

Research Article

Myelin Basic Protein in the Cerebrospinal Fluid within 24 Hours after a Traumatic Spinal Cord Injury

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- Biomarkers
- Trauma

Abstract

Study design: Prospective cohort study

Objective: To investigate the cerebrospinal fluid (CSF) concentration of myelin basic protein (MBP) in traumatic SCI within 24 hours post-injury and its correlation to the initial neurological severity according to the American Spinal Injury Association (ASIA) impairment scale (AIS).

Setting: Patients with a traumatic Spinal Cord Injury (SCI) between C2 and T12 were prospectively included in three level 1 trauma centers.

Methods: A lumbar puncture was performed to obtain CSF from sixteen acute traumatic SCI patients within 24 hours post-injury. Neurological examinations were performed within 24 hours of injury. Univariate analyses of variance were used to identify differences in CSF MBP concentrations between SCI patients with different AIS grades.

Results: Eleven of the 16 patients suffered from a motor complete SCI (AIS grade A or B). CSF MBP concentration did not consistently exceed the age-dependent upper limit of the reference ranges (p -value = 0.23). Moreover, no differences were observed in CSF MBP concentrations between patients with different AIS grades (p -value = 0.33) nor between motor incomplete and complete SCI (p -value = 0.42).

Conclusion: CSF MBP concentrations were not elevated in traumatic SCI patients. In addition, the CSF MBP appeared not to correspond with patients having a motor complete or incomplete SCI. Also no clear differences in CSF MBP concentrations were identified between the different AIS grades. Therefore it appears that CSF MBP is not a suitable CSF biomarker to predict outcome or prognosis after traumatic SCI within 24 hours post injury.

INTRODUCTION

Much effort has been expended to develop neuroprotective interventions that potentially reduce secondary injury after traumatic spinal cord injury (SCI) [1-3]. The neurologic benefits of such interventions in previously conducted human clinical trials have been equivocal [4]. An accurate characterization of the initial damage of the spinal cord that more exactly differentiates between the severities of SCI may help in the evaluation of novel pharmacologic or surgical neuroprotective interventions, by more precisely stratifying injury severity and reducing the variability in spontaneous neurologic recovery [5].

Following a traumatic SCI, the initial severity of neurologic

impairment is the best predictor of long-term neurologic outcome after traumatic SCI [6]. The assessment of neurologic impairment in accordance with the International Standards for Neurological Classification of Spinal Cord Injury (ISNCSCI) is considered to be most reliable and prognostic when conducted 72 hours after the initial trauma. Prior to the 72 hour post-injury mark, several factors such as spinal shock, medical instability, or concomitant injuries affect the reliability of the neurological examination [7]. Furthermore, even with a reliable baseline neurologic examination performed acutely after injury, the extent of spontaneous recovery amongst SCI patients with the same ASIA impairment scale (AIS) grade is extremely variable [8]. This variability in natural recovery forces investigators to

enrol large numbers of patients into clinical trials of acute SCI therapies. Therefore, an accurate diagnostic-prognostic test which more precisely predicts neurologic outcome would greatly facilitate the conduct of such clinical trials [5].

By its direct communication with the extracellular fluid surrounding cells of the spinal cord, the cerebrospinal fluid (CSF) reflects the pathological processes occurring at site of injury and may therefore provide a source for useful biomarkers of injury severity after SCI [9]. The potential of this approach in traumatic SCI was demonstrated by using the CSF concentration of several inflammatory cytokines and structural proteins such as S100 β , tau and glial fibrillary acidic protein (GFAP) in patients within 24 hours post-injury [5]. In addition, our previous study showed that structural biomarkers like neuron specific enolase (NSE), S100 β and neurofilament heavy (NFH) chain seem to be correlated with SCI patients having a motor complete or motor incomplete spinal cord lesion [10].

In the central nervous system (CNS, brain and spinal cord) myelin basic protein (MBP) plays an important role in maintaining the structural integrity of myelin sheaths [11]. As such, MBP may be released into the CSF in situations where myelinated axons are damaged. Provided that there is a relationship between the severity of axonal injury and the increase in its CSF concentrations, MBP may serve as a biomarker of the severity of axonal injury. In this regard, MBP has been shown to be elevated after traumatic brain injury [12] and in multiple sclerosis [13]. Moreover, MBP levels might be useful as a surrogate outcome measure for assessing the biological effect of interventions, particularly those that might spare white matter. In a study of 51 dogs with thoracic disc herniations, higher CSF levels of MBP were related to an unfavourable neurologic outcome and worse ambulation [14].

To our knowledge, CSF MBP has not been investigated in human traumatic SCI. The aim of the current study was to investigate the 24 hours post-injury CSF MBP concentrations and its relation to the initial neurologic impairment severity in patients with a traumatic SCI.

MATERIALS AND METHODS

Patients

Two level 1 trauma centers (Nijmegen, the Netherlands and Vancouver, Canada) prospectively recruited patients with complete or incomplete traumatic SCI between 2007 and 2011. Patients were recruited based on the following inclusion criteria: 18 years or older; blunt SCI between C2 and T12; presentation and operative decompression and/or stabilization within 24 hours of injury; and the ability to undergo a valid, reliable neurological examination according to the ISNCSCI [15]. Patients were excluded if they had concomitant major trauma to the chest, pelvis, and/or extremities requiring immediate invasive intervention, or if they suffered from pre-existent neurodegenerative disorders. Furthermore, eye opening and verbal response according to the Glasgow Coma Scale had to be 4 and 6, respectively. All patients provided informed consent (third-party assent was not allowed).

All patients underwent a neurological assessment according to the ISNCSCI by a certified physician or study nurse having at

least 1 year of experience in examining patients with SCI. They were classified as: AIS grade A (No motor or sensory function preservation in the sacral segments S4-S5), AIS grade B (Sensory but not motor function preservation below the neurological level of injury (NLI) and includes the sacral segments S4-S5), AIS grade C (Motor function preservation below the NLI, and more than half of key muscles below the NLI have a muscle grade less than 3) or AIS grade D (Motor function preservation below the NLI, and at least half of key muscles below the NLI have a muscle grade of 3 or more) [15].

The study protocols were approved by the respective local ethics committees and were registered within the Dutch or American clinical trial registries (trialregister.nl identifier NTR1381, clinicaltrials.gov identifier NCT00135278).

Analysis

CSF samples were obtained under supervision of the spine surgeon. Using strict aseptic technique in laterally positioned patients, lumbar punctures were performed at L3-L4 or L4-L5 and 3-5 millilitre of CSF was obtained in a polypropylene tube. In Vancouver, the lumbar puncture was followed by insertion of intrathecal catheter (PERIFIX® Custom Epidural Anesthesia Kit; B. Braun Medical Inc., Bethlehem, PA). Samples were drawn from this catheter using a strict sterile technique every 6-8 hours. The first samples from those patients, punctured within 24 hours post-injury, were included in this analysis. Within 1 hour of acquisition, samples were centrifuged at 3000 rpm for 5 minutes and the supernatant was immediately stored at -80°C until analyzed.

MBP analysis was performed using a homemade sandwich ELISA. In short, 96-well Nunc maxisorp flat-bottom ELISA plates were coated by an overnight (O/N) incubation at 4°C with a mixture of mouse monoclonal antibody directed against MBP (1:500; Santa Cruz sc-80875) and monoclonal rat anti-MBP (1:500 Millipore MAB395). After washing 5 times with PBST/0.05%Tween20 (PBST), plates were blocked with 1%BSA/PBS for 1 hour at room temperature (RT). Following washing five times, purified human MBP (a gift from Dr Sindic, Leuven, Belgium) in the range 0.1 to 6.25 μ g/L or CSF samples (diluted 1:1 in 1%BSA/Tween20/phosphate buffer) were added to wells and incubated for 2 hours at 25°C. After washing in PBST to remove unbound components, polyclonal rabbit anti human-MBP (1:3000; Dako DK-218) was added with incubation for 1 hour at 25°C. After removing unbound antibody, HRP-conjugated goat anti-rabbit antibody (1:5000; Jackson 111-A062301) was added. After a final PBST wash, a colour reaction was developed using 100 μ l of TMB substrate incubated for 10-30 minutes at RT, in the dark. A manuscript with an extensive description of this assay is currently in preparation.

The reference ranges for MBP in CSF were determined by analysis of patients who had CSF obtained as part of the workup of a non-traumatic neurological disorder (e.g., with tension-type headache or depression). Additionally, to be considered a 'control' patient, all routine analyses (e.g. cell count, glucose, lactate, total protein, blood pigments and oligoclonal IgG bands) had to be in the normal range [16]. A total of 111 control patients were used to establish normal CSF MBP concentrations. For each

age group, the number of patients were: age < 15 years: n = 37; age 15-50 years: n = 33; age > 50 years: n = 41. The reference ranges for CSF MBP are as follows: < 15 yrs, $\leq 1.0 \mu\text{g/L}$; 15-50 yrs, $\leq 1.3 \mu\text{g/L}$; > 50 yrs, $\leq 1.8 \mu\text{g/L}$.

Statistical Analysis

Statistical analyses were performed using SPSS 16.0 for windows. Data were presented as mean $\pm$ SD unless stated otherwise. First we tested for a correlation between CSF MBP concentration and both age as well as time from injury to lumbar puncture by calculating Pearson correlation coefficients. Next, the Fisher's exact test was used in order to test whether CSF MBP concentration was elevated in patients with SCI, taking age dependent reference ranges into account.

A univariate analysis of variance was used to test for differences in CSF MBP concentrations between patients of different AIS grades. Next, an independent student t-test was used to test for differences in CSF MBP concentrations between patients with complete motor SCI (AIS grade A and B) and incomplete motor SCI (AIS grade C and D).

RESULTS

A total of 19 patients who were admitted to one of the trauma centers following blunt traumatic SCI were considered. Three patients were excluded because the time of injury to CSF sampling was >24 hours. A total of sixteen patients were thus included.

The mean age of the included patients was 45 years (range, 18-72) and the mean time of injury to CSF sampling was 15 hours (range, 3-24 hours) (Table 1).

Mean time from injury to lumbar puncture was not correlated to CSF MBP concentration ($r = 0.253$; $p\text{-value} = 0.344$). Only 4

patients had a CSF MBP concentration that exceeded their age-dependent upper limit of the reference ranges ($p\text{-value} = 0.23$). Age was not correlated with CSF MBP concentrations ($r = 0.085$; $p\text{-value} = 0.755$). Univariate regression analysis showed no statistical difference in CSF MBP concentrations between patients with neurological injury of different AIS grades ($p\text{-value} = 0.33$) (Figure 1). Moreover, an independent t-test showed no significant difference in mean MBP concentrations between patients with motor complete, $1.43 \mu\text{g/l}$ or incomplete SCI $1.19 \mu\text{g/l}$ ($p\text{-value} = 0.42$).

DISCUSSION

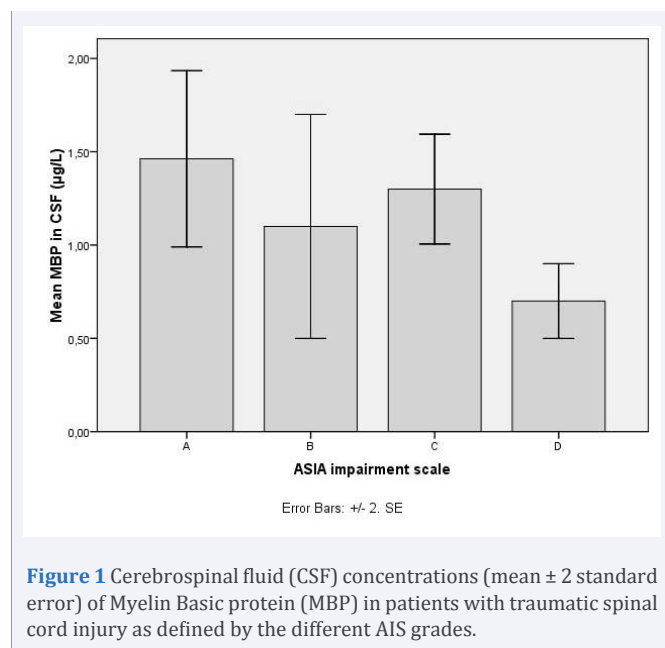
The objective of this study was to investigate the MBP CSF concentration in traumatic SCI patients within 24 hours post-injury and its relation to initial neurological severity according to the AIS grades. Our data demonstrated no significant differences in CSF MBP concentrations between patients with different AIS grades. These results suggest that CSF MBP is not a suitable biomarker for discriminating between severities in the first 24 hours after a SCI.

Our previous study showed that the CSF concentrations of GFAP, NSE, S100 β , tau and NFH neurofilament heavy chain were elevated in the SCI patients as compared with uninjured controls irrespective of the AIS severity [10]. CSF MBP, however, was not consistently elevated in the same study population. SCI is usually caused by a mechanical compression of the cord due to displacement of fractured vertebrae and/or discs extending into the spinal canal, inflicting a lesion site which spans over several segments. Griffiths and McCulloch examined the time course of changes in myelin sheaths of injured axons after a weight drop injury of the cat spinal cord [17]. Although some changes were observed in structure of myelin within the first 4 hours, it was only after 24 hours that many partially demyelinated axons

Table 1: Demographic data.

Case	Center	Age at injury (years)	Sex	Cause of injury	Interval injury-CSF sampling (hours)	NLI	AIS vac	MBP ($\mu\text{g/l}$)
1	Nijmegen	48	m	MVA	4.25	C6	A	2,0 +
2	Nijmegen	36	m	MVA	15.17	T9	A	0,7 -
3	Vancouver	47	m	MVA	19.67	T12	A	2,7 +
4	Nijmegen	46	f	Fall	7.45	C5	A	1,3 -
5	Nijmegen	20	m	Fall	3.00	T10	A	0,7 -
6	Vancouver	45	f	Fall	20.5	C6	A	1,3 -
7	Nijmegen	24	m	Fall	3.5	C6	A	1,3 -
8	Vancouver	55	m	Skiing accident	22.75	C6	A	1,7 -
9	Vancouver	34	m	Fall	23.42	C6	B	1,4 +
10	Vancouver	38	m	Hit by tree branch	16.58	C5	B	0,8 -
11	Vancouver	66	m	Fall	18.67	C4	C	1,3 -
12	Vancouver	59	m	Fall	24.00	T11	C	1,2 -
13	Vancouver	58	f	Fall	24.00	C6	C	1,7 -
14	Nijmegen	47	f	Fall	8.83	C4	C	1,0 -
15	Nijmegen	72	m	Fall	13.5	C5	D	0,6 -
16	Nijmegen	18	f	Fall	19.25	T12	D	1,5 +

Abbreviations: MVA: Motor Vehicle Accident; NLI: Neurological Level of Injury; AIS vac: ASIA Impairment Scale within 24 Hours Post-Injury; AIS ch: ASIA Impairment Scale After 6 or 12 Months; NSE: Neuron Specific Enolase; GFAP: Glial Fibrillary Acidic Protein; NFH: Neuro Filament Heavy Chain; NA: Not Applicable



were observed, progressing over following days. This delay in demyelination was attributed to secondary mechanisms responsible for the aggravation of the local damage in the spinal cord. The mechanical trauma inflicted in our traumatic SCI patients probably did not induce such damage to the myelin sheaths that it resulted in an elevation in CSF MBP concentrations within 24 hours post-injury. The acute mechanical injury clearly affects axons, as the levels of tau (a microtubule associated protein found in axons) were markedly elevated within CSF at this time [5]. This may indicate that in the very acute phase, the structural integrity of the myelin sheath remains grossly intact. However, as shown by the study of Griffith et al, secondary injury mechanisms may exacerbate the degeneration of myelin sheaths that could lead to higher CSF MBP levels more than 24 hours post-injury.

Our results should be interpreted in the context of specific study limitations. We presented the study results of 16 SCI patients. Although we used strict inclusion criteria, the small patient numbers in this study limits the interpretation of the results. Therefore, it cannot be excluded that in larger study cohorts, a correlation between AIS grades and CSF MBP will be found. However, despite these limitations, the normal or only marginally elevated MBP concentrations in our entire cohort suggest that overt degradation of myelin does not occur during the first 24 hours post-injury in SCI. In addition, the time between the injury and CSF sampling differed considerably among the patients in our study, ranging from 3 h to 24 h. Although no elevated concentrations were identified, our previous study showed that this variability in timing may influence biomarker concentrations [10]. Future studies therefore should perform CSF sampling on predetermined time intervals. The method of obtaining CSF differed between the two centers. As the purpose of this study was to analyze CSF samples obtained within 24 hours, we believe that this has not influenced our results. Future studies should determine whether CSF MBP does increase after more than 24 hours following the initial SCI. If so, hypothetically,

it could be used as a biological surrogate outcome measure to determine if acute interventions are protecting white matter from secondary injury processes.

CONCLUSION

In this study of 16 traumatic SCI patients, CSF MBP concentrations were not elevated in traumatic SCI patients. In addition, the CSF MBP appeared not to correspond with patients having a motor complete or incomplete SCI. Also no clear differences in CSF MBP concentrations were identified between the different AIS grades. Therefore it appears that CSF MBP is not a suitable CSF biomarker to predict outcome or prognosis after traumatic SCI within 24 hours post injury.

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