Case Report: *Aspergillus flavus* Brain Abscesses Associated with Hepatic Amebiasis in a Non-neutropenic Man in Senegal

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**Abstract.** A non-neutropenic man living in Senegal was repatriated to France for liver amebic abscesses associated with brain abscesses presumed to be of amebic origin. Surprisingly, the post-mortem examinations of brain abscesses showed *Aspergillus flavus*. The route of infection by *A. flavus* in this particular context is discussed.

**INTRODUCTION**

In an apparently immunocompetent man repatriated to France from Senegal for hepatic amebiasis associated with cerebral abscesses, the most likely diagnosis was diffusion of amebae to the brain. Unfortunately, in the case we report, the patient died eight days after his arrival in France despite an adapted treatment against visceral amebiasis. His necrotic and hemorrhagic cerebral lesions increased and diffused into almost the entire brain until a temporal engagement occurred. Post-mortem examination of brain abscesses showed the presence of *Aspergillus flavus* but not *Entamoeba histolytica*. Cerebral abscesses caused by *Aspergillus* species are observed mostly in immunosuppressed patients. The most frequent incriminated species is *A. fumigatus*. In immunocompetent persons, brain aspergillosis is most often of nasal and paranasal origin and the most frequent species encountered is *A. flavus*. In this report, *A. flavus* was responsible for brain abscesses in an apparently immunocompetent man who was not neutropenic or lymphopenic, had not received corticosteroids, and did not show paranasal sinus involvement. The route of infection by *A. flavus* in this particular context is discussed.

**CASE REPORT**

A 56-year-old man from France who had an unremarkable medical history and had lived in Senegal on a sailboat for four months was repatriated from Dakar to France after being diagnosed with multiple hepatic and cerebral amebic abscesses. He has been hospitalized 12 days before in the intensive care unit of a general hospital in Dakar for abdominal pain and diarrhea with bloody mucus. He showed clinical hepatomegaly and multiple liver abscesses documented by ultrasonography. He was treated intravenously with metronidazole (500 mg three times a day), ciprofloxacin (500 mg twice a day), and ampicillin (2 g three times a day) in addition to receiving rehydration.

Relevant laboratory data showed a leukocyte count of $15 \times 10^9$ cells/L, a platelet count of $8.8 \times 10^9$ cells/L, negative results for malaria on a thick blood smear, negative results for antibodies against human immunodeficiency virus, and positive serologic results for amebiasis. Ten days later, confusion associated with temporospatial disorientation developed in the patient. A cerebral computed tomography (CT) scan showed three low-density sustentorial lesions with a discreet periportal edema. An abdominal CT scan showed three low-density focal hepatic lesions associated with perihepatic, perisplenic, and right pleural effusions. New laboratory values demonstrated a 10-fold increase in transaminase and alkaline phosphatase levels associated with a prothrombin time of 32 seconds.

On his arrival at the Emergency Department of Groupe Hospitalier Pitié-Salpêtrière, Paris, on December 30, 2007, the patient had stable neurologic, hemodynamic, and respiratory functions. A CT scan of the brain showed a large abscess in the left parieto-occipital region and two abscesses in the right tempo-ro-occipital region with perifocal edema but without mass effect. An abundant right pleural effusion without pleural or parenchymatous lesion was noted on a chest CT, and an abdominal CT showed three voluminous hepatic abscesses with transdiaphragmatic rupture. Because rapid hemodynamic deterioration developed in the patient, he was transferred in the intensive care unit where intubation, fluid resuscitation, catecholamine treatment, and pleural drainage were initiated. Treatment with metronidazole and ampicillin was continued. The next day, because of severe anemia after an episode of melena, an emergency laparotomy was performed. A perforating duodenal ulcer was found and sutured. Cholecystectomy, peritoneal wash, and samples from hepatic abscesses and peritoneal liquid were obtained for analyses.

Among microbiologic findings, *Escherichia coli* and *Klebsiella* sp. with extended-spectrum β-lactamases and numerous colonies of *Candida albicans* were recovered from hepatic and peritoneal samples. Bacteriologic cultures were prepared on sheep blood agar incubated under aerobic and anaerobic conditions, on chocolate agar supplemented with PolyViteX (bioMérieux, Marcy l’Étoile, France) incubated in a 5% CO$_2$-air incubator, and in brain-heart infusion medium. Production of extended spectrum β-lactamase was demonstrated by the double-disk test. Fungal cultures were prepared in Sabouraud agar tubes with and without cycloheximide and incubated at 37°C.

Parasites were not found in pleural and peritoneal liquids by direct examination. However, genic amplification of small subunit ribosomal DNA of *Entamoeba histolytica* by a conventional polymerase chain reaction (PCR) showed a positive result. Results of serologic analysis for amebiasis were positive but parasitologic examination of stool was not performed. In addition, results of multiple bacteriologic and fungal blood cultures were negative. Thin and thick blood smears were negative for *Plasmodium* spp. Therefore, treatment with metronidazole was continued and the patient was then treated.

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Intravenously with imipenem (1 g four times a day), amikacin (1,250 mg once a day), and fluconazole (400 mg once a day).

The respiratory, hemodynamic, and infectious status of the patient improved, but his neurologic functions began to deteriorate on January 3 with progressive disappearance of brainstem reflexes. Magnetic resonance imaging (MRI) of the brain showed augmentation of lesions with voluminous necrotic and hemorrhagic areas prevailing in occipital regions associated with radiologic signs of intracranial hypertension (Figure 1). Two days later, a repeat cranial CT scan showed augmentation of lesions and perifocal edema associated with temporal engagement. In view of these extensive necrotic and diffuse lesions, he was considered inoperable and when he showed clinical signs of cerebral death, it was decided to stop resuscitation maneuvers. The patient died on January 7.

An autopsy was performed 36 hours after death and macroscopic examination showed a brain liquefied by multiple abscesses, pulmonary edema with green foam, multiple confluent hepatic abscesses, peritonitis, and pancreatic necrosis. Histologic examination of the liver showed mutilating fibrosis surrounding necrotic areas, but peripheral hepatic regions were devoid of fibrosis, which suggested no underlying liver cirrhosis. In addition, results of conventional PCR for *E. histolytica* in a hepatic abscess were positive. Histologic examination of cerebral necrotic areas showed abscesses with polynuclear infiltrates and diffuse vascular lesions with fibrinoid necrosis, thrombosis, and perivascular infarction. Results of a conventional PCR for *E. histolytica* in brain abscess and cerebrospinal fluid were negative.

In abscessed cerebral regions, periodic acid–Schiff and Grocott methanamin silver (GMS) staining showed some septated hyphae with acute angle branching suggestive of the genus *Aspergillus* (Figure 2). This finding was confirmed by Giemsa and GMS staining of brain abscesses and cerebrospinal fluid, which showed fungal septated hyphae, and by cultures of cerebrospinal fluid on Sabouraud dextrose agar, which yielded five colonies of *A. flavus* that were easily identified by macroscopic aspects and morphology by microscopic examination. Brain abscesses likely remained sterile because the fungus was not viable when the autopsy was performed. Antifungal in vitro susceptibility was not determined because of the death of the patient.

To support these results, a TaqMan-based real-time PCR was conducted with DNA extracted from cerebral abscesses, cerebrospinal fluid, and pulmonary fragment (the fragment was preserved in formaldehyde) from the post-mortem examination and from pleural fluid obtained on December 30. This assay was specific for mitochondrial DNA of *A. fumigatus*. The PCR, which was validated in our laboratory with pulmonary tissues from mice and was only genus specific, yielded PCR products exclusive for both cerebral samples. A retrospective search for galactomannan antigen (Platelia *Aspergillus*®; Bio-Rad, Marnes-la-Coquette, France) in a serum specimen obtained on January 2 showed an index of 0.4, which is considered negative but it close to the cutoff value of 0.5, and serologic results for the genus *Aspergillus* by coelectrosyneresis with *A. fumigatus* antigen (Bio-Rad) were negative. Periodic acid–Schiff and GMS staining of numerous lung specimens did not show fungal hyphae.

**DISCUSSION**

Invasive aspergillosis occurs mostly in immunosuppressed patients. However, in recent years, there has been a significant increase in *Aspergillus* infections in immunocompetent hosts in which a number of extrapulmonary infections have been observed, including central nervous system aspergillosis. Neuroaspergillosis is an uncommon infection accounting for 5% of all cranial infections but it is associated with an exceedingly high mortality rate that approaches 100%. In most cases, diagnosis is made after death or at the terminal stages of the disease. The patient in this case report had a progressive decline in neurologic function with increasing evidence of intracranial hypertension and associated edema, despite aggressive medical therapy. The presence of fungal hyphae in both the brain and lung specimens, along with positive results of conventional PCR for *A. fumigatus* and the presence of septate hyphae on histologic examination, strongly suggest a diagnosis of invasive aspergillosis. The use of real-time PCR for mitochondrial DNA of *A. fumigatus* provided additional evidence of active infection, although the lack of susceptibility testing limits the ability to determine the susceptibility of the fungus to antifungal agents. This case highlights the importance of considering invasive aspergillosis in the differential diagnosis of patients with neurologic symptoms, even in the absence of underlying immunosuppression.
stage of disease. This disease is caused by an opportunistic fungal infection that usually affects heavily immunocompromised hosts, typically patients with hematologic malignancy or bone marrow or solid organ transplantation in whom neutropenia and corticosteroid use are the major risk factors. Cerebral involvement commonly occurs by direct extension of invasive Aspergillus sinusitis or hematogenous spread from an occult source, usually the lungs. In immunocompetent patients, the disease has been described mostly in the setting of allergic rhinosinusitis caused by Aspergillus species after neurosurgical procedures, and in patients with significant comorbidities such as diabetes mellitus, severe malnutrition, or liver cirrhosis. Aspergillus fumigatus is the most frequently reported species in immunosuppressed hosts, but A. flavus has been isolated mostly from immunocompetent patients. Aspergillus flavus is the most common species isolated from cultures of nasal and paranasal specimens from immunocompetent patients with cerebral aspergillosis who do not have any risk factors but who live in hot and dry countries.

Aspergillus flavus has a worldwide distribution with an optimal growth temperature of 37°C. However, fungal growth can be observed at temperatures ranging from 12°C to 48°C. The fungus is a soil saprophyte that plays an important role as a nutrient recycler and is able to cause diseases in economically important crops such as maize and peanuts. Climatic conditions markedly influence the prevalence of A. flavus in the air. It is particularly prevalent in the air of some tropical countries with a dry and hot climate. In two studies in Iran, it was the most prevalent Aspergillus species isolated from the air of hospital wards and homes. In addition, surveys of fungi in drinking water have isolated many different taxa, including A. flavus, from water storage tanks.

To our knowledge, E. histolytica and Aspergillus spp. infections have never been reported concomitantly. We describe an apparently immunocompetent man (he was not neutropenic or lymphopenic and did not take any corticosteroids) who lived in Senegal and was repatriated to France for hepatic amebic abscesses associated with cerebral abscesses. These abscesses were presumably to be of amebic origin, but were eventually determined to be of fungal origin. Post-mortem examinations of brain abscesses and cerebrospinal fluid by histologic and mycologic staining showed fungal septated hyphae, five colonies of A. flavus isolated in cultures from cerebrospinal fluid, and positive genomic amplification of Aspergillus spp. by real-time PCR.

Our findings were surprising in an apparently immunocompetent man and in the context of liver amebiasis. Moreover, distinction between E. histolytica and Aspergillus spp. in cerebral abscesses is not easy because radioimaging features are not specific, and frequent ring-like enhancing lesions for both infections and other multiple patterns can be observed, especially in cerebral aspergillosis. However, E. histolytica was not identified by PCR in cerebrospinal fluid and brain abscesses. In addition, identification of hyaline, septated hyphae with dichotomous branching by histologic and mycologic staining of cerebrospinal fluid and brain abscesses, and isolation of five A. flavus colonies from cerebrospinal fluid culture implicate A. flavus as the causative agent of brain aspergillosis in this patient and exclude the possibility of sample contamination at the time of the autopsy. The negative result for Aspergillus spp. galactomannan antigen in serum does not exclude the diagnosis of invasive aspergillosis because this assay has a sensitivity of 97.4% for neutropenic patients. However, this assay has not been tested in immunocompetent patients.

The route of infection in our patient is unclear and no other sites of aspergillosis were detected. The patient had no evidence of sinus disease by CT scan and MRI, and the location of the cerebral abscesses did not suggest nasal or paranasal origin. Moreover, because a few reports described invasive pulmonary aspergillosis in immunocompetent patients, we searched for evidence to support this hypothesis. However, our patient had no evidence of invasive pulmonary aspergillosis by CT scan and histologic post-mortem examinations of the lungs. Galactomannan antigen was not detected in serum (bronchoalveolar lavage was not performed). In addition, his neutrophil and lymphocyte counts were normal; serologic results for antibodies against human immunodeficiency virus were negative; and factors predisposing the patient to invasive aspergillosis such as diabetes mellitus, steroid exposure, liver cirrhosis, intravenous drug abuse, or head trauma were not observed.

The digestive tract may have been a portal of entry for A. flavus in this patient. This route of invasive aspergillosis has been described in a few severely immunosuppressed patients, all with peritonitis, but never demonstrated. Aspergillus flavus is noted for causing crop disease in tropical countries and has been isolated from water, but the fungus was not isolated from peritoneal fluid from our patient who was not neutropenic or lymphopenic. Moreover, septated hyphae were not found in samples of digestive tract (stomach, ileum, and colon) that were examined.

In conclusion, we report a case of cerebral abscesses caused by A. flavus that led to the death of a non-neutropenic man who lived in Senegal and was repatriated to France for amebic liver abscesses associated with brain abscesses presumably of amebic origin. This case highlights the diagnostic challenge presented by central nervous system aspergillosis in non-neutropenic patients and underscores its grave prognosis. Of note is the species of Aspergillus involved, A. flavus, which is known to be more prevalent in immunocompetent patients and in hot and dry countries.

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