BRIEF REPORT

The Index Case for the Fungal Meningitis Outbreak in the United States

April C. Pettit, M.D., M.P.H., Jonathan A. Kropski, M.D., Jessica L. Castilho, M.D., M.P.H., Jonathan E. Schmitz, M.D., Ph.D., Carol A. Rauch, M.D., Ph.D., Bret C. Mobley, M.D., Xuan J. Wang, M.D., Steven S. Spires, M.D., and Meredith E. Pugh, M.D., M.S.C.I.

SUMMARY

Persistent neutrophilic meningitis presents a diagnostic challenge, because the differential diagnosis is broad and includes atypical infectious causes. We describe a case of persistent neutrophilic meningitis due to *Aspergillus fumigatus* in an immunocompetent man who had no evidence of sinopulmonary or cutaneous disease. An epidural glucocorticoid injection was identified as a potential route of entry for this organism into the central nervous system, and the case was reported to the state health department.

CASE REPORT

A man in his 50s with a history of degenerative lumbar-disk and joint disease presented with headache and neck pain that had become progressively worse over the course of 8 days. The associated symptoms included nausea, malaise, fatigue, chills, and decreased appetite. The patient reported no fevers, rash, photophobia, or vision changes. Four weeks before presentation, he had received the latest in a series of epidural injections of methylprednisolone for low back pain. The patient had no history of immunosuppressing conditions and was not taking any additional immunomodulatory medications.

Assessment of vital signs on presentation revealed a temperature of 36.7°C, pulse of 101 beats per minute, and blood pressure of 144/88 mm Hg. The physical examination was notable only for meningismus. Laboratory testing revealed a peripheral-blood leukocyte count of 7800 cells per cubic millimeter, with 88% polymorphonuclear cells. The remainder of the complete blood count and the comprehensive metabolic panel, including liver-function tests, were within normal limits. Computed tomography (CT) of the head without the administration of contrast material was unremarkable. A lumbar puncture was performed, and 8 ml of clear cerebrospinal fluid was removed. Analysis of the cerebrospinal fluid revealed an elevated protein level (147 mg per deciliter [reference range, 25 to 55]), low glucose concentration (31 mg per deciliter [1.7 mmol per liter], with a reference range of 45 to 75 mg per deciliter [2.5 to 4.2 mmol per liter]), and neutrophilic pleocytosis (2304 white cells per cubic millimeter; 72% polymorphonuclear cells) (Table 1). Gram's staining was negative for organisms. The patient was started on therapy with vancomycin, ceftriaxone, ampicillin, and glucocorticoids and was admitted to the hospital. Routine bacterial cultures of the blood and cerebrospinal fluid were negative, and the glucocorticoids were stopped. The patient's symptoms improved with antimicrobial therapy, as well as analgesia with opiate and nonste-

From the Divisions of Infectious Diseases (A.C.P., J.L.C., S.S.S.) and Allergy, Pulmonary, and Critical Care Medicine (J.A.K., M.E.P.), Department of Medicine, and the Department of Pathology, Microbiology, and Immunology (J.E.S., C.A.R., B.C.M., X.J.W.), Vanderbilt University School of Medicine, Nashville. Address reprint requests to Dr. Pettit at Vanderbilt University School of Medicine, 1161 21st Ave. S., A2200 Medical Center North, Nashville, TN 37232-2582, or at april.pettit@vanderbilt.edu.

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Table 1. Results of Laboratory Testing.

Variable	First Admission	Second Admission				
		Day 1	Day 6	Day 11	Day 13	Day 15
Cerebrospinal fluid						
Source of sample	L4–L5	L3–L4	L3–L4	EVD	EVD	EVD
Opening pressure (cm of water)		33	24	>30		
Protein (mg/dl)†	147	319	247	193	93	80
Glucose (mg/dl)‡	31	2	1	63	50	59
White cells (per mm^3)	2304	4422	5863	14	27	341
Polymorphonuclear cells (%)	72	89	92	71	90	97
Lymphocytes (%)	23	4	2	21	4	2
Red cells (per mm^3)	3	34	21	2225	2850	14,700
Gram's staining	No bacteria	No bacteria	No bacteria	No bacteria	No bacteria	No bacteria
Bacterial culture	No growth	No growth	No growth	No growth	No growth	No growth
Fungal culture	Not performed	*Aspergillus fumigatus*§	No growth to date	No growth to date		No growth to date
Aspergillus antigen index	9.14¶	9.52¶	9.51¶			9.65
Serum						
Glucose (mg/dl)	108	109	107	147	127	139
Aspergillus antigen index			0.23‖			

* Additional cerebrospinal fluid studies, including tests for herpes simplex viruses 1 and 2 (by means of polymerase-chain-reaction [PCR] assays); varicella–zoster virus (PCR); West Nile virus (IgM antibodies by enzyme-linked immunosorbent assay [ELISA] and PCR); enterovirus (PCR); *Mycobacterium tuberculosis* (PCR); histoplasma antigen and cryptococcus antigen, 14-3-3, and tau protein (ELISA); and acanthamoeba and naegleria (Giemsa staining), as well as India-ink staining, cytologic examination, and acid-fast and viral culturing, were performed and remain negative to date. To convert the values for glucose to millimoles per liter, multiply by 0.05551. EVD denotes external ventriculostomy drain.

† The reference range for protein is 25 to 55 mg per deciliter.

‡ The reference range for glucose is 45 to 75 mg per deciliter

§ The cerebrospinal fluid culture was positive on hospital day 7.

¶ Aspergillus antigen in the cerebrospinal fluid was assessed retrospectively on frozen cerebrospinal fluid samples obtained at the first admission and on hospital days 1 and 6 of the second admission.

‖ The serum aspergillus antigen index was assessed on hospital day 7. An index of <0.5 is considered to be negative.

roidal antiinflammatory drugs. He was discharged home to complete a course of vancomycin and ceftriaxone for presumed community-acquired meningitis.

The patient presented 1 week after discharge with symptoms of headache and low back pain that had been present and progressively worsening over the previous 2 days. On presentation, his temperature was 36.9°C and he appeared ill, uncomfortable, and agitated, with incomprehensible speech. No erythema or drainage was noted in the lower lumbar area. Neurologic examination was limited by the patient's inability to participate, but there were no gross deficits. Magnetic resonance imaging (MRI) of the brain with gadolinium contrast material revealed pial enhancement and ventriculitis; spinal imaging revealed thoracic and lumbar pial enhancement and an epidural collection at the L4 to L5 level that was less than 1 cm. A lumbar puncture was performed (Table 1). Findings included a protein level of 319 mg per deciliter, glucose concentration of 2 mg per deciliter (0.1 mmol per liter), and white-cell count of 4422 per cubic millimeter (89% polymorphonuclear cells). Treatment with intravenous vancomycin, meropenem, and levofloxacin was initiated. By hospital day 2, his mental status was markedly improved.

On hospital day 6, increased somnolence, intermittent staring spells, and a transient right facial droop developed. A head CT scan without the administration of contrast material showed

mild hydrocephalus. An electroencephalogram (EEG) did not reveal seizure activity. Lumbar puncture was repeated (Table 1), and empirical treatment with liposomal amphotericin B was initiated. The following day, the microbiology laboratory reported that the cerebrospinal fluid sample from hospital day 1 of the current admission was growing *Aspergillus fumigatus*. Intravenous voriconazole was administered, and liposomal amphotericin B was continued. A CT scan of the chest did not show findings consistent with pulmonary fungal infection. Aspergillus antigen (galactomannan) testing from the three available cerebrospinal fluid samples was performed (Table 1). Tests for aspergillus antigen (galactomannan) in the serum were negative. A repeat MRI of the brain revealed new infarcts in the midbrain and cerebellum; examination of the paranasal sinuses was unremarkable.

On hospital day 11, the patient abruptly became unresponsive, with rhythmic shaking of the head that was consistent with seizure activity. He was intubated and mechanical ventilation was initiated. A head CT scan showed intraventricular hemorrhage involving the lateral ventricles, subarachnoid hemorrhage in the perimesencephalic cistern, and worsening hydrocephalus (Fig. 1A and 1B). An external ventriculostomy drain was placed. The results of tests performed on samples of cerebrospinal fluid are shown in Table 1. Cerebral angiographic imaging showed extensive vasospasm and focal dilatation of the right superior cerebellar artery that was suggestive of a mycotic aneurysm (Fig. 1C and 1D) and was not amenable to intervention. The results of EEG monitoring were suggestive of seizure activity, and antiepileptic therapy was initiated. Findings from the repeat analysis of the cerebrospinal fluid are shown in Table 1.

Despite improving findings on cerebrospinal fluid testing and control of seizure activity, there was no meaningful neurologic recovery. On hospital day 15, a repeat brain MRI showed that additional cerebral and cerebellar infarcts had developed (Fig. 1E and 1F). Given the severity of the neurologic injury, the family elected not to pursue aggressive medical intervention, and life support was discontinued. The patient died on hospital day 22, and an autopsy was performed.

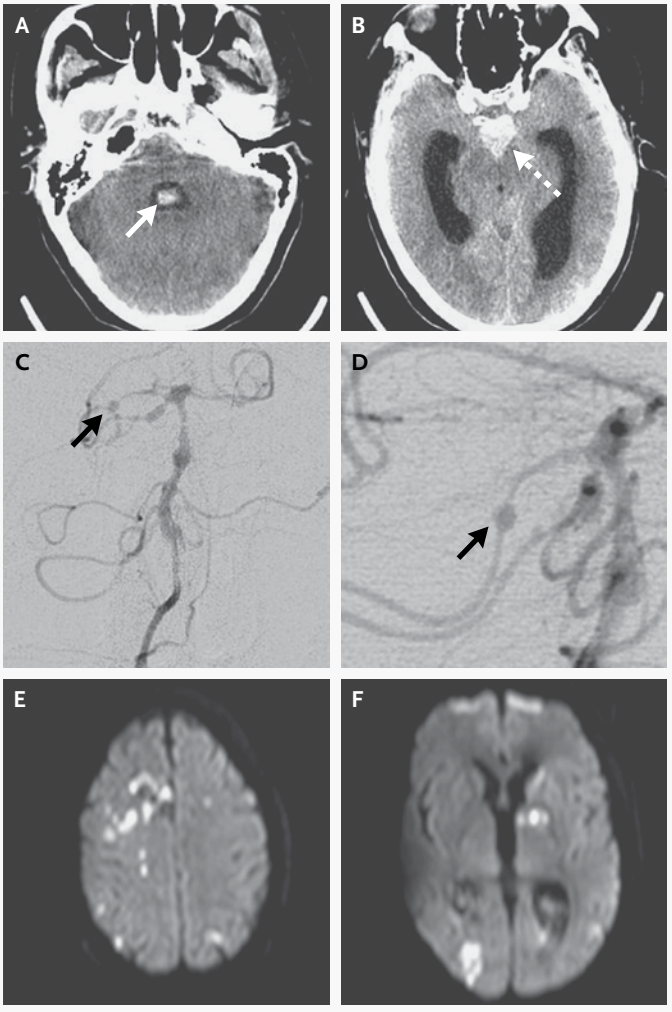

Figure 1. Imaging Studies.

Computed tomographic (CT) images of the head without the administration of contrast material, obtained on hospital day 11, show extensive hemorrhage into the fourth ventricle (Panel A, arrow) and subarachnoid hemorrhage in the perimesencephalic cistern (Panel B, dashed arrow). An angiographic image of the right vertebral artery (Panel C) shows focal segmental narrowing of the basilar artery that is consistent with vasospasm and a focal area of dilatation in the right superior cerebellar artery that is consistent with a mycotic aneurysm (arrow); a close-up view shows the mycotic aneurysm more clearly (Panel D). Diffusion-weighted images (Panels E and F) from magnetic resonance imaging of the brain performed on hospital day 16 show numerous areas of restricted diffusion (white) within cortical and deep structures, which are consistent with cerebral infarcts.

METHODS

CULTURE AND IDENTIFICATION OF FUNGUS

For fungal culturing, cerebrospinal fluid samples and tissue specimens obtained at autopsy were

inoculated directly onto solid agar mediums, including Sabouraud's dextrose agar; brain–heart infusion with 10% sheep's blood, chloramphenicol, and gentamicin; and Mycosel (BBL; Becton Dickinson). Slants were incubated at 30°C. Identification of mold isolates was made by means of macroscopic and microscopic morphologic methods (teasing preparation with lactophenol cotton-blue reagent), with an elevated temperature for growth.

ANTIGEN AND SUSCEPTIBILITY TESTING

Testing for aspergillus antigen (galactomannan) in the serum was performed with the use of an enzyme immunoassay at Associated Regional and University Pathologists (ARUP) laboratories, Salt Lake City. Testing for aspergillus antigen (galactomannan) in the cerebrospinal fluid was performed with the use of an enzyme immunoassay (Platelia, BioRad) at MiraVista Diagnostics, Indianapolis. In vitro susceptibility testing was performed with the use of broth microdilution methods for molds at ARUP laboratories.

AUTOPSY SPECIMENS

Formalin-fixed, paraffin-embedded tissue specimens were obtained from routine sites, as well as from the aneurysm at the right superior cerebellar artery, the spinal cord, and the lumbar spinal leptomeninges. Gomori methenamine silver and Fontana–Masson staining were performed in addition to routine hematoxylin and eosin staining.

RESULTS

FUNGAL CULTURE AND IDENTIFICATION OF MOLD

A mold was observed after 6 days of incubation in the fungal culture of the first cerebrospinal fluid sample obtained during the second hospital admission (Fig. 2A). This isolate was identified as *A. fumigatus*.[1] Extensive microbiologic sampling at autopsy yielded no growth of *A. fumigatus.* A single colony of cladosporium species, of unclear clinical significance, was isolated from an autopsy specimen of the dura overlying the frontal lobes.

ANTIGEN AND SUSCEPTIBILITY TESTING

Tests for aspergillus antigen (galactomannan) in the serum were negative (index, 0.23, with an index of <0.5 considered to be negative). The results of testing for galactomannan antigen in the cerebrospinal fluid are shown in Table 1. The isolate showed the following minimum inhibitory concentrations: amphotericin B, 1 μg per milliliter; itraconazole, 0.25 μg per milliliter; posaconazole, 0.06 μg per milliliter or less; and voriconazole, 0.5 μg per milliliter. The minimum effective concentration was 0.06 μg per milliliter or less for anidulafungin, caspofungin, and micafungin.

AUTOPSY SPECIMENS

Gross examination of the spine revealed gray discoloration of the left lumbar epidural compartment at the L4 to L5 level. No definitive epidural abscess was identified. A small amount of fluid was present in the L4 to L5 epidural space; a touch preparation and Gram's staining of the epidural fluid revealed septate, branching hyphal elements that were consistent with *A. fumigatus*. Dural puncture sites were not grossly evident for sampling. Incision of the dura revealed brown leptomeningeal discoloration spanning the length of the spinal cord.

Microscopic examination of the spinal cord at the T12 level revealed a focal infarction involving the white matter. Extensive leptomeningeal involvement of the spinal cord by hyphal elements was also identified (Fig. 2D). Results with Fontana–Masson staining were negative.

Gross examination of the brain revealed diffuse cerebral edema with markedly swollen gyri and diffuse, mild opacification of the cerebral meninges. Subarachnoid hemorrhage was present in the pons, midbrain, and superior aspect of the cerebellum. Coronal sections of the cerebral hemispheres revealed hemorrhage within the third and lateral ventricles and an infarct in the right frontal lobe. Two aneurysms of the right superior cerebellar artery were identified: a smaller aneurysm corresponding to that shown in Figure 1C and a second, larger aneurysm with evidence of rupture and an adherent blood clot.

Microscopic examination of the brain revealed multiple cerebral infarctions involving the frontal lobes, right occipital lobe, and left globus pallidus. The aneurysm in the superior cerebellar artery with gross evidence of rupture was examined microscopically and revealed necrotizing inflammation in the adventitia and hemorrhage (Fig. 2B). Rare foreign-body giant cells were identified. Gomori methenamine silver staining re-

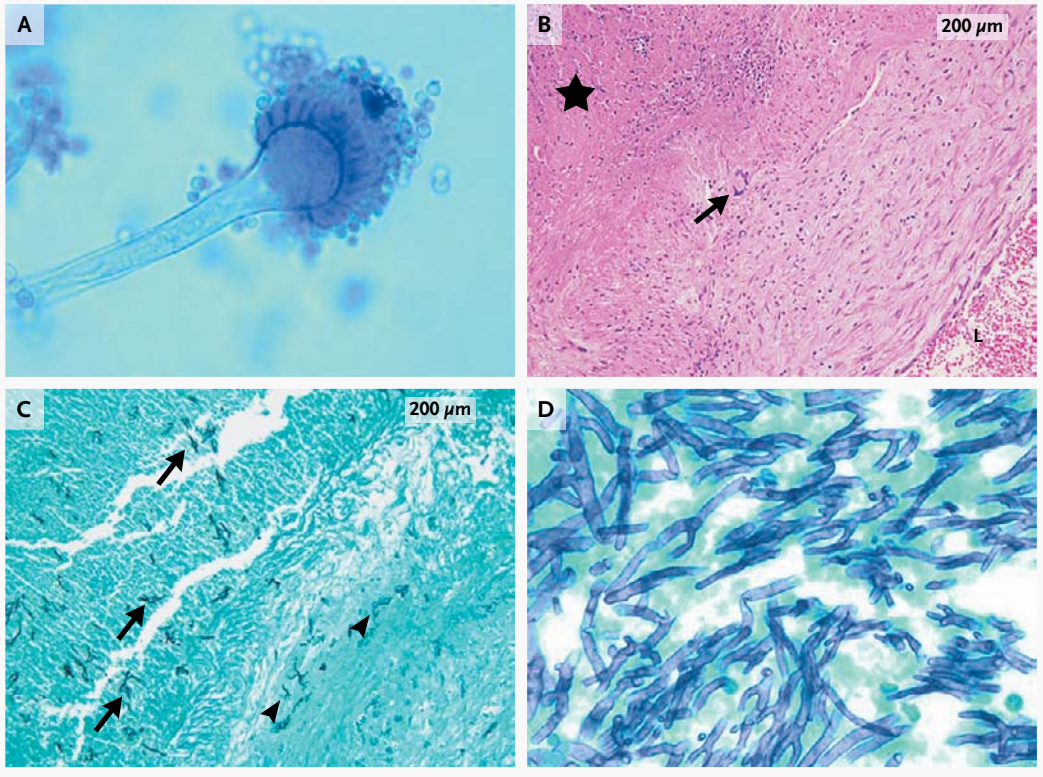

Figure 2. Pathological Studies.

Panel A shows the morphologic characteristics of *Aspergillus fumigatus* in the isolate from the first cerebrospinal fluid sample obtained during the second hospital admission. Panels B and C show sections of the right superior cerebellar artery. In Panel B (hematoxylin and eosin), necrotizing inflammation is evident in the adventitia (star), with foreign-body giant-cell reaction in the region of the aneurysm (arrow). L indicates the vascular lumen. In Panel C (Gomori methenamine silver stain), fungal hyphae can be seen within the wall of the artery (arrowheads), as well as within the area of necrotizing inflammation (arrows). Panel D (Gomori methenamine silver stain) shows a dense fungal hyphal mat in a section of the lumbar spinal leptomeninges.

vealed the presence of hyphae within the wall of the blood vessel as well as within the associated hemorrhage (Fig. 2C), a finding indicative of a mycotic aneurysm. On gross examination, there was no evidence of tissue infarction outside the central nervous system.

DISCUSSION

This case report describes the clinical presentation of neutrophilic meningitis in an immunocompetent man that did not improve despite broad-spectrum antibiotic therapy. Bacteria, particularly *Streptococcus pneumoniae* and *Neisseria meningitidis*, account for the vast majority of cases of acute neutrophilic meningitis.[2] Persistent neutrophilic meningitis is a syndrome defined by clinical meningitis, cerebrospinal fluid pleocytosis with more than 50% polymorphonuclear cells, elevated protein levels, and low glucose levels for more than 7 days despite appropriate empirical antimicrobial therapy.[3] The differential diagnosis of persistent neutrophilic meningitis is broad and includes both infectious and noninfectious causes. Among the most common infectious causes are atypical bacterial organisms, such as nocardia and actinomyces, and fungal organisms, including candida, aspergillus, and mucorales (formerly zygomycetes).[3] In this case, a broad evaluation for potential causes of persistent neutrophilic meningitis was conducted, and ultimately, fungal cultures of the cerebrospinal fluid from

the first day of the second hospital admission grew *A. fumigatus.*

Aspergillus species are ubiquitous in the air, soil, and organic matter.[4] Invasive disease, most commonly due to *A. fumigatus*, is rare among immunocompetent hosts.[5,6] The organism typically enters the body through the sinopulmonary tract or through a break in the skin. Invasion of the central nervous system can occur either through direct extension from the paranasal sinuses or by hematogenous dissemination though a pulmonary or cutaneous source.[7] In this case, the absence of evidence of infection at these sites led to the consideration of alternative portals of entry into the central nervous system. A dural puncture could permit direct transit of an organism from the epidural space into the intradural compartment. In this case, a dural puncture site was not grossly evident. However, the degree of involvement of the lumbar meninges by fungal hyphae is compatible with direct extension. In addition, the absence of infarcts in tissues outside the central nervous system is compatible with direct extension rather than hematogenous spread. Given the identification of a potential exposure through an epidural injection, the state health department was notified.

Aspergillosis in the central nervous system carries a poor prognosis, despite the availability of antifungal agents with good activity against aspergillus species and penetration of the central nervous system.[8,9] Premortem diagnosis requires a high index of clinical suspicion. Patients typically present with focal neurologic deficits; meningeal signs are rare.[10] Although radiographic imaging may be useful for identifying focal lesions or secondary complications, aspergillus meningitis is usually characterized by an absence of parenchymal lesions.[10] Angioinvasion by this organism is common and results in vascular thrombosis, tissue infarction, and hemorrhage.[10] Chemical testing of the cerebrospinal fluid is nonspecific, often showing pleocytosis with varying proportions of polymorphonuclear and mononuclear cells, elevated protein levels, and low-to-normal glucose levels.[10] Isolation of aspergillus from the cerebrospinal fluid is difficult and often requires repeated testing of large-volume samples.[11] The detection of aspergillus galactomannan in serum samples by means of an enzyme immunoassay has been validated for the diagnosis of invasive aspergillosis. This assay has also shown promise with cerebrospinal fluid specimens for the early diagnosis of central nervous system aspergillosis, although the threshold value for the diagnosis has not been determined.[12]

Early diagnosis and initiation of appropriate treatment can improve the outcomes of central nervous system aspergillosis.[13] Voriconazole is the primary recommended therapy for this condition. Intrathecal administration of antimicrobial agents is not recommended and may be complicated by chemical arachnoiditis, seizures, headache, and altered mental status.[7] Surgical resection of focal lesions, if present, should be considered.[14]

Diagnostic testing for aspergillus may not be performed routinely in cases of acute neutrophilic meningitis. Additional diagnostic testing for atypical pathogens should be pursued if the symptoms persist despite appropriate empirical therapy. If an atypical pathogen such as *A. fumigatus* is identified, a careful search for potential sources of exposure should be performed. In this case, the identification of potential exposure through epidural injection and the reporting of the case to the state health department led to an epidemiologic investigation that identified a multistate outbreak of fungal meningitis associated with epidural glucocorticoid injections.

Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

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