

Case Report

A Case of Balamuthia Amoebic Encephalitis Diagnosed with NGS in China

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Abstract

Background: Balamuthia amoebic encephalitis is caused by the invasion of Balamuthia mandrillaris, with a limited global incidence and no standardized treatment regimen currently available. The fatality rate exceeds 95%.

Case Presentation: A 64-year-old man from Shanxi, China, was admitted with abnormal mental behavior and weakness in the right lower limb. Cranial magnetic resonance imaging revealed multiple areas of abnormal signal intensity. High-throughput Next-Generation Sequencing (NGS) detected Balamuthia mandrillaris genomic DNA in the patient's cerebrospinal fluid. The patient was treated with albendazole 0.4g orally once daily, compound sulfamethoxazole tablets 0.48g×2 tablets orally every 8 hours, fluconazole sodium chloride injection 0.6g intravenously once daily, and flucytosine injection 2.5g intravenously every 8 hours for over four months. The patient's consciousness and lower limb muscle strength improved; unfortunately, the patient died from severe gastrointestinal bleeding and hemorrhagic shock despite resuscitation efforts.

Conclusions: This case involved a patient diagnosed with Balamuthia mandrillaris encephalitis through cerebrospinal fluid NGS testing. The patient was treated with a combination of four medications for over four months, demonstrating significant therapeutic effects. However, the patient ultimately died due to unexplained gastrointestinal bleeding. This case underscores the importance of closely monitoring the gastrointestinal system during treatment and performing gastroscopy as needed to ensure comprehensive assessment and timely intervention, thereby preventing potential complications.

Keywords: Encephalitis; Balamuthia mandrillaris; Next-generation sequencing; Treatment.

Background

Balamuthia mandrillaris is a free-living amoeba belonging to the genus *Balamuthia*, commonly found in natural water bodies, soil, and dust. It can occasionally invade the human central nervous system or other organs, causing granulomatous amoebic encephalitis, skin damage, and other complications. With a limited number of global cases and no standard treatment plan, *Balamuthia mandrillaris* is a rare and deadly disease. The case fatality rate exceeds 95%, and survivors with improved conditions after treatment are exceptionally rare. In this case, the *Balamuthia mandrillaris* sequence was detected in the cerebrospinal fluid via NGS. Four drugs were used in combination for anti-parasitic treatment over 4 months. The patient's symptoms improved and their condition stabilized, which holds important reference value for managing such cases.

Case Presentation

On July 22, 2024, our hospital admitted a 64-year-old male farmer from Shanxi, China. According to the family, the patient began exhibiting abnormal mental and behavioral symptoms on July 3 without any apparent cause, including impaired orientation, slowed reaction speed, and weakness in his right leg. During this period, the

patient did not experience fever, limb convulsions, nausea, vomiting, visual disturbances, loss of consciousness, or incontinence. On July 4th, the patient underwent blood glucose testing, which revealed elevated glucose levels and a positive result for urinary ketones. A cranial CT scan did not detect any hemorrhagic lesions. Based on these findings, the patient was diagnosed with diabetic ketoacidosis and received treatment involving glucose-lowering and fluid replacement therapies. However, during treatment, the patient's symptoms worsened, leading to the complication of urinary incontinence. Cranial Magnetic Resonance Imaging (MRI) results from July 8, 2021, showed multiple abnormal signals in the left cerebellar hemisphere, bilateral basal ganglia, bilateral periventricular regions, and bilateral frontal, parietal, and occipital lobes, as well as the right temporal lobe. The Cerebrospinal Fluid (CSF) examination on July 11 revealed normal pressure and normal Adenosine Deaminase (ADA) concentration of 3.4 U/L, glucose concentration of 4.6 mmol/L, chloride ion concentration of 121.1 mmol/L, protein concentration of 1253.0 mg/L, and IgG concentration of 250.3 mg/L. High-throughput Next-Generation Sequencing (NGS) technology detected *Balamuthia mandrillaris* in the CSF with a sequence copy number of 932. Thus,

based on the above diagnostic findings, the patient was diagnosed with Granulomatous Amoebic Encephalitis (GAE) caused by *Balamuthia mandrillaris*. After a week of anti-infective treatment with acyclovir, ceftazidime, tinidazole, co-trimoxazole, and fluconazole, the patient's symptoms did not show significant improvement. The patient was transferred to our hospital on July 22nd, and the treatment regimen was adjusted to include albendazole 0.4g po. Qd, compound sulfamethoxazole tablets 0.48g×2 tablets po. Q8h, fluconazole sodium chloride injection 0.6g iv drip Qd, flucytosine injection 2.5g iv drip Q8h, along with other supportive treatments.

During hospitalization, the patient's consciousness gradually improved, and after 10 days, they were able to respond correctly, albeit with slow reactions and occasional delirium. On August 1, a brain MRI revealed multiple abnormal signal shadows in the bilateral cerebellar hemispheres, caudate nucleus, right frontal, temporal, parietal, and occipital regions, hippocampus, insula, left cerebellar vermis, frontal lobe, left splenium of the corpus callosum, gyrus rectus, occipital lobe, and peritrigonal region of the lateral ventricles (Figure 1).

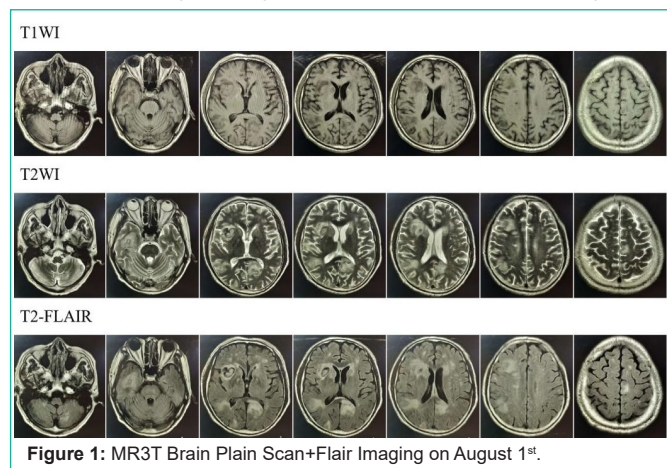


Figure 1: MR3T Brain Plain Scan+Flair Imaging on August 1st.

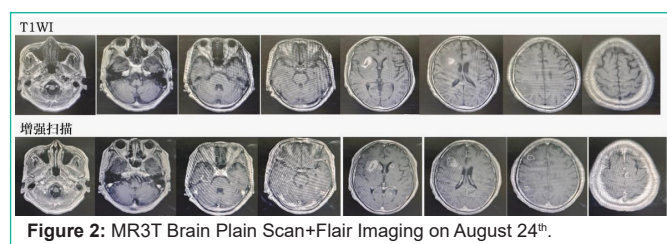


Figure 2: MR3T Brain Plain Scan+Flair Imaging on August 24th.

On August 8, the patient's gastric tube was removed successfully, and oral feeding was initiated. The patient was subsequently transferred from the intensive care unit to a general ward. By August 12, a repeat cranial MRI scan showed that the areas of multiple abnormal signal intensities in the brain had enlarged compared to previous results. Therefore, a contrast-enhanced MRI scan was recommended for further diagnostic confirmation. On August 17, the re-measured CSF pressure was 165 mmH₂O, and the routine biochemical tests of the CSF were also re-examined (Table 2). Meta CAP analysis of the CSF revealed the presence of *Balamuthia mandrillaris* (sequence number 57).

On August 19, the patient's urinary catheter was removed successfully, and the patient was able to walk with assistance. After reviewing the treatment plans of a few survivors, it was decided to change the patient's medication from co-trimoxazole to sulfadiazine tablets, taken orally every 8 hours at a dose of 1.5g. The other three medications remained unchanged. The enhanced cranial MRI performed on August 24 showed that the range of multiple abnormal signal shadows had decreased compared to previous scans (Figure 2). On August 28, during a lumbar puncture, the patient's cerebrospinal fluid pressure was 120 mm H₂O. Conventional and biochemical analyses of the cerebrospinal fluid were performed, and *Balamuthia mandrillaris* (sequence number 468) was detected through the Meta CAP assay. During this period, the patient exhibited clear consciousness and the ability to walk short distances independently. The patient's complete blood count, liver function, kidney function, coagulation profile, and blood glucose levels were all within normal ranges. The first follow-up examination, conducted on October 8th, showed that cerebrospinal fluid pressure measurements were within the normal range. Cerebrospinal fluid laboratory results indicated

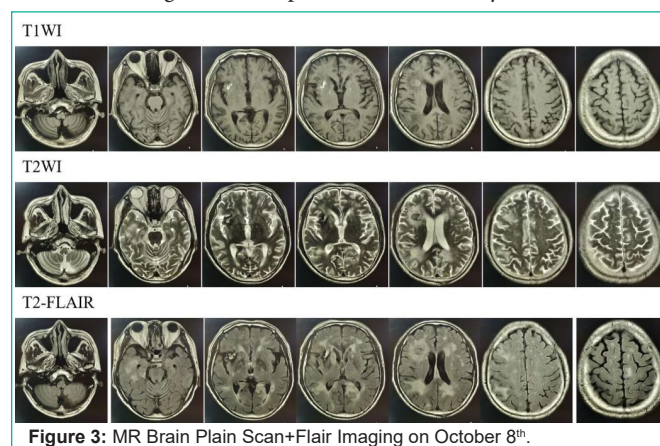


Figure 3: MR Brain Plain Scan+Flair Imaging on October 8th.

Table 1: The results of the Laboratory Indicators.

Time	Blood Routine Examination								CRP (mg/L)	PCT (ng/mL)	IL-6 (pg/mL)	LDH (U/L)	Coagulation series	
	WBC (10 ⁹ /L)	L%	M%	N%	E%	PLT (10 ⁹ /L)	RBC (10 ¹² /L)	HGB (g/L)					FDP (μg/ml)	D-D (μg/ml)
July 23	4.91	15.1	7.9	74.8	1.8	196	4.25	127	<5.0	0.08	—	362	31.7	11.4
July 24	5.02	15.7	3.8	80.3	0	214	4.49	136	<5.0	0.06	—	—	—	—
July 26	6.57	10.4	4.7	84.4	0.2	224	4.5	136	<5.0	0.03	9.42	—	23.9	10.23
July 28	4.26	10.8	4.5	83.8	0.7	169	4.57	134	<5.0	0.06	11.75	295	—	—
July 30	4.19	14.6	5.0	78.8	1.4	147	4.49	131	<5.0	0.04	11.41	—	—	—
August 1 st	3.53	15	6.5	76.5	1.4	143	4.48	132	<5.0	0.03	12.03	331	11.6	4.52
August 3 rd	4.3	12.1	7.0	79.8	0.9	141	4.2	122	6.95	0.06	—	327	8.3	3.202
August 5 th	5.57	11	6.1	81.8	0.7	140	4.1	120	28.29	0.06	—	310	5.29	1.809
August 8 th	4.14	22.5	9.4	66.5	1.4	149	3.4	101	—	—	—	—	8.9	3.57
August 11 th	3.52	23.9	9.1	64.4	2.0	190	3.23	95	27.11	0.05	23.49	—	—	—
August 16 th	3.82	26.7	6.8	63.8	2.4	253	3.06	90	<5.0	0.06	8.77	—	11	3.84
August 23 th	2.89	39.8	9.0	48.4	2.1	242	3.43	103	34.79	0.03	4.62	225	8.5	3.42
August 29 th	2.08	39.4	11.5	46.7	1.9	212	3.52	104	<5.0	0.03	5.1	214	8.4	3.114
September 2 nd	11.16	11.4	5.1	82.3	0.9	218	3.4	103	—	—	—	—	9.2	3.32

significant improvement. Meta CAP analysis of the cerebrospinal fluid revealed the presence of *Balamuthia mandrillaris* (sequence number 33). Additionally, cranial MRI results from the follow-up examination indicated that multiple abnormal signal shadows showed no significant change compared to the previous examination (Figure 3). The patient was capable of self-care in daily life, requiring only bed rest and occasionally experiencing symptoms of memory decline and spatial disorientation. Their Activity of Daily Living (ADL) scale score was 65, and they continued to follow the original treatment regimen with oral medication. However, on November 22nd, the patient suddenly developed melena without any obvious precipitating factors, accompanied by confusion. They were immediately rushed to a local hospital. Diagnosed with gastrointestinal bleeding and hemorrhagic shock, the patient unfortunately passed away on November 27th.

Discussion and Conclusions

Naegleria fowleri, *Balamuthia mandrillaris*, and *Acanthamoeba spp.* are three opportunistic pathogenic Free-Living Amoebae (FLA) that inhabit soil, water, and air worldwide [1,2]. The diseases caused by FLA can present with various clinical manifestations, including *Acanthamoeba keratitis* [2], skin and mucosal infections, as well as central nervous system diseases [1]. *Naegleria fowleri*, commonly known as the "brain-eating amoeba" [3,4], typically causes Primary Amebic Meningoencephalitis (PAM). From 1962 to 2022, the United States reported 157 cases, with only 4 survivors, resulting in a mortality rate of 97% [5]. Central nervous system diseases caused by *Balamuthia mandrillaris* and *Acanthamoeba spp.*, primarily manifesting as Granulomatous Amoebic Encephalitis (GAE) [1,6].

In 1990, a baboon at the San Diego Zoo in the United States died of encephalitis, and amoebae were found in its brain tissue. Subsequently, using rabbit anti-brain lysate serum for immunofluorescence testing, brain lysate amoebae were discovered in brain sections from numerous encephalitis cases worldwide and some cases of animal meningoencephalitis in the United States. This indicates that brain lysate amoebae are potential pathogens for fatal meningoencephalitis in both humans and animals [7].

There are currently approximately 200 reported cases of *Balamuthia mandrillaris* encephalitis worldwide [8], with the highest number of cases reported in the United States. From 1974 to 2016, the United States reported 109 cases of *Balamuthia mandrillaris* infection, with the majority affecting male patients, aged 36 on average. The primary manifestation is encephalitis, and the mortality rate is as high as 90% [6]. A systematic review analyzed 27 cases of *Balamuthia mandrillaris* encephalitis reported in China from 2018 to 2022, with 25 resulting in death and only 2 surviving cases [9]. From 1977 to 2009, Brazil reported two cases of *Balamuthia mandrillaris* encephalitis, both resulting in death [10]. In 2024, Pakistan also reported its first confirmed case [11]. GAE is commonly observed in individuals with compromised immune function [12]. Risk factors include AIDS, cancer, liver disease, diabetes, immunosuppression, and alcohol abuse [13]. Recently, cases of infection in individuals with normal immune function have also been reported [14,15].

This patient has a 15-year history of diabetes mellitus with poor glycemic control, a significant risk factor for the disease. Apart from a few cases with clear histories of floodwater, contaminated water,

or soil exposure, most patients cannot recall when or how they were infected [1]. As a farmer, this patient has frequent soil contact during fieldwork, a possible infection source. However, most reported Chinese cases are in southern regions, whereas this patient resides in northern China, making the case even more unusual.

There are only two species within the genus *Balamuthia*, namely *B. mandrillaris* and *B. spinosa* [16]. *B. spinosa* was discovered in 2022, and its pathogenicity remains unclear, with no reported cases to date [17]. *B. mandrillaris* was first isolated and cultured in 1986 and has been detected in environmental samples (water, soil, dust), as well as in human and animal cases [17]. Cases identified so far are primarily concentrated in the southern United States and South American countries [6].

The clinical manifestations are nonspecific, with common symptoms including fever, headache, nausea, vomiting, altered mental behavior, seizures, cranial nerve palsies, and altered consciousness [18]. Microscopic detection of the pathogen remains the primary method for definitive diagnosis. Given the rarity of the disease, both laboratory technicians and clinicians have limited experience in diagnosing and treating *Balamuthia mandrillaris*, making timely diagnosis challenging [1]. Early diagnosis in the course of the disease may potentially increase the likelihood of patient survival [19].

In recent years, PCR-based molecular diagnostic methods have been increasingly applied to diagnosis [20], and PET-CT has been used to detect brain stem lesions [21]. With the widespread use of NGS in clinical settings, there has been a growing trend in the diagnosis of *Balamuthia mandrillaris* infections through cerebrospinal fluid NGS [14,15,21-26]. In this case, the patient was definitively diagnosed based on cerebrospinal fluid NGS on the 8th day after symptom onset. Early and timely diagnosis in the course of the disease provided valuable time for subsequent treatment, which was a crucial factor in the patient's survival.

Although many drugs have demonstrated in vitro efficacy against amoebae, the clinical options for treating GAE are limited by the permeability constraints of the Blood-Brain Barrier (BBB) [27]. In recent years, the use of nanocarrier drugs for GAE treatment has garnered attention [28]. In 2023, the United States reported a successful case treated with nitroxoline [29], offering hope for future treatments, although nitroxoline is still in the clinical research phase in China. Babesiosis in baboons often requires a combination of antibiotics and antifungal medications [1]. Additionally, considering the life cycle of *Babesia*, the treatment regimen should address both trophozoites and cysts.

Successful cases have utilized the following drugs: artesunate inhibits membrane glutathione transferase, itraconazole inhibits ergosterol biosynthesis, metronidazole inhibits nucleic acid synthesis, 5-fluorocytosine inhibits RNA and DNA synthesis, amphotericin B targets ergosterol, leading to disruption of cell membrane integrity, fluconazole inhibits ergosterol synthesis, pentamidine inhibits DNA, RNA, phospholipids, and protein synthesis, trimethoprim-sulfamethoxazole is a folic acid biosynthesis inhibitor, trifluoperazine blocks central dopamine receptors, azithromycin inhibits protein synthesis, albendazole inhibits microtubule polymerization, clarithromycin inhibits protein synthesis, mitofloxone inhibits

cytochrome c oxidase, ketoconazole inhibits ergosterol synthesis, and methotrimoprazine [30]. In this case, the patient was treated with a combination of four drugs with different mechanisms of action: albendazole, compound sulfamethoxazole tablets, flucytosine, and fluconazole. The patient's symptoms improved, and the lesions on the cranial MRI showed a reduction in size.

There is no standard treatment regimen for *Balamuthia* amebiasis, and the duration of treatment is not fixed. A 26-year-old Hispanic male was diagnosed with BAE and received a treatment regimen that included miltefosine, azithromycin, sulfamethoxazole/trimethoprim, flucytosine, fluconazole, sulfadiazine, metronidazole, voriconazole, clarithromycin, pentamidine, albendazole, dexamethasone, and amphotericin B [31]. Miltefosine treatment continued for an additional 3 weeks after the cessation of the treatment regimen, while azithromycin and fluconazole treatments continued for an additional 87 weeks, and trimethoprim-sulfamethoxazole treatment continued for 39 weeks. Two years later, MRI imaging showed no signs of disease [31].

An 80-year-old man achieved favorable outcomes with a six-drug antibiotic regimen (metformin, azithromycin, fluconazole, flucytosine, sulfadiazine, and albendazole) [19]. Following discharge, the patient continued oral treatment with four medications. Currently, the patient's symptoms have alleviated, and he is able to manage daily activities independently. This case remains under ongoing follow-up. Although complete cure has not yet been achieved, the prolonged remission and stability of the patient's condition are noteworthy and can serve as a reference for peers.

Author Statements

Conflict of Interest Statement

All authors declare that they have no conflict of interest regarding the present study.

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