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Original Article

Correlation between brain injury biomarkers and Glasgow coma scale in pediatric sepsis

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Abstract

Background Sepsis in children, with or without multiple organ failure, causes increased susceptibility to brain injury due to systemic insults. Brain injury in sepsis is difficult to recognize clinically. Neuron-specific enolase (NSE) and S-100B have been extensively studied in brain injuries and appear to be promising alternative biomarkers.

Objectives To determine if there is a correlation between the Glasgow coma scale (GCS) and NSE as well as S-100B levels, in children with sepsis.

Methods We performed an analytical study on septic children aged > 2 years. GCS scores were assessed on the first and third days of admission. Blood specimens to test for NSE and S-100B were drawn on the first day of admission and stored at -70ºC for further analysis at the end of the study.

Results Out of 35 patients, 30 met the inclusion criteria. Post-analysis, one subject with NSE above the maximum level was excluded. Negative correlations were found between GCS score and NSE, as well as between GCS and S-100B levels. Analysis revealed a significant ROC for NSE, but not for S-100B. NSE concentration of 8.1 μg/L was the cut-off point for GCS scores below 12.

Conclusions There were negative correlations between GCS and NSE levels, as well as between GCS and S-100B levels. The predictive value of NSE level was a cut-off point of 8.1 μg/L for GCS scores below 12. **[Paediatr Indones. 2012;52:111-17]**.

Keywords*: pediatric sepsis, brain injury biomarkers, neuron specific enolase, protein S-100B, Glasgow coma scale*

Sepsis is a major health problem, causing high mortality rates in infants and children. Severe sepsis and septic shock are conditions with multi-organ failure, known to be a leading cause of mortality in pediatric sepsis.[1-3] Prevalence data vary among countries. In 1995, an epidemiologic study in the United States reported the prevalence of severe sepsis to be 0.56 per 1,000 people.[4] The highest prevalence was in the neonate and infant age group, while steep declines in prevalence were observed in the 10-14 year age group.

Multiple organ failure may involve any organ during sepsis.[5] Although the central nervous system (CNS), has selective protective barriers, it is also vulnerable to systemic insult during sepsis. However, the mechanisms are not fully understood. Septic encephalopathy accounts for most manifestations of CNS involvement, with a prevalence of 9-71%.[6] In contrast to adults, hemodynamic instability is common in pediatric sepsis, and may develop into a hypoxic-ischemic injury in the CNS.[7]

Clinical manifestations of CNS neuronal injury in septic children are challenging and difficult to

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analyze. GCS is an objective examination to assess the degree of CNS injury, although it is not very sensitive.[7] Electroencephalography (EEG) provides the most sensitive diagnostic work-up, and may be used for assessing neuronal damage, as well as having prognostic value in septic children.[8,9] However, the need for sophisticated infrastructure and expertise is cumbersome and limits the use of EEG in clinical settings. Imaging techniques (MRI and CT) are also sensitive for extensive structural damage, but they are not useful for detecting minor abnormalities.

As an alternative, biomarkers such as NSE and S100B are promising as objective and practical clinical tools for detecting early CNS insult. Both biomarkers have been extensively studied in pediatric neonatal populations with hypoxic-ischemic encephalopathy (HIE), head injuries, post-cardiac arrest and septic shock. The prognostic value of these biomarkers for neurologic deterioration and death has been established in several studies. [11-14]

Recent studies of CNS biomarkers in pediatric sepsis have been limited, so we aimed to find practical and academically feasible tools for assessing brain injury. As in some previous studies, we assessed CNS biomarkers' prognostic values for both neurologic and clinical outcomes.

Methods

The study was carried out in the intensive care units and pediatric wards of three state-owned hospitals in Central and West Jakarta and was approved by the Medical Ethics Committee, University of Indonesia. The duration of the study was 9 months, between July 2010 and March 2011. Subjects were obtained by consecutive sampling, and their parents/caregivers consented to their participation in this study. Inclusion criteria were patients with sepsis. Clinical and laboratory data were recorded on the first day of admission. We excluded patients with known or suspected meningitis, encephalitis, brain abscess, inflicted or non-inflicted traumatic brain injury, paroxysmal and brain disorders, mild to severe trauma, primary or secondary malignancy, as well as hemodialysed patients, due to its influence on S-100B clearance. All subjects were received proper management for their septic condition. Modified pediatric GCS scores and neurologic examinations were obtained on the first day of admission.

Table 1. Subjects' characteristics

Characteristics	Total (n = 30)
Median age, years (range)	6.4 (5.5 -16)
Sex, n	
Male	11
Female	19
Age group, n	
Toddler (2 – 5 years)	17
School-aged (6 - 12 years)	12
Adolescent (12 – 18 years)	1
Nutritional status, n	
Malnourished	1
Undernourished	13
Normal	12
Overweight	3
Obese	1
Septic severity, n	
Sepsis without organ dysfunction	19
Severe sepsis	5
Septic shock	6
Diagnosis, n	
Pneumonia/pleural effusion	13
Dengue hemorrhagic fever	5
Urinary tract infection	6
Abdominal infection	5
Abdominal post-operative	1
Underlying disease, n	
Tumor/malignancy	5
Post-operative	2
Guillain-Barre syndrome	1
Thalassemia	1
Cerebral palsy	1
HIV	1
Tuberculosis	1
Without underlying disease	18
Anemia (Hb < 12 g/dL), n	
No anemia	9
Anemia	21
Leukocyte count, n	
Normal	4
Leukocytosis	21
Leukopenia	5
Platelet count, n	
Normal	9
Thrombocytopenia	13
Thrombocytosis	8
NSE, n	
Normal	18
Abnormal	11
S-100B, n	
Normal	25
Abnormal	5
Outcome, n	
Survived	25
Died	5

Single specimens of NSE and S-100B were collected from eligible subjects on the first day of admission. All specimens were centrifuged, frozen (-70°C) and analyzed by the end of study. NSE measurement was performed using Nexus D_x ™ NSE (Nanogen Inc, San Diego, CA). Specimens with gross or visible hemolysis were discarded. NSE concentration < 15.6 μg/L was considered non-fatal. S-100B analysis was performed using Nexus D_x ™ S100 (Nanogen Inc, San Diego, CA). S-100B concentration < 0.5 μg/L was considered to be non-fatal.

Continuous data are represented as median and range, while categorical data are shown as counts. The clinical material was dichotomised using clinical endpoints of good versus bad outcomes. Chi-square and Fisher's exact test were used to analyze data. Specificity and sensitivity of NSE and S-100B were calculated. Correlation of NSE and S-100B to GCS were calculated using Spearman's coefficient analysis. Receiver-operating characterictic (ROC) analysis was also performed. All statistical analyses were performed using SPSS software version 15.0 (SPSS Inc., IL, USA).

Results

Thirty-five patients met the inclusion criteria, but three subjects' parents did not consent and two subjects had specimens with gross/visible hemolysis. Thus, data were obtained from 30 consecutive subjects. Serum specimen analysis by the end of study revealed one specimen without hemolysis had an NSE level above the maximum (> 100 mg/L) measurement capability of the Elisa Kit, so that subject was excluded. Therefore, the remaining data for subsequent correlation analysis of NSE was from 29 subjects. For S-100B, no subjects were excluded, for a total of 30 subjects available for S-100B analysis. Patient characteristics are summarized in **Table 1**.

The GCS is categorized into 3 levels of brain injury/decreased consciousness: mild to normal (13-15), moderate (8-12), and severe (<8). Of the 30 subjects, 19 subjects were in the mild to normal category, 2 were moderate, and 9 were severe. Six subjects in the severe category had fatal outcomes.

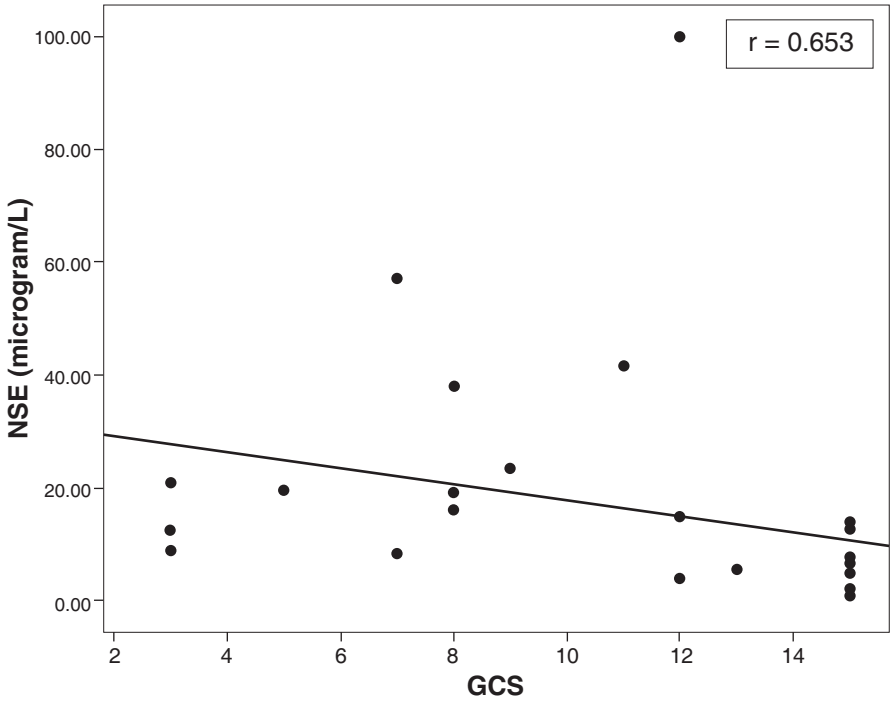

Figure 1. Correlation between GCS and NSE

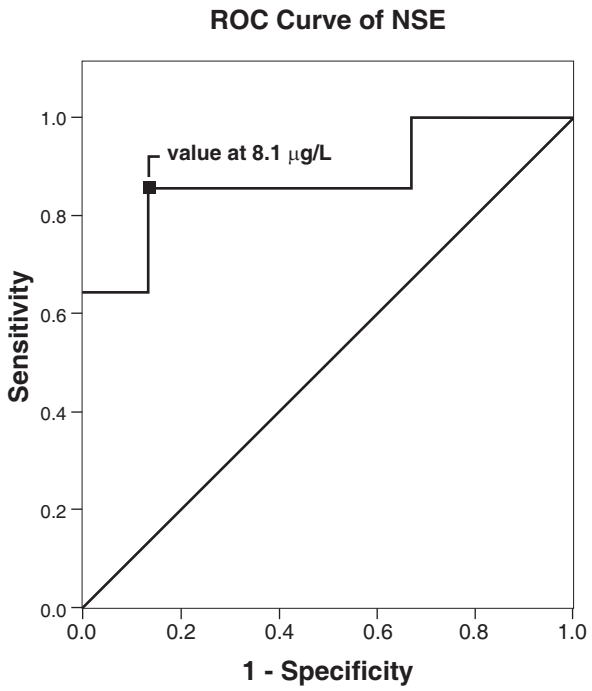

Figure 2. Receiver-operator characteristic for GCS score below 12. NSE value plotted at 8.1 ug/L with 85.7% sensitivity and 86.7% specificity (95% CI 0.71 to 1.00)

Abnormal NSE levels were found in 12 of 29 subjects on the first day of admission. Ten subjects were in the moderate/severe brain injury category, while two subjects were in the normal category. Chi-square test showed a significant relationship between level of consciousness and NSE level (RR 0.33 and 95% CI 0.15 to 0.73). Correlation analysis using Spearman Rho coefficient showed a negative correlation (r = -0.653 and P = 0.0001) as depicted in **Figure 1**. Receiver operator characteristics based on dichotomised data were identified. As presented in **Figure 2**, an NSE level of 8.1 μg/L was the cut-off point for GCS scores of < 12, with 85.7% sensitivity and 86.7% specificity. The area under curve (AUC) was 87.6% (95%CI 0.71 to 1.00).

Based on median level, 6 of 30 subjects had abnormal S-100B levels. Five of these subjects had moderately/severely decreased consciousness, one subject had normal consciousness. On day three, no improvement was observed in either of 5 subjects with moderately/severely decreased consciousness or mildly decreased consciousness. Bivariate analysis, using dichotomised clinical data of severity and mortality, revealed a significant relationship of S-100B

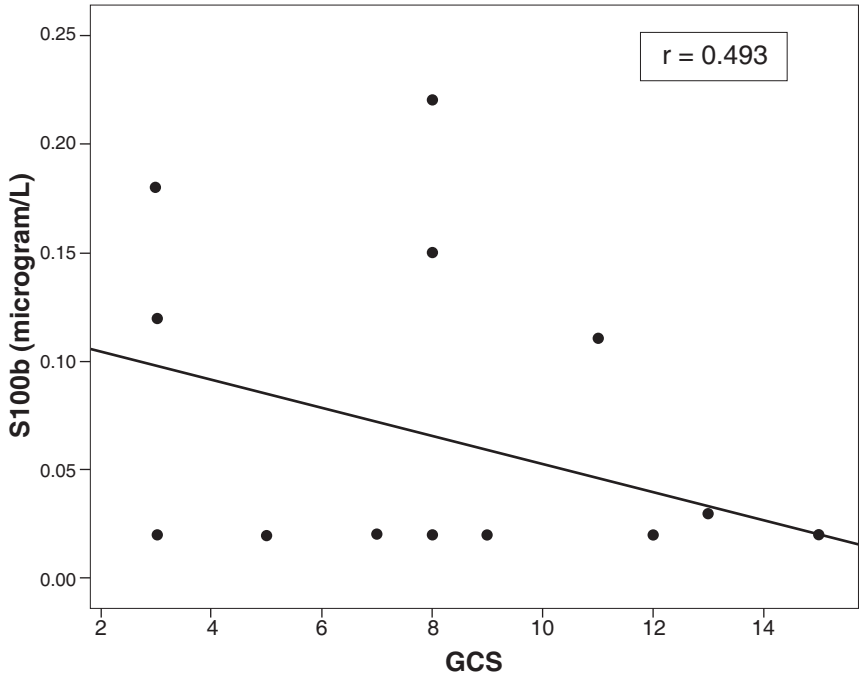

Figure 3. Correlation between GCS and S-100B

abnormality with both septic severity (RR=0.24, 95% CI 0.12 to 0.48) and mortality (RR=0.13, 95% CI 0.03 to 0.60). Correlation analysis using Spearman Rho coefficient showed a negative correlation (r = -0.493, P = 0.007) as depicted in **Figure 3**. Receiver-operator characteristics based on dichotomised data showed S-100B level of 0.03 μg/L to be the cut-off point for a GCS score < 12 with 38.5% sensitivity and 94.1% specificity. The AUC was 65.6% with 95% CI 0.45 to 0.86. However, since the P value was greater than 0.05, the ROC curve produced was statistically insignificant.

Discussion

This study aimed to determine if there were correlations between GCS, as a clinical parameter, and NSE and S-100B as brain injury markers in pediatric sepsis. To date, limited data is available regarding the properties of these two biomarkers in pediatric sepsis.

Neurologic manifestation in sepsis related to encephalopathy is generally seen as decreased level of consciousness, or decreased GCS scores. Eidelman *et al* described that if scores of 15 decreased to less than 8, mortality is increased from 18% to 63%.[14] In agreement with these findings, 6 of our 8 subjects with severely decreased consciousness had fatal outcomes in our trial. Dichotomous analysis performed with Chi-square test revealed a significant relationship between GCS and death (RR=0.07, 95% CI 0.01 to 0.45). Other neurological findings in sepsis are seizures, paralysis, and less commonly, asterixis, tremors, cranial nerve paralysis and nuchal rigidity.[15-17] Seizures and axonal-type lower motor neuron paralysis were observed in septic subjects. This finding may be related to sepsis as one form of septic encephalopathy, or may be related to the underlying disorder.[17] It is known that underlying diseases may contribute to the severity of sepsis and different clinical manifestations.

NSE concentrations were increased in all severe sepsis and septic subjects with multiple organ dysfunction. Some subjects with no evidence of organ dysfunction also had increased NSE concentration. This finding was in contrast to a study by Hsu *et al* in 37 children with severe sepsis and septic shock.[7] They reported higher NSE concentrations in all septic subjects compared to normal children as controls. Besides the difference in subjects' characteristics from the Hsu *et al* study, the increase of NSE concentration in some subjects may be due to early hemodynamic instability. Subsequently, these subjects may have developed hypoxic-ischemic brain injury before any clinical signs, such as decreased GCS score. However, erythrocytes and platelets are also an abundant source of defective NSE, due to hemolysis or immunologic mechanisms during sepsis. Thus, increased NSE concentration in serum is not solely from neuron or atroglial sources.[17-20] Correlation analysis using Spearman Rho coefficient revealed a correlation of -0.65 between GCS score and NSE concentration. Similar results were reported by Guan *et al* in children with traumatic brain injury. They found a correlation of -0.61 between GCS and serum NSE.[21] Ross *et al* noted a similar correlation coefficient of -0.61 between GCS and NSE in cerebrospinal fluid (CSF).[22] Further study to compare CSF and serum NSE to GCS is needed to determine the usefulness of CSF NSE as a biomarker of brain injury in children with sepsis. Receiver-operator characteristic curve was made to analyze the relationship of serum NSE concentration to GCS score at the time of admission. The predicted cut-off value was 8.1 μg/L for GCS score below 12. Few studies have investigated the relationship of GCS score as a clinical outcome to a single NSE measurement for predictive values.

S-100B protein is a calcium-binding enzyme found mostly in glial cells.[7,12,17] We found S-100B protein concentrations to be lower than the expected cut-off level. This finding hindered the real picture of S-100B properties, as a new cut-off point of 0.02 μg/L based on median value was used. Raw data analyzed using Spearman Rho coefficient, revealed a correlation of -0.493. A study by Elting *et al*, performed in children with traumatic brain injury, found a correlation of GCS score versus S-100B with a value of -0.81.[23] The lower correlation coefficient value compared to NSE values suggest that NSE is a more reliable predictor of neurologic outcomes than S-100B. Rundgren *et al*, studying patients after cardiac arrest, reported the superiority of NSE compared to S-100B in predicting neurological outcomes within 48 to 72 hours after cardiac arrest.[24] Unlike that of NSE, the resulting S-100B ROC curve was insignificant. However,

S-100B was significantly related to septic severity and death. S-100B concentrations in subjects with fatal outcomes varied from 1.5 to 10 times the normal cut-off level of 0.02 μg/L. In contrast to NSE, S-100B serum concentration is not influenced by hemolysis.[7,12] Glial cell death and injury contributes to its rise in serum and CSF. Cytokine activation, another mechanism related to the inflammation cascade, in turn disrupts the blood brain barrier integrity.[7,18] Fatality has been associated with high level of serum S-100B in some studies.[7,11-13] Given that S-100B is not glial specific, it has less of a relationship with severe clinical outcomes as compared to NSE. Heart and kidneys are the main organs producing significant amounts of S-100B in serum.[25] In cases of sepsis with multiple organ failure, vulnerable hemodynamic disturbances eventually raise serum S-100B concentration due to injury of these organs. Age, underlying and co-morbid diseases, and child developmental stage also influence serum S-100B concentration.[12]

The descriptive study design in this trial had some limitations. We had the required sample size for performing correlation analysis, but less than the required sample size for dichotomous analysis between clinical parameter and biomarkers, as indicated by the wide confidence intervals. Other limitations were single measurements instead of serial measurements, use of only clinical parameters rather than also using EEG and imaging (CT, MRI) findings. The former limitation was due to lack of parental consent, while the latter was due to lack of EEG availability in the ICU setting, transportation difficulties for both EEG and imaging procedures and unstable subject clinical conditions. Serial measurement of biomarkers would be ideal, as they can show multiple values within a certain time frame. Rundgren *et al* stated that serial biomarker measurement also decreased some technical errors in the laboratory.[24] EEG are sensitive tools to evaluate brain injury and are regarded as the gold standard, particularly for the detection of not only encephalopathy and severity of brain damage, but also subtle seizures in sedated or intubated subjects. [7,9,15,16]

Despite the limitations, some advantages must be noted. Single samples obtained from a regular clinical setting mimic routine daily bedside procedures. If future studies of different designs are to be carried out, more subjects should be recruited so that legal and ethical clearance can be easier to obtain from enough subjects.

Until recently, the use of brain injury biomarkers in pediatric sepsis was disputed and one study termed NSE and S-100B to be surrogate biomarkers. Our results show that NSE and S-100B are potentially useful biomarkers for use in pediatric sepsis cases.

In conclusion, consistent with previous studies,[21-23] we found a correlation between GCS score and biomarkers. NSE concentration of 8.1 ug/L for a GCS score below 12 had a predictive value. S-100B was associated with the clinical outcomes of disease severity and death.

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