New approach based on biomarkers in acute traumatic spinal cord injury

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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. 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 stabilisation 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.

Key words: lesional biomarker, phosphorylated neurofilament subunit, reactional biomarker, spinal cord injury

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 spinal cord injury leads to paraplegia or tetraplegia. Immediate traumatic spinal cord injury 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 hypo-perfusion 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.

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.

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 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.

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

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 must be compared with the nervous tissue injury and these can be biomarkers for the pathologic processes in spinal cord injury.

There are numerous experimental studies and a smaller number of clinical studies for determining and validating biomarkers in spinal cord injury: c-Tau, myelin basic protein – MBP, neuron-specific enolase – NSE, glial fibrillar acidic protein – GFAP etc. 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.

Ueno et al. (2011) presented a rat model of acute spinal cord injury 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. (2012) 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. The pNF-H concentration was measured by ELISA test in CSF in acute spinal cord injury patients and correlated the values of pNF-H with the clinical evolution. 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. 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.

Current status of biomarkers in traumatic spinal cord injury

The research in traumatic spinal cord injury 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 concerns the neurofilament subunit proteins (NF).

The phosphorylated neurofilament subunit NF-H (pNF-H) was measured in the cerebro-spinal fluid of patients with spinal cord injury and it was demonstrated the correlation between the pNF-H levels and the severity of the injury. The study included subjects with acute traumatic spinal cord injury who underwent surgery during the first

24 hours post injury (decompression, stabilization): patients with complete spinal cord injury (SCI) and patients with incomplete SCI. 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.

The patients with early surgery in complete spinal cord injury 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 spinal cord injury 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 (Figure2),

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

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 spinal cord injury (Figure 4).

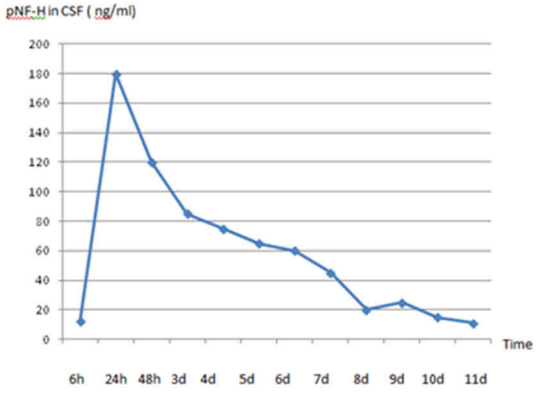

Figure 1 - Pattern of daily value of pNF-H in patients with favorable outcome

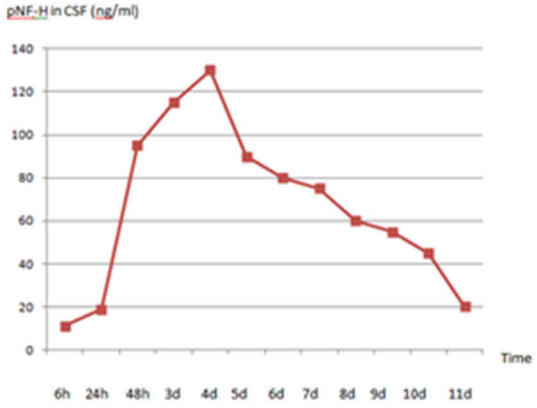

Figure 2 - Pattern with progressive increase of pNF-H

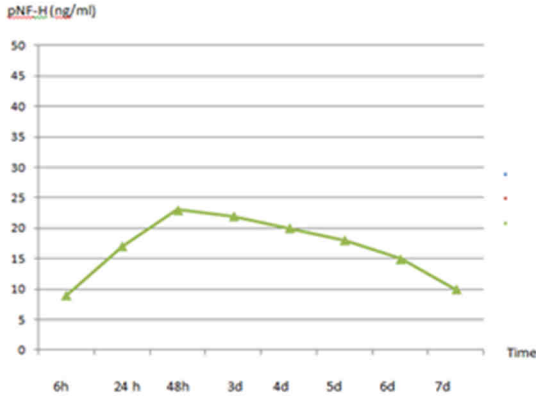

Figure 3 - Pattern with increase up to a plateau of pNF-H

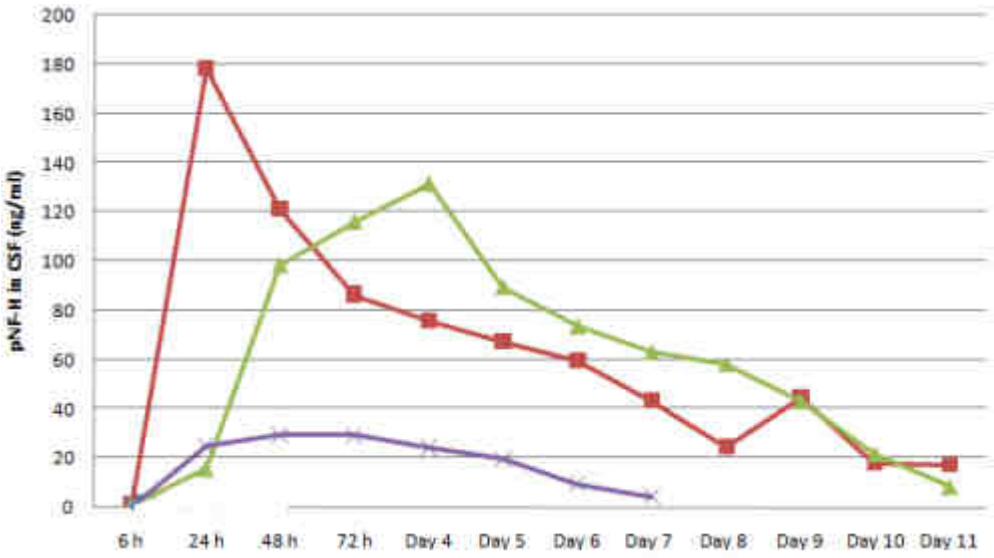

Figure 4 - The three specific and predictive pattern of daily values of pNF-H in traumatic SCI

Kato et al. (2015) 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. (2015) presented their results on a study of serum neurofilament light chain (pNF-L) in human spinal cord injury. 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 spinal cord injury is a specific lesional biomarker for spinal cord injury and it can distinguish the severity of SCI.

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.

Conclusion

These studies on biomarkers in spinal cord injuries highlights 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 spinal cord injury and they can distinguish the severity of SCI.

The heavy phosphorylated neurofilament subunit (pNF-H) is a predictive lesional biomarker; its values pattern show 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.

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