Acute Diagnostic Biomarkers for Spinal Cord Injury: Review of the Literature and Preliminary Research Report

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Key words
- Biomarker
- Pathophysiology
- Spinal cord injury
- Treatment

Abbreviations and Acronyms
AIS: American Spinal Injury Association Impairment Scale
BBB: Blood-brain barrier
BDP: Breakdown product
CNS: Central nervous system
CSF: Cerebrospinal fluid
GFAP: Glial fibrillary acidic protein
MAP2: Microtubule-associated protein 2
MBP: Myelin basic protein
MRI: Magnetic resonance imaging
NF: Neurofilament
NFH: Neurofilament, heavy chain
NFL: Neurofilament, light chain
NSE: Neuron-specific enolase
pNF: Phosphorylated neurofilament
SBDP: Spectrin breakdown product
SCI: Spinal cord injury
TBI: Traumatic brain injury
UCH-L1: Ubiquitin C-terminal hydrolase-L1

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Citation: World Neurosurg. (2015) 83, 5:867-878. http://dx.doi.org/10.1016/j.wneu.2013.03.012

Journal homepage: www.WORLDNEUROSURGERY.org

Available online: www.sciencedirect.com

OBJECTIVE: **Many efforts have been made to create new diagnostic technologies for use in the diagnosis of central nervous system injury. However, there is still no consensus for the use of biomarkers in clinical acute spinal cord injury (SCI). The aims of this review are (1) to evaluate the current status of neurochemical biomarkers and (2) to discuss their potential acute diagnostic role in SCI by reviewing the literature.**

METHODS: **PubMed (http://www.ncbi.nlm.nih.gov/pubmed) was searched up to 2012 to identify publications concerning diagnostic biomarkers in SCI. To support more knowledge, we also checked secondary references in the primarily retrieved literature.**

RESULTS: **Neurofilaments, cleaved-Tau, microtubule-associated protein 2, myelin basic protein, neuron-specific enolase, S100β, and glial fibrillary acidic protein were identified as structural protein biomarkers in SCI by this review process. We could not find reports relating ubiquitin C-terminal hydrolase-L1 and α-II spectrin breakdown products, which are widely researched in other central nervous system injuries. Therefore, we present our preliminary data relating to these two biomarkers. Some of biomarkers showed promising results for SCI diagnosis and outcome prediction; however, there were unresolved issues relating to accuracy and their accessibility.**

CONCLUSION: **Currently, there still are not many reports focused on diagnostic biomarkers in SCI. This fact warranted the need for greater efforts to innovate sensitive and reliable biomarkers for SCI.**

INTRODUCTION

Worldwide, the annual incidence of traumatic spinal cord injury (SCI) ranges from 12.1 to 57.8 cases per million, with motor vehicle accidents, falls, violence, and sports being the leading causes (1, 105). One-third of patients with SCI are reported to be tetraplegic, and 50% of patients with SCI to have a complete lesion (114). In the United States, an estimated 14,000 people suffer a SCI each year, 4200 die before reaching the hospital, and an additional 1500 patients die during the initial hospitalization (50). For better prognosis, apart from effort into the acute evaluation of the severity of the SCI, a diagnostic evaluation needs to be included (88).

The clinical, acute diagnosis of spinal trauma traditionally has been based mainly on the neurologic symptoms. More recently, magnetic resonance imaging (MRI) has been used for the acute diagnosis. Conventional MRI is currently the best imaging modality for evaluating SCI during the acute phase. However, in many locations and countries, MRI is still unavailable. Even when available, patients with concomitant multiple injuries may be too unstable and inaccessible for early MRI, where they are inaccessible to medical care for up to an hour. Also, MRI cannot be used in the patient with medical implants and devices, such as implantable cardioverter defibrillators, pacemakers, or deep brain stimulation devices, including bullets or shrapnel. These disadvantages may limit an MRI-based early diagnosis of SCI.

Many efforts have been made to innovate new acute diagnostic biomarkers in other central nervous system (CNS) injuries, including cerebral stroke (41) and traumatic brain injury (TBI) (40). However, there is still less consensus for the use of biomarkers for acute diagnosis of traumatic SCI. The aims of this review are to evaluate the current status of neurostructural protein biomarkers to discuss their potential diagnostic role in SCI by reviewing the previous literature.

METHODS

The PubMed database (http://www.ncbi.nlm.nih.gov/pubmed) was used to search up to 2012 to identify publications concerning diagnostic biomarkers in SCI. Keywords for search were "spinal cord injury," "biomarkers," "acute," and "diagnosis." All human and animal studies, case reports, and literature reviews were included. Literature with abstracts and/or full texts in English was included. The literature for neurostructural protein biomarkers was included. In contrast, articles that mentioned chronic SCI or SCI caused by internal diseases (e.g., infections) were excluded. Less-specific biomarkers such as general laboratory data (e.g., blood cell counts, cholesterol level, rheumatoid factor) and cytokine/chemokines also were excluded. The references of retrieved publications were checked manually for additional studies that could potentially meet the inclusion criteria.

RESULTS

In our first PubMed literature search, 118 articles from 1991 were retrieved. After a screening process, we could find only 12 articles that included the descriptions of protein and structural biomarkers for the evaluations of SCI (14, 26, 31, 52, 54, 62-64, 88, 99, 116, 120). In these primary searches of the literature, S100β was mentioned most frequently: S100β in nine articles, neuron-specific enolase (NSE) in five, neurofilaments (NF) in three, glial fibrillary acidic protein (GFAP) in two, Tau protein in two, myelin basic protein (MBP) in two, and microtubule-associated protein 2 (MAP2) in one. The references of these retrieved publications also were checked, and the following protein/structural biomarkers were chosen: NF, Tau, MAP2, MBP, NSE, S100β, and GFAP.

In our retrieved literature, we could not find any reports relating ubiquitin C-terminal hydrolase-L1 (UCH-L1) (69) or α-II spectrin breakdown products (SBDPs) (67), which are widely researched neurostructural proteins in CNS injury. Therefore, we also added our preliminary clinical/experimental data to supplement information for this topic.

In **Table 1**, we summarize current candidate SCI biomarkers, including our two preliminary candidates. We also introduce their biologic features and discuss their potential sensitivity/specificity as detailed in the sections to follow.

Neurofilaments

NFs are a major cytoskeletal component in axons. The NF heteropolymer consists of light chain (NFL, 68 kDa), medium chain (NFM, 150 kDa), heavy chain (NFH, 190–210 kDa), and α-internexin polypeptides (85). NF subunits are localized in the neuronal soma (29) and then travel by axonal transport, accumulating in disconnected axons after injury (17). After axonal injury, calcium influx activates calcineurin, altering the phosphorylation state and the repelling forces of the NF sidearms and forming areas of NF compaction with marked local loss of cytoskeletal integrity (39). Subsequently, activated calpain and caspase-3 mediate the dephosphorylation and proteolysis of phosphorylated NFs (pNFs), causing them to collapse into tightly packed bundles in the centers of axons (80). These modifications reduce the stability of pNFs and thus the caliber of injured axons. Subsequently, this processes promotes pNF degradation with axonal "retraction balls" and disconnection.

Experiments with a SCI rat model revealed that medium-chain NF (150 kDa) was up-regulated between 6 and 24 hours after injury (43). After spinal axonal injury in rats, significant serum levels of NFH fragments were seen at as early as 6 hours. The levels peaked between 12 and 48 hours and then decreased to baseline levels by 7 days (97). The two peaks in the serum levels of NFH fragments corresponded to primary and secondary axotomy, respectively. However, because the secondary axotomy was more severe than the primary, the second peak was sharper than the first (97). Because blood is quicker, safer, and more convenient to obtain than cerebrospinal fluid (CSF) sample collection, the assay of serum NFH has the potential to be a novel, specific, and convenient tool for assessing axonal damage in patients with SCI (4), as well as in diffuse axonal TBI.

Cleaved-Tau (c-Tau)

Tau is an intracellular, MAP that is highly enriched in axons. It is a microtubule-binding phosphoprotein with a molecular mass of 48–67 kDa. It assembles into stable axonal microtubule bundles and also participates in anterograde axoplasmic transport (75, 101). Under normal conditions, axonal tau is below the level of detection for immunostaining. After injury,

Table 1. Potential Spinal Cord Injury Biomarkers and Their Attributes

Candidate Marker	Marker Origin	Attributes
S100β	Glia	CNS injury marker benchmark
GFAP	Glia	Gliosis/glial cell injury
MBP	Oligodendrocytes/Schwann cells	Demyelination
SBDP150/SBDP145	Axon (calpain-generated)	Acute necrosis
SBDP120	Axon (caspase-3-generated)	Delayed apoptosis
UCH-L1	Neuronal cell body	Neuronal cell body injury
MAP2	Dendrites	Dendritic injury
IL-1β, TNF-α, IL-6, (and other cytokines	Microglia/infiltrating macrophage	Neuroinflammation

CNS, central nervous system; GFAP, glial fibrillary acidic protein; Il, interleukin; MAP2, microtubule-associated protein 2; MBP, myelin basic protein; SBDP, spectrin breakdown product; TNF, tumor necrosis factor; UCH-L1, ubiquitin C-terminal hydrolase-L1.