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# Utility of S100 Calcium Binding Protein B and Neuron-Specific Enolase in Meningitis

**Reem Mohsen Elkholy**[1], **Gehan Kamal ElSaeed**[1], **Moaz Mohammad Heikal**[2], **Asmaa Hasan Mansour**[2], **Noran Talaat Aboelkhair**[1*]

[1]Clinical Pathology Department, Faculty of Medicine, Menoufia University

[2]Infectious Diseases Department, Clinical Pathology Department, Shebin Elkom Fever Hospital, Ministry of Health

***Corresponding author**: Noran Talaat Aboelkhair, **Mobile:** (+2)01001470343),

**E-mail:** dr_naboelkhair@yahoo.com, **ORCID:** 0000-0002-2390-7364

## ABSTRACT

**Background:** Meningitis represents a critical medical situation that requires appropriate therapy to ensure the patient's survival and recovery. In such cases, differentiation between viral and bacterial meningitis is crucial to determine the most effective course of treatment. S100 calcium binding protein B (S100B) functions as a biomarker for the brain inflammatory process. Neuron-specific enolase (NSE) serves as a biomarker for neuronal stress and neurodegenerative disorders.

**Objectives:** The purpose of this research was to assess S100B and NSE in the serum and cerebrospinal fluid (CSF) of patients with acute meningitis and to assess the diagnostic utility of both markers to identify the cause of meningitis, whether bacterial or viral.

**Patients and methods:** This cross-sectional research was conducted on 44 patients, divided into two groups: bacterial meningitis and viral meningitis. S100B and NSE levels in the serum and CSF were determined by the enzyme-linked immunosorbent assay. Biochemical and cytological examination and cultures of CSF were performed.

**Results:** The paraclinical examinations revealed that the bacterial meningitis group exhibited significantly elevated levels of ESR, CRP, WBC count, neutrophils, and urea compared to the viral meningitis group ($p<0.05$). Furthermore, as compared to the viral meningitis group, the bacterial meningitis group exhibited significantly higher levels of CSF protein, CSF leukocyte count, and neutrophils ($p<0.05$). Bacterial meningitis was associated with significantly higher serum concentrations of S100B than viral meningitis ($p=0.047$).

**Conclusion:** Serum S100B is a simple, non-invasive biomarker that can be used for the early prediction and diagnosis of acute bacterial meningitis rather than acute viral meningitis.

**Keywords:** Neuron-specific enolase, S100 calcium binding protein B, Biomarkers, Viral meningitis, Bacterial meningitis.

## INTRODUCTION

Meningitis is defined as inflammation of the meninges, which wrap and safeguard the brain and spinal cord. This condition is typically evolved by bacterial, viral, fungal, or parasitic infection. The causative organism has an impact on the severity of meningitis [(1)]. Extremely high mortality rates are associated with acute meningitis; therefore, it constitutes a medical emergency. Antibiotic treatment requires prompt identification of bacterial meningitis [(2)]**.** The most prevalent etiological agents of bacterial meningitis in adults are *Neisseria meningitides*, *Streptococcus pneumonia*, and *Haemophilus influenza*. However, in infants, other agents, including group *B Escherichia coli*, *Listeria monocytogenes*, and *Streptococcus* are implicated. The antibiotics administration is critical for the survival and recovery of patients diagnosed with acute bacterial meningitis [(3, 4)].

Distinguishing between viral and bacterial meningitis is crucial in order to determine the most effective course of treatment. Antibiotics remain ineffective against viral infections [(5)]**.** Clinical characteristics and standard laboratory investigations succeed in identifying the causative agent in less than 60% of cases [(6)]**.** Determination of the etiologic bacterial organism occurs in less than one-third of the instances using conventional culturing techniques, however, it can take several days to produce results. Prompt and precise identification of the anticipated cause of the infection is critical to initiating appropriate treatment and improving patient prognosis [(7)]. Identification of blood biomarkers that most accurately predict the etiology of meningitis (viral or bacterial) can facilitate clues towards neuronal damage and bacterial pathogens that can be neutralized by antimicrobial therapy [(8)]**.**

Astrocytic cells in the central nervous system (CNS) contain elevated levels of S100 calcium-binding protein B (S100B); the secretion of S100B by these cells could potentially indicate a glial reaction to metabolic stress, ischemia, or inflammation. Leucocyte extravasation across the blood-brain barrier (BBB) and into the CNS parenchyma is more likely to occur in patients with a high systemic inflammatory burden, such as those who are critically ill [(9)]. Astrocytes are an essential element of the BBB, and the integrity of the barrier is determined by their interaction with the cerebrovascular endothelium. Consequently, if the BBB is disrupted due to inflammation, communication may occur between astrocytes and leucocytes, it could activate astrocytes and result in the release of S100B [(10)]**.** A variety of neurological disorders can be predicted using NSE, which is a biomarker of neuronal stress that is firmly established. A number of neurological diseases, such as hereditary spastic paraplegia, Friedreich ataxia, Alzheimer's disease and uncommon types of



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Parkinson's disease can all be properly identified by a high NSE. Brain lesions subsequent to myocardial infarction and cerebral infarction, as well as stroke, have been found to exhibit a significant correlation with elevated levels of NSE [(11)]. The objective of this research was to assess the diagnostic utility of S100B and NSE markers in determining whether meningitis is caused by a bacterial or viral pathogen, in addition to analyzing the serum and CSF concentrations of these biomarkers in patients affected with the condition.

## PATIENTS AND METHODS

### Study Patients:

This cross-sectional study was conducted from October 2022 to March 2023 at the Clinical Pathology Department, Menoufia University Hospitals, in collaboration with Shebin El-Kom Fever Hospital.

A cluster sample of all patients diagnosed with acute meningitis by clinical presentation and confirmed cytologically and biochemically by lumbar puncture (LP) was admitted to the Infectious Disease Department, Shebin Elkom Fever Hospital, in the period from October 2022 to March 2023. So, this study included forty-four patients.

The patients were divided into two groups: those with bacterial meningitis comprised 27 cases, while those with viral meningitis comprised 17 cases. Individuals who had undergone head trauma or neurodegenerative disorders, ventriculoperitoneal shunt placement, or neurosurgical intervention within one month prior to the onset of meningitis were ineligible to participate in the study. A comprehensive history-taking and clinical examination were performed on the patients, with particular attention given to symptoms indicative of meningeal irritation, including headache, photophobia, vomiting, fever, and irritability. Brudzinski's sign, neck rigidity, Kernig's sign, altered level of consciousness, and focal neurological signs were evaluated as indicators of meningeal irritation. High-pitched crying, weak suckling, and an anterior fontanel bulge were evaluated as symptoms in infants. Particular attention was devoted to the prior administration of antibiotics.

### Methods

### Blood sampling

Eight ml of blood were collected by sterile venipuncture and divided into three tubes: 1) Two ml of whole blood were transferred on an EDTA-containing tube for the complete blood count (CBC) assay, measured by Sysmex1 XN-1000 Automated Haematology Analyzer (Sysmex, Japan). 2) 1.6 ml of whole blood was added to a sodium citrate sterile tube for ESR, which was measured by the Westergren method. 3) 4.4 ml of blood were added to a sterile, plain tube and allowed to clot at 37°C. Serum was separated and used for assays of liver function tests (AST and ALT), kidney function tests (urea and creatinine), which were assayed by the automated chemistry analyzer Beckman Coulter AU 680 (Beckman, USA), and quantification of CRP, which was determined by the nephelometric method using Mispa-i2 (AGAPPE Diagnostics, Switzerland).

### CSF processing and culture

Ten ml of CSF were collected by LP under strict aseptic conditions. It was collected in three sterile containers, one for biochemical analysis, one for bacteriological examination, and one for cell count. The sample was transported immediately at ambient temperature to the laboratory, and if a delay was suspected, it was kept in the incubator or left at room temperature. The CSF was inspected for appearance and processed for differential cell count to detect an approximate percentage of neutrophils and lymphocytes using a slide-counting chamber. Glucose and protein levels were measured in CSF using biochemical methods. A microscopic examination of the Gram-stained smear of the sample was done. The CSF was centrifuged aseptically for 20 minutes at 1500 x g, and the sediment was cultured on chocolate agar with 5–10% $CO_2$, blood agar incubated (aerobically and anaerobically) and on MacConkey agar plates (Oxoid, UK). The culture was seeded heavily, and if no growth was detected after 24 hours, it was re-incubated for another day. Gram staining, colony morphology, and different biochemical reactions, including the oxidase test, were used to identify the colonies [(12)]. The identification was confirmed by the VITEK-2 compact system (BioMerieux, USA).

### Assessment of S100B and NSE levels

Aliquots of serum and CSF from all patients were separated and stored at -20°C for further estimation of S100B and NSE serum levels through the utilization of enzyme-linked immunosorbent assay (ELISA) kits provided by Sunred, China, in accordance with the guidelines of the manufacturer.

### Ethical consent

**The Research Ethics Committee at Menoufia Faculty of Medicine granted approval for this study in accordance with the 1964 Helsinki Declaration (IRB approval number: 10/2022CPATH16). Each participant or the guardian of child participant in the study provided informed consent after being briefed on the study's objectives and methodologies and receiving assurances regarding the confidentiality of their data. This work has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.**

***Statistical analysis:*** The data were tabulated and analyzed using SPSS v 26. Quantitative data, if parametric, were presented as mean and standard deviation (SD) and were

compared by t-test, and if non-parametric, were presented as median and interquartile range and were compared by Mann-Whitney (U) test. Qualitative data were presented as frequency (%). The Chi-Square test was used for comparison between categorical variables. The Fisher exact (FE) test was used to explore the relationship between two qualitative variables if any of estimated cells is less than five. Z test was used to compare between two proportions. The correlation between two non-parametric quantitative data sets was analyzed by Spearman's correlation. The area under the curve (AUC) and receiver operating characteristic (ROC) curve were designed for biomarker accuracy. P value ≤0.05 was considered statistically significant.

## RESULTS

The study comprised a total of 44 patients, consisting of 28 females and 16 males, whose ages ranged from 1 month to 75 years. Acute viral meningitis was identified in 17 patients, while bacterial meningitis was confirmed in 27 cases. Concerning age and gender, there were no significant differences between the groups in this study. The incidence of nausea, vomiting, photophobia, irritability, altered mental status, and Kernig's and Brudzinski's signs were significantly higher in bacterial meningitis. However, the triad of meningeal inflammation, consisting of fever, headache, and neck rigidity didn't show statistically significant distinction between the two groups **(Table 1).**

**Table 1: Demographic and clinical data of the studied groups.**

| Variable | Bacterial meningitis (n=27) | | Viral meningitis (n=17) | | Test of significance | p-value |
|---|---|---|---|---|---|---|
| | No. | % | No. | % | | |
| **Demographic data** | | | | | | |
| **Sex** | | | | | | |
| Male | 9 | 33.3 | 7 | 41.2 | $\chi^2$ =0.277 | 0.598 |
| Female | 18 | 66.7 | 10 | 58.8 | | |
| **Age (Years)** | | | | | | 0.250 |
| Median (IQR) | 23 (6.5-63) | | 7 (1-52) | | U=1.150 | |
| **Clinical data** | | | | | | |
| **Fever** | | | | | | |
| Present | 27 | 100.0 | 17 | 100.0 | ----- | ----- |
| Absent | 0 | 0.0 | 0 | 0.0 | | |
| **Headache** | | | | | | |
| Present | 22 | 81.5 | 11 | 64.7 | FE | 0.289 |
| Absent | 5 | 18.5 | 6 | 35.3 | | |
| **Nausea and vomiting** | | | | | | |
| Present | 27 | 100.0 | 0 | 0.0 | $\chi^2$ =44.000 | **<0.001*** |
| Absent | 0 | 0.0 | 17 | 100.0 | | |
| **Photophobia** | | | | | | |
| Present | 24 | 88.9 | 0 | 0.0 | $\chi^2$ =33.244 | **<0.001*** |
| Absent | 3 | 11.1 | 17 | 100.0 | | |
| **Irritability** | | | | | | |
| Present | 27 | 100.0 | 0 | 0.0 | $\chi^2$ =44.000 | **<0.001*** |
| Absent | 0 | 0.0 | 17 | 100.0 | | |
| **Altered mental status** | | | | | | |
| Present | 23 | 85.2 | 0 | 0.0 | $\chi^2$ =30.342 | **<0.001*** |
| Absent | 4 | 14.8 | 17 | 100.0 | | |
| **Neck rigidity** | | | | | | |
| Present | 22 | 81.5 | 11 | 64.7 | FE | 0.289 |
| Absent | 5 | 18.5 | 6 | 35.3 | | |
| **Kerning's sign** | | | | | | |
| Present | 27 | 100.0 | 3 | 17.6 | $\chi^2$ =32.612 | **<0.001*** |
| Absent | 0 | 0.0 | 14 | 82.4 | | |
| **Brudzinski's sign** | | | | | | |
| Present | 27 | 100.0 | 3 | 17.6 | $\chi^2$ =32.612 | **<0.001*** |
| Absent | 0 | 0.0 | 14 | 82.4 | | |

*: Statistically significant; IQR: Interquartile range; $\chi 2$: Chi-squared test; U: Mann-Whitney test; FE: Fisher exact test

The paraclinical tests of the bacterial meningitis group demonstrated significantly elevated levels of ESR, CRP, and urea in comparison to the viral group. Significant peripheral leukocytosis, accompanied by an increased percentage of neutrophils, was observed in the group with bacterial meningitis. It was characterized by a marked reduction in CSF glucose levels compared to viral cases. Patients diagnosed with bacterial meningitis exhibited notable CSF leukocytosis, with neutrophilic predominance and elevated CSF protein levels, which were significantly higher than in the viral meningitis group. However, viral meningitis showed a significantly higher lymphocytic predominance than bacterial meningitis cases **(Table 2).**

**Table 2: Paraclinical tests results of the studied groups.**

| Variable | Bacterial meningitis (n=27) | Viral meningitis (n=17) | Test of significance | p-value |
|---|---|---|---|---|
| **Blood laboratory tests** | | | | |
| **ESR (mm/hr)** Median (IQR) | 40 (25-55) | 20 (15-30) | U=2.896 | **0.004*** |
| **CRP (mg/dl)** Median (IQR) | 24 (12-48) | 12 (12-24) | U=2.348 | **0.019*** |
| **HB (g/dl)** Mean ±SD | 11.41 ±1.67 | 11.14 ±1.84 | t=0.505 | 0.616 |
| **Platelets ($X10^3/mm^3$)** Median (IQR) | 332 (262-436) | 232 (153-452.5) | U=1.097 | 0.273 |
| **WBCs ($X10^3/mm^3$)** Median (IQR) | 13.30 (11.50-18.20) | 10.50 (8.85-11.50) | U=3.473 | **<0.001*** |
| **PMN (%)** Mean ±SD | 75.40 ±9.02 | 53.79 ±4.49 | t=5.511 | **<0.001*** |
| **AST (U/L)** Median (IQR) | 20 (17-21) | 20 (17.5-21) | U=0.305 | 0.760 |
| **ALT (U/L)** Median (IQR) | 16 (12-18) | 17 (12.5-18) | U=0.218 | 0.827 |
| **Urea (mg/dl)** Median (IQR) | 20 (18-24) | 16 (14-19) | U=2.481 | **0.013*** |
| **Creatinine (mg/dl)** Median (IQR) | 0.8 (0.6-1) | 0.8 (0.55-0.9) | U=1.131 | 0.258 |
| **Glucose (mg/dl)** Mean ±SD | 134.30 ±28.28 | 130.65 ±21.53 | t=0.484 | 0.631 |
| **CSF laboratory tests** | | | | |
| **CSF glucose (mg/dl)** Median (IQR) | 40 (24-45) | 55 (40.5-75) | U=3.033 | **0.002*** |
| **CSF protein (mg/dl)** Median (IQR) | 139 (58-290) | 45 (43.5-55.5) | U=3.042 | **0.002*** |
| **CSF leucocyte count ($total/mm^3$)** Median (IQR) | 1160 (240-3200) | 150 (68.5-205) | U=3.701 | **<0.001*** |
| **CSF cells (No and %)** | | | | |
| Lymphocytes | 1 (3.7%) | 16 (94.1%) | Z=5.68 | **<0.001*** |
| Neutrophils | 26 (96.3%) | 1 (5.9%) | Z=5.68 | **<0.001*** |
| **Culture** | | | | |
| Positive | 8 (29.6%) | 0 (0.0%) | FE | **0.016*** |
| Negative | 19 (70.4%) | 17 (100.0%) | | |

*: Statistically significant, SD: Standard deviation; IQR: Interquartile range; U: Mann-Whitney U test, t: Student t-test, Z: Z test, ESR: Erythrocyte sedimentation rate, CRP: C-reactive protein, HB: Hemoglobin, WBCs: White blood cells, PMN: Polymorphonuclear cells, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, CSF: Cerebrospinal fluid.

Bacterial meningitis was associated with significantly higher levels in serum S100B in comparison to viral meningitis. While there were noticeable differences in the medians of the CSF NSE, serum NSE, and CSF S100B biomarkers between the two studied groups, these differences did not reach statistical significance **(Table 3).**

**Table 3: S100B and NSE values in the CSF and blood of the studied groups.**

| Biomarker | Bacterial meningitis (n=27) | Viral meningitis (n=17) | Test of significance | p-value |
|---|---|---|---|---|
| **Serum S100B (ng/l)** Median (IQR) | 730 (580-910) | 650 (450-680) | U=1.990 | **0.047*** |
| **CSF S100B (ng/l)** Median (IQR) | 1080 (800-1340) | 880 (430-1270) | U=1.314 | 0.189 |
| **Serum NSE (ng/ml)** Median (IQR) | 17.5 (15-21.5) | 16 (11.75-20.5) | U=1.158 | 0.247 |
| **CSF NSE (ng/ml)** Median (IQR) | 45.5 (34-58.5) | 34 (28.5-48.25) | U=1.085 | 0.278 |

*: Statistically significant, IQR: Interquartile range, U: Mann-Whitney U test; S100B: Calcium-binding protein B; NSE: neuron-specific enolase.

A positive correlation was observed between the platelets and the concentration of serum S100B **(Table 4)**, also between the NSE and protein levels of the CSF. However, a significant negative correlation existed between serum NSE and CSF glucose levels **(Table 5).**

**Table 4: Correlation between S100B and other studied parameters.**

| Variable | Serum S100B (ng/l) | | CSF S100B (ng/l) | |
|---|---|---|---|---|
| | ***R*** | **p-value** | ***r*** | **p-value** |
| **ESR (mm/hr)** | 0.045 | 0.770 | 0.107 | 0.488 |
| **CRP (mg/dl)** | -0.001 | 0.996 | 0.121 | 0.433 |
| **HB (g/dl)** | -0.217 | 0.157 | -0.115 | 0.458 |
| **Platelets ($X10^3/mm^3$)** | 0.461 | **0.002*** | -0.015 | 0.925 |
| **WBCs ($X10^3/mm^3$)** | 0.141 | 0.361 | 0.243 | 0.112 |
| **PMN (%)** | 0.148 | 0.337 | 0.173 | 0.263 |
| **AST (U/L)** | 0.237 | 0.121 | -0.016 | 0.919 |
| **ALT (U/L)** | 0.096 | 0.536 | -0.060 | 0.700 |
| **Urea (mg/dl)** | 0.232 | 0.130 | 0.039 | 0.801 |
| **Creatinine (mg/dl)** | 0.017 | 0.913 | 0.137 | 0.375 |
| **Glucose (mg/dl)** | -0.079 | 0.612 | -0.003 | 0.985 |
| **CSF glucose (mg/dl)** | -0.036 | 0.815 | -0.165 | 0.283 |
| **CSF protein (mg/dl)** | -0.020 | 0.895 | -0.008 | 0.957 |
| **CSF leukocyte count (total/$mm^3$)** | -0.045 | 0.770 | 0.087 | 0.574 |
| **Serum S100B (ng/l)** | | | -0.132 | 0.394 |
| **CSF S100B (ng/l)** | -0.132 | 0.394 | | |
| **Serum NSE (ng/ml)** | 0.182 | 0.236 | -0.265 | 0.082 |
| **CSF NSE (ng/ml)** | -0.205 | 0.181 | 0.083 | 0.593 |

*: Statistically significant, *r*: Correlation coefficient, ESR: Erythrocyte sedimentation rate, CRP: C-reactive protein, HB: Hemoglobin, WBCs: White blood cells, PMN: Polymorphonuclear cells, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, CSF: Cerebrospinal fluid, S100B: Calcium-binding protein B.

**Table 5: Correlation between NSE and other studied parameters.**

| Variable | Serum NSE (ng/ml) | | CSF NSE (ng/ml) | |
|---|---|---|---|---|
| | ***R*** | **p-value** | ***r*** | **p-value** |
| **ESR (mm/hr)** | 0.172 | 0.264 | 0.230 | 0.132 |
| **CRP (mg/dl)** | 0.171 | 0.268 | -0.066 | 0.672 |
| **HB (g/dl)** | 0.057 | 0.712 | -0.019 | 0.901 |
| **Platelets ($X10^3/mm^3$)** | 0.215 | 0.162 | 0.088 | 0.571 |
| **WBCs ($X10^3/mm^3$)** | 0.054 | 0.728 | -0.103 | 0.506 |
| **PMN (%)** | -0.020 | 0.896 | -0.063 | 0.686 |
| **AST (U/L)** | -0.149 | 0.336 | -0.233 | 0.128 |
| **ALT (U/L)** | -0.009 | 0.952 | -0.190 | 0.216 |
| **Urea (mg/dl)** | 0.226 | 0.140 | -0.102 | 0.512 |
| **Creatinine (mg/dl)** | 0.022 | 0.886 | -0.264 | 0.084 |
| **Glucose (mg/dl)** | -0.015 | 0.923 | -0.184 | 0.232 |
| **CSF glucose (mg/dl)** | -0.217 | 0.157 | -0.305 | **0.044*** |
| **CSF protein (mg/dl)** | 0.407 | **0.006*** | 0.291 | 0.056 |
| **CSF leukocyte count (total/$mm^3$)** | 0.084 | 0.587 | 0.192 | 0.212 |
| **Serum S100B (ng/l)** | 0.182 | 0.236 | -0.205 | 0.181 |
| **CSF S100B (ng/l)** | -0.265 | 0.082 | 0.083 | 0.593 |
| **Serum NSE (ng/ml)** | | | 0.203 | 0.187 |
| **CSF NSE (ng/ml)** | 0.203 | 0.187 | | |

*: Statistically significant, *r*: Correlation coefficient, ESR: Erythrocyte sedimentation rate, CRP: C-reactive protein, HB: Hemoglobin, WBCs: White blood cells, PMN: Polymorphonuclear cells, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, CSF: Cerebrospinal fluid, NSE: neuron-specific enolase.

The ROC curve demonstrated that serum concentrations of S100B had an AUC of 0.68 at a cutoff value of 700 ng/L. With respect to serum NSE concentrations, the AUC was 0.605, at the cutoff value of 14.25 ng/ml. When the para-clinical and serum S100 tests were combined, they gave an AUC of 0.802 **(Table 6).**

**Table 6: The diagnostic value of the studied serum biomarkers and paraclinical tests for early detection of acute bacterial meningitis against acute viral meningitis**

| Serum parameters | AUC | p-value | Cutoff point | Sensitivity | Specificity |
|---|---|---|---|---|---|
| **Serum S100B** | **0.680** | **0.047*** | **700 ng/l** | **56 %** | **82%** |
| **Serum NSE** | **0.605** | **0.247** | **14.25 ng/ml** | **93 %** | **35 %** |
| **Serum S100B and NSE** | **0.682** | **0.044*** | **-** | **52 %** | **82 %** |
| **Serum S100 and para-clinical tests** | **0.802** | **0.001*** | **-** | **63%** | **88%** |

*: Statistically significant; AUC: Area under curve; S100B: Calcium-binding protein B; NSE: neuron-specific enolase

As regards the S100B levels in the CSF, the AUC was 0.619 at the cutoff value of 625 ng/l. Meanwhile, NSE levels in CSF yielded an AUC of 0.680 at a cutoff of 38.5 ng/mL, whereas combining CSF S100 and para-clinical tests revealed an AUC of 0.821 **(Table 7).**

**Table 7: The diagnostic value of the studied CSF biomarkers and paraclinical tests for early detection of acute bacterial meningitis against acute viral meningitis.**

| CSF parameters | AUC | p-value | Cutoff point | Sensitivity | Specificity |
|---|---|---|---|---|---|
| **CSF S100B** | **0.619** | **0.189** | **625 ng/l** | **89 %** | **41%** |
| **CSF NSE** | **0.598** | **0.278** | **38.5 ng/ml** | **70 %** | **71 %** |
| **CSF S100B and NSE** | **0.619** | **0.189** | **-** | **93 %** | **41 %** |
| **CSF para-clinical tests** | **0.821** | **<0.001*** | **-** | **63%** | **100%** |

*: Statistically significant; AUC: Area under curve; S100B: Calcium-binding protein B; NSE: neuron-specific enolase.

Biomarkers from both CSF and blood were used simultaneously in the models that best predicted bacterial infection. The association of serum & and CSF S100B levels generated a ROC curve where the sensitivity was 96%, the specificity was 35%, and the AUC was 0.682. However, the association of serum & and CSF NSE levels generated a ROC curve where the sensitivity was 85%, the specificity was 47%, and the AUC was 0.623 **(Figure 1**).

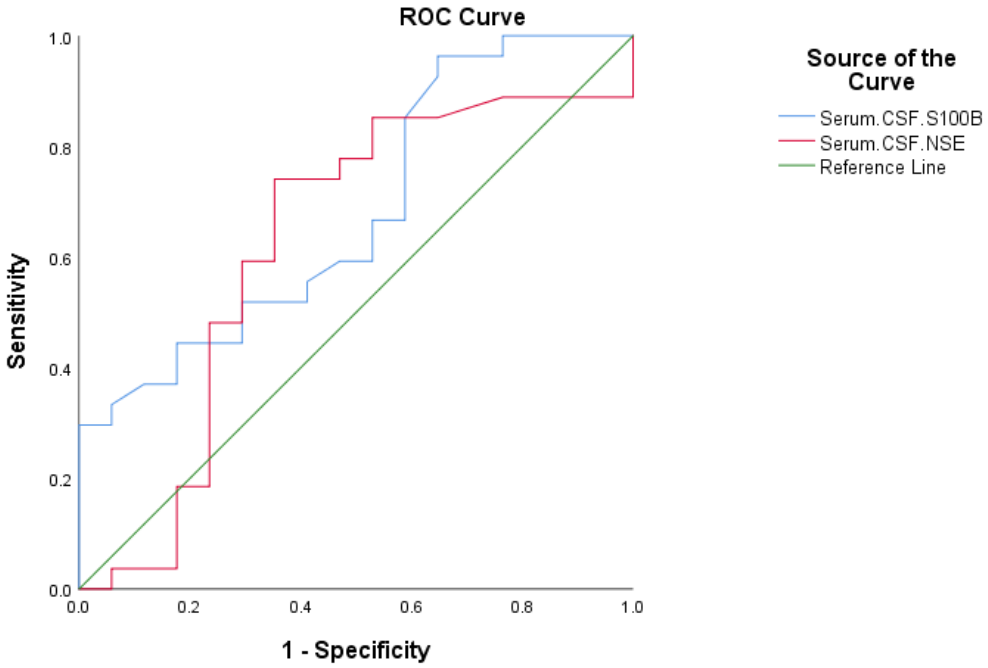


**Figure 1: ROC curve for combined serum and CSF biomarkers for early detection of acute bacterial meningitis against acute viral meningitis.**

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## DISCUSSION

Meningitis is a prevalent disease in Egypt, where the prevalence of bacterial meningitis has significantly elevated [13]. Failure to diagnose or treat acute bacterial meningitis (ABM) in its early stages may result in fatal consequences. Viral meningitis typically has a favorable prognosis and resolves spontaneously within one to two weeks [14]. Distinguishing between bacterial and viral meningitis presents a complicated task. Bacterial meningitis requires rapid diagnosis in order to decrease mortality and neurological complications. Due to the atypical presentation and the low sensitivity of standard diagnostic microbiology, the diagnosis may be delayed [6]. In CNS infections, the assessment of CSF parameters via repetitive LP is essential for both diagnosis and therapeutic strategy control. Neuronal and glial lesion quantity influence the clinical course and prognosis. Serum parameters monitoring may aid in decreasing the incidence of LP in meningitis patients [15]. The purpose of this research was to assess the diagnostic utility of S100B and NSE biomarkers in the serum and CSF in determining whether the infection was bacterial or viral in nature.

Bacterial meningitis comprised 61.4% of the population under investigation in the current study, while viral meningitis comprised the remaining 38.6%. There was variation in the proportion of bacterial to viral cases across multiple studies [16, 17]. This difference could potentially be attributed to differences in the study sites, times, and administration of anticapsular vaccines [18].

A statistically significant difference was observed between both groups in terms of the incidence of photophobia, irritability, nausea, and vomiting, with the bacterial group exhibiting a higher frequency. The impact of altered mental status was more pronounced in patients with bacterial meningitis compared to those with viral meningitis. As stated earlier, focal neurologic deficits are uncommon in cases of aseptic meningitis [19]. Typically, the duration until hospital arrival and the severity of the disease are correlated with these neurologic manifestations. Patients with bacterial meningitis exhibited statistically significant Kernig's and Brudzinski's signs. However, **Fouad *et al.*** observed that Brudzinski's sign and nuchal rigidity could not effectively differentiate between patients who had meningitis caused by bacteria compared to those who did not [20].

The paraclinical investigations revealed significantly higher median values for CRP and ESR in the bacterial group than viral meningitis. In the bacterial meningitis group, there was a significant increase in peripheral leukocytosis, accompanied by a high percentage of neutrophils. Plasma inflammatory markers, including CRP and peripheral blood leukocyte count, can be of great assistance in differentiating bacterial meningitis from non-bacterial meningitis [21]. As a further method for differentiating bacterial meningitis from viral meningitis, CRP was suggested in a meta-analysis of 35 studies [22]. In comparison to the viral meningitis group, our patients with bacterial meningitis exhibited significant CSF leukocytosis with neutrophil predominance and elevated CSF protein.

Contrary to bacterial meningitis, which was characterized by the predominance of neutrophils, viral meningitis was characterized by clear CSF, elevated lymphocytes, and normal or slightly elevated protein. Early during bacterial meningitis, CSF leukocyte counts below 1,000/mm$^3$ may be detected in patients who have undergone partial treatment, immune-suppressed patients, and leucopenic patients [23]. In contrast to viral cases, patients with bacterial meningitis exhibited a significant decrease in CSF glucose concentration. In cases of enterovirus, HSV-2, and VZV meningitis, CSF glucose levels may be reduced. However, in the case of aseptic meningitis, CSF glucose levels are typically normal. For the diagnosis of bacterial meningitis, a prior investigation demonstrated that a CSF-to-serum glucose ratio below 0.4 had 80% sensitivity and 98% specificity [24].

Our findings indicated a minor incidence of culture-positive disease, which could be attributed to multiple factors, such as the pre-admission administration of antibiotics, a prevalent issue in numerous countries [25]. Furthermore, we employ a broad clinical case definition to enhance the surveillance's sensitivity. Additional logistical challenges associated with the rapid processing of CSF cultures may have significantly reduced the yield of CSF cultures. This was in agreement with **Men *et al.,*** who reported that the utilization of antibiotics and the size of the sample are constraints on bacterial identification and culture methods. The majority of bacteria are undetectable, leading to negative culture results [26]. Although C-reactive protein and ESR levels have been employed to evaluate the inflammatory syndrome and provide some degree of differentiation, they lack the specificity required to identify neuromeningeal infections [27]. Therefore, we were interested in analysing the blood and CSF of the patients for more specific indicators of neuromeningeal affection and inflammatory markers, such as S100B and NSE.

According to the study results, serum S100B levels were significantly elevated in cases of bacterial meningitis relative to viral meningitis. Furthermore, CSF S100B, serum NSE, and CSF NSE were all greater in bacterial meningitis compared to viral meningitis; however, this difference was not statistically significant. In accordance with these results, **Lins *et al.*** observed significant differences in S100B levels in serum and CSF between viral and bacterial meningitis; where the highest levels in both CSF and serum were detected during the initial LP upon admission and subsequently decreased and returned to normal levels. They stated that NSE levels in serum and CSF did not increase significantly [28]. In patients where sequential LPs are undesirable or contraindicated, S-100B levels in CSF and serum could be utilized as a monitoring parameter to evaluate the activity and severity of the disease process, as well as to track the efficacy of treatment [29]. Also, **Obreja *et al.*** revealed that the group with bacterial meningitis had significantly higher blood levels of S100B than the group with viral meningitis [21].

The study of **Mahalini *et al.*** [30] partially agreed with our results as they demonstrated that the concentrations of S100B in serum and CSF were significantly higher in patients with bacterial meningitis who had a positive culture compared to those who had a negative culture. Moreover, they found no significant difference in the AUC value between S100B in serum and CSF. They reported that the diagnostic biomarker S100B protein has a moderate degree of accuracy in identifying bacterial meningitis in children. In addition, **Wang *et al.*** [31] discovered that the concentrations of S100 protein and NSE were significantly lower in the aseptic meningitis group compared to the purulent meningitis group. This suggests that the degree of neuronal damage is considerably less severe in the aseptic meningitis group.

Meningitis is characterised by pathophysiological mechanisms that include a systemic inflammatory reaction leading to leukocyte invasion into the CNS via the BBB, as well as local inflammatory responses induced by resident macrophages and glial cells that induce apoptosis. Patients who exhibited focal lesions on CT or MRI demonstrated increased S-100B levels, suggesting that the release of S-100B into CSF and serum is due to cerebral damage [28]. Although the clinical utility of NSE as a prognostic biomarker is restricted by its lack of specificity, systemic NSE levels can be influenced by various factors. For instance, hemolysis, which may develop in cases of traumatic brain injury and ABM complicated by a septic state, can affect NSE levels [32].

In the present study, serum concentrations of S100B had 56% sensitivity, 82% specificity, and an AUC of 0.68 at a cutoff value of 700 ng/L. In addition, regarding S100B levels in the CSF, the AUC was 0.619, sensitivity was 89%, and specificity was 41% at the cutoff value of 625 ng/l. Also, **Mahalini *et al***. [30] deemed that S100B levels of 177 ng/L or higher in blood (98% specificity, 19% sensitivity) and 54 ng/L or higher in CSF were useful. An AUC of 0.655 was reported for S100B in blood, and 0.523 was reported for S100B in CSF. Serum S100B may also be employed as a supplementary diagnostic tool in cases where LP is not feasible to validate the diagnosis of bacterial meningitis in children. They reported that S100B in the CSF and serum possess promising utility and high specificity in confirming the diagnosis of bacterial meningitis in children. By comparison, **Obreja *et al.*** [21] determined that S100B levels in CSF had an AUC of 0.591 with 50% specificity and 63.88% sensitivity at a cutoff value of 540.99 ng/mL, whereas those in the patients' blood had an AUC of 0.652 with 57.14% specificity and 65.90% sensitivity at a cutoff

value of 36.24 ng/ml. However, **Wang *et al.*** [31] noticed the CSF S100 threshold at 0.4315 had a specificity of 100% and a sensitivity of 92.1%.

The results of this study show that regarding serum NSE concentrations, the AUC was 0.605, sensitivity was 93%, specificity was 35%, and the cutoff value was 14.25 ng/ml. Meanwhile, NSE levels in CSF yielded 70% sensitivity, 71% specificity, and an AUC of 0.680 at a cutoff of 38.5 ng/ml. Meanwhile**, Wang *et al.*,** [31] discovered that the threshold for CSF NSE was 9.325, with a specificity of 90% and a sensitivity of 92.1%. They stated that aseptic meningitis and purulent meningitis may be distinguished through the combined detection of NSE and S100B levels in the CSF.

Extracellular S100B protein concentrations can have a trophic effect on cellular function; nevertheless, pathological concentrations can induce apoptosis and glial stimulation. Apoptosis is induced in vitro through a neurotoxic effect of extracellular S100B, which also stimulates the expression of proinflammatory cytokines [33]**.** By interacting with the receptor for advanced glycation end products, micromolar concentrations of S100B have the potential to induce apoptosis, increase the levels of reactive oxygen species, release cytochrome C, and activate the caspase cascade. Excessive concentrations of S100B may induce astrocytes to secrete nitric oxide (NO), which may cause neuronal damage [34]**.** S100B has an approximately 30-minute biological half-life; therefore, a sustained increase in S100B concentration in the bloodstream indicates that S100B is continuously secreted from the injured tissue. There is a relationship between the concentration of S100B protein and both the extent of brain damage and the prognosis subsequent to brain damage. In addition, it was demonstrated to indicate BBB damage [32]**.** In cases of bacterial meningitis, the elevated concentration of CSF S100B may indicate the presence of infections that have been linked to the activation of proinflammatory cytokine cascades, thereby stimulating glial cells to generate an excessive amount of S100B. It has been demonstrated that high concentrations of S100B in extracellular fluid are neurotoxic, inducing apoptosis and NO-mediated neuronal cell death [35]**.** Relative heterogeneity and small sample sizes impeded the interpretation and analysis of correlations between biochemical and clinical outcome data, constituting study limitations. We recommend replicating in a larger group of individuals to confirm the role of S100B and other biomarkers in early diagnosis and prediction of ABM in order to introduce them in routine tests for patients suspected of meningitis and critically ill meningitis patients instead of repeated LP or where LP is contraindicated.

**Conclusion:**

Serum S100B was significantly higher in ABM than in acute viral meningitis, particularly when paired with paraclinical tests that enable prompt confirmation of the bacterial etiology and guide the decision to treat patients with anticipated meningitis before conventional culture results are obtained. So, S100B is a simple serum biomarker for the diagnosis and early prediction of ABM.

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**REFERENCES**

1. **Le D, Liaw D, Naterelli N *et al.* (2023):** Recent developments in the treatment of bacterial meningitis. Infectious Diseases Drug Delivery Systems, 19: 349-381.
2. **Martín-Cerezuela M, Aseginolaza-Lizarazu M, Boronat-García P *et al.* (2023):** Severe community-acquired Streptococcus pneumoniae bacterial meningitis: clinical and prognostic picture from the intensive care unit. Critical Care, 27(1): 72.
3. **Nauclér P, Huttner A, Van Werkhoven C *et al.* (2021):** Impact of time to antibiotic therapy on clinical outcome in patients with bacterial infections in the emergency department: implications for antimicrobial stewardship. Clinical Microbiology and Infection, 27(2): 175-181.
4. **Tigabu A, Jember A, Nega T *et al.* (2021):** Bacterial meningitis among adult patients at University of Gondar Comprehensive Specialized Referral Hospital. Infection and Drug Resistance, 15: 565-574.
5. **Goodlet K, Tan E, Knutson L *et al.* (2021):** Impact of the Film Array meningitis/encephalitis panel on antimicrobial duration among patients with suspected central nervous system infection. Diagnostic Microbiology and Infectious Disease, 100(4): 115394.
6. **Roux A, Houcke S, Sanna A *et al.* (2019):** Clinical features, diagnosis, and outcome of encephalitis in French Guiana. The American Journal of Tropical Medicine and Hygiene, 100(2): 452.
7. **Sulaiman T, Salazar L, Hasbun R (2017):** Acute versus subacute community-acquired meningitis: analysis of 611 patients. Medicine, 96(36): e7984
8. **Kyriazopoulou E, Giamarellos-Bourboulis E (2022):** Antimicrobial stewardship using biomarkers: Accumulating evidence for the critically ill. Antibiotics, 11(3): 367.
9. **Rauf A, Badoni H, Abu-Izneid T *et al.* (2022):** Neuroinflammatory markers: key indicators in the pathology of neurodegenerative diseases. Molecules, 27(10): 3194.
10. **Zhang W, Zhu L, An C *et al.* (2020):** The blood brain barrier in cerebral ischemic injury–Disruption and repair. Brain Hemorrhages, 1(1): 34-53.
11. **Haque A, Polcyn R, Matzelle D *et al.* (2018):** New insights into the role of neuron-specific enolase in neuro-inflammation, neurodegeneration, and neuroprotection. Brain Sciences, 8(2): 33.
12. **Kennedy W, Chang S, Purdy K *et al.* (2007):** Incidence of bacterial meningitis in Asia using enhanced CSF testing:

polymerase chain reaction, latex agglutination and culture. Epidemiology and Infection, 135(7): 1217-1226.

13. **Abdelkader A, Gad A, Mohamed E *et al.* (2023):** The Efficacy of bacterial meningitis score in predicting bacterial meningitis at Mansoura Fever Hospital. Afro-Egyptian Journal of Infectious and Endemic Diseases, 13(1): 43-52.
14. **de Almeida S, Furlan S, Cretella A *et al.* (2020):** Comparison of cerebrospinal fluid biomarkers for differential diagnosis of acute bacterial and viral meningitis with atypical cerebrospinal fluid characteristics. Medical Principles and Practice, 29(3): 244-254.
15. **Tumani H, Petereit H, Gerritzen A *et al.* (2020):** S1 guidelines "lumbar puncture and cerebrospinal fluid analysis" (abridged and translated version). Neurological Research and Practice, 2: 1-28.
16. **Chanteau S, Dartevelle S, Mahamane A *et al.* (2006):** New rapid diagnostic tests for Neisseria meningitidis serogroups A, W135, C, and Y. PLoS Medicine, 3(9): e337.
17. **Amarilyo G, Alper A, Ben-Tov A *et al.* (2011):** Diagnostic accuracy of clinical symptoms and signs in children with meningitis. Pediatric Emergency Care, 27(3): 196-199.
18. **Bertolini D, Costa L, van der Heijden I *et al.* (2012):** Immunogenicity of a meningococcal serogroup C conjugate vaccine in HIV-infected children, adolescents, and young adults. Vaccine, 30 (37):5482-6.
19. **Davis L (2018):** Acute bacterial meningitis. Contin Lifelong Learn Neurol., 24 (5): 1264-83.
20. **Fouad R, Khairy M, Fathalah W *et al.* (2014):** Role of clinical presentations and routine CSF analysis in the rapid diagnosis of acute bacterial meningitis in cases of negative gram-stained smears. Journal of Tropical Medicine, 2014: 1-7.
21. **Obreja M, Miftode E, Stoleriu I *et al.* (2022):** Heparin-binding protein (HBP), neutrophil gelatinase-associated lipocalin (NGAL) and S100 calcium-binding protein B (S100B) can confirm bacterial meningitis and inform adequate antibiotic treatment. Antibiotics, 11(6): 824.
22. **Gerdes L, Jørgenseny P, Nexø E *et al.* (1998):** C-reactive protein and bacterial meningitis: a meta-analysis. Scandinavian Journal of Clinical and Laboratory Investigation, 58(5): 383-394.
23. **Brouwer M, Van De Beek D (2018):** Epidemiology of community-acquired bacterial meningitis. Current Opinion in Infectious Diseases, 31(1): 78-84.
24. **Tunkel A, Hartman B, Kaplan S *et al.* (2004):** Practice guidelines for the management of bacterial meningitis. Clinical Infectious Diseases, 39(9): 1267-1284.
25. **Wagner K, Springer B, Pires V *et al.* (2018):** Pathogen identification by multiplex LightMix real-time PCR assay in patients with meningitis and culture-negative cerebrospinal fluid specimens. Journal of Clinical Microbiology, 56(2): 10-1128.
26. **Men X, Zhao G, Zhao W *et al.* (2020):** Pathogen identification in culture-negative cerebrospinal fluid specimens of patients with purulent meningitis using next-generation sequencing technology. International Journal of Clinical and Experimental Pathology, 13(9): 2427.
27. **Al-Qahtani S, Shati A, Alqahtani Y *et al.* (2022):** Etiology, clinical phenotypes, epidemiological correlates, laboratory biomarkers and diagnostic challenges of pediatric viral meningitis: descriptive review. Frontiers in Pediatrics, 10: 923125.
28. **Lins H, Wallesch C, Wunderlich M (2005):** Sequential analyses of neurobiochemical markers of cerebral damage in cerebrospinal fluid and serum in CNS infections. Acta Neurologica Scandinavica, 112(5): 303-308.
29. **Undén J, Christensson B, Bellner J *et al.* (2004):** Serum S100B levels in patients with cerebral and extracerebral infectious disease. Scandinavian Journal of Infectious Diseases, 36(1): 10-13.
30. **Mahalini D, Sudewi A, Soetjiningsih S *et al.* (2018):** Accuracy of cerebrospinal fluid and serum S100B protein in the diagnosis of bacterial meningitis in children. Bali Medical Journal, 7(3): 601-606.
31. **Wang J, Chen S, Wang X *et al.* (2019):** Value of NSE and S100 protein of Kawasaki disease with aseptic meningitis in infant. Open Life Sciences, 14(1): 358-362.
32. **Grønhøj M, Sejbaek T, Hansen R *et al.* (2021):** Serum levels of neurofilament light chain, neuron-specific enolase and S100 calcium-binding protein B during acute bacterial meningitis: a prospective cohort study. Infectious Diseases, 53(6): 409-419.
33. **D'Ambrosi N, Milani M, Apolloni S (2021):** S100A4 in the physiology and pathology of the central and peripheral nervous system. Cells, 10(4): 798.
34. **Tsoporis J, Izhar S, Leong-Poi H *et al.* (2010):** S100B interaction with the receptor for advanced glycation end products (RAGE): a novel receptor-mediated mechanism for myocyte apoptosis postinfarction. Circulation Research, 106(1): 93-101.
35. **Prager O, Friedman A, Nebenzahl Y (2017):** Role of neural barriers in the pathogenesis and outcome of Streptococcus pneumoniae meningitis. Experimental and Therapeutic Medicine, 13(3): 799-809.