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Clinical Value of Assessing Cytokine Levels for the Differential Diagnosis of Bacterial Meningitis in a Pediatric Population

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Abstract: We performed a prospective observational study to evaluate the utility of measuring inflammatory cytokine levels to discriminate bacterial meningitis from similar common pediatric diseases.

Inflammatory cytokine levels and other cerebrospinal fluid (CSF) physicochemical indicators were evaluated in 140 patients who were diagnosed with bacterial meningitis via microbiological culture or PCR assay.

The CSF concentrations of interleukin (IL)-6 and IL-10, CSF/blood IL-6 and IL-10 ratios, CSF white blood cell count, and CSF micro total protein were significantly elevated in bacterial meningitis patients compared with healthy children or patients with viral encephalitis, epilepsy, or febrile convulsions ($P < 0.001$). The area under the curve values for CSF concentrations of IL-6 and IL-10, CSF/blood IL-6 and IL-10 ratios, CSF white blood cell count, and CSF micro total protein to identify bacterial meningitis episodes by receiver-operating characteristic analysis were 0.988, 0.949, 0.995, 0.924, 0.945, and 0.928, respectively. The area under the curve for the combination of CSF IL-6 and CSF/blood IL-6 ratio was larger than that for either parameter alone, and the combination exhibited enhanced specificity and positive predictive value. After effective meningitis treatment, CSF IL-6 levels dropped significantly.

These results suggest that CSF IL-6 and CSF/blood IL-6 ratio are good biomarkers in discriminating bacterial meningitis. Evaluating CSF IL-6 and CSF/blood IL-6 ratio in combination can improve diagnostic efficiency. Additionally, CSF IL-6 levels can be used to monitor the effects of bacterial meningitis treatment.

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Abbreviations: AUC = area under the curve, BBB = blood–brain barrier, CSF = cerebrospinal fluid, IFN = interferon, IL = interleukin, M-TP = micro total protein, NPV = negative predictive value, PPV = positive predictive value, ROC = receiver-operating characteristic, TNF = tumor necrosis factor, WBC = white blood cell.

INTRODUCTION

Bacterial meningitis is an acute bacterial infection of the central nervous system that is common among children. Because antibiotic treatments are available, the morbidity and mortality of bacterial meningitis are significantly lower than in the past. However, the condition still results in a 5% to 10% mortality rate and causes permanent neurologic deficits in 5% to 40% of survivors.[1–5] Early diagnosis and early treatment with appropriate antibiotics are of vital importance. As approximately one-third of pediatric patients do not present with typical clinical features, it is difficult to diagnose the disease based on clinical features alone.[6,7] Etiological examination of cerebrospinal fluid is the gold standard for diagnosing bacterial meningitis; however, the low positive rate of CSF bacterial cultivation has greatly restricted its value. In the early stages of bacterial meningitis or after antibiotic treatment, cases with atypical CSF physicochemical indicators are often encountered. As a result, early diagnosis and differential diagnosis are difficult, and the efficacy of clinical treatment is seriously affected.[8,9]

T helper cell (Th) 1/Th2 balance theory is widely accepted in academia and has been clinically confirmed. Th1/Th2 cytokines play very important roles in anti-infection immunity.[10,11] We hypothesized that CSF Th1/Th2 cytokine profiles changed after the contraction of bacterial meningitis. Therefore, we investigated the clinical value of assessing Th1/Th2 cytokine levels to facilitate the differential diagnosis of bacterial meningitis.

METHODS

Patients and Controls

The current prospective observational study was conducted from January 2012 through August 2015 at the Department of Neurology in the Children's Hospital of Zhejiang University School of Medicine in China. The study was approved by the medical ethics committee of the Children's Hospital of Zhejiang University School of Medicine. Informed written consent was obtained from the guardians of the minor/child participants who were enrolled in the study. Patients who met the following

criteria were included: younger than 18 years old, and diagnosed with bacterial meningitis. Bacterial meningitis was diagnosed based on clinical presentation and abnormal laboratory tests .[12] All cases of bacterial meningitis were further confirmed by CSF culture or PCR.

To assess the predictive power of cytokine levels, patients diagnosed with bacterial meningitis between January 2012 and December 2013 were assigned to a derivation cohort, whereas those diagnosed with bacterial meningitis between January 2014 and August 2015 were assigned to a validation cohort. Healthy children and patients with viral encephalitis, epilepsy, or febrile convulsions admitted to the hospital during the study period were included as a control group. When patients with bacterial meningitis, viral encephalitis, epilepsy, or febrile convulsions were admitted to the hospital, blood samples and CSF specimens were immediately taken for microbiological analyses, biochemical analysis, and Th1/Th2 cytokine detection. A lumbar puncture was performed in healthy children because of suspicion of a neurological disease.

Cytokine Assay

Clotted blood samples and CSF specimens were centrifuged at 4000 rpm for 5 minutes. Flow cytometry-based quantification (320 flow cytometer, Becton Dickinson, San Jose, CA) of cytokine Th1/Th2 levels were measured in the harvested sera and CSF supernatants, using a dye-labeled bead kit (BDTM CBA Human Th1/Th2 Cytokine Kit II; BD Biosciences, San Jose, CA). Six bead populations with distinct fluorescence intensities were coated with capture antibodies specific to the cytokine of interest, such as interleukin (IL)-2, IL-4, IL-6, IL-10, tumor necrosis factor (TNF)-α, and interferon (IFN)-γ proteins. CSF or serum samples, phycoerythrin-conjugated detection antibodies, and the cytokine capture beads were incubated together to form sandwich complexes. Sample data were acquired by flow cytometry, and sample results were generated using BD CBA Analysis Software (BD Biosciences, San Jose, CA).

Statistical Analysis

Intergroup comparisons were performed using a chi-square test for categorical variables and the Mann–Whitney *U* test for continuous variables. All statistical analyses were performed using SPSS Statistics 18.0 software. $P < 0.05$ was statistically significant. Receiver-operating characteristic (ROC) curves were used to assess the value of cytokines and other laboratory indexes in the differential diagnosis of bacterial meningitis using MedCalc 9.4.2.0 software. Logistic regression was used to calculate the predicted probabilities of various combinations of cytokines. The predicted probabilities were saved as a new indicator to assess the diagnostic values of different combinations using ROC curves. The diagnosis critical value was determined on the basis of weighted Youden index and our clinical experience.

RESULTS

Patient Characteristics

A total of 140 patients with bacterial meningitis confirmed by microbiological culture or PCR assay in CSF were evaluated between January 2012 and August 2015. The median patient age was 3.9 years (range 0.1–10.1 years), and the male-to-female ratio was 1.9. The main causative organisms were Gram-positive bacteria, which accounted for 34.3% of the cases. The majority of the bacteria were enterococcus (n = 28) and *Streptococcus pneumoniae* (n = 20). Gram-negative bacteria accounted for 57.1% of the cases; the 2 primary organisms were *Escherichia coli* (n = 55) and *Klebsiella pneumoniae* (n = 25). The remaining 12 cases were confirmed by PCR assay, but had negative CSF cultures; these accounted for 8.6% of the cases. For the control group, 182 viral encephalitis patients, 166 febrile convulsion patients, 146 epilepsy patients, and 180 healthy children were enrolled. Detailed patient information is provided in Table 1.

Th1/Th2 Cytokine Levels and Other CSF Physicochemical Indicators of Bacterial Meningitis

No differences were found in CSF levels of IL-2, IL-4, IL-6, IL-10, IFN-γ, or TNF-α between healthy children and patients with viral encephalitis, epilepsy or febrile convulsions; however, CSF levels of IL-6 and IL-10 were significantly elevated in bacterial meningitis patients ($P < 0.001$; Table 1, Figure 1). In addition, CSF/blood IL-6 and IL-10 ratios, CSF white blood cell (WBC) count, and CSF micro total protein (M-TP) were all significantly higher in bacterial meningitis patients versus the control groups ($P < 0.001$; Table 1, Figure 1).

Establishment of a Model for Bacterial Meningitis Identification

We divided the bacterial meningitis patients into derivation and validation cohorts to evaluate the performance of inflammatory cytokines and other CSF physicochemical indicators in discriminating bacterial meningitis from other similar diseases. As discussed above, CSF concentrations of IL-6 and IL-10, CSF/blood IL-6 and IL-10 ratios, CSF WBC count, and CSF M-TP were markedly elevated in patients with bacterial meningitis compared with those with similar diseases. We evaluated the utility of assessing these parameters to identify bacterial meningitis by performing ROC analysis of the derivation cohort. The area under the curves (AUCs) for CSF concentrations of IL-6 and IL-10, CSF/blood IL-6 and IL-10 ratios, CSF WBC count, and CSF M-TP were 0.988, 0.949, 0.995, 0.924, 0.945, and 0.928, respectively. A CSF IL-6 level equal to 38.2 pg/mL or greater had 100.0% sensitivity and 91.0% specificity in discriminating bacterial meningitis. The positive predictive value (PPV) and negative predictive value (NPV) were 92.4% and 100.0%, respectively. At an optimal cut-off value of 11.3, CSF/blood IL-6 ratio showed both higher specificity (96.5%) and PPV (96.5%) than CSF IL-6 for discriminating bacterial meningitis. The AUC for the combination of CSF IL-6 and CSF/Blood IL-6 ratio was larger than that for either parameter alone, and the specificity and PPV of the combination were better. These results indicate that the combination of CSF IL-6 and CSF/blood IL-6 ratio is an effective biomarker for discriminating bacterial meningitis (Table 2, Figure 2).

Utilizing Inflammatory Cytokines to Identify Bacterial Meningitis in the Validation Set

We assessed the utility of evaluating CSF concentrations of IL-6 and IL-10, CSF/blood IL-6 and IL-10 ratios, CSF WBC count, and CSF M-TP to identify bacterial meningitis in the validation cohort, which consisted of 69 patients. The AUCs for CSF concentrations of IL-6 and IL-10, CSF/blood IL-6 and IL-

TABLE 1. CSF and Blood Date in Healthy Children and Patients

Parameters	Bacterial Meningitis	Viral Encephalitis	Febrile Convulsion	Epilepsy	Healthy Control
Number	140	182	166	146	180
Sex (male/female)	91/49	119/63	104/62	57/89	120/60
Age, years	3.9 (0.1–10.1)	3.8 (0.1–13.3)	1.4 (0.7–3.3)	1.5 (0.2–13.6)	2.4 (0.1–12.8)
Hospital stays, days	19.2 (10.5–29.0)†	8.5 (2.0–27.0)	5.0 (2.0–8.0)	6.0 (2.0–16.0)	—
CSF IL-2, pg/mL	3.5 (1.2–8.0)	3.4 (1.0–7.1)	3.8 (1.1–6.8)	2.8 (1.0–5.6)	3.2 (1.8–5.0)
CSF IL-4, pg/mL	3.6 (1.7–4.6)	3.0 (0.7–5.7)	3.2 (0.5–4.3)	2.8 (0.8–5.4)	2.6 (1.0–3.0)
CSF IL-6, pg/mL	812.3 (46.3–3683.0)†	6.3 (1.6–359.8)	6.1 (3.2–19.2)	3.4 (1.8–20.1)	5.3 (2.1–15.7)
CSF IL-10, pg/mL	6.8 (5.0–472.6)†	2.3 (1.2–78.6)	2.1 (1.3–4.3)	1.8 (1.0–3.6)	2.3 (1.5–4.4)
CSF TNF-α, pg/mL	2.5 (1.5–49.1)	1.9 (1.0–608.0)	4.5 (1.0–3.3)	1.9 (1.0–3.7)	2.0 (1.0–3.7)
CSF IFN-γ, pg/mL	7.5 (2.5–988.0)	6.3 (1.6–208.8)	5.8 (1.6–9.5)	4.7 (1.7–7.5)	6.1 (2.9–8.0)
Blood IL-2, pg/mL	3.3 (1.1–5.4)	4.1 (1.9–6.7)	4.0 (2.3–6.0)	4.2 (1.9–7.5)	4.8 (2.7–7.2)
Blood IL-4, pg/mL	3.1 (1.5–68.3)	3.4 (1.1–5.4)*	3.1 (2.7–5.1)*	3.5 (1.8–5.8)	2.7 (1.1–2.9)
Blood IL-6, pg/mL	15.4 (2.5–68.3)	20.5 (2.2–1368.9)*	5.6 (1.5–507.8)	5.0 (2.1–1031.7)	3.9 (1.2–6.7)
Blood IL-10, pg/mL	4.3 (1.9–6.1)	4.1 (1.6–46.0)*	3.7 (2.0–25.9)	3.0 (1.5–16.0)*	2.1 (1.3–3.4)
Blood TNF-α, pg/mL	2.4 (1.1–7.9)	2.1 (1.0–86.1)	2.2 (1.2–4.2)	2.4 (1.0–10.8)	2.2 (1.3–2.6)
Blood IFN-γ, pg/mL	7.4 (3.6–741.4)	8.0 (3.4–50.5)*	9.6 (5.0–41.8)*	6.5 (4.3–25.6)*	4.7 (3.8–7.8)
CSF/blood IL-2 ratio	1.2 (0.5–4.5)	0.9 (0.2–2.7)	1.0 (0.2–2.1)	0.7 (0.2–1.8)	0.7 (0.4–1.5)
CSF/blood IL-4 ratio	1.0 (0.3–2.6)	1.0 (0.1–2.9)	0.9 (0.1–1.6)	0.9 (0.2–2)	0.9 (0.6–1.6)
CSF/blood IL-6 ratio	47.8 (11.2–345.4)†	0.4 (0.1–44.8)	0.7 (0.1–8.3)	0.7 (0.2–8.7)*	1.4 (0.4–6.1)
CSF/blood IL-10 ratio	1.8 (1.0–116.3)†	0.6 (0.4–18.6)	0.6 (0.1–1.3)*	0.5 (0.1–1.3)*	1.0 (0.4–2.2)
CSF/blood TNF-α ratio	1.4 (0.5–7.7)†	0.9 (0.2–4.1)	0.8 (0.2–1.3)	0.9 (0.2–2.8)	1.0 (0.4–2.1)
CSF/blood IFN-γ ratio	1.6 (0.4–96.2)†	0.8 (0.1–44.4)	0.5 (0.1–1.4)*	0.7 (0.2–1.5)*	1.3 (0.4–1.6)
ADA, U/L	2.2 (0.2–8.0)	0.8 (0.1–8.5)*	6.8 (0.1–4.4)	0.4 (0.1–5.1)	0.3 (0.1–0.8)
LDH, U/L	49.8 (10.0–286.0)†	20.0 (4.0–269.0)	19.5 (5.0–32.0)	17.0 (6.0–40.0)	25.0 (12.0–36.0)
CK, U/L	3.7 (1.2–51.6)†	2.0 (1.0–72.0)	2.5 (1.0–11.0)	2.0 (1.0–26.0)	1.0 (1.0–5.0)
GLU, mmol/L	3.8 (1.6–5.8)	3.8 (2.7–6.3)	3.9 (3–8.9)	3.7 (2.9–5.1)	3.6 (2.9–4.3)
Cl, mmol/L	122.6 (101.2–133.0)	122.5 (101.8–136.3)	122.4 (115.5–123.7)	121.3 (108.9–130.2)	123.1 (119.7–128.0)
M-TP, mg/L	579.1 (289.4–2872.5)†	185.9 (76.1–1970.9)*	130.3 (84.8–240.9)	144.7 (63.9–352.3)	91.0 (33.0–222.0)
WBC, $\times 10^6$/L	75 (5–2250)†	2 (2–286)	2 (2–4)	2 (2–4)	2 (2–4)

Except number and sex, range and median values are represented for each group.

ADA = adenosine deaminase, CK = creatine kinase, Cl = chlorine, CSF = cerebrospinal fluid, GLU = glucose, IFN = interferon, IL = interleukin, LDH = lactic dehydrogenase, M-TP = micro total protein, TNF = tumor necrosis factor, WBC = white blood cell.

*$P < 0.001$ (compared to healthy control group).

†$P < 0.001$ (compared to other groups).

10 ratios, CSF WBC count, and CSF M-TP were 0.985, 0.938, 0.993, 0.912, 0.946, and 0.934, respectively. CSF IL-6 level and CSF/blood IL-6 ratio, both individually and in combination, had similar sensitivities, specificities, PPVs, and NPVs, with the same cut-off values in the validation cohort and the derivation cohort (Table 2). These results indicated the stable power of these parameters in identifying bacterial meningitis. Of these indicators, the combination of CSF IL-6 and CSF/blood IL-6 ratio was the most effective biomarker in discriminating bacterial meningitis (Table 2, Figure 2).

Value of CSF IL-6 Measurements in Monitoring the Curative Effect of Bacterial Meningitis Treatment

To clarify whether CSF IL-6 levels in patients with bacterial meningitis change with the condition of the illness, we tracked fluctuations in these levels in 85 bacterial meningitis patients. Antibiotic treatment was effective in 73 cases and ineffective in 12 cases. CSF IL-6 levels were detected before and after treatment. Among the 12 patients for whom the treatment was ineffective, the average pretreatment CSF IL-6 level was 823.0 (100.1–3683.0) pg/mL, and the average posttreatment CSF IL-6 level was 980 (120.0–3725.0) pg/mL. The median level of IL-6 after treatment increased slightly, but no significant change was observed ($P = 0.435$). Among the 73 patients for whom the treatment was effective, the average pretreatment CSF IL-6 level was 1001.0 (157.0–2751.0) pg/mL, and the average posttreatment CSF IL-6 level was 35.0 (12.0–120.0) pg/mL. After effective treatment, CSF IL-6 levels declined significantly ($P < 0.001$) (Figure 3).

DISCUSSION

Etiological examination of CSF is the gold standard for diagnosing bacterial meningitis. However, owing to the limited number of pathogen types that can be detected in the laboratory and the extensive use of antibiotics, the positive rate of bacterial cultivation is quite low, decreasing the effectiveness of etiological diagnosis.[13] Increases in total WBC count in CSF are caused by stimulation of the meninges by bacteria and their metabolites. Previous studies have suggested that increases in CSF total WBC count indicate a potential diagnosis of bacterial meningitis. However, in a previous study, total WBC counts in some bacterial meningitis patients were lower than the diagnostic standard.[14] Moreover, total WBC count in the CSF increased in some viral meningitis patients upon initial lumbar

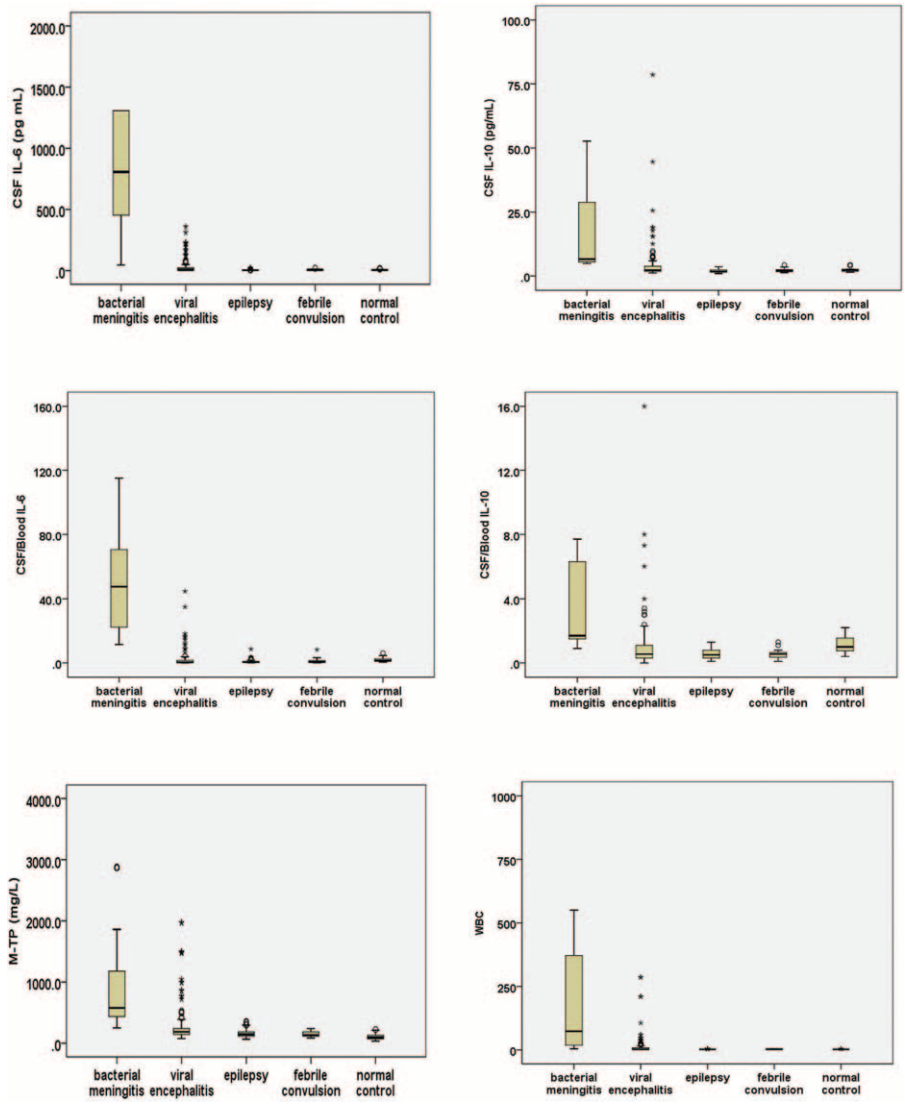

FIGURE 1. CSF concentrations of IL-6 and IL-10, CSF/blood IL-6 and IL-10 ratios, CSF WBC count, and CSF M-TP in the normal control group and in patients with bacterial meningitis, viral encephalitis, febrile convulsions, or epilepsy. CSF = cerebrospinal fluid, IL = interleukin, M-TP = micro total protein, WBC = white blood cell.

puncture. Therefore, CSF WBC count is not always accurate in diagnosing bacterial meningitis.[8]

As bacterial meningitis is associated with uncontrolled and excessive cytokine release, early diagnosis is crucial for patient survival. In the current study, we quantified IL-2, IL-4, IL-6, IL-10, TNF-α, and IFN-γ levels in bacterial meningitis patients using a CBA kit (BDTM CBA Human Th1/Th2 Cytokine Kit II; BD Biosciences, San Jose, CA). Because viral encephalitis, epilepsy, and febrile convulsions are common pediatric disorders with similar clinical features to bacterial meningitis, it is typically difficult to distinguish these conditions.[14] Therefore, patients with the above conditions were selected to serve as a control group for the differential diagnosis of bacterial meningitis. Although CSF levels of IL-2, IL-4, TNF-α, and IFN-γ in the bacterial meningitis patients were comparable with those in the control group, CSF levels of IL-6 and IL-10 were significantly elevated in the bacterial meningitis patients (both $P < 0.001$).

Interleukin-6 can initiate the acute-phase response of inflammation,[15,16] a rise in acute phase proteins,[17] and systemic inflammatory responses[18] (eg, fever and leukocytosis). IL-6 also contributes to the transition from acute to chronic inflammation.[19] The pleiotropic cytokine IL-10 has been shown to play an important role in the anti-inflammatory activities, which leads to a significant suppression in the release the proinflammatory chemokines and thereby limits antigen-presenting cell function.[20,21]

Interleukin-10 can also directly inhibit T-cell activation, proliferation,[22] and chemotaxis,[23] and also cytokine production,[24] to limit inflammation.

Based on the biological functions of IL-6 and IL-10 in inflammation and the abnormal CSF levels of these cytokines in bacterial meningitis patients, we evaluated the abilities of these 2 indicators in identifying bacterial meningitis using ROC analysis. The AUCs for IL-6 and IL-10 were 0.988 and 0.949, respectively, indicating that they were both effective

TABLE 2. Performances of Inflammatory Cytokines and Other CSF Physicochemical Indicators in Discriminating Bacterial Meningitis

Diagnostic Indicators	AUC	Cut-off	Sensitivity, %	Specificity, %	PPV, %	NPV, %
Derivation cohort (n = 71)						
CSF IL-6	0.988	38.2 (pg/mL)	100.0	91.0	92.4	100.0
CSF IL-10	0.949	5.0 (pg/mL)	85.6	91.7	89.9	87.2
CSF/blood IL-6 ratio	0.995	11.3	100.0	96.5	96.5	100.0
CSF/blood IL-10 ratio	0.924	1.3	95.2	86.4	87.2	95.1
M-TP	0.928	501.5 (mg/L)	65.1	92.3	88.7	73.5
CSF WBC count	0.945	30.0 ($\times 10^6$/L)	70.0	93.1	90.5	76.3
Logregr_Pred (IL-6 + CSF/blood IL-6)	0.996		100.0	96.7	96.7	100.0
Validation cohort (n = 69)						
CSF IL-6	0.985	38.2 (pg/mL)	100.0	89.4	91.6	100.0
CSF IL-10	0.938	5.0 (pg/mL)	85.4	87.7	88.9	84.5
CSF/blood IL-6 ratio	0.993	11.3	95.9	94.8	95.4	94.7
CSF/blood IL-10 ratio	0.912	1.3	95.3	82.4	85.7	93.9
M-TP	0.934	501.5 (mg/L)	65.4	93.2	91.6	70.8
CSF WBC count	0.946	30.0 ($\times 10^6$/L)	70.5	92.9	91.9	73.2
Logregr_Pred (IL-6 + CSF/blood IL-6)	0.994		100.0	94.7	95.6	100.0

Logregr_Pred (IL-6 + CSF/blood IL-6) indicates the combination of IL-6 and CSF/blood IL-6 ratio.

AUC = area under the curve, CSF = cerebrospinal fluid, IL = interleukin, M-TP = micro total protein, NPV = negative predictive value, PPV = positive predictive value, WBC = white blood cell.

biomarkers for identifying bacterial meningitis. CSF IL-6 was more effective in identifying bacterial meningitis than CSF IL-10. In addition, the diagnostic efficiency of IL-6 was higher than conventional CSF physicochemical indexes, such as CSF WBC count and M-TP. Under similar diagnostic specificities, the sensitivity of IL-6 in the differential diagnosis of bacterial meningitis was significantly higher than the sensitivities of conventional physicochemical indexes in CSF. When CSF IL-6 levels exceeded 38.2 pg/mL, the diagnostic sensitivity of this biomarker was 100.0%, compared with 70.0% for

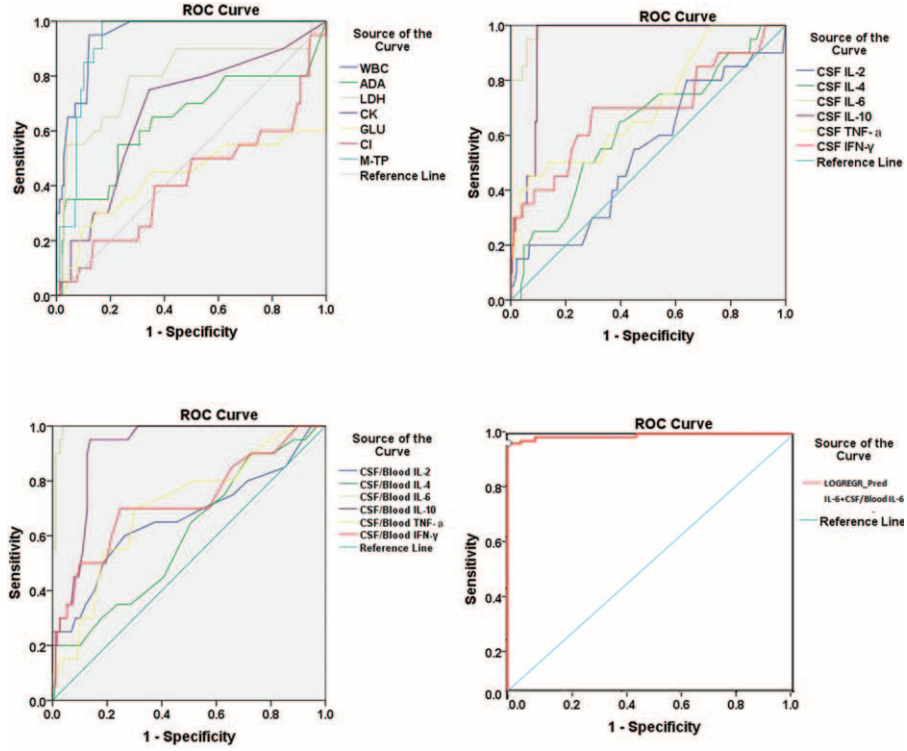

FIGURE 2. ROC curve analysis to evaluate the utility of evaluating inflammatory cytokines and other CSF physicochemical indicators to discriminate between bacterial meningitis and other similar diseases. CSF = cerebrospinal fluid, ROC = receiver-operating characteristic.

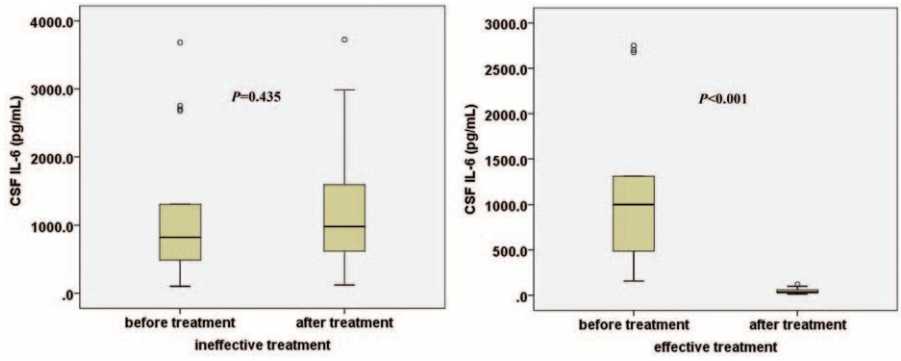

FIGURE 3. The value of CSF IL-6 detection in monitoring the effects bacterial meningitis treatment. CSF = cerebrospinal fluid, IL = interleukin.

CSF WBC count and 65.1% for M-TP. These results were in agreement with reported experimental results from adult patients. Because the immune system of an adult is mature, IL-6 levels tend to be higher in adult bacterial meningitis patients.[25]

Cytokines are small molecules, and some of them can penetrate the blood–brain barrier (BBB). Thus, cytokine levels in the blood affect cytokine levels in CSF. When bacterial meningitis is accompanied by other bacterial infections, such as sepsis, a large quantity of cytokines will penetrate the BBB from the blood, leading to increased cytokine levels in CSF. A false appearance of intracranial infection will result. To eliminate the influence of cytokine levels in the blood on cytokine levels in CSF, cytokine levels in these 2 sites were detected in parallel, and the ratio between these levels was calculated. According to the experimental results, CSF/blood IL-6 ratio has a higher diagnostic efficiency than the independent detection of CSF IL-6. With consistent diagnostic sensitivity, the specificity of diagnosing bacterial meningitis with CSF/blood IL-6 ratio improved.

Based on the high sensitivity of CSF IL-6 and high specificity of CSF/blood IL-6 ratio in diagnosing bacterial meningitis, we considered evaluating these parameters in combination. Logistic regression was used to calculate the predicted probabilities of the combination, and these were saved as a new indicator to assess the diagnostic value of the combination using ROC curve analysis. Our study shows that the combination of CSF IL-6 and the CSF/blood IL-6 ratio (Logregr_Pred IL-6+CSF/blood IL-6) is the most effective biomarker in the differential diagnosis of bacterial meningitis.

Many IL-6 detection methods are currently available. The reagent cost associated with ELISA is low, and it does not require the use of expensive instruments; therefore, this technique can be generalized in hospitals at various levels. Flow cytometry is convenient and extremely efficient in detecting IL-6 and is therefore a useful method for the emergency laboratory. The detection can be performed within 1 day. In addition, a Th1/Th2 kit including IL-6 has been regularly used for many years in our laboratory, and our experience indicates that the reagents in this kit are stable, and the obtained results are consistent. However, IL-6 detection is not a substitution for CSF examination and the measurement of other CSF biochemical indexes (such as WBC count, glucose, and protein). When diagnosing bacterial meningitis, a patient's clinical features cannot be ignored. The use of the above biomarkers is simply a beneficial supplement when a definitive diagnosis cannot be made. The results of biomarker analysis should be interpreted in parallel with the results of routine tests and the evaluation of clinical symptoms. Additionally, meningitis patients with coagulase-negative staphylococci were not included in the study. Because these bacteria are likely to be caused by pollution and biofilm/device-associated infections with them are less inflammatory than parenchymal infection which can significantly skew the inflammatory profile.

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