

Application of cerebrospinal fluid cytokine refinement model in identifying common infectious meningitis in children

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Abstract

Objective

To explore the cytokine profiles in cerebrospinal fluid of Common Pediatric Infectious Meningitis and to construct a refined multi-parameter model for pathogen identification.

Methods

The model was developed using binary logistic regression in a retrospective cohort from May 2020 to June 2024 of 70 Bacterial Meningitis (BM) and 90 Viral Meningitis (VM) combining discriminatory cerebrospinal fluid (CSF) cytokines with routine parameters. For the purpose of validating the constructed model's pathogen differentiation capability in Common Pediatric Infectious Meningitis, calibrating its accuracy, and assessing its clinical practical value, an additional 75 clinically diagnosed cases from July 2024 to April 2025 were collected.

Results

1. Compared with VM, IL-2, IL-6, IL-8, IL-10, IL-17 and INF- γ were elevated significantly in BM, difference means significant ($P < 0.05$). 2. Although they could be single index for differential diagnosis BM or VM ($P < 0.01$), IL-6 and IL-17 were superior ($AUC_6=0.712$, $AUC_{17}=0.762$). The key indicator was IL-17. 3. The goodness of fit was supreme for the model combining IL-6, IL-17 and routine biochemical indexes, and the calibration curve along with internal structure analyses indicated strong predictive accuracy. 4. The model combining IL-6, IL-17 and routine biochemical indexes achieved the optimal efficiency for differential diagnosis ($AUC = 0.872$, $SEN = 78.39\%$, $SPE = 87.50\%$). 5. In validation for differential diagnosis, the model combining IL-6, IL-17 and routine biochemical indexes was still outstanding ($AUC = 0.812$, $SEN = 76.09\%$, $SPE = 85.30\%$). The model showed good calibration, high accuracy, and stronger early predictive ability.

Conclusions

The CSF cytokine-based prediction model serves as a valuable adjunct for the early diagnosis and differential diagnosis of Common Infectious Meningitis in children.

Introduction

Central nervous system infectious meningitis in children represents a medical emergency. According to WHO data from 2014 to 2019, the mortality rate of BM in children under five years of age ranged from 7.9% to 11%, with approximately 4% to 5% suffering from irreversible neurological sequelae¹. VM often

presents with acute onset, meanwhile viruses can be transmitted or reactivated through multiple pathways². In infants, limited expressive ability makes early manifestations including fever and headache easily confused with simple respiratory infection, complicating diagnosis and treatment³. Moreover, widespread use of meningitis vaccines and strains with reduced antimicrobial sensitivity have led to more patients lacking the classic triad (fever, headache, meningeal irritation), further hindering etiological identification⁴. Consequently, the CSF examination is the main basis for laboratory diagnosis. Yet, qua the gold standard, the positive rate of pathogen culture is only 25.6% -33.6%. Polymerase Chain Reaction (PCR) need to eliminate interference by pollutant or microbial flora colonization. Positive results of Next-Generation Sequencing (NGS) should be carefully explained after excluding the influence of operation and quality control. Negative results cannot exclude infection⁵. Based on the existing molecular diagnostic techniques cannot seek out the etiology nearly 50%-60%⁶, in addition a certain proportion of pseudomeningitis with similar clinical manifestations⁷. To sum up, urgent need new technological indexes to compensate for laboratory diagnosis.

Although the confirmed mechanism of the neuroimmune axis, which releases cytokines to influence disease progression in children with meningitis⁸, has made pediatric neuroimmune research a new focus, relevant information remains limited, particularly regarding cerebrospinal fluid studies covering children of all ages. Therefore, based on existing adult studies using combined detection of immune cells and cytokines to identify meningitis infection types⁹, and in light of research demonstrating that combined detection of traditional inflammatory markers and cytokines in CSF yields high accuracy for diagnosing Central Nervous System Infections¹⁰, this study establishes and validates a combined prediction model of CSF cytokines and routine biochemical parameters in a pediatric cohort covering all ages. Pediatric-specific identification markers are provided to offer an optimal strategy for diagnosing and differentiating the etiological types of Common Infectious Meningitis in children.

Materials and Methods

1. Research subjects

1.1 Children with infectious meningitis diagnosed in our hospital from May 2020 to June 2024 were included in the Disease Group

70 of BM Group, male to female ratio was 1:0.6; ranging from 1 month to 15 years and 8 months old, median age was 3 years and 1 month old. Diagnostic standard^{11,12} include: ☐Changes in consciousness and mental state, fullness and tense of bregma or positive meningeal irritation sign, headache, convulsion, or focal neurological symptoms, with or without fever and respiratory infections. ☐Inflammatory manifestations in peripheral blood routine and positive blood culture. ☐Imaging examination shows brain parenchymal lesions, or abnormal electroencephalogram conforms to the characteristics of encephalitis. ☐Routine examination of CSF: The appearance is cloudy, with increased pressure, elevated protein, low glucose, or degressive ratio to blood glucose. More than $1000 \times 10^6/L$

WBC or below $100 \times 10^6/L$, main of neutrophilic segmented granulocyte. Pathogenic bacteria were detected in microbiological detection of CSF. Compliance with ~ indicates clinical diagnosis, while compliance with indicates pathogenic diagnosis.

90 of VM Group, male to female ratio was 1:0.5, ranging from 1 years to 15 years and 8 months old, median age was 5 years and 3 months old. Diagnostic standard^{13,14} include: Clinical manifestation: Changes in consciousness and mental state, fullness and tense of bregma or positive meningeal irritation sign, headache or nuchal rigidity, with or without symptoms of fever and respiratory infections. Inflammatory manifestations in peripheral blood routine and positive blood culture. Clinical imaging examination showed the presence or absence of meningeal changes and hydrocephalus, and EEG findings were consistent with the characteristics of meningitis. Routine examination of CSF: The appearance is clear, with normal or increased pressure, normal or slightly increased protein, and normal glucose content. More than $100 \times 10^6/L$ WBC or normal, early stage is main of neutrophilic segmented granulocyte, later stage became lymphocytes. Virus strains were detected in microbiological detection of CSF. Compliance with ~ indicates clinical diagnosis, while compliance with indicates pathogenic diagnosis.

Exclusion criteria of Common Infectious Meningitis in Children: Meningitis caused by fungus, chlamydia or else, other Central System Infectious diseases without meningitis infection. Clinical diagnosis is Autoimmune Nervous System Disease. Immunodeficiency related infectious diseases. Merge hematological diseases or tumors. With clarify diagnosis, but without 12 cytokines measurement or routine biochemical indexes detection of CSF in BM or VM.

1.2 Validation of the model was performed using a cohort of 75 children who met the aforementioned diagnostic criteria for common infectious meningitis between July 2024 and January 2026.

2. Reagents and Instruments

2.1 Cytokine Profiling: Levels of 12 cytokines were quantified using a CYTOMICS FC 500 flow cytometer (Beckman Coulter, Indianapolis, Indiana, America) in conjunction with a multiplex bead-based flow immunofluorescence assay kit (Raisecare, Qingdao, China).

2.2 CSF Biochemistry: Analysis was performed using a Siemens fully automated biochemical analyzer (Siemens, Flanders, New Jersey, America) and its corresponding reagents.

2.3 Cell Counting: White blood cell counts in cerebrospinal fluid were performed using the Sysmex XN-3000 fully automated hematology analyzer with proprietary reagents (Sysmex Corporation, Kobe, Japan).

3. Sample processing and testing

3.1 Flow cytometer detected cytokines: Centrifugation by 1000g for 10 minutes, add 25μL experimental buffer to the 10mL tube. Add centrifuged basal CSF 25μL. Then add 25μL capture microspheres and 25μL detection antibodies, vibrate ($400-500 \text{ r} \cdot \text{min}^{-1}$) and incubate at room temperature (25 ± 1) °C for 2 hours in dark place. Add SA-PE fluorescent chromogenic agent 25μL, vibrate ($400-500 \text{ r} \cdot \text{min}^{-1}$) and incubate at room temperature (25 ± 1) °C for 0.5 hours in dark place. Join 1×9 Washing buffer 800μL. Vortex for seconds, centrifuge by 400g for 5 minutes, pour the liquid, invert and absorb water. Finally, add 1×9 Washing buffer 300μL, vortex for seconds and gauge within 4 hours.

3.2 Biochemical analyzer and numeration of WBC: Non-anticoagulant tube, clinical standard operation to collect CSF and keep at least 1mL, within 1 hour at room temperature or within 2 hours at $4-8^{\circ}\text{C}$ ¹⁵. Detecting glucose (GLU), chloride ion (CL), and CSF protein (CFP) and numeration of WBC within 2 hours.

4. Statistical Analysis

All statistical analyses were performed using SPSS (version 22.0). The pathogen distribution across the 160 cases (collected from May 2020 to June 2024) was summarized. As all continuous variables deviated from a normal distribution, data are presented as median and interquartile range (IQR; M, Q1, Q3). Differences between the Bacterial Meningitis (BM) and Viral Meningitis (VM) were conducted using the Mann-Whitney U test with a significance level of $P < 0.05$. Three distinct models were developed using binomial logistic regression: a cytokine-only model, a routine biochemical index model, and a combined model incorporating both cytokine and biochemical data. R was employed to generate radar charts for the comparative assessment of multiple models in the discrimination between bacterial and viral meningitis, while the Bootstrap, Hosmer-Lemeshow test and the clinical decision curve. ROC analysis was performed using MEDCALC 11 to assess the discriminatory performance of cytokines and each model for distinguishing bacterial from viral meningitis. Figures were generated using GraphPad Prism (version 5).

Results

1. Summary of clinical manifestation, clinical iconography changes, and microbiological detection in groups

Owing to the similar clinical symptoms and imaging manifestations between BM and VM, differential diagnosis based solely on these findings was difficult as Table 1 ($P > 0.05$). Microbiological analysis (pathogen culture, PCR and NGS) effectively identified etiological types ($P < 0.05$), but had long detection cycles or high costs. With discharge diagnosis as the reference standard, each method had a missed diagnosis rate exceeding 35% for cerebrospinal fluid detection.

Table 1

Clinical manifestation, clinical iconography changes, and microbiological detection analysis results

Basic information	BM (n = 70,)	VM (n = 90,)	χ^2	P	omission diagnose rate	
					BM (%)	VM (%)
fever	62(88.57)	79(87.78)	3.162	> 0.05	11.43	12.22
headache	50(71.43)	66(73.33)	0.740	> 0.05	28.57	26.67
vomiting	38(54.29)	44(48.89)	0.459	> 0.05	45.71	51.11
consciousness Disorder	53(75.71)	65(72.22)	0.248	> 0.05	24.29	27.78
meningeal irritation sign	45(64.28)	53(58.89)	0.483	> 0.05	35.72	41.11
history of upper respiratory infections	57(81.43)	64(71.11)	2.274	> 0.05	18.57	28.89
clinical iconography changes	51(72.86)	57(63.33)	1.628	> 0.05	27.14	36.67
pathogen culture	30(42.86)	5 (5.56)	32.058	< 0.05	57.14	84.44
polymerase chain reaction	43(61.43)	40(44.44)	4.550	< 0.05	38.57	55.56
next-generation sequencing	45(64.29)	39(43.33)	6.932	< 0.05	35.71	56.67

2.Differences in cytokines of CSF between BM and VM

A comparison between BM and VM was presented in Table 2, Except for INF- α , the median of cytokines in the BM group was higher than in VM group. Furthermore, a comparison between the BM and VM groups revealed statistically significant differences for IL-2, IL-6, IL-8, IL-10, IL-17 and INF- γ ($P < 0.05$).

Table 2
The level and difference in cytokines of CSF between BM and VM groups (pg/mL, $M(Q_1, Q_3)$)

project	BM (n = 70)	VE (n = 90)	<i>U</i>	<i>P</i>
IL-1 β	6.16(0.14, 51.54)	0.45(0.04, 16.96)	2502.50	0.06
IL-2	2.51(1.44, 8.09)	2.02(0.68, 4.22)	2442.50	< 0.05
IL-4	1.45(0.85, 2.13)	1.38(0.77, 2.05)	2880.50	0.35
IL-5	1.80(0.59, 3.76)	1.39(0.45, 2.88)	2882.00	0.36
IL-6	135.74(12.81, 819.34)	14.74(3.76, 63.32)	1762.50	< 0.001
IL-8	489.07(154.16, 1170.06)	152.54(76.54, 387.05)	2030.50	< 0.001
IL-10	5.58(1.64, 17.86)	1.66(0.89, 3.41)	1824.50	< 0.001
IL-12P70	1.99(1.27, 3.12)	1.41(0.81, 2.52)	2635.50	0.08
IL-17	4.80(1.58, 37.35)	1.33(0.44, 2.23)	1456.50	< 0.001
TNF- α	1.80(0.08, 4.45)	0.29(0.03, 3.21)	2583.50	0.05
IFN- α	1.84(0.45, 4.25)	1.95(0.53, 3.72)	3139.00	0.97
IFN- γ	7.64(1.09, 21.69)	0.14(0.12, 9.21)	2118.00	< 0.001

3. Analysis of the differential diagnosis efficacy of cytokines between BM and VM

As shown in Fig. 1, ROC analysis with the VM group as controls revealed that IL-2, IL-6, IL-8, IL-10, IL-17 and INF- γ , as individual cytokines, could discriminate between BM and VM ($P < 0.01$). Among them, the AUC values for IL-6 (0.712) and IL-17 (0.762) were significantly higher than those of the others, indicating excellent diagnostic performance. However, although the sensitivity of INF- γ was 83.33%, meanwhile the specificity of IL-17 was 95.45%, all cytokines as standalone indicators showed limited discriminatory power (Youden < 0.5). Overall, the screening efficacy of a single indicator remained suboptimal.

Table 3
ROC analysis results of cytokines between BM and VM Groups

project	AUC	Z	cut-off (pg/mL)	SEN (%)	SPE (%)	NPV (%)	PPV (%)	Youden
IL-2	0.611 (0.531,0.687)	2.468	5.80	29.17	90.91	61.10	72.40	0.2008
IL-6	0.712 (0.635,0.781)	5.158	87.91	54.17	80.68	68.30	69.60	0.3485
IL-8	0.672 (0.593,0.744)	4.003	474.68	50.00	80.68	66.40	67.90	0.3068
IL-10	0.693 (0.609,0.774)	4.709	3.04	53.50	78.43	67.50	68.10	0.3193
IL-17	0.762 (0.688,0.826)	6.835	4.89	48.61	95.45	69.40	89.70	0.4406
IFN-γ	0.677 (0.599,0.749)	4.208	0.18	83.33	52.27	79.30	58.80	0.3560

(Among the sheet, all of Z means $P < 0.01$)

4. Binomial logistic regression analysis of cytokines and routine biochemical indicators of CSF

Given the limited diagnostic efficacy of individual cytokines, we constructed Model 1 using a panel of cytokines (IL-2, IL-6, IL-8, IL-10, IL-17, INF-γ) and Model 2 using routine biochemical indexes combined in CSF. As shown in Tables 4.A/ 4.B, Model 1 showed a goodness-of-fit ($R^2=0.409$) lower than that of Model 2($R^2=0.428$), and both models had a predictive accuracy below 80%. We therefore developed Model 3 by incorporating IL-6 and IL-17 with the routine biochemical indexes combined in CSF, which had high individual AUC. This combined model yielded the highest goodness-of-fit ($R^2=0.564$) and achieved a discriminatory accuracy of 81.3% for distinguishing BM from VM (Table 4.C). IL-17 was associated with the development of BM and, as such, served as a critical indicator for distinguishing it from VM.

Table 4A

Model 1 Binary logistic regression for differential diagnosis between BM and VM Groups

project	B	SE	OR(95%CI)	P
IL-2	0.049	0.039	1.050(0.973,1.133)	0.21
IL-6	0.002	0.002	1.001(1.000,1.002)	0.17
IL-8	0.001	0.001	1.000(0.009,1.001)	0.35
IL-10	-0.015	0.013	0.985(0.961,1.010)	0.25
IL-17	0.183	0.070	1.201(1.046,1.379)	0.009
IFN- γ	0.003	0.003	1.001(0.999,1.002)	0.54
constant	-1.372	0.305	0.254	< 0.001
Equation	Logit(P)=1.372 + 0.049×IL-2 + 0.002×IL-6 + 0.001×IL-8-0.015×IL-10 + 0.183×IL-17 + 0.003×INF- γ			
R ²	0.409			
predictive efficiency(%)	75.00			

Table 4B. Model 2 Binary logistic regression for differential diagnosis between BM and VM Groups

project	B	SE	OR95%CI	P
CSF-GLU	-1.524	0.380	0.2180.1030.459	<0.001
CSF-CL	-0.059	0.044	0.9420.8651.027	0.17
CSF-CFP	0.001	0.001	1.0011.0011.002	0.063
CSF-WBC	0.001	0.001	1.0010.9991.003	0.46
constant	11.040	5.414	62315.582	0.041
Equation	Logit(P)=11.040-1.524×GLU-0.059×CL+0.001×CFP+0.001×WBC			
R ²	0.428			
predictive efficiency%	79.40			

Table 4C. Model 3 Binary logistic regression for differential diagnosis between BM and VM Groups

project	B	SE	OR95%CI	P
IL-6	0.001	0.001	1.0011.0011.002	0.13
IL-17	0.130	0.061	1.1391.0111.282	0.032
CSF-GLU	-1.463	0.442	0.2320.0970.551	0.001
CSF-CL	-0.065	0.052	0.9370.8471.037	0.21
CSF-CFP	0.001	0.001	1.0011.0001.001	0.10
CSF-WBC	-0.001	0.002	0.9990.9971.001	0.43
constant	10.944	6.322	56585.376	0.049
Equation	LogitP=10.944+0.001×IL-6+0.130×IL-17-1.463×GLU-0.065×CL+0.001×CFP-0.001×WBC			
R ²	0.564			
predictive efficiency %	81.30			

5. Differential diagnostic efficacy of the three models for discriminating between BM and VM groups

ROC curve analysis as the Table 5 revealed, that Model 3 demonstrated the superior discriminatory performance, with an AUC of 0.872. It achieved a specificity of nearly 80.00% and a sensitivity surpassing 85.00%. Both the positive and negative predictive values exceeded 80.00%, indicating a powerful capacity to correct false positives and false negatives. As the unique Youden index exceeding 0.650, Model 3 could serve as a reliable auxiliary modeling for differential diagnosis between BM and VM. As shown in Fig. 2, Model 3 exhibited the best performance among the three models for differentiating etiological types.

Table 5
Analysis results of ROC for three models between BM and VM Groups

project	AUC	Z	SEN (%)	SPE (%)	NPV (%)	PPV (%)	Youden
Model 1	0.781 (0.709–0.843)	7.532	63.89	84.09	74.00	76.70	0.4798
Model 2	0.839 (0.772–0.892)	10.388	77.78	80.68	81.60	76.70	0.5845
Model 3	0.872 (0.810–0.919)	12.520	78.39	87.50	82.90	83.30	0.6589
(Model 1 = IL2 + 6 + 8+10 + 17+IFN-γ, Model 2 = GLU + CL+ CFP + WBC, Model 3 = IL6+17 + GLU + CL+ CFP + WBC)							

(Among the sheet, all of Z means $P < 0.01$)

6. Internal structure evaluation and calibration analysis of the Model 3

As indicated in the left panel of Fig. 3, Model 3 demonstrated superior fit, evidenced by the lowest Akaike Information Criterion (AIC), Bayesian Information Criterion (BIC), Root Mean Square Error (RMSE) and Logarithmic Loss (Log Loss). Meanwhile, the coefficient of determination(R^2), probability of correct prediction (PCP), and correlation coefficient (core) reached their highest values, reflecting both strong explanatory power and robust predictive ability. Meanwhile, as indicated in the right panel of Fig. 3, Model 3 showed excellent calibration, as evidenced by a very low Brier score and a non-significant Hosmer-Lemeshow test result ($\chi^2=5.44$, $P > 0.05$), indicating strong predictive accuracy and high reliability.

7.Validation and calibration analysis of Model 3

Additional 75 CSF etiological test results were obtained, with 35 bacterial and 40 viral infections identified. The model validation was illustrated in the left panel of Fig. 4, the AUC of Model 3 yielded a maximum sensitivity of 76.09%, a specificity of 85.30%, negative and positive predictive values closed to or above 80.00%, and a Youden's index of 0.6139. Internal validation was performed using Bootstrap resampling ($B = 200$), as shown in the right panel of Fig. 4, the C-statistic of the model was 0.845, indicating satisfactory discriminative ability. The calibration slope was 0.953, and the Brier score was 0.095, reflecting low prediction error. The Hosmer-Lemeshow test yielded $\chi^2=3.26$ ($P > 0.05$), indicating that there was no significant deviation between predicted probabilities and observed outcomes, and that the risk of overfitting was low.

8. Evaluation of the clinical application value of Model 3

As indicated in the left panel of Fig. 5, in the decision curve analysis (DCA), Model 3 consistently outperformed the treat-all strategy across the 10%–40% threshold range, yielding net positive clinical benefits. As shown in Table 6A, these benefits were most prominent at lower thresholds (10%–20%), and the advantage over the treat-all strategy widened at intermediate thresholds (20%–30%). Although the threshold intervals were relatively wide, this limitation could be addressed by expanding the validation sample size in future studies.

As indicated in the right panel of Fig. 5, the DCA comparing Model 3 with clinical judgment alone showed that Model 3 had the highest net benefit at a threshold of 0%, with a gradual decline as the threshold increased. The net benefit remained positive across thresholds from 0% to 30%. Although the gap between Model 3 and clinical judgment narrowed at higher thresholds, Model 3 consistently demonstrated a superior net benefit compared with clinical judgment across the evaluated range, as shown in Table 6B, indicating a positive net clinical benefit in disease diagnosis and management.

Table 6A
Analysis results of DCA for model 3 between BM and VM Groups

Threshold probability	Net benefit	95% CI	antibiotic therapy all	antibiotic therapy none
10%	0.425	0.302–0.545	0.423	0
20%	0.366	0.245–0.490	0.347	0
30%	0.260	0.124–0.400	0.238	0
40%	0.228	0.087–0.365	0.119	0

Table 6B
Comparison of DCA between model 3 and clinical discrimination methods

Threshold probability	antibiotic therapy all	antibiotic therapy none	cancerpredmarker	famhistory
10%	0.050	0	0.080	0.028
20%	0	0	0.068	0.012
30%	-0.010	0	0.056	-0.050

Discussion

Meningitis is the leading infectious cause of neurological disability worldwide, with nearly 30% of affected children developing long-term sequelae, including emotional disorders, hearing loss, or visual impairment¹⁶. In 2023, an estimated 2.54 million new cases and 259,000 deaths were attributed to meningitis globally, with children under 5 accounting for more than one-third of fatalities¹⁷. Recent advances in molecular diagnostics have enhanced pathogen identification and antimicrobial stewardship, facilitating accurate diagnosis and rational therapy. However, polymerase chain reaction (PCR) can only detect predetermined pathogens, requires high gene copy numbers¹⁸, has an average annual ineffective test rate of 1.60%¹⁹, and cannot distinguish between viable and non-viable bacteria. The accuracy of mass spectrometry heavily depends on sufficient and purified samples, with significant inter-instrument variability, technical reproducibility of 55%–86%, and large measurement errors²⁰. Although next-generation sequencing (NGS) outperforms many molecular detection methods, its methodological limitations include 91% human-derived sequences and only 75% sensitivity in CSF²¹. Furthermore, sample collection after antibiotic administration, pathogen loads below the detection limit in early disease stages, and the influence of certain bacterial groups or localized lesions²² collectively reduce its diagnostic accuracy to 40.4% in CSF infections²³. Therefore, NGS is more appropriate for ruling out infection or broadening the range of detectable pathogens than for serving as a standalone diagnostic tool²⁴. In this study, its average diagnostic omission rate exceeded 46%, and negative results necessitated comprehensive interpretation in conjunction with other clinical findings.

To improve the accuracy of early differential diagnosis and promote rational drug use, this study established an early differential model between BM and VM, with the aim of mitigating the interference of lymphocyte-derived cytokines in the discrimination of BM. We explored the recruitment and release of neutrophils in the CSF during BM, a process that exacerbates blood–brain barrier injury and augments the extent of neuroinflammation²⁵. What cannot be ignored is that both cerebrospinal fluid mononuclear macrophages and dural macrophages play a substantial role in the subsequent course of bacterial meningitis^{26,27}. This study demonstrated that IL-8 levels are highest in BM, which held clinical value in differentiating it from VM. These results indicate that the bacterial inflammatory response can stimulate monocyte-derived macrophages to cross the blood-brain barrier, secrete large amounts of IL-8, and promote neutrophil recruitment into the subarachnoid space, where neutrophils become activated, secrete IL-6, and undergo degranulation, ultimately leading to disruption of the blood-brain barrier in acute BM²⁸. At the onset of bacterial invasion, cerebrovascular endothelial cells promote astrocyte activation via two parallel pathways: one direct, involving soluble factors and the CK2–Myosin 9–NF-KB axis²⁹; the other indirect, where in endothelial-derived IL-6 and other cytokines activate microglia to produce MyD88/NF-KB-dependent IL-6 and IL-8^{30,31}, which in turn trigger astrocyte activation and complement-enhancing effects³². Microglia and astrocytes concurrently produce IL-6, with a delayed secretion of IL-10³³. The observation that IL-10 levels were higher in BM than in VM suggests an imbalance between pro-inflammatory and anti-inflammatory responses^{34,35}. Moreover, cerebrovascular endothelial cells, through IL-6 secretion, promoted Th1 polarization of central nervous system infiltrating T cells, thereby enhancing the production of Th1 associated IFN- γ and IL-2³⁰. IL-2 mediated regulation

relies on astrocytes to exert its functions³⁶, whereas elevated IFN- γ activates astrocytes and disrupts cerebral vascular endothelial tight junctions³⁷. Therefore, increases in IL-6, IL-10 and IFN- γ in the CSF of patients with suspected BM who are negative on bacterial culture can be used to predict the disease³⁸.

In this study, IL-2, IL-6, IL-8, IL-10, IL-17 and IFN- γ all showed significant value in differentiating BM from VM. Among them, IL-6 and IL-17 demonstrated the highest diagnostic accuracy based on their AUC values. This is attributable to the additive effects of various cells during the inflammatory response in BM, which collectively result in substantial IL-6 secretion. Upon neural injury, mononuclear phagocytes activate the NF- κ B signaling pathway to upregulate IL-6 expression in adjacent microglia, and also trigger the PERK pathway to enhance IL-23 secretion by microglia³⁹. IL-6 and IL-23 can directly induce neutrophils expressing ROR γ t transcription factor to produce IL-17, and significantly enhance the killing ability of neutrophils⁴⁰. This study showed that IL-6 and IL-17 were sensitive and synergistic indicators for the occurrence and development of BM, with etiological direction. Some scholars also pointed out that higher levels of IL-6 and IL-17 were associated with acute complications of BM and neurological sequelae⁴¹.

Nevertheless, given the insufficient discriminatory capacity of any single biomarker to distinguish between BM and VM, this study established a combined detection model for cytokines and conducted model evaluation and performance evaluation in comparison with conventional cerebrospinal fluid biochemical parameters. Because the goodness-of-fit of the combined detection model for cytokines was inferior to that of conventional cerebrospinal fluid biochemical parameters, and its area under the curve for evaluating the differential efficacy in identifying the etiological types of common pediatric infectious meningitis was lower than that of conventional cerebrospinal fluid routine biochemical parameters, whereas IL-17 served as a key indicator for distinguishing between BM and VM, a combined detection model incorporating IL-6 and IL-17 with conventional cerebrospinal fluid biochemical parameters (Model 3) was established, with reference to the area under the curve of each single indicator.

In the analysis of the internal results, the Model 3 showed stronger explanatory power in data analysis, as well as the ability of disease prediction. This was also confirmed in the evaluation of differential diagnosis effectiveness for BM or VM. In this research, Model 3 showed the high AUC, with sensitivity and specificity close to or exceeding 80%, by the high Youden index indicated a robust model for clinical differential screening. Unlike the cytokine combination model proposed by Sibel Yorulmaz Goktas et al for suspected meningitis screening⁴², our study focuses specifically on differentiating between BM or VM, rather than merely identifying cases from general populations. It also differs from the suspected case identification model by Sabine E. Olie et al. in adult hospitals, which only focuses on different inflammatory indicators⁴³. Qua the recommended indicators for the diagnosis and treatment of BM or VM in current expert consensus and laboratory, traditional routine biochemical indexes in CSF have been widely used for a long time. Recently, the importance of traditional routine biochemical indexes in CSF have been established in the domestic research on the identification of Tuberculous meningitis and

BM⁴⁴. This study focused on the metabolic effects following cytokine action, added IL-6 and IL-17 to optimize the existing cerebrospinal fluid biochemical detection technology. The specificity and positive predictive value were increased by about 7%, which also hinted that the Model 3 in CSF could better reduce the clinical misdiagnosis rate. Moreover, reverse validation demonstrated that IL-6 and IL-17 exhibited characteristics indicative of BM infection.

This study is the first to consider the difficulty of cerebrospinal fluid collection in young children, to reduce the required sample volume, to optimize and integrate existing mature cerebrospinal fluid detection parameters, and to screen for the optimal predictive model for the purpose of accurate disease type identification. In this study, the combined detection model incorporating IL-6 and IL-17 with conventional cerebrospinal fluid biochemical parameters demonstrated robust discriminative ability, suggesting its potential as a reliable tool for differentiating bacterial from viral meningitis in pediatric patients. The superior performance of this combined detection strategy, as reflected by the C-statistic value of 0.845, was likely attributable to its ability to capture complementary pathophysiological information from both inflammatory cytokines and routine parameters. The near-ideal calibration further ensured that predicted probabilities closely match observed risks. This holds particular value in settings where bacterial cultures remain negative or delayed, Model 3 provides a rapid adjunctive tool to support early diagnosis, potentially reducing misdiagnosis and guiding appropriate therapy.

In the actual evaluation of clinical application benefit, the combined detection Model 3 assisted clinicians in the whole process of disease from "vigilant observation" to "active medication", and it had stronger predictive ability at an early stage, which could effectively promote the rational use of drugs in clinical practice and prevent the adverse effects caused by the abuse of antibiotics. Compared with the method relying solely on the judgment of clinicians, Model 3 has the advantage of accurate discrimination in the absence of clinical evidence for determining disease type. In the early and rapid identification of the disease, it can effectively assist the clinical evaluation of the etiological types of common infectious meningitis in children, and provides a new laboratory test method for clinical practice.

This study has several limitations. First, it is a single-center study with cases collected only from 2020 onward, without inclusion of earlier data or extension to the full pediatric age range. Additionally, the number of newborns included was insufficient. The predictive value of this model for disease occurrence and progression warrants further investigation. Moreover, external validation in a large, multicenter prospective cohort is essential to confirm the model's generalizability across different populations and clinical settings, and several steps remain to be completed before clinical implementation.

Conclusion

Collectively, the analysis of cerebrospinal fluid cytokines refines the diagnostic value of conventional CSF testing, thereby enhancing the early etiological diagnosis and differentiation of common infectious meningitis in children. The combined detection model incorporating IL-6 and IL-17 with conventional

cerebrospinal fluid biochemical parameters constitutes a complementary laboratory screening strategy to standard pathogen detection. Its implementation in cases with negative etiological findings enables the effective correction of clinical misdiagnosis.

Declarations

Ethics Approval and Consent to Participate

This study was conducted in accordance with the WMA Declaration of Helsinki (2024 revision) and the CIOMS International Ethical Guidelines (2016). The protocol was approved by the ethics committee of Capital Institute of Pediatrics, batch number SHERLLM2024032. This ethics committee is affiliated with the Capital Institute of Pediatrics and is responsible for conducting ethical review of all research involving human subjects at the Capital Center for Children's Health, Capital Medical University. All procedures complied with the Measures for Ethical Review of Life Science and Medical Research Involving Human Beings (2023). Written informed consent was obtained from all participants. As the study involved only routine biochemical and immunological analyses without generating or utilizing human genetic resource information, the Implementing Rules for the Regulations for the Administration of Human Genetic Resources (2023) were not applicable.

Written informed consent to participate was obtained from the parents or legal guardians of all participants under the age of 16. In addition, the research process was explained to each child in age-appropriate language, and assent was obtained from the minors themselves prior to participation. Assent was documented in writing using an assent form previously approved by the parents or legal guardians and the ethics committee. Participants were informed that their participation was voluntary and that they could withdraw at any time without consequence.

Consent for publication

Not applicable.

Consent statement

Informed consent was obtained from all participants prior to their involvement in the study.

Competing interest

The authors have no conflicts of interest relevant to this article to disclose.

Data Sharing Statement

All data generated or analysed during this study are included in this published article and its supplementary information files.

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Author Contributions

Shuping Wang: Writing – original draft, Conceptualization, Validation, Formal analysis, Data curation, Methodology, Supervision, Visualization.

Lijuan Ma: Writing – review & editing, Conceptualization, Funding acquisition, Supervision.

Jing Li: Writing – review & editing, Conceptualization, Methodology, Supervision, Validation.

Wen Zhang: Data curation, Resources, Formal analysis, Investigation.

Weiya Wang: Data curation, Resources, Formal analysis, Investigation.

Shuzhi Dai: Resources, Formal analysis, Investigation.

All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

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Figures

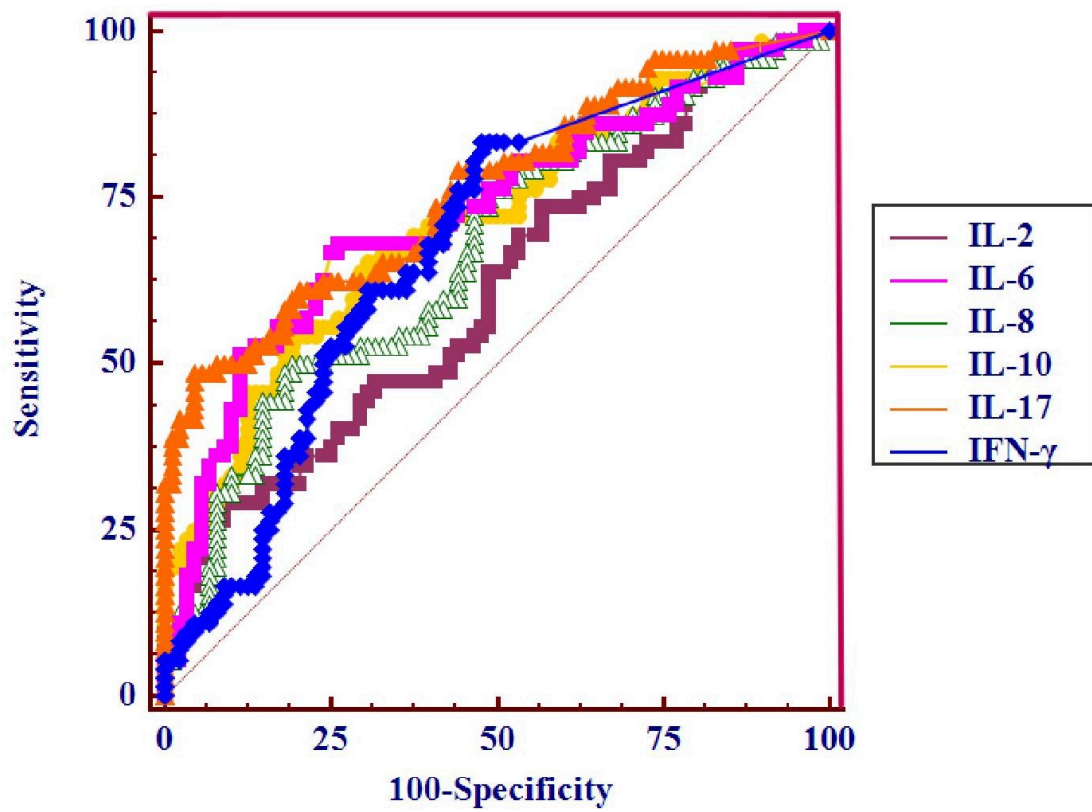


Figure 1

ROC curve of cytokines between BM and VM Groups

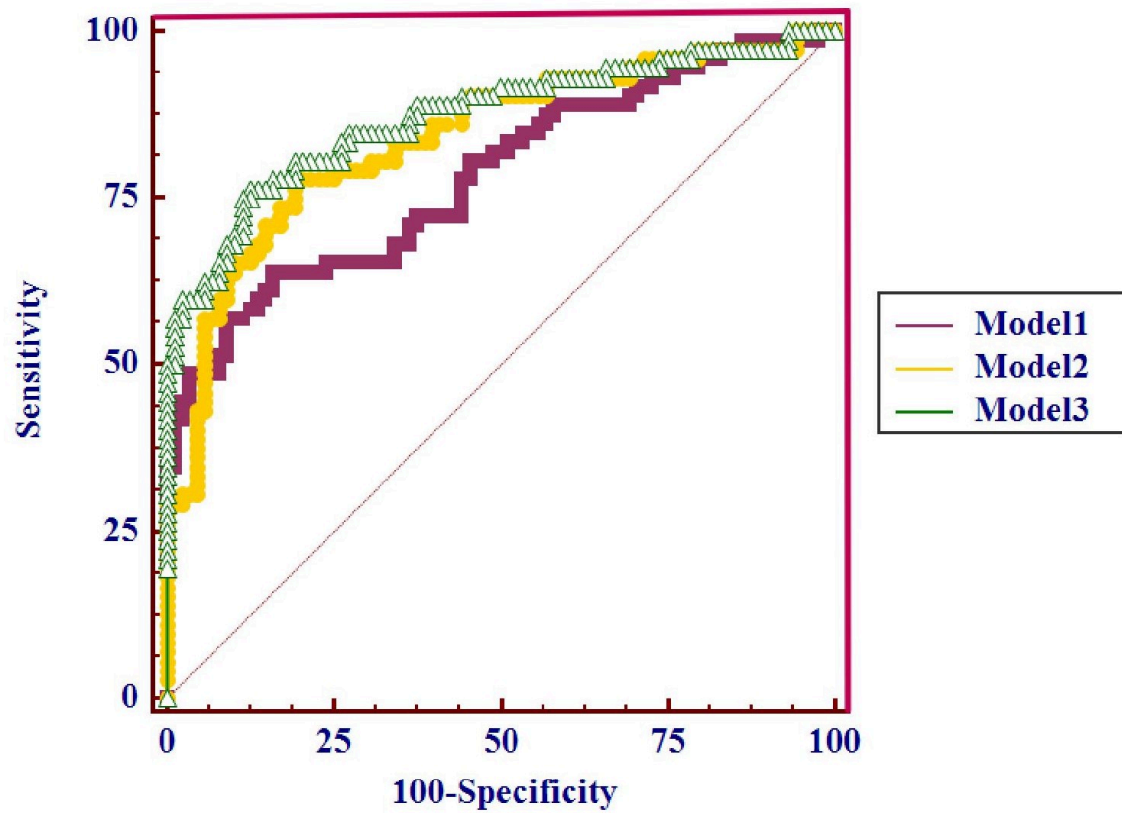


Figure 2

ROC curve of three models for Identification

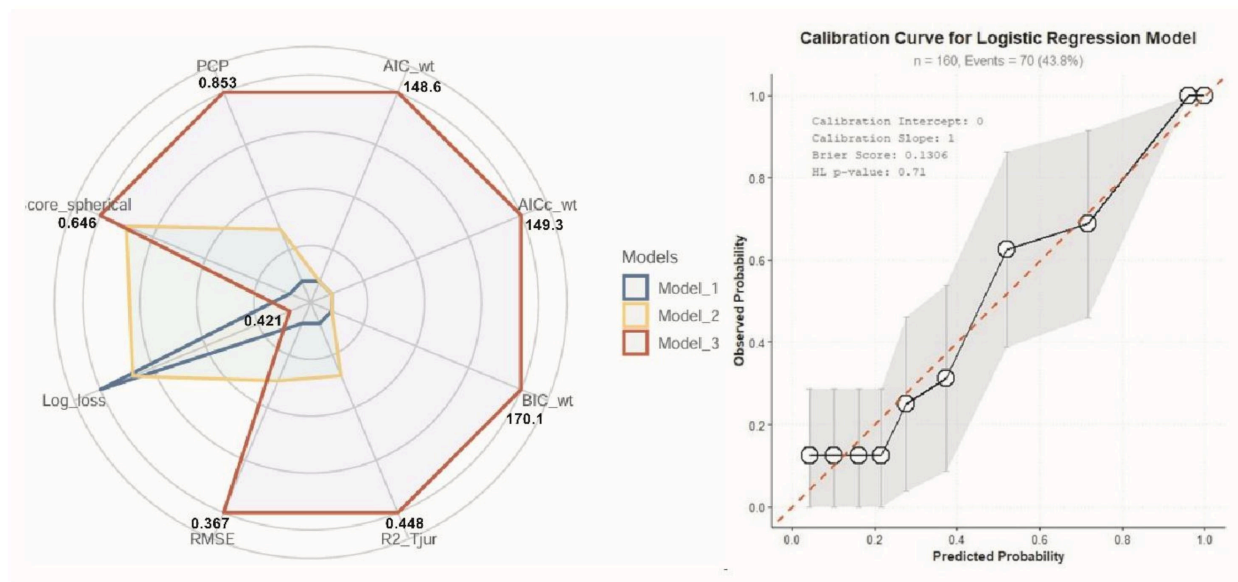


Figure 3

Radar chart for evaluation of three models and correction curve of Model 3

The radar chart on the left presents seven performance metrics used to evaluate the three models: Akaike Information Criterion (AIC), Bayesian Information Criterion (BIC), Root Mean Square Error (RMSE), Logarithmic Loss (Log Loss), coefficient of determination (R^2), probability of correct prediction (PCP), and correlation coefficient (corr). As shown in the radar chart, Model 3 (red) exhibited the largest coverage area and thus demonstrated the best overall performance among the three models.

The calibration curve on the right closely approximates the ideal curve. Model calibration was evaluated using the Brier score and the Hosmer-Lemeshow test, with the latter yielding a χ^2 statistic.

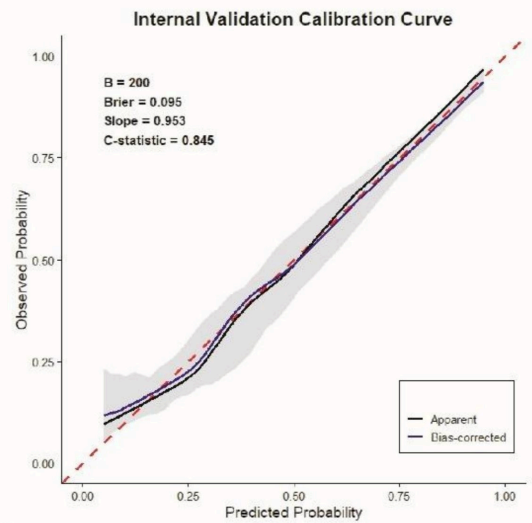
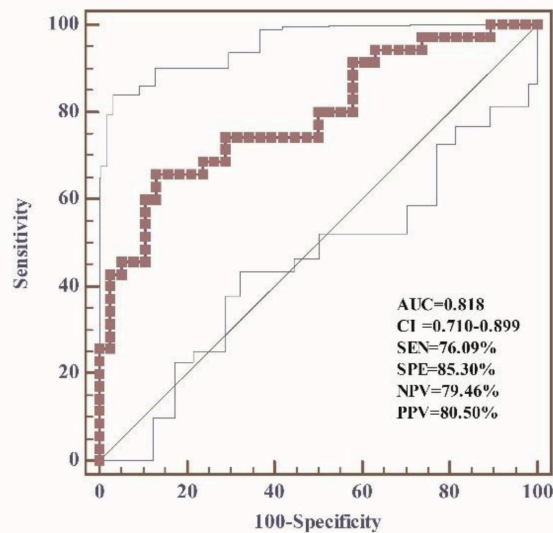


Figure 4

ROC curve and calibration curve of model validation

The left panel presents the ROC curve of Model 3 on the validation dataset, while the right panel shows the corresponding calibration curve. Model performance was evaluated using the calibration slope, the Brier score, and the Hosmer-Lemeshow test (with χ^2 statistic). The model demonstrated good performance without evidence of overfitting.

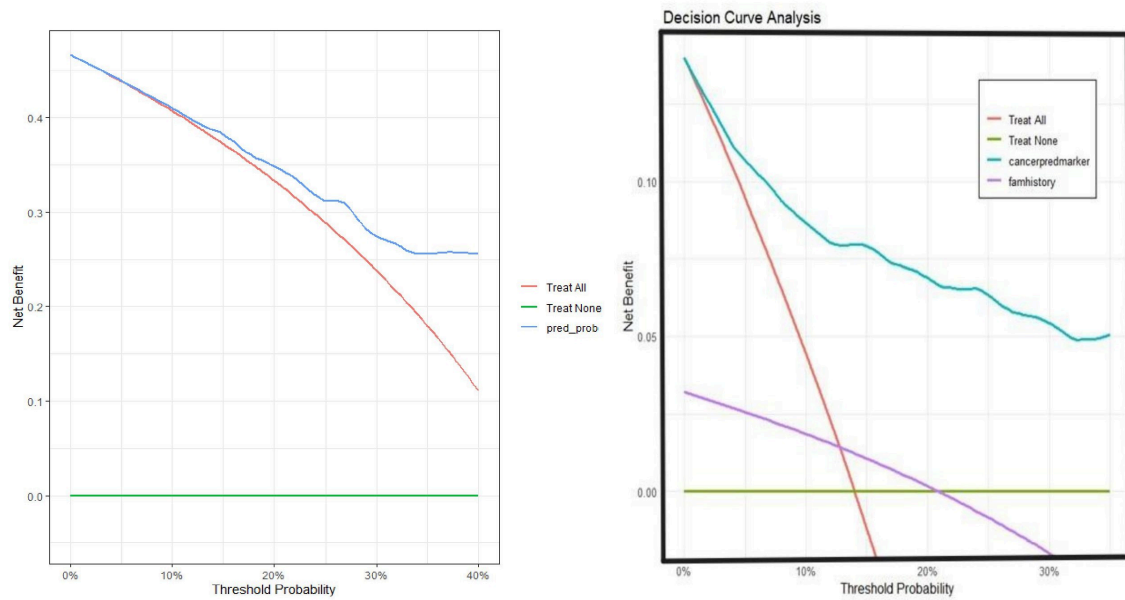


Figure 5

Decision curve analysis and calibration curve compares with clinical judgment in model validation

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