Metagenomic Next-Generation Sequencing in Central Nervous System Angiostrongyliasis

Angiostrongylus cantonensis is a zoonotic pathogen that is the most common cause of eosinophilic meningitis worldwide. Due to the lack of a valid diagnostic method, most cases are diagnosed clinically. Although reverse transcription-polymerase chain reaction (RT-PCR) is recognized increasingly as the diagnostic method of choice, when it is not available, a serum enzyme linked immunosorbent assay (ELISA) is preferred. When these tests are negative, the diagnosis is difficult. Although *A. cantonensis* presents as eosinophilic meningitis and has a good outcome, there are still a few cases that may develop fatal meningoencephalitis. Metagenomic next-generation sequencing (mNGS) is an emerging method with a potential for pan-pathogen screening. We report two brothers with eosinophilic meningitis. Traditional microbiological and cytopathological detection methods failed to make a clear diagnosis. We detected *A. cantonensis* in the CSF of one of them using mNGS, enabling clear diagnoses for both and ensuring that they were cured in a timely manner.

A 8-year-4-month-old boy was admitted to our tertiary teaching hospital in western China with the chief complaint of paroxysmal headache for 22 days. It was a frontal lobe headache and accompanied by malaise. While he had no fever, he had blurred vision, paresthesia, vomiting, convulsions, and an altered state of consciousness. He was diagnosed with rhinosinusitis in two different hospitals and administered oral or intravenous anti-biotics empirically; however, there was no relief of symptoms.

Simultaneously, his 10-year-9-month-old elder brother developed dizziness and was admitted to our hospital. Both reported having consumed uncooked snails one week prior to the onset of symptoms, as part of traditional Chinese medicine.

On examination of the older child, temperature was 36.4°C, heart rate 116/min, respiration rate 24/min, and blood pressure 120/76 mmHg. He was conscious and cooperative. No peripheral lymphadenopathy was observed. Systemic examination of respiratory and cardiovascular system, and abdominal examinations were normal. There were no signs of cranial nerve palsy or focal neurologic deficits. Meningeal signs, including Kernig sign, Brudzinski sign, and nuchal rigidity were absent. Babinski sign was also absent. The hematological tests indicated a white blood cell count of 13.9×10^9/L, an eosinophil count of 54.0%, a hemoglobin level of 134 g/L, and a platelet count of 379×10^9/L. The C-reactive protein level was less than 0.5 mg/L. The liver and kidney function tests, and stool microscopy were normal. The magnetic resonance imaging (MRI) of the brain demonstrated scattered abnormal signals in the bilateral cerebral hemispheres and the left cerebellum. An abnormal signal was also noted in the right parieto-occipital sulcus. Cerebrospinal fluid (CSF) exami-nation revealed a white blood count of 560 cells per mm^3 (75% eosinophils), an elevated protein level (760 mg/L), and a decreased glucose level of (25 mg/dL). The chloride and adenosine deaminase concentrations were normal. The Gram stain, India ink stain, and acid-fast stain of the CSF were all negative. The CSF cultures for bacteria and fungi were negative. The results of the physical examination, blood tests, and CSF findings in the younger child were similar to the results of the older brother.

A diagnosis of eosinophilic meningitis was made based on the presence of CSF eosinophilia. *A. cantonensis* infection was highly suspected, as it is the most common parasitic etiology for eosinophilic meningitis and is due to known exposure to infective larvae. The serum antibodies for the common parasites were negative. The CSF sample was sent for mNGS. The specific DNA of *A. cantonensis* was detected in the CSF with mNGS, and 106 unique reads were identified. The coverage of the identified parasite genome, calculated by mapping the detected reads, was 0.0039%. Therefore, the younger brother was diagnosed definitively with CNS angiostrongyliasis.

The two patients were administered albendazole (15 mg/kg/day) in combination with prednisone (1 mg/kg/day, which was gradually discontinued over two weeks). The symptoms were relieved noticeably, and the patient did not complain of any headache or dizziness. One month later, a repeat routine blood test showed a white blood cell 6.6×10^9/L (8% eosinophils), and a repeated CSF analysis showed a decreased cell count of 165 cells per mm^3 (45% eosinophils), and normal levels of protein and glucose.

The existing literature shows that the severity of *A. cantonensis* disease and mortality in children is significantly higher than that in adults [2]. In humans, ingested third-phase larvae are not mature but are aggressive migrators. Neurological symptoms occur 2–35 days after infection. Eosinophilic meningitis caused by *A. cantonensis* is a self-limiting illness in which headaches, non-focal neurologic findings, and cranial nerve involvement are the most common symptoms and signs. Encephalitis is a relatively rare condition [3]. In a case report in which an infant with infection presented with long-term fever without other symptoms, the CSF also showed characteristic eosinophilia [4]. There have also been cases of transverse myelitis being reported [5]. In a prospective study that followed up on three previous studies, Chotmongkol, et al. [6] confirmed that a 2-week course of corticosteroids shortened the duration of headache and reduced the need for repeated lumbar punctures. The study concluded that corticosteroids plus albendazole were no better than corticosteroids alone. However, some scholars believe that corticosteroids are beneficial for severe patients, and that the dose can be increased when warranted [2]. The cases reported were not from an area that is knwon to be endemic to this parasite. The confirmation of the diagnosis underscores the utility of mNGS for CNS infections with unknown pathogens. Given the expanding endemic regions of *A. cantonensis* due to transportation logistics and global warming, clinicians should be aware of the possibility of the occurrence of *A. cantonensis* and

the utility of mNGS when etiological diagnosis is difficult.

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MIS-C Triggered by Omicron Variant of SARS-CoV-2

World Health Organization (WHO) designated the new variant of SARS-CoV-2 (B.1.1.529) as Omicron on November 26, 2021 [1]. Analyzing the initial cases of Omicron in South Africa to assess the clinical severity of cases, Walter and colleagues concluded that compared to Delta variant, the odds of hospitalization due to severe disease were less [2]. Even though the severity is likely to be mild, its impact on children and subsequent development of MIS-C is unknown.

Pediatric hospitalization due to Omicron in Gauteng Province of South Africa, was noted to be more when compared to the previous waves. During a six-week period, there were nearly 6,287 children with Omicron and four children in their series died, not because of COVID-19, but due to underlying comorbidity [3]. No case of MIS-C was reported in their series. India detected its first Omicron case on December 2, 2021, in Karnataka. We report what we believe to be the first case of MIS-C due to Omicron in India.

A 3-year-old male child presented to us on January 4, 2022 with fever for 6 days and maculopapular rash over the trunk and extremities, bilateral non purulent conjunctival congestion and abdominal pain with vomiting. Both the parents of this child had PCR confirmed mild COVID-19 a week before. Clinical examination did not reveal any features of tropical infections such as dengue or enteric fever. Since child had fever >3 days with mucocutaneous and gastrointestinal involvement, MIS-C was considered and further investigations were done. Complete blood count and inflammatory markers revealed leukocytosis and significantly elevated CRP and hypoalbuminemia (**Table I**). Given the epidemiology, reverse transcriptase polymerase chain reaction for COVID-19 was done, which was positive (Ct value – 12.9). Child had all criteria for WHO case definition for MIS-C [5]. ECHO and ECG were normal. He was started on intravenous immunoglobulin (2 g/kg) and intravenous steroids (methyl prednisolone 10 mg/kg/day for 3 days initially) which was then tapered and stopped over 2 weeks, and was also started on aspirin (5 mg/kg/day). He became afebrile within 24 hours and was well on follow-up after 2 weeks. Repeat ECHO at 2 weeks was normal.

This child presented to us after a lag period of around 4 weeks after the first case detection in our country. Whole genome sequencing of the SARS-CoV-2 from the nasopharyngeal aspirate confirmed it to be an Omicron variant (**Web Fig.1**).

There is a steep rise in the number of SARS-CoV-2 infections in South Africa, US and Europe and CDC has reported a proportionate surge in MIS-C with the increase in the number

Table I Laboratory Parameters in the Index Case

Laboratory parameters	*Value*
Leukocyte count	1.53×10^9 /L (N- 59%)
Hemoglobin	10.8 g/dL
Platelet count	271×10^9/L
C-reactive protein	64.2 mg/L
Serum sodium	130 mmol/L
Serum albumin	2.7 g/dL
Urine microscopy	Normal
D-dimer	2453 ng/mL
NT- Pro BNP	2774 pg/mL