Balamuthia mandrillaris and Acanthamoeba Amebic Encephalitis with Neurotoxoplasmosis Coinfection in a Patient with Advanced HIV Infection

Paula Pietrucha-Dilanchian, a Joseph C. Chan, b Amilcar Castellano-Sanchez, b Alicia Hirzel, b Panthipa Laowansiri, a Claudia Tuda, c Govinda S. Visvesvara, d Yvonne Qvarnstrom, e and Kenneth R. Ratzan f

Division of Infectious Diseases, Jackson Memorial Hospital, University of Miami, Miami, Florida, USA; a Division of Pathology, Mount Sinai Medical Center of Greater Miami, Miami Beach, Florida, USA; b Division of Infectious Disease, Mount Sinai Medical Center of Greater Miami, Miami Beach, Florida, USA; c Centers for Disease Control and Prevention, National Center for Emerging and Zoonotic Infectious Diseases Division of Foodborne, Waterborne & Environmental Diseases Waterborne Disease Prevention Branch; d and Centers for Disease Control and Prevention, Center for Global Health, Division of Parasitic Diseases and Malaria, Parasitic Diseases Branch, e Atlanta, Georgia, USA

We describe a patient with advanced HIV infection and Balamuthia mandrillaris and Acanthamoeba amebic encephalitis with Toxoplasma gondii coinfection. A multidisciplinary effort and state-of-the-art diagnostic techniques were required for diagnosis. Our patient is the first reported case of an HIV-infected person with dual Balamuthia mandrillaris and Acanthamoeba amebic encephalitis with neurotoxoplasmosis coinfection.

CASE REPORT

A 53-year-old Cuban man diagnosed with HIV in 2008 was brought to a local community hospital emergency room (ER) on 3 August 2010 with progressive weakness and confusion after falling at home. While in the ER, the patient had a generalized seizure which prompted further evaluation. A computed tomography (CT) brain scan with contrast revealed a single mass (2.3 by 2.4 cm) in the left parietal region with surrounding edema and mass effect on the left lateral ventricle. The patient was transferred to our hospital for further management.

The patient resided in an assisted living facility in Key West, FL, and was previously homeless. He used alcohol and tobacco daily, and he denied illicit drug use. He was admitted to an outside hospital in July 2009, where magnetic resonance imaging (MRI) showed a 2.5-cm ring-enhancing lesion in the right thalamus/parietal convexity region with surrounding edema not present in imaging conducted in August 2010. At that time, the patient received treatment with sulfadiazine and pyrimethamine, which he continued to take upon discharge until December 2009 for presumptive neurotoxoplasmosis. After December 2009 he subsequently took prophylactic trimethoprim-sulfamethoxazole up until the 2010 admission. However, his medication adherence was unknown, and he was not taking antiretroviral therapy (ART).

On examination the patient was afebrile, with stable vital signs. He was cachectic, alert, and oriented to person and place only, with no head trauma or nuchal rigidity. There were no skin lesions present. The remainder of the exam was unremarkable, with no obvious neurological deficits.

His white blood cell count was 7,120 cells/µl (86% neutrophils, 8% lymphocytes, 6.3% monocytes, 0% eosinophils). His hemoglobin was 11.6 g/dl, his hematocrit was 38%, and his platelets were 206,000/µl. His alkaline phosphatase was 179 IU/liter, with a normal total bilirubin, and his aspartate transaminase (AST) was 114 IU/liter. His toxoplasma IgG was positive at >5. His CD4 count was 25 T cells/µl, his CD4% was 4%, his CD4/CD8 ratio was 0.05, and his HIV-1 RNA was 74,900 copies. An MRI of the brain with gadolinium contrast showed a ring-enhancing lesion (2.3 by 2.3 by 2.4 cm) in the left parietal/occipital lobe with surrounding vasogenic edema and mass effect on the left ventricle (Fig. 1).

Intravenous dexamethasone was given for 6 days. The patient refused a brain biopsy, left against medical advice, and was discharged on sulfadiazine, pyrimethamine, and weekly azithromycin. Three weeks later, on 23 August 2010, he returned with right upper extremity weakness.

On 25 August 2010, the patient underwent a left parietal craniotomy, ultrasound guided biopsy, and subtotal removal of the lesion. An intraoperative cytologic exam revealed necrosis and reactive astrocytes. Additional samples showed necrotic tissue with a focal infiltrate of lymphoid and plasma cells. Deeper sections with Giemsa stain showed rare organisms consistent with Toxoplasma bradyzoites and vacuolated structures resembling amebae (Fig. 2).

Stains for acid-fast bacilli (AFB), bacteria, and fungi were unrevealing, and cultures for AFB were negative, as was the DNA PCR for Mycobacterium tuberculosis. Brain biopsy and serum samples were sent to the Centers for Disease Control and Prevention (CDC) for further evaluation. The patient was started on tenofovir, emtricitabine, and efavirenz. The right-sided weakness slowly resolved, and the patient left prior to receiving confirmatory results from the CDC; therefore, he was not offered treatment other than for toxoplasmosis.

Indirect immunofluorescence antibody (IFA) for Balamuthia mandrillaris from three serum samples collected 5 days apart...
showed a titer of 1:64. Titers of 1/32 to 1/64 were seen in one out of three samples and 1:64 in two out of three samples for *Acanthamoeba* spp. Although examination of the hematoxylin and eosin (H&E)-stained slides did not reveal intact amebae, broken-down vacuolated cells reminiscent of amebic organisms were seen around blood vessels. These structures reacted heavily with both rabbit antisera (anti-*Acanthamoeba* and anti-*Balamuthia*). Additionally, DNA from the brain tissue tested positive for both *Balamuthia mandrillaris* in two independent real-time PCR assays (11, 19) and *Acanthamoeba* spp. in one real-time and one endpoint PCR assay (19, 21). However, brain tissue examination clearly identified *Toxoplasma gondii* (see Fig. 3), and PCR testing (12) performed at the CDC also confirmed the presence of *Toxoplasma gondii* in the brain lesion.

On 13 November 2010, the patient was readmitted to our hospital and was found to be increasingly lethargic and confused. He was taking sulfadiazine, pyrimethamine, and his ART continuously for 2 months after discharge. During this admission he was pancytopenic, with an absolute neutrophil count of 500/µl, a hemoglobin of 7.9 g/dl, a hematocrit of 22.6%, and a platelet count of 21,000/µl. In addition, his CD4 count was 21 T cells/µl and his HIV-1 RNA was 81,900 copies/ml.

A CT brain scan with contrast showed a ring-enhancing lesion (2.6 by 1.9 by 1.8 cm) with surrounding vasogenic edema in the left parietal lobe, with a slight increase in mass effect compared to the prior MRI. Fluconazole was added for treatment of central nervous system (CNS) *Balamuthia mandrillaris* infection. The patient died 1 month after discharge. An autopsy was not permitted.

*Acanthamoeba* spp., *Naegleria fowleri*, and *Balamuthia mandrillaris* are pathogenic free-living amebae that cause human disease (17, 25). *Balamuthia mandrillaris* was first classified as a leptomyxid ameba when isolated from the brain of an infected pregnant mandrill in 1986 (26). Later, *Balamuthia mandrillaris*
was found to be phyllogenetically related to *Acanthamoeba* spp. and was moved from the Leptomyxidae family to the Acanthamoebidae family (18). The first confirmed human case of *Balamuthia mandrillaris* infection was reported in 1990; however, analyses of brain biopsy samples from patients with suspected *Acanthamoeba* sp. infections were found to be *Balamuthia mandrillaris* infections by retrospective indirect immunofluorescence (IIF) assays, with the earliest case seen in 1975 (26).

Primary amebic meningoencephalitis (PAM) is an acute, often fatal disease caused by *Naegleria fowleri* seen in young, healthy people with recent exposure to warm freshwater while participating in recreational activities. *Acanthamoeba* spp. and *Balamuthia mandrillaris* both cause chronic granulomatous amebic encephalitis (GAE), which progresses less rapidly than PAM. *Acanthamoeba* spp. infections are seen mostly in immunocompromised people, including those with HIV, diabetes, systemic lupus erythematosus, and malignancies (14), while GAE from *Balamuthia mandrillaris* is seen in both immunocompetent and immunocompromised people (5, 18, 26). An estimated 400 cases of *Acanthamoeba* spp. and *Balamuthia mandrillaris* GAE have occurred worldwide (14, 18, 22). Most recently, *Balamuthia mandrillaris* GAE has been reported in solid organ transplant recipients from two separate donors (3, 4).

Both *Acanthamoeba* spp. and *Balamuthia mandrillaris* infections are associated with soil exposure from occupational and recreational sources, in addition to freshwater sources, and have the potential of causing disseminated disease (7, 14, 16, 22). Both amebae are commonly associated with cutaneous lesions, especially in HIV patients with *Acanthamoeba* infections, and are seen preceding GAE (4, 14, 16). Imaging shows unifocal or multifocal ring-enhancing lesions throughout the brain, including the cerebellum, cerebral hemispheres, pons, and brain stem, with hydrocephalus or edema (8, 9).

Microscopic examination of brain tissue shows mostly a granulomatous reaction surrounding cortical necrotic areas (14, 20). Hematoxylin-eosin-stained (HES) tissue shows trophozoites and cysts for both species that are indistinguishable from each other. Additionally, cysts may be visualized with Gomori methenamine-silver or periodic acid-Schiff (PAS) stains (9, 10). The trophozoites tend to be angiotropic, demonstrating perivascular accumulation and vessel invasion within tissue (9).

It is not possible to morphologically distinguish *Acanthamoeba* spp. from *Balamuthia mandrillaris*; therefore, the diagnosis must be confirmed with indirect immunofluorescence techniques and PCR (9, 10, 22). Serum IFA identifies exposure to the amebae, and confirmation by PCR is being used more frequently, as with our patient (13). *Acanthamoeba* spp. can be easily cultured on bacteria-coated nonnutrient agar plates. *Balamuthia mandrillaris*, however, is cultured on mammalian cell cultures (10, 26).

Although there are more than 30 cases of *Acanthamoeba* and HIV coinfections reported in the literature (14), our case is one of the few reported cases of *Balamuthia mandrillaris* GAE in an HIV-infected person. The first case was reported in 1988, occurring in a 26-year-old man who presented with CNS disease and multiple skin abscesses and had disseminated disease involving the adrenal glands and kidneys (2). Another case reported in 1998 presented with GAE and disseminated disease involving the adrenal glands, kidneys, and thyroid (24). Both of these patients died soon after their initial presentation. We present the first reported case of *Toxoplasma gondii*, *Balamuthia mandrillaris*, and *Acanthamoeba* spp. triple infection.

To our knowledge, there are only a few reported cases of patients, including immunocompromised patients and those with HIV infection, surviving GAE due to *Acanthamoeba* and *Balamuthia mandrillaris* (1, 3, 4, 5, 6, 7, 10, 14, 15, 16, 25). Although there is no accepted treatment regimen for *Acanthamoeba* spp. and *Balamuthia mandrillaris* GAE, pentamidine, fluconazole, sulfadiazine, flucytosine, and the macrolides have been used in various combinations, in addition to miltefosine and thiouridazole. Interestingly, our patient did not have a progressive disease, according to imaging, despite receiving glucocorticoids, which have been associated with a more rapid progression of disease (23).

This challenging case required a multidisciplinary effort among infectious disease, microbiology, and pathology experts. Realizing the limitations of our laboratory, we solicited the expertise of the CDC, which discovered three etiological agents using state-of-the-art diagnostic techniques. This report, and others describing GAE from *Balamuthia mandrillaris* and *Acanthamoeba* sp. infections, should make clinicians more aware of this lethal disease and diagnostic options. Also, further investigation of a presumptive diagnosis, such as neurotoxoplasmosis, that does not respond to conventional therapy should prompt the physician to pursue additional diagnostic interventions.
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REFERENCES