The comparisons of blood plasma and cerebrospinal fluid S100B protein concentrations in patients with Alzheimer`s disease, amyotrophic lateral sclerosis, and multiple sclerosis

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ABSTRACT

Introduction: S100 calcium-binding protein B (S100B) is a biochemical marker of astroglial damage.
Purpose: To assess the pathophysiological implications of S100B concentrations in blood plasma and cerebrospinal fluid of patients with neurodegenerative central nervous system disorders.
Materials and Methods: In this study, we determined and compare S100B concentrations in blood plasma and cerebrospinal fluid obtained from subjects diagnosed with Alzheimer's disease (n=20), amyotrophic lateral sclerosis (n=12), multiple sclerosis (n=40) and the reference group (n=20), using enzyme-linked immunosorbent assay.
Results: Concentrations of S100B in plasma collected from patients diagnosed with Alzheimer's disease (252,38±183,50 pg/mL) and multiple sclerosis (164,92±250,14 pg/mL) were above laboratory standards, but in patients with amyotrophic lateral sclerosis (53,96±56,92 pg/mL) and the reference group (2,12 pg/mL) were below laboratory norms (N>75 pg/mL). Concentrations of S100B in plasma collected from patients with Alzheimer's disease (252,38±183,50 pg/mL) were significantly higher than in patients with amyotrophic lateral sclerosis (53,96±56,92 pg/mL) ($p<0,029$). Concentrations of S100B in CSF collected from the reference group (546,96±236,62 pg/mL) and from patients with Alzheimer's disease (587,53±189,57 pg/mL), amyotrophic lateral sclerosis (404,41±179,56 pg/mL), multiple sclerosis (462,03±146,01 pg/mL) were very similar, and none of pairwise comparisons reached statistical significance.
Conclusions: Results of our studies indicate the importance of S100B protein concentration assessment in blood in central nervous system disorders differential diagnostics.
Keywords: S100, blood, cerebrospinal fluid, Alzheimer's disease, Amyotrophic lateral sclerosis, Multiple sclerosis

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INTRODUCTION

S100 calcium-binding protein B (S100B) is a member of the S100 protein family, synthesized in astrocytes and Schwann cells, adipocytes, chondrocytes, lymphocytes, bone marrow and melanoma cells [1]. The gene encoding S100B is located at 21q22.3 chromosome. Intracellularly, S100B is a normal part of calcium hemostasis, thereby transferring signals from second messengers. S100B is also involved in cell differentiation and cell cycle progression, and it has been shown to inhibit apoptosis if applied in experimental conditions. Extracellularly, in both normal physiology and during traumatic conditions, administered S100B promotes neurogenesis and neuronal plasticity. Some authors claim that S100B is released into the serum through the disrupted the blood-brain barrier. S100B also regulates astrocyte and microglia migration and acts as a neurotrophic or neurotoxic molecule. Increased S100B concentration in blood plasma and CSF is a biochemical marker of traumatic brain injury, stroke, multiple sclerosis, neurodegenerative diseases, CNS infections and tumors [2-6].

The aim of the study was to determine and compare the S100B concentration in blood plasma and cerebrospinal fluid (CSF) patients with:

- neurodegenerative diseases including Alzheimer`s disease (AD) and amyotrophic lateral sclerosis (ALS);
- disease with inflammatory and neurodegenerative etiopathogenesis – multiple sclerosis (MS);
- the reference group consisting of patients suffering from the conditions which do not alter standard parameters of CSF and blood, such as idiopathic headache and idiopathic facial nerve palsy.

MATERIAL AND METHODS

Patients

Blood and CSF samples were obtained from patients admitted to the Department of Neurology, Medical University of Bialystok. The study was approved by the Medical University of Bialystok Ethics Committee for Research on Humans and Animals (R-I-002/382/2009) and written consent was obtained from all subjects. All individuals underwent lumbar puncture for diagnostic purposes and MR imaging of the brain was done to exclude organic lesions such as tumors, ischemic or hemorrhagic stroke. The clinical characteristics of patients are shown in Table I.

Table I. The clinical characteristics of patients

				CSF	
Diagnosis	**Number of patients (women)**	**Age (years)**	**Q(Alb)**	**Total protein (15 – 45 µg/mL)**	**Lymphocytes (N≤ 5 cell/µL)**
AD	**20 (13)**	69.9±10.4	5.7±1.3	38.3±17.2	1.25±1
ALS	**12 (9)**	57.5±10.3	6.1±2.3	39,7±15,3	2.2±2.3
MS *	**40 (25)**	35.5±10.5	6.3±1.5	375±125	3.8±2.1
Idiopathic headache	**13 (11)**	40.2 ±20.4	6.5±1.3	409 ± 155	3.0 ± 2.1
Idiopathic facial nerve palsy	**7 (5)**	48.4 ±15.3	7.5±0.6	357 ± 179	5.1 ± 2.1

*AD - Alzheimer's disease; ALS - amyotrophic lateral sclerosis; MS - multiple sclerosis; QAlb - coefficient of albumin; * all patients with MS in CSF presented oligoclonal bands of IgG (type 2 or 3) and mean EDSS (Expanded Disability Status Scale) score in MS group was 1.5±0.5;*

The diagnosis of AD was based on NINCDS-ADRDA criteria (National Institute of Neurologic, Communicative Disorders and Stroke/Alzheimer's Disease and Related Disorders Association) [7].

All patients had progressive, significant episodic memory and cognitive functions impairment, lasting more than six months, validated by validated by Mini Mental State Examination (MMSE) [8].

All patients with AD have been performed magnetic resonance imaging of the brain, which excluded organic lesions in the brain and showed brain atrophy. The diagnosis of relapsing-remitting MS was based on the McDonald criteria [9]. The degree of

disability of MS patients was assessed using the Expanded Disability Status Scale (EDSS), with a mean score of 1.5 ± 0.6 [10]. All patients underwent head MRI study, which demonstrated multiple disseminated demyelinating plaques, with no gadolinium enhancement and a diagnostic lumbar puncture. All patients presented oligoclonal bands of IgG type 2 or 3 in CSF. None of patients was during the relapse of disease and none was treated with corticosteroids or immunomodulating drugs (beta-interferon, glatiramer acetate, natalizumab). The clinically definite ALS was diagnosed on the basis of Airlie House/El Escorial Revisited World Federation of Neurology criteria [11]. All patients with ALS showed features of damage to upper and lower motor neuron confirmed by electromyography (EMG) studies. None of patients were treated with riluzol. The reference group consisted of patients with idiopathic headache and idiopathic facial nerve palsy (Bell's palsy), who underwent lumbar puncture and CSF analysis to exclude CNS infection or subarachnoid hemorrhage. No one of patient with idiopathic facial nerve palsy was immunocompromised and had herpes simplex virus 1 antibodies in blood in ELISA tests (ELISA kit; Genzyme Virotech GmbH, Rüsselsheim, Germany).

Samples preparation

Samples of anticoagulated blood were centrifuged and collected plasma were frozen (-80ºC). After collection, the CSF underwent a standard examination. Then samples of CSF were centrifuged (2000 x g, 20 min) and supernatants were subjected to total protein analysis and frozen (-80ºC). CSF analysis included physical properties, cytosis, total protein concentration and Q Alb ratio (Q Alb = albumin in CSF (mg) / serum albumin (g) x 1000) indicating efficiency of the blood-CSF barrier. The S100B concentration was measured with ELISA kit provided by LDN Labor Diagnostika Nord Gmbh & Co KG, Germany.

Statistical analysis

The differences between the groups were evaluated with an unpaired Student's t-test. The results were analyzed statistically using the Kruskal-Wallis and Dwass-Steel-Critchlow-Fligner test. The statistically significant p value was < 0.05.

RESULTS

Concentrations of S100B in plasma collected from patients diagnosed with Alzheimer's disease (252,38±183,50 pg/mL) were significantly higher than in patients with amyotrophic lateral sclerosis (53,96±56,92 pg/mL) ($p < 0,029$). Concentrations of S100B in plasma collected from patients diagnosed with Alzheimer's disease (252,38±183,50 pg/mL) and multiple sclerosis (164,92±250,14 pg/mL) were above laboratory standards, and in patients with amyotrophic lateral sclerosis (53,96±56,92 pg/mL) and the reference group (2,12 pg/mL) were below laboratory norms included in instruction for use of S100B ELISA kit provided by LDN Labor Diagnostika Nord Gmbh & Co KG, Germany (N>75 pg/mL) (Figure 1).

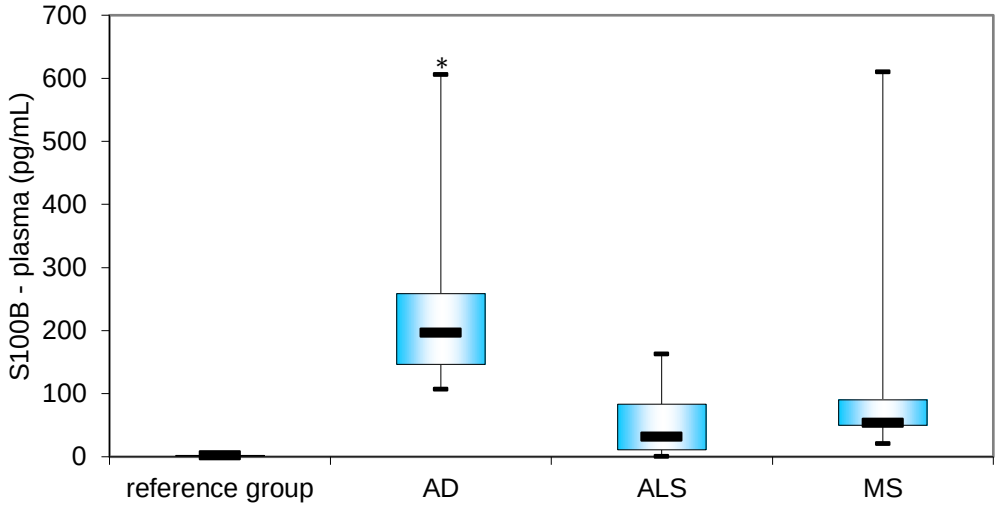

Figure 1. Comparison of S100B concentration in blood plasma *p < 0.029 comparing to patients with ALS; AD - Alzheimer's disease; ALS - amyotrophic lateral sclerosis; MS -multiple sclerosis; Kruskal-Wallis test with post hoc Dwass-Steele-Critchlow-Fligner test

Concentrations of S100B in CSF collected from the reference group (546,96±236,62 pg/mL) and from patients with Alzheimer's disease (587,53±189,57 pg/mL), amyotrophic lateral sclerosis (404,41±179,56 pg/mL), and multiple sclerosis (462,03±146,01 pg/mL) were very similar, and none of pairwise comparisons reached statistical significance (Figure 2).

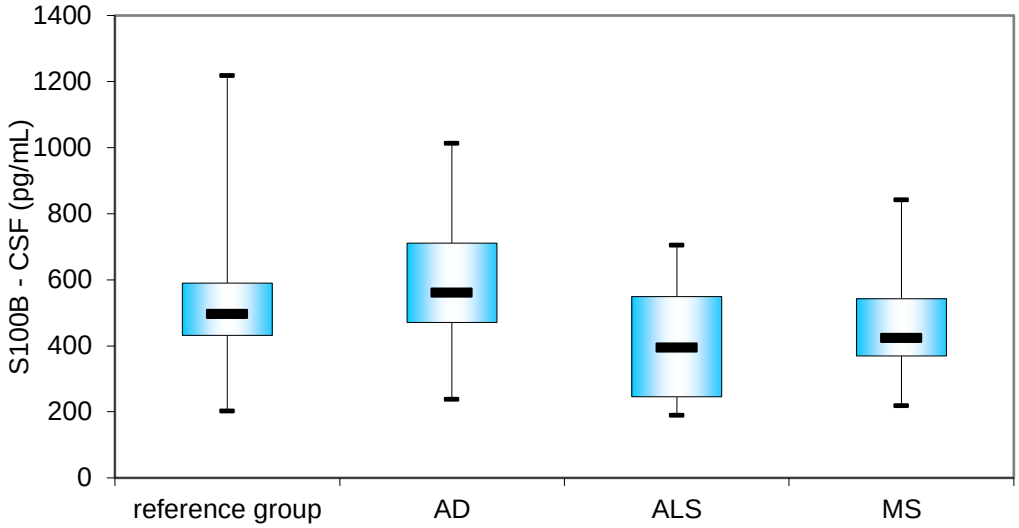

Figure 2. Comparison of S100B concentration in cerebrospinal fluid (CSF);
AD - Alzheimer's disease; ALS - amyotrophic lateral sclerosis; MS - multiple sclerosis; Kruskal-Wallis test with post hoc Dwass-Steele-Critchlow-Fligner test

DISCUSSION

In our studies concentrations of S100B in plasma and CSF collected from patients diagnosed with AD were the highest among all evaluated diseases and in plasma were significantly higher than in patients with ALS. It is consistent with previous studies which showed highly increased concentrations of S100B in plasma and CSF of patients with AD [12,13]. AD is a neurodegenerative disease in which etiopathogenesis takes place activation and destruction of astrocytes causing S100B release. S100B triggers the formation of amyloid plaques and increases intraneuronal free calcium and nitric oxide concentration, which results in activation of inflammatory process and leads to neuronal injury. It has also been shown that beta-amyloid stimulates the synthesis of S100B production in astrocytes. The most probably the high concentration of S100B in plasma of patients with AD results from its high concentration in CSF and secondary diffusion from CSF to blood due to increased permeability of the blood-CSF-barrier in the course of this disease [13,14].

The concentrations of S100B in plasma and CSF of patients with ALS are respectively decreased, which is also in agreement with previous studies. It results from the fact that ALS is a slowly progressing neurodegenerative disease characterized, first of all, by motor neuron loss and astrogliosis with ongoing consumption and downregulation of S100B in the course of disease. S100b is also accumulated locally near damaged astrocytes and is not released into the CSF compartment [15]. In the course of ALS, the presence of S100B in blood plasma also most probably results from the alternation in blood-CSF-barrier permeability which is ongoing in the course of disease [16].

The concentrations of S100B in plasma and CSF collected from patients diagnosed with multiple sclerosis were moderately increased, which is in agreement with previous studies [17].

Neurodegeneration and inflammatory process plays an important role in the pathogenesis of MS and causes excessive CNS cell destruction with secondary release of S100B [17,18-23].

The AD, ALS and MS patients results of plasma S100B concentration were compared with laboratory norms included in instruction for use of S100B ELISA kit provided by LDN Labor Diagnostika Nord Gmbh & Co KG, Germany. There are no laboratory norms for S100B concentration in CSF that wise the obtained results of CSF S100B

concentration had to be compared with reference group. Since, there are no indications to perform lumbar puncture in healthy subjects, our reference group consisted of patients with idiopathic headache and idiopathic facial nerve palsy (Bell's palsy). The concentration of S100B in this reference group was very similar to the results of other examined groups. It may be an evidence that so called "idiopathic headaches" or "idiopathic facial nerve palsy" are not exactly idiopathic but some "organic" pathological processes are engaged in their pathogenesis. This is in agreement with previous studies which showed that S100B contributes to idiopathic headache pathology by participation in a glial activation leading to neuroinflammation [24,25].

Since S100B protein is synthesized among others by astrocytes and Schwann cells, the protein present in blood comes also from the CSF origin. Astrocytes envelop cerebral capillaries and secreted S100B can cross a damaged BBB to the blood where it is detected in plasma. Additionally, S100B is abundantly expressed by myeloid cells and adipocytes. Despite normal values of Q(Alb) ratio, the marker of the blood-CSF-barrier permeability, counted for all study groups, pathologically increased S100B protein penetration from CSF to blood due to ongoing neurodegeneration seems to be the most probable explanation of its high serum concentration in AD, ALS and MS patients. Results of our study suggest the blood-CSF-barrier is altered the most in AD patients. The S100B protein penetration in MS and especially in ALS are less affected and there were almost no protein diffusion in reference group. The AD patients were the oldest ones and a dependency of the blood-CSF-barrier permeability on age has been previously described [6,26].

CONCLUSIONS

In conclusion, results of our preliminary study suggest that concentrations of S100B in plasma and CSF of patients suffering from neurodegenerative disorders may result from their pathogenesis.

Results of our studies indicate the importance of S100B protein concentration assessment in blood serum in CNS diseases differential diagnostics.

Conflict of interests

None declared.

Financial disclosure

None declared.

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