Inability To Make a Premortem Diagnosis of *Acanthamoeba* Species Infection in a Patient with Fatal Granulomatous Amebic Encephalitis

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Granulomatous amebic encephalitis (GAE), an infection of immunocompromised hosts, is almost uniformly fatal. A case of GAE in a patient who failed to mount a serologic response to *Acanthamoeba polyphaga* is presented. Although *Acanthamoeba polyphaga* that is sensitive to multiple antimicrobials grew from brain tissue, an inability to make a premortem diagnosis precluded therapy.

**CASE REPORT**

A 70-year-old female presented for medical attention with lethargy and seizures. Symptoms began 36 days earlier when she was hospitalized with syncope. An evaluation at that time included magnetic resonance imaging (MRI), revealing a focal area of edema in the right temporal lobe consistent with an acute infarction, and she was discharged to a rehabilitation facility with a diagnosis of cerebrovascular accident. She was readmitted with worsening ataxia, lethargy, and generalized seizures. Her past medical history included chronic neutropenia secondary to myelodysplastic syndrome, hypogammaglobulinemia treated with monthly intravenous gamma globulin, asplenia, steroid-dependent discoid lupus (prednisone, 20 mg twice a day), and diabetes mellitus.

On exam, the patient was afebrile and hemodynamically stable. An ocular exam did not reveal papilledema. Her neck was supple. She was oriented only to person. A neurological examination was otherwise nonfocal. Laboratory studies included a platelet count of 355 × 10⁹/liter, a hematocrit of 32.8%, and a white blood cell count of 2.8 × 10⁹/liter, which was unchanged from her baseline leukopenia. A lumbar puncture (Table 1) was significant for lymphocytic pleocytosis and a neutrophilic infiltrate in the leptomeninges, but no amebae or granulomas, and negative bacterial, fungal, and mycologic cultures.

The patient’s hospital course was significant for progressive obtundation, requiring intubation for airway protection. The results of repeat lumbar punctures are provided in Table 1. Serial MRI revealed persistent abnormal signal intensity in the temporal lobes, with new areas of uptake in the right basal ganglia, pons, and left occiput. Extensive diagnostic evaluation was unrevealing except for a stable elevation in titers of antibodies to *Mycoplasma pneumoniae* (1.425 in the acute phase and 1.645 in the convalescent phase) and *Ehrlichia chaffeensis* (1:128 in the acute phase and 1:128 in the convalescent phase) and evidence of prior Epstein-Barr virus infection (viral capsid antigen immunoglobulin G titer of >10, viral capsid antigen immunoglobulin M titer of <10, and EBNA titer of >10).

Despite empirical treatment with acyclovir, decadron, and intravenous gamma globulin, the patient was transferred to our facility with a diagnosis of cerebrovascular accident. She was admitted with an acute infarction, and she was discharged to a rehabilitation facility with a diagnosis of cerebrovascular accident.

Granulomatous amebic encephalitis (GAE), an infection of immunocompromised hosts, is almost uniformly fatal. A case of GAE in a patient who failed to mount a serologic response to *Acanthamoeba polyphaga* is presented. Although *Acanthamoeba polyphaga* that is sensitive to multiple antimicrobials grew from brain tissue, an inability to make a premortem diagnosis precluded therapy.

**TABLE 1. Cerebrospinal fluid parameters**

| Day after initial presentation | WBC/mm³ | RBC/mm³ | Differential (%) | Glucose (mg/dl) | Protein (mg/dl) |
|------------------------------|---------|---------|-----------------|----------------|----------------|
| 38                           | 25      | 1       | 10              | 90             | 19             | 86           |
| 48                           | 27      | 0       | 19              | 79             | 2              | 14           | 88           |
| 58                           | 48      | 115     | 89              | 9              | 124            |
| 65                           | 26      | 0       | 89              | 7              | 166            |

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from the patient were applied to the amebae in dilutions of 1:2 to 1:4,096 as previously described (35). The wells were then treated with goat antihuman fluorescein isothiocyanate serum conjugate, followed by washing, mounting, and observation with a fluorescence microscope. Serum obtained 48 days after the patient's initial presentation yielded antibody titers of 1:16 to \( A. \text{castellanii} \), 1:8 to \( A. \text{polyphaga} \), and 1:16 to \( A. \text{culbertsoni} \). A subsequent sample, obtained 64 days after the patient's presentation, yielded an antibody titer of 1:32 to \( A. \text{castellanii} \). Serum from an asymptomatic control run in parallel with the paired specimens demonstrated a titer of antibody to \( A. \text{cas-tellani} \) of 1:32.

Amebae were isolated from brain tissue obtained at autopsy. A sample of brain tissue was macerated in sterile phosphate-buffered saline, and the suspension was applied to the surface of a nonnutrient agar petri plate that had been streaked with a suspension of \( \text{Escherichia coli} \). After 2 days of incubation at 30°C, inverted microscopy revealed the presence of amebae feeding on the bacteria and moving over the agar surface, ultimately undergoing encystment to produce thick-walled cysts. Bacterium-free (axenic) cultures of trophic amebae were subsequently established by transfer to proteose-peptone-yeast extract-glucose medium supplemented with fetal calf serum and a vitamin supplement at 30°C (22). Penicillin-streptomycin was added to kill any bacteria that were carried forward into the axenic medium. The isolate was identified as \( Acanthamoeba \text{polyphaga} \) based on the cyst morphology. When tested for growth at different temperatures (30°C, 33°C, and 37°C), the amebae grew best at 30°C. Similar attempts to isolate the ameba from cerebrospinal fluid obtained on day 65 after the patient’s presentation, both in the presence of bacteria and into axenic proteose-peptone-yeast extract-glucose medium, were unsuccessful.

Cultured amebae were tested for sensitivity to the following antimicrobial agents at 1, 5, and 10 \( \mu \text{g/ml} \): amphotericin B, azithromycin, clarithromycin, fluconazole, flucytosine, pentamidine isethionate, and sulfadiazine. Sensitivity was determined by the growth or the absence of growth of amebae on monkey kidney cells (23). Ameba growth was strongly inhibited by fluconazole (all concentrations), azithromycin (all concentrations), and pentamidine (all concentrations) and less so by amphotericin B (5 and 10 \( \mu \text{g/ml} \) only). No inhibition was seen with clarithromycin, flucytosine, and sulfadiazine.

\( Acanthamoeba \) spp. are ubiquitous in the environment and are highly tolerant of a wide range of growth conditions from sea to tap waters, tropical to arctic soils, aquatic waste dump sites, and cooling towers of air conditioning systems. In the home, they can be recovered from humidifiers, aquariums, biofilms in sink drains and water faucets, and soil in potted plants. Amebae have been isolated from the nasal mucosa of various groups of healthy individuals, including military recruits, students, and children, suggesting that while colonization and subclinical infections are relatively common, invasive disease is, fortunately, a rarity (3, 12, 14).

Granulomatous amebic encephalitis (GAE) presents as a subacute but progressive meningoencephalitis that is almost universally fatal (15). While there are reports of infections in immunocompetent individuals (27, 28), the majority of cases of GAE have occurred in immunocompromised hosts. Case reports of GAE in patients with human immunodeficiency virus/AIDS (18, 26, 33) and patients who have undergone organ transplantation (2, 16, 29, 30) likely reflect an increased incidence of GAE due to a larger population of susceptible individuals. The preponderance of GAE among patients with impaired T-cell immunity, coupled with experimental data showing T-lymphocyte proliferation among healthy volunteers exposed to \( Acanthamoeba \) antigens (32), implicates deficits in cell-mediated immunity as an important risk factor for GAE. The patient in this report had impaired T-cell immunity based on chronic steroid use for systemic lupus erythematosus, which has been reported in previous fatal cases of GAE (9, 10, 20).

Diagnosis of amebic meningoencephalitis is typically made by recognition of trophozoites and cysts on examination of brain tissue. In this case, a stereotactic biopsy performed pre-mortem was nondiagnostic. Granuloma formation, the patho-
logical hallmark of GAE, may be absent or diminished in immunocompromised individuals (13, 30). False-negative biopsy results have previously been reported due to sampling error (19), failure to recognize amebae on the initial review (8, 30), or misidentification of the organisms as reactive histiocytes (25) or yeasts (31). Because the pathological diagnosis of acanthamoebiasis may be elusive, particularly if limited specimens are obtained, alternative diagnostic methods are needed.

Low-level antibody titers to Acanthamoeba spp. are found in 50 to 100% of asymptomatic individuals, suggesting that occult infection is common (4, 5, 7). Significant elevations in titers have been reported among patients with acanthamoebic keratitis (1) and Acanthamoeba meningoencephalitis (9), raising the possibility that serology may provide a noninvasive method for early diagnosis of GAE in a clinically compatible case. Positive antibody titers in Acanthamoeba GAE are typically 1:128 and higher (G. S. Visvesvara, personal communication). In this study, testing for serum antibodies using three different species of Acanthamoeba gave consistently low titers (A. castellani, 1:16; A. polyphaga, 1:8; and A. culbertsoni, 1:16) comparable to the levels of titers detected in the asymptomatic control. There was no significant increase in titers (acute phase, 1:16; convalescent phase, 1:32) despite sufficient time between the serial samples for a rise in antibodies to develop. We postulate that the failure to develop a serologic response may have been due to the underlying diagnosis of hypogammaglobulinemia and that treatment with high-dose corticosteroids may have blunted the humoral immune response, as has been previously reported (14). Of note, however, is that detection of stably elevated titers of antibodies to Mycoplasma pneumoniae and Ehrlichia chaffeensis suggests that this patient was able to mount an immunologic response to other infectious agents in the past.

Culture of Acanthamoeba has a limited role in diagnosis but is useful for speciation and determination of antimicrobial susceptibility. The ameba was successfully isolated from macedrate brain tissue obtained at biopsy and identified as A. polyphaga based on cyst morphology. Optimal growth of the organism in vitro was at 30°C, consistent with other clinical isolates of Acanthamoeba which grow best below mammalian body temperature (23). As was the case in this report, isolation of amebae from cerebrospinal fluid is uncommon (11, 28).

PCR of corneal scrapings has been reported as both sensitive and specific for the diagnosis of Acanthamoeba keratitis (17); however, the role of molecular testing on either cerebrospinal fluid or brain tissue for a diagnosis of amoebic infection has not been defined. In the current report, rRNA gene sequencing was used to identify the isolate as a member of the T4 group of Acanthamoeba spp. (Gregory C. Booton, personal communication). Of note, amebas of the T4 group of Acanthamoeba spp. are detected in the majority of systemic and ocular infections (24), suggesting either (i) that T4 amebae may be more virulent than members of other groups of acanthamoebae found in the environment or (ii) that they are more commonly encountered in the environment and, therefore, are more likely to infect immunocompromised hosts.

Could earlier diagnosis leading to the initiation of treatment have altered the fatal outcome? Antimicrobial treatment of GAE is largely empirical, and as yet, there are no standardized treatment recommendations. The rare reports of long-term survivors among patients with GAE (26–28) and disseminated acanthamoebiasis (16, 25, 29) who are treated with a combination of antibiotic regimens support aggressive therapy. Often, however, the same antibiotic regimens have been used unsuccessfully in other patients, suggesting that early diagnosis, virulence of the agent, infective dose, and host immune factors all play a role in determining the outcome of GAE. Among the drugs that have been used with success in treating GAE cases are pentamidine isethionate, imidazoles, triazoles, fluoroxytine, amphotericin B, sulfa-containing antibiotics, and macrolides (21). The isolate grown from our patient was resistant to a number of these agents, making determination of susceptibility critical for optimizing the antibiotic regimen.

The current report illustrates the difficulty in making a diagnosis of GAE premortem. While GAE should be included in the differential diagnosis of any immunocompromised patient presenting with a subacute and progressive central nervous system syndrome, in this case, serologic testing for Acanthamoeba spp. performed on premortem specimens and stereotactic brain biopsy were nondiagnostic, precluding initiation of empirical antibiotic therapy. The antimicrobial resistance pattern of the isolate ultimately cultured from the patient’s brain underscores the need for both early diagnosis and standardized methods for testing antimicrobial susceptibility if progress is to be made in decreasing the case fatality rate of GAE.

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REFERENCES

1. Alizadeh, H., S. Apte, M. S. El-Agha, L. Li, M. Hurt, K. Howard, H. D. Cavanagh, J. P. McCulley, and J. Y. Niederkorn. 2001. Tear IgA and serum IgG antibodies against Acanthamoeba keratitis. Cornea 20:622–627.
2. Anderlini, P., D. Przepiorka, and M. Luna. 1994. Acanthamoeba meningoencephalitis after bone marrow transplantation. Bone Marrow Transplant. 14:459–461.
3. Badenoch, P., T. Grimmond, J. Cadwgan, S. Deayton, M. Essery, and B. Hill. 1988. Nasal carriage of free-living amebae. Microb. Ecol. Health Dis. 1:209–211.
4. Cerva, L. 1989. Acanthamoeba culbertsoni and Naegleria fowleri: occurrence of antibodies in man. J. Hyg. Epidemiol. Microbiol. Immunol. 33:99–103.
5. Chappell, C. L., J. A. Wright, M. Coletta, and A. L. Newsome. 2001. Standardized methods of measuring Acanthamoeba antibodies in sera from healthy human subjects. Clin. Diagn. Lab. Immunol. 8:724–730.
6. Culbertson, C. G., and K. Harper. 1977. Immunoperoxidase staining of E. histolytica and soil amebas in formalin-fixed tissue. Am. J. Clin. Pathol. 68:529–530.
7. Cursons, R. T. M., T. J. Brown, E. A. Keys, K. M. Moriarty, and D. Till. 1980. Immunity to pathogenic free-living amebae: role of humoral antibody. Infect. Immun. 29:401–407.
8. Deetz, T., M. Sawyer, G. Billman, F. Schuster, and G. Visvesvara. 2003. Successful treatment of Balamuthia ameobic encephalitis: presentation of 2 cases.Clin. Infect. Dis. 37:1304–1312.
9. Gruenert, M., G. Cannon, and J. Kushner. 1981. Fulminant amebic meningoencephalitis due to Acanthamoeba. Neurology 31:174–177.
10. Koide, J., E. Okusawa, T. Ito, S. Mori, T. Takeuchi, S. Itoyama, and T. Abe.
1998. Granulomatous amoebic encephalitis caused by Acanthamoeba in patient with systemic lupus erythematosus. Clin. Rheumatol. 17:329–332.

11. Lalitha, M. K., V. Anandi, A. Srivastava, K. Thomas, A. M. Cherian, and S. M. Chandi. 1985. Isolation of Acanthamoeba culbertsoni from a patient with meningitis. J. Clin. Microbiol. 21:666–667.

12. Lawande, R., S. Abraham, I. John, and L. Egler. 1979. Recovery of soil amebas from the nasal passages of children during the dusty harmattan period in Zaria. Am. J. Pathol. 101:201–205.

13. Marciano-Cabral, F., and G. Cabral. 2003. Acanthamoeba spp. as agents of disease in humans. Clin. Microbiol. Rev. 16:273–307.

14. Martinez, A. 1982. Acanthamoebiasis and immunosuppression. Case report. J. Neuropathol. Exp. Neurol. 41:548–557.

15. Martinez, A. 1985. Free-living amebas: natural history, prevention, diagnosis, pathology, and treatment of disease. CRC Press, Boca Raton, Fla.

16. Oliva, S., M. Jantz, D. Tiernan, D. Cook, and M. Judson. 1993. Successful treatment of widely disseminated Acanthamoebiasis. South. Med. J. 92:55–57.

17. Pasricha, G., S. Sharma, P. Garg, and R. K. Aggarwal. 2003. Use of 18S rRNA gene-based PCR assay for diagnosis of Acanthamoeba keratitis in non-contact lens wearers in India. J. Clin. Microbiol. 41:3206–3211.

18. Rivera, M., and T. Padhya. 2002. Acanthamoeba: a rare primary case of rhinosinusitis. Laryngoscope 112:1201–1203.

19. Rowen, J., C. Doerr, H. Vogel, and C. Baker. 1995. Balamuthia mandrillaris: a newly recognized agent for amebic meningoencephalitis. Pediatr. Infect. Dis. J. 14:705–710.

20. Sangruchi, T., A. J. Martinez, and G. Visvesvara. 1994. Spontaneous granulomatous amoebic encephalitis: report of four cases from Thailand. South-East Asian J. Trop. Med. Public Health 25:309–313.

21. Schuster, F., and G. Visvesvara. 2003. Amebic encephalitides and amebic keratitis caused by pathogenic and opportunistic free-living amebas. Curr. Treat. Options Infect. Dis. 5:273–282.

22. Schuster, F. L. 2002. Cultivation of pathogenic and opportunistic free-living amebas. Clin. Microbiol. Rev. 15:342–354.

23. Schuster, F. L., and G. S. Visvesvara. 1998. Efficacy of novel antimicrobials against clinical isolates of opportunistic amebas. J. Eukaryot. Microbiol. 45:612–618.

24. Schuster, F. L., and G. S. Visvesvara. 2004. Free-living amoeae as opportunistic pathogens of humans and animals. Int. J. Parasitol. 34:1001–1027.

25. Schwarzwald, H., P. Shah, J. Hicks, M. Levy, M. L. Wagner, and M. W. Kline. 2003. Disseminated Acanthamoeba infection in a human immunodeficiency virus-infected infant. Pediatr. Infect. Dis. J. 22:197–199.

26. Seijo Martínez, M., G. González-Mediero, P. Santiago, A. Rodríguez de Lope, J. Díaz, C. Conde, and G. S. Visvesvara. 2000. Granulomatous amebic encephalitis in a patient with AIDS: isolation of Acanthamoeba sp. group II from brain tissue and successful treatment with sulfadiazine and fluconazole. J. Clin. Microbiol. 38:3892–3895.

27. Sharma, P., P. Gupta, M. Murahi, and V. Ramachandran. 1993. Primary amebic meningoencephalitis caused by Acanthamoeba: successfully treated with cotrimoxazole. Indian Pediatr. 30:1219–1222.

28. Singhal, T., A. Bajpai, and V. Kalra. 2001. Successful treatment of Acanthamoeba meningitis with combination oral antimicrobials. Pediatr. Infect. Dis. J. 20:623–627.

29. Slater, C., J. Sickel, G. Visvesvara, R. Pabico, and A. Gaspari. 1994. Successful treatment of disseminated Acanthamoeba infection in an immunocompromised patient. N. Engl. J. Med. 331:85–87.

30. Steinberg, J. P., R. L. Galindo, E. S. Kraus, and K. G. Ghanem. 2002. Disseminated Acanthamoebiasis in a renal transplant recipient with osteomyelitis and cutaneous lesions: case report and literature review. Clin. Infect. Dis. 35:e43–e49.

31. Tan, B., M. Weldon-Linne, D. Rhone, C. Penning, and G. Visvesvara. 1993. Acanthamoeba infection presenting as skin lesions in patients with the acquired immunodeficiency syndrome. Arch. Pathol. Lab. Med. 117:1043–1046.

32. Tanaka, Y., S. Suguri, M. Harada, T. Hayabara, K. Suzumori, and N. Ohta. 1994. Acanthamoeba-specific human T-cell clones isolated from healthy individuals. Parasitol. Res. 80:549–553.

33. Torno, M. S., Jr., R. Babapour, A. Gurevitch, and M. D. Witt. 2000. Cutaneous Acanthamoebiasis in AIDS. J. Am. Acad. Dermatol. 42:351–354.

34. Uschuplich, V., D. Mileusnic, and M. Johnson. 2004. Progressive fatal encephalopathy in an immunosuppressed patient with a history of discoid lupus erythematosus. Arch. Pathol. Lab. Med. 128:e109–e111.

35. Visvesvara, G., F. Schuster, and A. Martinez. 1993. Balamuthia mandrillaris, N.G., N. Sp., agent of amebic meningoencephalitis in humans and other animals. J. Eukaryot. Microbiol. 40:504–514.