Molecular Diagnosis of Severe Angiostrongylus cantonensis-Induced Eosinophilic Meningitis: A Case Report Emphasizing the Need for Accurate Detection Methods

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Patient: Male, 66-year-old
Final Diagnosis: Severe angiostrongylus cantonensis-induced eosinophilic meningitis
Symptoms: Fever and conscious change
Clinical Procedure: —
Specialty: Infectious Diseases

Objective: Rare disease
Background: Angiostrongylus cantonensis, also known as the rat lungworm, is the most common parasitic cause of human eosinophilic meningitis. A. cantonensis infection is an emergent disease causing permanent neurological injury or even death when not diagnosed and treated promptly. Usually, human infection occurs through ingestion of food contaminated by intermediate hosts or the third stage larvae of A. cantonensis. Indicators for diagnosis include clinical signs of meningitis; contact history, such as that from eating raw or improperly cooked intermediate hosts or contaminated vegetables; and cerebrospinal fluid (CSF) eosinophilia. However, diagnosis is now primarily defined through polymerase chain reaction (PCR) assay of CSF or serum.

Case Report: A 66-year-old homeless man with unclear exposure history presented with fever and conscious change. The initial hemogram showed eosinophilia without neutrophilic leukocytosis. Non-contrast computed tomography (CT) and magnetic resonance imaging (MRI) of the head revealed no evidence of stroke. A lumbar puncture was performed and showed eosinophilic meningitis. The patient was ultimately diagnosed through PCR and sequencing for A. cantonensis infection, and dexamethasone treatment was started immediately. Although his general condition improved after dexamethasone treatment, his mental status did not improve completely.

Conclusions: Our report highlights the importance of applying molecular techniques in diagnosis of angiostrongylus, especially in individuals who have unknown contact history.

Keywords: Angiostrongyliasis • Angiostrongylus cantonensis • Meningitis • Polymerase Chain Reaction

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Background

Angiostrongylus cantonensis is a parasite that is primarily responsible for eosinophilic meningitis [1]. Humans become infected by eating raw or undercooked intermediate hosts or food contaminated with the larvae of A. cantonensis. Common symptoms include fever, headache, vomiting, nausea, and malaise. The larvae of A. cantonensis, which produce mucus-containing secretions, are highly neurotropic and cause varying degrees of neurological dysfunction [2]. Eosinophilic meningitis is the most common manifestation [3]. A. cantonensis infection is diagnosed based on several factors, including travel and diet history, clinical symptoms, and the presence of eosinophilic pleocytosis in CSF. Serological confirmation of the disease may yield negative results during the early stages of the disease, making it a less than ideal method [4]. The PCR assay used for diagnosis of A. cantonensis has high sensitivity and specificity, and can aid in early diagnosis [5-7]. This study presents a homeless man with fever and unclear contact history who was diagnosed as having A. cantonensis infection through PCR and direct sequencing methods.

Case Report

A 66-year-old homeless man experiencing fever, dizziness, cough, and lethargy for 1 day visited our hospital. Upon arrival, the patient presented with a fever of 39.2°C, which subsequently subsided after he took acetaminophen. The hemogram showed eosinophilia without neutrophilic leukocytosis (white cell count 9090 mm³ with 9% eosinophils and 63.9% neutrophils). The COVID-19 PCR test was negative. Following these findings, the patient was discharged. However, the following day, he returned to our ED complaining of chest tightness, headache, and fever. His cardiac enzyme readings were within their normal ranges, and his electrocardiogram findings indicated no myocardial infarction. Eosinophilia without neutrophilic leukocytosis persisted in the hemogram. Blood cultures yielded negative results, and urinalysis was negative for leukocyte esterase and nitrites. Biochemical blood profiles showed elevated creatine kinase (CK) level and myoglobin level. Follow-up biochemical blood profiles revealed decreased CK and myoglobin levels, and he was discharged with improved symptoms. However, he returned to our ED on the same day for a resurgence of fever reaching 37.9°C. His consciousness then deteriorated. Since we were unable to reach any of his family members and his consciousness dramatically deteriorated, the contact history remained unclear.

On examination, he had a Glasgow Coma Scale score of E1V2M4, with spontaneous movement in his arms and legs. His pupils were equal, round, and mildly reactive to light, with a decrease in diameter from 4 to 3 mm. The remainder of the examination revealed normal findings. The white cell count was 10 150 mm³ with 12.5% eosinophils, the blood level of sodium was 128 mmol/L, his C-reactive protein level was 0.82 mg/dL, and the CK level was 457 U/L. His hematocrit results, hemoglobin count, other white cell differential count, platelet count, and anion gap were normal. Similarly, the blood levels of potassium, ethyl alcohol, ammonia, glucose, lactate, and tropinin-I were all normal. Additionally, the results of renal and liver function tests were also normal. The urine toxicologic screening was negative. Non-contrast computed tomography (CT) and magnetic resonance imaging (MRI) of the head revealed no evidence of stroke. Lumbar puncture revealed clear CSF and an opening pressure of 24 cm of water. The CSF white blood cell count was 373 cells/mL, and eosinophils were predominant (54%); the glucose level was 107.2 mg/dL; and the protein level was 55 mg/dL. The corresponding serum glucose level was 176 mg/dL. The CSF PCR assays were negative for of cytomegalovirus, enterovirus, herpes simplex virus 1, herpes simplex virus 2, human herpesvirus 6, human parechovirus, varicella zoster virus, Haemophilus influenzae, Escherichia coli K1, Listeria monocytogenes, Neisseria meningitidis, Streptococcus agalactiae, Streptococcus pneumoniae, Cryptococcus neoformans, and Cryptococcus gattii were negative. India ink staining, Gram staining of CSF, and bacterial culture were negative. Screenings for human immunodeficiency virus, syphilis, and Toxoplasma gondii. Toxocariasis was not tested in this case. The patient was then admitted to the ward of the infectious disease department for further survey and treatment.

Throughout hospitalization, CSF specimen was sent to the Taiwan Centers for Disease Control for testing of leptospirosis, Q fever, and scrub typhus. The results of these tests were negative. Subsequently, we began treatment with intravenous ceftriaxone and doxycycline. However, the patient’s level of consciousness did not improve despite antibiotic treatment. Because the CSF cell count remained eosinophil predominant (36%), we decided to survey A. cantonensis, a common cause of eosinophilic meningitis in Taiwan.

The CSF sample from this patient was tested by standard PCR. A 213 base pairs amplicon of the mitochondrial cytochrome oxidase subunit 1 gene was amplified and sequenced. Mixture for PCR amplification consisted of 0.2 μM CO1ACF7 primer (5’-TGC TCT TTT TCC AGA TCA AAG C-3’), 0.2 μM CO1ACR7 primer (5’-TCA TCT CCG TAG GAA CCG CA-3’), the KAPA2G Fast HotStart ReadyMix (KAPA Biosystems, Cape town, South Africa), and 5 μL of the DNA sample in the DNAzol DIRECT solution (Molecular Research Center Inc., Ohio, U. S. A.) in a total volume of 50 μL [6]. Following an initial incubation at 95°C for 10 minutes to activate the thermoactive polymerase, the PCR program was as follows: 94°C for 30 seconds, 50°C for 30 seconds, and 72°C for 30 seconds, repeated for 45 cycles. The PCR amplicons were then subjected to DNA sequencing, later
compared to National Center for Biotechnology Information (NCBI) database. Both PCR and sequencing were performed in the biosafety cabinet with BSL 2 facilities of Medical Science and Technology Building of Veterans General Hospital to ensure the safety and security of the personnel. Figure 1 demonstrates the CSF PCR results, show a positive band at 213 bp, revealing positivity for *A. cantonensis*. Figure 2 depicts the confirmation of *A. cantonensis* infection by sequencing result compared to NCBI database.

**Figure 1.** Agarose gel electrophoresis of PCR amplification for mitochondrial cytochrome oxidase subunit 1 (CO1) gene. P – CSF pellet mixed with 2 primers; S5 – 5 μL supernatant of CSF mixed with 2 primers; S10 – 10 μL CSF supernatant mixed with 2 primers. The first lane indicates a 100-bp ladder DNA size marker and the arrow indicates the size (213 bp) of the expected PCR products.

**Figure 2.** Sequence alignment results of the PCR amplicon, which showed 100% identical to the *A. cantonensis*.

**Figure 3.** Brain T2-weighted MRI shows increased signal intensity in the bilateral periventricular and subcortical white matter.
Gnathostoma spinigerum is an approved gally proof of migrating larva die over time, leading to local inflammation and neural angiostrongyliasis. Although Angiostrongylus eosinophilic meningitis is typically self-limited, neural angiostrongyliasis can cause severe outcomes, including the possibility of death.

The diagnosis of *A. cantonensis* infection is traditionally determined through clinical signs, meningitis symptoms, high-risk behaviors, and eosinophilic pleocytosis presence in CSF. Recently, confirmatory diagnostic methods have been developed, including PCR for detecting *A. cantonensis* DNA in the CSF and immunoassay of *A. cantonensis* antibodies. Immunodiagnostic methods, such as western blot and enzyme-linked immunosorbent assay (ELISA), may differ in their diagnostic performance due to the characteristics of the antigens [6]. Nonetheless, the PCR assay has a sensitivity of approximately 75% and a specificity of 99%, which is higher than those of western blot and ELISA [7]. The PCR assay allows for parasite DNA detection in CSF during the acute phase, meaning it can obtain a diagnosis faster than serological testing. PCR has now become the primary confirmatory diagnostic method for confirming *A. cantonensis* infection [4-6,8,9].

The management of cerebral angiostrongyliasis primarily involves supportive measures, with the infection typically resolving spontaneously. In severe cases, corticosteroids and repeated lumbar puncture is recommended to control inflammation and decrease intracranial pressure. The efficacy and safety of anthelmintic therapy, such as benzimidazoles and ivermectin, remain controversial, as it can exacerbate neurological symptoms by provoking an inflammatory reaction as the worms die off [4,7,8].

Patients with neurological symptoms may undergo head CT or MRI to exclude other non-parasitic causes. Head CT without focal lesions is a sign of neuroangiostrongyliasis rather than gnathostomiasis or neurocysticercosis. A study reported that MRI helps to distinguish between different parasitic causes of eosinophilic meningitis [10]. *Gnathostoma spinigerum* is another main cause of eosinophilic meningoencephalitis. It can be differentiated from *A. cantonensis* based on differences in contact history and certain clinical presentations, such as cutaneous gnathostomiasis. Brain CT revealing intraparenchymal brain hemorrhagic lesions and MRI displaying myelitis patterns are suggestive of gnathostomiasis. In contrast, increased signal intensities over the white matter on T1-weighted MRI and the subcortical white matter on T2-weighted MRI indicate neuroangiostrongyliasis [7,11-13]. Leptomeningeal enhancement and punctuated or linear hemorrhagic lesions within the cerebral and cerebellar hemisphere can be identified through SWI [7,12,14,15]. However, SWI lacks the necessary specificity for clinical diagnosis, making PCR for parasite DNA the most powerful and accurate diagnostic method. In our patient, increased activity was noted in the bilateral periventricular, subcortical white matter on T2-weighted MRI, and multiple linear

**Discussion**

Eosinophilic meningitis is a rare but potentially fatal disease if not promptly diagnosed. The etiology of eosinophilic meningitis can be divided into non-parasitic and parasitic. Non-parasitic etiology, which is rare, includes fungal infection, such as coccidioidomycosis and cryptococcosis, hyperesinophilic syndromes, and neoplastic diseases. Among the parasitic causes of eosinophilic meningitis, *A. cantonensis* is the most common pathogen. Angiostrongylus eosinophilic meningitis is mainly prevalent in the Asia-Pacific region, including Taiwan. Because humans are not proper hosts for *A. cantonensis*, the migrating larva die over time, leading to local inflammation and neural angiostrongyliasis. Although Angiostrongylus eosinophilic meningitis is typically self-limited, neural angiostrongyliasis can cause severe outcomes, including the possibility of death.

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**Figure 4.** Brain SWI MRI showed linear hypointensities (arrows) over bilateral cerebral hemispheres.

Brain MRI revealed increased signal intensity in the bilateral periventricular and subcortical white matter on T2-weighted imaging (Figure 3). Susceptibility-weighted imaging (SWI) revealed linear hypointensities over bilateral cerebral hemispheres, suggesting small arterial occlusion compatible with larvae occlusion in the small arteries (Figure 4) [7].

After *A. cantonensis* infection was diagnosed, dexamethasone 10 mg was initiated for 1 week and the dose was gradually tapered off. The 1-month follow-up CSF eosinophil percentage decreased to 6%, but the patient’s clinical condition did not improve. He was discharged in late September 2022 in relatively stable condition and transferred to a long-term care institution. He was then lost to follow-up.
hypointensities were noted over bilateral cerebrum on SWI, suggestive of small arterial occlusion compatible with larvae occlusion in the small arteries.

**Conclusions**

This report highlights that when assessing a conscious impaired patient with unknown contact history, eosinophilia, or eosinophilic meningitis, clinicians must consider *A. cantonensis* as a possible differential diagnosis. PCR assay with DNA sequencing is recommended as a confirmatory diagnosis approach.

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**References:**