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EVALUATION OF CEREBROSPINAL FLUID S100B PROTEIN CONCENTRATION IN PATIENTS WITH PURULENT, BACTERIAL MENINGITIS – OWN OBSERVATIONS

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ABSTRACT

THE AIM of the study was evaluation of usefulness of cerebrospinal fluid (CSF) S100 B protein concentration assessment in adults with purulent, bacterial meningoencephalitis.
MATERIAL AND METHODS. The investigation was performed in 16 subjects hospitalized at the Department of Infectious Diseases of Medical University of Silesia in Bytom in 2008 – 2012 due to purulent, bacterial meningoencephalitis. All patients were divided into two groups according to the severity of their clinical condition: I group – very severe course of the disease, II group – moderate and mild course of the disease. In all individuals CSF S100 B protein concentration was evaluated during the first 24 hours of hospitalization.
RESULTS. Mean CSF S100 B protein concentration in patients in very severe clinical condition (group I) was 1215.63 pg/mL compared to 419.56 pg/mL in subjects of group II with moderate and mild course of disease. The difference between CSF mean concentration of this protein was statistically significant ($p<0.01$). No correlations were assessed between CSF S100 B protein concentrations and other CSF inflammatory parameters. Control assays performed in 7 patients from group I revealed only slightly decrease of CSF S100 B protein level in fatal course of the disease. In survivals with recovery CSF concentration of this protein was evident decreased compared to initial level.
CONCLUSIONS. The obtained results indicate the usefulness of CSF S100 B protein concentration assessment in estimation of severity of the patient's clinical condition. The level of this protein concentration also seems to be helpful as prognostic marker in purulent, bacterial meningoencephalitis.

Key words : *S100 B protein, cerebrospinal fluid, purulent, bacterial meningoencephalitis*

INTRODUCTION

Bacterial infections of the central nervous system (CNS) remain a serious problem of contemporary medicine. Despite advances of pharmacotherapy and intensive care, bacterial purulent meningoencephalitis remain the disease of an uncertain prognosis and relatively high mortality rate and in many cases it leads to permanent, neurological sequelae (1). The results of routinely performed cerebrospinal fluid (CSF) tests, i.e. CSF leukocyte count and cytogram, concentration of protein, glucose, chloride and, rarely, lactic acid do not always seem to fully reflect the actual intensity of brain tissue inflammatory process in these diseases. (1,2). The aim of this study was to evaluate the usefulness of CSF S100 B protein levels determination, for the diagnosis of purulent bacterial meningoencephalitis in adults.

MATERIAL AND METHOD

The study was conducted in group of 16 patients hospitalized at the Department of Infectious Diseases of Medical University of Silesia in Bytom in 2008-2012. A total of 11 males (68.75%) and 5 females (31.25%) were included, the youngest patient was 19 years old, the oldest - 67, and mean age was 42 years. Patients were admitted to the Department with suspected meningoencephalitis. In all cases based on the CSF test results purulent bacterial meningoencephalitis was diagnosed. The following etiological factors of disease were identified: *Streptococcus pneumoniae* in 5 patients (31.25%), *Neisseria meningitidis* in 2 cases (12.5%), in the remaining nine patients (56.25%) the etiological agent of meningoencephalitis was not determined.

Due to clinical severity of the condition assessed on the day of admission to the Department, the patients were divided into two groups:

1) Group I - 9 patients in very severe conditions (6 men and 3 women, mean age 55 years) with altered consciousness, focal neurological deficits, generalized seizures (in the period prior to hospitalization or at the first day), Glasgow Coma Scale score (GCS) did not exceed 8; etiological factors were: *Streptococcus pneumoniae* in 4 cases, *Neisseria meningitidis* in one case, in the remaining four cases the etiology was not established.
2) Group II - 7 patients in mild to moderately-severe condition (5 men and 2 women; mean age 40 years) who had no significant disturbances of consciousness, no focal neurological deficits or seizures, Glasgow Coma Scale score exceed 9; etiological factors of meningioencephalitis were: *Streptococcus pneumoniae* (1 case), *Neisseria meningitidis* (1 case), in the other five cases, the etiology was not established.

In all patients lumbar puncture and CSF examination were made at the day of admission, including leukocyte count and cytogram, concentration of proteins, glucose, lactic acid and S100 B protein (S100 B). To measure the concentration of S100 B, enzyme immunoassay kits was used : Human S100B ELISA, BioVendor, Research and Diagnostic Products GmbH (Germany).

Furthermore, at the 10 th day of treatment in seven patients in group I control CSF tests were made. Of these, four people were successfully treated, in one deafness occurred as a consequence of disease and two patients died.

The comparison of average count of leukocytes, concentrations of protein, glucose, lactic acid and protein S100 B in the two groups of patients were performed using Student's t test. Significance was defined as $p(\alpha) \leq .05$ and $p(\alpha) < 0{,}01$. The correlation between the parameters of CSF in both groups using Pearson's correlation coefficient was also evaluated.

RESULTS

Results of CSF examination in patients with purulent bacterial meningoencephalitis obtained on admission day are shown in Table I.

In group I, the average pleocytosis was 663 cells / mm 3, in all patients in cytogram PNMs prevailed (from 70% to 100% of the cells), the mean protein concentration 1840 mg / L, glucose - 0.62 mmol / L, lactic acid - 10 , 46 mmol / L, and concentration of protein S100B - 1,215.63 pg / mL.

The condition of these patients assessed on the admission day and course of the disease was very severe. In three cases, there was an ARDS, intubation or a tracheotomy was necessary, patients were treated with mechanical ventilation on the Intensive Care Unit, two of them died. Overall, three patients of this group died, one had a persistent neurological sequelae as a deafness, 5 patients were cured. The highest CSF concentration of protein, lactic acid, and protein S100 B was observed in fatal cases.

In group II the average pleocytosis was 498 cells /mm3, in all patients' cytogram also PNMs prevailed (from 59% to 87% of all cells). Mean CSF levels of other parameters were as follows: protein 906 mg / L, glucose 0.79 mmol / L, lactic acid 3.21 mmol / L, and the concentration of protein S100 B - 419.56 pg / mL. Condition of the patients and course of disease in this group was moderately-severe or mild, and outcomes compared with the group I were definitely better. Complete recovery was achieved in 6 cases, in one patient hearing loss occured as a consequences of neuroinfections. There was no respiratory distress in this group, none of the patients died.

Results of CSF control examinations performed in seven patients in group I were as follows: in 4 patients who were cured, there was a marked reduction of the concentration of S100 B protein. In all cases reduction in the average count of leukocytes, CSF protein and lactic acid in comparison to the initial results were observed. In a patient with a deafness as a consequence decrease of S100 B protein concentration in control examination was less pronounced.

Whereas in two patients who died, there was insignificant decrease in the concentration of S100 B protein in CSF. In both cases there was a persistently high PNMs pleocytosis, a high CSF protein and lactic acid concentration. Results of control determinations of CSF S100 B protein in the course of disease in patients of group I are shown in Table II.

Table 1. The results of CSF examination in patients with purulent, bacterial meningoencephalitis on the day of admission to the ward

Patient group	Pleocytosis (cell/mm^3)	Protein (mg/L)*	Glucose (mmol/L)	Lactic acid (mmol/L)**	S100B protein (pg/mL)**
Group I (n = 9)	663 ± 478 (88 – 1214)	1840 ± 780 (754 – 2400)	0.62 ± 0.39 (0 – 1.4)	10.46 ± 5.11 (2.8 – 19.0)	1215.63 ± 943.67 (848.67 –2125.72)
Group II (n = 7)	498 ± 323 (69 – 533)	906 ± 311 (480 – 1698)	0.79 ± 0.58 (0.3 – 2.4)	3.21 ± 0.91 (2.5 – 4.8)	419.56 ± 301.44 (219.54 – 971.49)

The table shows the mean values of examined parameters,

* - Statistically significant difference ($p < 0.05$)

** - Statistically significant difference ($p < 0.01$)

Table II. CSF S100 B protein levels in the course of the disease in group I

Patient	S100 B protein (pg/mL)		Outcome
	I examination	II examination	
1.	924.66	239.32	recovery
2.	990.25	524.23	- „ -
3.	1021.21	611.19	- „ -
4.	1074.14	618.17	- „ -
5.	1149.55	845.17	deafness
6.	2125.72	2017.93	decease
7.	1717.37	1617.22	- „ -

Differences in average count of leukocytes and CSF glucose concentrations between the two groups of patients were not statistically significant. However, there were statistically significant differences in mean CSF concentrations of protein ($p < 0.05$), lactic acid ($p < 0.01$) and S100 B protein ($p < 0.01$) between Group I and II.

DISCUSSION

The basic test in the diagnosis of CNS infections is analysis of the cerebrospinal fluid. In most laboratories CSF is routinely examined for count and types of leukocytes, protein concentration, glucose and chloride levels, rarely - lactic acid (1.2).

For many years, attempts were made to extend the scope of CSF parameters for diagnosis of the CNS infections. The following parameters were determined, i.a., the concentration of lysozyme, immunoglobulins, inflammatory cytokines, chemokines, arachidonic acid derivatives (prostaglandins, thromboxanes, leukotrienes), procalcitonin (PCT), lactate dehydrogenase (LDH), creatine kinase (CK), neuron-specific enolase (NSE) and ciliary neurotrophic factor (CNTF).These tests allowed for a more accurate assessment of the actual intensity and course of inflammatory processes in patient's subarachnoid space, but their performing often requires significant financial outlays and a well-equipped laboratory (3-7).

S100 B protein belongs to a calcium-binding protein family. It occurs in the CNS, mainly in the astroglia, oligodendrocytes and Schwann cells, in the cytosol or attached to cell membranes. It is synthesized and secreted primarily by astrocytes of the brain.

S100 B protein is a regulatory protein modulating activity of effector cells and is involved in maintaining calcium homeostasis and the regulation of proteins phosphorylation. S100 B intracellular activity involve regulatory effect on cell growth, differentiation, modeling and energy metabolism. And extracellularly stimulates neuronal survival and differentiation, astrocyte proliferation, neuronal death by apoptosis, stimulate or inhibit the activity of inflammatory cells (8-11).

Depending on the concentration protein S100 B exhibits a neurotrophic and trophic or toxic to brain tissue. At physiological concentrations it has neurotrophic effects on development and regeneration of nerves, in high concentrations is neurotoxic and participates in neurodegenerative disorders. S100 B protein concentration is therefore considered to be one of the biochemical markers of brain damage (10,11).

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Experimental studies revealed that S100 B protein is involved in various disease processes of CNS, including meningitis and encephalitis. In the course of meningoencephalitis damage to the nerve and glial cells occurs, which leads to the release of specific intracellular proteins into the extracellular space and CSF. The concentration of S100 B protein in CSF may be a marker of glial activation and damage in cerebral white matter (2,12,13).

S100 B levels in serum and CSF were tested in various CNS diseases, in particular vascular brain diseases . In ischemic stroke the increased serum concentration of this protein was shown (14,15) and the extent of S100B elevation may help to identify patients with an increased risk of specific early neurological complications (16). In patients with cerebral hemorrhage, the elevated S100 B levels in serum were also observed(17).

There was also elevated serum and CSF levels of S100 B in the course of traumatic brain injury (18). According to Vos et al extent of S100 B elevation in the serum can be considered as a single strongest predictor of poor outcome of disease (19).

Studies in patients with neurodegenerative diseases, including Alzheimer's disease have shown elevated levels of S100 protein B in CSF and serum (20). Measuring of S100 B concentration demonstrated its usefulness in the diagnosis of the schizophrenia and depression (21).

There is relatively little reports about the research of S100B role in the course of purulent bacterial meningoencephalitis. In pathophysiology of bacterial CNS infections ischemia and hypoxia of brain tissue is an important issue.

In the course of inflammation in the subarachnoid space initially activation occurs and then - the damage and death of glial cells. Glial cells and CNS resident macrophages are involved in the inflammatory response; then apoptosis induction (nerve and glial cells), and damage to brain tissue occurs. Reflection of these processes is the release of S100B from glial cells

and neuron-specific enolase (NSE) from neurons into the CSF. The clinical course and outcome of bacterial meningoencephalitis depend on severity and extent of neuronal and glial damage (1,2,6,13,22,23).

Studies in children with bacterial meningoencephalitis showed an increase the concentration of S100B in serum and CSF. The concentration, both in CSF and in the serum, in the early stage of the disease correlated with the clinical severity, as well as with occurrence neurological complications after disease. Elevated levels of this protein in serum and CSF in the acute phase of the disease were reduced in control tests in successfully treated cases (24-27). Lins et al point the usefulness of S100B in serum in monitoring of the course of bacterial meningoencephalitis, which may be of great practical importance in case of contraindications for performing control lumbar puncture (23).

There was no clear relation between concentration of S100B protein in the CSF and the etiology of bacterial meningoencephalitis (24,25,27,28). We also have not found such relation in patients involved in the study .

There has not also been shown clear correlation between the concentration of the protein in CSF and others routinely tested CSF parameters. In successfully treated cases were observed reductions of S100 B levels in control tests parallel with the normalization of other CSF parameters (pleocytosis, proteins and glucose) (24,26).

A positive correlation between concentrations of S100B and lactic acid level in CSF was found in Group I of observed patients, whereas there was no correlation between S100B and PMNs count, protein and glucose levels. In the second group, a correlation between concentrations of S100B and other parameters of CSF was not observed.

An interesting issue is relation between concentration of S100B in CSF and clinical severity in patients with bacterial purulent meningoencephalitis . The highest concentrations of S100 B in CSF was observed in patients with the most severe clinical course.

This seems to indicate the substantial damage of brain tissue, white matter glial cells, resulting from bacterial infection. However, changes in concentration of S100B in the course of bacterial meningoencephalitis have a certain relationship with the further progression and course of the disease. Control tests performed during hospitalization showed significantly reduced levels of S100 B protein in CSF in patients with clinical improvement and normalization of routine CSF tests. In patients whose clinical condition did not improve, the concentration of this protein in CSF remained high, comparable to that of the first test (23,24,26).

In our studies we observed the highest concentrations of S100B in the CSF in patients in a very severe clinical condition (group I). There were no statistically significant differences in average PMNs count and glucose in CSF between the groups I and II. However, the highest mean concentrations of protein and lactic acid observed in patients with most severe course, particularly those who died.

The results indicate that concentration of S100 B protein clearly correlated with the clinical severity on admission to the Department and with the further course of the disease. Control tests showed a realtion between concentration of S100 B protein in CSF and outcome of the disease. In successfully treated cases S100 B levels were decreased, in most cases before clinical improvement and normalization of other CSF parameters.

In contrast, in patients who died, the concentration of S100B virtually were not reduced compared to the first examination. At the same time maintained a high CSF inflammatory parameters and clinical condition did not improve. Relatively small number of studied patients hampers a more detailed statistical analysis of results and to draw unequivocal, far-reaching conclusions, but justifies the desirability of further research.

SUMMARY

Concentration of S100B protein in CSF seems largely reflect the intensity of brain damage caused by bacterial infection. Elevated levels of S100B in CSF initially points to the activation of glial cells in the course of meningoencephalitis, and then - the white matter of brain damage. S100B protein is considered an indicator of damage to white matter in a number of pathological processes of the central nervous system, including bacterial meningoencephalitis (13,22-24,26,28)

Determination of S100 B in CSF in patients with purulent bacterial meningoencephalitis may therefore be important both in assessing the actual intensity of glial damage, which are relevant to the course and outcome of the disease, as well as to predict fatal outcome. This can be useful in monitoring the course and treatment of purulent meningoencephalitis and have some prognostic value.

REFERENCES

1. Roos KL, Tunkel AR, Scheld WM. Acute bacterial meningitis. W: Scheld WM, Whitley RJ, Marra CM, red. Infections of the Central Nervous System. Philadelphia: Lippincott, Williams and Wilkins;2004:346-422.
2. Leib SL, Täuber MG. Pathogenesis and pathophysiology of bacterial infections. W: Scheld WM, Whitley RJ, Marra CM, red. Infections of the Central Nervous System. Philadelphia: Lippincott, Williams and Wilkins;2004:331-346.

3. Kępa L, Oczko-Grzesik B, Błędowski D. Prokalcytonina (PCT) w płynie mózgowo-rdzeniowym i w surowicy chorych z bakteryjnymi ropnymi i limfocytarnymi zapaleniami opon i mózgu u dorosłych – obserwacje własne. Przegl Epidemiol 2005;59,3:703-709.
4. Kępa L, Oczko-Grzesik B, Błędowski D. Ocena aktywności dehydrogenazy mleczanowej (LDH) w płynie mózgowo-rdzeniowym i surowicy chorych z ropnymi, bakteryjnymi zapaleniami opon i mózgu. Przegl Epidemiol 2006;60,1:291-298.
5. Kępa L, Oczko-Grzesik B, Błędowski D. Ocena aktywności kinazy kreatynowej (CK) w płynie mózgowo-rdzeniowym i w surowicy chorych z ropnymi, bakteryjnymi zapaleniami opon i mózgu. Przegl Epidemiol 2007;61(4):693-700.
6. Kępa L. Ocena stężenia enolazy neuronowo-swoistej (NSE) w płynie mózgowo-rdzeniowym i w surowicy chorych z ropnymi, bakteryjnymi zapaleniami opon i mózgu. Przegl Epidemiol 2009;63(1):23-25.
7. Kępa L. Ocena stężenia rzęskowego czynnika neurotropowego (CNTF) w płynie mózgowo-rdzeniowym chorych z ropnymi, bakteryjnymi zapaleniami opon i mózgu.-obserwacje własne. Przegl Epidemiol 2012;66(3):425-430.
8. Marenholz I, Heizmann CW, Fritz G. S100 protein in mouse and man: from evolution to function and pathology (including on update of the nomenclature. Biochem Biophys Res Commun 2004;332:1111-1122.
9. Donato R. S100: a multigenic family of calcium-modulated proteins of the EF-hand type with intracellular and extracellular functional roles. Int J Biochem Cell Biol 2001;33:637-668.
10. Sen J, Belli A. S100B in neuropathologic states: the CRP of the brain? J Neurol Res 2007;15,85(7):1373-1380.
11. Van Eldik LJ, Wainwright MS. The Janus face of glial-derived S-100B, beneficial and detrimental function in the brain. Restor Neurol Neurosci 2003;21:97-108.
12. Ellis EF, Willoughby KA, Sparks SA, i in. S100B protein is released from rat neonatal neurons, astrocytes and microglia by in situ trauma anti-Sl00 increases trauma-induced delayed neuronal injury and negates the protective effect of exogenous S100B on neurons. J Neurochem 2007;101(6):1463-1470.
13. Schmidt H, Gerber J, Stuertz K, i in. S100B in cerebrospinal fluid – A marker for glial damage in the rabbit model of pneumococcal meningits. Neurosci Lett 2010;475:104-107.
14. Beer C, Blacker D, Bynevelt M, i in. Systemic markers of inflammation are independently associated with S100B concentration: result of an observational study in subjects with acute ischaemic stroke. J Neuroinflammation 2010;7:71-81.
15. Laskowitz DT, Kasner SE, Saver J, i in. Clinical usefulness of a biomarker-based diagnostic test for acute stroke: the Biomarker Rapid Assessment in Ischaemic Injury (BRAIN) study. Stroke 2009;40(1):77-85.
16. Dassan P, Keir G, Brown MM. Criteria for a clinically informative serum biomarker in acute ischaemic stroke: a review of S100B. Cerebrovasc Dis 2009;27(3):295-302.
17. James ML, Blessing R, Phillips-Bute BG, i in. S100B and brain natriuretic peptide predict functional neurological outcome after intracerebral haemorrhage. Biomarkers 2009;14(6):388-394.
18. Gonçalves CA, Leite MC, Nordin P. Biological and methodological features of the measurement of S100B, a putative marker of brain injury. Clin Biochem 2008;41(10-11):755-763.
19. Vos PE, Jacobs B, Andriessen TM, i in. GFAP and S100B are biomarkers of traumatic brain injury: an observational cohort study. Neurology 2010;75(20):1786-1793.
20. Steiner J, Bogerts B, Schroeter ML, i in. S100B protein in neurodegenerative disorders. Clin Chem Lab Med 2011;49(3):409-424.
21. Rothermundt M, Ahu JN, Jörgens S. S100B in schizophrenia: an update. Gen Physiol Biophys 2009;28:F76-81.
22. Infante JR, Martines A, Ochoa J, i in. Cerebrospinal fluid s100 protein levels in neurological pathologies. J Physiol Biochem 2003;59:255-261.
23. Lins H, Wallesch C-W, Wunderlich MT. Sequential analyses of neurobiochemical markers of cerebral damage in cerebrospinal fluid and serum in CNS infections. Acta Neurol Scand 2005;112:303-308.
24. Gazzolo D, Grutzfeld D, Michetti F, i in. Increased S100B in cerebrospinal fluid of infants with bacterial meningitis: relationship to brain damage and routine cerebrospinal fluid findings. Clin Chem 2004;50:941-944.
25. Hamed SA, Hamed EA, Zakary MM. Oxidative stress and s100b protein in children with bacterial meningitis. BMC Neurol 2009;9:51-55.
26. Spinella PC, Donaghue A, Rajendra A, i in. Cerebrospinal fluid levels of s-100beta in children and its elevation in pediatric meningitis. Pediatr Clin Care Med 2004;5:53-57.
27. Unden J, Christensson B, Bellner J, i in. Serum s100B levels in patients with cerebral and extracerebral infectious diseases. Scand J Infect Dis 2004;36:10-13.
28. Jung K, Goerdt C, Lange P, i in. The Use of S100B and Tau Protein Concentrations in the Cerebrospinal Fluid for the Differential Diagnosis of Bacterial Meningitis: A Retrospective Analysis. Eur J Neurol 2011;66:128-132.

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