

Can Admission S-100 β Predict the Extent of Brain Damage in Head Trauma Patients?

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ABSTRACT: Background: As has been shown previously, S-100 β levels in serum can be a useful predictor of brain damage after head trauma. This pilot study was designed to investigate whether urine samples, which are much easier to obtain, could be used for the same purpose instead of serum samples. **Methods:** Ninety-six consecutive patients admitted with head trauma were recruited in the study. After exclusion of 54 patients, mostly because of significant additional trauma, S-100 β levels were analyzed in serum and urine of 42 patients using a luminometric assay. A range for normal values was established based on samples from ten healthy volunteers. **Results:** S-100 β serum levels increased proportional to the severity of the head trauma, as had been previously shown by several other groups. In many patients, initial increases in urine S-100 β levels were seen later than in serum, after which the kinetics of S-100 β levels in urine seemed to follow that established for serum levels. S-100 β values in urine were on average about 54% lower in urine than in serum. **Conclusions:** S-100 β levels in urine obtained on admission to the hospital are not a good indicator for the extent of brain damage. However, urine S-100 β levels obtained at later time points might be a useful indicator for the development of secondary brain injury.

RÉSUMÉ: Le niveau de S-100 β au moment de l'admission peut-il prédire l'étendue du dommage cérébral chez les traumatisés crâniens?

Contexte : Tel que démontré antérieurement, les niveaux sériques de S-100 β peuvent être utiles pour prédire le dommage cérébral après un traumatisme crânien. Cette étude pilote a été conçue afin d'étudier si des échantillons d'urine, qui sont beaucoup plus faciles à obtenir, pourraient être utilisés à cet effet plutôt que des échantillons de sérum. **Méthodes :** Quatre-vingt-seize patients consécutifs admis pour traumatisme crânien ont été inclus dans l'étude. Cinquante-quatre patients ont été exclus de l'étude, la plupart du temps à cause de la présence d'autres traumatismes importants. Les niveaux de S-100 β ont été mesurés dans le sérum et l'urine de 42 patients au moyen d'un test luminométrique. Nous avons établi l'écart normal à l'aide d'échantillons prélevés chez 10 volontaires sains. **Résultats :** Les niveaux sériques de S-100 β augmentaient proportionnellement à la sévérité du traumatisme crânien, tel que démontré antérieurement par plusieurs autres équipes de chercheurs. Chez plusieurs patients, l'augmentation initiale des niveaux de S-100 β dans l'urine était observée plus tardivement que dans le sérum et la cinétique des niveaux de S-100 β dans l'urine semblait suivre celle déjà connue des niveaux de S-100 β dans le sérum. Les valeurs urinaires de S-100 β étaient en moyenne environ 54% plus basses que les valeurs sériques. **Conclusions :** Les niveaux urinaires de S-100 β obtenus au moment de l'admission à l'hôpital ne sont pas un bon indicateur de l'importance du dommage cérébral. Cependant, les niveaux urinaires de S-100 β obtenus par la suite pourraient être un indicateur utile de l'apparition de lésions cérébrales secondaires.

Can. J. Neurol. Sci. 2009; 36: 612-616

S-100 β , a small monomer of the calcium-binding S-100 protein family, is present in high concentrations in astrocytes and Schwann cells. S-100 β levels in serum were initially identified as a valuable marker in the assessment of cerebral injury during cardiovascular surgery and stroke.¹⁻⁴ Over the last decade, several groups have published their results correlating S-100 β levels in serum to the severity of brain damage and patient outcome. Positive correlation of S-100 β levels with computerized tomography (CT) and magnetic resonance imaging (MRI) findings were documented.⁵⁻¹⁰ In patients who had suffered mild head trauma, S-100 β levels in serum were found to be a specific but not very sensitive predictor for post-concussion symptoms¹¹, while no correlation was found between S-100 β serum levels and cognitive performance in the early stage of recovery.¹² S-100 β serum levels were found to be a

reliable predictor of survival and quality of outcome for patients with moderate and severe head trauma.^{7,13-21} Raabe and colleagues found that in head injured patients with similar CT scan findings it was possible to predict better or worse outcome according to S-100 β levels in serum.⁵

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RECEIVED MARCH 9, 2009. FINAL REVISIONS SUBMITTED MAY 13, 2009.
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This project was designed to measure S-100 β levels in serum and urine with the aim to test whether the easier to obtain urine samples could be substituted for serum samples. S-100 β protein is a relatively small protein with a molecular weight of about 10 kDa.²² Therefore, the expectation to detect the molecule in urine is well justified.

MATERIALS AND METHODS

Ninety-six consecutive patients admitted to the Royal University Hospital at the University of Saskatchewan with the diagnosis 'head trauma' were originally enrolled in this study. Appropriate institution ethics approval was obtained. Informed consent was obtained from either the patient or the next of kin. It has been shown that injury other than head trauma can be the cause of increased S-100 β levels.²³ Anderson and colleagues have demonstrated that S-100 β levels can be increased up to 40 times as a consequence of soft tissue trauma and increased by two orders of magnitude after bone fractures.²⁴ In order to exclude extracranial injury as confounding factor in our study, we excluded 49 of the originally recruited patients because of significant additional trauma other than head injury, most commonly fractures of the extremities (n = 14), serial rib fractures (n = 11) and pneumothorax (n = 6). Five patients were excluded because of insufficient documentation. Thus, only the results of 42 patients (aged 3-90 years) were found suitable for further analysis (Table). Based on their admission Glasgow Coma Scores (GCS) scores, patients were grouped into mild (13-15), moderate (9-12) and severe head injury (scores equal to and less than 8).

Table: Patient stratification based on severity of head injury

	Mild head injury (GCS 13-14)		Moderate head injury (GCS 9-12)		Severe head injury GCS (3-8)	
	n	average age	n	average age	n	average age
male	19	28.5 y (15-44 y)	5	34.5 y (16-44 y)	3	37.5 (3-47 y)
female	11	24.5 y (6-33 y)	2	34 y (17-51 y)	2	58 y (26-90 y)
total	30	26.5 y (6-44 y)	7	34.25 y (16-51 y)	5	47.75 y (3-90 y)

(n = 42); GCS: Glasgow Coma Score, y: years

Base values for healthy patients were established with the help of ten volunteer donors, five males (ages 35-51, average 40 years) and five females (ages 25-44, average age 37 years), donated both blood and urine to establish a range of control values. Arterial blood samples from the patients were taken with the routine blood samples on admission or once a next of kin became available to consent to the patient's inclusion into the study. In some of the patients, further samples were acquired up to 96 hours after admission. Urine was collected at the same time as blood samples.

Arterial blood was collected in heparinized test tubes, centrifuged and the serum was collected. The serum and urine samples were then stored at -70°C until analysis was performed. An in-vitro immunoassay specific for quantitative determination of the S-100 β protein subunit (LIA-mat@Sangtec@100 kits, AB Sangtec Medical, Bromma, Sweden) was used for analysis of both blood and urine. Briefly, 100 μ l of diluent and 100 μ l of sample were added to each test tube containing a solid-phase antibody and incubated at room temperature. One hour later, the samples were washed three times, using the washing solution provided with the kit, and analyzed in a luminometer with two programmable injectors and automatic readout (Berthold Detection Systems, Germany). The injectors added 300 μ l alkaline peroxide solution and 300 μ l catalyst solution to each sample. The measurement period was five seconds. Samples were run in duplicate and the mean values were used for further analysis. The detection limit for this monoclonal antibody assay directed against the β -chain of the S-100 molecule is 0.02 μ g / litre. The luminometric assay is the most sensitive method available to measure S-100 β levels in fluid, and therefore was chosen for our project. Luminometric determination of S-100 β serum levels were performed in the Department of Anatomy and Cell Biology at the University of Saskatchewan.

RESULTS

S-100 β levels were detectable in the urine of seven and in the serum of all of the ten healthy volunteers (Figure 1). Mean values were 0.035 μ g / l (SD \pm 0.019) in urine and 0.06 μ g / l (SD \pm 0.027) in serum for male volunteers and 0.042 μ g / l (SD \pm 0.017) in urine and 0.096 μ g / l (SD \pm 0.034) in serum for

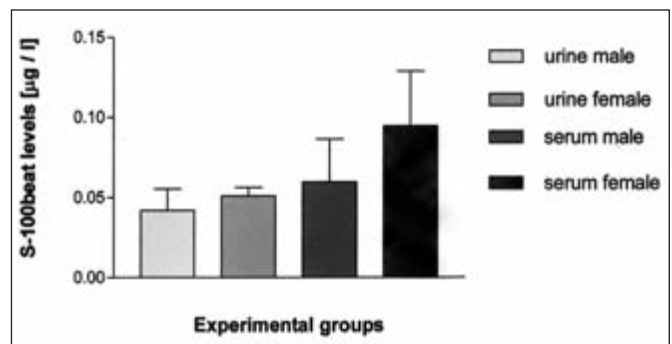


Figure 1: S-100 β levels in urine and serum of healthy volunteers.

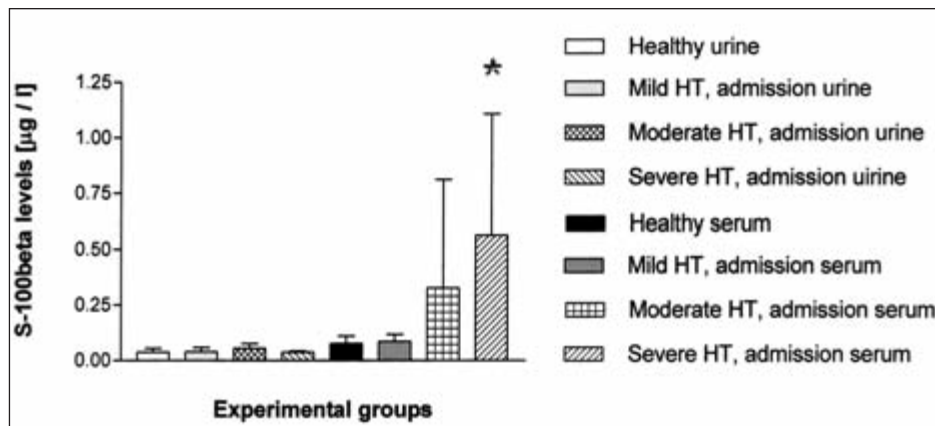


Figure 2: Admission S-100 β levels in urine and serum for all groups. A significant ($p < 0.05$) increase of S-100 β levels was seen in serum (asterisk) but not urine of patients with severe head trauma on admission compared to values obtained from healthy volunteers.

female volunteers. Thus, S-100 β levels were about 54% lower in urine than in serum (51.7% for men, 56.2% for women) and thus often close to the detection limit of the assay. There was a trend towards higher S-100 β levels in female vs. male volunteers. However, this was not statistically significant in either urine ($p = 0.56$) or serum ($p = 0.11$). Therefore, we have not separated the patient analysis in male and female subgroups. All serum S-100 β values were within the limits expected in the absence of brain damage, based on the disclosure of the test set instructions and published work.²⁴ Since volunteer samples were acquired either during short breaks in the daily work routine or at the end of the work day, the difference in activity states could have contributed to a larger standard deviation than expected if all samples had been acquired under the same rest conditions.²⁵

The mean admission values of S-100 β in urine of patients were 0.04 $\mu\text{g/l}$ (SD ± 0.019) for mild head trauma ($p = 0.51$), 0.055 $\mu\text{g/l}$ (SD ± 0.021) for moderate head trauma ($p = 0.1$) and 0.038 $\mu\text{g/l}$ (SD ± 0.006) for severe head trauma ($p = 0.92$). Thus, admission values in all three patient groups differed only insignificantly from urine levels found in healthy volunteers (0.038 $\mu\text{g/l}$, SD ± 0.018). On the contrary, with a mean of 0.64 $\mu\text{g/l}$ (SD ± 0.55) the difference in serum S-100 levels to normal values was significant for severe head trauma ($p = 0.01$) and a strong trend, albeit not statistically significant, was seen with a mean of 0.33 $\mu\text{g/l}$ (SD ± 0.49) in patients with moderate head trauma ($p = 0.12$). In the serum of patients with mild head trauma, a mean of 0.086 $\mu\text{g/l}$ (SD ± 0.032) demonstrated only a slight increase compared to values found in healthy volunteers (0.077 $\mu\text{g/l}$, SD ± 0.034), which was not statistically significant ($p = 0.86$). In four patients with mild head trauma, S-100 β urine levels were below the detection limit.

When ratios between group averages (mean values) in serum and urine levels of S-100 β were calculated, we found that ratio to be 2:1 in healthy patients and increasing with severity of the trauma. In patients with mild head trauma, the ratio was 2.2:1, while ratios of 5.9:1 were calculated for patients with moderate and 15:1 for patients with severe head trauma. Thus, excretion of

S-100 β in urine did not increase in a linear fashion with the rise in serum levels.

The values between the S-100 β urine levels on admission were not significantly different between patients with mild, moderate and severe head trauma, while S-100 β levels in serum were significantly different between mild and moderate head trauma ($p = 0.035$). Differences in serum levels were not significant between moderate and severe head trauma ($p = 0.621$). The p-values for the comparison of S-100 β urine levels between mild and moderate head trauma were 0.138, and 0.15 for the comparison of moderate and severe head trauma (Figure 2).

Three case studies are presented to illustrate the kinetics of S-100 β levels in urine compared to those in the patients' serum.

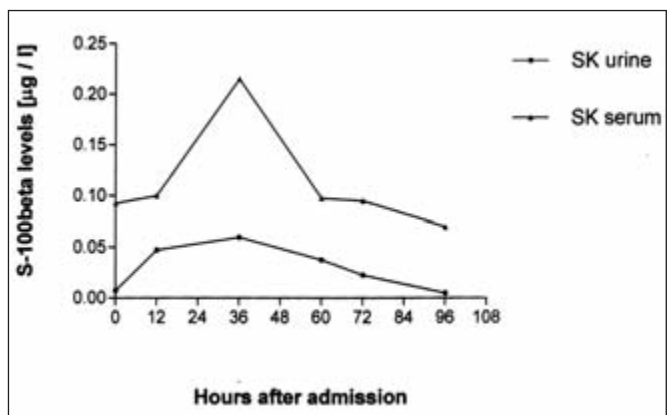


Figure 3: S-100 β levels in urine and serum followed over 96 hrs after admission in a 37-year-old male patient, admitted with a GCS of 10 and small contusions in the frontal and temporal lobes seen on CT. The rise and fall of S-100 β levels in urine follows the kinetics seen in the serum values although, contrary to serum, in urine the peak value is not significantly different from preceding and subsequent values.

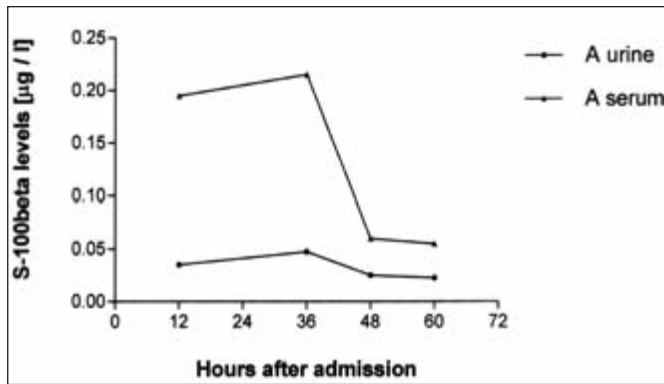


Figure 4: S-100 β levels in urine and serum followed over 60 hrs after admission. The 90-year-old female patient had been involved in a motor vehicle accident and discharged home. Two days later, she was admitted to the hospital with a GCS of 8 and the diagnosis of a left acute subdural haematoma. Her GCS deteriorated to 7 before the haematoma could be evacuated. Blood and urine were collected just before surgery. The S-100 β levels correspond to a GCS of 14 at 36 hrs and GCS of 15 at 48 hrs.

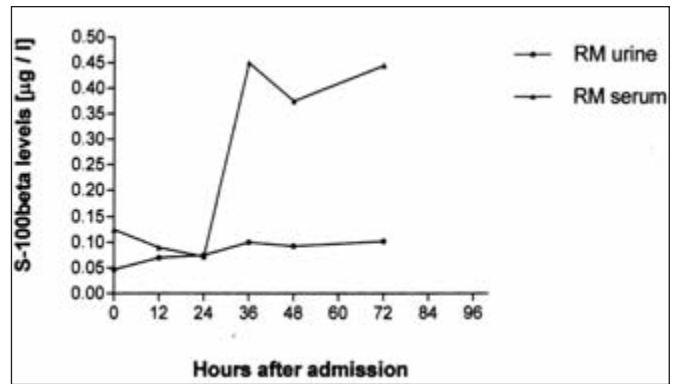


Figure 5: S-100 β levels in urine and serum followed over 72 hrs after admission in a 47-year-old male patient, admitted with a GCS of 13. A CT on admission showed a frontal linear fracture and a small epidural haematoma. A follow-up CT about 72 hrs after admission showed the development of bilateral frontal contusions and the patient developed increasingly severe headaches over the next 14 hrs.

Figure 3 illustrates the case of a patient with moderate head trauma (admission GCS 10) and small cerebral contusions who made a continuous and uneventful recovery. Figure 4 illustrates the case of a patient with acute subdural haematoma. In this patient the symptoms worsened two days after the trauma necessitating admission to the hospital (GCS 8). Improvement rapidly followed evacuation of the haematoma, with GCS scores of 15 being recorded within less than 48 hrs after surgery. Figure 5 illustrates the case of a patient admitted with a GCS of 13. The CT scan showed a frontal linear fracture, diffuse subarachnoid haemorrhage and a small frontal epidural haematoma. After initial improvement, contusions and headache developed, preceded by a rise in both serum and urine S-100 β levels. The patient was discharged ten days after admission and his initially impaired short-term memory improved to almost normal over the following week. Although the patient's admission S-100 β urine level was above the mean, it was within the range of values seen in healthy volunteers.

DISCUSSION

At the time of admission, a correlation between S-100 β levels in serum and GCS scores was seen in all patients, while a correlation between S-100 β levels in urine and GCS scores was seen only in the moderate head injury group. In the mild head injury group most, and in the severe head injury group all urine S-100 β levels were in the range of values seen in healthy volunteers.

Although the admission S-100 β urine level was above the mean in the patient of the case study illustrated in Figure 5, it was not out of the range of values seen in healthy volunteers. Thus, one could say that, in the setting of head trauma, urine S-100 β levels at the upper end of the normal range should raise suspicion of brain tissue damage, but the positive predictive value would be very low. On the other hand, although our patient series was

small, it appears that the negative predictive value of S-100 β levels in urine is 100%, since the CT was without pathology in all patients with undetectable S-100 β levels in urine. The primary path of excretion for S-100 β is through the kidneys.²⁶ Thus, one might speculate that the absence of an increase in admission S-100 β urine levels in patients with severe head trauma is associated with impaired renal function secondary to systemic shock. However, the kinetics shown in Figure 4 make that a less likely explanation, since the patient in that case had been initially deemed well enough to return home. The deterioration mirrored in a drop of GCS only developed on the second day after the trauma and the patient had normal renal function.

The case study illustrated in Figure 5 supports the observation made by other authors that S-100 β serum levels rise before the onset of clinical symptoms. The kinetics of S-100 β levels observed in Figures 4 indicate that the rise might also be sustained beyond the duration of the symptoms. This further supports the claim made by other authors that S-100 β levels in serum are an extremely sensitive indicator of cerebral tissue damage.

With the method used in our study, neither admission values nor values obtained at later time points for S-100 β levels in urine were found to be reliable predictors of brain tissue damage. Although we were able to show that the kinetics of S-100 β levels in urine follow those observed in the serum, the changes in the urine values were not sufficiently significant to be used as reliable indicator. The lack of a correlation between GCS scores and urine S-100 β levels has previously been described in paediatric patients.²⁷ However, it has been suggested by the results of a more recent study that the use of an S-100 β assay with a lower detection limit and antibodies targeting different epitopes in the β -chain might improve the correlation between urine S-100 β levels and the severity of brain damage.²⁸

Furthermore, it might be worthwhile to investigate whether a ratio of S-100 β levels to other urine components or to the specific weight of the urine, for example, might prove more suitable than absolute S-100 β values.

With our method, S-100 β levels in urine obtained from head injured patients on hospital admission were not a good indicator for the extent of brain damage. Correlation of urine levels with increased serum levels were seen at later time points, and the kinetic pattern seems to follow that seen in serum levels. However, the increases in S-100 β urine levels were too insignificant to be used as a reliable diagnostic parameter. The exploration of a more sensitive test kit based on antibodies to different epitopes in the S-100 β -chain could be useful for future work.

ACKNOWLEDGMENTS

The authors thank all our patients and their relatives, who agreed to participate in this study; our dedicated nurses who, besides taking care of our patients, also collected the necessary samples for our study; and the healthy volunteers who donated samples for this study. Our work was supported by grants from the College of Medicine Teaching and Research Fund and by the Royal University Hospital Foundation of Saskatchewan.

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