Clinical Case Report

Medicine®

OPEN

Next-generation sequencing specifies *Angiostrongylus* eosinophilic meningoencephalitis in infants

Two case reports

Mei Xie, MD[a,*], Zhen Zhou, MD[a,*], Suhua Guo, MD[a], Zengqing Li, MD[a], Hui Zhao, MD[a], Jiusheng Deng, PhD[b]

Abstract

Rationale: *Angiostrongylus cantonensis*-induced eosinophilic meningoencephalitis (AEM) in infants is a very rare but fatal disease. Utilization of genetic assay to detect the cerebral parasite plays an important role for the treatment of the infection.

Patient concerns: Two infants (<2 years) presented with cough, intermittent fever, mental fatigue, and poor diet.

Diagnosis: The patients were under clinical examination and laboratory test including cardiac ultrasound, chest X-ray, blood or cerebrospinal fluid (CSF) cell counting, serum enzyme-linked immunosorbent assay (ELISA), head magnetic resonance imaging (MRI) and next-generation sequencing (NGS) on DNA from CSF. Due to hypereosinophils in patients' peripheral blood and CSF, and abundant DNA sequences from *A cantonensis* in CSF, the patients were diagnosed with *Angiostrongylus* eosinophilic meningoencephalitis.

Interventions: The patients were treated with albendazole to deworm, and methylprednisolone to reduce inflammation.

Outcome: The patients were completely recovered from AEM without relapse after 10-day treatment.

Lessons: ELISA and MRI are not sufficiently accurate for the diagnosis of AEM in infants. NGS can specify the infection by the cerebral parasite and offers a new effective approach for the early and precise diagnosis of AEM in infants.

Abbreviations: AEM = *Angiostrongylus cantonensis*-induced eosinophilic meningoencephalitis, CSF = cerebrospinal fluid, ELISA = enzyme-linked immunosorbent assay, MRI = magnetic resonance imaging, NGS = next-generation sequencing, RBC = red blood cell, WBC = white blood cell.

Keywords: *Angiostrongylus cantonensis*, diagnosis, eosinophilic meningoencephalitis, infants, next-generation sequencing

1. Introduction

Angiostrongylus cantonensis-induced eosinophilic meningoencephalitis (AEM) is a parasitic disease mostly in adults, with rare cases in infants.[1,2] The primary host of *A cantonensis* is rat. However, the disease in human is mainly caused by eating uncooked contaminated foods such as lettuce, snail, freshwater prawn, crabs, mollusks, crickets, and fish, which are the intermediate host or the host material of the parasite[3] or by drinking parasitic larvae-contaminated water or fruit juice.[4,5] The parasitic infection in children is usually by direct contact with the infected mollusk when crawling or playing on the ground. Since the discovery of *A cantonensis*, infections by the parasite have been reported in Asia, islands of the Pacific and the Indian Ocean, Australia, the North, Central, and South America; and has become an emerging global infectious disease.[6–8]

Manifestations of AEM include headache, nausea, cough, vomiting, lethargy or apathy, fever, neck stiffness, muscle twitching, paresthesia, weakness, diarrhea, etc.[9] However, AEM in infants has very inconsistent symptom different from that in adults.[2,10] The presence of infiltrated eosinophils and the 3rd stage larvae of *A cantonensis* in patient's cerebrospinal fluid (CSF) are the 2 major pathologic characters of AEM. Eosinophils have exocytotic capability to degrade parasites in CSF through the extrusion of cellular granules and contents.[11] On the contrary, a large amount of eosinophils infiltrated around the worm, together with other leukocytes, can form eosinophilic granuloma or abscess, leading to the pathologic inflammation and cytotrophic meningoencephalitis.[12,13]

The immune system of infants is not fully developed,[14] the larva of *A cantonensis* can migrate to the pulmonary artery and develop into an adult worm, causing pulmonary embolism, respiratory failure, and even death in infants. Thus precise diagnosis of AEM in infants is critical for the urgent treatment of the disease. In this case reports, we tested next-generation

Editor: N/A.

This study was approved by the Research Ethics Committee of Guangdong Women and Children Hospital, Guangzhou, China.

Written informed consents were obtained from the parents of the patients or healthy donors for publication of this case report.

The authors have no funding and conflicts of interest to disclose.

[a] Department of Pediatrics, Guangdong Women and Children Hospital, Guangzhou, China, [b] Department of Pathology and Laboratory Medicine, Emory University School of Medicine, Atlanta, GA.

[*] Correspondence: Mei Xie, Zhen Zhou, Department of Pediatrics, Guangdong Women and Children Hospital, 521 Xingnan Avenue, Guangzhou 511400, China (e-mails: xiem020@126.com, zhouzhen5229@163.com).

Medicine (2019) 98:35(e16985)

Received: 4 January 2019 / Received in final form: 27 June 2019 / Accepted: 7 August 2019

http://dx.doi.org/10.1097/MD.0000000000016985

sequencing (NGS)[15] as a new molecular method for the precise diagnosis of AEM in infants.

2. Methods

2.1. Patients

Two patients (under 2 years old) were on admission into hospital due to cough, fever, mental fatigue, and poor diet in the summer of 2018; and subjected to clinical examination including cardiac ultrasound with the PHILIPS iE33 Ultrasound Machine (Philips, Charlotte), chest X-ray with United Imaging UDR 770i X-Ray Scanner (Shanghai Lianying Medical Technology Co, Ltd, Shanghai, China), blood test, head magnetic resonance imaging (MRI),[16] and NGS on CSF DNA. The recruitment of human subjects in this case reports was approved by the Research Ethics Committee of Guangdong Women and Children Hospital, and the informed consent was obtained from the parents of the patients.

2.2. Blood and CSF collection

Peripheral blood (5 mL) was collected from patients via elbow vein for blood test before and after treatment, or from healthy donors (1–2 years old; n = 3) as controls. Serum was isolated from blood samples (before treatment) after centrifugation for 15 minutes at 3000 rpm at 4°C, and stored at −80°C for use. Two microliters of CSF was extracted at the 3 to 4 lumbar intervertebral space from patients or from one of the healthy donors by lumbar puncture, and stored at −80°C for use.

2.3. Blood analyses

Blood cell counting including red blood cells and white blood cells (WBCs) in peripheral blood or CSF samples was performed on the Sysmex XN2000 Automatic Hematology Analyzer (Sysmex, Lincolnshire). Eosinophils were additionally stained as orange-red granules. The percentage of eosinophils in WBC and the absolute number of eosinophils in the samples were then calculated.

2.4. Enzyme-linked immunosorbent assay

Serum or CSF was subjected to enzyme-linked immunosorbent assay (ELISA) with rapid cerebral parasite diagnostic kits (JiYu Inspection Center, Guangzhou, China) following its instruction. The kits contained reagents to detect antibodies against cerebral parasites including *A cantonensis*, *Taenia solium*, *Paragonimus westermani*, *Spirometra*, *Schistosoma japonicum*, and *Toxoplasma gondii*.

2.5. Luminex assay

Luminex assay was performed on serum isolated from peripheral blood of the patients with kits (JiYu Inspection Center, Guangzhou, China) to detect common tumor markers, including carcinoembryonic antigen (CEA), alpha-fetoprotein (AFP), carbohydrate antigen 125 (CA125) and 242 (CA242), cell keratin fragment antigen 21-1 (CKFA21-1), and nerve-specific enolase (NSE).[17]

2.6. Magnetic resonance imaging

The MRI examination on patients' head was performed on a Brivo MR355 superconducting scanner (GE, Boston). Images were collected from the system with a FLAIR sequence (The repetition time = 8044 milliseconds, The echo time = 140.9 milliseconds, field-of-view = 24 × 24) with 8 mm thickness and 0.8 mm gap.

2.7. NGS

The NGS was performed as described.[18] Briefly, DNA was extracted from CSF of each patient with Micro DNA kit (Tiangen Biotech, Beijing, China), and fragmented into 200 to 300 bp fragments with a Bioruptor Pico for the construction of DNA libraries. The libraries were then sequenced on a BGISEQ-100 platform. The sequencing data were aligned to the NCBI microbial genome database (ftp://ftp.ncbi.nlm.nih.gov/genomes/) containing 1494 bacteria, 2700 viruses, 73 fungi, and 48 parasites. Genomic coverage of sequence reads for targeted pathogen was calculated.

3. Case presentation

3.1. Case 1

The patient (1 year and 10 months old, male) was admitted into hospital in July 2018 due to cough, intermittent fever (37.5–39.5°C), mental fatigue, and poor diet. Before admission, the patients received routine immunization and had no history of hepatitis, thalassemia, glucose-6-phosphate dehydrogenase deficiency, tuberculosis, and genetic or metabolic diseases, or eating uncooked food. After admission, the patients were under clinical examination and laboratory test including cardiac ultrasound, chest X-ray, peripheral blood, CSF cell counting, serum luminex assay, ELISA, head MRI, and NGS. Cardiac ultrasound showed that the patient had normal heart structure and function. Chest radiography revealed that the patient had bronchial pneumonia. Blood or CSF cell counting and eosinophil staining demonstrated that the patient had marked increase of eosinophils and the percentage of eosinophils in WBCs in peripheral blood or CSF, compared to healthy subjects (Table 1). The percentage of eosinophils in WBC in blood and CSF was 23.2% and 68.9% (Table 1). Luminex assay excluded the existence of common tumor markers including CEA, AFP, CA125 and CA242, cell keratin fragment antigen 21-1, and NSE in the patient (data not shown). ELISA with rapid cerebral parasite diagnostic kits showed that the patient had anti-*A cantonensis* IgG in serum. There was no 3rd stage larva found in the eyes of the patient. However, head MRI showed that the patient had T2 FLAIR abnormal signal in the right cerebellar hemisphere (Fig. 1A) and the bilateral occipital lobe (Fig. 1B). NGS on CSF DNA

Table 1

Cell profile in peripheral blood and cerebrospinal fluid.

	Patient 1	Patient 2	Healthy subjects
Peripheral blood			
RBC, $\times 10^{12}$/L	4.9	4.1	4.3
WBC, $\times 10^{9}$/L	14.2	9.7	9.5
Eosinophils, $\times 10^{9}$/L	3.3	3.2	0.1
Eosinophils in WBC, %	23.2	33	1.1
Cerebrospinal fluid			
RBC, $\times 10^{6}$/L	0	0	0
WBC, $\times 10^{6}$/L	274	174	0.8
Eosinophils, $\times 10^{6}$/L	189	57	0
Eosinophils in WBC, %	68.9	32.7	0

RBC = red blood cell, WBC = white blood cells.

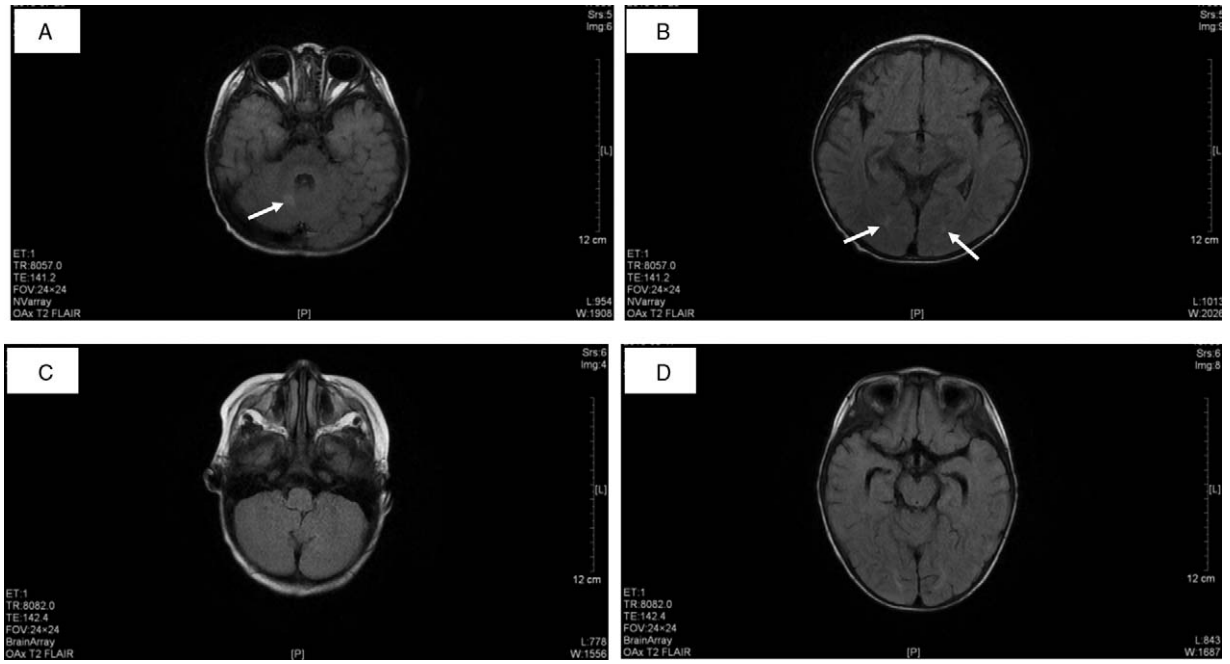

Figure 1. Magnetic resonance imaging on the heads of the patients after admission. T2 FLAIR signal in the right cerebellar hemisphere (A) and signal in the bilateral occipital lobe (B) of patient 1. T2 FLAIR signal in the right cerebellar hemisphere (C) and in the bilateral occipital lobe (D) of patient 2.

demonstrated that there were 3204 DNA sequence reads from *A cantonensis* genome in patient's CSF (Fig. 2A), but not in nontemplate controls (data not shown). The percentage of gene reads corresponding to *A cantonensis* in total reads on the patient's sample was 99.4% (Table 2); and the genomic coverage of NGS sequence reads from *A cantonensis* was 0.0786% (Table 2). There were no detectable DNA sequences from virus, bacteria, fungi, and mycoplasma pneumoniae (data not shown). Sequences were aligned to the NCBI microbial genome database (ftp://ftp.ncbi.nlm.nih.gov/genomes, containing 1494 bacteria, 2700 viruses, 73 fungi, and 48 parasites).

Based on the evidences that the patients had hypereosinophils in peripheral blood and CSF, and there were exclusive DNA sequences of *A cantonensis* in patient's CSF, we treated the patient as AEM with albendazole and methylprednisolone.[19] The dose of albendazole was 0.1 g/time, oral, twice a day to deworm; and the dose of methylprednisolone was 10 mg/time via intravenous injection, twice a day to reduce inflammation. After 10-day treatment, eosinophils in patient's CSF (Fig. 3A) and peripheral blood (Fig. 3B) rapidly decreased, and WBC in patient's CSF (Fig. 3C) also markedly reduced. WBC in patient's peripheral blood decreased as well (Fig. 3D). The patient was absent from the

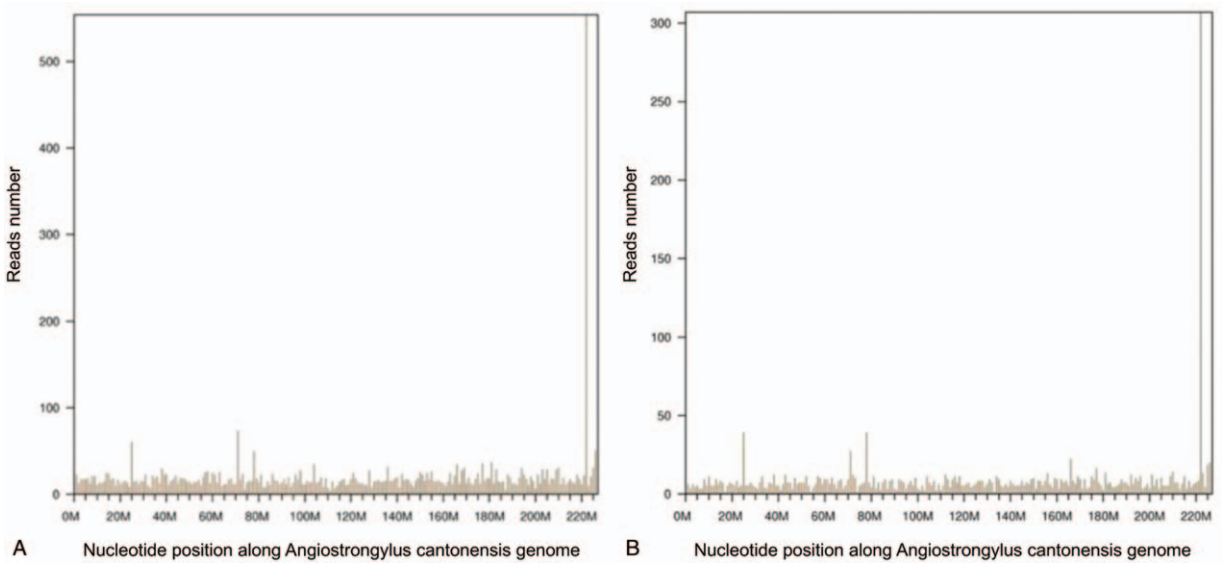

Figure 2. Next-generation sequencing on patients' cerebrospinal fluid. (A) Identified DNA sequences corresponding to *Angiostrongylus cantonensis* in patient 1. (B) Identified DNA sequences corresponding to *A cantonensis* in patient 2.

Table 2

Next-generation sequencing sequence reads from *Angiostrongylus cantonensis* on patients' cerebrospinal fluid.

	Patient 1	Patient 2
Total sequence reads	3224	892
A cantonensis reads	3204	876
Percentage of *A cantonensis* reads	99.4%	98.2%
Coverage of *A cantonensis* genome	0.0786%	0.0345%

symptoms of AEM after 10-day treatment. There were no eosinophils in patient's CSF on days 30 and 60 after treatment (Fig. 3A). The number of eosinophils in patient's peripheral blood (Fig. 3B) and the number of WBC in both CSF (Fig. 3C) and peripheral blood (Fig. 3D) of the patient returned to the levels similar to healthy infants. MRI further showed there were normal signals in the right cerebellar hemisphere and the bilateral occipital lobe of the patient (Fig. 4A and B). After treatment, the patient was completely recovered from AEM without relapse.

3.2. Case 2

The patient (1 year and 3 months old, male) was admitted into hospital in August 2018 due to intermittent fever (37.7–38.8°C), cough, vomiting, mucosal herpes, and startle. The patient had routine immunization, and no history of genetic or metabolic diseases, or eating raw food. The patient was subjected to clinical examination and laboratory test after admission. Cardiac ultrasound showed the patient had normal heart structure and function. Chest radiography showed bronchial pneumonia in the patient. Blood or CSF cell analyses demonstrated that there was a marked increase of eosinophils and the percentage of eosinophils in WBCs in the patient in comparison with healthy subjects (Table 1). The percentage of eosinophils in WBC in blood and CSF was 32.7% and 33% in the patient (Table 1). Luminex assay showed that there were undetectable common tumor markers in the patient's serum (data not shown). ELISA showed that there was anti-*T gondii* IgG in the patient's CSF. There was no presence of *Toxoplasma* or *Angiostrongylus* in CSF of the patient. MRI showed normal signal in the patient's skull MRIs (Fig. 1C and D). However, NGS on CSF DNA demonstrated that there were 876 DNA sequence reads from *A cantonensis* genome in patient's CSF (Fig. 2A and B), but not in nontemplate controls (data not shown). The percentages of gene reads corresponding to *A cantonensis* in total reads were 98.2% (Table 2); and the genomic coverages of NGS sequence reads from *A cantonensis* were 0.0345% (Table 2). However, there were no detectable DNA sequences from *T gondii* genome, as well as from the genomes of virus, bacteria, fungi, and mycoplasma pneumoniae (data not shown).

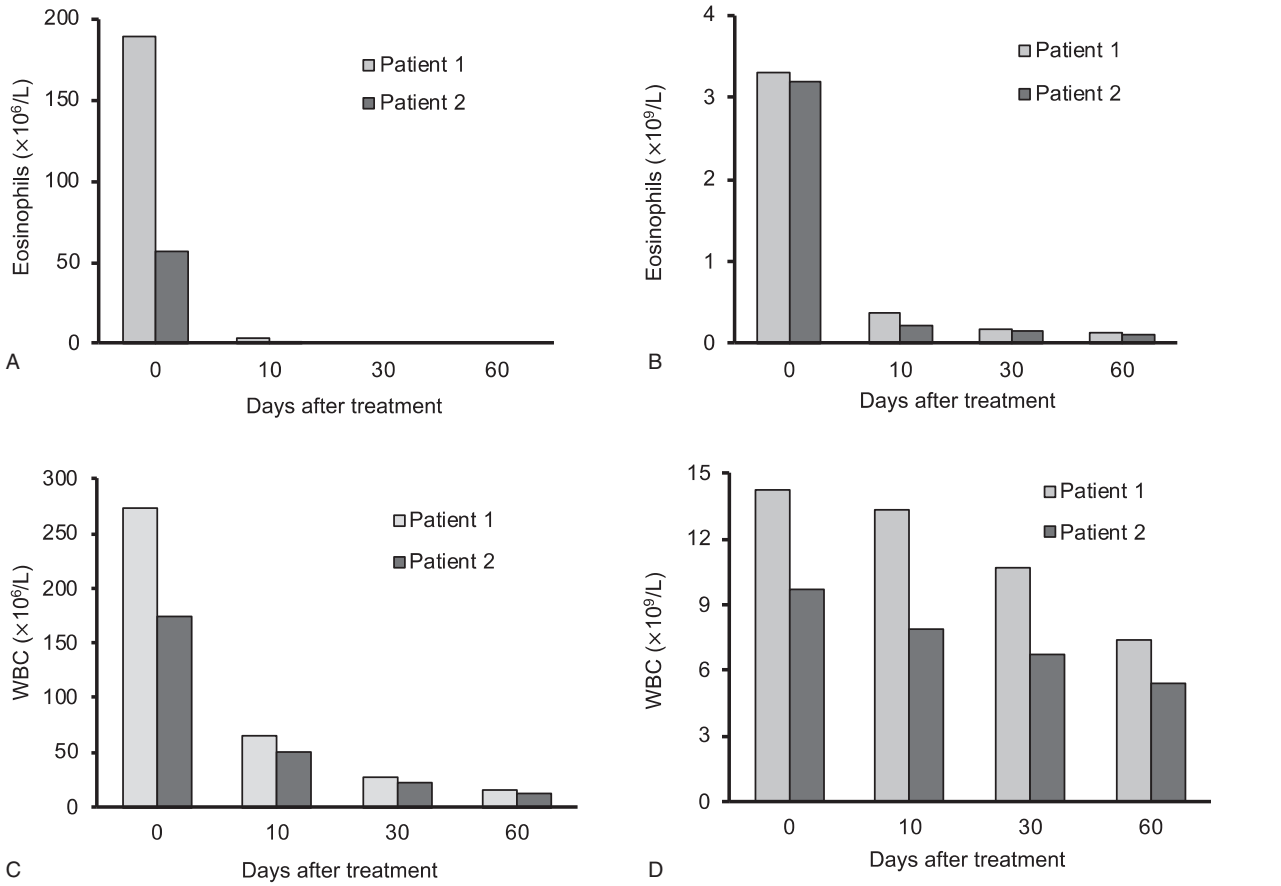

Figure 3. The profile of eosinophils and white blood cell (WBC) in cerebrospinal fluid (CSF) and peripheral blood of the patients after treatment. The changes of eosinophils in CSF (A) and in peripheral blood (B) of the patients after treatment. The changes of WBC in CSF (C) and in peripheral blood (D) of the patients after treatment.

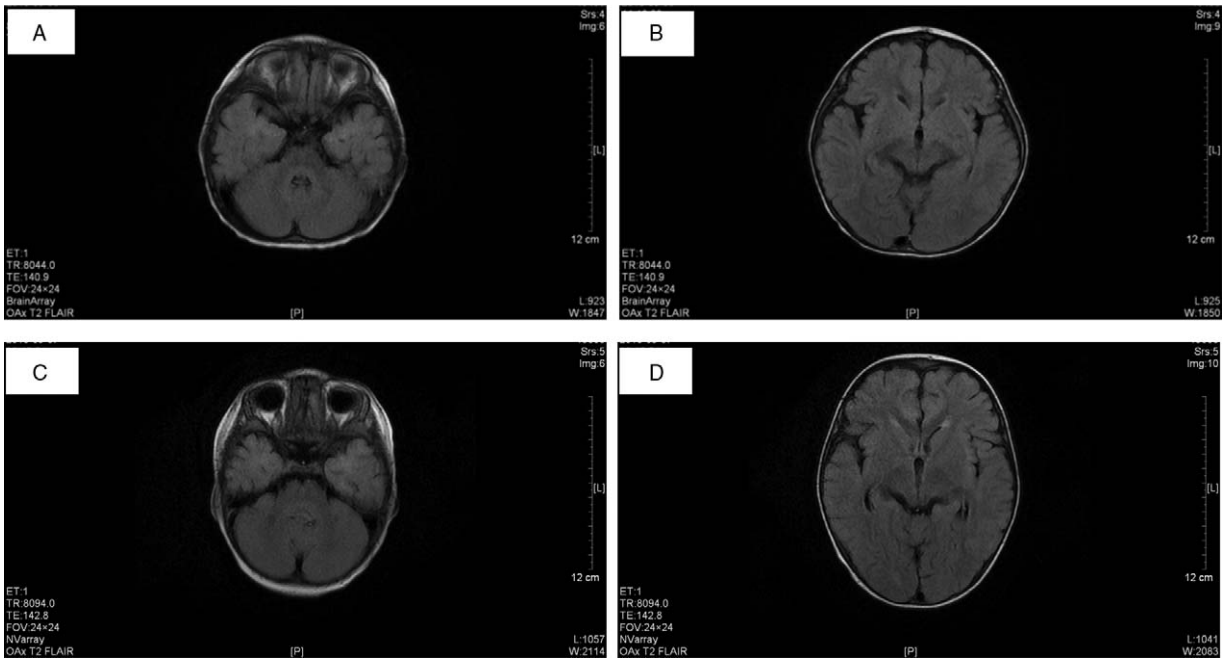

Figure 4. Head magnetic resonance imaging of the patients after treatment. T2 FLAIR signal in the right cerebellar hemisphere (A) and in the bilateral occipital lobe (B) of patient 1 on day 30 after treatment. T2 FLAIR signal in the right cerebellar hemisphere (C) and in the bilateral occipital lobe (D) of patient 2 on day 30 after treatment.

Based on hypereosinophils in peripheral blood and CSF of the patient, and the unique DNA sequences of *A cantonensis* in the patient's CSF, the patient was diagnosed with AEM and treated with albendazole and methylprednisolone as in case 1. After 10-day treatment, eosinophils in CSF (Fig. 3A) and peripheral blood (Fig. 3B) of the patient rapidly decreased; WBC in patient's CSF (Fig. 3C) also markedly reduced. WBC in peripheral blood decreased as well (Fig. 3D). There were also no eosinophils in the patient's CSF on days 30 and 60 after treatment (Fig. 3A). The number of eosinophils in peripheral blood (Fig. 3B) and the number of WBC in both CSF (Fig. 3C) and peripheral blood (Fig. 3D) returned to normal levels. The patient was completely recovered without symptoms of AEM after 10-day treatment.

4. Discussion

In this case report, we showed NGS as a new molecular diagnostic technique for AEM in infants, and demonstrated that NGS can exclusively detect *A cantonensis* DNA sequences in patients' CSF and specify cerebral infection by the parasite. To our knowledge, it is the 1st report that NGS could provide precise diagnosis of AEM in infants.

The diagnosis of AEM is mostly based on clinical manifestations, microscopic identification of eosinophils present in CSF, ELISA detection of anti-*A cantonensis* antibody, and patient's skull MRI.[20] Due to its atypical symptom, AEM in infants is easily misdiagnosed as viral encephalitis or bronchial pneumonia, in particular when the patients had no clear parasite-contact history. Elevated eosinophils in peripheral blood and CSF are associated with parasitic infections, allergies, and even cancers.[11,12,21] Eosinophil profile in patients' peripheral blood and CSF examined by microscopy or blood counting facilitates the diagnosis of AEM.[12] In this case report, the patients with AEM indeed had hypereosinophils in peripheral blood and CSF, and the percentages of eosinophils in WBC were ranged from 23.2% to 68.9%. ELISA has been widely used to detect antibodies against infectious pathogens in the serum of patients. However, antibody detection could not reveal the early stage of infection,[22,23] and the antibodies detected could be due to cross-reactions with other parasites.[24–26] In this case report, there was indeed anti-*A cantonensis* antibodies detected by ELISA in the serum, but in only one of the patients with AEM. Unexpectedly, 1 patient with AEM had no detectable anti-*A cantonensis* antibodies in the serum, but had anti-*T gondii* antibodies in his CSF as detected by ELISA, suggesting that ELISA is not sufficiently accurate for the diagnosis of AEM in infants. MRI can reveal the interval development of nodular brain lesions and increased intramedullary spinal cord involvement caused by the infection of *A cantonensis*.[10,27,28] In this report, only one of the patients with AEM had abnormal MRI signals in his right cerebellar hemisphere and bilateral occipital lobe of brain, together with previous report,[28,29] indicating that MRI could not provide strong evidence for the precise diagnosis of AEM in infants.

The NGS is an advanced molecular technique that can detect all the infectious pathogens within 1 clinical sample.[30–33] Specific DNA sequences or reads corresponding to a parasite from NGS has a crucial diagnostic value for prediction of the parasitic infection.[34] As a new precise diagnostic technique, NGS is more specific and sensitive than traditional diagnostic methods such as microscopy, serologic test, or MRI. For instance, NGS detected genes from cerebral parasite in patients' CSF, but there were no infectious pathogens detectable by routine methods.[34,35] In this case reports, NGS indeed detected abundant DNA sequences corresponding to *A cantonensis* in the CSF sample from each patient, with more than 98% of reads corresponding to the parasite; however, the results from ELISA or MRI could support the parasitic infection in case 2. Our findings

strongly suggest that NGS offers a new approach for the precise diagnosis of AEM in infants.

5. Conclusion

The ELISA and MRI are not sufficiently accurate for the diagnosis of AEM in infants. NGS on DNA from patient's CSF specifies cerebral infection by parasite *A cantonensis*, which offers a new effective approach for the early and precise diagnosis of AEM in infants.

Acknowledgment

The authors appreciate both the patients and healthy donors for their support in this study.

Author contributions

Conceptualization: Mei Xie, Zhen Zhou.
Data curation: Mei Xie, Zhen Zhou, Suhua Guo, Zengqing Li, Hui Zhao.
Formal analysis: Mei Xie, Zhen Zhou, Zengqing Li, Hui Zhao, Jiusheng Deng.
Investigation: Mei Xie, Zhen Zhou, Suhua Guo, Zengqing Li, Hui Zhao.
Methodology: Zhen Zhou, Suhua Guo, Zengqing Li, Hui Zhao.
Project administration: Mei Xie.
Writing – original draft: Mei Xie, Zhen Zhou, Jiusheng Deng.
Writing – review & editing: Mei Xie, Zhen Zhou, Jiusheng Deng.

References

[1] Lombard F, Basset D, Cambonie G, et al. Pediatric angiostrongyliasis. Med Sante Trop 2018;28:76–81.
[2] Ma M, Zhang M, Qiu Z. Eosinophilic meningitis caused by Angiostrongylus cantonensis *in an infant: a case report. Medicine 2018;97:e10975*.
[3] Eamsobhana P. Eosinophilic meningitis caused by Angiostrongylus cantonensis–*a neglected disease with escalating importance. Trop Biomed 2014;31:569–78*.
[4] Slom TJ, Cortese MM, Gerber SI, et al. An outbreak of eosinophilic meningitis caused by Angiostrongylus cantonensis *in travelers returning from the Caribbean. N Eng J Med 2002;346:668–75*.
[5] Tsai HC, Lee SS, Huang CK, et al. Outbreak of eosinophilic meningitis associated with drinking raw vegetable juice in southern Taiwan. Am J Ttropl Med Hyg 2004;71:222–6.
[6] Binder S, Levitt AM, Sacks JJ, et al. Emerging infectious diseases: public health issues for the 21st century. Science 1999;284:1311–3.
[7] Cowie RH. Angiostrongylus cantonensis*: agent of a sometimes fatal globally emerging infectious disease (rat lungworm disease). ACS Chem Neurosci 2017;8:2102–4*.
[8] Wang QP, Wu ZD, Wei J, et al. Human Angiostrongylus cantonensis*: an update. Eur J Clin Microbiol Infect Dis 2012;31:389–95*.
[9] Sawanyawisuth K, Chindaprasirt J, Senthong V, et al. Clinical manifestations of Eosinophilic meningitis due to infection with Angiostrongylus cantonensis *in children. Korean J Parasitol 2013;51: 735–8*.
[10] Foster CE, Nicholson EG, Chun AC, et al. Angiostrongylus cantonensis *infection: a cause of fever of unknown origin in pediatric patients. Clin Infect Dis 2016;63:1475–8*.
[11] Cadman ET, Thysse KA, Bearder S, et al. Eosinophils are important for protection, immunoregulation and pathology during infection with nematode microfilariae. PLoS Pathog 2014;10:e1003988.
[12] Graeff-Teixeira C, da Silva AC, Yoshimura K. Update on eosinophilic meningoencephalitis and its clinical relevance. Clin Microbiol Rev 2009;22:322–48.
[13] Gosnell WL, Kramer KJ. The role of eosinophils in angiostrongyliasis: multiple roles for a versatile cell? Hawaii J Med Public Health 2013;72:49–51.
[14] Simon AK, Hollander GA, McMichael A. Evolution of the immune system in humans from infancy to old age. Proc Biol Sci 2015;282:20143085.
[15] Behjati S, Tarpey PS. What is next generation sequencing? Arch Dis Child Educ Pract Ed 2013;98:236–8.
[16] Rodriguez Carnero P, Hernandez Mateo P, Martin-Garre S, et al. Unexpected hosts: imaging parasitic diseases. Insights Imaging 2017;8:101–25.
[17] Jacobs EL, Haskell CM. Clinical use of tumor markers in oncology. Curr Probl Cancer 1991;15:299–360.
[18] Fan S, Qiao X, Liu L, et al. Next-generation sequencing of cerebrospinal fluid for the diagnosis of neurocysticercosis. Front Neurol 2018;9:471.
[19] Murphy GS, Johnson S. Clinical aspects of eosinophilic meningitis and meningoencephalitis caused by *Angiostrongylus cantonensis*, the rat lungworm. Hawaii J Med Public health 2013;72:35–40.
[20] Kanpittaya J, Jitpimolmard S, Tiamkao S, et al. MR findings of eosinophilic meningoencephalitis attributed to *Angiostrongylus cantonensis*. AJNR Am J Neuroradiol 2000;21:1090–4.
[21] Khoury P, Bochner BS. Consultation for elevated blood eosinophils: clinical presentations, high value diagnostic tests, and treatment options. J Allergy Clin Immunol Pract 2018;6:1446–53.
[22] Liang SH, Huang HC, Pan CW, et al. Detection of *Angiostrongylus cantonensis* circulating antigen by monoclonal antibodies. Zhonghua Yi Xue Za Zhi 2005;85:3057–61.
[23] Wang LC, Wan YL. Alteration of antibodies against the fifth-stage larvae and changes in brain magnetic resonance images in experimentally infected rabbits with *Angiostrongylus cantonensis*. J Parasitol 2004;90: 1193–6.
[24] Eamsobhana P, Ongrotchanakun J, Yoolek A, et al. Multi-immunodot for rapid differential diagnosis of eosinophilic meningitis due to parasitic infections. J Helminthol 2006;80:249–54.
[25] Fan CK, Su KE. Cross-reactions with Ascaris suum antigens of sera from mice infected with *A. suum*, *Toxocara canis*, and *Angiostrongylus cantonensis*. Parasitol Int 2004;53:263–71.
[26] Yen CM, Chen ER, Kojima S, et al. Preparation of monoclonal antibody against *Angiostrongylus cantonensis* antigen. Southeast Asian J Trop Med Public Health 1989;20:119–24.
[27] Rai S, Madi D, Pai S, et al. Unusual larva in the CSF and unique MRI findings in a case of eosinophilic meningitis. J Clin Imaging Sci 2014;4:76.
[28] Tsai HC, Chen YS, Yen CM. Human parasitic meningitis caused by *Angiostrongylus cantonensis* infection in Taiwan. Hawaii J Med Public health 2013;72:26–7.
[29] Tsai HC, Tseng YT, Yen CM, et al. Brain magnetic resonance imaging abnormalities in eosinophilic meningitis caused by *Angiostrongylus cantonensis* infection. Vector Borne Zoonotic Dis 2012;12:161–6.
[30] Wylie KM, Mihindukulasuriya KA, Sodergren E, et al. Sequence analysis of the human virome in febrile and afebrile children. PLoS One 2012;7: e27735.
[31] Mizrahi H, Peretz A, Lesnik R, et al. Comparison of sputum microbiome of legionellosis-associated patients and other pneumonia patients: indications for polybacterial infections. Sci Rep 2017;7:40114.
[32] Kawada J, Okuno Y, Torii Y, et al. Identification of viruses in cases of pediatric acute encephalitis and encephalopathy using next-generation sequencing. Sci Rep 2016;6:33452.
[33] Long Y, Zhang Y, Gong Y, et al. Diagnosis of sepsis with cell-free DNA by next-generation sequencing technology in ICU patients. Arch Med Res 2016;47:365–71.
[34] Wilson MR, Naccache SN, Samayoa E, et al. Actionable diagnosis of neuroleptospirosis by next-generation sequencing. N Eng J Med 2014;370:2408–17.
[35] Yao M, Zhou J, Zhu Y, et al. Detection of *Listeria* monocytogenes in CSF from three patients with meningoencephalitis by next-generation sequencing. J Clin Neurol 2016;12:446–51.