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Case Report

Clinical application of metagenomic next-generation sequencing in purulent meningitis: a case series

Min Ding, Chunfeng Yang, Yumei Li

Department of Pediatric Intensive Care Unit, The First Hospital of Jilin University, Changchun, China.

ABSTRACT

Background. Purulent meningitis remains an important cause of mortality and morbidity among children worldwide. An immediate diagnosis of the causative microorganism is critical to significantly improving the outcome of this condition.

Case. In this study, we collected cerebrospinal fluid (CSF) samples from four patients clinically diagnosed with purulent meningitis. Patients with purulent meningitis may present with a variety of clinical symptoms or laboratory results. Infectious microorganisms including *Pseudomonas aeruginosa, Staphylococcus aureus, Streptococcus pneumonia,* and *Haemophilus influenzae* were identified in the CSF samples via metagenomic next-generation sequencing (mNGS).

Conclusions. mNGS is effective for the immediate detection of pathogens, which can in turn facilitate prompt diagnosis and treatment among individuals with purulent meningitis, especially if conventional CSF results (such as CSF culture and polymerase chain reaction) are negative.

Key words: purulent meningitis, metagenomic next-generation sequencing, pediatrics, diagnosis, case series.

Purulent meningitis is an inflammation of the meninges affecting the pia, arachnoid, and subarachnoid space caused by a purulent bacterial infection. It commonly has an acute onset and is characterized by high fever, headache, meningeal irritation, and other symptoms. The etiology and clinical characteristics of purulent meningitis with onset at different ages in children significantly differ.[1] Moreover, it is an extremely serious intracranial infectious disease. If timely treatment is not provided, it can be life-threatening or cause severe neurological sequelae. The morbidity of purulent meningitis in developed countries is 1.4–6.0 per 100,000 individuals, and the mortality rate is approximately 5.2% in newborns. The morbidity and mortality rates in developing countries are higher than those in developed countries, thereby showing multiple growth.[2-4]

✉ Yumei Li
ym_li@jlu.edu.cn

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Due to a lack of knowledge about its causative pathogen, strain variation, and unreasonable application of antibiotics, purulent meningitis has a high mortality rate, and survivors present with severe neurological sequelae. Therefore, early diagnosis and timely administration of optimal antimicrobial therapy are important.

Metagenomic next-generation sequencing (mNGS) is a newly developed technology for the immediate, efficient, and unbiased collection of nucleic acid sequence information for all microorganisms. In 2014, a case of *Leptospira* infection was diagnosed via mNGS. Hence, its diagnostic value has been rapidly recognized.[5] A multicenter prospective study was conducted to investigate the efficacy of mNGS in the cerebrospinal fluid (CSF). In total, 204 patients with primary encephalitis, meningitis, or myelitis from eight hospitals in the United States were included in this research. Compared with conventional methods (such as CSF culture and polymerase chain reaction [PCR]), the positive and negative coincidence rates of mNGS were

80% and 98.2%, respectively. Further, 13 (22%) cases of infections were detected via mNGS alone.[6] Although mNGS is more expensive and takes longer to detect, it can simultaneously sequence billions of nucleic acid fragments using high-throughput technology, unlike traditional PCR tests, which require specific primers.[7,8] At present, mNGS has been increasingly used for the diagnosis of infectious diseases, particularly in cases with limitations in conventional diagnostic methods. Herein, we report four patients with complicated purulent meningitis in which the pathogens were identified via mNGS.

Case

Case 1

A 3-year-old boy was admitted to our hospital due to fever with an unknown cause and was diagnosed with acute lymphoblastic leukemia. After receiving chemotherapy and blood transfusion, his body temperature gradually normalized. One month after admission, the patient again presented with fever (temperature: 38.4°C), and he developed chills, headache, stiff-neck, and left upper gingival pain and redness with localized ulceration. *Pseudomonas aeruginosa* was isolated from blood and catheter cultures. He was further clinically diagnosed with gingivitis and sepsis. Treatment was switched to meropenem and micafungin, and chemotherapy was discontinued. Severe sepsis and septic shock subsequently developed, and the patient received fluid resuscitation, continuous positive airway pressure-assisted ventilation, and antimicrobial (meropenem, amikacin, and micafungin) therapy. However, the child was always drowsy, and he presented with a stiff-neck, grade 3 muscle strength of both upper limbs, and grade 2 muscle strength of both lower limbs. The routine blood tests revealed that the patient's C-reactive protein (CRP) and procalcitonin (PCT) levels were significantly elevated (CRP: 241 mg/L; PCT: 1.56 ng/ml). Brain magnetic resonance imaging (MRI) showed subdural effusion on the right frontotemporal top and brain tissue compression (Fig. 1). Hence, we needed to pay high attention to an intracranial infection. However, the routine CSF biochemical examination result was normal. The routine CSF bacterial culture (culture medium: 5% sheep blood agar and enriched chocolate agar, culture time: 72 h) and PCR had negative results. Furthermore, *P. aeruginosa* was detected in the CSF sample via mNGS (BGI Group [Beijing, China]). The patient was further diagnosed with purulent meningitis and subdural effusion. Antimicrobial therapy was continued. After treatment, the patient's vital signs stabilized, and his general condition improved. The patient was transferred to the department of pediatric hematology.

Case 2

A 9-year-old boy underwent a cerebellar hemisphere lesion resection smoothly. The patient had a fever on day 2 of hospitalization (postoperative period), with the highest body temperature reaching 40°C. While on tracheal intubation, the boy was unconscious, and

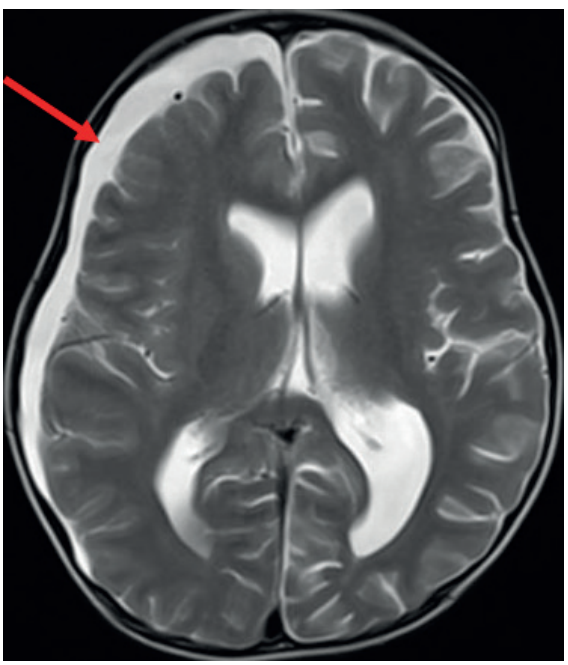

Fig. 1. Imaging changes of the brain in case 1.

he presented with occasional spontaneous breathing and anisocoria. Moreover, his pupils did not react to light. The boy had a persistent high fever, and he underwent multiple routine blood tests, which revealed a white blood cell (WBC) count of 24.78 × 10^9/L with 91% neutrophils. The CRP and PCT levels were 214.53 mg/L and 7.68 ng/mL, respectively, and both values were significantly higher than normal. CSF analysis revealed that the total WBC count was 403.00 × 10^6/L (with 74% multiple nuclear cells) and the red blood cell (RBC) count was 60.00 × 10^6/L. The protein level was elevated at 1.66 g/L. Meanwhile, the CSF chloride level decreased to 117.7 mmol/L, and the CSF glucose level decreased to 4.14 mmol/L. The level of blood glucose was 6.0 mmol/L. Based on the test results, we considered the presence of an intracranial infection, which was treated with vancomycin and meropenem. However, the microbial diagnostic examinations, including repetitive routine CSF culture, blood culture and PCR, had negative results. Furthermore, the CSF sample was collected and sent for pathogen detection via mNGS, and the examination detected *Staphylococcus aureus*. Moreover, surgical pathological results showed pilocytic astrocytoma. During hospitalization, the boy's general condition was poor, with repeated and persistent high fever. Craniocerebral computed tomography (CT) scan revealed changes in the right cerebellar hemisphere, intracranial pneumatocele, left frontal epidural hematoma, hydrocephalus, right frontal subdural hematoma, and epidural hematoma after cranial surgery. The boy had severe brain damage. On the 30th day of admission, the boy's condition did not improve, and routine CSF examination was re-performed. CSF analysis revealed that the WBC count was 704.00 × 10^6/L (with 56% multiple nuclear cells). The protein level was significantly elevated. Meanwhile, CSF glucose and chloride levels decreased. The CSF culture tested positive for *S. aureus*, and this was consistent with the result of mNGS upon admission. On the 33rd day of admission, the boy's condition still did not improve, and his family refused treatment.

Case 3

A 4-month-old-boy presented to the hospital due to complaints of fever for 4 days and intermittent convulsions with unconsciousness for 6 hours. The child was born at 35 + 5/7 weeks of gestation. His family history included seizures. A physical examination revealed poor response and drowsiness. Laboratory tests showed the following: WBC count, 15.69 × 10^9/L; 79% neutrophils; CRP level, 132.49 mg/L; and PCT level, 0.554 ng/mL. The CSF pressure was 160 mmH_2O, and the CSF looked like rice soup. The CSF test revealed that the WBC count was 19617.00 × 10^6/L and the RBC count was 100.00 × 10^6/L. CSF analysis revealed that the glucose, chloride, and protein levels were 0.13 mmol/L, 114.5 mmol/L, and 1.59 g/L, respectively. The level of blood glucose was 5.5 mmol/L. Brain MRI revealed bilateral frontotemporal subarachnoid space widening, which can indicate subdural effusion or extracerebral hydrocephalus. With consideration of the available information, the patient was diagnosed with purulent meningitis and subdural fluid. Then, the patient was treated with vancomycin combined with meropenem. The patient's CSF sample was immediately taken to BGI Group (Beijing, China) for mNGS. The result showed that *Streptococcus pneumonia* was detected in the CSF sample. Then, the CSF and blood culture results indicated the presence of *S. pneumoniae*, which was consistent with the results of mNGS. The patient continually received vancomycin treatment, and meropenem was discontinued. On the 8th day of admission, the state of the child gradually improved with intermittent fever and occasional convulsions. Electroencephalogram (EEG) results were abnormal with slow background activity and predominantly (multiple) sharp slow waves in the bilateral posterior temporal regions. Treatment with oral topiramate for epilepsy was added to the regimen. On the 36th day of admission, brain CT scan showed bilateral frontotemporal subdural effusion, right frontotemporal and subdural hematoma, hydrocephalus, and periventricular edema. Hence, the supplementary clinical

diagnosis was hydrocephalus. Compared with previous findings, the recent CT scan result showed aggravation. Considering that the child had a serious intracranial infection complicated with hydrocephalus, subdural effusion, and hematocele, there might be neuro-related sequelae. The family chose to transfer the patient to another hospital for treatment, and we then lost contact with the patient's family.

Case 4

A 9-year-old girl was admitted to the hospital due to a 2-day history of headache, fever, and vomiting accompanied by seizures for 4 hours. She received ceftazidime for 2 days prior to admission. Physical examination showed that her vital signs were stable while she was comatose. Her Glasgow Coma Scale score was 9. Both eyelids, more prominently on the left side, were drooping. She had double vision and was not able to move her eyeballs. The muscle strength of both upper limbs was grade 3, and the tendon reflexes were weak. The muscle strength of both lower limbs was grade 0, and the tendon reflexes were absent. The sensory plane was located at the level of the bilateral groin. Laboratory blood tests showed that the WBC count was 40.05×10^9/L with 92% neutrophils. The CRP level was 220.58 mg/L, and the PCT level was 30.69 ng/mL. Both values were higher than normal. CSF analysis revealed that the total WBC count was 29374.00×10^6/L and the RBC count was 300.00×10^6/L. The protein level was elevated at 1.70 g/L. Meanwhile, the CSF glucose level (1.26 mmol/L) and chloride level (116.7 mmol/L) decreased. The level of blood glucose is 4.5 mmol/L. A brain CT scan revealed that the cerebral falx and cerebellar tentorium had a higher density, with consideration of adenoid hypertrophy and sinusitis. Therefore, the patient was diagnosed with purulent meningitis and was immediately treated with meropenem, intrathecal dexamethasone, and vancomycin. The CSF and blood cultures did not detect any microorganisms and PCR examinations were also negative. The CSF and blood samples were collected and sent for pathogen detection via mNGS. Results showed the presence of *Haemophilus influenzae* in the CSF and blood. Therefore, vancomycin was discontinued, and amikacin was added as an antimicrobial treatment. MRI of the thoracic spine revealed a suspected abnormal signal shadow in the spinal cord at the 7th thoracic vertebrae to the 1st lumbar vertebrae. An additional clinical diagnosis of myelitis was made, and antimicrobial treatment, immune modulation, and other therapies were continued. The patient's clinical symptoms improved gradually. She had clear consciousness and no fever. However, the muscle strength was still abnormal. On the 74th day of admission, the patient had a significant improvement in her clinical condition and laboratory test results. Further, there were no abnormalities on thoracic and lumbar spine MRI. Her condition improved, and she was then discharged from the hospital.

Discussion

Purulent meningitis is a common disease with sudden onset and has a high mortality rate in the pediatric population. Timely diagnosis and appropriate antibiotic therapy are effective in achieving complete recovery. This report showed the identification of pathogens via mNGS in four patients with purulent meningitis. Notably, mNGS was found to have an important role in the early identification of pathogens particularly among patients with critical illnessess.

Purulent meningitis is one of the most serious infections in childhood, and is associated with serious complications. In this report, there were multiple types of complications including subdural effusion, hydrocephalus, brain abscess, and myelitis. These could lead to severe permanent sequelae if not treated promptly. The main clinical signs of purulent meningitis are fever, change in consciousness, vomiting, convulsion, and headache.[9] Epileptic seizures are the most common clinical symptom of acute-stage purulent meningitis. Purulent meningitis accompanied by acute epileptic

seizures may be a risk factor for epilepsy and nervous system sequela or mortality.[10,11] Yang et al.[12] showed that the rate of abnormal EEG patterns in patients with purulent meningitis was relatively low. Pomeroy et al.[13] monitored the EEG of 58 children with purulent meningitis accompanied by an acute epileptic attack. In case 3 of the current study, the patient presented with convulsions and abnormal brain waves, and antiepileptic drugs were provided. However, due to late complications including hydrocephalus, subdural effusion, and hematocele, nervous system sequelae may develop. Therefore, children with purulent meningitis should be diagnosed promptly, and appropriate and full-course antibiotic treatment should be provided immediately to reduce the development of complications. Meanwhile, in recent years, the proportion of typical cases has been gradually decreasing, and treatment based on experience can lead to misdiagnosis and delayed management. Therefore, in the diagnosis and treatment of purulent meningitis, accurate treatment must be provided based on the pathogen.

Conventional testing methods used in clinical microbiology laboratories include PCR, culture, and CSF antigen and antibody detection. Although these methods are currently applied, they have limitations in detecting the range of pathogens, particularly those that are uncommon. PCR has been a great advancement in numerous individual techniques that specifically target organisms. However, a rare organism can still be missed, or limited primers containing mismatches to the microbial strain involved will be used, which decreases the sensitivity of detection.[14] CSF culture is the gold standard for diagnosing purulent meningitis. The Infectious Diseases Society of America guidelines state that if acute bacterial meningitis is suspected, a sample must be obtained immediately for blood culture, and lumbar puncture for CSF biochemical examination, routine examination, and culture should be performed to confirm the diagnosis.[15] Nonetheless, a positive culture result is challenging to obtain due to the administration of broad-spectrum or prophylactic antimicrobial drugs prior to lumbar puncture, as well as the presence of organisms that are fastidious or slow growing.[16] The specificity of CSF bacterial culture is up to 97%. However, the sensitivity is only 25%–90%.[17] Due to the early application of antibiotics, the lowest positive rate of CSF culture was only 5.3%.[18] Meanwhile the yield of CSF culture in suspected cases is also low.[19] In the current study, patients 1 and 4 received antimicrobial drugs prior to lumbar puncture, and they had negative CSF culture results. The pathogenic factor of purulent meningitis is pathogen infection, and its nucleic acid is often detected in the CSF or brain tissue. Hence, it is theoretically feasible to sequence the metagenome of the CSF.

In recent years, mNGS has gradually been applied in the diagnosis of clinical infectious diseases. In our setting, we sent samples of blood and CSF to BGI Group (Beijing, China) for mNGS. BGI Group conducted mNGS as described previously[20], collecting 2-3 mL sample. They then extracted DNA following standard procedures. DNA libraries were constructed through DNA fragmentation, end repair, adapter ligation, and PCR amplification. They sequenced the qualified libraries by using the BGISEQ-100 platform.[21] They screen high-quality sequencing data and exclude low-quality reads, then performed computational subtraction of human host sequences mapped to the human reference genome (hg19) using Burrows-Wheeler alignment.[22] They classified the remaining data and simultaneously aligning the sequences to microbial genome databases for bacteria, viruses, fungi, and parasites downloaded from the US National Center for Biotechnology Information (ftp://ftp.ncbi.nlm.nih.gov/genomes). Experts work together to assess the patient's condition and interpret the mNGS results to identify possible etiologic agents. Finally, we identified the causative agent through mNGS.

Compared with conventional detection methods, mNGS has several advantages. For

example, it is not based on traditional culture methods and can detect pathogenic bacteria in samples after the application of antibiotics. In a multicenter study conducted by Wilson et al.[6], 32 (55.17%) of 58 patients were diagnosed with intracranial infection via mNGS. Thus, the positive rate of mNGS in purulent meningitis was significantly higher than that of traditional etiological detection. Of the four patients in this study, only one had a positive CSF culture result. Meanwhile, all patients underwent mNGS to confirm pathogenic infection. Table I shows the causative microorganism in four cases. This is primarily attributed to the fact that mNGS detection is an unbiased calculation of information about microbial DNA fragments, which is not associated with bacterial survival. Unlike traditional bacterial culture, a certain number of living bacteria is required. Therefore, whether antibiotics are used before sampling has little influence. All patients presented with complex purulent meningitis with different characteristics. That is, in case 1, the patient was immune deficient, and he developed purulent meningitis caused by sepsis. mNGS has evident advantages in the detection of different complex purulent meningitis. Unlike bacterial culture, which requires up to 72 h of final identification, the detection time of mNGS is short.[23] Clinicians can get timely pathogenic results via mNGS, which can improve clinical treatment level. The mNGS results of our patients were obtained after 1 day. In cases 4, antibiotic therapy was modified. Nevertheless, culture is still the gold standard and should not be neglected, because it alone allows for an antibiogram.

Several types of bacteria cause purulent meningitis. *S. pneumoniae*, *N. meningitidis*, *H. influenzae*, and *Listeria* are the most common pathogens of community acquired purulent meningitis.[17,24-26] In addition to the common pathogenic bacteria, *Coliform bacillus*, *Deform bacillus*, *Enterococcus*, and *P. aeruginosa* can cause purulent meningitis. mNGS can find both common pathogenic bacteria and other uncommon pathogenic bacteria. In case 1, *P. aeruginosa* infection was identified. *P. aeruginosa* is an opportunistic pathogen and the main pathogen of nosocomial infection. The patient presented with acute lymphoblastic leukemia, and the routine CSF biochemistry had normal results. However, the CRP level was high, and subdural effusion was observed. The CSF culture result was negative, and *P. aeruginosa* infection was confirmed via mNGS. Therefore, mNGS has an advantage in detecting pathogenic bacteria in purulent meningitis. However, it also has disadvantages. That is, mNGS is associated with a risk of contamination by environmental species during the routine collection of clinical samples. This can then result in misdiagnoses. In clinical diagnosis and treatment, mNGS detection results must be used in combination with clinical data, imaging characteristics, and routine laboratory test findings to confirm the presence of pathogenic, colonization, and contaminated bacteria.

In conclusion, the current series showed that mNGS could be used to diagnose purulent meningitis. Nevertheless, this preliminary finding should be examined in diagnostic trials in the future.

Table I. Culture, PCR, and mNGS result of of the four patients.

Case	Culture			PCR		mNGS		
	Blood	CSF	Time	CSF	Time	Blood	CSF	Time
1	*P. aeruginosa*	negative	3d	negative	1d	Not tested	*P. aeruginosa*	1d
2	*S. Aureus*	negative		negative		Not tested	S. Aureus	
3	*S. pneumoniae*	*S. pneumoniae*		negative		Not tested	*S. pneumoniae*	
4	negative	negative		negative		*H.influenzae*	*H.influenzae*	

CSF: cerebrospinal fluid, mNGS: metagenomic next-generation sequencing, PCR: polymerase chain reaction,
CSF: cerebrospinal fluid, *P. aeruginosa: Pseudomonas aeruginosa, S. Aureus: Staphylococcus aureus;*
S. pneumoniae: Streptococcus pneumonia, H.influenzae: Haemophilus influenzae.

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Ethical approval

The First Hospital of Jilin University Institutional Review Board approved this study, with the need for informed consent waived.

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: MD, CY; data collection: MD; analysis and interpretation of results: MD, CY, YL; draft manuscript preparation: MD, CY. All authors reviewed the results and approved the final version of the manuscript.

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Conflict of interest

The authors declare that there is no conflict of interest.

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