

Neuroprotein Dynamics in the Cerebrospinal Fluid: Intraindividual Concomitant Ventricular and Lumbar Measurements

Sebastian Brandner^a Christian Thaler^a Piotr Lewczuk^b Natalia Lelental^b
Michael Buchfelder^a Andrea Kleindienst^a

Departments of ^aNeurosurgery, and ^bPsychiatry and Psychotherapy, University of Erlangen-Nürnberg, Erlangen, Germany

Key Words

Biomarker · S100B · Neuron-specific enolase · β -Trace · Neurotrauma · Neuroproteins · Cerebrospinal fluid · Ventricular drain

Abstract

Objective: The measurement of neuromarker/neuroproteins in the cerebrospinal fluid (CSF) is gaining increased popularity. However, insufficient information is available on the rostrocaudal distribution of neuroproteins in the CSF to guarantee an appropriate interpretation of ventricular versus lumbar concentrations. **Methods:** In 10 patients treated with both an external ventricular and a lumbar CSF drain, we collected concomitant CSF samples. We measured CSF concentrations of the glial S100B protein, the neuron-specific enolase (Cobas e411[®]; Roche Diagnostics), the leptomeningeal β -trace protein (BN Pro Spec[®]; Dade Behring/Siemens), and the blood-derived albumin (Immage; Beckman Coulter). Statistical analysis was performed with a paired Wilcoxon signed ranks test. **Results:** In patients with a free CSF circulation without any recent neurosurgical procedure, S100B and neuron-specific enolase concentrations did not differ between the ventricular and lumbar CSF while β -trace and albumin levels were significantly higher in the lumbar than in the ventricular CSF ($p = 0.008$ and $p = 0.005$). Following posterior

fossa tumor surgery, all proteins accumulate in the lumbar CSF. **Conclusion:** For brain-derived proteins, we could not confirm a rostrocaudal CSF gradient while lepto-meningeal and blood-derived proteins accumulate in the lumbar CSF. We conclude that for the interpretation of protein CSF concentrations, the source of the sample is of crucial importance.

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Introduction

The measurement of neuroproteins in the cerebrospinal fluid (CSF) has gained clinical attraction [1] and is hoped to improve outcome prediction and to guide therapy [2, 3]. However, a detailed knowledge of protein dynamics in the CSF is required for an appropriate interpretation. The current concept of brain-derived protein dynamics in the CSF has originally been described by Reiber [4] calculating a ventricular to lumbar (V/L) concentration ratio. His theory suggests that brain-derived proteins, once released into the CSF, are reabsorbed cranio-caudally resulting in a decline between the ventricular and lumbar CSF concentration. However, during a decade studying the relevance of S100B following brain injury and collecting lumbar CSF samples of patients without any intracerebral pathology, we failed to confirm con-

sistently the lumbar S100B accumulation [5]. On the other hand, due to very few indications for intraindividual concomitant ventricular and lumbar CSF drainage, there is only a limited access to such sample pairs.

To the best of our knowledge, no data have been published yet about intraindividual comparisons of neuroprotein concentrations collected simultaneously from the ventricular and lumbar CSF, confirming the concept of a rostrocaudal CSF gradient. Here, we present concomitant ventricular and lumbar concentrations of different CSF proteins, i.e. the glial S100B protein, the neuron-specific enolase (NSE), the leptomeningeal β -trace protein and the blood-derived albumin.

S100B is a 20-kDa neurotrophic protein that is released from astrocytes into the extracellular fluid. Numerous reports indicate that S100B is released after brain insults and serum levels are positively correlated with the degree of injury and negatively correlated with outcome [6–8] or may allow the detection of secondary insults following brain injury [6, 9]. However, since serum measurements do not acknowledge the multifaceted active and passive S100B release pattern and the effect of the blood-brain barrier, S100B measurements in the CSF have gained interest allowing a more accurate determination of the true cerebral S100B levels following various types of brain insults [10–13]. However, ‘normal’ S100B values in the CSF vary among publications [5, 14], and lumbar values were supposed to be corrected by a factor of 3.5 based on Reiber’s study [15, 16].

NSE is a 78-kDa protein considered to originate from neurons. NSE concentrations in serum and CSF have been assessed for outcome prediction in neurotrauma or stroke patients [17, 18]. According to the above-mentioned concept of a rostrocaudal concentration CSF gradient for brain-derived proteins, a NSE V/L gradient of 2:1 has been postulated [4]. β -Trace is a 25-kDa protein, predominantly secreted by the leptomeninges and the choroid plexus, and is therefore used for the diagnosis of CSF leakage [19–21]. For β -trace, a V/L gradient of 1:11 was postulated reflecting its accumulation during its rostrocaudal flow path [4]. Similarly, for albumin, a V/L ratio of 1:2.5 has been described for patients with an unimpaired blood-brain barrier [4].

We compared the concentration of neuroproteins collected at the same time from the ventricular and lumbar CSF in patients with a free CSF circulation without any recent neurosurgical procedure, i.e. a communicating hydrocephalus due to subarachnoid hemorrhage (SAH), traumatic brain injury (TBI) and meningitis. We could

not confirm a ventricular-lumbar gradient as reported in the literature, while following posterior fossa surgery, all proteins accumulate in the lumbar CSF.

Material and Methods

Subjects

The protocol was approved by the local ethical committee and informed consent was obtained from an appropriate member of each patient’s family. Ventricular and lumbar CSF samples were obtained from 10 patients (4 male, 6 female, mean age 55.5 ± 20.0 years).

In 7 patients who developed a communicating hydrocephalus following SAH, TBI or meningitis, an external ventricular drain (EVD) was placed. To assess the requirement for a permanent shunt placement to treat a persisting hydrocephalus following the acute phase, a lumbar drain was inserted 8–12 days after the initial EVD placement [22]. The EVD was kept close, but remained in place for up to 2 days (SAH $n = 2$, TBI $n = 1$) to monitor the intracranial pressure.

In 3 patients with a tumor in the posterior fossa, the EVD was placed to monitor the intracranial pressure postoperatively, and to allow an immediate CSF drainage whenever necessary. Simultaneously, 10–15 ml CSF was drained by a lumbar puncture for prophylaxis of a CSF fistula on the second postoperative day.

Sample Collection and Processing

In those in patients with a free CSF circulation without any recent neurosurgical procedure, i.e. SAH, TBI and meningitis, the CSF samples were collected at 8 a.m. In order to assess the neuro-marker/protein concentrations in samples reflecting the actual patient CSF concentration as accurate as possible, we collected the CSF from the drain close to the patient after discarding 2 ml. The ventricular CSF samples were collected first (2–3 ml), and after closure of the ventricular drain, the lumbar CSF samples (5 ml) were collected. In patients after posterior fossa surgery the ventricular CSF samples were collected at 8 a.m. Subsequently, the patient underwent a lumbar puncture and 5 ml of the lumbar CSF were collected for the protein analysis. The samples were processed immediately for measurement of S100B and β -trace and albumin. For detection of NSE, the samples were stored at -80°C and the measurement was performed later.

S100B and NSE were measured with the Cobas e411[®] electrochemiluminescence assay using commercial test kits from Roche Diagnostics (Roche Elecsys[®] 2010 S100 reagent kit, lower detection limit $0.005 \mu\text{g/l}$, and Roche Elecsys[®] 2010 NSE reagent kit, lower detection limit $0.015 \mu\text{g/l}$). β -Trace and albumin were measured by immunochemical nephelometry using the commercially available nephelometric research assay N-latex- β -TP for β -trace (BN Pro Spec[®] nephelometer; Dade Behring/Siemens) and the Immage Immunochemistry Systems-ALB test kit for albumin (IMMAGE nephelometer; Beckman Coulter).

Statistical Analysis

Statistical analysis was performed with the Wilcoxon paired signed ranks test to assess the difference of the central tendency of values between the ventricular and lumbar concentrations (SPSS software 20.0). Statistical significance was accepted at $p < 0.05$.

Fig. 1. Ventricular (V) and lumbar (L) CSF concentrations of S100B, NSE, β -trace and albumin in patients with a free CSF communication. **a** Ventricular and lumbar S100B CSF concentrations were not significantly different. **b** Ventricular and lumbar NSE CSF concentrations were not significantly different. **c** Lumbar β -trace CSF concentrations were significantly higher in comparison to ventricular concentrations ($p = 0.008$). **d** Lumbar albumin CSF concentrations were significantly higher in comparison to ventricular concentrations ($p = 0.005$). ° = outlier (more than 2-times the interquartile distance); * = extreme outlier (more than 3-times the interquartile distance).

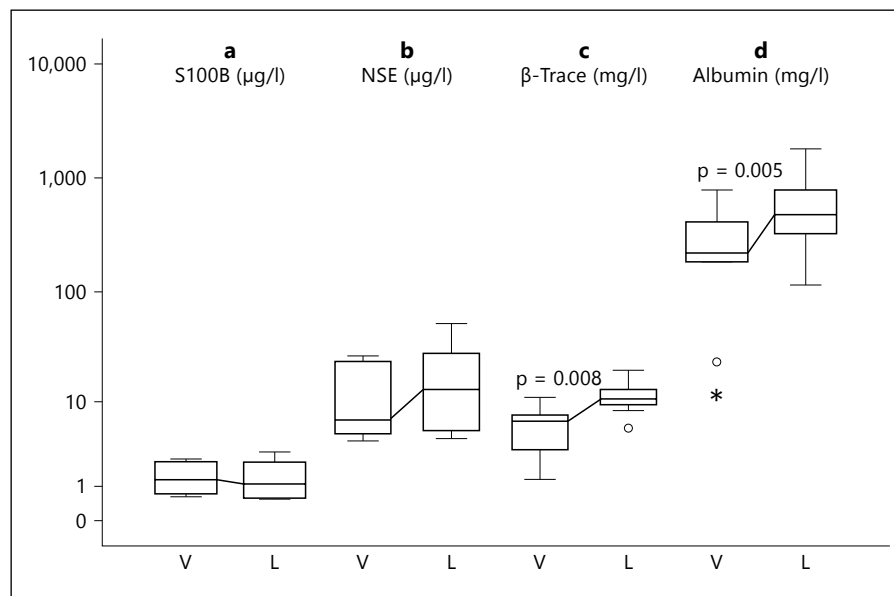


Table 1. Patient characteristics and their S100B, NSE, β -trace and albumin concentrations in the ventricular and lumbar CSF

No.	Age, years	Sex	Diagnosis	S100B			NSE			β -Trace			Albumin		
				ventricular, $\mu\text{g/l}$	lumbar, $\mu\text{g/l}$	V/L ratio	ventricular, $\mu\text{g/l}$	lumbar, $\mu\text{g/l}$	V/L ratio	ventricular, mg/l	lumbar, mg/l	V/L ratio	ventricular, mg/l	lumbar, mg/l	V/L ratio
1	77	m	meningitis	1.62	1.67	0.97	4.29	5.17	0.83	11.0	19.8	0.56	785	1,800	0.44
2	45	f	meningitis	0.73	0.57	1.28	4.84	4.24	1.14	3.19	5.44	0.59	232	467	0.50
3	74	f	SAH	2.49	2.89	0.86	26.87	39.71	0.68	7.39	12.3	0.60	482	1,070	0.45
4	72	f	SAH	2.41	3.00	0.80	25.77	51.92	0.50	6.81	10.7	0.63	412	785	0.52
				0.67	0.55	1.22	4.79	4.78	1.00	6.44	8.25	0.78	184	324	0.57
5	50	f	SAH	0.62	0.58	1.07	–	–	–	–	–	–	208	343	0.60
				2.25	2.25	1.00	23.87	28.02	0.85	5.52	9.45	0.58	194	524	0.37
6	70	m	TBI	1.46	1.18	1.24	16.08	13.13	1.22	10.8	18.3	0.59	254	489	0.52
7	42	m	TBI	1.13	0.74	1.53	6.61	11.7	0.56	1.3	9.42	0.14	23.7	115	0.21
				0.98	1.03	0.95	4.01	17.35	0.23	1.7	13.2	0.13	11.7	164	0.07
8	71	m	PFTS	21.56	47.5	0.45	22.9	112.3	0.20	3.9	35.9	0.11	221	1,740	0.12
9	35	f	PFTS	3.61	147.5	0.02	3.1	104.3	0.02	1.1	19.3	0.06	170	1,270	0.13
10	19	f	PFTS	8.74	113	0.08	11.09	133.4	0.08	0.7	20.5	0.03	17.4	968	0.02

Two sample pairs were available in 2 SAH patients and 1 TBI patient. PFTS = Posterior fossa tumor surgery.

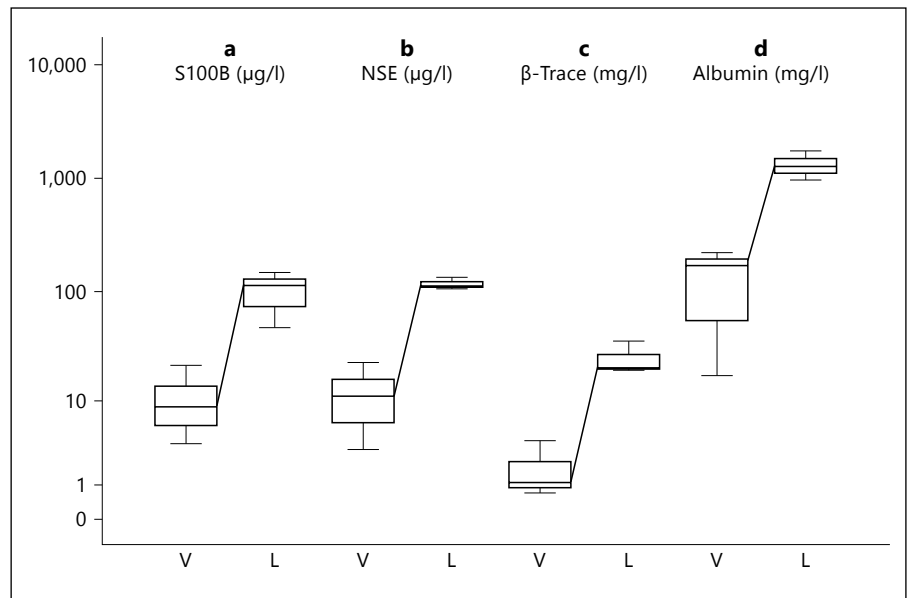
Results

The patient characteristics are summarized in table 1. A total of 10 patients were included into the study (4 male, 6 female), and the mean age was 55.5 (± 20.0) years.

In all patients with a free CSF communication, i.e. following SAH, TBI and meningitis, the ventricular and

lumbar S100B CSF levels were within the normal range [14] and the V/L ratio ranged from 0.8 to 1.53 (table 1). The statistical analysis revealed no difference of the central tendency of the ventricular and lumbar S100B CSF concentration ($Z = -0.059$ based on positive ranks, $p = 0.953$; fig. 1a). In all patients who underwent posterior fossa tumor surgery, both ventricular and lumbar S100B CSF concentrations were above the normal levels, with

Fig. 2. Ventricular (V) and lumbar (L) CSF concentrations of S100B (a), NSE (b), β -trace (c) and albumin (d) in patients following posterior fossa tumor surgery demonstrating an accumulation of all proteins in the lumbar CSF.



an accumulation in the lumbar compartment resulting in a median V/L ratio of 0.07 (fig. 2a).

In 4 patients with a free CSF communication, the ventricular and lumbar NSE CSF levels were within the age-related range [23], while in 3 patients, the NSE concentrations were slightly increased with NSE concentrations between 23.97 and 51.92 $\mu\text{g/l}$ (normal range 3.8–16.0). The lumbar NSE CSF concentrations were slightly increased as compared to the ventricular ones (table 1). However, the statistical analysis revealed no difference of the central tendency of the ventricular and lumbar NSE CSF concentration in patients with a free CSF communication ($Z = -1.836$ based on negative ranks, $p = 0.066$; fig. 1b). Following posterior fossa tumor surgery, NSE accumulated in the lumbar CSF resulting in a median V/L ratio of 0.08 (fig. 2b).

In the patients with a free CSF communication, the lumbar β -trace CSF concentrations were higher than the ventricular ones resulting in a median V/L ratio of 0.59. The statistical analysis revealed the central tendency of the lumbar β -trace CSF concentration to be higher than the ventricular one ($Z = -2.666$ based on negative ranks, $p = 0.008$; fig. 1c). In patients who underwent posterior fossa tumor surgery, β -trace accumulated in the lumbar CSF resulting in a low median V/L ratio of 0.06 (fig. 2c).

In the patients with a free CSF communication, the lumbar CSF concentrations were higher than the ventricular ones resulting in a median V/L ratio of 0.47. The sta-

tistical analysis revealed the central tendency of the lumbar albumin CSF concentration to be higher than the ventricular one ($Z = -2.805$ based on negative ranks, $p = 0.005$; fig. 1d). In patients who underwent posterior fossa tumor surgery, albumin accumulated in the lumbar CSF resulting in a median V/L ratio of 0.13 (fig. 2d).

Discussion

The quantification of neuroproteins in the CSF in different neurological conditions is gaining increased popularity. The glial S100B protein has been promoted for monitoring neurological and neurotrauma patients [6, 8, 12], whereas NSE has been evaluated for monitoring stroke patients [17]. While lumbar CSF sampling is performed more often by neurologists, neurosurgeons are used to collecting ventricular CSF samples. Hence, there remains the controversy whether the measured concentrations in these samples – ventricular versus lumbar – are comparable or whether a multiplier has to be applied correcting for the CSF dynamics of the respective protein [4]. Due to a limited access to simultaneously collected lumbar and ventricular CSF samples, no information is available about the rostrocaudal distribution of neuroproteins in the CSF. So far, ventricular and lumbar CSF samples from different populations have been used to characterize the protein distribution in the CSF [4, 24].

This concept of different protein dynamics in the CSF, as established by Reiber [4] in 2001, differentiated brain-derived proteins originating from neurons or glial cells and decreasing in the concentration from ventricular to lumbar, and leptomeningeal or blood-derived proteins increasing from ventricular to lumbar due to a continuous influx into the CSF along its craniocaudal flow path. However, the lumbar CSF samples in Reiber's study were collected in neurological patients, while his ventricular ones originated from neurosurgical patients not accounting for the reason and extent of the surgical intervention.

We compared the CSF concentrations of the glial S100B protein and the neuronal protein NSE in simultaneously collected ventricular and lumbar samples of patients with a free CSF communication, i.e. a communicating hydrocephalus due to SAH, TBI or meningitis. The samples were collected in the postacute period when the S100B CSF levels stabilized within the normal range. By then, any contribution by the surgical trauma of EVD insertion could be excluded [25]. We found no difference of the brain-derived proteins S100B and NSE between the ventricular and lumbar CSF compartment and could not confirm the negative craniocaudal gradient as suggested by Reiber. We suggest that the previously described negative rostrocaudal gradients for glial and neuronal proteins might have been a procedure-related artifact caused by the ventriculostomy.

In contrast, we could confirm the accumulation of the leptomeningeal β -trace protein and blood-derived albumin in the lumbar CSF resulting in a positive rostrocaudal gradient [4]. One limitation of our study is the small number of included individuals. On the other hand, the indication of a simultaneous ventricular and lumbar CSF drainage is very limited and obviously the reason that none of these comparisons have been performed yet. Al-

though including a small number of patients with a communicating hydrocephalus, the statistical analysis was robust and the albumin V/L ratio of 0.42 found in our study corresponds exactly to the V/L ratio of 0.4 reported in the initial study [4].

In the present series, we demonstrated elevated lumbar protein CSF levels following surgery for posterior fossa tumors – most likely due to the infratentorial surgical manipulation and protein influx into the CSF below the level of the lateral ventricles where the EVD was placed. A neuroprotein release has been demonstrated even for minor procedures for up to 48 h [25]. However, the number of individuals included in the analysis following posterior fossa tumor surgery is too small to result in any statistical implication.

Taken together, we could not confirm a negative craniocaudal concentration gradient for brain-derived glial and neuronal proteins whenever the CSF communication is free, while leptomeningeal and blood-derived proteins accumulate in the lumbar compartment. Surgical manipulations do result in a substantial protein release into the CSF and cause artifacts. However, whether our findings in patients with a communicating hydrocephalus translate into a normal population has to be investigated further.

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Disclosure Statement

The authors have no conflicts of interest to disclose.

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