Non-granulomatous cerebellar infection by Acanthamoeba spp. in an immunocompetent host

Infection

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Abstract:	Acanthamoeba spp is a free-living amoeba, frequently involved in keratitis by contact lens in immunocompetent hosts. Anecdotal reports associate Acanthamoeba spp as a cause of severe granulomatous encephalitis in immunocompromised and, less frequently, in immunocompetent subjects. Data regarding clinical and therapeutic management are scanty and no defined therapeutic guidelines are available. We describe an unusual case of non-granulomatous Acanthamoeba cerebellitis in an immunocompetent adult male, with abrupt onset of neurological impairment, subtle hemorrhagic infarction at magnetic resonance imaging, and initial suspicion of cerebellar neoplasm. Histopathological findings of excised cerebellar mass revealed the presence of necrosis and inflammation with structure resembling amoebic

	trophozoites, but without granulomas. Polymerase chain reaction from cerebellar tissue was positive for Acanthamoeba T4 genotype. Due to gastrointestinal intolerance to miltefosine, the patient was treated with long-term course of fluconazole and trimethoprim/sulphamethoxazole, obtaining complete clinical and neuroradiological resolution.
Author Comments:	

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Dipartimento di Biotecnologie Mediche

To the Editor in Chief of Infection

Siena, September-19-2018

Dear Sir,

We submit to your attention our revised paper titled "**Non-granulomatous cerebellar infection by *Acanthamoeba* spp. in an immunocompetent host**".

We thank you and the Reviewers for the interest and the constructive criticism regarding our paper: we modified text accordingly to suggestions and we performed a point by point reply.

Hoping this new version will be suitable for publication in Infection,

best regards,

Francesca Montagnani

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POINT by POINT REPLY

Reviewer #1: The article deals with the important topic of diagnosis and treatment of Acanthamoeba spp. Infections in humans. The article can be helpful in similar cases of infection.

Thank you for your comment and appreciation.

Reviewer #2: Very interesting case that reminds that when an infection is caused by a rare pathogen the diagnosis may be very difficult

Thank you for your useful suggestions, we have changed text accordingly.

1) Page 3 line 2

I would suggest to add: are ubiquitous protozoa and are commonly found in lakes, swimming pools, tap water, and heating and air conditioning units.

Added as request

2) Page 3 line 59 and page 4 line 0

Due to the fact that the first suspect is linked to the haematoxylin and eosin tissue staining and microscopy, I would suggest to describe better what you have seen in Figure 2

This is what other colleagues must look for and keep in mind (as described at the CDC link https://www.cdc.gov/dpdx/freelivingamebic/index.html) :

The cysts of Acanthamoeba spp. are typically 10-25 µm in diameter. The cysts have two walls: a wrinkled fibrous outer wall (exocyst) and an inner wall (endocyst) that may be hexagonal, spherical, star-shaped or polygonal. Cysts contain only one nucleus with a large karyosome. Trophozoites of Acanthamoeba spp. are pleomorphic and measure approximately 15-45 µm. They often produce many spine-like processes called acanthapodia. Trophozoites contain a large nucleus with a large, centrally-located karyosome but no peripheral chromatin.

Thank you for this suggestion. To better clarify staining description, we specify "and they do not show peripheral chromatin." into the text (page 4 line 0), we revised graphic of Figure 2 (see Figure 2_REV file), we added an inset, to emphasize the difference in size of amoeba-like structures, and we modify related legend, as follow:

"Haemorrages and necrosis in the white matter (a). Variably-sized amoeba-like structures (b, arrows). Small (b, upper inset, thin arrows) and large (b, lower inset, thin arrow) (bar = 5 micron) amoeba-like structures admixed to small cerebellar granules (thick arrows); the asterisk on a macrophage; Unlike macrophages, they do not show peripheral chromatin. Numerous macrophages (b, thick arrow), positive for CD68 (c, thick arrow), which instead does not stain the ameboid forms (c, thin arrows).
a, Haematoxylin and eosin, original magnification (OM): x 100;
b, Haematoxylin and Eosin, OM: x 200; b, upper and lower insets, Haematoxylin and eosin, OM: x 1000;
c, CD68 immunohistochemistry, Leica bond III automated stainer (Band Polymer Refine Detection system); chromogen: diaminobenzidine; OM: x 400"

3) Page 5 line 24

Due to the lack of experience and the difficulties of microscopic diagnosis, in case of hemorrhagic cerebellitis or cerebellar abscesses without any diagnosis it could be useful to remind that a sample of cerebellar lesion or purulent fluid should be preserved frozen together with two paired serum specimen for additional molecular or serological tests in selected reference diagnostic laboratories.

Useful remind: we added the following text "Moreover, due to the lack of experience and the difficulties of microscopic diagnosis, in case of hemorrhagic cerebellitis or cerebellar abscesses without any diagnosis it could be useful to remind that a sample of cerebellar lesion or purulent fluid should be preserved frozen together with two paired serum specimens for

additional molecular or serological tests in selected reference diagnostic laboratories. An appropriately preserved tissue sample is also essential to perform a culture, which is often considered gold standard for Acanthamoeba detection [12] in the absence of molecular testing." Moreover, in order to better underline the difficult clinical and radiological diagnosis at onset, a minor adjustment to the abstract was added: "We describe an unusual case of non-granulomatous *Acanthamoeba* cerebellitis in an immunocompetent adult male, with abrupt onset of neurological impairment, subtle hemorrhagic infarction at magnetic resonance imaging, and initial suspicion of cerebellar neoplasm."

4) Page 5 line 24 after note 3

Some authors (An update on Acanthamoeba keratitis: diagnosis, pathogenesis and treatment Jacob Lorenzo-Morales1,a,*, Naveed A. Khan2,a, and Julia Walochnik3,a; Parasite 2015, 22, 10 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4330640/pdf/parasite-22-10.pdf) still wrote: The gold standard for Acanthamoeba detection is still the plate culture technique . The material (corneal scrapings/biopsies or transport medium/contact lenses/swabs, etc.) is applied centrally onto a 90 mm 1.5% non-nutrient (NN) agar plate covered with a lawn (100 lL) of a 24 h old culture of non-mucous bacteria (e.g. Escherichia coli). Plates are sealed with Parafilm , incubated at 30 C and screened daily for amoebae, optimally by inverted phase contrast microscopy. In cases of severe infection, amoebae are usually already visible after 24-48 h. However, samples should be observed for up to 1 week to reliably prove a negative result. Alternatively, amoebae can be cultured in tissue culture flasks in a suspension of bacteria in PBS.

We provided to insert suggested reference and the following sentence was added into discussion "An appropriately preserved tissue sample is also essential to perform a culture, which is often considered gold standard for Acanthamoeba detection [12] in the absence of molecular testing."

TITLE PAGE

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TITLE: Non-granulomatous cerebellar infection by *Acanthamoeba* spp. in an immunocompetent host

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KEYWORDS

Acanthamoeba; Free living amoebas; Encephalitis; Immunocompetent host.

ABSTRACT

Acanthamoeba spp is a free-living amoeba, frequently involved in keratitis by contact lens in immunocompetent hosts. Anecdotal reports associate *Acanthamoeba* spp as a cause of severe granulomatous encephalitis in immunocompromised and, less frequently, in immunocompetent subjects. Data regarding clinical and therapeutic management are scanty and no defined therapeutic guidelines are available.

We describe ~~a~~an unusual case of non-granulomatous *Acanthamoeba* ~~encephalitis~~ cerebellitis in an immunocompetent adult male, with abrupt onset of neurological impairment, subtle hemorrhagic infarction at magnetic resonance imaging, and initial suspicion of cerebellar neoplasm. H~~The h~~istopathological findings of excised cerebellar mass revealed the presence of necrosis and inflammation with structure resembling amoebic trophozoites, but without granulomas. Polymerase chain reaction from cerebellar tissue was positive for *Acanthamoeba* T4 genotype. Due to gastrointestinal intolerance to miltefosine, the patient was treated with long-term course of fluconazole and trimethoprim/sulphamethoxazole, obtaining complete clinical and neuroradiological resolution.

INTRODUCTION

Free living amoebas (FLAs) are ubiquitous protozoa~~.~~ and are commonly found in lakes, swimming pools, tap water, and heating and air conditioning units. Four genera of FLAs are actually identified as cause of human infections: *Balamuthia*, *Naegleria*, *Sappinia* and *Acanthamoeba* [1]. Although the infections of these FLAs are thought to be very rare, they may cause severe diseases in humans. *Acanthamoeba* spp are usually involved in eye-blinding keratitis in immunocompetent contact lens wearers and, less frequently, they are the cause of granulomatous amoebic encephalitis (GAE), mainly in immunocompromised subjects [1, 2]. GAE is a severe life-threating disease, with a case fatality rate of >90%. The poor outcome of GAE is associated with the lack of a defined therapeutic management strategy, which results from scarcity of reports and insufficient clinical evidence. Since 1968, only 23 cases of GAE by *Acanthamoeba* spp. have been reported in immunocompetent patients [2–4]. Diagnosis of GAE is challenging, and misdiagnosis is frequent [5, 6]. Neuroimaging studies typically reveal a nonspecific focal brain lesion, which is not helpful in suggesting *Acanthamoeba* etiology. Search of amoebas in brain biopsies by expert microscopists is useful, but it may not resolve amoeba identification unless the characteristic amoebic cyst structures are observed [7].

CASE

On March 2016, a previously healthy 35 years-old man complained of a recent history of speech disturbance, gait instability, and clumsy hands, followed by abrupt onset of left hemiparesis, left hemihypoesthesia, dysarthria, and drowsiness. His medical history was formerly silent, except for recurrent headache in the last 20 years. The patient was born in Santo Domingo, Dominican Republic, and had moved to Italy two years prior to symptoms onset. At the Emergency Room, brain computed tomography and magnetic resonance imaging (MRI) (Figure 1, a-c) revealed a inhomogeneous contrast-enhancing right cerebellar lesion with subtle areas of hemorrhagic infarction. The patient was admitted to the Neurosurgery Unit of the Siena University Hospital (Central Italy) and a lumbar puncture was performed. The cerebrospinal fluid (CSF) showed normal chemical-physical features and was sterile. The patient was afebrile, and blood cell counts and standard blood chemistry were normal. A neoplasm was suspected and an initial neurological improvement was obtained with steroids. After 2 weeks, neurological deterioration occurred with ataxia, right dysmetria, and left VI cranial nerve palsy and surgical excision was planned. Preoperative MRI (Figure 1, d) showed restricted diffusion within the cerebellar lesion, consistent with suppuration. At surgery on April 12, the cerebellar mass grossly resembled a tumor but a macroscopically purulent fluid was also found. Intraoperative inspection of squash smears demonstrated inflammatory cells, hemorrhage and necrosis. The lesion was drained and a biopsy was performed. A haematoxylin and eosin (H&E) tissue staining revealed numerous macrophages that were further confirmed by CD68 staining (Figure 2). In addition, throughout the inflammatory areas, a few round and variably-sized structures with single nucleus and vacuolated cytoplasm, resembling amoebic trophozoites, were seen:

these did not stain with CD68 and they do not show peripheral chromatin. There were no neutrophils, epithelioid cells or giant cells, and macrophages did not aggregate to form organized granulomas.

Postoperative course was uneventful, except for wound revision, with gradual neurological improvement. The patient was transferred to the Infectious Diseases Unit and empiric intravenous (i.v.) ceftriaxone 2 gr q12h and metronidazole 750 mg q8h were started. Complete physical examination was confirmed to be negative except for previously described neurological alteration. Negative serology for *Entamoeba histolytica*, *Echinococcus* spp., cysticercosis, *Trypanosoma cruzi*, HTLV, *Treponema pallidum*, and HIV was obtained; *Toxoplasma gondii* IgG were positive. No alteration was noted by complete hematologic and immunologic evaluation. The chest X-ray and a complete ultrasound study of the abdomen were normal.

In our setting, immunohistochemisty (IHC) assays specific for amoebas such as *Acanthamoeba* spp. were not available. Therefore, DNA extracted from the paraffin-embedded biopsy tissue was sent to the Microbiology Unit, for molecular testing, which detected *Acanthamoeba* spp. DNA by polymerase chain reaction (PCR) [8]. Sequencing of the 18S ribosomal DNA gene fragment revealed 98% identity with an *Acanthamoeba* T4 genotype (GenBank Accession number KJ652989.1). Additionally, two paired serum specimens (collected on May 10 and May 30, respectively) and paraffin embedded blocks of biopsy samples were sent to the Free-Living and Intestinal Amoebas Laboratory, CDC, Atlanta, GA, USA for further confirmation. Both sera were positive for *Acanthamoeba* antibodies by an indirect immunofluorescence assay, with a titer of 1:64 and 1:128, respectively. Moreover, at the FLIA Laboratory, further molecular analysis by a multiplex real-time PCR [8] of the brain tissues was positive for *Acanthamoeba* spp. and negative for *Balamuthia mandrillaris* and *Naegleria fowleri* DNA.

On May 27, specific therapy was initiated with fluconazole 400 mg/day i.v, trimethoprim/sulphamethoxazole 15/75 mg/kg/day i.v. and oral miltefosine 50 mg q8h. The latter was discontinued after 14 days and the dosage of trimethoprim/sulphamethoxazole was reduced due to gastrointestinal intolerance. On June 30, the patient was discharged with oral fluconazole 400 mg/day and trimethoprim/sulphamethoxazole 8/40 mg/kg/day. No major alterations of laboratory parameters were observed during the treatment except for occasional hyperkaliemia.

Forty-five and seventy-five day-postoperative follow-up MRI documented a progressive reduction of the size and of the gadolinium enhancement of the purulent foci, (Figure 1, e-f). On October 26, a new serum sample was negative for *Acanthamoeba* antibodies. The treatment was discontinued in January 2017.

In May 2017, brain MRI was negative for purulent foci, showing scarring evolution. The neurological examination on July 2017 was normal.

DISCUSSION

GAE due to *Acanthamoeba* species occurs predominantly in immunocompromised hosts. In this study, the patient was immunocompetent. MRI showed evolution from hemorrhagic cerebellitis to cerebellar abscesses. Histopathological findings, although suggestive of amoebic cerebellitis, did not allow a certain histopathological diagnosis: there were no specific and classical diagnostic clues, *Acanthamoeba* immunohistochemistry was not available, and brain granulomas were absent, which is unusual for *Acanthamoeba* brain infections and described in few reports in immunocompromised hosts [9-11].

The accurate pathological diagnosis of amoebic brain infections is challenging. This is partly because the amoebas are not considered in the initial differential diagnosis due to their rare role in causing infections in humans, and because of the inherent complexity in distinguishing amoebas from the amoeba-shaped host macrophages. In our case, the exclusion of other causes, along with a combination of several diagnostic approaches, including histopathology, nucleic acid amplification and serology and the therapeutic response, lead to the final diagnosis. This case emphasizes the complexity of the diagnosis of certain brain ~~*Acantamoeba*~~*Acanthamoeba* infections and suggests how molecular techniques and serology may be helpful. Moreover, due to the lack of experience and the difficulties of microscopic diagnosis, in case of hemorrhagic cerebellitis or cerebellar abscesses without any diagnosis it could be useful to remind that a sample of cerebellar lesion or purulent fluid should be preserved frozen together with two paired serum specimens for additional molecular or serological tests in selected reference diagnostic laboratories. An appropriately preserved tissue sample is also essential to perform a culture, which is often considered gold standard for *Acanthamoeba* detection [12] in the absence of molecular testing.

Therapeutic management in our case was problematic due to tolerability issues, however a complete recovery was obtained using two antimicrobials for 7 months. Overall, there are no established antimicrobial regimens against *Acanthamoeba* brain infections. Recently, miltefosine has been added to the treatment armamentarium, in combination with other antimicrobials, and almost all recent *Acanthamoeba* brain infection survivors received miltefosine in their treatment regimens. However its effectiveness remains unclear since the same drug regimens containing miltefosine were effective only in a part of the treated patients [1, 2]. In our case, miltefosine treatment probably played a marginal role, given its short duration, which was due to drug intolerance. Therefore, we conclude that a “patient-customized” treatment supported by the available literature evidence appears to be the most effective way to manage such rare parasitic infections.

NOTES

Compliance with ethical standards

Funding sources None

Acknowledgments We thank Marguerite Kelly Keating, formerly Infectious Disease Pathology Branch, Centers for Disease Control and Prevention (CDC), for critical revision of the manuscript.

Conflict of interest SM, CM, MGC, GC, VFM, FI, CN, IKMA, SR, AC, GZ: No conflict.

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FIGURE CAPTIONS

Fig. 1 Neuroradiological work-up At diagnosis, unenhanced nonconsecutive axial computed tomography (a), gradient-echo axial (b, upper row), T2-weighted coronal (b, lower row), and gadolinium-enhanced T1-weighted axial (c, upper row) and coronal (c, lower row) magnetic resonance (MR) images show an irregularly gadolinium-enhancing cortico-subcortical right cerebellar hemisphere lesion (white arrows), with subtle areas of hemorrhagic infarction (black arrows) resulting in mass effect. Preoperative axial diffusion-weighted (b: 1000 sec/mm^2) MR images obtained four days later (d, upper row) and at six-day follow-up (d, lower row) clearly show appearance of cortico-subcortical restricted diffusion (black open arrowheads), consistent with purulent cerebellitis. Nonconsecutive axial diffusion-weighted (b: 1000 sec/mm^2) MR images obtained 45 days after surgery (e) show clear-cut multiple foci (black open arrowheads) of restricted diffusion, consistent with abscesses, which are less evident one month later (f)

Fig. 2 Histopathologic findings

Haemorrages and necrosis in the white matter (a). Variably-sized amoeba-like structures (b, arrows). Small (b, upper inset, thin arrows) and large (b, lower inset, thin arrow) (bar = 5 micron) amoeba-like structures admixed to small cerebellar granules (thick arrows); the asterisk on a macrophage. Unlike macrophages, they do not show peripheral chromatin. Numerous macrophages (b, thick arrow), positive for CD68 (c, thick arrow), which instead does not stain the ameboid forms (c, thin arrows).

a, Haematoxylin and eosin, original magnification (OM): x 100;

b, Haematoxylin and Eosin, OM: x 200; b, upper and lower insets, Haematoxylin and eosin, OM: x 1000;

c, CD68 immunohistochemistry, Leica bond III automated stainer (Band Polymer Refine Detection system); chromogen: diaminobenzidine; OM: x 400~~Haemorrages and necrosis in the white matter (a). Amoeba-like structures (b, inset, thin arrows) admixed to small cerebellar granules (b, inset, thick arrow). Numerous macrophages (b, thick arrow), positive for CD68 (c, thick arrow), which instead does not stain the ameboid forms (c, thin arrows).~~

~~a, Haematoxylin and eosin, original magnification (OM): x 100;~~

~~b, Haemotoxylin and eosin, OM: x 200; C-D inset, Haematoxylin and eosin, OM: x 1000;~~

~~c, CD68 immunohistochemistry, Leica bond III automated stainer (Bond Polymer Refine Detection system); chromogen: diaminobenzidine; OM: x 400~~

Figure 1

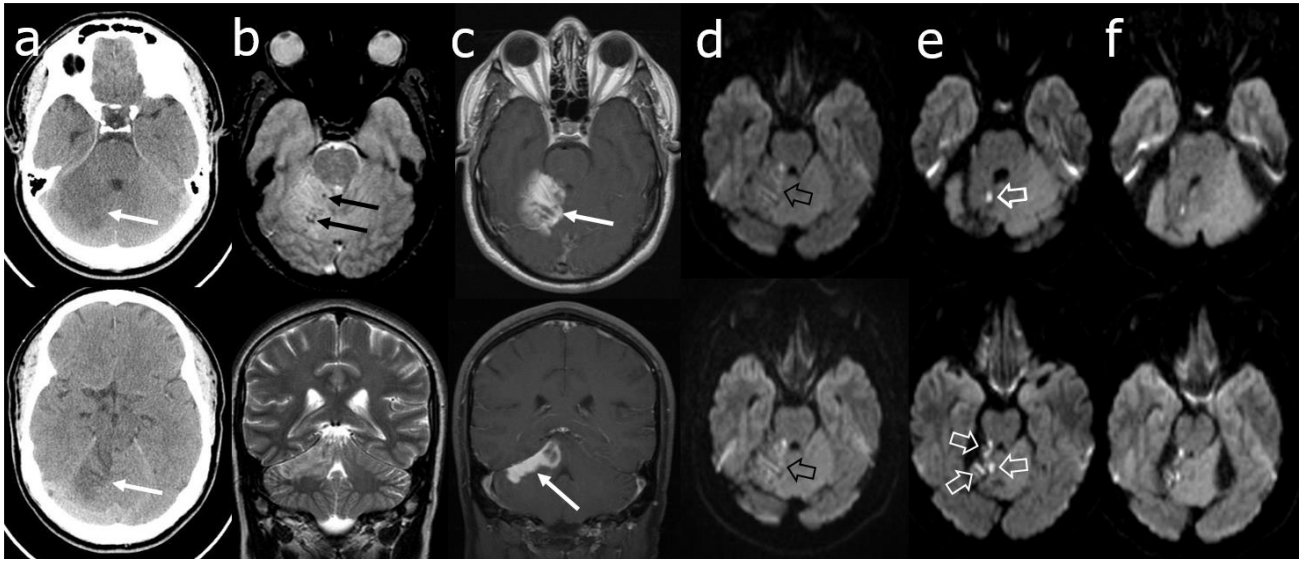

Figure 2

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