Diagnostic Value of Elecsys S100 as a Marker of Acute Brain Injury in the Emergency Department

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The aim of this study was to evaluate the screening and diagnostic validity of the initial serum S100 levels by Elecsys S100 immunoassay in an emergency department. We measured serum S100 in 101 acute brain injury (ABI) patients, in 40 healthy subjects, and in 41 chronic brain injury (CBI) patients (the control group). The ABI patients were divided in several groups according to the Glasgow Coma Scale (GCS), injury type and findings of brain imaging. Of the 101 patients, the 66 cranial computed tomography (CCT) or brain magnetic resonance imaging (MRI)-positive patients showed higher S100

levels than did the 35 CCT/MRI-negative patients ($P = 0.028$). At a cutoff of $0.105 \mu\text{g/L}$, the sensitivity was 84.8% and the specificity 74.3% for all of the ABI patients, and the sensitivity was increased to 96.9% for the traumatic ABI patients for detecting image-positive ABI. There was no significant difference of S100 levels among the groups on the CCT or MRI pathological findings ($P = 0.478$). In conclusion, serum S100 levels can be used in the emergency department as an additional screening tool to identify CCT- or MRI-positive ABI. *J. Clin. Lab. Anal.* 21:387–392, 2007.

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INTRODUCTION

Early estimation of the acute brain injury (ABI) in the patients arriving to the emergency department is critical to identify those patients who may require operative intervention, and to avoid posttraumatic complications and secondary brain damage (1). The clinical decision for performing brain imaging such as a cranial computed tomography (CCT) or brain magnetic resonance imaging (MRI) is dependent on the Glasgow Coma Scale (GCS) (2,3), which is based on clinical criteria. Because the clinical symptoms and GCS have low efficiency for discriminating between CCT-positive and CCT-negative findings, and alcohol intoxication can be combined in patients' presentations (3,4), having additional objective parameters would be quite useful to optimize clinical decisions for conducting an initial brain image test.

Dysfunction and damage of the brain can be detected with biochemical markers. S100 is a calcium binding protein that is highly expressed in the astroglial cells of the brain tissue (5). If these cells are structurally

or ischemically damaged, then S100 is released into the cerebrospinal fluid and it crosses the blood-brain barrier (6). Although S100 has been investigated as a possible marker of brain injury (2–4,7–11), real-time assessment of serum S100 in the patients admitting emergency department under the suspicion of both traumatic or nontraumatic ABI has not been evaluated.

The Elecsys S100 automatic immunoassay has recently been introduced for the real-time detection of serum S100 β chain (S100 $\alpha\beta$ and S100 $\beta\beta$) levels in patients with minor head injury (3). However, its relation to severe traumatic brain damage is controversial (12). The aim of this study was to evaluate

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the screening and diagnostic validity of the initial serum Elecsys S100 levels obtained at an emergency department for the detection of CCT- or MRI-positive ABI.

MATERIALS AND METHODS

Patients and Clinical Characteristics

This study consisted of 101 patients who were admitted to our emergency department within 6 hr after the onset of ABI symptoms. All patients underwent initial CCT or MRI testing. The patients who showed negative findings in CCT were confirmed by MRI. There were 45 patients with traumatic ABI and 56 patients with nontraumatic ABI. The neurological examination and the assessment of the level of consciousness were performed with the GCS. The patients were stratified into three subgroups on the GCS: mild (13–15), moderate (8–12), and severe (≤ 8). The patients with metabolic disorders or seizure did not take imaging test and were excluded in this study. After the initial brain image examination, the patients were divided into two groups: the CCT- or MRI-positive group ($n = 66$) and the CCT/MRI-negative group ($n = 35$). The CCT- or MRI-positive patients were classified to one of four subgroups according to the image finding: intracerebral hemorrhage, skull fracture, cerebral infarction, and brain edema. We included control groups for comparing the data, and this consisted of serum samples from a healthy control group ($n = 40$) and a chronic brain injury (CBI) group ($n = 41$). The CBI group included patients who had undergone brain surgery 10 days or more ago. The CBI patients received surgery for the intracranial tumor ($n = 21$), traumatic hemorrhage ($n = 4$), nontraumatic hemorrhage ($n = 11$), and aneurysm ($n = 5$).

Measurement of Serum S100

The serum S100 levels were determined by automatic electrochemiluminescence immunoassay (Elecsys S100[®]; Roche Diagnostics, Penzberg, Germany) on the Roche Elecsys 2010 according to the manufacturer's instruction. The measuring time was 18 min and the analytical range was 0.005–39 $\mu\text{g/L}$. All the blood specimens were processed to serum, then centrifuged at 3,000 rpm for 10 min at room temperature and the aliquots of serum were stored at -80°C before being analyzed. The assay is unaffected by icterus (bilirubin $< 25 \text{ mg/dL}$), hemolysis (hemoglobin $< 1.0 \text{ g/dL}$), lipemia (lipid $< 1500 \text{ mg/dL}$), and biotin ($< 50 \text{ ng/mL}$).

The data are presented as medians and interquartile ranges (IQR) (25th–75th percentiles). The statistics were performed with Windows SPSS 10.1 (SPSS, Inc., Chicago, IL) and the differences between the groups

were evaluated by the Mann-Whitney U-test or the Kruskal-Wallis test. The receiver operating characteristic (ROC) curve were used to evaluate the cutoff points for detecting CCT- or MRI-positive ABI. A two-tailed P value of < 0.05 was considered to be statistically significant.

RESULTS

The serum S100 levels of the ABI patients ($n = 101$), the healthy control group ($n = 40$), and the CBI control group ($n = 41$) are given in Table 1. The 101 ABI patients (45 years [31–59]; 57.9% male) had higher serum S100 levels than did the 40 healthy control subjects (58 years [34–82], 46.3% male) or the 41 patients with CBI (58 years [34–82], 46.3%) ($P = 0.024$, 0.034 , respectively). Of the 101 patients with ABI that were studied, the 66 (65.3%) CCT- or MRI-positive patients showed higher S100 levels than did the 35 (34.7%) CCT/MRI-negative patients ($P = 0.028$) (Fig. 1A). However, there was no difference of serum S100 concentrations among the healthy, CBI control, and CCT/MRI-negative ABI patients ($P > 0.05$). When we classified the 101 ABI patients according to the type of injury, 56 patients (55.4%) were proved to have nontraumatic brain injury and 45 (44.6%) were proved to have traumatic brain injury. In both groups, the S100 serum concentrations were significantly higher in the CCT- or MRI-positive patients than those in the CCT/MRI-negative patients ($P = 0.037$ for the nontraumatic ABI group, $P = 0.028$ for the traumatic ABI group) (Table 2, Fig. 1B).

Analysis of the ROC curves for the S100 serum levels showed a maximum area under the ROC curve (AUC) of 0.839 (95% confidence interval [CI], 0.748–0.929) for detecting CCT- or MRI-positive ABI (Fig. 2). A cutoff

TABLE 1. Serum S100 concentrations in the patients with acute brain injury and the controls

Study group	n	S100 ($\mu\text{g/L}$) median (interquartile range)
Healthy	40	0.080 (0.049–0.094)
Acute brain injury		
Whole group	101	0.150 (0.088–0.358)
GCS		
13–15	82	0.136 (0.173–0.292)
9–12	9	0.478 (0.186–0.974)
≤ 8	10	0.179 (0.099–2.060)
Subgroup by CCT or MRI result		
Hemorrhage	30	0.209 (0.131–0.622)
Fracture	7	0.275 (0.167–1.910)
Infarction	13	0.208 (0.099–0.395)
Edema	16	0.206 (0.135–0.578)
Chronic brain injury	41	0.070 (0.039–0.083)

TABLE 2. Comparative results of the serum S100 levels in the CCT- or MRI-positive patients and the CCT/MRI-negative patients*

Group	n	CCT- or MRI-positiveS100 (µg/L)	n	CCT/MRI-negativeS100 (µg/L)	P ^a
Whole group	66	0.208 (0.131–0.478)	35	0.065 (0.049–0.104)	0.028
Subgroup by injury type					
Nontraumatic ABI	34	0.179 (0.099–0.417)	22	0.057 (0.045–0.095)	0.037
Traumatic ABI	32	0.253 (0.168–1.086)	13	0.098 (0.075–0.246)	0.028
Subgroup by GCS					
Mild (13–15)	48	0.199 (0.135–0.388)	34	0.064 (0.049–0.103)	0.010
Moderate (9–12)	8	0.491 (0.181–1.292)	1		NA
Severe (≤8)	10	0.179 (0.099–2.06)	–		NA

*Data are presented as medians and interquartile ranges (25th–75th percentiles).

^aP-value describes the statistical difference on the Mann-Whitney U test for the differentiation between the CCT- or MRI-positive patients and the CCT/MRI-negative patients.

NA, not available.

Fig. 1. S100 concentrations in the study groups. CCT- or MRI-positive (+) patients showed higher S100 levels than did the CCT/MRI-negative (-) patients ($P = 0.028$ for total ABI group (A), $P = 0.037$ for the nontraumatic ABI group, and $P = 0.028$ for the traumatic ABI group (B)). Data are given as box plots (median, interquartile range) and CIs (5–95%).

Fig. 2. ROC curve of the S100 measurements for the detection of CCT- or MRI-positive ABI (CCT- or MRI-positive vs. CCT/MRI-negative, AUC = 0.839 (95% CI, 0.748–0.929; $P < 0.001$)).

TABLE 3. Characteristics of the Elecsys S100 concentration using a 0.105 µg/L cutoff value for detecting CCT- or MRI-positive acute head injury

Study group	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Whole group (n = 101)	84.8	74.3	86.2	72.2
Nontraumatic ABI (n = 56)	73.5	86.4	89.3	67.9
Traumatic ABI (n = 45)	96.9	53.8	83.8	87.5

level of 0.105 µg/L S100 identified the patients with ABI correctly as CCT- or MRI-positive with a sensitivity of 84.8%, a specificity of 74.3%, a positive predictive value (PPV) of 86.2%, and a negative predictive value (NPV) of 72.2% (Table 3). Of 66 patients with image-positive ABI, 10 patients revealed serum S100 levels below the cutoff of 0.105 µg/L. Of these 10 patients who showed false-negative results, nine patients had nontraumatic brain injury: hemorrhage (n = 4), infarction (n = 3), and edema (n = 2). The one traumatic ABI patient with false-negative results showed a S100 concentration of 0.093 µg/L and that patient demonstrated epidural hemorrhage on CCT scan. When we evaluated the analytical performance of S100 for detecting traumatic ABI patients, the sensitivity for detecting CCT- or MRI-positive ABI increased to 96.9% (31/32).

When comparing the serum S100 concentrations of ABI patients with respect to their GCS score on admission, the patients with moderate or severe brain injury had higher serum S100 levels than did the patients having mild brain injury ($P = 0.039$ and $P < 0.001$,

respectively) (Fig. 3A). All the patients with a GCS < 12 (moderate and severe), except for one patient with moderate ABI (GCS = 10), demonstrated a CCT- or MRI-positive result. However, there was no difference of the serum S100 levels between the patients with moderate and severe brain injury ($P = 0.361$). Of the 82 patients with mild brain injury, the 48 patients who were CCT- or MRI-positive showed higher serum S100 levels than did the 34 CCT/MRI-negative patients ($P = 0.010$).

The CCT- or MRI-positive patients (n = 66) were stratified according to the pathological findings: intracerebral hemorrhage (n = 30), skull fracture (n = 7), cerebral infarction (n = 13), and brain edema (n = 16). Each group showed higher serum S100 level in CCT- or MRI-positive patients than the CCT/MRI-negative patients. However, there was no significant difference of the S100 levels between the groups according to the CCT or MRI pathological findings ($P = 0.478$; Kruskal-Wallis test) (Fig. 3B).

DISCUSSION

Despite its poor value for diagnosing diffuse brain injury, brain imaging such as CCT or MRI is generally recommended as the method of the choice for making the initial diagnosis of ABI (12,13). The main result of the present study is that a single measurement of the serum Elecsys S100 levels obtained within 6 hr after symptom onset can identify positive intracranial lesions such as hemorrhage, diffuse brain edema, skull fracture, or infarction. Of the 41 CBI patients, seven patients (two intracranial tumor, four nontraumatic hemorrhage, and one traumatic hemorrhage) showed S100 levels above 0.105 µg/L; however, we could not compare the S100 levels among the CBI patients in age and natural diseases due to the small sample size. Since the patients with CBI did not show increased serum S100 levels, persistently elevated Elecsys S100 serum levels seem to be related with prolonged neurological deficit. Because nontraumatic ABI such as stroke is the second leading cause of all deaths worldwide (14), and both hemorrhagic and ischemic stroke can lead to cerebrovascular accidents involving cell death and disruption of the blood-brain barrier, we examined both traumatic and nontraumatic ABI patients in this study.

With respect to an identified cutoff value of 0.105 µg/L, the CCT- or MRI-positive brain damage was diagnosed with a sensitivity of 84.8% and a specificity of 74.3% for all the ABI patients. Our findings fit well with and confirmed a previous report that S100 could be used to select patients for CCT scanning (2,3). In comparison with the previous study on patients with mild head trauma (3), in which the CCT-positive

Fig. 3. Serum S100 concentrations for the ABI patients on the GCS (A), and according to the intracerebral lesions as detected on the initial CCT or MRI (B). Data are given as box plots (median, interquartile range) and CIs (5–95%).

patients were identified with a sensitivity of 99% and a specificity of 30%, our results showed a sensitivity of 96.8% for detecting the traumatic ABI patients who were CCT- or MRI-positive.

As small amounts of S100 protein have been found in fat tissue, skin, bone, and skeletal muscle tissue, as well as in melanoma and glioblastoma cells (6,15–17), the S100 may be false positive in severe traumatic injury (18), including soft tissue trauma or bone fracture. In this study, we had a similar result, as the specificity was lower for the patients with traumatic ABI than the specificity for the patients with nontraumatic ABI (specificity of 53.8% vs. 86.4%, respectively). Although significant correlation between the S100 levels and the

incidence of intracerebral pathological findings on CCT or MRI was shown, some patients (nontraumatic [n = 9], traumatic [n = 1]) with CCT- or MRI-positive findings exhibited S100 concentrations below the reference value. As the previous study reported that the patients suffering from ischemic stroke showed serum S100 values with the maximum levels being reached 2 to 4 days after onset (18), taking repeated measurements would probably provide higher diagnostic accuracy for this group of patients.

When we compared the serum S100 levels among the subgroups according to the GCS, a low GCS score was associated with increased serum S100 levels. However, there was no statistical difference in this study between

the moderate (GCS = 9–12) and severe (GCS ≤ 8) ABI subgroups, as is shown in Fig. 3A. These results may be due to the small sample size; therefore, further studies are required to confirm the validity of the given cutoff points according to the subgroups. Because the previous reports showed continuous release of S100 with secondary brain injury and a prolonged half-life in the severely injured cases (19), repeated measurements of the serum S100 may help identify those patients with poor outcomes.

When classifying the subgroups according to the CCT or MRI findings, no significant differences were identified in the present study among the subgroups (hemorrhage, fracture, infarct, and edema). This result suggests that only the presence of brain damage, and not the injury type, was significantly related with the initial serum S100 level.

In summary, this study revealed that a single serum S100 measurement performed within 6 hr after symptom onset can predict CCT- or MRI-positive ABI. An MRI or CCT study is complex and costly, and the serum S100 test is objective and easy to use. Further, the automatic analyzer used in the present study was able to analyze the S100 serum results within 30 min and this machine is available for use 24 hr in most hospitals. Therefore, the serum S100 levels can be used in the emergency department as an additional screening tool to identify CCT- or MRI-positive ABI patients. Further studies should be performed that focus on the predictive value of S100 according to the treatment strategies employed in emergency departments.

REFERENCES

- Graham ID, Stiell IG, Laupacis A, O'Connor AM, Wells GA. Emergency physicians' attitudes toward and use of clinical decision rules for radiography. *Acad Emerg Med* 1998;5:134–140.
- Ingebrigtsen T, Romner B, Marup-Jensen S, et al. The clinical value of serum S-100 protein measurements in minor head injury: a Scandinavian multicentre study. *Brain Inj* 2000;14:1047–1055.
- Biberthaler P, Linsenmeier U, Pfeifer KJ, et al. Serum S-100B concentration provides additional information for the indication of computed tomography in patients after minor head injury: a prospective multicenter study. *Shock* 2006;25:446–453.
- Biberthaler P, Mussack T, Wiedemann E, et al. Elevated serum levels of S-100B reflect the extent of brain injury in alcohol intoxicated patients after mild head trauma. *Shock* 2001;16:97–101.
- Schafer BW, Heizmann CW. The S100 family of EF-hand calcium-binding proteins: functions and pathology. *Trends Biochem Sci* 1996;21:134–140.
- Haimoto H, Hosoda S, Kato K. Differential distribution of immunoreactive S100-alpha and S100-beta proteins in normal nonnervous human tissues. *Lab Invest* 1987;57:489–498.
- Rothoerl RD, Woertgen C, Holzschuh M, Metz C, Brawanski A. S-100 serum levels after minor and major head injury. *J Trauma* 1998;45:765–767.
- Persson L, Hardemark HG, Gustafsson J, et al. S-100 protein and neuron-specific enolase in cerebrospinal fluid and serum: markers of cell damage in human central nervous system. *Stroke* 1987;18:911–918.
- Buttner T, Weyers S, Postert T, Sprengelmeyer R, Kuhn W. S-100 protein: serum marker of focal brain damage after ischemic territorial MCA infarction. *Stroke* 1997;28:1961–1965.
- Wiesmann M, Missler U, Hagenstrom H, Gottmann D. S-100 protein plasma levels after aneurysmal subarachnoid haemorrhage. *Acta Neurochir (Wien)* 1997;139:1155–1160.
- Griffin WS, Yeralan O, Sheng JG, et al. Overexpression of the neurotrophic cytokine S100 beta in human temporal lobe epilepsy. *J Neurochem* 1995;65:228–233.
- Shackford SR, Wald SL, Ross SE, et al. The clinical utility of computed tomographic scanning and neurologic examination in the management of patients with minor head injuries. *J Trauma* 1992;33:385–394.
- Stein SC, Ross SE. The value of computed tomographic scans in patients with low-risk head injuries. *Neurosurgery* 1990;26:638–640.
- Murray CJ, Lopez AD. Mortality by cause for eight regions of the world: global burden of disease study. *Lancet* 1997;349:1269–1276.
- Kahn HJ, Bauml R, Van Eldik LJ, Dunn RJ, Marks A. Immunoreactivity of S100 beta in heart, skeletal muscle, and kidney in chronic lung disease: possible induction by cAMP. *Mod Pathol* 1991;4:698–701.
- Alber B, Hein R, Garbe C, Caroli U, Lippa PB. Multicenter evaluation of the analytical and clinical performance of the Elecsys S100 immunoassay in patients with malignant melanoma. *Clin Chem Lab Med* 2005;43:557–563.
- Anderson RE, Hansson LO, Nilsson O, Liska J, Settergren G, Vaage J. Increase in serum S100A1-B and S100BB during cardiac surgery arises from extracerebral sources. *Ann Thorac Surg* 2001;71:1512–1517.
- Abraham HD, Butterworth RJ, Bath PM, Wassif WS, Garthwaite J, Sherwood RA. Serum S-100 protein, relationship to clinical outcome in acute stroke. *Ann Clin Biochem* 1997;34:366–370.
- Mussack T, Kirchhoff C, Buhmann S, et al. Significance of Elecsys S100 immunoassay for real-time assessment of traumatic brain damage in multiple trauma patients. *Clin Chem Lab Med* 2006;44:1140–1145.