Case Reports

Journal of INTERNATIONAL MEDICAL RESEARCH

Journal of International Medical Research
50(5) 1–8

DOI: 10.1177/03000605221093217
journals.sagepub.com/home/imr
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Subacute *Balamuthia mandrillaris* encephalitis in an immunocompetent patient diagnosed by next-generation sequencing

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Abstract

Balamuthia mandrillaris is a free-living heterotrophic amoeba found in soil that causes a rare and usually fatal granulomatous amebic encephalitis. We report an immunocompetent patient infected with *B. mandrillaris* encephalitis diagnosed by next-generation sequencing (NGS). Clinical manifestations included sudden headache and epilepsy with disturbance of consciousness. The opening pressure of cerebrospinal fluid (CSF) was 220 mmH_2O, with mildly elevated white blood cell numbers and elevated protein levels. Cranial magnetic resonance imaging revealed abnormal signals in the right frontal lobe, left parietal lobe, and left occipital lobe. CSF NGS detected *B. mandrillaris*. Albendazole and metronidazole combined with fluconazole were administered to the patient immediately, but his condition deteriorated and he eventually died. Encephalitis caused by *B. mandrillaris* is rare and has a high mortality rate. Clinical manifestations are complex and diverse, but early diagnosis is very important for successful treatment. This can be aided by the metagenomic NGS of CSF.

Keywords

Balamuthia mandrillaris, encephalitis, next-generation sequencing, cerebrospinal fluid, amoeba, central nervous system

Date received: 26 July 2021; accepted: 17 March 2022

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Introduction

Balamuthia mandrillaris was originally isolated from the brain of a female baboon that died of meningoencephalitis at the San Diego Zoo in 1986.[1] Balamuthia amebic encephalitis (BAE) has been reported in more than 200 cases worldwide, and predominantly occurs in South America and the United States.[2]

B. mandrillaris is a widely distributed, free-living amoeba found in the soil, fresh and salty water, swimming pools, humidifiers, and air conditioning units.[2] Unlike other amebic protozoa, *B. mandrillaris* can cause a rare but fatal granulomatous amebic encephalitis (GAE) in both immunocompetent and immunocompromised patients.[3] Encephalitis caused by *B. mandrillaris* is rare in the clinic, but its current mortality rate is above 90%, despite antimicrobial therapy.[4] Early diagnosis is extremely important for prognosis, but clinical manifestations of BAE are complex and diverse, making diagnosis difficult. Fortunately, the application of next-generation sequencing (NGS) has enabled more BAE cases to be diagnosed.[5–7]

Here, we present a case of BAE in an immunocompetent male patient diagnosed by NGS in Guangzhou, China. The reporting of this study conforms to CARE guidelines.[8]

Case presentation

A 54-year-old man presented with headache and dizziness on 2August 2020. The headache was persistent and mainly localized to the bilateral temples, with no nausea, vomiting, blurred vision, limb weakness, or convulsions. Two days later, he experienced twitching of his right limb and face, which lasted for about 2 minutes without loss of consciousness. The patient lived alone. His medical history was unremarkable, except for the presence of skin lesions, which he had not received treatment for. Family members were not sure when the lesions had developed.

The patient was admitted to a local hospital on 2 August 2020 where cranial computed tomography (CT) and magnetic resonance imaging (MRI) were performed (Figure 1). Skin erythema on the medial side of his right knee joint and the curved side of his upper right thigh was noticed. A skin biopsy was conducted and non-specific inflammation was observed. Mannitol and glycerol fructose were administered for suspected intracranial hypertension, but his neurological status deteriorated.

On 7 August 2020, the patient was comatose and the twitching of his right limbs became aggravated, mainly in his right leg. He was transferred to the neurological intensive care unit of Nanfang Hospital on 12 August 2020. On admission, his body temperature was 37.8°C, and skin lesions on his right leg were observed (Figure 2). Neurological examination showed that he was comatose with a Glasgow coma score (GCS) of 6 (E1V1M4). Neck stiffness was obvious, but other physical examinations were unremarkable. Lumbar puncture, cranial CT, and positron emission tomography (PET) were arranged promptly. Cerebrospinal fluid (CSF) showed an elevated opening pressure of 220 mmH_2O (normal range, 80–180 mmH_2O), with a mildly elevated white blood cell (WBC) count of 66 cells/μL (normal range, 0–8 cells/μL), normal glucose level of 3.96 mmol/L (normal range, 2.8–4.5 mmol/L), slightly decreased chloride level of 117 mmol/L (normal range, 120–130 mmol/L), and increased protein level of 1.05 g/L (normal range, 0.15–0.45 g/L). CSF cytology revealed a dramatically elevated WBC count of 8000 cells/0.5 mL (normal range, <200 cells/mL), of which lymphocytes accounted for 80%.

Two weeks after the onset of headache and dizziness, repeated cranial CT revealed an increase in patchy, low-density lesions in

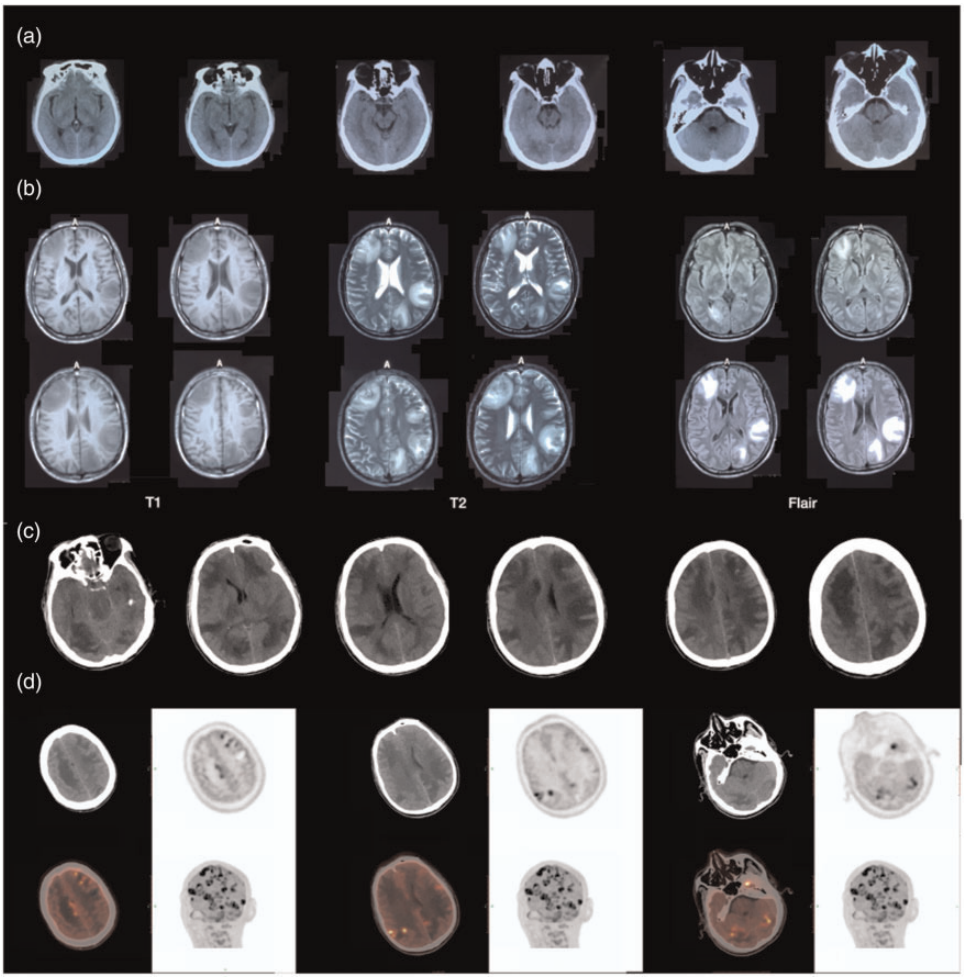

Figure 1. Cranial images of the patient. A. CT scan of the brain performed on August 6, 2020 showing multiple, patchy, low-density shadows. B MRI performed on August 7, 2020 showing multiple lesions with edema in the right frontal lobe, left parietal lobe, and left occipital lobe. C Cranial CT performed on August 16, 2020 showing diffuse low-density shadows. D PET examination of the brain performed on August 13, 2020 showing multiple nodular and strip hypermetabolic lesions with patchy edema.
CT, computed tomography; MRI, magnetic resonance imaging; PET, positron emission tomography.

the brain (Figure 1), and PET examination showed nodular and strip hypermetabolic lesions with patchy edema (Figure 1). Laboratory screening for autoimmune diseases, HIV infection, syphilis, and pathogens such as herpes simplex virus ([HSV]-1 and HSV-2), cytomegalovirus, *Mycobacterium tuberculosis*, *Taenia solium*, *Mycoplasma*, and *Legionella pneumonia* were all negative. Smears and cultures of CSF using the Alcian blue stain, acid-fast stain, and liquid-based cytology were also negative for pathogens. Chest CT revealed multiple nodules in the posterior segment of the upper lobe and the posterior basal segment of the lower lobe.

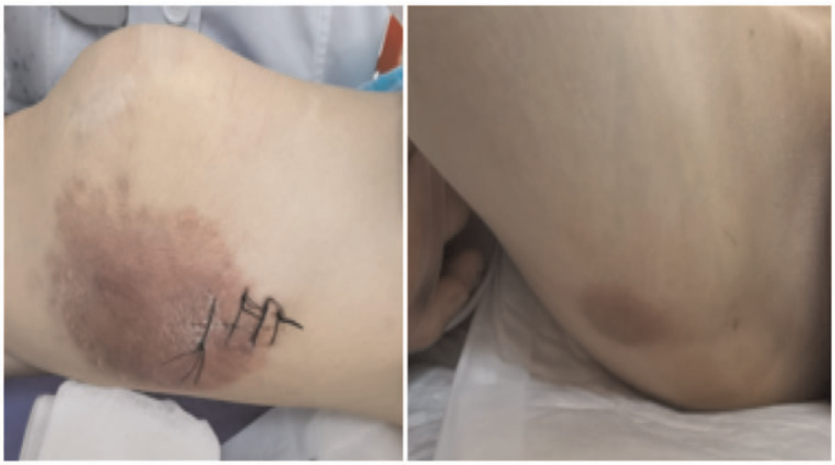

Figure 2. Round erythema (4 × 5 cm) on the inside face of the right knee joint. The boundary was unclear, and the rash was dry, prominent, raised, and circular. Another round erythema with clear boundaries can be seen on the curved side of the right lower extremity.

Given the possibility of a brain abscess, meropenem 2 g every 8 hours (q8h) was administrated from 13 to 17 August 2020, and vancomycin 1 g q12h was administrated from 13 to 15 August 2020. The patient developed carbon dioxide retention and required intratracheal intubation on 14 August 2020. Physical examination showed a high fever of 39.4°C, and deep coma with a GCS of 3 (E1V1M1). Considering the possibility of fungal infection, voriconazole 0.2 g q12h was administered from 14 to 15 August 2020 (Figure 3). At midnight on 14 August 2020, the patient developed a brain herniation with bilaterally dilated pupils. The next morning, he developed hypoxemia and hemodynamic instability, and required 12 mg noradrenaline to maintain his blood pressure. Physical examination revealed bilateral dilated and fixed pupils. On 15 August 2020, NGS of DNA extracted from a CSF sample performed by Vision Medicals (Guangzhou, China) identified 399 reads of *B. mandrillaris*. Albendazole 0.4 g twice daily, metronidazole 0.6 g q8h, and fluconazole 0.4 g daily were administered from 15 to 17 August 2020 according to published guidelines (Figure 3).[9] The patient died on 17 August 2020.

Discussion

BAE is a rare, subacute chronic infection of the central nervous system (CNS).[1] Risk factors include AIDS, cancer, liver disease or diabetes, immunosuppression, and alcoholism.[10] BAE CNS symptoms are caused by thromboangiitis obliterans, leading to hemorrhage, infarction, and necrosis.[11] Initial symptoms include regression, unilateral headaches, focal seizures, cranial nerve dysfunction, or localized motor deficits.[12] Signs of increased intracranial pressure may be present with lymphocytic pleocytosis, mild to severe elevation of protein concentrations, and normal to low glucose concentrations, followed by progressive loss of consciousness.[13] This progression generally occurs over 2 to 12 weeks.[14,15] Brain CT or MRI typically demonstrates multiple lesions ranging from small, solid lesions to large, nodular lesions with ring enhancement. Intralesional hemorrhage is

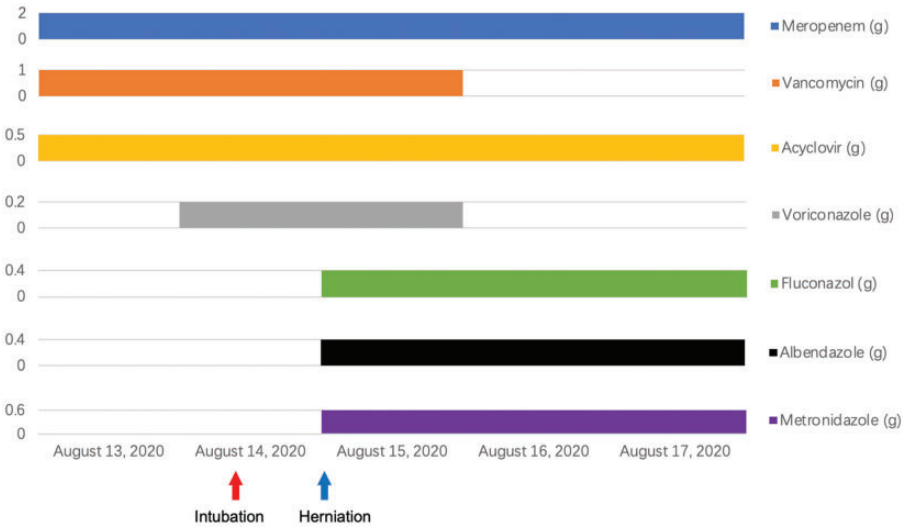

Figure 3. Graphical timeline of drug administration.

an important radiological sign.[15] Patients with cutaneous involvement commonly present with a non-ulcerative asymptomatic plaque, and either single or occasional satellite lesions.[16]

Clinicians have a certain understanding of the skin lesions typical of BAE, which may facilitate an early diagnosis and initiate prompt anti-amoeba therapy. In our case, the patient had typical skin lesions before developing CNS symptoms, but his skin biopsy did not identify the pathogen. There are no clear diagnostic criteria for BAE, and its symptoms and imaging findings are complex and diverse, making a diagnosis challenging. In a case series from the United States between 1974 and 2016, a diagnosis of BAE was primarily made through brain tissue biopsies. Most (88%) cases required a brain biopsy to assist with the diagnosis, while 9% of cases were diagnosed by skin biopsy.[12] A definitive diagnosis requires the visualization of a trophozoite or cyst,[17] but amebic trophozoites are rare and can be difficult to distinguish from histiocytes. Indirect immunofluorescence and PCR are species-specific assays with a high sensitivity for detecting amebae in tissue.[17] However, laboratory tests in most hospitals are limited to commonly known and suspected pathogens, and most countries lack these tests for *B. mandrillaris*.

NGS technology uses the parallel sequencing of multiple small DNA fragments. This has allowed a dramatic increase in the speed and a decrease in the cost at which an individual's genome can be sequenced, making a diagnosis very convenient and efficient.[18–20] Our patient was diagnosed using NGS, which was consistent with the case reported by Greninger et al.[5] Multiple reports suggest that it has great potential in the early diagnosis of BAE.[21–26] However, there is a lack of evidence-based standardized treatment. Experience in the treatment of BAE is very limited, often because of patient mortality or extensive disease at the time of diagnosis, and the lack of well-developed protocols. In 2017, the CDC of the United States Centers for Disease Control and Prevention recommended the use of pentamidine, miltefosine, fluconazole, flucytosine, sulfadiazine, and

macrolides (azithromycin and/or clarithromycin) in addition to symptomatic treatment for BAE.[27–29] Our patient deteriorated rapidly after the administration of albendazole, metronidazole, and fluconazole, which could reflect rapid disease progression or a severe inflammatory reaction. Indeed, *B. mandrillaris* is known to release multiple antigens,[27–29] which might induce inflammatory reactions, brain edema, and intracranial hypertension, and eventually accelerate the progression of death. Therefore, including corticosteroids in the initial treatment of BAE should be considered.

Conclusions

Herein, we present a case of BAE in an immunocompetent Chinese adult male diagnosed by NGS of a CSF sample. Although the diagnosis was quick and prompt therapy was initiated, the patient's neurologic symptoms deteriorated, and an inflammatory response to the treatment was suspected. More research is warranted to improve treatment for BAE. Moreover, clinicians should be aware of the skin lesions associated with *B. mandrillaris* and consider NGS as a useful tool to help in the diagnosis of rare pathogens.

Ethics approval and consent to participate

This study was approved by the local ethics committee of Nanfang Hospital, Southern Medical University.

Author contributions

SW, DW, and YW contributed to study conception and design. CX and XW participated in data analysis and drafted the manuscript. MT was involved with patient care and collected clinical data. All authors made substantial contributions to the study, and read and approved the final version of the manuscript.

Consent for publication

We obtained written consent for publication from the patient's wife and daughter, including the publication of identifying images. Copies of written consent forms are available for review from the Editor of this journal.

Availability of data and material

Not applicable.

Competing interests

The authors declare no competing interests.

Funding

The authors disclosed receipt of the following financial support for the research, authorship, and publication of this article: This study was supported by the Guangdong Key Research and Development Project Fund (Grant No. 2019B010109001), the National Natural Science Foundation of China (Grant No. 82071484), and the National Science Foundation of Guangdong Province of China (Grant No. 2019A151 5011760).

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