

New approach in cervical spine compression.

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Abstract

Cervical spine compression with cervical radicular or cervical spinal cord compression is a common neck condition caused by inflammatory diseases, cervical disk degeneration, neck injuries or other less common causes (tumours, infections etc.). The phosphorylated form of the High-Molecular-Weight Neurofilament Subunit NF-H (pNF-H) in Cerebro-Spinal Fluid (CSF) is a specific biomarker for spinal cord lesion. The current study included 5 subjects with acute traumatic cervical spinal cord injury; the patients were classified according to the American Spinal Injury Association Impairment Scale (ASIA) and all patients underwent surgery during the first 24 hours post injury (decompression, stabilization). We measured daily the heavy Phosphorylated Neurofilament Subunit (pNF-H) concentration by sandwich ELISA test in CSF in all patients and we compared the values of pNF-H with the patients' clinical evolution. The evolution of the daily values in the levels of CSF pNF-H showed three patterns of this biomarker in the cervical spinal cord compression. This new approach in cervical spine compression is based on pNF-H; the heavy phosphorylated neurofilament subunit concentration can be a predictive lesional biomarker because the pattern in its values can show the reduction or stoppage of the secondary lesion with a favourable result.

Keywords: Lesional biomarker, Cervical spinal cord compression, Spinal cord injury, Phosphorylated neurofilament subunit.

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Introduction

Complete traumatic cervical spinal cord compression can lead to tetraplegia. Many traumatic spinal cord injuries are initially incomplete and the secondary damage completes the destruction of the spinal cord later. It has been accepted that the severity of the primary mechanical injury causes the amplitude of the secondary damage. The primary spinal cord injury at the time of trauma can be: contusion, compression, laceration, shearing and even distraction with transection of the spinal cord. Immediately after the injury event, the secondary injury mechanisms start and these lesions consist of haemorrhages in the central grey matter, spinal cord oedema and vasospasm with hypo perfusion of the spinal cord causing tissue hypoxia. This secondary injury extension of the spinal cord caused by inflammation, hypoxia and excitotoxicity can be reduced by an early efficient therapy. We cannot act on the primary lesion, but the secondary damage can be stopped or reduced and it is assumed that such interventions are efficient early so one needs to act with a neuroprotection in the very acute stage; also the therapeutical answer may differ in different patients depending of the severity of the injury [1,2].

The aim of this study was to determine the correlation between the pNF-H levels in cerebro-spinal fluid as lesional biomarker and the severity of the compression in cases of cervical spinal cord injury, and based on this to propose a second micro neurosurgery in the spinal cord compression site.

Materials and Methods

We performed a nine months study of acute traumatic spinal cord injury patients including five complete cervical traumatic compressions, classified according to the American Spinal Injury Association Impairment Scale (ASIA) as A grade with tetraplegia. The diagnosis was established by plain radiographs supplemented with CT and/or MRI for poorly visualized or suspicious areas. Cerebral CT and MRI excluded simultaneous traumatic brain injuries and other chronic CNS pathologies that can lead to the presence of pNF-H in CSF. All patients underwent surgery during the first 24 hours with anterior approach with decompression, reduction and anterior arthrodesis with plate fixation. Table 1 presents the patients' characteristics. We measured the Heavy Phosphorylated Neurofilament Subunit (pNF-H) concentration by standard

sandwich ELISA test in CSF in all patients and we correlated the values of pNF-H with the clinical evolution; we also measured the normal values in samples obtained by lumbar puncture from individuals with no neurologic disorders. We used a sandwich Enzyme Immunoassay (EIA) which measures pNF-H in CSF (Phosphorylated Neurofilament Sandwich

ELISA Kit). The period of dosing for pNF-H was different due to the different durations of hospitalization for each patient or in some cases for subjective reasons. All patients or their relatives, as relevant to each situation, gave their written informed consent before the start of the study and the hospital ethics committee approved the study protocol.

Table 1. Patients' characteristics.

Case	Age	Sex	Level of injury	Mechanism of injury	ASIA initial	Steroid treatment	Surgery decompression instrumentation	Follow-up period	ASIA final
1	53	M	C4C5	FW	A	yes	yes	1 year	A
2	42	M	C7	FW	A	no	yes	2 years	B
3	34	F	C5C6	FW	A	yes	yes	18 months	C
4	32	M	C4	MVA	A	no	yes	1 year	A
5	24	M	C6C7	FW	A	yes	yes	2 years	C

MVA: Motor Vehicle Accident; FW: Fall from Farm or Hay Wagon or from Horse Cart.

Results

The results are presented in Table 1 and Table 2, both the patients' characteristics and the values of pNF-H for each patient relative to the clinical evolution. There were five patients with complete spinal cord compression and among these in terms of gender distribution there was only a female, therefore an evidence of male dominance, related to the type of activity having caused the injury. For all patients, pNF-H was detectable in CSF samples; the normal level of CSF pNF-H was determined in samples obtained by lumbar puncture from three individuals with no neurologic disorders and these values were similar to the normal level set by Petzold and Shaw in 2007: 0 ng/ml to 0.9 ng/ml [3]. We found the level of CSF pNF-H of ten to a hundred times higher in these complete cervical traumatic compression cases; also we found that the increased level of CSF pNF-H remained elevated up to five to twelve days after injury. The graphical form of the values of CSF pNF-H in these cases shows the correlation with the

clinical evolution and the improvement or with the same neurological status. The daily values of CSF pNF-H in case 1 (C4C5 spinal cord injury level), which neurological status remained unchanged after treatment, were five to six times higher than normal and develop to a plateau with a quasi-stationary level of pNF-H values. Cases 2, 3 and 5, which had a favourable neurological evolution after treatment, had a different pattern of pNF-H daily values: a sudden increase up to a maximum value then a gradual decrease to normal. The maximum values were different in each case, from 10 times up to 170 times higher than normal. Case 4, with unchanged neurological status, had an increase of daily pNF-H values with a different pattern: a gradual increase with a peak, related to the secondary lesion, then a gradual decrease. In all cases the elevated values of pNF-H in cerebro-spinal fluid stayed elevated for different periods of time, probably due to individual variations but possibly correlated with the severity of injury.

Table 2. Values of pNF-H in cerebro-spinal fluid (ng/mL).

No/ASIA	Level	6 h	24 h	48 h	72 h	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	1 y/18 m/2 y
Case 1 A	C4C5	4.5	5.5	5.9	6.5	5.6								A
Case 2 A	C7	1.17	178.2	121.2	86.4	75.6	67.2	59.4	43.2	24.6	44.4	18	17.4	B
Case 3 A	C5C6	0.6	163.2	116.4	69	33.6	9.6							C
Case 4 A	C4	0.99	34.5	57	64.2	60	45	33						A
Case 5 A	C6C7	0.6	9.6	8.4	3.6	1.2	0.82							C
Normal 1	-	0	0											E
Normal 2	-	0.1	0.1	0.1										E
Normal 3	-	0	0											E

Discussion

Traumatic cervical spinal cord compression is one of the most devastating traumas for an individual because it leads to tetraplegia [1,2]. 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 start simultaneously and the substances that are produced 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.

Several published reports concerning the nervous tissue markers as indicators of tissue injury in central nervous system damage have indicated that the levels of specific proteins in the CSF and serum may be used as biomarkers [4-6]. Also 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; they consist of five subunits of neurofilaments, named based on molecular weight. The direct axonal destruction in SCI causes the appearance of free neurofilament subunits and they can be considered lesional biomarkers [7,8].

The report of Guez et al., analysed the entire protein complex of Neurofilament Protein (NFL) levels in CSF after trauma to the cervical spine in human. They found significantly increased levels of NFL in all patients with tetrapareses relative to those with incomplete injuries and they noted that it is possible to quantify the degree of the spinal cord compression by assessing the nervous tissue markers in CSF [9].

In this study we analysed the values of the phosphorylated form of the high-molecular-weight neurofilament subunit NF-H (pNF-H) as a specific marker for axonal injury in the cerebrospinal fluid samples from five patients with complete cervical traumatic compression.

Cerebral CT and MRI excluded both acute brain injuries and chronic CNS pathologies, such as multiple sclerosis, amyotrophic lateral sclerosis etc. that can lead to the presence of pNF-H in CSF. More studies have shown that increased pNF-H in the CSF or plasma may correlate to axonal damage: increased pNF-H in the plasma and CSF of Amyotrophic Lateral Sclerosis (ALS) patients; increased levels of pNFH have been detected in the CSF of multiple sclerosis correlated with the diseases' progression [10-12]; also in mild and severe traumatic brain injury [13].

For our patients the increased pNF-H levels were detectable in CSF samples after trauma and the values had grown to a peak or they increased to a plateau and then the values decreased. Hayakawa et al., noted that the prolonged elevation of plasma

pNF-H suggests the involvement of a continuous axonal degeneration, or secondary axonal damage and the pNF-H level in blood represents the amount of axonal degeneration as a "hole" [14].

In our study we found some patterns of increased pNF-H levels strongly correlated with the clinical evolution of patients with complete spinal cord injury. The cases with a favourable neurological evolution after early surgery in complete spinal cord injury had a specific pattern of daily 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. We found two patterns in cases with unfavourable outcome or with unchanged neurological status after the same treatment: an increase to a plateau of pNF-H values, also with increased values of five or ten times higher than normal, or the second unfavourable pattern had a gradual increase up to a peak, followed then by a gradual decrease to normal values; the peak was a hundred times higher than normal. With respect to these types of pNF-H patterns we assume that in cases with favourable evolution, after the initial increase, the gradual decrease of pNF-H values, without extension of the increased values in plateau or without of a second peak, shows a reduction or even stopping of the secondary lesion with evident effect on the favourable evolution of the spinal cord injury.

We believe that the phosphorylated form of the High-Molecular-Weight Neurofilament Subunit NF-H (pNF-H) could be a specific lesional biomarker for spinal cord injury in the cerebrospinal fluid samples. CSF pNF-H is specific to axonal injury, therefore in relation to the spinal cord injury; the values of pNF-H describe early on the severity of spinal cord injury. These values have a characteristic pattern consistent with the progression of the injury and the pNF-H values confirm the results of other investigations (spinal MRI, tractography). The CSF pNF-H measurement in patients with SCI is relatively inexpensive, easy to use and the results are reproducible [8,15].

The comparison between degenerative diseases of the nervous system (amyotrophic lateral sclerosis or multiple sclerosis) and traumatic injuries shows that the same biomarker: pNF-H can be a lesional biomarker in traumatic injuries and is a reaction biomarker in degenerative diseases, because of the different pathophysiological mechanisms: direct injury or secondary response caused by other factors.

Our outcome assessments are limited by the small sample size of only five patients; also we believe that the future studies should be extended to other regions of the spinal cord and they must include a higher number of patients.

But based on these results, in cases of acute traumatic spinal cord compression with a predictive pattern of unfavourable outcome after decompression and stabilization during the first 24 hours, a new approach is proposed based on the predictive pattern of daily pNF-H values. If the clinical neurological evolution is unfavourable and imaging techniques (MRI) show a complete spinal cord injury and the daily pNF-H values as a

lesional biomarker form a predictive unfavourable pattern, a second micro neurosurgery in the SCI site can create favourable conditions for functional recovery of the remaining spinal cord: opening the spinal cord in the midline and microsurgical debridement of the necrotic tissue.

Conclusion

Although the study includes a small number of enrolled patients, we can consider that the phosphorylated form of the High-Molecular-Weight Neurofilament Subunit NF-H (pNF-H) can be a specific lesional biomarker for spinal cord injury in patients without traumatic brain injury or degenerative diseases of the nervous system. CSF pNF-H can be a lesional biomarker that can indicate the severity of SCI and it can be a predictive biomarker because the CSF pNF-H values pattern can show the reduction or stoppage of the secondary lesion and the favourable result. The CSF pNF-H values pattern in a plateau or with a latter peak is consistent with a progressive secondary lesion and therefore with an unfavourable result.

We propose a new approach in cervical spinal cord compression based on these three predictive patterns of CSF pNF-H values post spinal cord compression which are correlated with the severity of the lesion. These results are promising although there were only a few patients in this study; the issue of biomarkers in spinal cord compression is extremely important and requires further research in this area.

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