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# S-100β PROTEIN IN PATIENTS WITH SEVERE SEPSIS

## PROTEIN S-100β KOD PACIJENATA SA TEŠKOM SEPSOM

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**Summary:** The effects of sepsis on the brain are not fully elucidated. This study investigated the serum levels of S100β protein in severe sepsis, as a biomarker of brain damage. The aim was to determine whether the levels of S100β are increased early, at the onset of sepsis, and if this protein is a good early predictor of outcome. We studied 30 patients with severe sepsis, divided into the survivors (n=8) and norsurvivors (n=22). Blood was sampled within the first 24h after the onset of symptoms. The concentrations of S100β were measured using an electrochemiluminiscence immunoassay (Elecsys 2010, Roche Diagnostics). Also, we measured the levels of C-reactive protein (CRP) using the immunonephelometric assay. Out of 30 patients, 74.4% had increased levels of S100β, while 25.6% had values within the reference range. A total of 30 patients had increased levels of CRP. The mean values of S100β and CRP did not differ significantly between the survivors and nonsurvivors (0.390±0.515 vs. 0.415± 0.508 μg/L; 98.76±69.94 vs. 161.68±118.38 mg/L). Correlation between S100β and outcome was not found. The increased levels of S100β indicate possible occult diffuse brain injury, that can be reversible. Moreover, the study showed S100β protein not to be a good early predictor of outcome in severe sepsis.

**Keywords:** brain damage, C-reactive protein, sepsis, S100β

**Kratak sadržaj:** Uticaj sepse na mozak nije u potpunosti razjašnjen. U ovom radu, ispitivali smo vrednosti proteina S100β, kao biomarkera oštećenja mozga, kod teške sepse. Cilj rada bio je da se utvrdi da li su vrednosti proteina S100β povišene na samom početku bolesti i da li se na osnovu njih može predvideti ishod. Ispitano je 30 pacijenata sa teškom sepsom, koji su bili podeljeni na preživele (n=8) i umrle (n=22). Za analizu, uzimana je krv u prvih 24h od pojave simptoma. Koncentracija proteina S100β merena je pomoću imunološkog testa metodom elektrohemiluminiscencije (Elecsys 2010, Roche Diagnostics). Takođe, meren je i nivo C-reaktivnog proteina (CRP) imunonefelometrijskim testom. Od 30 ispitanih pacijenata, 74,4% imalo je povišene vrednosti proteina S100β, dok je 25,6% imalo vrednosti u okviru referentnog opsega. Povišene vrednosti CRP imalo je svih 30 pacijenata. Između preživelih i umrlih nije bilo statistički značajne razlike u srednjoj vrednosti proteina S100β i CRP (0,390±0,515 vs. 0,415±0,508 μg/L; 98,76±69,94 vs. 161,68±118,38 mg/L). Korelacija između proteina S100β i ishoda nije nađena. Povišene vrednosti S100β ukazuju na verovatna difuzna okultna oštećenja mozga, koja mogu biti i reverzibilna. Osim toga, protein S100β nije dovoljno pouzdan marker za rano predviđanje ishoda kod pacijenata obolelih od teške sepse.

**Ključne reči:** oštećenje mozga, C-reaktivni protein, sepsa, S100β

### Introduction

The effects of sepsis on the brain are not fully elucidated. The interplay between direct effects resulting from toxic mediators and indirect effects, such as hypotension, hyperthermia and increased intracranial pressure, contribute to the unclear image of the brain during sepsis. Despite recent advances in the rapid diagnosis and treatment of sepsis, the neurologic sequelae of severe sepsis remain poorly understood. Neurologic dysfunction in the form of encephalopathy occurs frequently in patients with severe sepsis and is associated with increased morbidity and mortality (1–4). Ischemic and hemorrhagic brain lesions are described in autopsied patients who died of sepsis and septic shock (5–7). The diagnosis of septic

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encephalopathy can be difficult, because many patients are sedated for various reasons. Very often sedation precedes the onset of detectable neurologic symptoms, thus masking the diagnosis. Also, the central nervous system imaging studies such as computed tomography and magnetic resonance cannot be easily performed and may pose further risk during transport of a critically ill patient. Biomarkers of brain injury can be useful to evaluate brain dysfunction in sepsis. S100 is a small dimeric protein with molecular weight of approx. 10.5 kD. It belongs to a large family of calcium-binding proteins, and is composed of hetero- or homo-dimers of the α- and β-subunit. S100A1 (αα) and S100B (ββ) are predominantly expressed by cells of the central nervous system, mainly astroglial cells, but are also expressed in melanoma cells and to some extent in other tissues (8). As a marker of brain injury it has been used in various conditions such as trauma, ischemia, stroke, cardiac arrest, cardiac and carotid artery surgery, malignant metastases (8–15).

The aim of the study was to determine whether the levels of S100β are increased early, at the onset of sepsis, and if this protein is a good early predictor of outcome.

## Materials and Methods

The study was approved by the Ethics Committee of the Military Medical Academy (MMA), Belgrade. We studied 30 patients with severe sepsis, 18 males and 12 females, treated in the Intensive Therapy Unit (ITU). The patients were divided into the survivors (n=8) and nonsurvivors (n=22). The control group comprised 10 healthy volunteers. Blood was sampled within the first 24h after the onset of symptoms. Serum was separated by centrifugation and stored at –20 °C until analyzed (at least three months). The concentrations of S100β protein were measured using the electrochemiluminiscence immunoassay on an Elecsys 2010 analyzer (Roche Diagnostics). The minimum level of detection was 0.005 μg/L, and the reference range was <0.105 μg/L. Hemolysis does not interfere with S100β determinations (16). Also, we measured at the same time levels of high sensitivity C-reactive protein (CRP) as an acute phase reactant using the immunonephelometric assay on a Behring Nephelometer II.

The values were expressed as mean ± standard deviation. Statistical tests were performed by the statistical package Statistic for Windows (Stat for Windows, R. 4.5, USA). The difference between groups was determined by the Student's t test. The correlation was analyzed by the Pearson linear regression test. Values of $p<0.05$ were taken as statistically significant (17).

## Results

Peritonitis was the most common cause of sepsis (56.6%, n=17), followed by pancreatitis (20%, n=6), trauma (16.6%, n=5) and other infections (6.6%, n=2). ITU mortality was very high, 74.4%. In the sepsis group the mean values of S100β and CRP were higher ($p<0.01$) than in the control group (0.408±0.501 vs. 0.045±0.020 μg/L; 144.9±110.1 vs. 1.91±0.90 mg/L). However, out of 30 patients, 74.4% (n=22) had increased levels of S100β protein, while 25.6% (n=8) had values within the reference range. The data are shown in *Table I*. Elevated levels of S100β were found in 6 survivors and 16 nonsurvivors, and 2 survivors and 6 nonsurvivors had normal values. The mean value of S100β protein did not differ significantly between the survivors and nonsurvivors (0.390±0.515 vs. 0.415±0.508 μg/L). Four patients had levels of S100β higher than 1.0 μg/L (tenfold higher than the upper limit of the reference range), one of them survived. This patient had a late onset of sepsis relative to primary insult (cholecistectomy). After one month she developed diffuse peritonitis and severe sepsis. The positive predictive value of S100β was 72.7% and the negative predictive value was 25%. Correlation between S100β and outcome was not found.

A total of 30 patients had increased levels of CRP. Although the mean value was lower in the survivor group, between the survivors and nonsurvivors significant difference was not found (98.76±69.94 vs. 161.68±118.38 mg/L). It is interesting that we found a very strong correlation between S100β and CRP, r=0.537, p=0.002 (*Figure 1*).

## Discussion

The main result of this study is that early in the course of sepsis increased levels of S100β do occur in 74.4% patients. In addition, baseline concentrations of S100β were not predictive of the outcome in severe sepsis. Our results are in agreement with the results of other authors (12, 15, 18). Dr Joana and Moren Panni (19) suggest that S100β changes rather than absolute values may be a better marker of severity of sepsis-associated encephalopathy. Although S100β originates from the central nervous system (CNS) and increases in cerebrospinal fluid (CSF) after injury (8, 20), it remains unclear whether elevation of serum levels of S100β is a sign of blood–brain barrier (BBB) dysfunction, neuronal damage or both. Some authors suggest that serum S100 β represents a marker of BBB integrity in patients with brain lesions rather than brain neuronal damage (21). Also, we found a strong association between S100β and CRP. C-reactive protein is an acute phase reactant synthesized by the liver upon stimulation by proinflammatory cytokines reflecting both the acute and chronic inflammatory states (22, 23). Acute phase reactant changes reflect the presence and intensity of inflammation, and have been

**Table I** Serum values of S100 and CRP in the control and sepsis group.

| | Control N=10 | Sepsis-survivors N=8 | Sepsis-nonsurvivors N=22 |
|---|---|---|---|
| S100β, μg/L | 0.045±0.020 | 0.390±0.515 | 0.415±0.508** |
| CRP, mg/L | 1.91±0.90 | 98.76±69.94* | 161.68±118.38*** |

*p<0.05, **p<0.01, ***p<0.001 vs. control group

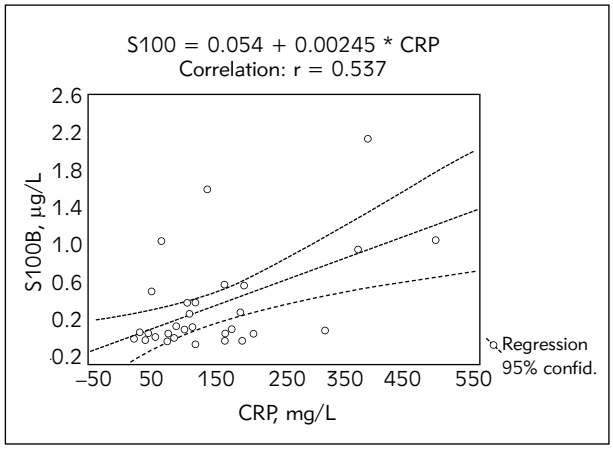


**Figure 1** Correlation between serum S100β and CRP in patients with severe sepsis.

used as a clinical guide to diagnosis and therapeutic management (24). CRP has many pathophysiologic roles in the inflammatory process. A major function of CRP is its ability to bind phosphocholine and thus recognize some foreign pathogens as well as phospholipid constituents of damaged cells. On the other hand, S100β is a functional protein, which is implicated in a variety of intra- and extracellular regulatory activities. In a recently reported review, authors describe S100β as »the CRP of the brain« (25).

Brain dysfunction is a severe complication of sepsis with an incidence ranging from 9% to 71% and is associated with increased morbidity and mortality (1, 26). Sepsis associated encephalopathy (SAE) is defined as a diffuse cerebral dysfunction induced by the systemic response to infection without any clinical or laboratory evidence of direct infections involvement of the central nervous system (18). The mechanism of sepsis-associated encephalopathy involves inflammatory and noninflammatory processes that affect endothelial cells, glial cells, and neurons and induce BBB breakdown, derangements of intracellular metabolism and cell death (26). There is a frequent occurrence of occult diffuse brain injury in sepsis (20, 27).

The increased levels of S100β protein indicate possible occult diffuse brain injury, that can be reversible. Moreover, the study showed S100β protein not to be a good early predictor of outcome in severe sepsis.

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