Validation of *Angiostrongylus cantonensis* combined with herpes simplex virus type 1 in cerebrospinal fluid by next-generation sequencing

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*To the Editor:* *Angiostrongylus cantonensis* is one of the major causes of eosinophilic meningitis/encephalitis, due to eating raw or undercooked paratenic or intermediate hosts like mollusks. Here, we described a case of combined infection of *A. cantonensis* and herpes simplex virus type 1 (HSV-1) based on the results of next-generation sequencing (NGS).

A 59-year-old male patient had paroxysmal dizziness, followed by fatigue and low fever of 38.1°C for 18 days. Eighteen days later, he developed severe headache with nausea, vomiting, and high fever of 39.0°C. Then, he was sent to the local hospital. He lived in Guangdong Province and was used to eating online-shopped restoratives. Brain magnetic resonance imaging (MRI) revealed slight leukoencephalopathy [Figure 1A]. He was diagnosed and treated as presumed cerebral infarction while the symptoms worsened consequently. Thus, he was transferred to the center hospital, where the serum HSV-1 IgM was found positive and antiviral drugs were administered. However, the clinical manifestations deteriorated and he got unconsciousness and was transferred to our hospital 1 month later since the onset of disease.

On admission, the patient was febrile (38.0°C) with a Glasgow Coma Scale (GCS) of 7 (E1V1M5). He had neck stiffness and bilaterally positive Kernig signs. Laboratory findings revealed prominent acidophilus with an eosinophil ratio of 21.9% in peripheral blood. Electroencephalogram showed 3–4 Hz 30 to 50 μV δ wave on the background. Lumbar puncture yielded clear cerebrospinal fluid (CSF), with moderate pleocytosis (cell count of 310/μL) and abnormally elevated eosinophil of 40%. Examination for autoimmune encephalitis-related antibodies, including anti-N-methyl-D-aspartate receptor, aquaporin 4, myelin oligodendrocyte glycoprotein, and glial fibrillary acidic protein antibodies in serum and CSF were all negative.

Polymerase chain reactions of viruses (HSV-1, HSV-2, Varicella-zoster virus, Epstein-Barr virus, CytoMegalovirus) in CSF were all negative. NGS (Illumina NextSeq 550, Vision Medicals Co., Ltd, USA) for pathogens in CSF was performed as well. A repeated brain MRI revealed obvious leukoencephalopathy on T2 weighted and fluid-attenuated inversion recovery images (T2W-FLAIR) [Figure 1B]. Considering the elevated eosinophil in both blood and CSF, parasite antibodies were also tested. Based on the positive serum HSV-1 IgM and elevated C-reactive protein/procalcitonin (CRP/PCT), Acyclovir and Ceftriaxone were empirically administered at admission. Three days later, the NGS reported positive reads for *A. cantonensis* and HSV-1 in CSF, with 17,202 and 16 reads respectively [Figure 1D]. In addition, the serum IgG of *A. cantonensis* turned out to be positive. Albendazole and methylprednisolone were added. His temperature and the eosinophil became normal gradually. His consciousness improved with GCS of 10 (E4V1M5). A repeated NGS of CSF after 2-week anthelmintic therapy showed 6416 reads of *A. cantonensis* without reads of HSV-1 [Figure 1E]. After a 3-week intermission, he started another round of anthelmintic therapy. A third NGS of CSF showed no reads of either *A. cantonensis* or HSV-1. His consciousness further improved with GCS of 14 (E4V4M6), and then discharged for rehabilitation. The brain MRI after two rounds of anthelmintic therapy showed distinct recovery of leukoencephalopathy, as shown in Figure 1C. Six months later, modified Rankin Scale of the patient was 0 via follow-up.

*A. cantonensis* is one of the major causes of eosinophilic meningitis and meningoencephalitis. The imaging findings of the brain are diverse but relatively nonspecific. The MRI findings of the lesions mainly reveal multiple nodular enhancing lesions in the brain and linear enhancement in the pia. Some retrospective studies found leptomeningeal enhancement and increased signal intensity in the
subcortical white matter of the cerebrum and cerebellum on T2W- FLAIR images. Kanpittaya et al\textsuperscript{[1]} thought the abnormal signal intensity in the deep white matter may indicate gliosis or worm migratory tracks. The movement of the migrating worm may probably result in the separation of the axon from the nutrient cell. But no treatment information concerned about leukoencephalopathy. The serial images of our patient suggested the leukoencephalopathy due to neural-angiostrongyliasis could be alleviated after therapy.

NGS, a method of simultaneously sequencing millions of fragments of DNA has been recently applied to brain infections since the first diagnosis of neuroleptospirosis infection in 2014.\textsuperscript{[2]} Thereafter, a few cases were reported with intracranial α-herpes virus infection diagnosed by NGS.\textsuperscript{[3,4]} It has been suggested that NGS will provide more comprehensive, timely and actionable information for patients with multiple or rare infections. In the present study, NGS results correlated with traditional tests and treatment response, confirming the diagnosis of combined infection \textit{A. cantonensis} with HSV-1.

The mechanism of combined infection might be related to the blood-brain barrier disruption caused by \textit{Angiostrongylus}. \textit{A. cantonensis} initiated a series of responses in the central nervous system and broke the blood-brain barrier. Matrix metallopeptidase 9 is a protease that degrades extracellular matrix proteins and deteriorates blood-brain barrier. It increases possibly due to the damage inflicted by migrating worms. Animal studies of \textit{A. cantonensis} indicate that eosinophils release matrix metallopeptidase 9 into the subarachnoid space, activating a proteolytic cascade that disrupts the blood-brain barrier.\textsuperscript{[5]} But the concrete pathology is incompletely understood.

In clinical practice, pathogenic detection mostly depends on smear, the culture and pathology, which are short in...
sensitivity and/or time-consuming. NGS, which could detect a wide range of central nervous system pathogens within 48 h, is useful for the early diagnosis, especially in multi-infection.

Declaration of patient consent
The authors certify that they have obtained all appropriate patient consent forms. In the form the patient has given his consent for his images and other clinical information to be reported in the journal. The patient understands that his name and initials will not be published and efforts will be made to conceal the identity of the patient, although anonymity cannot be guaranteed.

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Conflicts of interest
None.

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