Serum S100B protein concentration in brain-dead organ donors: a pilot study

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Abstract

Background: Protein S100B is considered to be a marker of brain damage, but there is a paucity of data regarding the utility of its assessment in brain-dead organ donors. The aim of the study was to compare serum protein S100B concentrations between brain-dead organ donors and patients with a confirmed permanent neurological deficit but without signs of brain death.

Methods: The concentration of serum S100B protein was measured in 12 brain-dead organ donors (including 7 males with a median age of 40 years). All measurements were taken when brain death was confirmed by the commission. Twenty-nine patients (including 13 males with a median age of 63 years) who died in the medical ICU with confirmed permanent brain injury without signs of brain death acted as controls. In these patients, S-100B protein measurements were performed upon ICU admission.

Results: In brain-dead organ donors, the median values of serum S100B protein were much higher in comparison to the control group (median and IQR, respectively: 5.04 µg L⁻¹; 1.775–6.765 vs 0.897 µg L⁻¹; 0.324–1.880, \( P < 0.001 \)). S100B serum values > 1.81 µg L⁻¹ predicted brain death with the highest accuracy (AUROC = 0.83; 95% CI 0.68–0.93; \( P < 0.001 \)).

Conclusion: Concentrations of serum S100B protein in brain-dead organ donors are extremely high and may support the diagnosis of brain death. This fact may be of value when the presence of reflex movements (frequently reported despite brain death) might delay determination of brain death and result in the failure of organ donation.

Key words: S100B protein; organ donors; brain death

The S100B protein is a member of small-molecule calcium binding proteins [1]. It is established as a biomarker that is used in diagnosing central nervous system (CNS) damage resulting from different aetiologies [1, 2]. Diagnosis is based on the role of S100B, which is a protein of astrocyte-like glia that participates in astrocyte proliferation as well as interactions between the glia and surrounding nervous tissue. The release of S100B protein from a glia could induce apoptosis through the production of nitric oxide (NO). This release results from blood-brain barrier damage, which is observed in each case of brain structure damage [1, 2].

The diagnostic utility of S100B protein in prognosis assessments was confirmed in patients after injuries of CNS, subarachnoid haemorrhages, strokes and cardiac arrest [1, 3–5]. Serum S100B protein concentration after severe brain damage is correlated with the GOS (Glasgow Outcome Score) score of neurological damage [6] and with the GCS (Glasgow Coma Scale) score of neurological state [7].

Additionally, serum or cerebrospinal fluid (CSF) S100B protein concentration predicts both hard endpoints (death) and cognitive function impairment [5, 8]. Because therapeutic hypothermia does not influence S100B protein concentration [9, 10], its assessment after brain damage occurrence seems to be a direct indicator of the destruction of neuron cells.

There is a paucity of data in the literature on the usefulness of serum S100B concentration assessment in the procedure of brain death confirmation. Thus, the aim of the
present study was an evaluation of serum S100B concentration in brain-dead patients (GOS=1) in comparison with patients after confirmed permanent neurological damage (GOS=2) but without features of brain death.

**METHODS**

Assuming that the results of serum S100B protein concentration assessments did not change the management and the general state of the majority of patients who were precluded from giving an informed consent to obtain blood samples, the Ethics Committee decided to abolish that requirement in the study protocol.

The project was designed as a case-control study. Between 2009 and 2011, serum S100B protein concentrations were assessed with enzyme-linked immunosorbent assay (ELISA), using a Cobas e411 analyzer (Hitachi, Japan) and reagents made by Boehringer-Manheim (Germany). Five millilitres of venous blood was obtained, centrifuged and frozen at a temperature of −70°C. The samples were collectively unfrozen and analysed once per quarter.

The evaluated group consisted of brain dead patients as confirmed by the committee (GOS=1), according to existing rules and regulations [11]. The blood sample was obtained at the donor site, upon the transplant coordinator’s request after brain death ascertainment and before the organ removal procedure. The control group consisted of patients who were treated in the intensive care unit with an internal and surgical profile with confirmed permanent neurological damage (GOS=2) but who did not meet the brain-dead criteria. In this group, the blood samples were taken during admission.

The analyses were performed based on procedures available in MedCalc v14. Quantitative variables were presented as medians and interquartile ranges. Qualitative variables were presented as absolute numbers. The U Mann-Whitney test was used to analyse the difference between qualitative variables, and the exact Fisher test was used for evaluation of qualitative variables. A plot with an ROC curve was performed to present a brain death prediction using the serum S100B protein concentration. A P value of less than 0.05 was treated as statistically significant.

**RESULTS**

Table 1 presents baseline characteristics of the groups.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Study group</th>
<th>Control group</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (male/female)</td>
<td>7 / 5</td>
<td>13 / 16</td>
<td>0.5</td>
</tr>
<tr>
<td>Age (median, interquartile range) [years]</td>
<td>40 (28.5–49.5)</td>
<td>63 (56–73)</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

Age differences between the groups were statistically significant; the brain-dead patients were significantly younger than patients with permanent neurological damage.

The serum S100B protein concentrations were statistically higher in brain-dead patients than in patients with permanent neurological damage (median with corresponding interquartile range: 5.04 µg L⁻¹; 1.775–6.765 and 0.897 µg L⁻¹; 0.324–1.880, respectively) (Fig. 1). There were no statistically significant differences between serum S100B protein concentrations in female and male patients (P = 0.9). Similarly, there was no correlation between serum S100B concentration and age (R= −0.17; P = 0.3).

The brain deaths could be predicted with the highest accuracy (AUROC=0.83; 95% CI: 0.68–0.93; P < 0.001) with the values of serum S100B protein concentration > 1.81 µg L⁻¹ (sensitivity: 75%, specificity: 76%, Youden index = 0.49) (Fig. 2). The odds ratio (OR) of a brain death diagnosis with such a S100B protein concentration was 9.43 (95% CI: 1.93–44.83). With a cut-off point > 0.749 µg L⁻¹, sensitivity was 100% (with a specificity of 48%) (Youden index = 0.52); therefore, for an S100B protein concentration > 6.56 µg L⁻¹, specificity was 100% (with a sensitivity of 25%) (Youden index = 0.75).

**DISCUSSION**

The aim of our study was the evaluation of serum S100B protein concentration in patients with confirmed brain death. The median S100B protein concentration in the study group was 5.04 µg L⁻¹, and it was more than 5.5-fold higher than in the control subjects. This is a very valuable observation, as the previously published values in brain-dead patients ranged from less than 0.5 to greater than 10 µg L⁻¹ [5]. The results are influenced by different variables, such as age, sex, brain damage cause and blood sample obtainment timing [6, 7, 12–14]. Because the baseline characteristics...
In our study, we conclude that a serum S100B protein concentration > 1.81 µg L⁻¹ could expedite the decision about initiating the procedure of brain death confirmation because the odds of brain death with lower concentrations are nearly 10 times higher. Hence, demonstrating high serum S100B protein concentrations justifies the initiation of the brain death diagnosis process.

Diagnostic accuracy, as expressed by the area under the curve (AUC) of the ROC, was 0.83 with an optimal Youden index of 0.49. Total specificity of the measurement was achieved exclusively with S100B protein concentrations > 6.56 µg L⁻¹. Similar results were obtained by Egea-Gurererro et al. in patients after severe brain damage with brain death confirmation; with a cut-off point of 2.0 µg L⁻¹, the value of the AUROC was 0.92 with a specificity of 100%, a sensitivity of 60% and an odds ratio of OR = 8.38 [12]. In previous studies with lower cut-off thresholds (0.365 µg L⁻¹ [13], 0.372 µg L⁻¹ [14]), the diagnostic accuracy was also lower (0.75 [13] and 0.78 [14], respectively). In a meta-analysis involving 39 cohort studies, the cut-off point for death prediction (with a specificity of 100%) ranged from 1.38 to 10.50 µg L⁻¹, and for predicting impaired neurological state (GCS ≤ 3 points), the cut-off point ranged from 2.16 to 14 µg L⁻¹ [5]. With such cut-off thresholds, the weighted odds ratios for death prediction, impaired neurological state (GCS ≤ 3 points) and brain death were as follows: 2.55 (95% CI: 2.02–3.21), 2.62 (95% CI: 2.01–3.42) and 2.9 (95% CI: 2.3–3.5), respectively [5]. The ability to predict brain death could be increased by adding other variables to the statistical model, such as patient age and underlying reason for cardiac arrest [15–17].

Shortening the time to brain death confirmation is very important for family members as well as for transplantation procedures. The earlier a patient’s family becomes aware of suspected brain death in the patient, the shorter the period of uncertainty, which could accelerate the psychological process of bereavement. Organs that are taken early are less vulnerable to damage resulting from the complications of a prolonged stay in the intensive care unit [18], and early diagnosis of brain death increases the chance to take more organs with sufficient function. The measurements of serum S100B protein concentrations could be extremely important in patients with spinal and pupillary reflexes. Spinal reflexes could be present in up to 75% of patients after brain death confirmation [19] involving pronation reflex, the flexion of upper extremities, abdominal reflexes or flexion movements of lower extremities. In contrast, simultaneous assessment of pupillary reflex and serum S100B protein concentration comprise an algorithm for an early appointment with the commission within the procedure of brain death prediction that is suggested in the literature [14].

The present study has some limitations that influence the generalization of the obtained results. First, there is a significant heterogeneity in the enrolled patients. Because it was already proved that serum S100B protein concentration could significantly differ according to the underlying reason for neurological impairment (or the reason for baseline death) [12, 15], the relevant subgroup analysis should be performed. Unfortunately, the numerosness of such subgroups is not efficient to build adequate mathematical models. This is connected to a second limitation, i.e., a small number of studied groups. Nevertheless, the obtained results seemed to be interesting enough that it was decided to present them in the form of a preliminary report. Third, in the present study, a single measurement of serum S100B protein concentration was used; previous publications showed that the concentration of that protein differs over time after an injury [7, 14]. Finally, serum S100B protein concentration correlates with scores in the GOS scale [6]; thus, the difference between the studied group and the control subjects could result at least partially from that fact. Notably, the importance of this fact for the interpretation of the results is not likely a result of the deep neurological impairment that was observed in our study.

CONCLUSIONS

Serum S100B protein concentration in brain-dead organ donors is significantly higher than in patients with
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3. The preliminary results of the presented study were presented during the Annual Congress of European Society of Intensive Care Medicine (ESICM) in Berlin, Germany, 1–5 of October 2011 and as a poster during the International Congress of Polish Society of Anaesthesiology and Intensive Therapy, in Wisła, 14–18 of September 2014.

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