SEPTEMBER 2004: A 6-YEAR-OLD GIRL WITH HEADACHE AND STIFF NECK

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Clinical history, radiology and microscopic description. A previously healthy 6-year-old girl from the southern Vermont presented to the emergency department with gradually worsening headache, stiff neck, nausea and vomiting, and low-grade fever for several days in the early October. There was no history of trauma, chills, night sweating or upper respiratory tract infection. Physical and neurological examinations as well as routine laboratory tests and chest radiograph were within normal limits. Blood culture for microorganisms was negative. A head MRI with contrast demonstrated a 2.0-cm ring-enhancing lesion in the right parietotemporal region, consistent with abscess formation (Figure 1; MRI with contrast). The CSF analysis showed WBC at 43/mm^3 with 96% of lymphocytes, RBC at 1/mm^3, glucose at 52 mg/dl and protein at 37 mg/dl. CSF Gram stain and microorganism culture were negative. The patient was treated empirically with Acyclovir, Oxacillin and Flagyl. The abscess appeared to have responded to the therapy based on a repeat imaging study, although the patient's symptoms were not relieved. A brain biopsy revealed tissue necrosis, acute inflammatory infiltrate as well as granulomatous response (Figure 2A; arrows indicating multinucleated giant cells). The stains for bacteria, fungi, acid-fast bacilli, herpes virus, cytomegalovirus and toxoplasma were all negative. On the follow up CT and MRI, multiple cystic lesions were noted in both cerebral hemispheres. The second brain biopsy was performed showing multiple foci of microorganisms arranged mostly in perivascular spaces (Figure 2B). Occasional binucleolus form was observed (Figure 3A, B; original magnification ×100). Some of these microorganisms were in cystic form with an outer wall of either smooth or wrinkled contour best demonstrated on Trichrome or PAS stains (Figure 4A, B: Trichrome, and Figure 4C: PAS. Original magnification ×100). An immunofluorescence antibody stain was performed for further classification of the microorganisms. The therapy was then switched to pentamidine, sulfadiazine, intraconazole and azithromycin; however, the patient's condition kept deteriorating and she died in the late-January, 75 days after admission. The blood and brain tissue cultures remained negative. At autopsy, the brain sections showed edema, necrosis, tremendous reactive astrogliosis, acute and chronic inflammation and foci of multinucleated giant cell reaction. Scant eosinophils were also present. Partially degenerated microorganisms were focally abundant (not shown).

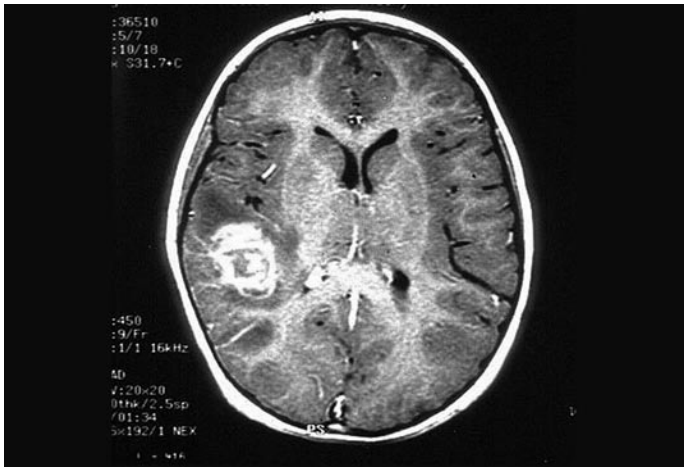

Figure 1.

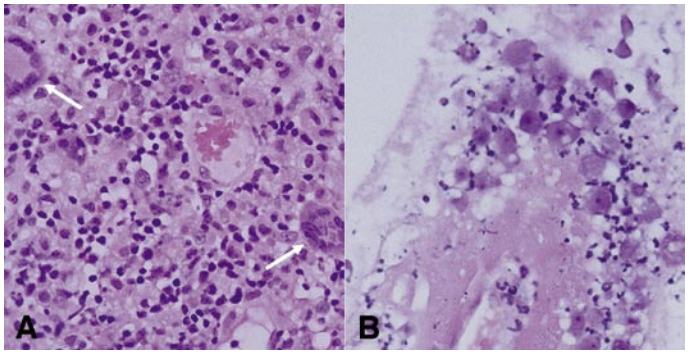

Figure 2.

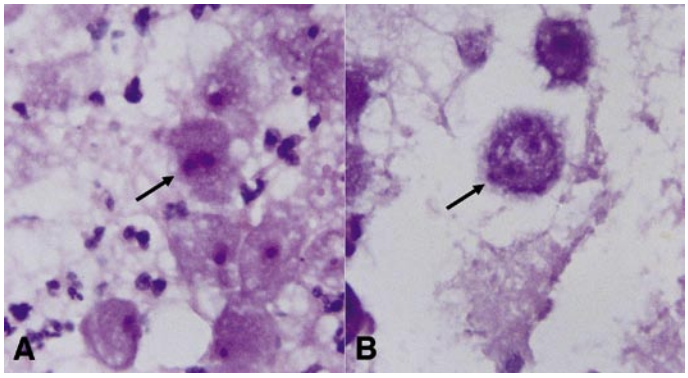

Figure 3.

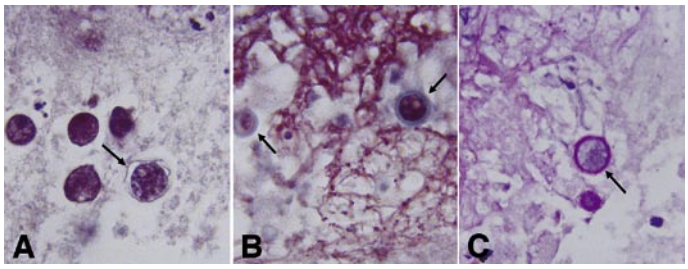

Figure 4.

Diagnosis. Granulomatous amebic meningoencephalitis caused by Balamuthia mandrillaris.

Discussion. *Balamuthia mandrillars* is a relatively newly described pathogen related to the free-living ameba superfamily (4), which also includes *Naegleria fowleri* and *Acanthamoeba* species. These free-living amebas, except for *B. mandrillaris*, have been isolated from the soil, fresh water of swimming pool, and air conditioning unit (4, 6). They can cause infections of skin, respiratory tract and brain in both human and animals. The first few cases of human CNS infection due to free-living amebas were described in 1965 (1). Since then nearly 400 cases have been reported worldwide (3, 4). Although the route of invasion into the brain is still unclear, hematogenous spread of amebas through a skin lesion or the respiratory tract has been postulated. Individuals infected by amebas, especially *N. fowleri*, are usually with a recent history of fresh water-related activities. The patient in our report did have a history of swimming in a freshwater pond in her backyard during summer time.

N. fowleri causes primary amebic meningoencephalitis (PAM), which has an abrupt onset and a fulminate, rapid progression to coma and death (5). Both *Acanthamoeba* species and *B. mandrillaris* cause granulomatous amebic encephalitis (GAE), which has a subacute to chronic course with an almost invariably fatal outcome (1, 4). Since its first isolation from the brain of a mandrill baboon at the San Diego Wild Animal Park in 1990 (8), *B. mandrillaris* has caused human CNS infection in more than 85 cases worldwide, including 40 cases in United States (4).

No characteristic clinical symptoms, laboratory or neuroimaging findings have been found to be diagnostic of *B. mandrillaris* GAE. The infected patient may present with headache, stiff neck, low-grade fever, personality and mental status changes, and cranial nerve palsies mainly affecting the third and sixth cranial nerves. Cerebellar ataxia and diplopia have also been reported in some cases. The infection may mimic space-occupying lesions in CNS, and the infected patient may present with hemiparesis, aphasia or seizures. In such instances, head CT and MRI may reveal ring-enhancing lesion suggestive of a brain abscess or tumor. The CSF analysis may show lymphocytes, normal or slightly low glucose level, and normal or mild elevation of proteins. In most cases, the CSF study resembles that of aseptic meningitis. Clinically, the symptoms and laboratory data closely mimic and are often mistaken for a bacterial or viral encephalitis/leptomeningitis or tuberculous meningitis. The direct cause of death is usually acute bronchopneumonia, hepatic or renal failure, septicemia and brain edema leading to uncal and cerebellar tonsillar herniation (4, 8).

Histopathologically, the CNS infection is characterized by brain edema, hemorrhage and a subacute necrotizing meningoencephalitis with associated vasculitis and/or thrombosis. A modest inflammatory infiltration composed of lymphocytes, plasma cells and macrophages is often present. The lymphocytes may be the predominant inflammatory cells depending on the immunological status of the patient. Giant cells may also be present, but well-formed granulomas may not be a prominent feature. Like *Acanthamoeba* species, *B. mandrillaris* has a vegetative trophic stage and a dormant cyst stage in its life cycle, both of which can be seen in tissue section. The trophozoites measure 15 to 60 mm in diameter and have a round nucleus with a large nucleolus. The nuclei containing more than one nucleolus can be seen, which distinguishes *B. mandrillaris* from *Acanthamoeba*. The cysts of *B. Mandrillaris* are usually spherical and measure 6 to 30 µm in diameter, with a mean of 15 µm. They consist of an outer wrinkled wall (ectocyst) and an inner thin wall (endocyst). There is also a refractile granule layer beneath the inner cyst wall. Ultrastructurally, an additional thick, amorphous, fibrillar middle layer (mesocyst) can also be seen (4). Amebic trophozoites and cysts are usually present within perivascular spaces and within the necrotic CNS parenchyma.

The identification of the organism in tissue section is the key to make the diagnosis of amebic meningoencephalitis. The presence or absence of a cyst wall of trophozoites can be used to distinguish *Balamuthia* and *Acanthamoeba* from *N. fowleri* since the latter does not produce cyst forms in tissue. Although bi- and multi-nucleolus trophozoites are indicative of *Balamuthia*, the distinction between the *Balamuthia* and *Acanthamoeba* usually requires special techniques such as immunofluorescence antibody stain, as in our case. In infected brain specimen, marked necrosis due to tissue destruction either directly by ameba or indirectly by infarct secondary to vascular thrombosis can often mask the trophozoites, and the degenerated parasites can mimic macrophages, making detection challenging. Therefore, a high index of suspicion is needed in any brain infection with granulomatous feature. Retrospective review of the first biopsy identified several degenerated structures within necrotic background that were thought to be macrophages, but were in fact the degenerated trophozoites.

There is no specific treatment for *B. mandrillaris* infections (4, 8). Some in vitro studies demonstrated that *B. mandrillaris* is sensitive to pentamidine methionate, azithromycin and clarithromycin, but none are amebicidal at nontoxic concentrations (2, 7). In fact, the treatment for CNS amebic infection is in general non-specific and late, mainly due to difficulty of diagnosis.

Although *Balamuthia* encephalitis is rare, human infection resulting from *B. mandrillaris* has increased significantly (4). It should be considered in the differential diagnosis in a patient with subacute or chronic granulomatous meningoencephalitis, even if CSF glucose or protein concentration suggests a bacterial or viral pathogen, or when the patient has poor response to the antibiotic therapy.

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CASE OF THE MONTH: ABSTRACT

July 2004. **A 40-year-old man had a 6-week history of severe frontal headaches and dry cough. Chest x-ray showed hilar adenopathy with bilateral parenchymal infiltrates. A diagnosis of atypical pneumonia was made. Four weeks later he was admitted with persistent headache. Infectious screen was negative. Brain MR post contrast, revealed cerebellar enhancement and swelling with moderate tonsillar herniation; findings which precluded the performance of a lumbar puncture. High resolution CT thorax confirmed hilar abnormalities; shown by microscopy to represent non caseating granulomata. A presumptive diagnosis of sarcoidosis was reached. Despite an initial symptomatic improvement his headache persisted. Repeat MRI, eleven days after admission, showed reduced cerebellar enhancement and swelling with no change in the degree of tonsillar herniation. He deteriorated acutely and died two weeks after admission. Autopsy revealed cerebral oedema with tonsillar herniation secondary to cryptococcal meningitis variety neoformans. There was no evidence of neurosarcoid. Active and inactive sarcoid was identified in the lungs and hilar nodes with no evidence of systemic sarcoid. Focal evidence of cryptococcal pneumonitis was present in the lung as a necrotic focus. A strong index of clinical suspicion is necessary to diagnose the rare association of cryptococcus complicating sarcoidosis.**

August 2004. **A 64-year-old-man had a 2-year history beginning with a sense of abdominal "constriction." Additional slowly rising symptoms, such as tingling of the legs, mild gait ataxia and painful micturition, led to MRI investigation of the spinal cord. A fusiform enlargement of the cord extending from T5 to T8 was shown. The space occupying lesion infiltrated diffusely the spinal cord. A contrast medium enhancing exophytic tumor pellet was approached via a 2-level laminoplasty and resected. Biopsies were taken from different exophytic tumor areas whereas the intramedullary part was spared. The histologic examination confirmed the typical pattern of a pilocytic astrocytoma in all specimens. In our surgical experience with 226 intramedullary tumors and with 117 patients affected by intracranial pilocytic astrocytoma this case is unique because of its combination of tumor location, growth pattern and age of the patient.**

September 2004. **Free-living amebas in the genera *Naegleria*, *Acanthamoeba* and *Balamuthia* are known to cause CNS infections. Here we report a case of fatal granulomatous amebic meningoencephalitis (GAE) caused by Balamuthia mandrillaris in a 6-year-old previously healthy girl who presented with headache and stiff neck. She was treated medically for brain abscess after a CT scan identified a ring-enhancing lesion in the right temporo-parietal area. A brain biopsy showed necrosis and granulomatous inflammation. Subsequently, multiple new lesions appeared in the brain bilaterally. A second brain biopsy revealed viable amebic trophozoites that were most abundant in perivascular spaces, accompanied by neutrophils, macrophages and eosinophils. Immunofluorescence study confirmed the amoeba as Balamuthia mandrillaris. This case demonstrates that making diagnosis of GAE pre-mortem requires a high index of suspicion. Amebic infection should be included in the differential diagnosis of any granulomatous lesion in CNS; and careful search for amebic parasites should be carried out especially when necrosis predominates in the pathological material.**

For a more complete discussion of these cases, additional micrographs, and information regarding submission of cases, please access the *Brain Pathology* web site at *http://www.brainpathology.com*. We welcome comments about these or similar cases our readers may have encountered.