REVIEW

Biomarkers in spinal cord injury

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Study design: Literature review.

Objectives: In traumatic spinal cord injury (SCI), much effort has been put into the evaluation of SCI severity and the prediction of recovery potential. An accurate prediction of the initial damage of the spinal cord that differentiates between the severities of SCI however, may help physicians in choosing a particular neuroprotective treatment in the acute phase. Neurochemical biomarkers may possibly fulfil these requirements. The aim of this review was to describe (1) the current status of neurochemical biomarkers in SCI; (2) their potential diagnostic role in SCI.

Methods: MEDLINE was searched from 1966 to 2008 to identify publications concerning biomarkers in traumatic SCI.

Results: The biomarkers S-100 β , neuron-specific enolase, neurofilament light chain, and Glial fibrillary acidic protein are significantly increased in cases of (experimental) spinal cord injury. Furthermore, increased serum concentrations of S-100 β have been correlated with an unfavourable functional outcome. Although biomarkers in SCI show promising results, considerations and shortcomings, such as polytrauma, haemolysis, extracerebral sources, and poor resuscitation, must be studied in greater detail before biomarkers can be utilised in the clinical care of SCI.

Conclusions: Quantitative standards for determining the extent of SCI during the acute phase must be developed and validated. Even though increased concentrations of neurochemical biomarkers have been identified in patients with SCI, these do not yet provide a sensitive prognostic tool. Considering the limited availability of sensitive prognostic tools, neurochemical biomarkers of SCI should be evaluated and validated in future clinical trials.

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Introduction

In traumatic spinal cord injury (SCI), much effort has been put into the evaluation of SCI severity and prediction of recovery potential. Interventions for the recovery of function following SCI include a combination of pharmacological,¹ surgical² and rehabilitation³ approaches. The benefits of these interventions, however, are not univocal in clinical trials. It is assumed that patients with more severe SCI respond differently to neuroprotective interventions than do patients with less severe SCI.³ An accurate prediction of the initial damage of the spinal cord that differentiates between the different severities of SCI may help physicians in

choosing an available or experimental neuroprotective intervention in the very acute phase.

Conventional magnetic resonance imaging (MRI) is currently the best imaging modality for evaluating traumatic SCI during the very acute phase.⁴ Although several MRI findings such as parenchymal hemorrhage, transection and longer lesion length correlate with less favorable neurological outcomes, the findings on neurological examinations are most predictive of outcomes.⁵ Prediction of functional outcome by means of the American Spinal Injury Association (ASIA) motor scores is considered to be reliable and prognostic in patients with motor complete SCI when tested 72 h after the initial trauma.^{6,7} Combining the results of MRI and initial neurological examination results in an even better prediction of the recovery of motor scores.^{8,9} However, early ASIA examinations (for example, within 72 h post-injury) are considered unreliable.⁶

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Therefore, an early, accurate diagnostic test with the purpose of indicating neuroprotective interventions is preferable. Conventional MRI appears to be restricted to assessing macroscopic changes in the injured spinal cord, as it does not adequately address axonal injury in the white matter. As the degree and localization of injured and spared white matter primarily determine functioning after SCI, MRI has limited success as a prognostic tool.⁵ MRI is largely a qualitative measure and quantitative standards, in relation to functional SCI outcomes, will need to be developed and validated.³

A new approach for evaluating the primary cord damage in the acute phase may be the assessment of biomarker concentrations in the cerebrospinal fluid (CSF). Trauma to the spinal cord causes an acute physical injury with neuronal necrosis. This is followed by a secondary axonal degeneration and further degeneration or death of nerve cells by either apoptosis or necrosis, processes that may last between days and weeks. As the spinal cord is surrounded by the CSF, damage to the spinal cord may lead to the release of proteins and metabolites from the nervous tissue into the CSF. This process allows for the study of 'biomarkers' of SCI in the CSF.^{10–12} A biomarker is a characteristic that is objectively measured and evaluated as an indicator of normal or pathologic processes or pharmacologic responses to a therapeutic intervention.¹³

Biomarkers of SCI can be approached in two ways: (1) a direct survey of primary structural damage using a specific unique marker (or markers) of tissue damage, and (2) measure aspects of the cellular, biochemical or molecular cascades in the secondary injury (or repair) response phase.¹⁴ An ideal prognostic central nervous system (CNS) biochemical marker should have all of the following properties:¹⁵

1. central nervous system specificity;
2. rapid and significant release into blood or CSF after injury;
3. readily obtainable assay results;
4. predictability of serious injury from an early sample;
5. relationship of marker concentration with the degree of injury;
6. inexpensive;
7. minimally influenced by confounding factors;
8. reproducible.

There has been growing interest in biomarkers as indicators of tissue destruction in CNS diseases. Several studies have indicated that monitoring the levels of neuron-, myelin- or astroglia-specific proteins in the serum and CSF may be a useful approach for evaluating the severity of non-traumatic CNS injury.^{11,12,16,17}

Several studies have been published concerning the use of biomarkers in CSF and serum of patients with traumatic brain injury.^{18–21} A recent review discussed the role of biomarkers in traumatic brain injury.¹⁴ In the field of SCI, however, there is no review on the value of biomarkers.

The aim of this literature review is to describe the current status of neurochemical biomarkers and their potential

diagnostic value from either experimental models or patient series of acute SCI. This review focuses on the markers that survey direct structural damage.

Methods

Clinical and experimental studies that included biomarkers investigating direct structural damage in serum and/or CSF after an SCI in humans or animals were eligible for this review.

We searched MEDLINE (PubMed interface), EMBASE and reference lists of the included articles published between 1966 and November 2008. In MEDLINE, we used a combination of the following search terms: diagnosis, spinal cord, trauma, biomarker, cerebrospinal fluid and serum. Furthermore, the references of retrieved publications were checked manually for additional studies that could potentially meet the inclusion criteria.

Results

The search strategy resulted in 250 potentially relevant articles from MEDLINE. The search in other databases yielded no additional relevant articles. A review of the titles, abstract and the full text resulted in the inclusion of 18 articles. Twelve studies investigated biomarkers in humans^{22–32} and six studies investigated biomarkers in animals.^{33–38} Relatively few studies have been performed in which biomarkers in serum and/or CSF after SCI were investigated. For those that have been conducted, S-100 β and neuron-specific enolase (NSE) have especially received attention. An overview is shown in Tables 1 and 2.

Biomarkers in animal studies after SCI

S-100 β . S-100 β is a calcium-binding protein localized predominantly in astroglial and Schwann cells.³⁹ In a model using Sprague–Dawley rats with SCI (induced by a weight-drop device), significantly increased serum and CSF levels of S-100 β were observed compared with the control group without SCI at 6 h after the induced SCI.³⁷ Another model using Sprague–Dawley rats identified a significant increase of S-100 β serum concentrations after an SCI or a plexus avulsion injury compared with a control group.³⁴

Neuron-specific enolase. Neuron-specific enolase is a glycolytic enzyme predominantly localized in the cytoplasm of neurons and cells of neuroendocrine lineage.⁴⁰ Loy *et al.*³⁷ assessed the NSE serum and CSF levels in a rat model using a weight-drop device to simulate SCI. Compared with the control group, the NSE levels in serum and CSF were significantly increased at 6 h after the induced SCI. The NSE levels in the two graded injured groups did not differ significantly. Another study simulating ischemic SCI using thoracoabdominal aortic cross-clamping in 10 dogs identified significantly elevated NSE levels in CSF during clamping and reperfusion.³⁵

Table 1 Included animal studies

Study	Biomarker investigated	CSF/serum	Details of study (sub) groups	Results
Skouen <i>et al.</i> ³³	S-100 β , NFL, NSE and GFAP	CSF	18 pigs underwent an experimental nerve root injury and 18 pigs were used as sham operated animals. Control: 5 pigs without nerve root injury	At 1 week after the experimental nerve root injury, NFL concentrations were elevated ($P < 0.001$). Nonsignificant differences of S-100 β , NSE and GFAP concentrations were identified after the induced nerve root injury.
Ma <i>et al.</i> ³⁴	S-100 β	Serum	40 Sprague–Dawley rats in a weight-drop model and 66 Sprague–Dawley rats with induced lumbar plexus avulsion injury. Control: 28 Sprague–Dawley rats without SCI or plexus injury.	At 3, 12 and 72 h after SCI, increased levels of S-100 β ($P < 0.05$) were identified.
Nagy <i>et al.</i> ³⁵	NSE	CSF	10 dogs who underwent thoracoabdominal cross-clamping.	At 55 min of clamping, increased levels of NSE were identified ($P < 0.05$).
Cornefjord <i>et al.</i> ³⁶	S-100 β , NFL, NSE and GFAP	CSF	20 pigs were divided in four groups ($n = 5$ each): (1) experimental nerve root injury, (2) autologous nucleus pulposus application, (3) experimental nerve root injury and nucleus pulposus application. Control: (4) sham operation.	At 1 week after the experimental nerve root injury, NFL concentrations were elevated ($P < 0.01$). Nonsignificant differences of S-100 β , NSE and GFAP concentrations were identified after the induced nerve root injury.
Loy <i>et al.</i> ³⁷	S-100 β and NSE	Serum	34 Sprague–Dawley rats in a weight-drop model. Control: 6 Sprague–Dawley rats without SCI.	At 6 h after SCI, increased levels of S-100 β ($P < 0.05$) and NSE ($P < 0.001$) were identified.
Cao <i>et al.</i> ³⁸	S-100 β and NSE	Both	60 Sprague–Dawley rats in a weight-drop model. Control: 20 Sprague–Dawley rats without SCI.	At 2 h after the force-defined SCI, serum and CSF concentrations were elevated ($P < 0.05$).

Abbreviations: CSF, cerebrospinal fluid; GFAP, Glial fibrillary acidic protein; NFL, neurofilament protein; NSE, neuron-specific enolase; SCI, spinal cord injury.

Time-dependency of biomarkers

Cao *et al.*³⁸ evaluated the relationship between the protein levels of NSE and S-100 β in serum and CSF and the severity of acute SCI in an animal model. Eighty Sprague–Dawley rats were divided into four groups: control group, mild SCI group, moderate SCI group and severe SCI group. Graded SCI was provided using a weight-drop model from different heights. Serum and CSF samples were collected at different time points. When compared with the control group, the concentrations of NSE and S-100 β were significantly ($P < 0.05$) increased at 2 h after SCI and reached maximum levels at 6 h. The NSE and S-100 β levels in the moderate and severe SCI groups were significantly higher ($P < 0.05$) than the NSE and S-100 β levels in the mild SCI group. At 24 h after injury, the NSE and S-100 β levels in the serum and CSF were still significantly higher than those in the control group. However, after 24 h, the graded SCI groups did not differ significantly.

It is hypothesized that the peak in the concentration of NSE and S-100 β after 6 h reflects a mechanical disruption of the spinal cord. Furthermore, the concentration of NSE and S-100 β is positively correlated with the grade of the SCI. This study indicates that the NSE and S-100 β alterations are time dependent and positively correlated with the severity of the trauma. Although the correlations between the neurophysiological findings and/or radiographic abnormalities and changes of biomarkers after trauma were not assessed,

this study suggests that both biomarkers reflect neuronal and glial damage induced during the acute phase of SCI.

Nerve root injury. Although a nerve root compression is not a CNS injury, the role of biomarkers in nerve root injury is addressed. From earlier studies, it is known that a breakdown of the blood–nerve barrier occurs in compressed nerve roots.⁴¹ This may result in a detectable elevation of biomarkers in CSF.

In a pig model investigating biomarkers after experimental nerve root injury, unilateral compression of the nerve root of S1 was obtained by means of an ameroid constrictor.³³ Compared with the control group, pigs with an experimental nerve root injury had elevated NFL levels in CSF 1 week after the induced injury.³³ Another pig model also investigated several biomarkers in CSF in an experimental nerve root injury model of S1.³⁶ Twenty pigs were divided into four groups: (1) slow-onset mechanical compression with an ameroid constrictor; (2) harvested autologous nucleus pulposus applied to the nerve root; (3) mechanical compression plus harvested autologous nucleus pulposus; (4) sham operation. Significantly increased concentrations of neurofilament protein (NFL) in CSF after mechanical compression on spinal nerve roots were identified compared with the nucleus pulposus group and sham group after 1 week.³⁶ The results of these

Table 2 Included human studies

Study	Biomarker investigated	CSF/serum	Details of study (sub) groups	Results
Van Dongen <i>et al.</i> ²³	S-100 β	Both	8 patients who underwent elective TAAA surgery.	Nonsignificantly elevated S-100 β CSF concentrations. Serum concentrations were not elevated.
Van Dongen <i>et al.</i> ²²	S-100 β	CSF	19 patients who underwent elective TAAA surgery.	Non-significantly elevated S-100 β concentrations.
Brisby <i>et al.</i> ²⁵	S-100 β and NSE	CSF	15 patients who underwent surgery due to a lumbar disc herniation. Control: 7 patients without lumbar disc herniation.	Concentrations of NFL ($P < 0.01$) and S-100 β ($P < 0.05$) were elevated in patients with lumbar disc herniation. NSE and GFAP concentrations were nonsignificantly elevated.
Kunihara <i>et al.</i> ⁴²	S-100 β	Both	23 patients who underwent thoracic aorta or TAAA surgery.	At 6 h after surgery, increased levels of S-100 β ($P < 0.01$) were identified in four patients with post-operative SCI.
Ohta <i>et al.</i> ²⁶	MBP	Both	36 patients with tropical spastic paraparesis. Control: 45 patients with non-neurological diseases and 70 healthy subjects.	Elevated CSF MBP concentrations were identified in patients with tropical spastic paraparesis ($P < 0.001$). Nonsignificantly elevated serum MBP concentrations were detected.
Guez <i>et al.</i> ²⁷	GFAP and NFL	CSF	6 patients with traumatic SCI and 17 patients with a whiplash injury Control: 24 neurologically healthy individuals.	Nonsignificantly elevated levels of NFL and GFAP were identified in all SCI patients.
Shiyya <i>et al.</i> ²⁸	Tau and S-100 β	CSF	28 patients who underwent elective prosthetic replacement of the descending thoracic aorta or TAAA.	Nonsignificantly elevated levels of Tau. At 6 h after surgery, elevated levels of S-100 β ($P < 0.05$) were identified in three patients with post-operative SCI.
Marquardt <i>et al.</i> ²⁹	S-100 β	Serum	11 patients with spinal epidural empyema; S-100 β measurements were correlated with clinical outcome.	Patients with increased levels for a minimum of 3 days had unfavorable outcome ($P < 0.003$).
Marquardt <i>et al.</i> ²⁹	S-100 β	Serum	34 patients with paresis due to spinal metastasis; S-100 β measurements were correlated with clinical outcome.	Patients with increased levels for a minimum of 10 days had unfavorable outcome ($P < 0.0001$).
Brunnekreef <i>et al.</i> ²⁴	S-100 β	CSF	8 patients who underwent elective TAAA surgery.	Non-significantly increased levels of S-100 β were detected.
Winnerkvist <i>et al.</i> ³¹	GFAP, NFL and S-100 β	CSF	39 patients who underwent elective TAAA surgery.	At 24 h after surgery, increased levels of S-100 β ($P < 0.05$), NFL ($P < 0.05$), and GFAP ($P < 0.001$) were identified in five patients with post-operative SCI.
Khaladj <i>et al.</i> ³²	S-100 β	Both	13 patients who underwent elective TAAA surgery.	At 6 h after surgery, increased levels of S-100 β ($P < 0.001$) were identified in two patients with post-operative SCI.

Abbreviations: CSF, cerebrospinal fluid; GFAP, Glial fibrillary acidic protein; MBP, myelin basic protein; NFL, neurofilament protein; NSE, neuron-specific enolase; SCI, spinal cord injury; TAAA, thoracoabdominal aortic aneurysm.

studies indicate that the compression of nerve roots can induce a significant increase in NFL, indicating nerve tissue damage.^{33,36}

Biomarkers in patients after SCI

S-100 β . In patients with thoracic and thoracoabdominal aortic aneurysms (TAAAs), surgical treatment may be associated with a significant risk of perioperative morbidity, including paraplegia. Several studies investigated the concentrations of S-100 β in serum and CSF during and after thoracic endovascular stent grafting. Van Dongen *et al.*^{22,23} identified elevated CSF concentrations of S-100 β in 19 patients undergoing TAAA surgery. The highest concentrations of S-100 β were found in CSF samples taken 5 min after reperfusion. The authors suggest that the increased CSF S-100 β after unclamping of the aorta indicates that there is a continuous release of S-100 β from ischemic neural

tissue.^{22–24} Another study reevaluated the potential impact of S-100 β in serum and CSF in 13 patients undergoing TAAA surgery. Compared with the patients without SCI, significantly elevated ($P < 0.001$) CSF concentrations of S-100 β were identified in two patients with ischemic SCI 6 h after unclamping.³² In another study, significantly elevated serum and CSF concentrations of S-100 β have also been identified 6 h after unclamping, compared with patients without SCI.⁴² Brunnekreef *et al.*²⁴ evaluated S-100 β CSF concentrations in eight patients who underwent TAAA surgery. However, there was no significant increase in S-100 β CSF concentrations. It was suggested that an increased concentration of S-100 β in CSF is a marker of spinal cord ischemia.^{22–24} In addition, the release of the biomarker S-100 β , neurofilament light chain protein (NFL), and Glial fibrillary acidic protein (GFAP) in CSF was investigated in another study in which 39 patients underwent TAAA surgery.³¹ Glial fibrillary acidic protein is a filament protein localized predominantly in astroglial

cells.⁴³ NFL is a structural protein of neurons and it is predominantly localized in the axons.⁴⁴ In this study, CSF concentrations of S-100 β , NFL and GFAP were increased significantly in five patients ($P < 0.05$) with ischemic SCI compared with patients without SCI.³¹

Furthermore, the predictive potential of S-100 β concentrations in serum has been analyzed in patients with spinal cord compression caused by epidural empyema and spinal metastasis.^{29,30} Clinical outcome was considered favorable in cases of motor score improvement and preservation or retrieval of walking ability, whereas no improvement or further neurologic deterioration without restoration of gait function was regarded to be unfavorable. Motor function was based on the strongest muscle group in the lower extremities using the 0–5 rating system. Patients with epidural empyema and persistently increased S-100 β levels for a minimum of 3 days after operative decompression had unfavorable functional outcome ($P < 0.003$), as measured by motor function.³⁰ Patients with spinal metastasis and persistently increased S-100 β levels for a minimum of 10 days after operative decompression had unfavorable functional outcome ($P < 0.0001$), as measured by motor function.²⁹ As motor scores are not equal to a direct functional ambulation outcome measurement,⁴⁵ patients with increased S-100 β levels in these studies have unfavorable motor function, not unfavorable functional outcome.

Traumatic SCI. To our knowledge, Guez *et al.*²⁷ conducted the only study that investigated biomarkers in patients with traumatic SCI. In this prospective study, 6 patients with traumatic SCI and 17 patients with a severe whiplash injury were compared with a control group of 24 neurologically healthy individuals. All individuals underwent a lumbar puncture. CSF concentrations of NFL and GFAP were analyzed. Nonsignificantly increased concentrations of NFL and GFAP were identified in CSF of all SCI patients compared with the control group of neurologically healthy individuals.

Myelin basic protein. Myelin basic protein (MBP) is a protein produced by oligodendrocytes and it is the major constituent of the myelin sheath of axons.^{46,47} No study has specifically investigated the role of MBP in traumatic SCI; however, a single study retrospectively identified higher CSF concentrations of MBP in patients with tropical spastic paraparesis compared with CSF of patients with non-neurological diseases.²⁶

Tau. Tau, a protein localized primarily in the neurons and especially in the axonal compartments,⁴⁸ has been correlated with outcome in patients with traumatic brain injury.⁴⁹ Alterations of CSF Tau levels were evaluated in a study with 28 patients undergoing aortic surgery. However, when compared with the group without any neurologic complications, the Tau levels were not significantly elevated in patients with postoperative SCI.²⁸

Nerve root injury. The pathophysiologic mechanisms of disc herniation are not fully understood; however, herniated

discs are believed to have direct mechanical effects on the nerve root. Brisby *et al.*²⁵ assessed biomarkers in 15 patients with disc herniation who underwent surgery because of a lumbar disc herniation. Increased CSF concentrations of NFL and S-100 β were identified when compared with the control group. Furthermore, patients with less than 3 months' duration of symptoms before surgery had significantly higher levels of NFL than did patients with more than 3 months' duration ($P < 0.05$). This may be consistent with a release of NFL from the damaged nerve of the compressed nerve root during the acute phase. The lower level of NFL in patients with long-standing chronic pain may have been caused by atrophy of the nerve root, which results in a lesser release of NFL.²⁵

Considerations

In contrast to the large number of reports on biomarkers in structural brain damage, only a few studies have investigated the role of biomarkers in patients with SCI. Although studies investigating biomarkers in traumatic brain injury and SCI are promising, several considerations must be kept in mind before using them in the clinical care of SCI.

Most biomarker assays are not widely available in a standard clinical chemistry laboratory as these are 'specialty analyses' and are therefore available only in designated laboratories. However, standardized commercial assays are available for S-100 β and NSE. Commercial assays are also available for Tau, GFAP and MBP. There is no commercial assay for NFL. It is expected that faster techniques will cause neurochemical biomarkers to be more readily obtainable; however, at this stage, most biomarker assays must be performed in designated laboratories.

The studies of Ohta *et al.*²⁶ and Guez *et al.*²⁷ showed that biomarker concentrations are not elevated in neurologically healthy subjects. In contrast, other studies showed that biomarker levels in CSF can be elevated in patients without SCI.^{22,27,31,32} For example, patients with a period of aortic clamping and patients with a whiplash trauma may have elevated biomarker levels in CSF without neurologic deficits. It is possible that these patients have a subclinical injury of the spinal cord with absent neurological deficits. For instance, in the aortic clamping group, there may have been ischemic spinal cord damage to some extent with concomitant elevation of biomarkers, but without neurological deficits.^{22,31,32} This means that there is a 'false positive' range of biomarker elevations. Considering the absence of neurologic deficits as the 'gold standard' for the absence of SCI, a certain elevation of biomarker concentration should be regarded as normal. Furthermore, the NSE and S-100 β serum levels can become artificially elevated after polytrauma, hemolysis and poor resuscitation.^{13,37,50,51} The role of NSE and S-100 β in polytraumatized patients as markers of nervous tissue damage is therefore questionable as increased serum levels of NSE and S-100 β are also identified in critically ill patients without brain injury. Traumatized fat, muscle, bone marrow and several abdominal organs have been identified as the source for these increased serum levels of NSE and S-100 β in trauma patients without CNS injury.^{50,51}

Increased concentrations of biomarkers S-100 β , NSE, NFL and GFAP have been identified in serum and CSF after SCI, but how these concentrations relate to neurological outcome remains unclear. Only two studies have identified patients as having an unfavorable motor function in cases of persistently increased serum levels of S-100 β .^{29,30} The studies of Marquardt *et al.*^{29,30} identified that a longer period of persistently elevated S-100 β concentrations in serum was significantly related to lower extremity muscle power outcome.

To our knowledge, only one study has investigated biomarkers in traumatic SCI.²⁷ This study, however, has several limitations. For example, this study included only six SCI patients, the lumbar puncture was performed with a range of 1–21 days after the initial trauma, no statistical analysis was performed, and concentrations were not correlated to neurologic deficit or functional outcome. Although this study is the first to suggest the possibility of quantifying the degree of nerve cell damage after traumatic SCI, no definitive conclusions can be drawn about the diagnostic role of biomarkers in traumatic SCI patients.

Conclusion

Although several studies have identified increased concentrations of neurochemical biomarkers, most studies identified these biomarkers in patients at risk for ischemic SCI during and after TAAA surgery. To date, only one study investigating biomarkers in traumatic SCI has been performed. Moreover, none of the available human studies correlated the concentrations of biomarkers with adequate measures of neurological outcome. Therefore, care must be taken to control for several clinical variables to ensure accurate results and to prevent invalid conclusions.

Considering the limited availability of sensitive prognostic modalities for the evaluation of traumatic SCI, it is our opinion that further clinical trials in SCI are necessary to evaluate the applicability of biomarkers as a diagnostic tool in patients with traumatic SCI. We are currently performing a multicenter study to investigate the diagnostic role of neurochemical biomarkers in traumatic SCI within the consortium of the European Multicentre Study on Human Spinal Cord Injury (EM-SCI; www.emsci.org).

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