sciendo Revista Română de Medicină de Laborator 2023;31(2):107-112 RESEARCH ARTICLE
DOI:10.2478/rrlm-2023-0009

Diagnostic values of glial fibrillary acidic protein, neuron-specific enolase and protein S100β for sepsis-associated encephalopathy

Zhigang Cao[1], Xiang Huang[2], Feixiang Chen[3*]

1. Intensive Care Unit, Affiliated Renhe Hospital of China Three Gorges University, China
2. Intensive Care Unit, Changshu Hospital Affiliated to Nanjing University of Chinese Medicine, China
3. Intensive Care Unit, 903rd Hospital of PLA, Hangzhou 310013, China

ABSTRACT

Background: To investigate the expressions of glial fibrillary acidic protein (GFAP), neuron-specific enolase (NSE) and protein S100β and their diagnostic values for sepsis-associated encephalopathy (SAE).

Methods: One hundred patients with sepsis treated from August 2021 to August 2022 were included. They were assigned to a sepsis group (n=65) and an SAE group (n=35), while 50 healthy volunteers physically examined in the same period were enrolled as a control group. The levels of GFAP and NSE were detected by enzyme-linked immunosorbent assay, and that of S100β was determined by transmitted immunoturbidimetric assay. The expressions of GFAP, NSE and S100β in patients with SAE were detected, and their correlations and diagnostic values were analyzed.

Results: Compared to patients with mild and moderate SAE, those with severe SAE had higher levels of GFAP, NSE and S100β (P<0.05). The levels of GFAP, NSE and S100β were higher in coma patients than those with consciousness disturbance, and they were higher in patients with a poor prognosis than those with a good prognosis (P<0.05). Positive correlations were identified between GFAP and NSE (r=0.573, P=0.001), GFAP and S100β (r=0.468, P=0.005), and NSE and S100β (r=0.540, P=0.001) expression in patients with SAE. Compared with GFAP, NSE and S100β alone, their combination had higher sensitivity and lower specificity for diagnosing SAE (P<0.05).

Conclusions: There are correlations among GFAP, NSE and S100β, and the combined detection of these three indicators is highly valuable for the diagnosis of SAE.

Keywords: glial fibrillary acidic protein, neuron-specific enolase, protein S100β, diagnostic value, sepsis-associated encephalopathy

Received: 2 December 2022; Accepted: 3 March 2023; Published: 3 March 2023

INTRODUCTION

Sepsis is a common systemic inflammatory response syndrome resulting from microbial infections and mostly manifested as rapid heartbeat, high fever and tachypnea [1,2]. If the condition of patients with sepsis is not promptly controlled, the spread of inflammation may cause a series of complications, the more common of which is sepsis-associated encephalopathy (SAE) [3,4]. Epidemiological data show that about 70% of patients with sepsis develop SAE, whose symptoms mainly include changes in consciousness and cognition, giving rise to irreversible cognitive impairment, or even greatly affecting the patients' prognosis [5-7]. The annual number of new cases of SAE has stayed high in China, and such patients have a relatively high mortality rate. Since there is still no effective therapeutic regimen in clinic due to its complex pathogenesis, investigating the pathogenesis of SAE and exploring new diagnosis and treatment targets are essential for diagnosing, treating, and improving the prognosis of SAE. In this study, therefore, the levels of glial fibrillary acidic protein (GFAP), neuron-specific enolase (NSE) and protein S100β in patients with SAE were detected to analyze their correlations and diagnostic value for SAE.

MATERIALS AND METHODS

General data

One hundred patients with sepsis who were treated in our hospital from August 2021 to August 2022 were included and assigned to the sepsis group (n=65) and SAE group (n=35), according to the presence or absence of

* Correspondence to: Feixiang Chen, Intensive Care Unit, 903rd Hospital of PLA, Hangzhou 310013, China. E-mail: chenfx903hpla@peak-edu.cn

SAE. In the sepsis group, there were 31 males and 34 females aged (57.69±8.69) years, with an average body mass index (BMI) of (22.72±2.37) kg/m^2. Thirty-nine patients had a hypertension history, and there were 26 patients with a drinking history and 30 patients with a smoking history. The SAE group was composed of 15 males and 20 females aged (58.51±8.25) years, with an average BMI of (23.51±3.16) kg/m^2. Twenty-one patients had a hypertension history, and there were 12 patients with a drinking history and 17 patients with a smoking history. Moreover, 50 healthy volunteers physically examined in our hospital during the same period were enrolled in the control group, including 23 males and 27 females aged (56.37±8.13) years, with an average BMI of (23.62±2.11) kg/m^2. There were 32 participants with a hypertension history, 19 participants with a drinking history, and 24 participants with a smoking history. No significant differences were observed in general data among the three groups (P>0.05), which were comparable (Table 1). Informed consent was obtained from the family members of all subjects in this study.

Inclusion criteria were as follows: 1) subjects (sepsis and SAE groups) who met the diagnostic criteria for sepsis in the ***Guidelines for Emergency Treatment of Sepsis and Septic Shock in China (2018)***, 2) those (SAE group) who were diagnosed with SAE in our hospital, and 3) those (control group) who had no history of cerebrovascular diseases. All subjects had complete medical records.

Exclusion criteria involved: 1) subjects with incomplete medical records, 2) patients with a history of cerebrovascular surgery, 3) those with a history of sepsis, cerebral hemorrhage or intracranial infection, 4) those with immune diseases, 5) those with heart or lung dysfunction, or 6) those with head or neck malignancies.

Collection of samples

On the day of the medical visit, 8 mL of fasting venous blood was drawn from each subject in the morning, centrifuged at 3,000 rpm for 15 min and stored at -30°C for examination.

Detection of GFAP and NSE levels by enzyme-linked immunosorbent assay (ELISA)

The ELISA plate was labeled, the standards were diluted, and the levels of GFAP and NSE were detected using a microplate reader. To be specific, blank well 1, blank well 2 and test well were set, the stop buffer and development reagent were added to blank well 1, the diluted standard was added to blank well 2, and the serum specimen and antibody were added to the test well. Next, the membrane was blocked, shaken and incubated in an incubator. With blank well 1 as the control, the GFAP and NSE levels were calculated by measuring absorbance values at 450 nm using an ELISA analyzer (model: ML-dr3518, Shanghai Enzyme-linked Biotechnology Co., Ltd.).

Detection of S100β level by transmitted immunoturbidimetric assay

Three clean test tubes labeled as reference A, reference B, and testing tube were each added with 350 μL of buffer solution. Then the distilled water, standard solution and 20 μL of serum specimen were added, shaken well and stored in an incubator at 37°C for 20 min. Subsequently, the S100β level in the specimen was detected by the turbidimetric assay.

Analysis of indicators

The patients with SAE were grouped according to the severity, consciousness state and prognosis, and the correlations of GFAP, NSE and S100β levels with the clinical features of patients were analyzed. Criteria for assessing SAE severity: The Glasgow Coma Scale (GCS) was used, with a total score of 15 points. 9-15 points: mild and moderate SAE; 0-8 points: severe SAE. Criteria for assessing consciousness state: GCS was used, with a total score of 15 points. 8-15 points: consciousness disturbance; 0-7 points: coma. Criteria for assessing prognosis: The prognosis was evaluated according to the "Guidelines for the Treatment of Severe Sepsis/Septic Shock in China (2014)" [8]. Good prognosis: with symptom alleviation after treatment; poor prognosis: without symptom alleviation or even with aggravation after treatment.

Statistical analysis

SPSS 26.0 software was employed for statistical analysis. The measurement data were expressed as mean ± standard deviation (`x ± s) and compared among groups by the F-test, and compared between two groups by the independent-samples t-test. Pearson's analysis was used to determine correlations. The receiver operating characteristic (ROC) curve was plotted to obtain the area under the curve (AUC), confidence interval (CI), sensitivity and specificity. P<0.05 was considered statistically significant.

RESULTS

General data of subjects

There were no significant differences in general data (including gender ratio, age, average BMI, hypertension history, drinking history and smoking history) among the three groups (P>0.05). The levels of white blood cell (WBC) and C-reactive protein (CRP) in sepsis and

SAE groups were higher than those in the control group (P<0.05) (**Table 1**).

GFAP, NSE and S100β levels

Sepsis and SAE groups displayed higher levels of GFAP, NSE and S100β than the control group (P<0.05). The levels of GFAP, NSE and S100β in the SAE group were higher than those in the sepsis group (P<0.05) (**Table 2**).

Correlations of GFAP, NSE and S100β levels with clinical features of patients with SAE

In contrast to patients with mild and moderate SAE, those with severe SAE showed higher levels of GFAP, NSE and S100β, and the differences were of statistical significance (P<0.05). The levels of GFAP, NSE and S100β were higher in coma patients than those with consciousness disturbance (P<0.05). Besides, the levels of GFAP, NSE and S100β in patients with a poor prognosis were higher than those with a good prognosis (P<0.05) (**Table 3**).

Correlations of GFAP, NSE and S100β levels in patients with SAE

The correlations of GFAP, NSE and S100β levels in patients with SAE were analyzed. The results revealed that there were positive correlations between GFAP and NSE (*r*=0.573, P=0.001), GFAP and S100β (*r*=0.468, P=0.005), and NSE and S100β (*r*=0.540, P=0.001) (**Figure 1**).

Diagnostic values of GFAP, NSE and S100β for SAE

In comparison with the single detection of GFAP, NSE and S100β, the combined detection of these three indicators exhibited higher sensitivity and lower specificity in diagnosing SAE (P<0.05) (**Table 4** and **Figure 2**).

DISCUSSION

As a common complication of sepsis, SAE characterized by a high incidence rate, high severity and a poor prog-

Table 1. General data of subjects in the three groups

General data	Control group (n=50)	Sepsis group (n=65)	SAE group (n=35)	F/χ2	P
Gender (male/female)	23/27	31/34	15/20	0.213	1.035
Age (year)	56.37±8.13	57.69±8.69	58.51±8.25	0.912	0.328
BMI (kg/m2)	23.62±2.11	22.72±2.37	23.51±3.16	1.326	0.211
Hypertension history	32 (64.00)	39 (60.00)	21 (60.00)	0.219	1.028
Drinking history	19 (38.00)	26 (40.00)	12 (34.29)	0.321	0.985
Smoking history	24 (48.00)	30 (46.15)	17 (48.57)	0.072	1.365
WBC ($\times10^9$/L)	7.35±1.37	15.32±2.31	16.77±2.15	25.316	0.001
CRP (mg/L)	8.32±1.21	68.35±7.33	75.12±7.91	62.352	0.001

Table 2. GFAP, NSE and S100β levels in the three groups of subjects (`x ± s)

Group	Number of cases (n)	GFAP (μg/L)	NSE (ng/mL)	S100β (ng/L)
Control	50	16.58±2.34	8.26±1.58	58.63±6.51
Sepsis	65	42.69±5.02	19.81±2.26	151.32±17.28
SAE	35	123.16±14.33	25.33±2.57	203.51±21.35
F		49.136	19.537	22.652
P		0.001	0.001	0.001

Table 3. Correlations of GFAP, NSE and S100β levels with clinical features of patients with SAE (`x ± s)

Clinical feature		Number of cases (n)	GFAP (μg/L)	NSE (ng/mL)	S100β (ng/L)
Severity	Mild and moderate	12	93.31±11.29	23.26±2.31	183.62±19.63
	Severe	23	139.25±15.66	27.49±2.82	222.51±21.77
t			8.989	4.464	5.180
P			0.001	0.001	0.001
Consciousness state	Consciousness disturbance	15	95.37±10.53	22.40±2.23	185.62±18.67
	Coma	20	137.51±15.21	28.56±2.91	220.39±20.64
t			9.190	6.824	5.134
P			0.001	0.001	0.001
Prognosis	Good	14	90.27±10.51	22.61±2.19	182.35±18.62
	Poor	21	140.33±16.21	28.11±2.85	223.16±22.19
t			10.140	6.066	5.627
P			0.001	0.001	0.001

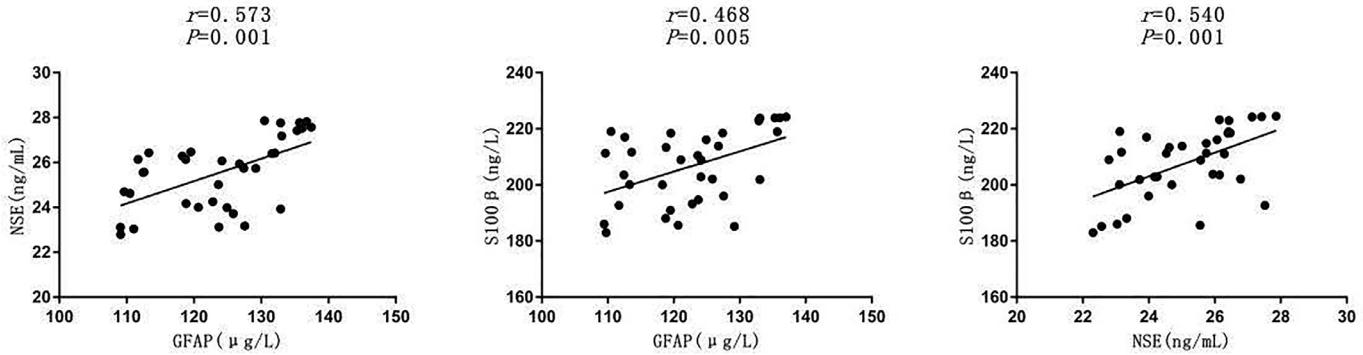

Fig. 1. Correlations of GFAP, NSE and S100β levels in patients with SAE.

Table 4. Diagnostic value of GFAP, NSE and S100β for SAE

Indicator	AUC	P	Sensitivity (%)	Specificity (%)	95% CI
GFAP	0.702	0.007	78.62	85.23	0.570-0.834
NSE	0.712	0.005	79.03	83.29	0.582-0.841
S100β	0.703	0.007	78.61	85.23	0.567-0.838
Combined detection	0.911	0.001	88.37	71.39	0.843-0.980

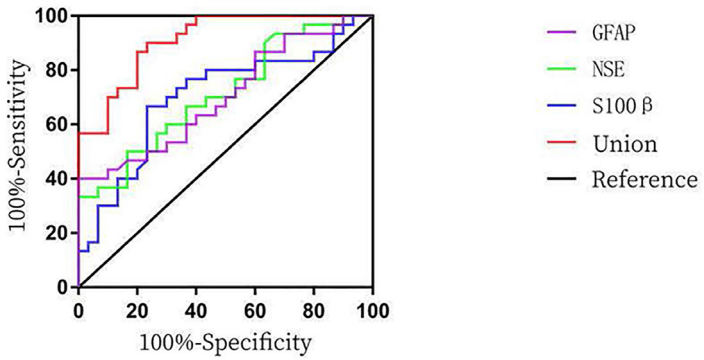

Fig. 2. ROC curves of diagnostic values of GFAP, NSE and S100β for SAE.

nosis may occur at any stage of the progression of sepsis, and such patients mainly present with consciousness, cognition and behavior disorders [9,10]. The pathogenesis of SAE is complex. Inflammatory response, blood-brain barrier damage and cerebral blood flow abnormalities are viewed as risk factors for SAE. At present, there is still no effective regimen for treating SAE and the prognosis of patients is poor. Hence, an increasing number of experts and scholars have done research on the pathogenesis and diagnosis and treatment targets of SAE from the perspective of molecular biology [11,12].

GFAP is a type III intermediate filament protein that is identified mainly as a monomer in astrocytes [13]. GFAP has been reported to be a marker with high specificity in the nervous system and is excessively released when astrocytes are damaged, so it can serve as a specific marker for brain tissue damage [14]. Some scholars hold that GFAP is principally present in astrocytes and not detected outside the brain tissues, so GFAP has high specificity as a marker for brain tissue damage [15]. Studies have reported a markedly high level of GFAP in patients with SAE. Nonetheless, there are few studies regarding the diagnostic value of GFAP for SAE. The results of this study indicated that the level of GFAP was relatively high in patients with SAE, particularly in those with higher severity, coma or a poor prognosis, signifying that the level of GFAP rises abnormally during the progression of SAE, and the change in GFAP level is relevant to the severity, consciousness and prognosis of patients. As such, GFAP may serve as a specific marker for the diagnosis of SAE, and its level is highly valuable for predicting the patients' prognosis.

As an acid protease, NSE mostly exists in neurons and neuroendocrine cells of the body. According to reports, NSE is a specific marker for brain diseases such as brain injury and stroke [16,17]. Besides, NSE has been proven to be widely present in nerve cells, and it may diffuse to the intercellular space and cerebrospinal fluid when brain tissues are damaged [18]. The results of this study revealed that patients with SAE showed a higher level of

NSE than those with sepsis, indicating that NSE is abnormally high in the progression of SAE. Meng ***et al***. [18] reported a higher level of NSE in patients with SAE, in line with the findings in this study. Additionally, it was found that the NSE level was relatively high in patients with SAE who had higher severity, coma or a poor prognosis, indicating that the change in NSE level is associated with the severity, consciousness state and prognosis of patients. Thus, NSE can be utilized as a diagnostic marker for SAE.

S100β, as a brain injury marker that has been most studied, is highly valuable in diagnosing brain tissue injury diseases [19,20]. As previously reported, S100β is abundant in the nervous system and acts as a crucial player in the energy metabolism of brain cells and the differentiation of neurons [21]. In this study, the results manifested that patients with SAE had a higher level of S100β, particularly those with higher severity, consciousness or a poor prognosis, indicating that the change in GFAP level has a certain correlation with the severity, consciousness and prognosis. Thus, it has a certain diagnostic value for SAE.

Besides, it was also discovered that GFAP, NSE and S100β were positively correlated in SAE. The combined detection of the three was of high diagnostic value for SAE. Thus, it is speculated that GFAP, NSE and S100β may be jointly implicated in the occurrence and progression of SAE, and combined detection of these three indicators has high diagnostic value for SAE.

In conclusion, the levels of GFAP, NSE and S100β are significantly abnormal in patients with SAE, and they are closely correlated with the patients' clinical features. In addition, the correlations are identified among GFAP, NSE and S100β, and the combined detection of these three indicators is highly valuable for the diagnosis of SAE.

ACKNOWLEDGEMENTS

This study was financially supported by Joint Fund of Hubei Provincial Health Commission (No. WJ2019H540).

COMPETING INTERESTS

The authors declare that they have no competing interests.

REFERENCES

1. Chiu C, Legrand M. Epidemiology of sepsis and septic shock. Curr Opin Anaesthesiol. 2021;34(2):71-6. DOI: 10.1097/ACO.0000000000000958
2. Pierrakos C, Velissaris D, Bisdorff M, Marshall JC, Vincent JL. Biomarkers of sepsis: time for a reappraisal. Crit Care. 2020;24(1):287. DOI: 10.1186/s13054-020-02993-5
3. Tauber SC, Djukic M, Gossner J, Eiffert H, Brück W, Nau R. Sepsis-associated encephalopathy and septic encephalitis: an update. Expert Rev Anti Infect Ther. 2021;19(2):215-31. DOI: 10.1080/14787210.2020.1812384
4. Gao Q, Hernandes MS. Sepsis-Associated Encephalopathy and Blood-Brain Barrier Dysfunction. Inflammation. 2021;44(6):2143-50. DOI: 10.1007/s10753-021-01501-3
5. Chung HY, Wickel J, Brunkhorst FM, Geis C. Sepsis-Associated Encephalopathy: From Delirium to Dementia? J Clin Med. 2020;9(3):703. DOI: 10.3390/jcm9030703
6. Ren C, Yao RQ, Zhang H, Feng YW, Yao YM. Sepsis-associated encephalopathy: a vicious cycle of immunosuppression. J Neuroinflammation. 2020;17(1):14. DOI: 10.1186/s12974-020-1701-3
7. Ren C, Yao RQ, Zhang H, Feng YW, Yao YM. Pathogenesis of sepsis-associated encephalopathy: more than blood-brain barrier dysfunction. Mol Biol Rep. 2022;49(10):10091-9. DOI: 10.1007/s11033-022-07592-x
8. Society of Critical Care Medicine, Chinese Medical Association. Guidelines for the treatment of severe sepsis/septic shock in China (2014). Chin Crit Care Med. 2015;27:401-26. DOI: 10.3760/cma.j.issn.2095-4352.2015.06.001
9. Zhao L, Gao Y, Guo S, et al. Sepsis-Associated Encephalopathy: Insight into Injury and Pathogenesis. CNS Neurol Disord Drug Targets. 2021;20(2):112-24. DOI: 10.2174/18715273MTExrNTka3
10. Mazeraud A, Bozza FA, Sharshar T. Sepsis-associated Encephalopathy Is Septic. Am J Respir Crit Care Med. 2018;197(2):698-9. DOI: 10.1164/rccm.201712-2593ED
11. Wei XB, Jiang WQ, Zeng JH, Huang LQ, Ding HG, Jing YW, et al. Exosome-Derived lncRNA NEAT1 Exacerbates Sepsis-Associated Encephalopathy by Promoting Ferroptosis Through Regulating miR-9-5p/TFRC and GOT1 Axis. Mol Neurobiol. 2022;59(3):1954-69. DOI: 10.1007/s12035-022-02738-1
12. Kikuchi DS, Campos ACP, Qu H, Forrester SJ, Pagano RL, Lassègue B, et al. Poldip2 mediates blood-brain barrier disruption in a model of sepsis-associated encephalopathy. J Neuroinflammation. 2019;16(1):241. DOI: 10.1186/s12974-019-1575-4
13. Wu L, Ai ML, Feng Q, Deng S, Liu ZY, Zhang LN, et al. Serum glial fibrillary acidic protein and ubiquitin C-terminal hydrolase-L1 for diagnosis of sepsis-associated encephalopathy and outcome prognostication. J Crit Care. 2019,52:172-9. DOI: 10.1016/j.jcrc.2019.04.018
14. Liu X, Wen M, Han Y, Ding H, Chen S, Li Y, et al. Mechanism of resveratrol on ameliorating the cognitive dysfunction induced by sepsis associated encephalopathy in rats. Zhonghua Wei Zhong Bing Ji Jiu Yi Xue. 2020;32(10):1189-93. DOI: 10.3760/cma.j.cn121430-20200720-00531
15. Yan S, Gao M, Chen H, Jin X, Yang M. Expression level of glial fibrillary acidic protein and its clinical significance in patients with sepsis-associated encephalopathy. Zhong Nan Da Xue Xue Bao Yi Xue Ban. 2019;44(10):1137-42. DOI: 10.11817/j.issn.1672-7347.2019.190180
16. Li XL, Xie JF, Ye XY, Li Y, Li YG, Feng K, et al. Value of cerebral hypoxic-ischemic injury markers in the early diagnosis of

sepsis associated encephalopathy in burn patients with sepsis. Zhonghua Shao Shang Za Zhi. 2022;38(1):21-8. DOI: 10.3760/cma.j.cn501120-20211006-00346

17. Zheng SM, Zhao FL, Luo YY, Lin XF, Wen MY. Clinical effect of electroacupuncture at Baihui and Shuigou points in treatment of brain injury in patients with sepsis-associated encephalopathy. Zhen Ci Yan Jiu. 2020;45(5):402-6. DOI: 10.13702/j.1000-0607.190781
18. Meng JF, Li YP, Tan DM, Chen MJ, Chen J. [Diagnosis value of combined detection of serum TNF-α, NSE and MCP-1 in early sepsis-related encephalopathy]. Hebei Medicine. 2020;26(10):1596-600.
19. Guo W, Li Y, Li Q. Relationship between miR-29a levels in the peripheral blood and sepsis-related encephalopathy. Am J Transl Res. 2021;13(7):7715-22.
20. Ehler J, Saller T, Wittstock M, Rommer PS, Chappell D, Zwissler B, et al. Diagnostic value of NT-proCNP compared to NSE and S100B in cerebrospinal fluid and plasma of patients with sepsis-associated encephalopathy. Neurosci Lett. 2019;692:167-73.. DOI: 10.1016/j.neulet.2018.11.014
21. Wu L, Feng Q, Ai ML, Deng SY, Liu ZY, Huang L, et al. The dynamic change of serum S100B levels from day 1 to day 3 is more associated with sepsis-associated encephalopathy. Sci Rep. 2020;10(1):7718. DOI: 10.1038/s41598-020-64200-3