allows sampling from the brain side of the blood–brain barrier, it should be remembered that only 20–30% of the CSF volume is derived directly from the brain; 70–80% is a choroid plexus-derived filtrate of plasma.

2.2. Blood

The other major biofluid for the measurement of TBI markers is blood (serum or plasma). Blood is more accessible than CSF but most CNS-specific markers are present in blood at very low concentrations that necessitate the employment of ultra-sensitive techniques that can measure in the femtomolar range (most standard immunochemical techniques cannot reach this analytical sensitivity). The blood–brain barrier also poses a challenge in the analysis of CNS injury markers in blood. Further, most intra-cellular proteins released into the bloodstream undergo degradation and/or modification by proteases and other enzymes. The dilution of CNS proteins into 4 L blood instead of 150 mL CSF may also contribute to the low concentrations of CNS-derived molecules in the blood.

2.3. Saliva, urine and tears

It is possible that some CNS-derived proteins are eventually excreted into body fluids other than CSF and blood. The presence of the CNS-specific protein tau in saliva has been demonstrated using mass spectrometry (Shi et al., 2011). The same research group has also detected Parkinson-related α-synuclein and DJ-1 in this body fluid (Devic et al., 2011). However, the relationship between salivary concentrations of these proteins to processes within the CNS is far from clear and no conclusive data on disease association has been reported so far. At present, it is hard to imagine how a test based on sampling of saliva, urine or tears could produce results with a clear link to changes in the brain, given the many barriers and compartments the marker would have to cross on its way to the sampling site. Since these measures are unlikely to yield useful markers, there will not be any further consideration of them in the review.

3. Measurement techniques

Most fluid markers of CNS injury are proteins or protein fragments. Such proteins may be visualized on Western blots in which proteins in the sample are separated on a gel, transferred to a membrane and visualized using labeled antibodies to the protein of interest. This technique is often sensitive but not really quantitative and is not suitable for use in clinical laboratory practice.

Enzyme-linked immunosorbent assay (ELISA) and variants thereof have become established as the method of choice for the measurement of specific proteins in biofluids. The general principle is that a capture antibody directed against one epitope on the target analyte is immobilized on a surface, whereafter sample and labeled detector antibody (directed against another epitope on the same analyte) are added sequentially between washing steps to remove unspecific signal. Capture and detector antibodies are in molar excess so that most of the target analyte is captured in a sandwich between the antibody pair. Many ELISAs and ELISA-like techniques can reach lower limits of quantification of 10–100 pg/mL, but getting lower, as is needed for most brain-specific proteins in the blood, is a challenge.

To that end, two new techniques have entered the market: Erenna and Simoa (Blennow and Zetterberg, in press). The magnetic bead-based Erenna system can detect molecules at femtogram/mL concentrations using Single Molecule Counting (SMC) technology in which labeled detector antibodies are released from the captured immunocomplexes and counted one by one. Simoa is based on the isolation of individual immunocomplexes on magnetic beads using standard ELISA reagents. The main difference between Simoa and conventional immunoassays lies in the ability to trap single beads in femtoliter volume wells, allowing

Table 1
Candidate markers of acute mild traumatic brain injury.

Biomarker	Process
Neurofilament light (NF-L)	An intra-axonal structural protein highly expressed in large caliber axons
Tau	An intra-axonal structural protein highly expressed in thin, unmyelinated axons
α-Spectrin N-terminal fragment (SNTF)	An axonal injury marker generated by the calpain family of calcium-activated proteases
Neuron-specific enolase (NSE)	A protein highly expressed in the neuronal soma, but also in red blood cells
Ubiquitin C-terminal hydrolase L1 (UCHL1)	A deubiquitinating enzyme highly expressed in neurons, but also in gonads and lung tissue
S100B	An astroglial protein which is CNS-enriched but not CNS-specific
Glial fibrillary acidic protein (GFAP)	An astroglial protein which is CNS-specific

Please cite this article as: Zetterberg, H., Blennow, K., Fluid markers of traumatic brain injury, Mol. Cell. Neurosci. (2015), http://dx.doi.org/10.1016/j.mcn.2015.02.003