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Exploring the appropriate situation of performing CSF mNGS in patients with proposed intracranial infections

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Abstract

Background Identifying the responsible pathogen is crucial for precision medicine in intracranial infections, and Cerebrospinal Fluid (CSF) Metagenomic Next-Generation Sequencing (mNGS) is a reliable method for this detection. However, the indiscriminate utilization of this approach may impose a financial burden on both patients and society. The study aims to investigate the optimal conditions for applying CSF mNGS in patients with suspected intracranial infections, offering valuable references for precision medicine of intracranial infections.

Methods A total of 175 hospitalized patients presenting with suspected intracranial infections were selected for retrospective analysis. Base on the detection of responsible pathogens using CSF mNGS, the patients were categorized into two groups, responsible pathogens in Group A were detected but not in Group B. The types of responsible pathogens in group A and the final diagnosis of patients in group B were analyzed. Demographic data, clinical presentation, CSF analysis, imaging results, and electroencephalography (EEG) findings were analyzed for both groups. Finally, a scoring system was established to promptly assess the appropriateness of CSF mNGS for patients with suspected intracranial infections. Each independent predictor was assigned a score of 1, and the patients were subsequently scored. We advocate sending patients' CSF for mNGS when the cumulative score is ≥ 2.

Results In Group A, the predominant responsible pathogen was the varicella-zoster virus (VZV), while Group B exhibited the highest proportion of final diagnoses related to epilepsy. The logistic regression model indicates that headache [OR = 2.982, 95% CI (1.204–7.383), $p = 0.018$], increased cerebrospinal fluid white cell count [OR = 4.022, 95% CI (1.331–12.156), $p = 0.014$], and decreased cerebrospinal fluid glucose levels [OR = 9.006, 95% CI (2.778–29.194), $P < 0.001$] are independent predictive factors for intracranial infection pathogens detected by CSF mNGS. Under this scoring system, the sensitivity for detecting the responsible pathogen was 57.5%, and the specificity was 87.4%.

Conclusion The likelihood of detecting the responsible pathogen through CSF mNGS in patients with suspected intracranial infections can be evaluated using the scoring system. Furthermore, it is crucial to consider the possibility

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of another condition, such as epilepsy, when the responsible pathogen is not detected using cerebrospinal fluid mNGS.

Keywords Intracranial infection, Metagenomic next-generation sequencing, Scoring system, Cerebrospinal fluid, Responsible pathogen, Precision medicine

Introduction

Intracranial infections pose a significant health burden with diverse pathogen involvement, leading to high mortality and disability rates [1, 2]. Global reports indicate approximately 1.3 million cases of meningitis annually, while encephalitis has an incidence ranging from 1.7 to 7.4 cases per 100,000 people [3, 4]. However, epidemiological studies have revealed that nearly 30-50% of encephalitis cases lack a definitive etiological diagnosis [5]. In a retrospective cohort study conducted by Michael A. Hansen and colleagues, examining 340 cases of encephalitis in 19 hospitals in New Orleans, Louisiana, and Houston, Texas, from 2000 to 2017, it was revealed that 194 cases (57%) had an unknown etiology [6]. In a retrospective study by Timothy A. Erickson and colleagues, medical records of 231 pediatric patients diagnosed with encephalitis in urban and rural areas of Houston were reviewed from 2010 to 2017. The results revealed that 42% of the pediatric patients had an unclear etiology [7]. Although traditional methods like cerebrospinal fluid (CSF) culture and PCR play a crucial role in pathogens detection, their sensitivity and efficiency still need to be improved. Metagenomic Next-Generation sequencing (mNGS) is an unbiased assay that allows simultaneous sequencing of number DNA or RNA fragments, enabling the identification of responsible pathogens [8, 9]. In theory, mNGS is believed to be capable of detecting nearly all pathogens in a single test, significantly enhancing the breadth and sensitivity of pathogen detection. Multiple studies have demonstrated the diagnostic value of CSF mNGS for central nervous system (CNS) infectious diseases, and it is increasingly being implemented in clinical settings [10–12].

However, not all suspected intracranial infection patients undergoing CSF mNGS testing can identify the pathogenic pathogen. Possible reasons for this situation may include improper sample collection, misdiagnosis of intracranial infection, low pathogen load, or limited extent of the infection. While a negative result from CSF mNGS can still provide valuable information to clinicians [13], the indiscriminate use of mNGS may impose a financial burden on patients and society due to its high cost. However, the relevant information about identifying the suitable situation of using CSF mNGS is incomplete. Therefore, a retrospective study was conducted on a clinical cohort of patients with suspected intracranial infections to determine the appropriate circumstances for utilizing CSF mNGS. This study aims to compare the differences between the two groups based on the detection of the responsible pathogen and to develop a scoring system to assist in decision-making for CSF mNGS. Additionally, the study also described and analyzed the responsible pathogens identified through CSF mNGS and the final diagnosis in cases where CSF mNGS failed to identify the responsible pathogens.

Materials and methods

This study focused on patients admitted to the Harrison International Peace Hospital in Hengshui, Hebei Province, who presented with suspected intracranial infections between April 1st, 2021, and February 23rd, 2023. All data in this study were gathered throughout clinical treatment and documented in medical records. The retrospective analysis was conducted using this selected population as the study sample. The inclusion criteria consisted of the following: (1) presence of one or more clinical manifestations associated with intracranial infection, such as headache, fever, nausea, vomiting, psychosis, decreased level of consciousness, seizures, cognitive impairment, focal neurological deficit, positive meningeal irritation signs, among others; (2) availability of both CSF mNGS and CSF analysis. The exclusion criteria encompassed cases with incomplete CSF data, including leukocyte, protein, glucose, and chloride content.

This study analyzed various aspects, including demographics, clinical presentation, cerebrospinal fluid analysis, imaging findings, and electroencephalography (EEG) results, within this patient cohort. In addition, this study also established a simple scoring system to assess whether patients should be recommended for diagnostic testing using CSF mNGS.

mNGS

Approximately 1–2 ml of patient's CSF was collected via lumbar puncture and promptly stored in a test tube within 30 min in a −80 °C refrigerator for subsequent mNGS analysis. DNA extracted was performed using a micro-sample genomic DNA extraction kit (Tiangen Biotech, Beijing, China), followed by fragmentation of the DNA into 200 to 300 bp base pair fragments using a DNA cutting ultrasound fragmentation machine (Bioruptor Pico protocols, Diagenode, Liege, Belgium). DNA libraries were generated once the samples passed quality control and size control assessments. This process involved quantitative polymerase chain reaction (PCR) detection, followed by end-repair, A-tailing, articulator ligation,

and PCR amplification using NGS Library Construction Kit (Genskey, Tianjin, China). The amplified DNA was subjected to rolling loop amplification, and the resulting nucleic acid fragments were loaded onto a sequencing chip for sequenced using the equipment and services (Genskey, Tianjin, China).

RNA was extracted from the CSF to construct an RNA-seq library. The extracted RNA underwent assessments for purity, integrity, and content. Subsequently, DNA and ribosomal RNA were removed from the RNA sample, and the remaining RNA was fragmented and prepared for mRNA library construction. The fragmented RNA was then subjected to reverse transcription into complementary DNA (cDNA), followed by end repair, A-tailing, and articulator ligation steps. After purifying the ligation products and performing amplification, the resulting library was sequenced using the sequencing services (Genskey, Tianjin, China).

Statistics

We utilized IBM SPSS Statistics version 21.0 to conduct the statistical analysis, and GraphPad Prism version 8.0.1 was employed to create graphs. We initially assessed continuous variables' normality using the Kolmogorov-Smirnov (K-S) test. If the variables conformed to a normal distribution, they were presented as mean (standard deviation, SD); otherwise, they were reported as median (interquartile range, IQR). Descriptive statistics were used to present count data in terms of frequency percentages. To compare continuous variables, we employed either the t-test or the Mann-Whitney U test based on the normality and homoscedasticity of the variables. The χ^2 test or Fisher's exact test was used for comparing count data. Furthermore, we conducted univariate logistic regression analysis on the count data to identify statistically significant indicators. Subsequently, multivariate logistic regression analysis was performed on these indicators. The statistically significant indicators from the multivariate logistic regression analysis were selected as independent predictors for identifying responsible pathogens through CSF mNGS. Based on these independent predictors, a scoring system was established. The scoring system was validated using ROC curves, and the Jorden index was employed to determine the appropriate score for distinguish when patients should be recommended for CSF mNGS. The internal validation of this scoring system was conducted by boostrap-resampling with R package "tidyverse" and "caret" (R version is 4.2.2).

Table 1 Responsible pathogens for encephalitis or meningitis in Group A patients

Pathogens responsible for intracranial infections	Number
Varicella zoster virus	10
Mycobacterium tuberculosis	4
Human herpesvirus type 6 A	4
Klebsiella pneumoniae	3
Cytomegalovirus	2
Herpes simplex virus	2
Listeria monocytogenes	2
Streptococcus pneumoniae	2
Hepatitis B virus	2
Cryptococcus neoformans	2
Acinetobacter baumannii	1
Brucella spp.	1
Escherichia coli	1
Aspergillus oryzae	1
Human Microvirus B19	1
Streptococcus constellatus	1
Serratia marcescens	1
Total	40

Results

Grouping

The cohort comprised 175 patients, aged 13 to 85, with a median of 56 years. Among the study, 52% (91/175) were male. The results of the CSF mNGS test were analyzed, and based on the detection of responsible pathogen, the patients were divided into two groups. Group A consisted of patients in whom CSF mNGS identified responsible pathogens, while Group B consisted of patients in whom CSF mNGS detected no responsible pathogens.

Clinical features

Clinical information of patients in group A

In group A, there were 40 individuals with a mean age of 55.30 years. Among these patients, 20 (50%) were male. The responsible pathogens identified in group A included viruses in 21 cases, bacteria in 16 cases, and fungi in 3 cases. Among all the pathogens detected, varicella zoster virus (VZV) was the most prevalent, accounting for 25% (10/40) of cases. The most commonly identified bacterial responsible pathogen was Mycobacterium tuberculosis, which accounting for 10% (4/40) of cases. Fungal intracranial infections were primarily associated with Cryptococcus neoformans, representing 5% (2/40) cases. The specific responsible pathogens are shown in Table 1; Fig. 1.

The most frequent clinical manifestations observed in patients from Group A were headache (47.50%), decreased level of consciousness (47.50%), and fever (42.50%). Nine patients (22.5%) presented with one or more signs of meningeal irritation. Among the patients in Group A, 27 underwent brain magnetic resonance imaging (MRI), and 7 exhibited findings suggestive of intracranial inflammation. Thirteen patients underwent electroencephalography (EEG), and 11 displayed abnormal EEG results. CSF pressure was measured in 34 patients, with a mean value of 177.79 mmH2O, and 15 patients exhibiting abnormal CSF pressure readings. In group A, the median CSF leukocyte count was

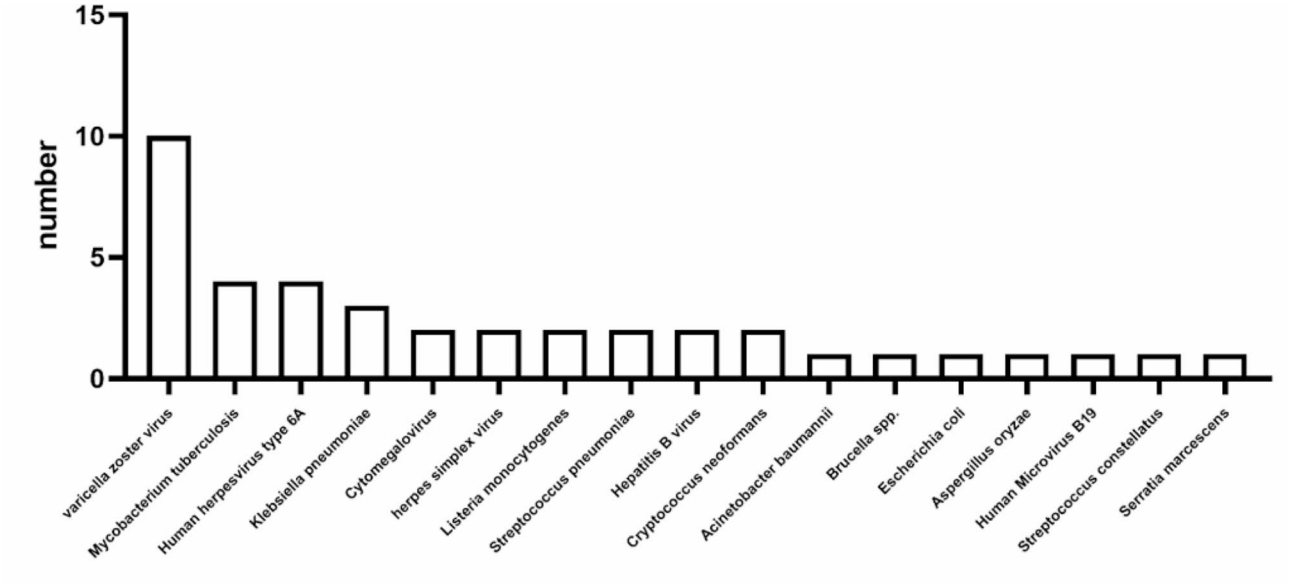

Fig. 1 Responsible pathogens for intracranial infections in Group A patients

Table 2 Comparison between clinical characteristics of patients with proposed encephalitis or meningitis and ancillary tests

	Group A		Group B		
Variable groups	**Number**	**Mean (SD)/ Median (IQR)/ Percentage**	**Number**	**Mean (SD)/ Median (IQR)/ Percentage**	**P-value**
Age (years)		55.30(20.55)		55(32)	0.076
Gender (male)	20	50%	71	52.6%	0.773
Fever	17	42.5%	33	24.4%	0.026*
Headaches	19	47.5%	36	26.7%	0.013*
Decreased level of consciousness	19	47.5%	43	31.9%	0.069
Psychosis			15	11.11%	
Seizures	6	15%	25	18.5%	0.609
Cognitive impairment	1	2.5%	7	5.2%	0.777
Nauseating	4	10%	12	8.9%	1
Vomiting	3	7.5%	14	10.4%	0.815
Focal neurological deficit	10	25%	40	29.6%	0.569
Meningeal irritation sign	9	22.5%	23	17%	0.432
Intracranial inflammation in MRI	7($N=27$)	25.9%	10($N=100$)	10%	0.031*
Abnormal EEG	11($N=13$)	84.6%	36($N=51$)	70.6%	0.503
CSF Leukocytosis	29	72.5%	42	31.1%	< 0.001**
CSF protein increased	26	65%	41	30.4%	< 0.001**
CSF glucose decreased	17	42.5%	11	8.1%	< 0.001**
CSF chloride decreased	23	57.5%	41	30.4%	0.002**
CSF pressure abnormal	15($N=34$)	44.1%	49($N=128$)	38.3%	0.536

IQR: interquartile range; SD: standard deviation. *: $P<0.05$, **: $P<0.01$

130.5×10^6/L, with 29 patients (72.50%) having elevated CSF leukocyte levels. The median CSF protein was 1068.5 mg/L, and 26 patients (65%) had elevated CSF protein levels. For CSF glucose, the median value was 2.70 mmol/L, and 17 patients (42.50%) exhibited decreased CSF glucose levels. Regarding CSF chloride, the median concentration was 118.13mmol/L, and 23 patients (57.50%) had decreased CSF chloride levels. More detailed clinical information can be found in Table 2.

Clinical information of patients in group B

In group B, there were 135 individuals with a median age of 55; among them, 71 patients (52.6%) were male. The most common clinical manifestations in group B patients were decreased level of consciousness (31.9%), focal neurological deficit (29.6%), and 23 (17%) patients presented with one or more signs of meningeal irritation. Out of the group B patients, 100 underwent brain MRI, and 10 displayed findings suggestive of intracranial inflammation. Additionally, 51 patients underwent EEG, with

36 exhibiting abnormal EEG results. CSF pressure was measured in 128 individuals, and 49 had abnormal CSF pressure readings. The median CSF pressure in B group was 150 mmH2O. Regarding CSF analysis, the median leukocyte count was 3×10^6/L, and 42 patients (31.1%) had elevated CSF leukocyte levels. The median CSF protein levels was 341 mg/L, with 41 patients (30.4%) exhibiting elevated CSF protein levels. The median CSF glucose level was 3.97 mmol/L, and 11 patients (8.1%) had decreased CSF glucose levels. The median CSF chloride level was 122.90 mmol/L, and 41 patients (30.4%) had decreased levels. More detailed clinical information can be found in Table 2.

We analyzed the final diagnosis of patients in group B and discovered that 22 patients had epilepsy, making it the most prevalent diagnosis. Additionally, 19 patients were diagnosed with cerebrovascular disease, 9 had autoimmune encephalitis, 14 had non-neurological diseases, and 36 had an unknown diagnosis. The final diagnosis of the patients in group B is described in Table 3; Fig. 2.

Comparison of clinical data between the two groups

After analyzing both groups' demographic characteristics and clinical manifestations, we observed no statistical difference in the age and gender composition. However, when considering clinical manifestations, we found a significantly higher occurrence of headache and fever in group A patients than in group B ($P<0.05$). Furthermore, 15 patients in group B exhibited psychosis, whereas none were observed in group A. Additionally, although a higher percentage of patients in group A displayed meningeal irritation signs compared to group B, no statistical significance was observed. Regarding ancillary examinations, we identified statistically significant differences ($P<0.01$) between groups A and B in the incidence of increased cerebrospinal fluid protein content, increased leukocytes, decreased glucose content, and decreased chloride content. These abnormalities were more prevalent in Group A than in group B. However, the proportion of cerebrospinal fluid pressure abnormalities did not show significant differences ($P=0.536$) between the two groups. Moreover, the proportion of patients in Group A exhibiting intracranial inflammation on brain MRI was significantly higher than in Group B, with a statistically significant difference ($P=0.031$). Although, the incidence of abnormal EEG was higher in group A than in group B, it did not reach statistical significance. For detailed information on the demographic characteristics, clinical manifestations, and findings from ancillary tests in both groups (Table 2).

Table 3 Final diagnosis of patients in group B

Final diagnosis	Number
Epilepsy	22
non-neurological diseases	14
Ischemic cerebrovascular disease	14
Autoimmune encephalitis	9
Primary headache	8
Parainfectious encephalitis	6
Hemorrhagic cerebrovascular disease	5
Tumours of the CNS	3
Hypercranial pressure syndrome	2
Neuronal intranuclear inclusion body disease	2
Hypocranial pressure syndrome	1
Multiple Sclerosis	1
Radiation encephalopathy	1
Paraneoplastic syndrome	1
Acute Grimballi syndrome	1
Facial nerve palsy	1
Meningeal bulge	1
Vestibular nerve vascular- compression syndrome	1
Mild cerebral white matter lesions	1
heat strode	1
Vaso-vagal syncope	1
Late onset encephalopathy from carbon-monoxide poisoning	1
Peripheral vertigo	1
Autoimmune myelitis	1
Diagnosis unknown	36
Total	135

Scoring system established

A scoring system was established to explore the appropriate circumstances of CSF mNGS in patients with a proposed intracranial infection. The logistic regression model indicates that headache [OR=2.982, 95% CI (1.204–7.383), $p=0.018$], increased cerebrospinal fluid white cell count [OR=4.022, 95% CI (1.331–12.156), $p=0.014$], and decreased cerebrospinal fluid glucose levels [OR=9.006, 95% CI (2.778–29.194), $P<0.001$] are independent predictive factors for intracranial infection pathogens detected by CSF mNGS (Table 4). We assigned a score of 1 to each independent predictor and scored the patients in the cohort. The proportion of patients in Group A and Group B in each score and the proportion of patients in each score in two groups are shown in Fig. 3. We recommend sending patients' cerebrospinal fluid for mNGS when the score is ≥2. The sensitivity for detecting the responsible pathogen at this juncture stood at 57.5%, while the specificity reached 87.4%. Furthermore, a notable difference was observed in the proportion of patients with a cumulative score of ≥2 between Groups A and B. We demonstrated the accuracy of this scoring system using the ROC curve (AUC=0.78, $P<0.001$), which is detailed in Fig. 3. We additionally performed internal validation through bootstrap resampling, demonstrating that this scoring system exhibits higher accuracy (accuracy: 0.789).

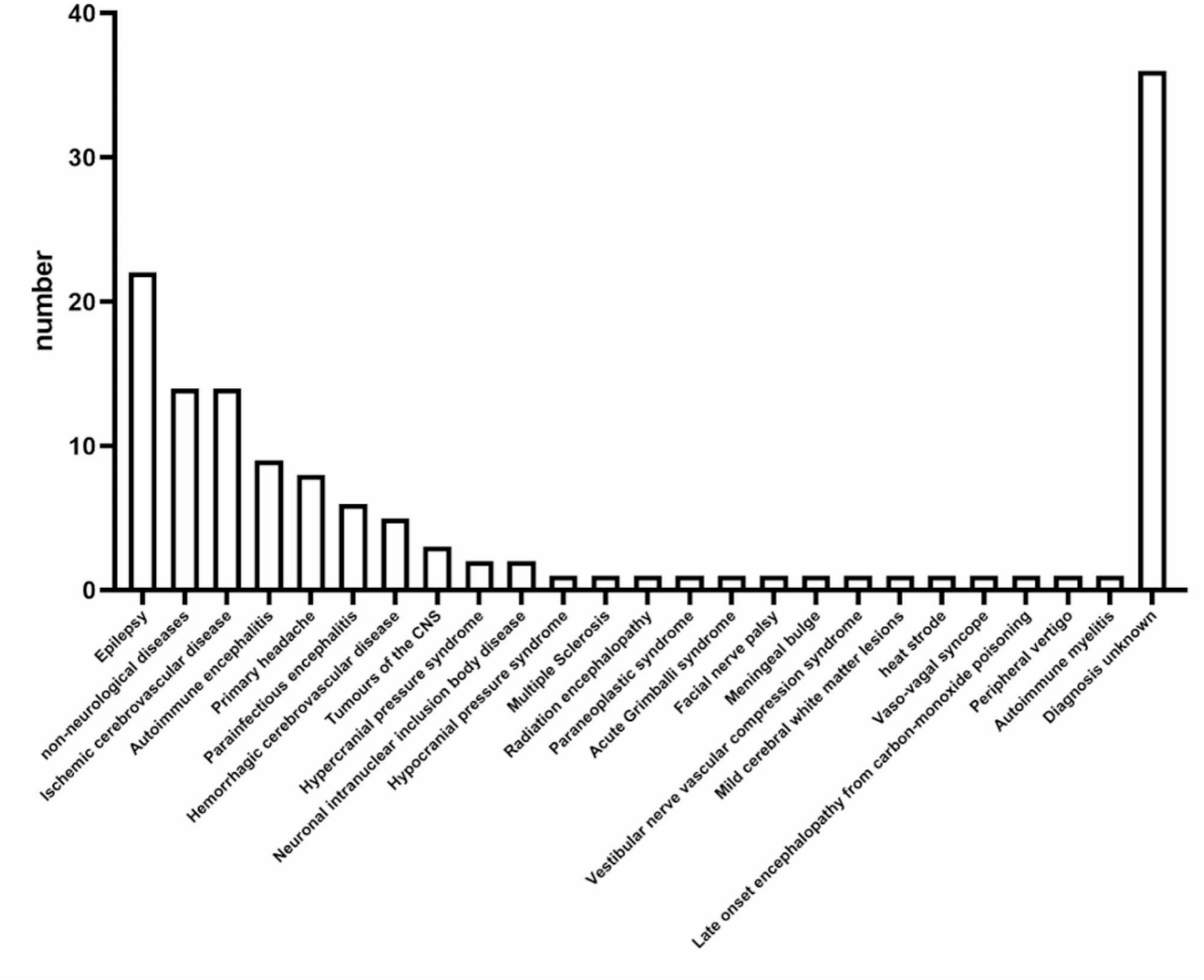

Fig. 2 Final diagnosis of patients in group B

Discussion

Intracranial infection refers to invading pathogens into the central nervous system, leading to acute or chronic diseases. Various pathogens, such as bacteria, virus, fungi, parasites, and other pathogens can be responsible for intracranial infections. Common clinical presentations include headache, fever, neck straightening, and, in cases where the inflammation involves the brain parenchyma, epilepsy and a decreased level of consciousness may occur [14]. However, approximately half of all CNS infections fail to identify the specific pathogen. To address this, mNGS is employed as a non-targeted assay to detect known and unknown pathogens analyzing nucleic acids in clinical samples and comparing them to known pathogens. Due to its unbiased nature, mNGS has the potential to identify almost all pathogens, making it a valuable tool in clinical practice. CSF mNGS has been increasingly used in China for diagnosing neurological infections, with several studies confirming its effectiveness [10, 15, 16]. However, the indiscriminate use of mNGS can be costly, posing a financial burden on patients and society. Therefore, selecting suitable patients for cerebrospinal fluid mNGS is essential. This study analyzed a cohort of 175 patients suspected of having intracranial infections who underwent CSF mNGS. Based on the results, the patients were divided into two groups: Groups A, where a responsible pathogen was detected by CSF mNGS, and group B, where no responsible pathogen was identified.

In the clinical diagnosis of intracranial infection, a comprehensive analysis of the patient's clinical manifestations, laboratory tests, imaging examinations, and electro-encephalography plays a crucial role. The collection and analysis of these clinical data revealed that a higher proportion of patients in group A exhibited fever and headache, and this difference was statistically significant. This suggests that when patients with symptoms of headache and fever, there is a greater likelihood of

Table 4 The results of Univariate and Multivariate Logistic regression analysis

	Univariate regression		Multivariate regression	
Variable groups	**OR (95% CI)**	**P value**	**OR (95% CI)**	**P value**
Gender	1.109(0.548–2.247)	0.773		
Fever	**2.285(1.090–4.787)**	**0.029**	1.271(0.503–3.212)	0.613
Headaches	**2.488(1.201–5.155)**	**0.014**	**2.982(1.204–7.383)**	**0.018**
Decreased level of consciousness	1.936(0.944–3.971)	0.072		
Seizure	0.776(0.294–2.049)	0.609		
Cognitive impairment	0.469(0.056–3.929)	0.485		
Nauseating	1.139(0.346–3.747)	0.831		
Vomiting	0.701(0.191–2.572)	0.592		
focal neurological deficit	0.792(0.354–1.771)	0.570		
Meningeal irritation sign	1.414(0.594–3.365)	0.434		
CSF leukocytosis	**5.838(2.666–12.784)**	**<0.001**	**4.022(1.331–12.156)**	**0.014**
Reduced glucose levels in CSF	**8.332(3.458–20.074)**	**<0.001**	**9.006(2.778–29.194)**	**<0.001**
Increased protein levels in CSF	**4.258(2.019–8.979)**	**<0.001**	1.517(0.550–4.187)	0.421
Reduced chloride levels in CSF	**3.102(1.500-6.413)**	**0.002**	1.058(0.381–2.944)	0.913
CSF pressure anomaly	1.273(0.592–2.735)	0.537		
Intracranial inflammation in MRI	**3.150(1.069–9.281)**	**0.037**	2.572(0.711–9.304)	0.150
Abnormal EEG	2.292(0.452–11.610)	0.316		

Statistically significant results are bolded in type

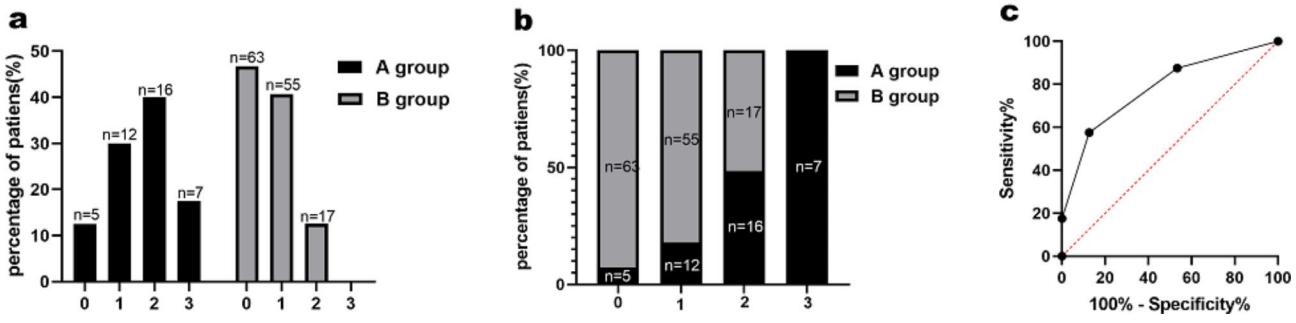

Fig. 3 Scoring system established. (**a**) Proportion of patients in each score in two groups (**b**) Proportion of patients in Group A versus Group B in each score (**c**) ROC curve of the scoring system

detecting the responsible pathogen through CSF mNGS. However, it is essential to note that headache and fever are non-specific symptoms as they can be present in various diseases. Therefore, clinicians need to interpret these symptoms in conjunction with other ancillary tests to arrive at an accurate diagnosis.

The analysis of CSF changes plays a crucial role in diagnosing intracranial infection. This study comprehensively examined the CSF indicators of patients. However, it is essential to note that different pathogens can cause distinct CSF manifestations in intracranial infections. Virus infections often result in mildly increased CSF leukocytes and elevated CSF proteins levels [17]. On the other hand, intracranial bacterial infections exhibit different CSF characteristics, characterized by markedly elevated CSF leukocyte ($>1000\times10^6$/L), along with increased CSF protein and decreased CSF glucose levels [18]. Reduced CSF chloride levels often accompany intracranial infections caused by Mycobacterium tuberculosis. Furthermore, some patients with intracranial infections may have normal CSF, making diagnosis through CSF analysis more challenging [4]. This study observed significant differences in CSF indicators, except for CSF pressure, between patients in groups A and B. These findings suggest that CSF indicators can serve as screening criteria to determine whether patients with suspected intracranial infections should undergo CSF mNGS.

MRI could be the first choice in imaging methods for diagnosing intracranial infections [19]. The proportion of intracranial inflammation found by brain MRI was significantly higher in group A than in group B. This suggests that brain MRI screening can be an initial assessment of the need for CSF mNGS in patients with a proposed intracranial infection. It is noteworthy that in some Group B patients, intracranial inflammation also be found by brain MRI, which may be due to the presence of autoimmune diseases, early infections that are difficult to detect, limited infections, or post-infection course [7].

Electroencephalography (EEG) is also an ancillary test to diagnose intracranial infections. The manifestations of EEG abnormalities in intracranial infections are diverse, and intracranial infections caused by different pathogens may cause specific EEG changes. Herpes simplex virus encephalitis typically manifests with periodic lateralized epileptiform discharges [17] and generalized periodic complexes, which consist of generalized and synchronous bursts of sharp-slow wave discharges in EEG. This pattern is characteristic of subacute sclerosing panencephalitis [18]. However, non-specific EEG alterations are more commonly observed in intracranial infections [19]. Our analysis of all abnormal EEGs results demonstrated a higher proportion of abnormal EEGs in group A compared to group B, although the difference did not reach statistical significance. Non-specific EEG abnormalities do not help screen the need for CSF mNGS in patients with suspected intracranial infections. However, specific EEG alterations related to different pathogenic infections or diseases may hold significance in screening, but such analysis was not conducted in this study.

In our analysis, we delved deeper into the responsible pathogens identified in the intracranial infections of group A patients and the final diagnosis of group B patients. Among the 40 patients in group A, virus infections were found to be responsible in 21 cases, bacterial infections in 16 cases, and fungal infections in 3 cases. Notably, VZV emerged as the most common pathogen detected in group A. Mycobacterium tuberculosis infection is the second most common type of this cohort, suggesting that tuberculous intracranial infections may be more prevalent than expected. Metagenomic next-generation sequencing (mNGS) may aid in the early diagnosis and treatment of such infections. Furthermore, some rare pathogens, such as Streptococcus constellatus and Serratia marcescens, have been detected, which are relatively rare in cases of intracranial infections. Therefore, when searching for the pathogens responsible for intracranial infections, doctors are less likely to consider them initially. These findings provide clinicians with valuable insights into precision medicine and contribute to our understanding of the epidemiological characteristics of intracranial infections in the Hengshui area.

In group B, most patients were diagnosed with epilepsy, which might have initially been considered a result of an infection involving the brain parenchyma. Furthermore, cerebrovascular disease, autoimmune encephalitis, and even non-neurological diseases can be misdiagnosed as intracranial infections during the initial diagnosis. This lack of specificity in the clinical presentation of intracranial infections could contribute to such misdiagnoses. However, the diagnosis remained unclear for 36 patients, out of which 15 were still suspected of having intracranial infection. Only one patient in this group had a suspected pathogen (Acinetobacter baumannii) detected through cerebrospinal fluid culture. These are several possible reasons for these uncertain diagnoses. Firstly, some patients may have yet to be detected by mNGS due to low pathogen load in the cerebrospinal fluid, the high background of host nucleic, or restricted infection site within the intracranial infection [20, 24]. Secondly, some patients may have autoimmune diseases that have not yet produced detectable antibodies, making it challenging to reach a definitive diagnosis. This study provides clinicians with critical diagnostic considerations when CSF mNGS results rule out intracranial infections.

Detection of responsible pathogens is crucial for the diagnosis and treatment of intracranial infections. PCR as a well-established traditional method, has played a significant role in identifying these pathogens. The development of multiple PCR and meningitis/encephalitis (ME) panels has led to substantial progress in the etiological diagnosis of intracranial infections based on PCR. With its simple and mature detection technology and short result time, such method is suitable for urgent scenarios. However, as the understanding of intracranial infections grows, the pathogen spectrum of intracranial infections continues to expand. Consequently, the limitations of multiple PCR or BioFire FilmArray meningitis/encephalitis (ME) panels are gradually becoming apparent, particularly the need to predict the responsible pathogen before detection [21, 22]. A significant advantage of CSF mNGS as an unbiased assay is its ability to detect rare or unknown pathogens that are difficult to identify based on PCR. One study demonstrated that CSF mNGS was able to identify pathogens in 22% of samples that had not been detected by traditional methods [12]. And in our study, there is 37.5% responsible pathogens aren't involved in the pathogens which could be identified by BioFire FilmArray meningitis/encephalitis (ME) panels. In certain cases, the condition may be complex, atypical, and lacking complete clinical data, making it difficult to predict the responsible pathogens. However, CSF mNGS possesses significant advantages in handling such situations due to its inherent characteristics [24]. In the detection of conventional pathogens, CSF mNGS also has advantages. A study indicated that for the detection of VZV, CSF mNGS exhibits higher sensitivity compared to PCR. Furthermore, the study found that CSF mNGS identified a patient with both VZV infection and Human herpesvirus type 6 A (HHV-6 A) infection, suggesting that CSF mNGS plays a crucial role in uncovering co-infections. This is of great significance for comprehensively understanding intracranial infections [23]. Despite its advantages, CSF mNGS also has some limitations: 1) Cost: Generally, one CSF mNGS test, which includes both DNA and RNA analysis, will cost around 4000 CNY. Although the cost of CSF mNGS is decreasing, it remains

relatively expensive. 2)Maturity: Compared to traditional test methods, CSF mNGS is still considered immature. Insufficient depth of sequencing may lead to false negatives. After CSF collection, the result of CSF mNGS can be returned within 72 h. We recommend that hospitals carry out CSF mNGS testing if it is feasible, or establish rapid response mechanisms with appropriate testing institutions to reduce the testing time. To expand the efficiency, we have developed a scoring system for CSF mNGS to assist clinicians in determining when the use of CSF mNGS is recommended for pathogen identification. As CSF mNGS technology continues to evolve, these limitations are expected to be overcome. Furthermore, the data obtained from CSF mNGS can be utilized for various secondary analyses, including testing for antimicrobial resistance genes of pathogens and determining whether the infection is linked to a disease outbreak in a specific location. Therefore, CSF mNGS is not only valuable for pathogen detection but also plays a crucial role in the treatment and management of patients, as well as in public health efforts [24].

Based on the univariate and multivariate logistic regression analyses, we identified headache, elevated CSF leukocyte levels, and decreased CSF glucose levels as independent predictors for detecting responsible pathogens through CSF mNGS. Each predictor was assigned a score of 1 to establish a scoring system. The higher the score in this scoring system, the greater the probability of detecting responsible pathogen through CSF mNGS. We recommend performing CSF mNGS for patients with a score of ≥2. Headache is a common manifestation of intracranial infection and can often be the initial symptom [24]. Our findings confirm that headache could serve as an independent predictor, further emphasizing the predictive significance of this symptom. However, this study did not analyze the severity, duration, and other headache characteristics, which should be explored in future studies. The results of CSF analysis hold significant value in assessing the need for CSF mNGS-previous studies by Yan. L [8] and Laura. M [25] have highlighted that higher CSF protein content and increased CSF leukocyte count are associated with a higher detection rate of responsible pathogens through CSF mNGS. In this study, elevated CSF leukocytes count and decreased CSF glucose levels were also identified as independent predictors, consistent with the conclusions drawn by these two researchers. This further underscores the critical indicative value of CSF test results. Since our study did not restrict the suspected responsible pathogens types, this scoring system aligns closely with the clinical characteristics encountered when initially diagnosing and treating patients with proposed intracranial infections. It can aid clinicians in making quick judgments on whether to proceed with CSF mNGS when the etiology of such patients is still unclear. However, due to the significant heterogenicity among patients with intracranial infections, it is essential to make informed decisions by considering the scoring system alongside the patient's overall condition.

This study offers valuable insights for the screening of appropriate patients with suspected intracranial infections for CSF mNGS and the clinical management of these patients. However, there are several limitations that should be acknowledged: (1) The number of patients included in this cohort study is relatively small; conducting larger-scale and multicenter cohort studies would enhance the robustness of this diagnostic scoring system. (2) In this study, not all participating patients underwent cerebrospinal fluid testing, brain MRI, and electroencephalogram examinations. The potential data loss may introduce a certain degree of bias to the results. (3) The scoring system developed in this study has yet to undergo external validation in other cohort studies. Therefore, further validation for its generalizability and reliability in different patient populations and optimization of the scoring system is still needed.

Conclusion

We developed a scoring system to guide the decision to perform CSF mNGS in patients with suspected intracranial infections. The scoring system recommends obtaining CSF for mNGS when patients exhibit two or more conditions: headache, elevated CSF leukocyte levels, and decreased CSF glucose levels. The scoring system, established by referencing clinical guidelines, is a rapid assessment tool for suspected intracranial infections, offering a quicker alternative to traditional clinical guidelines. Furthermore, our analysis of patients with identified responsible pathogens revealed that VZV infection was the most prevalent, providing valuable epidemiological insights into intracranial infections. It is essential to consider other potential diagnoses, such as epilepsy, cerebrovascular disease, autoimmune encephalitis, non-neurological diseases, etc., in cases where the responsible pathogen is not detected by cerebrospinal fluid mNGS in patients with suspected intracranial infections. The study provided a reference for precision medicine of intracranial infections by investigating the suitable circumstances for CSF mNGS. We hope there will be more extra cohorts to make an external validation of the scoring system, which is needed. With more and more attention paid to intracranial infection, developing a suitable clinical pathway to diagnosis and treatment is an aim of further study, and our study could contribute to this aim. Moreover, the cost of NGS should be further reduced, and the readiness of NGS at medical facilities needs to be improved.

Abbreviations

CSF	Cerebrospinal fluid
mNGS	Metagenomic Next-Generation sequencing
VZV	Varicella zoster virus
EEG	Electroencephalography

MRI	Magnetic resonance imaging
PCR	Polymerase chain reaction
HHV-6A	Human herpesvirus type 6 A

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Author contributions
JD and XC have contributed to desigh or conceptualization of the study, analysis or interpretation of the data and drafting the initial draft. YB and JZ have contributed to acquisition of the data and revising the manuscript. JH has contributed to acquisition of the data, supervision and funding acquisition. All authors contributed to the article and approved the submitted version.

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Data availability
The data generated or analyzed during this study are included in this published article and the datasets generated or analyzed during the current study are available in the NCBI repository, the accession number to datasets is PRJNA1021775. Further inquiries can be directed to the corresponding author.

Declarations

Ethics approval and consent to participate
The studies involving human participants were reviewed and approved by the Ethics Committee of the Harrison International Peace Hospital. The approval number is 2019-1-024.And all methods were performed in accordance with Declaration of Helsinki. The patients provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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