

Primary Amoebic Meningoencephalitis

Franklin S. Fuda, DO, Ellen Sigauke, MD, Dominick Cavuoti, DO, Rita Gander, PhD, Steven Kroft, MD, Monte S. Willis, MD, PhD

Department of Pathology, University of Texas Southwestern Medical School, Dallas, TX

- ▶ Etiology of Primary Amoebic Meningoencephalitis (PAM)
- ▶ Laboratory diagnosis of PAM
- ▶ The need for early detection of PAM
- ▶ Differentiating *Naegleria fowleri* from *Acanthamoeba* species
- ▶ Treatment and Prognosis

Case Presentation

A 9-year-old female with an unremarkable past medical history presented to a Tyler, Texas emergency department with a 7-day history of worsening frontal headache and bilateral lower extremity “aches.” During the previous 4 days she had also experienced a cough, nasal congestion, and rhinorrhea. Her condition rapidly progressed to include an increasingly severe headache, photophobia, non-bloody emesis, neck pain, fever (103.6°F), irritability, and disorientation along with episodes of verbal outbursts, purposeless movements, and eye rolling.

The patient underwent a lumbar puncture, and cerebral spinal fluid was analyzed with the following results: glu-

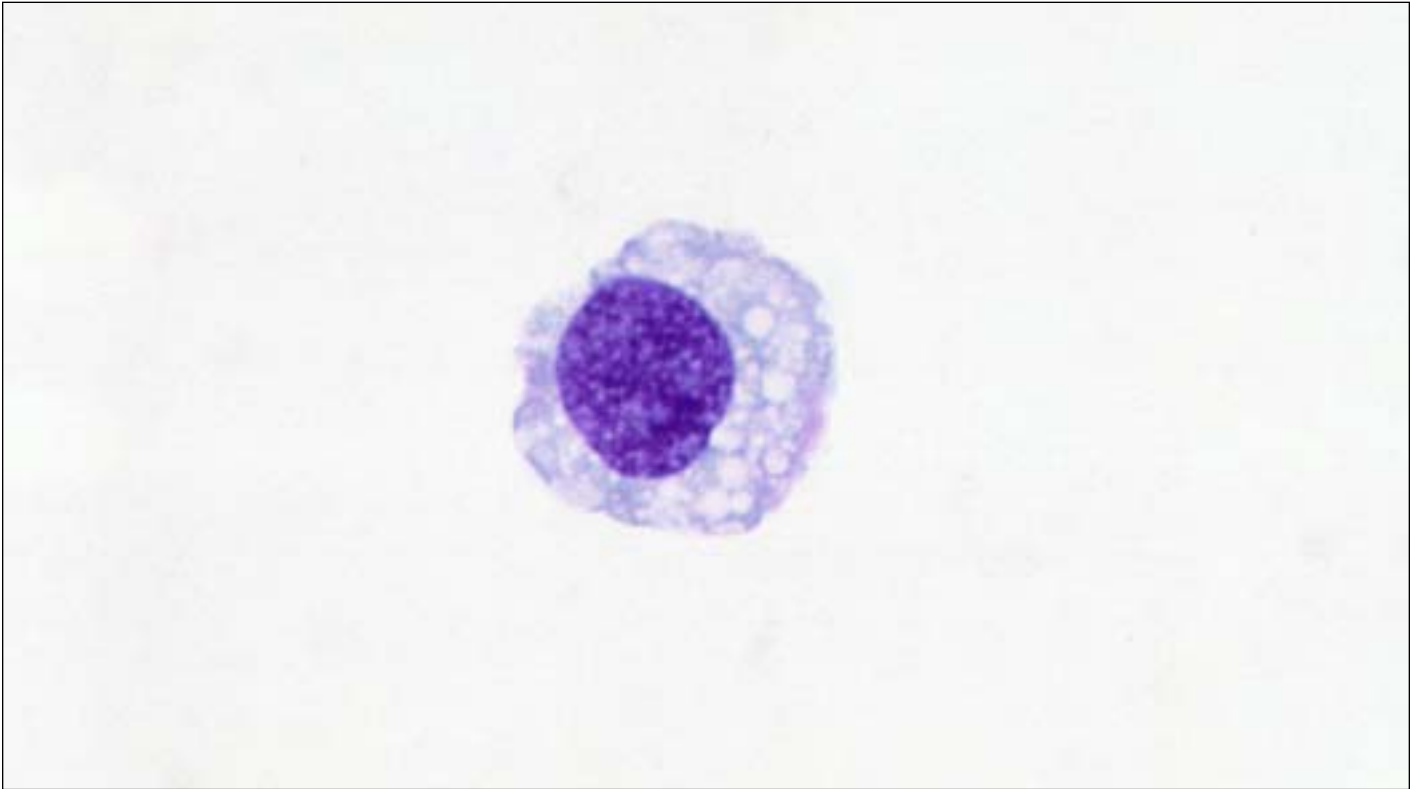
cose 71 mg/dL (Normal 40-70 mg/dL), protein 148 mg/dL (Normal 15-45 mg/dL), nucleated cells 1080 cell/μL consisting of 69% neutrophils, 25% lymphocytes, and 6% monocytes (Normal up to 20 lymphocytes/μL in children), red blood cells 126 cells/μL (Normal no RBCs).

A presumed diagnosis of bacterial meningitis was made and ceftriaxone was started. The patient was transferred to Children’s Medical Center of Dallas where her medical condition continued to deteriorate. A head-computed tomography was performed and revealed no abnormalities. Upon further questioning it was discovered that the patient had been swimming in an eastern Texas lake shortly before the onset of symptoms. This increased the suspicion for primary amoebic meningoencephalitis (PAM). Empiric intrathecal amphotericin B and systemic rifampin was initiated. Subsequent wet mount analysis of the CSF revealed *Naegleria fowleri* trophozoites, the etiologic agent of PAM. The patient’s condition continued to worsen and she died several days later.

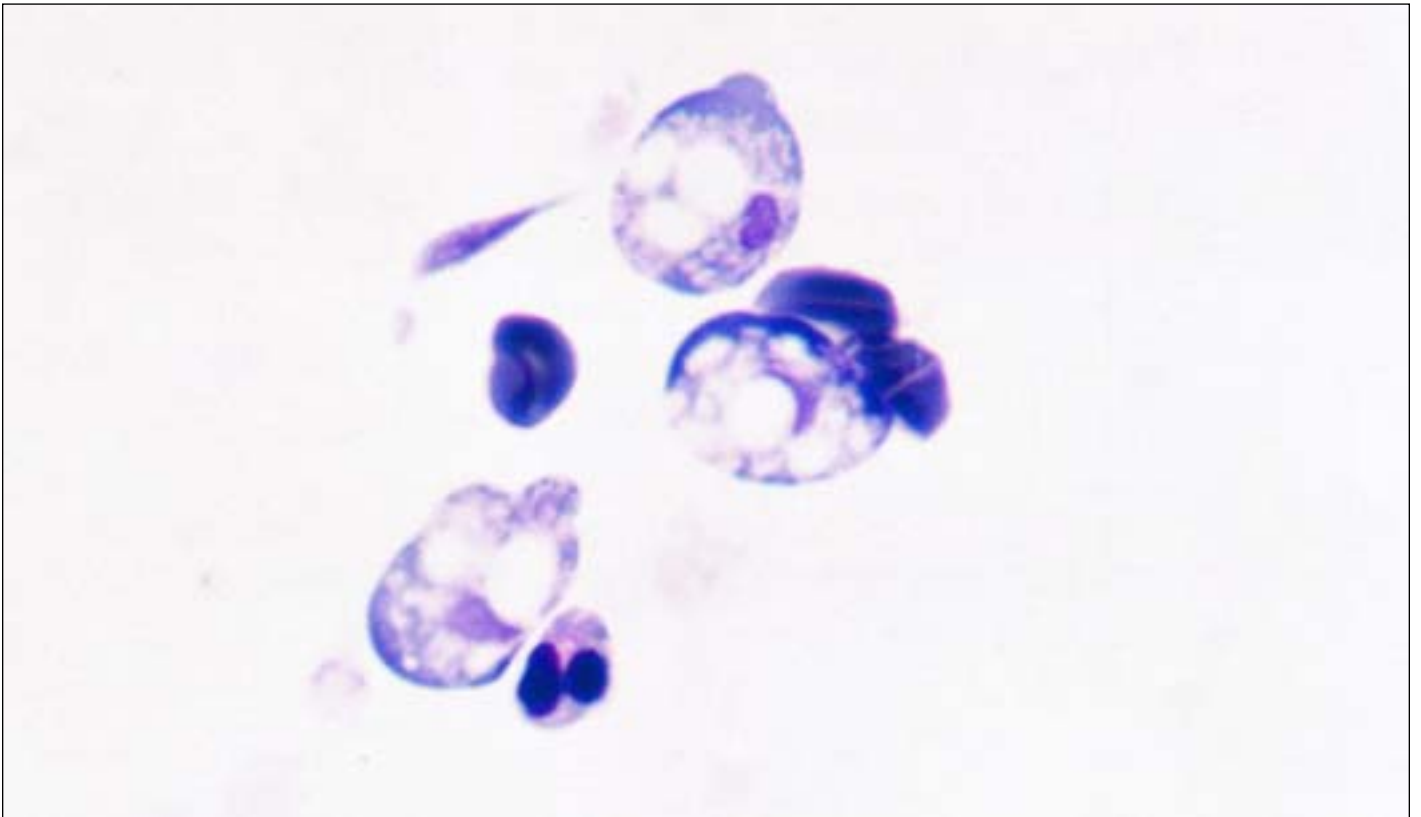
Clinical Background Etiology of PAM

Primary amoebic meningoencephalitis is a rapidly progressive disease that generally results in death within 7 to 10 days of symptom onset. The etiologic agent is the free-living amoeba *Naegleria fowleri*. Infection occurs most commonly in young, healthy individuals following exposure to contaminated water sources. Inoculation occurs when contaminated water is forced into the nasal cavity. Access to the CNS is achieved by invasion into the olfactory neuroepithelium with subsequent penetration of the cribriform plate. Once in the CNS, the organisms colonize cerebral gray matter leading to a rapidly fatal meningoencephalitis.

The genus *Naegleria* contains several species of which *N fowleri* is the only known human pathogen. These protozoa are found ubiquitously in the environment and are distributed worldwide. They can be found in warm, stagnant bodies of water, and even chlorinated swimming pools, making contact with humans inevitable.



[1] Monocyte in CSF (100x magnification). Note the uniform and slightly ropey chromatin pattern of the nucleus as well as the higher nuclear to cytoplasmic ratio relative to that of the amoeba shown in [2].



[2] Naegleria fowleri trophozoites in CSF (Wright-Giemsa stained; 100x magnification).

The progression to a disease state, however, is rare with a total of 179 cases reported worldwide through 1997.³ The protective factors that prevent infection by *N fowleri* have not yet been elucidated, but serologic surveys of healthy adults indicate that many adults have been exposed to *N fowleri* without having PAM.¹

Naegleria fowleri exist in trophozoite, flagellate, and cyst forms, of which the former two are the most common. They can survive inclement conditions in the cyst form for up to 8 months.⁹ *N fowleri* are most numerous in water exceeding 80°F.¹⁷ Although the cyst form can withstand lower temperatures, they survive only 5 minutes with desiccation.² This reliance upon an aqueous environment for survival explains the strong association of *N fowleri* infection and activity in warm water lakes, ponds, swimming pools, and springs. Most cases of *N fowleri* occur in the southeastern United States and Australia after swimming or water-skiing.

Pathogenesis and Symptomatology of PAM

In vitro and animal studies suggest several mechanisms for damage once penetration of the CNS is achieved. *N fowleri* produce and release several degradation enzymes that potentially lead to demyelination and/or lysis of the neuronal cells.⁴⁻⁷ Additionally, they possess amoebastomes that have been shown to phagocytize neurons in a “piecemeal fashion.”^{7,8} Lastly, the amoebae harbor pathogenic bacteria in a symbiotic fashion, which potentially plays a role in nerve cell destruction.¹⁰

Gross cerebral edema with uncal or cerebellar herniation as well as diffusely hyperemic and scantily purulent meninges can be seen in the brains of patients with PAM.^{1,2} Microscopic examination reveals trophozoites, mononuclear cells, and polymorphonuclear cells in focal areas of necrosis and hemorrhage.¹ The olfactory bulbs, perivascular spaces of small to mid-sized arteries, and the cerebral gray matter of the frontal and temporal lobe bases are most frequently affected. Outside of the CNS there appears to be a sterile myocarditis associated with approximately 50% of autopsied cases.¹

Typically, infection with *N fowleri* has a 3 to 7 day incubation period. An inflammatory response to invasion of the neuroepithelium leads to a change in taste and smell followed by rhinitis, mild fever, and malaise.¹ These signs and symptoms rapidly progress to a marked fever accompanied by severe headache, vomiting, and neck rigidity. By the third day, severe disorientation, seizures, and hallucinations may occur with eventual progression to coma and death within 7 to 10 days of symptom onset.

Laboratory Diagnosis of PAM

Due to the rapidity of disease progression, early diagnosis is imperative. This is often difficult because PAM is rare and closely resembles meningitis of other etiologies. In PAM, the peripheral white blood cell count is usually elevated with a neutrophilia. A CSF analysis reveals an elevated pressure, a high neutrophil count, a high red blood cell count, an increased protein level, and a low to normal glucose level. Such findings cannot definitively distinguish PAM from bacterial or certain viral meningitis. Therefore, diagnosis needs to be made by direct visualization of the amoebae in a spinal fluid wet mount.

Naegleria fowleri trophozoites vary between 7 to 15 µm in size when observed in a CSF wet mount. Classically, trophozoite nuclei appear to lack chromatin, are clear with thin membranes, and have a single large, rounded, refringent nucleolus.² Numerous cytoplasmic vacuoles can be seen aggregated around each organism’s nucleus, and pseudopodia may be identified projecting away from the cells.^{1,2} Distinguishing the features of amoebic nuclei from those of monocytes is particularly important in diagnosing PAM, as the two cell types can be confused. Monocyte nuclei are significantly larger with higher nuclear to cytoplasmic ratios than those of amoebae. Furthermore, monocyte nuclei do not appear clear but rather have a uniform and slightly ropey chromatin pattern without distinct nucleoli [1].

Staining specimens with Giemsa or Trichrome stains can enhance *N fowleri* trophozoite features after cytocentrifugation [12].² Gram staining, on the other hand, destroys the amoebae making it

useless in diagnosing *N fowleri*.² Time constraints require that the organisms be identified as early as possible. The increased sensitivity of staining, therefore, does not preclude the necessity to investigate a wet mount preparation. Lowering the microscope condenser may help in identifying trophozoites in unstained wet mounts.² Resuspending centrifuged samples in water may also reveal flagellate forms, which is an easier stage to identify.²

In addition to appearing morphologically similar to monocytes, *N fowleri* can be mistaken for other amoebae rarely found in the CSF. *Acanthamoeba* species, for example, cause a granulomatous amoebic encephalitis and can be identified on CSF wet mounts on rare occasions.² The distinguishing morphological features of *Acanthamoeba* species, including acanthopods (small, filamentous cytoplasmic projections), size (12 to 60 µm), and the lack of a flagellate stage, differ from *N fowleri* but are often too subtle to recognize. Therefore, morphology should be used in conjunction with clinical correlation to make a tentative diagnosis. Clinically, granulomatous amoebic encephalitis occurs primarily in immunocompromised individuals, is not associated with water activities, has an insidious onset with a relatively long duration of CNS disease before death (average 39 days), and presents with focal neurological deficits.¹ This clinical course differs dramatically from that of PAM and should be sufficient evidence to differentiate the two diseases.

Definitive diagnosis is usually determined upon autopsy. The typical postmortem and histological findings in PAM were discussed earlier. *Naegleria fowleri* trophozoites exhibit the same morphological features in tissue as in CSF wet mounts but are usually smaller (7 to 9 µm).² Unlike PAM, granulomatous amoebic encephalitis shows extensive cerebral necrosis with granuloma formation around trophozoites in early stages and cysts in later stages.² Further specificity for diagnosis can be achieved through molecular and immunologic methods (polymerase chain reaction, restriction fragment length polymorphism, isoelectric focusing, and monoclonal antibody testing).^{10,18}

Treatment of PAM

After identifying amoebae on CSF wet mount, treatment must be initiated immediately in patients suspected of having PAM. Successful treatments have included high dose administration of systemic and intrathecal Amphotericin B with or without miconazole, rifampin, and sulfisoxazole.¹ Other agents, currently under investigation, include the use of antibodies as an adjunct to antimicrobials.¹¹ Despite treatment efforts, the mortality of PAM remains high (95%), and only 6 survivors have been documented.¹²⁻¹⁶ In each of these cases, treatment was instituted early in the disease course emphasizing the importance of early diagnosis.

Conclusion

Primary amoebic meningoencephalitis is a rare, rapidly fatal disease of children and young adults. Early diagnosis and treatment increase the chance for survival, so particular attention to a recent history of swimming and/or water exposure should be made in determining the differential diagnoses. Since the survival rate is so low (5%), prevention is also important. Warm,

stagnant, polluted waters and swimming pools not properly chlorinated (1 to 2 ppm) should be avoided. Nose plugs may be of some benefit particularly while diving, water-skiing, or participating in any activity that could force contaminated water into the nasal cavity. Although detection methods are available for *N fowleri* in the environment, the low prevalence of disease progression as well as its ubiquitous distribution probably precludes routine surveillance as a public health measure.¹

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