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The accuracy of cerebrospinal fluid and serum S100B protein to diagnose bacterial meningitis in children at pediatric ward Department of Child's Health, Sanglah Hospital Denpasar, Bali-Indonesia

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ABSTRACT

Background: The diagnosis of bacterial meningitis is challenging because of the vague clinical presentation primarily in infants and children. The S100B protein is a biomarker of the inflammatory process in the brain. It is thought to have a relationship with neuron damage and oxidative stress in bacterial meningitis.

Methods: In this study, we measured the levels of S100B protein in cerebrospinal fluid (CSF) and serum by using ELISA method, in children with suspected bacterial meningitis. The diagnosis of proven bacterial meningitis is based on a positive culture that confirmed the causing bacteria. We analyzed the data by using MedCalc-version 17.6 programme.

Results: Eighty patients suspected of bacterial meningitis included in this study. As many as 47 (58.8%) are males. The mean of age was 29.8 months (SD ± 32.1). Prevalence of proven bacterial meningitis was 21 out of 80 (26.25%). The primary clinical symptoms were seizures, decreased consciousness, clinically sepsis, positive meningeal sign, vomiting and focal neurologic deficits. The comparison of CSF S100B levels between a positive culture and negative culture were 31.4 (SD ± 32.81) and 29.2 (SD ± 27.13). The serum S100B level in a positive culture was 114.1 (SD ± 95.67) and in a negative culture was 74.9 (SD ± 75.84). The area under the curve (AUC) of CSF S100B and serum S100B were 0.523 and 0.655, respectively. For diagnosing bacterial meningitis, the optimal level of CSF S100B protein is ≥ 54 ng/L (sensitivity 29%, specificity 98%), while the optimal level of serum S100B is ≥ 177 ng/L (sensitivity 19%, specificity 98%).

Conclusion: CSF S100B and serum S100B protein have prospective value with high specificity to confirm the diagnosis of bacterial meningitis in children. Serum S100B may be used as an additional test for diagnosing bacterial meningitis in children when there is a contraindication for lumbar puncture.

Keywords: Bacterial meningitis, biomarker, cerebrospinal fluid, diagnostic test, S100B

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BACKGROUND

Meningitis is an inflammation of the meninges and or cerebrospinal fluid (CSF) which surround and protect the brain and spinal cord. Bacterial meningitis is a bacterial infection on meninges which can be proved by the positive result of the culture, Polymerase Chain Reaction (PCR), Gram stains, or antigen test on the CSF.[1,2]

The incidence of meningitis is varied between countries. Moreover, without adequate treatment, the mortality rate of bacterial meningitis could be up to 70%. The incidence of meningitis was up to 35-40 cases per year in Sanglah Public General Hospital, Bali.

Bacterial detection methods with CSF or blood Gram stain and culture are not always able to determine the diagnosis, especially in patients who have received antibiotics therapy prior to the culture and lumbar puncture examination. The results of the CSF analysis on bacterial meningitis are not always specific and not consistently can be used to establish the diagnosis.[1,3]

The S100B protein is a calcium-binding protein that physiologically produced, primarily by astrocytes in the central nervous system (CNS). It is involved in the development and maintenance of the nervous system. The S100B protein is one of the biomarkers known to be indicating neural damage in the process of circulation failure, stroke, and head injury. The S100B protein is also reported to contribute to the damage of the neurons and oxidative stress on sepsis encephalopathy and bacterial meningitis in children.[4,5]

In the research, we measured the level of S100B protein in CSF and serum from patients with suspected bacterial meningitis. The diagnosis of proven bacterial meningitis is established based on positive clinical signs of meningitis, positive CSF analysis, and positive CSF culture. The main objective

of this research is to determine the accuracy of S100B protein levels in the CSF and serum in diagnosing bacterial meningitis in children. The secondary purpose is to compare the Area Under the Curve (AUC) between the CSF and serum S100B protein in diagnosing bacterial meningitis in children.

MATERIALS AND METHODS

The type of the research is cross sectional. This diagnostic test accuracy study was done from 1 February 2016 to 31 March 2017 in the Department of Pediatrics, Faculty of Medicine, Udayana University/Sanglah Hospital, Denpasar, Bali.

Subjects

The subject of this research were children ≤ 12 years who were treated in our pediatric ward suspected with bacterial meningitis and met other criteria for sample selection. The inclusion criteria were: age ≤ 12 years, positive clinical signs and symptoms of meningitis, CSF analysis showed leukocytes >10 cell/mm^3, peripheral blood leukocytes ≥10.000 cell/uL, and the parents agreed for the patient to participate in this study. The clinical symptoms of meningitis are considered positive when there is a fever accompanied by the presence of at least one of the following symptoms: meningeal irritation sign, decreased consciousness, seizure, vomiting, headache, and or bulging of the anterior fontanels in children under 18 months. The subject was proven to have bacterial meningitis when the clinical signs and symptoms are accompanied by positive CSF culture. The exclusion criteria were: previous nerve surgery or V-P shunt placement, the patient or parents refused lumbar puncture and the presence of local bacterial infections on the lumbar puncture area.

The nutritional status is calculated based on the criteria of Waterlow: body weight according to age. Data on gender are collected according to CDC standards.

Laboratory Tests

Complete blood count (CBC), CSF analysis and culture were done as routine laboratory tests in Sanglah General Hospital. Human S100B Protein ELISA Kit (E3669Hu) by Bioassay Technology Laboratory was used to examine the level of S100B protein in CSF and serum used. The ELISA test was based on the Biotin double antibody sandwich technology to assay the human S100B protein for in vitro quantitative measurement of S100B in human serum and other biological fluids.

Sample size

The sample size was 80, calculated using the AUC test formula, with AUC estimation of the serum S100B protein 0.80 and CSF S100B protein 0.95, $\alpha = 0.05$ and $\beta = 0.10$ and two-tailed hypothesis. The eligible subjects were enrolled by consecutive sampling.

Statistical Analysis

The analysis of the comparison of AUC and diagnostic accuracy of CSF S100B and serum S100B used MedCalc-version 17.6 programme.

Ethics

The research was approved by the Research Ethics Commission of Medical Faculty of Udayana University and Sanglah Hospital in Denpasar. The ethical clearance number was 129/UN.14.2/Litbang/2016.

RESULTS

Eighty subjects were examined for S100B protein in the CSF and serum. The data distribution was normal. The sample characteristics, the results of CBC and CSF analysis from the unproven and the proven bacterial meningitis patients, are shown in Table 1. The mean age was 29.8 months (SD ± 32.1). The sample was equally distributed to the under 18 months old group and the older than 18 months old (f = 40). The nutritional status was categorized based on the Waterlow criteria (body weight for age). The undernourished subject was 45%, well-nourished 36.2%, and overweight 18.8%.

The clinical symptoms found were seizures, decreased consciousness, sepsis, vomiting, meningeal signs, paresis or paralysis, and headache.

Mean levels of CSF S100B protein were 31.40 ng/L in bacterial meningitis and 29.22 ng/L in non-bacterial meningitis (p = 0.767; 95% CI: -16.69–12.34). Mean levels of serum S100B protein were 114.12 ng/L in bacterial meningitis and 74.12 ng/L in non-bacterial meningitis (p=0.062; 95%CI: -80.34.48–2.01).

Receiver operating characteristic (ROC) curve of CSF S100B was shown in Picture 1. Prevalence of the disease was 26.25%, and the AUC was 0.523 (95% CI: 0.408–0.636) and not significantly different with the diagonal lines (p = 0.768). The Youden Index with the associated criterion of CSF S100B protein > 54.20 ng/L had a sensitivity of 28.57% and specificity 86.44%.

The accuracy of CSF S100B is shown in Table 2. Using cut-point above 54 ng/L, the sensitivity was 28.57 and specificity 86.44%. The positive predictive

Table 1 Characteristics of the subjects

Subject Characteristics	Non-Bacterial meningitis n = 59 mean (± SD)	Proven Bacterial meningitis n = 21 mean (± SD)	Total n = 80 mean (± SD)
Age (in month)	29.9 (33.3)	29.5 (29.2)	29.8(32.1)
Gender, male	36 (61.0%)	11 (52.4%)	47 (58.8%)
Nutritional status			
Undernourished	24 (40.7)	12 (57.1)	36 (45.0)
Well nourished	24 (40.7)	5 (23.8)	29 (36.2)
Overweight	11 (18.6)	4 (19.0)	15 (18.8)
Peripheral blood			
Leucocytes (10^3/uL)	12.1 (6.6)	16.9 (15.3)	13.3(9.8)
Neutrophils (%)	61.5 (18.9)	59.3 (24.8)	60.9(20.5)
Lymphocytes (%)	28.2 (18.2)	29.9 (22.7)	28.7(19.4)
CSF analysis			
Cells	46.6 (115.4)	343.3 (872.5)	124.5 (468.8)
Neutrophils (%)	21.4 (27.2)	25.2 (26.2)	22.4(26.8)
Lymphocytes (%)	78.2 (27.2)	74.7 (26.2)	77.3(26.8)
Glucose (mg/dL)	70.5 (34.5)	59.1 (33.8)	65.5(35.2)
Protein (mg/dL)	62.9 (71.0)	117.2 (93.3)	77.17 (80.55)
CSF S100B (ng/L)	29.22 (27.13)	31.40 (32.81)	29.79 (28.52)
Serum S100B (ng/L)	74.95 (75.84)	114.12 (95.67)	85.23 (82.71)

Table 2 Accuracy of S100BCSF for bacterial meningitis diagnosis

CSF S100B cut-point	Sensitivity (CI 95%)	Specificity (CI 95%)	PPV (CI 95%)	NPV (CI 95%)	+LR	-LR
>2.23	95.24 (76.2-99.9)	1.69 (0.04-9.1)	25.6 (23.8-27.6)	50 (6.1-93.9)	0.97	2.81
>5.22	90.48 (69.6-98.8)	10.17 (3.8-20.8)	26.4 (23.3-29.7)	75 (39.6-93.2)	1.01	0.94
>7.10	80.96 (58.1-94.6)	27.12 (16.4-40.3)	28.3 (23.4-33.9)	80 (60.1-91.4)	1.11	0.7
>8.24	66.67 (43.0-85.4)	35.59 (23.6-49.1)	26.9 (20.5-34.5)	75 (59.9-85.7)	1.04	0.94
>54.21*	28.57 (11.3-52.2)	86.44 (75.0-94.0)	42.9 (22.8-65.6)	77.3 (71.8-81.9)	2.11	0.83
>86.22**	9.52 (1.2-30.4)	98.31 (90.9-100)	66.7 (16.0-95.4)	75.3 (72.6-77.9)	5.62	0.92

*Youden Index, **Optimal Criteria

value (PPV) was 42.9%, and the negative predictive value (NPV) was 77.3%. The likelihood ratio for a positive test (LR+) was 2.11, and the likelihood ratio for a negative test (LR-) was 0.83. The optimal cut off point for CSF S100B was more than 86 ng/L with a specificity exceeding 98%. It means that CSF S100B more than 86 ng/L can be used in helping to establish the diagnosis of bacterial meningitis with high accuracy, but not good enough for screening purposes.

ROC curve of serum S100B can also be seen in Picture 1. The value of the AUC was 0.655

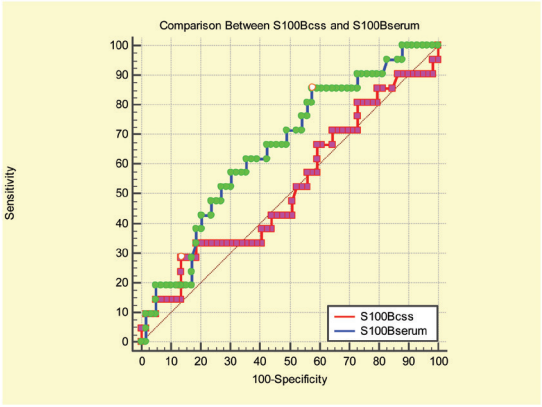
Table 3 The accuracy of serum S100B protein to diagnose bacterial meningitis in children

Serum S100B cut-point	Sensitivity (CI 95%)	Specificity (CI 95%)	PPV (CI 95%)	NPV (CI 95%)	+LR	-LR
>14.79	95.24 (76.2-99.9)	11.86 (4.9-22.9)	27.8 (25.2-30.5)	87.5 (47.8-98.2)	1.08	0.40
>28.61	90.48 (69.6-98.8)	27.12 (16.4-40.3)	30.6 (26.4-35.2)	88.9 (66.7-97.0)	1.24	0.35
>40.67*	85.71 (63.7-97.0)	42.37 (29.6-55.9)	34.6 (28.6-41.2)	89.3 (73.7-96.1)	1.49	0.34
>128.12	33.33 (14.6-57.0)	81.36 (69.1-90.3)	38.9 (22.1-58.8)	77.4 (71.2-82.6)	1.79	0.82
>177.17	19.05 (5.4-41.9)	91.53 (81.3-97.2)	44.4 (19.2-73.0)	76.1 (71.8-79.9)	2.25	0.88
>297.22**	9.52 (1.2-30.4)	98.31 (90.9-100)	66.7 (16.0-95.4)	75.3 (72.6-77.9)	5.62	0.92

*Youden Index, **Optimal Criteria

Table 4 The value of AUC and the difference

Biomarker Parameters	AUC	CI 95%	Difference	SE (CI 95%)	p-value
CSF S100B	0.523	0.636–0.408	0.132	-0.0693 (0.334–0.103)	0.1984
Serum S100B	0.655	0.758–0.541			



Picture 1 CSF and serum S100B ROC in diagnosing bacterial meningitis

(p = 0.023; 95%CI: 0.541–0.758). The value of the Youden Index (J) was 0.2809 at the associated criterion more than 40.67 ng/L.

Table 3 showed that the accuracy of serum S100B protein. Using cut-point above 28 ng/L, the sensitivity was above 90% and with cut-point over 40 ng/L, the sensitivity was 85.71%, specificity 42.37%, PPV 34.6%, NPV 89.3%, LR+ 1.49, and LR- 0.34. The optimal cut-point of serum S100B was above 297 ng/L with specificity 98.31%.

The level of serum S100B more than 28 ng/L can be used for screening purpose with high accuracy in patients suspected of meningitis prior to lumbar puncture. The cut-point serum S100B above 177 ng/L can be used to increase diagnostic value of bacterial meningitis when lumbar puncture could not be done because of contraindications or other reasons.

The AUC difference of the CSF S100B and serum S100B was not significant (p = 0.132; SE = 0.103; 95%CI: -0.069 –0.334; statistic Z = 1.286; p = 0.1984) as shown in Table 4.

DISCUSSIONS

The S100B protein is considered as the C-reactive protein (CRP) of the brain.[6,7] A concentration of extracellular S100B protein may cause trophic effect on the cellular function. But, a pathological concentration can cause glial activation and apoptosis. In vitro, extracellular S100B stimulate proinflammatory cytokine expression and produce neurotoxic effect that induces apoptosis. A recent study showed that S100B on micromolar concentrations could cause apoptosis by interaction with the receptor for advanced glycation end products (RAGE), which can increase reactive oxygen species (ROS), release of cytochrome C, and activate caspase cascade. The high concentrations of S100B may cause neuronal damage through the secretion of nitric oxide (NO) by astrocytes.[4,8]

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Biological half-time of S100B is approximately 30 minutes. Persistent increase of S100B concentration in serum indicates continuous secretion of S100B from injured tissue. The concentration of S100B protein is related to the severity of the brain damage and the outcome after brain damage. It also has been proven as a marker of the blood-brain barrier damage. The concentration of S100B usually becomes normal in 24 hours after acute injury. If S100B protein increases continuously, it may reflect a cellular damage in progress or a secondary cellular injury. Therefore, the increase of S100B concentration not only reflects the tissue damage, but can also exacerbate the damage.[4]

Spinella et al. reported the level of CSF S100B in children with meningitis was significantly different compared to S100B in healthy children.[9] Another study in adult patients with purulent meningoencephalitis showed the comparison of CSF S100B between patients with severe to very severe meningitis and mild to moderate meningitis indicates that the level of S100BCSF can be used to assess the severity of the clinical condition.[10]

Our study is the first diagnostic test of S100B levels in suspected bacterial meningitis cases. The results showed AUC value of serum and CSF S100B in bacterial meningitis was above 0.5. But, there was no significant difference between the two AUC values. It can be inferred that serum S100B has similar diagnostic value to CSF S100B. In a condition where there is a contraindication for lumbar puncture, high concentration of serum S100B may be used in exchange of CSF S100B to confirm the diagnosis. S100B protein may have additional diagnostic value, even though AUC values less than 0.8.

A retrospective study that compared CSF S100B in children with CNS infection (encephalitis and meningitis grouped based on brain parenchymal damage) and a control group without CNS pathology showed CSF S100B in encephalitis was higher than in meningitis.[12] An increased level of CSF S100B had high specificity in predicting a poor outcome in children with CNS infections.[12] Concentration of S100B protein is related to the severity of the brain damage and the outcome after brain damage.[12] Brain injury causes leakage of the S100B into the CSF. The protein enters the blood flow through the damaged blood-brain barrier or CSF circulation.[12] Our study did not examine the involvement of the brain parenchyma. The literature showed mild to extensive damage on the brain parenchyma could be found in bacterial meningitis as well as in non bacterial meningitis. Therefore, it cannot be distinguished whether S100B is increased because of bacterial or non bacterial infection, or inflammation, or whether it is caused by meningitis or not. Increased level of S100B is related to the level of brain parenchyma damage and severity of the illness. Therefore, the CSF S100B appears to be a useful biomarker to determine the prognosis on purulent meningitis or meningoencephalitis.[10,11]

The S100B protein level has a significant negative correlation with age. A higher level of S100B in serum is found before the age of 2 years, the highest level found in neonates, and a basic concentration appears to be stable after 20 years of age. The pattern differences of S100B related to age causing interpretation difficulty.[12-14] In the future, research of S100B should include a certain age group to represent the appropriate population group.

The high level of CSF S100B in bacterial meningitis may reflect infections that have been known to induce the cascade activation of proinflammatory cytokines, which trigger glial cells to produce S100B excessively. The high level of S100B extracellular fluid has been proved to be neurotoxic, triggered apoptosis and neurons cell death mediated by NO.[15]

There have been only a few studies exploring the correlation of serum S100B protein to CNS infection. In our study, the mean serum S100B was higher compared to CSF S100B. In addition, the mean S100B in patients with confirmed bacterial meningitis was higher than in patients with nonbacterial meningitis. However, the difference is not statistically significant. A study showed the increase of serum S100B in bacterial meningitis was higher than in viral meningitis.[16] The highest increase occurred in encephalitis patients. The concentration of serum S100B in patients with CNS infections were generally higher than non-CNS infections.[16]

The limitation of this study is that the control group were subjects with clinical symptoms of meningitis but had negative culture. Further research is needed to prove the role of the S100B protein in bacterial meningitis infections. Research to test the validity of the scoring system of the clinical symptoms by adding the value of serum S100B protein to prove the accuracy of S100B protein for diagnosis of bacterial meningitis may be recommended.

CONCLUSION

The study showed that the S100B in CSF and serum were consistently in a higher concentration in patients with bacterial meningitis with positive culture compared to meningitis with negative culture. There was no significant difference in the AUC value between S100B in CSF and serum. S100B protein has moderate accuracy as a diagnostic biomarker for diagnosing bacterial meningitis in children.

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