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Biomarkers in Traumatic Spinal Cord Injury

Stefan Mircea Iencean and Andrei Stefan Iencean

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Abstract

Spinal cord injury (SCI) is one of the most devastating traumas for an individual because the complete traumatic spinal cord injury leads to paraplegia or tetraplegia. The mechanical injuries directly cause axonal destruction in fiber tracts, destruction of the neurons, and of the glial cells, and their destruction releases substances whose presence, quantity, and dynamics can be lesional biomarkers. The reactions of partially injured cells simultaneously start and the occurring substances and their quantity may be reaction biomarkers. The lesional biomarkers appear immediately post injury and after several hours there are both lesional biomarkers and reaction biomarkers.

In recent years, a number of protein biomarkers have been evaluated to detect neuronal injury and recently there have been studies about their potential diagnostic and predictive value for spinal cord injuries. The most important lesional biomarkers are the phosphorylated neurofilament subunits resulting from the axonal neurofilament destruction. The heavy phosphorylated neurofilament subunit (pNF-H) is a predictive lesional biomarker because its values pattern can show the reducing or stopping of the secondary lesions and the favorable outcome. The complete SCI patients with a favorable development had a specific pattern of daily values of pNF-H: a sudden increase up to a maximum value then a progressive decrease to normal. The patients with unfavorable outcome or neurological stabilization had two patterns: an increase to a plateau of pNF-H values or a progressive increase up to a peak followed by a progressive decrease to quasi-normal values.

Keywords: lesional biomarker, microneurosurgery, phosphorylated neurofilament subunit, reactional biomarker, spinal cord injury

1. Introduction

Spinal cord injury (SCI) is one of the most devastating traumas for an individual and their family because, depending on the level of injury, the complete traumatic SCI leads to paraplegia or tetraplegia [1].

Immediate traumatic SCI is the primary mechanical injury caused through the direct injury of the neurons, axons, and blood vessels (compression, laceration, shearing, and even transection of the spinal cord). After the injury event, the secondary injury mechanisms begin immediately and the secondary spinal cord lesions consist of hemorrhages, spinal cord edema, vasospasm, and hypoperfusion of the spinal cord and the damage of the spinal cord continues to progress for several days to weeks, and leads to the death of neurons and the interruption of the axonal tracts [2].

Many traumatic spinal cord injuries can be initially incomplete and the secondary damage completes the lesion of the spinal cord. Spinal cord injuries are difficult to treat because of these secondary injuries. Current therapy is unable to act on the primary mechanically lesion, but the secondary injury extension of the spinal cord could be stopped or reduced by an early efficient therapy.

It is necessary to know the type and the evolution of the secondary spinal cord lesions: complete destruction of spinal cord or an injury with potential recovery. To this end it is ideal to have complete and extensive information about the injury and this information must reveal the treatment required to ensure a favorable outcome.

In SCI, the neurological examination brings the first very important information about the lesion and this directs imaging procedures to confirm the lesion. But because of the spinal shock, unstable condition of the patient, attendant injuries, alcohol or drugs etc., the clinical examination immediately following injury, even using the American Spinal Injury Association (ASIA) motor scores or other scales, cannot be considered reliable. These clinical examinations must be repeated, but they offer only static clinical states and no data about possible future development.

The noninvasive imaging techniques used in SCI are the radiographies of the spine, spinal computed tomography (CT) or/and spinal magnetic resonance imaging (MRI), even functional MRI, tractography, or the recent structural volumetric and microstructural MRI protocols of the site of SCI.

All these offer only static images of the stage of the lesion and not an accurate prediction of the severity of SCI and about the development of the secondary spinal cord lesions.

We need a dynamic approach for the lesions; hence, biomarkers were evaluated for their capacity to be sensitive and accurate tools to measure the neuronal injuries and to predict the evolution of these injuries. Biomarkers are measurable features that can be used to confirm the presence or to predict the severity of the disorders. Biomarkers as biochemical indicators in SCI can allow detection of the secondary lesion, can monitor its progress and predict the severity of SCI, and can also indicate the specific treatments required. In SCI, biomarkers detect

the severity of injury within the first few hours and can direct the best patient care in a timely manner.

In acute traumatic SCI, the mechanical injuries directly cause axonal destruction in fiber tracts, destruction of the neurons in gray matter, and of the glial cells. Their destruction releases substances—cellular constituents, whose presence, quantity, and dynamics can be lesional biomarkers.

The reactions of partially injured cells and of uninjured cells around the site of injury simultaneously start as response to the biochemical substances released by the destruction. The responses of these cells, that is, the synthesis of new proteins, are secondary to changes in mRNA. Detecting these changes allows us to determine their role at the site of SCI, to stabilize the damaged cells, to stop the spinal cord scar formation, and so on. The correlation of these changes with copied, or transcribed mRNA, from DNA, will establish the responsible genes intervening in the response to the SCI [3].

The assessment of these changes in the damaged spinal cord will allow therapeutic responses, for example:

- to stop the mechanisms by which secondary spinal cord lesion occurs and its progression,

- to understand the mechanisms of formation of a spinal cord scar as mechanical barrier that obstructs the axonal regeneration processes,

- even genetically targeted therapy to stimulate the genes in DNA responsible for neuronal regeneration, stimulating mRNA or to use the necessary proteins for SCI healing, and so on.

Detecting these protein changes, their quantity and dynamics may be biomarkers of response, or reaction biomarkers.

Correlating the lesional biomarkers and the reaction biomarkers with the clinical outcome and with the imaging techniques will enable understanding the complexity of the biological response to SCI and the establishment of appropriate therapies. Obtaining cells from the site of SCI is problematic in patients, therefore most research evaluated the lesional biomarkers in human spinal cord injuries. There are new approaches in the management of acute traumatic SCI that could enable obtaining cells from the site of SCI without adverse consequences for the patient.

The lesional biomarkers appear immediately post injury and their dynamics show the extension of the SCI and after several hours there are both lesional biomarkers and reaction biomarkers, involving the secondary cellular response to injury.

2. Current status of biomarkers and the diagnostic value in SCI

In recent years, a number of protein biomarkers have been evaluated to detect neuronal injury and recently there have been studies about their potential diagnostic and predictive value for spinal cord injuries. The concentration of specific proteins in blood or in the cerebrospinal fluid

(CSF) must be compared with the nervous tissue injury and these can be biomarkers for the pathologic processes in SCI.

There are numerous experimental studies and a smaller number of clinical studies for determining and validating biomarkers in SCI: c-Tau, myelin basic protein (MBP), neuron-specific enolase (NSE), glial fibrillar acidic protein (GFAP), and so on [4].

The researches have not been systematized and because the studies have been done at different time interval from the moment of the trauma, they did not differentiate between lesional biomarkers and reaction biomarkers and many of them were not followed to verify the clinical usefulness.

A brief overview of the evolution of these researches is shown below.

van Dongen et al. [5, 6] correlated the concentration of S-100 protein in the CSF with the results of somatosensory and motor-evoked potential monitoring indicating spinal cord ischemia during and after thoracoabdominal aortic aneurysm (TAAA) surgery and concluded that the S-100 protein in CSF seems to be a marker to detect spinal cord ischemia [5, 6], and in 2001 Kunihara et al. [7] found increased levels of S-100β in patients with post-operative SCI caused by spinal cord ischemia too. Basu et al. [8] presented free radicals as an inflammatory response indicator with the role of biomarker during spinal cord ischemia.

In 2003, Guéz et al. evaluated the CSF concentration of light subunits of neurofilaments (NF-L, 68 kDa) and of glial fibrillary acid protein (GFAP) in after trauma to the cervical spine: patients with acute traumatic cervical SCI and whiplash cases, compared to a control group of normal cases. The CSF concentrations of both light subunits of neurofilaments and GFAPs were significantly higher in all the cases with cervical SCI and pronounced neurological deficits [9].

In 2005, Loy et al. [10] reported that serum levels of NSE and S-100β protein are biomarkers in an animal model of traumatic SCI.

Kwon et al. [11] studied as possible biomarkers other inflammatory cytokines and structural proteins: S-100β (a glial-specific calcium-binding β protein), glial fibrillary acidic protein (GFAP), and interleukin 8 (IL-8, also known as neutrophil chemotactic factor), in patients within 24 hours post-SCI. Their concentration in the CSF and blood samples in patients with complete and incomplete SCI showed they could be potential biomarkers to diagnose the severity of SCI [11, 12].

In a literature review from 1966 to 2008, Pouw et al. [13] identified the biomarkers S-100β, NSE, neurofilament light chain, and glial fibrillary acidic protein as significantly higher in cases of experimental SCI in animal models.

New potential biomarkers were reported: the neurofilaments, the major cytoskeletal components in axon fibers. The most important are neurofilament subunit proteins (NF) that coassemble forming the cytoskeletal of axon fibers and they consist of five subunits of neurofilaments, named on the basis of molecular weight: heavy or highest (NF-H, 200–220 kDa), medium or middle (NF-M, 145–160 kDa), and light or lowest (NF-L, 68–70 kDa) subunits,

also alpha-internexin subunit (NF66) discovered later than NF and the intermediate filament protein subunit peripherin [4].

Ueno et al. [14] presented a rat model of acute SCI and they showed that the high molecular weight neurofilament subunit levels in plasma could be a biomarker for evaluating the efficacy of therapies for SCI.

Hayakawa et al. [15] studied the concentration of the phosphorylated neurofilament subunit NF-H (pNF-H) in plasma in patients with acute cervical SCI and concluded pNF-H may be a prognostic biomarker for SCI.

Iencean et al. [16] measured pNF-H concentration by enzyme-linked immunosorbent assay (ELISA) test in CSF in acute SCI patients and correlated the values of pNF-H with the clinical evolution, also they measured the normal values in samples obtained by lumbar puncture from individuals without neurologic disorders. They showed the phosphorylated form of the neurofilament subunit NF-H (pNF-H) is a biomarker in SCI in humans and its increased values are consistent with an unfavorable outcome. The neurofilament subunit NF-H (pNF-H) is a lesional biomarker, it appears after the mechanical injury by axonal destruction in the fibers tracts [16].

By now these studies have identified some potential biomarkers, but these biomarkers have not been validated and they still cannot be used in the clinical setting, for diagnosis, prognosis, and evaluating therapeutic interventions.

3. New research on biomarkers in traumatic SCI

The research in traumatic SCI has been focused on the discovery of lesional biomarkers and lesser for reaction biomarkers. Lesional biomarkers can be studied in patients with acute traumatic SCI immediately after injury; reaction biomarkers occur after a short period post injury and after several hours post injury these two types of biomarkers coexist, and it is difficult to differentiate them. The study of reaction biomarkers involves cells around the lesion, which is not possible in patients with SCI. Therefore research is conducted on nerve cell cultures and there are experimental animal models, but the translation into human medicine is difficult because there are important differences. The most important studies on lesional biomarkers concern the neurofilament subunit proteins (NF).

Pouw et al. [17] in a prospective cohort study obtained CSF from sixteen acute traumatic SCI patients within 24 hours post injury and found that the concentrations of glial fibrillary acidic protein, NSE, S-100β, tau and neurofilament heavy chain (NFH) in motor complete patients was significantly higher compared with motor incomplete patients.

Takahashi et al. [18] conducted a study to evaluate pNF-H levels in the CSF of patients with worsening symptoms of cervical compression myelopathy and their results suggest that pNF-H in CSF can act as a biomarker that reflects the severity of acutely worsening compression myelopathy.
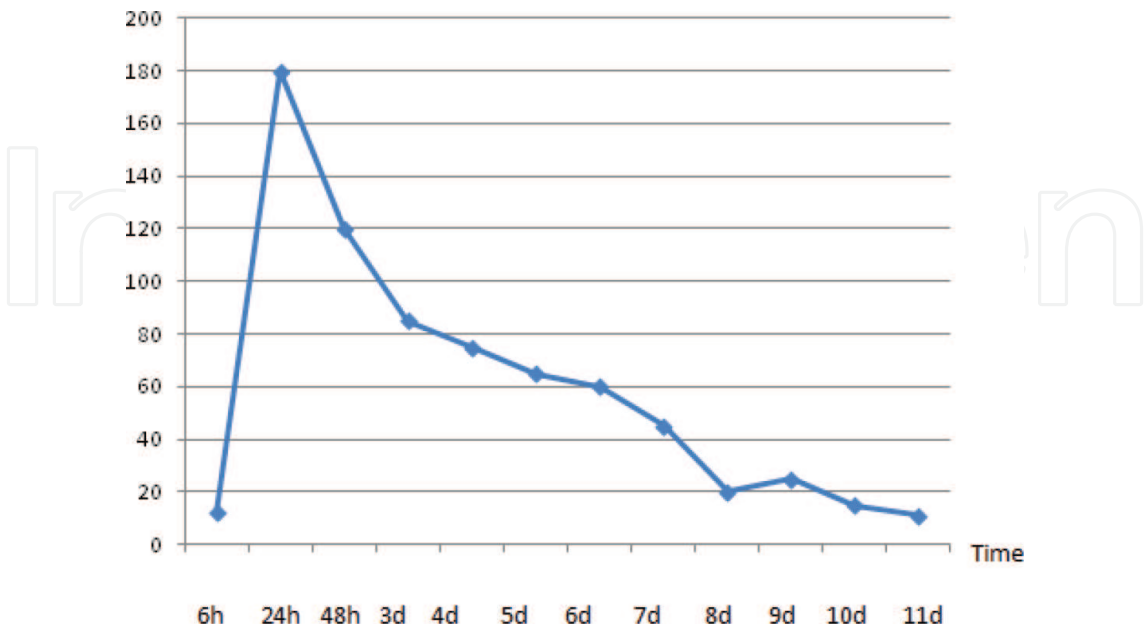
Iencean et al. measured the phosphorylated neurofilament subunit NF-H (pNF-H) in the CSF of patients with SCI and demonstrated the correlation between the pNF-H levels and the severity of the injury. They studied 15 subjects with acute traumatic SCI who underwent surgery during the first 24 hours post injury (decompression, stabilization): eight patients with complete SCI and seven patients with incomplete SCI. They measured daily the heavy phosphorylated neurofilament subunit (pNF-H) concentration by sandwich ELISA test in CSF in all patients. The level of CSF pNF-H was ten to a hundred times higher in complete SCI than the level of CSF pNF-H in cases with incomplete SCI, where the level of this biomarker was close to normal [19, 20].

The patients with early surgery in complete SCI and with a favorable outcome had a specific pattern of daily values of pNF-H: a sudden increase up to a maximum value then a gradual decrease to normal; the peak was different in each case, from 10 times up to 170 times higher than normal (**Figure 1**).
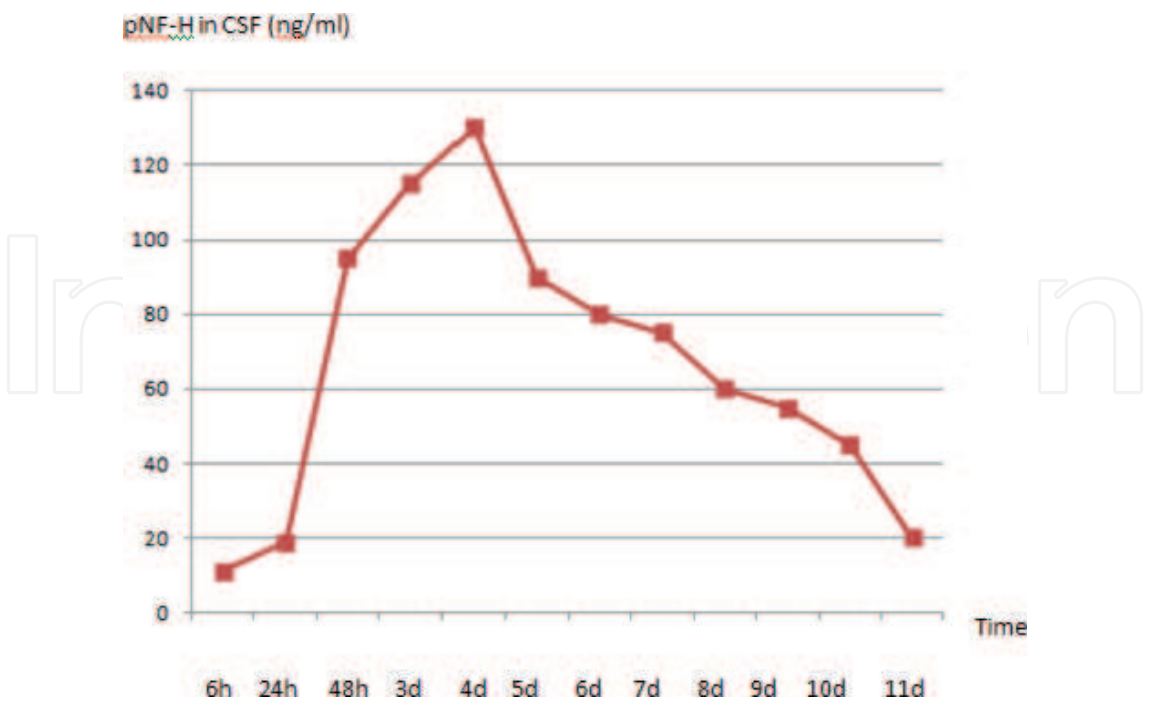
The same type of the pattern for the values of pNF-H appears in the incomplete SCI with favorable outcome, but with smaller values of pNF-H.

There are two patterns in cases with unfavorable outcome or neurological stationary after the same early surgery and treatment:
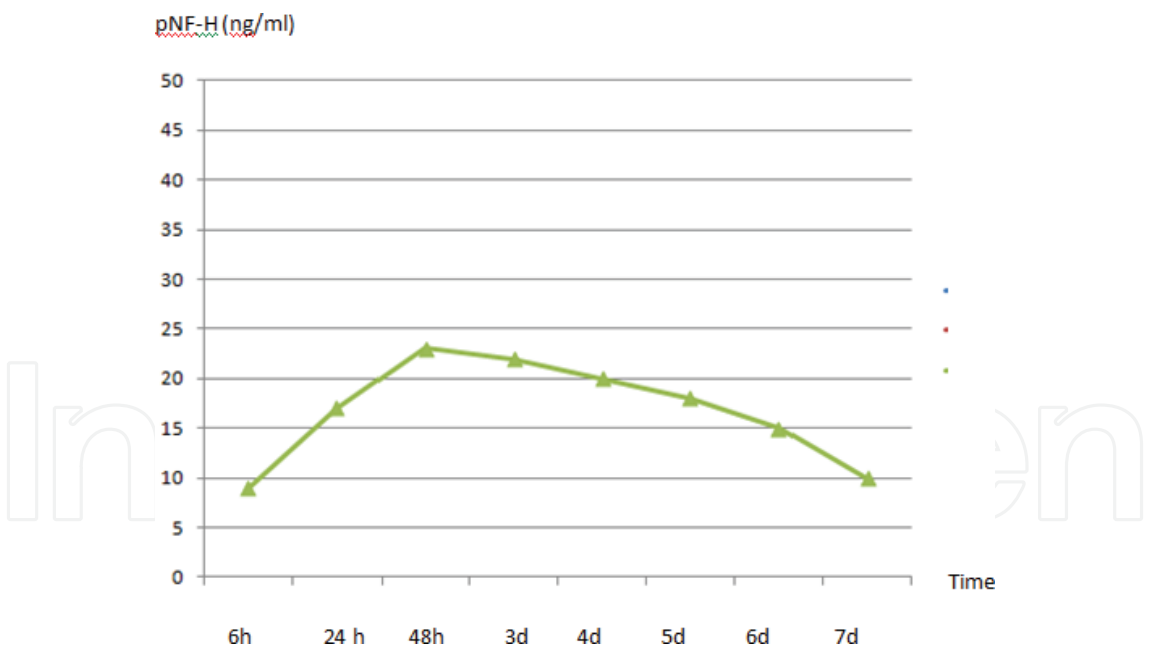
- the second unfavorable pattern had a progressive increase up to a peak and then was followed by a progressive decrease to normal values, the peak was a hundred times higher than normal values (**Figure 2**),

- an increase to a plateau of pNF-H values, with increased values five or ten times higher than normal (**Figure 3**).

Figure 1. Pattern of daily value of pNF-H in patients with favorable outcome.
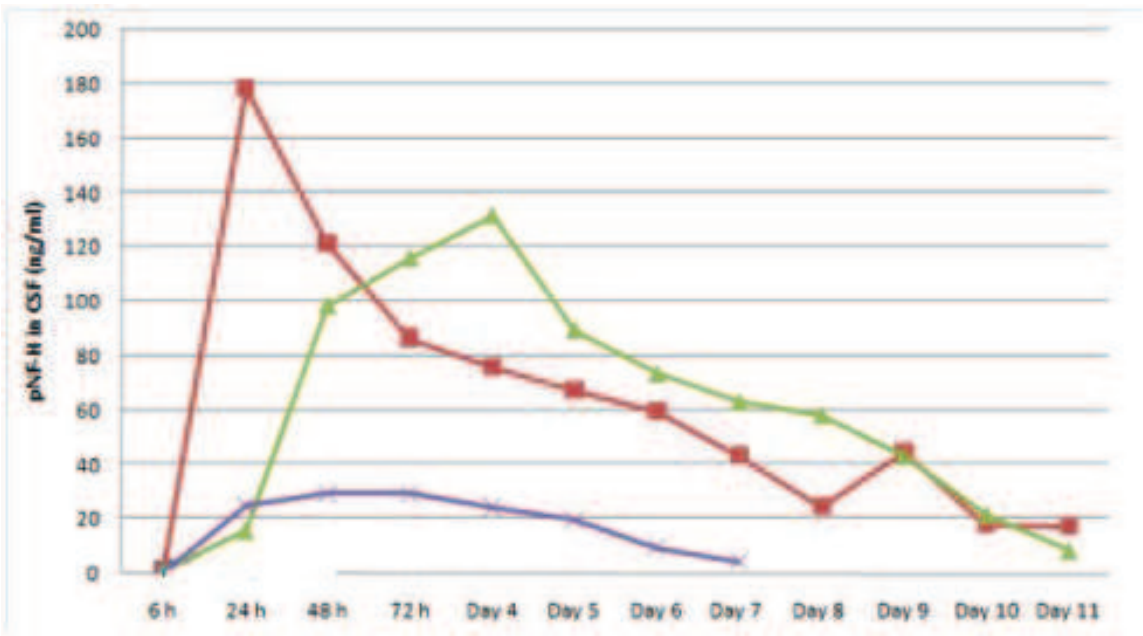
Figure 2. Pattern with progressive increase of pNF-H.

Figure 3. Pattern with increase up to a plateau of pNF-H.

The authors found that in patients with favorable development the progressive decrease of pNF-H values after the initial sudden increase, without extension of increased values in plateau or without a second peak, signifies a reduction or even a stop of the secondary lesion with evident effect on the favorable outcome in the SCI (**Figure 4**).

Figure 4. The three specific and predictive patterns of daily values of pNF-H in traumatic SCI.

Kato et al. [21] investigated the phosphorylated form of the high molecular weight neurofilament subunit (pNF-H) levels in the serum in patients with cervical compressive myelopathy and they found an elevated serum level of pNF-H only in acute worsening of myelopathy and this study confirms that pNF-H is a lesional biomarker.

Kuhle et al. [22] presented their results on a study of serum neurofilament light chain (pNF-L) in human SCI. They concluded that serum neurofilament light subunit (pNF-L) concentration in SCI patients has a close correlation with acute severity and neurological outcome and it is of predictive value in SCI patients.

The presentation of these studies on biomarkers in SCI highlights that the most important ones and those with significant results relate to lesional biomarkers, and first are the phosphorylated neurofilament subunits, light or heavy (pNF-L or pNF-H), resulting from the axonal neurofilament destruction. The research showed that the phosphorylated neurofilament subunit, light or heavy (pNF-L or pNF-H) in SCI is a specific lesional biomarker for SCI and it can distinguish the severity of SCI (Hayakawa, Iencean, and Kuhle).

The heavy phosphorylated neurofilament subunit (pNF-H) is a predictive lesional biomarker because its values pattern can show the reducing or stopping of the secondary lesions and the favorable outcome. The complete SCI patients with a favorable development had a specific pattern of daily values of pNF-H: a sudden increase up to a maximum value then a progressive decrease to normal. The patients with unfavorable outcome or neurological stabilization had two patterns: an increase to a plateau of pNF-H values or a progressive increase up to a peak followed by a progressive decrease to quasi-normal values.

4. Conclusion and future perspectives. Ethics

These studies on biomarkers in spinal cord injuries highlight that the most important lesional biomarkers are the phosphorylated neurofilament subunits, light or heavy (pNF-L or pNF-H). The phosphorylated neurofilament subunits (pNF-L or pNF-H) are specific lesional biomarkers for SCI and they can distinguish the severity of SCI.

The heavy phosphorylated neurofilament subunit (pNF-H) is a predictive lesional biomarker; its values pattern shows the reducing or stopping of the secondary lesions and the favorable outcome.

There is a specific pattern of daily values of pNF-H in complete SCI patients with a favorable outcome: a sudden increase up to a maximum value then a progressive decrease to normal. Also there are two patterns in the patients with unfavorable outcome: an increase to a plateau of pNF-H values or a progressive increase up to a peak followed by a progressive decrease to quasi-normal values.

These specific patterns could be used to aid clinicians with making a diagnosis and establishing a prognosis, and evaluating therapeutic interventions. These studies should continue on larger groups of patients to prove the clinical usefulness.

Also the studies on reaction biomarkers are very important, but obtaining cells from the site of SCI is problematic in humans. A new approach in the management of acute traumatic SCI has been proposed that could enable obtaining cells from the site of SCI without adverse consequences for the patient. In the cases with a predictive pattern of unfavorable outcome or neurological stationary after decompression and stabilization during the first 24 hours, a new approach was proposed based on the predictive pattern of daily values of pNF-H. If the clinical neurologic evolution is unfavorable and imaging techniques (MRI) show a complete SCI and the daily values of NFP-H as lesional biomarker form predictive unfavorable pattern, a second microneurosurgery in the SCI site can create favorable conditions for functional recovery of the remaining spinal cord: opening the spinal cord in the midline and microsurgical debridement of the necrotic tissue. At the same time this second microneurosurgical approach in the SCI site could enable obtaining cells from the site of SCI without adverse consequences for the patient. The use of these cells (neurons and glial cells around the lesion) for cell culture techniques will allow the study of the changes in the spinal cord at the molecular and structural levels in humans.

Diagnosis, prognosis, and treatment guidance based on biomarker used as a predictive indicator can determine ethical difficulties by differentiated therapies in patients with SCI.

It is difficult to stop or to limit the treatment of neurological recovery in patients with complete SCI, with paraplegia or tetraplegia, with complete spinal cord lesions on imaging techniques and unfavorable patterns of predictive lesional biomarkers. We do not currently know the value of the lesional predictive biomarkers for the neurological outcome several years after the injury. At the moment, we cannot take a decision limiting the treatment of neurological recovery in patients with complete SCI because we do not know the complexity of the biological response to SCI.

This requires extensive and profound research both on lesional biomarkers and on reaction biomarkers correlated with genetic and molecular response in SCI and we hope further research will deliver effective treatments.

Acknowledgements

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