**Aspergillus Meningitis: Diagnosis by Non-Culture-Based Microbiological Methods and Management**

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Meningitis caused by *Aspergillus* species is very rare. This syndrome often manifests itself with fever and headache that may be present for several weeks before a diagnosis is established. The management of central nervous system (CNS) aspergillosis, including meningitis, is problematic since diagnosing the infection is difficult and treatment should include a drug with activity against *Aspergillus* species that also penetrates into the cerebrospinal fluid (CSF). We report a case of fungal meningitis in an immunocompetent woman caused by *Aspergillus fumigatus* and describe the performance of noncultural diagnostic tests with CSF samples obtained from this patient.

**Case report.** A 73-year-old woman with a history of left- and right-side chronic otitis media for which bilateral mastoidectomy had been performed became unwell with headache and vomiting. She developed a temperature of 39°C and was admitted to the hospital under the suspicion of bacterial meningitis. Neurological examination showed drowsiness and symptoms of meningismus. Eye, nose, and throat examination, including paranasal sinuses, showed no abnormalities, but both mastoid cavities showed inflammation. A computed tomography (CT) of the brain showed no parenchymal lesions. CSF analysis revealed 2,130 leukocytes per μl, 1.5 mmol of glucose per liter (2.6 mmol of blood glucose per liter), 1,582 mg of protein per liter, and no microorganisms in Gram’s stain. Under the suspicion of chronic bacterial otitis media with extension to the brain, intravenous amoxicillin and cefazidime were started. *A. fumigatus* was cultured from the right mastoid cavity. CSF obtained by two additional lumbar punctures failed to reveal a causative microorganism, but it showed a decrease of the number of leukocytes to 300/μl (67% lymphocytes and 33% neutrophils) and glucose to 0.6 mmol/liter and an increase in protein to 4,500 mg/liter. Because severe inflammation was present in the right mastoid cavity and a small section of the dura mater was visible at that side, the possibility of meningitis due to *A. fumigatus* was considered. High levels of the *Aspergillus* antigen galactomannan were detected by enzyme-linked immunosorbent assay (ELISA) in the first three CSF samples obtained from this patient. Amoxicillin was discontinued and oral itraconazol (Jansen-Cilag B.V., Tilburg, The Netherlands) at a dosage of 300 mg twice daily was added to the regimen for presumed *Aspergillus* meningitis. After 1 week of antifungal treatment, the patient showed no improvement, and since levels of itraconazol in blood were not available at that time, itraconazol was discontinued and intravenous treatment with amphotericin B desoxycholate (Bristol-Myers Squib B.V., Woerden, The Netherlands) was started at a dosage of 1 mg/kg of body weight/day. After 16 days of treatment with amphotericin B, a repeat CT brain scan showed asymmetrical third ventricles suggestive of hydrocephalus. An intraventricular catheter was inserted for external drainage. A smear of CSF obtained via the catheter revealed the presence of fungal hyphae, and culturing yielded *A. fumigatus*. Local intraventricular instillation of amphotericin B at a dosage of 0.5 mg in 5% glucose every other day was added to the regimen. The clinical condition of the patient was stable despite occasional episodes of somnolence and persistent fever. Because of nephrotoxicity, the intravenous amphotericin B was discontinued (cumulative dose, 1,450 mg), and treatment with voriconazol (Pfizer Central Research, Sandwich, United Kingdom) at a dosage of 12 mg/kg/day was begun after informed consent was obtained. The intrathecal administration of amphotericin B had to be discontinued (cumulative dose, 4 mg) because of intraventricular-catheter-associated infection. The clinical condition of the patient improved gradually, although she remained febrile for 8 weeks. Repeat CT brain scans showed no evidence of parenchymal lesions, but a ventriculoperitoneal shunt was inserted because of a recurrence of increased intracranial pressure. The clinical condition of the patient improved further, and after 14 weeks of treatment with voriconazol, the drug was discontinued. Antifungal prophylaxis was not given since the defect near the dura mater in the right mastoid cavity had been closed during hospitalization. Currently, 12 months after cessation of voriconazol treatment, the patient remains well, although a cognitive decline compared with the premorbid state has been noted.
Sample collection and culture. A total of 26 CSF samples were obtained by lumbar puncture or via an intraventricular catheter. The samples were centrifuged at 10,000 × g for 10 min. The CSF sediment was stained with Gram’s stain, methylene blue, and calcofluor white for direct microscopy. Besides routine bacteriological culture, fungal culturing was performed by plating the CSF sediment onto Sabouraud glucose medium containing 10% chloramphenicol and onto blood agar plates and incubating the sediment with Sabouraud broth at 30°C for 3 weeks. The supernatant of each CSF sample was stored at −70°C until testing. Aspergillus isolates were identified by their cultural characteristics, their ability to grow at 48°C, and the appearances of their conidiophores and conidia. During hospitalization, serum and plasma samples were collected weekly.

In order to test the specificity of the noncultural methods, 30 CSF samples were randomly collected from patients admitted to our hospital with either neurological signs and symptoms or suspected meningitis. Only one eventually had a proven diagnosis of bacterial meningitis.

Antigen detection. The presence of the Aspergillus antigen galactomannan was measured in the serum and CSF with a commercial sandwich ELISA (Platelia Aspergillus; Sanofi Diagnostics Pasteur, Marnes-La Coquette, France) (30).

Antibody detection. The presence of immunoglobulin G (IgG) antibodies in serum and CSF against Aspergillus species was determined with a commercial ELISA (Genzyme Virotech GmbH, Rüsselsheim, Germany).

Aspergillus PCR. Aspergillus DNA was detected by genus-specific hot-start PCR, as described previously for bronchoalveolar lavage fluid (17, 32), with two Aspergillus genus-specific primers, Asp1 and Asp2, and hybridization with the internal Aspergillus-specific oligonucleotide probe Asp-p (17).

In vitro susceptibility testing. The MICs of amphotericin B, itraconazole, and voriconazole for the *A. fumigatus* isolates were determined by a microdilution method with RPMI 1640 medium according to the National Committee for Clinical Laboratory Standards-recommended guidelines for in vitro susceptibility testing of filamentous fungi (11). Itraconazole-susceptible (AF71; MIC = 1.0 μg/ml) and -resistant (AF90; MIC > 64 μg/ml) *A. fumigatus* isolates were included in each test. For itraconazole and voriconazole, 75% growth inhibition was used as a MIC endpoint (11).

Drug levels in blood and CSF. The concentrations of itraconazole and the hydroxy metabolite in plasma and CSF were determined by high-performance liquid chromatography (HPLC). The concentrations of voriconazole in plasma and CSF were measured by an agar diffusion method and compared with HPLC results.

Data analysis. The mean optical density and standard deviation were calculated for the 30 control CSF samples. The cutoff value for positives was defined as the mean optical density plus five times the standard deviation. The cutoff ratio for CSF (CSF ratio) was calculated by dividing the cutoff optical density by the optical density of the threshold control.

Twenty-eight of the 30 control CSF samples were culture negative. One of the remaining samples grew *Streptococcus pneumoniae*, and a *Propionibacterium* species was recovered from the other. For antigen detection, the mean optical density was 0.19 (range, 0.16 to 0.33), and the calculated cutoff value was 0.36, which is equivalent to a CSF ratio of 0.6. For antibody detection the mean optical density was 0.14 (range, 0.04 to 0.52), and a cutoff value of 0.76 was calculated, which corresponds with a CSF ratio of 10.2. Aspergillus DNA was not detected in the 30 CSF samples from control patients.

Performance of the noncultural methods. Twenty-three consecutive CSF samples were available for the detection of *A. fumigatus* antibodies by ELISA. The mean optical density of the patient’s CSF samples was 0.23 (range, 0.125 to 0.368), which was not significantly higher than that of controls (P = 0.14). All CSF samples from controls were negative, and 1 of the 23 CSF samples from our patient was positive, with a titer of 11.8.

The sandwich ELISA showed reactivity with all 26 CSF samples, and Aspergillus DNA was detected in 4 of 26 samples (Table 1). The galactomannan titer remained high during therapy with antibacterial agents, itraconazole, and intravenous amphotericin B (Fig. 1). A significant decrease of the antigen titer occurred after intraventricular administration of amphotericin B was begun, and the titer continued to decrease during therapy with voriconazole. Neither galactomannan nor IgG antibodies directed against *Aspergillus* species were detected in 13 consecutive serum samples.

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# Drug levels in blood and CSF

The trough and peak concentrations of itraconazole in blood that were obtained after 1 week of therapy were 0.55 and 0.64 mg/liter, respectively, and the hydroxy metabolite levels were 1.5 and 1.6 mg/liter, respectively. The itraconazole and hydroxy metabolite concentrations in CSF were 0.07 and 0.13 mg/liter, respectively, which are 12 and 8% of the mean level in blood. These results suggest that therapeutically active levels were present in the blood but not in the CSF. The trough and peak concentrations of voriconazole in blood varied from 1.4 to 4.8 mg/liter and from 1.8 to 5.8 mg/liter, respectively. Voriconazole concentrations in CSF were between 0.8 and 3.1 mg/liter, which is 38 to 55% of the mean levels in blood. Concentrations of voriconazole in both blood and CSF were above the MIC during treatment.

Establishing a diagnosis of *Aspergillus* meningitis is difficult, and many cases have been diagnosed only at autopsy (10, 14, 20). Clinical findings such as headache and meningismus may
be present but are nonspecific. Typically only a small number of fungal cells are present in CSF (16). A positive culture may be obtained if a large volume of CSF, preferably 5 ml or more, is cultured, if CSF is cultured repeatedly (16), or if ventricular fluid is obtained for culture (15). CSF cell counts are usually elevated, showing pleocytosis (16, 21). Glucose concentrations may be decreased and protein levels are usually elevated (21), but none of these findings are specific for fungal disease. Although CT may help to diagnose fungal brain abscesses, Aspergillus meningitis is characterized by the absence of parenchymal lesions (14, 20, 31). CT may be useful for documenting the concurrent presence of a local focus of infection or secondary complications (3, 20) or in identifying coexisting parenchymal brain abscesses (23). In addition, gadolinium-enhanced magnetic resonance imaging may contribute to establishing a diagnosis, but this procedure has been used with only a limited number of patients with Aspergillus meningitis (18, 21).

Significant levels of antibodies were not detected by ELISA in the CSF or serum. Detection of serum antibodies to Aspergillus has been documented for at least four patients with Aspergillus meningitis (2, 3, 12, 20), but for one patient no antibodies were detected in serum (31). In the last patient precipitating antibodies were detected in the CSF, but they were not detected in the CSF of another patient with meningitis due to Aspergillus flavus (1). Although different assays were used to detect antibodies, the variable results found in these cases and the failure to detect antibodies in our patient indicate that antibody detection in serum or CSF is not useful.

To obtain a positive PCR result, either fungal cells or fungal DNA should be present in the CSF sample. The number of cells present in a CSF specimen is extremely low, and the rate of clearance of DNA from the CSF is unknown. Therefore, even if the PCR assay is very sensitive, the assay may not be positive because fungal cells or fungal DNA is not present in the CSF sample. For our patient, DNA was detected only in samples obtained via the intraventricular catheter. However, we were unable to test the sediment of the CSF sample, which may yield a higher sensitivity than CSF supernatant. Nevertheless, we have previously detected Aspergillus DNA in the supernatant of a single CSF sample of a patient with disseminated invasive aspergillosis and cerebral abscesses (33), which indicates that PCR may be useful for diagnosing cerebral involvement in invasive infections due to Aspergillus species.

Previous studies have demonstrated the presence of antigens in the CSF of patients with CNS aspergillosis. Aspergillus antigen was detected by radioimmunoassay of ventricular CSF from a patient with Aspergillus terreus meningoencephalitis (34) and by Western blotting of CSF of three patients infected by A. flavus and A. terreus (26). For our patient, the detection of the Aspergillus antigen galactomannan was the earliest indicator for the presence of Aspergillus meningitis.

In addition to early diagnosis of the infection, the course of the antigen titer corresponded with the clinical response to treatment. The antigen titer showed a significant decline only after local instillation of amphotericin B was begun. The antigen titer continued to decline after amphotericin B was replaced by voriconazole. Concentrations of galactomannan in serum have been found to correspond with the fungal burden (30), and in a number of patients, the course of the antigen titer in the serum corresponded with the clinical response to treatment (22, 33). Monitoring of CSF galactomannan levels during therapy may help to evaluate the response to treatment.

Given the rarity of Aspergillus meningitis, there is no standard therapy. Intravenous amphotericin B desoxycholate has been used successfully (1, 3), although several treatment failures have been documented (3, 19, 23, 29). The difficulty in achieving high drug levels in CSF has resulted in the use of combination therapy, such as intravenous amphotericin B with flucytosine (8, 12, 19, 31) and rifampin (31). Local intracranial instillation of amphotericin B has been used successfully for treating patients with Aspergillus brain abscesses and has produced no long-term side effects or neurological sequelae (4),...
although amphotericin B-induced myelopathy has been described (5). Lipid formulations of amphotericin B have not been evaluated in patients with Aspergillus meningitis, but liposomal amphotericin B has been used successfully in treating Aspergillus brain abscesses (6).

Itraconazole has been used successfully to treat patients with Aspergillus brain abscesses (13, 27) as well as a patient with Aspergillus meningitis (18), despite the fact that the drug penetrates poorly into the CSF (24). Our patient was treated with itraconazole capsules, which resulted in concentrations of itraconazole in CSF that were below the MIC after 1 week of therapy. Treatment with itraconazole oral solution may have resulted in higher concentrations in CSF since the bioavailability of this formulation is increased (25). Nevertheless, treatment with itraconazole was discontinued in our patient because her clinical condition did not improve and, more importantly, concentrations of itraconazole in blood were not directly available. Therefore, the duration of treatment was too short to allow evaluation of clinical response.

Voriconazole is a new antifungal azole which has fungicidal activity against Aspergillus fumigatus (9) and penetrates into the CSF. In one patient with Aspergillus brain abscesses, the concentrations of voriconazole in CSF were between 40 and 70% of the concentrations in plasma (28). In our patient, similar CSF levels were achieved. Several patients with Aspergillus brain abscesses have been treated successfully with voriconazole (7, 28), and therefore this drug should be considered for the treatment of patients with CNS aspergillosis.

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REFERENCES