The Laryngoscope

Case Report

Fungal Skull Base Osteomyelitis: Emerging Microbial Identification Techniques

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Culture-based pathogen identification in skull base osteomyelitis, particularly for fungi, is often inaccurate. We report the case of patient with fungal skull base osteomyelitis cured by sustained antifungal therapy after 16 months of debilitating illness. Due to medical complications, a strong clinical rationale was needed to justify long-term antifungal therapy. The offending fungus was identified by experimental molecular technology (Ibis T5000 universal biosensor); invasive fungal disease was corroborated by biochemical assays. Our discussion will help familiarize the otolaryngologist with existing biochemical and molecular diagnostics for invasive fungal disease. We encourage future investigators to study their application in cases of skull base osteomyelitis.

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INTRODUCTION

Contemporary series report that fungi are responsible for 10% of cases of skull base osteomyelitis (SBO).[1,2] This contrasts with historical paradigms, which implicate *P. aeruginosa* in 99% of cases and fungi only rarely—and among severely immunocompromised patients.[3] The true incidence of fungal SBO may even be higher; 21% of SBO cases fail to grow organisms in culture (culture-negative),[4] and 60% of histologically confirmed invasive Aspergilloses are culture-negative.[5] These data, and the observation that invasive fungal disease is on the rise,[6] suggest that historical paradigms of the microbiology of SBO fail to explain the observations of current practice. For example, in the senior neurotologist's (T.A.H.) practice, fungal SBO was diagnosed in four diabetic patients over the last 5 years, including one case attributed to *C. parapsilosis* (a commensal organism in humans and never reported as an SBO pathogen).

If the incidence of fungal SBO is truly higher than expected (and rising), culture-based methods of diagnosis are a hurdle to timely and accurate management. In fact, diagnostic delays of about 7 months are typical for cases of fungal SBO.[4] In immunocompromised hosts, such delays should be alarming, particularly because this disease is mortal in 27% to 36% of patients.[5,7] Fortunately, other laboratory tests are available for invasive fungal disease.[6]

We report the use of a novel molecular technology for pathogen identification in SBO, applied in compassionate use. In this case, cultures failed to meaningfully guide treatment, and the disease relapsed and progressed. Because we were able to identify a fungal pathogen with this experimental method and corroborate the presence of invasive fungal disease via approved biochemical assays, physicians were more willing to commit to long-term antifungal therapy and the patient was cured.

CASE REPORT

A 45-year-old diabetic male was treated for right otitis externa with otic and oral ciprofloxacin without resolution (months 0–3). The patient was referred to our subspecialty practice. Technetium-99m medronate scintigraphy revealed increased turnover in the right temporal bone but no osteomyelitis. Diagnosis of necrotizing otitis externa was made and empiric treatment was continued. Culture results from the ear canal on two occasions yielded few coagulase-negative *Staphylococci*.

On month 5, headaches, weight loss, and intractable nausea led to hospital admission. Magnetic resonance (MR) imaging revealed osteomyelitis involving the right temporal bone and clivus, and sigmoid sinus thrombosis (confirmed on MR venography) without hydrocephalus. Treatment was initiated with IV (intravenous) ciprofloxacin, cefepime, and vancomycin. Lumbar puncture opening pressure was 16 cm H_2O; cerebrospinal fluid chemistries and cell counts were normal; and no bacteria or fungus were isolated after 25

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days of incubation. Diagnostic right mastoidectomy specimens revealed chronic inflammation and no organisms using Grocott's methenamine silver stain. Sigmoid sinus patency returned. Titers for Lyme disease and human immunodeficiency virus were undetectable, and purified protein derivative was nonreactive. The patient's pain led him to suicidal ideation and he was treated for major depression. With initiation of empiric IV voriconazole and hyperbaric oxygen therapy, the patient's pain improved, as well as oral intake. Simultaneously, the patient developed a right cranial nerve (CN) VI palsy for which no intraorbital etiology was identified. At hospital discharge (month 6), the patient was receiving IV ciprofloxacin, vancomycin, and (empiric) voriconazole for right skull base osteomyelitis. While on this antimicrobial regimen and hyperbaric oxygen therapy, the abducens weakness resolved. However, Gallium (Ga)-67 uptake remained elevated in the right temporal bone. The patient's pain continued to improve.

Four weeks after hospital discharge (month 7), the patient developed acute kidney injury, and all antimicrobials and hyperbaric dives were discontinued. Gallium-67 scan appeared improved, but by month 8 was again increased in the right skull base. Antimicrobial monotherapy with daptomycin was initiated based on the only available culture data (coagulase-negative staphylococci). Glycemic control was strict.

On month 11, disease progressed and the patient was hospitalized with new *left* (contralateral) otorrhea, dysphagia, aspiration, and palsies of left CN VII (grade 4/6) and X. The patient's condition was guarded; he had lost 80 lbs since diagnosis. Otomicroscopy revealed new mycotic debris in the left ear canal and bilateral serous otitis media. Swabs were obtained from the left ear canal, and bilateral tympanocentesis was performed. The swab was sent for culture. There was enough tympanic fluid for two specimens from each ear: one used for culture and another, with the patient's permission, submitted to the Allegheny-Singer Research Institute (Pittsburgh, PA) for DNA analysis using the Ibis T-5000 universal biosensor. This technology was made available for compassionate use. Molecular results revealed *Aspergillus* spp. in the right ear and *P. acnes* and methicillin-resistant *S. epidermidis* in the left ear. Cultures revealed no growth. Infectious disease consultation was obtained. Serum tests for 1,3-Beta-D-glucan (Fungitell, Associates of Cape Cod, Inc., East Falmouth, MA)) and galactomannan were performed, yielding 189 pg/mL (positive) and 0.22, respectively. Voriconazole IV was added to daptomycin IV. The patient was discharged after 1 week; erythrocyte sedimentation rate had decreased from 68 to 55. Cranial nerve palsies persisted.

At month 14, Ga-67 uptake was decreased in the left skull base and normal on the right. Left facial nerve improved to grade 2/6 and the patient was gaining weight. The patient remained symptom-free, corroborated by a clear Ga-67 scan on month 16, and antimicrobial therapy was discontinued. Now over 4 years since initial diagnosis, the patient is back to work; has normal facial function, voice, and deglutition; and no signs of osteomyelitis.

DISCUSSION

This particular case reveals a waxing/waning clinical course, with several possible causative microorganisms. However, given the molecular and biochemical evidence for fungi and the patient's improvement (and cure) only while receiving antifungal therapy, a hypothesis of fungal pathogenicity is well supported.

Numerous molecular techniques utilizing polymerase chain reaction (PCR) can detect fungi, but their clinical utility has been challenged.[6] A technical enhancement to strict PCR emerged in 2005, utilizing mass spectrometry of amplified PCR products to rapidly identify the genomic fingerprint of individual species present in a sample. This technology, originally developed for biological weapons defense, was refined for research as the Ibis T-5000 universal biosensor.[8,9] Because the biosensor technology does not require organisms to grow (and compete for nutrients) in culture, it can detect indolent pathogens. This is particularly relevant to the study of biofilms and chronic infections.[10] The Ibis system can process a variety of specimens, including skin and mucosal swabs, lavage fluid, sputum, and blood.[8] The cellular content in a given sample is then lysed to release the nucleic acids for analysis. Although the universal biosensor cannot provide information about the behavior of an organism present in a sample, it can accurately determine its concentration, thereby distinguishing commensal flora from pathogens.[9]

The universal biosensor technology developed by Ibis Biosciences, Inc., was acquired by Abbott in 2009. It is now marketed under the name IRIDICA and is CE (European Conformity) marked for in vitro diagnostics. Although it has been proven sensitive and highly accurate compared to conventional methods of pathogen identification, it is approved in the United States for experimental use only. As of this writing, the only U.S. laboratories that have operational Ibis machines are the Allegheny Singer Research Institute in Pittsburgh, Pennsylvania, and the Ibis Biosciences facility in Carlsbad, California. The ASRI currently charges about $300 for analysis of specimens.

Several commercially available biochemical assays can diagnose invasive fungal infections. Fungitell (Associates of Cape Cod, Inc.) is a U.S. Food and Drug Administration-approved panfungal assay for the detection of the cell wall polysaccharide 1,3-Beta-D-glucan (BDG). It requires 3 to 5 mL of whole blood, centrifuged to yield 0.5 mL serum, and refrigerated at 2°C to 8°C or frozen for transport. Cerebrospinal fluid must be frozen for transport. The test exhibits 70% to 100% sensitivity and 75% to 90% specificity in the detection of fungal pathogens[6] and takes less than 1 hour. It is not, however, species-specific, because BDG is present in cell walls of *Candida*, *Aspergillus*, and several other genera (except, notably, Mucorales and *Cryptococcus*); false positives may occur in the presence of certain antibiotics (including cefepime, cefotaxime, and amoxicillin-clavulanate), bacteria, materials, or blood products.[6] U.S. Food and Drug Administration-approved assays for galactomannan (GM), which is specific to *Aspergillus* cell wall,

are also available (Platelia Aspergillus EIA, Bio-Rad, Hercules, CA).[6] This test also requires a whole blood sample, with a minimum volume of 1 mL serum after centrifugation, and refrigeration or freezing for transport. The BDG and GM assays are considered valid detection methods for invasive fungal infections; our laboratory bills insurance $96.00 and $207.00, respectively, for these tests (and provides a discounted price for self-pay patients).

CONCLUSION

Patients with SBO and unyielding cultures should raise the index of suspicion for fungal pathogens. Although biopsy and culture are standard of care, current data indicates these may not be sufficient for diagnosis. Rapid biochemical assays can identify the presence of invasive fungi, and in refractory or severe cases, the (currently) off-label use of biosensor technology may identify the offending organism(s). This technology has been proven sensitive and accurate for other infected tissues; however, its validity for skull base infections is unknown and only a few labs have the equipment. Consequently, research studies of the biosensor technology and SBO would require a multi-institutional effort to clarify the problem of culture negativity in the microbiology of this disease. For off-label clinical use, in the authors' opinion, an understanding must exist between the treating physician and the laboratory—and the patient must give their informed consent. The authors hope that this knowledge will inspire further study into the microbiology of SBO. In the meantime, selective application of molecular diagnostics in the care of SBO patients may reduce treatment delays and avoid lengthy, morbid courses of illness.

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BIBLIOGRAPHY

1. Spielmann PM, Yu R, Neeff M. Skull base osteomyelitis: current microbiology and management. *J Laryngol Otol.* 2013; 127(S1):S8–S12.
2. LeClerc N, Verillaud B, Duet M, et al. Skull base osteomyelitis: incidence of resistance, morbidity, and treatment strategy. *Laryngoscope* 2014;124: 2013–2016.
3. Hirsch BE. Otogenic skull base osteomyelitis. In: Jackler RK, Brackmann DE, eds. Neurotology. 2nd ed. Philadelphia, PA: Mosby; 2005: 1096–1106.
4. Jacobsen LM, Antonelli PJ. Errors in the diagnosis and management of necrotizing otitis externa. *Otolaryngol Head Neck Surg* 2010;143: 506–509.
5. Stodulski D, Kowalska B, Stankiewicz C. Otogenic skull base osteomyelitis caused by invasive fungal infection. *Eur Arch Otorhinolaryngol* 2006; 263:1070–1076.
6. Baskova L, Buchta V. Laboratory diagnostics of invasive fungal infections: an overview with emphasis on molecular approach. *Folia Microbiol* 2012;57:421–430.
7. Blyth CC, Gomes L, Sorrell TC, et al. Skull-base osteomyelitis: fungal vs. bacterial infection. *Clin Microbiol Infect* 2011;17:306–311.
8. Ecker DJ, Drader JJ, Gutierrez J, et al. The Ibis T5000 universal biosensor: an automated platform for pathogen identification and strain typing. *J Lab Autom* 2006;11:341–351.
9. Ecker DJ, Sampath R, Massire C, et al. Ibis T5000: a universal biosensor approach for microbiology. *Nat Rev Microbiol* 2008;6:553–558.
10. Boase S, Foreman A, Cleland E. The microbiome of chronic rhinosinusitis: culture, molecular diagnostics and biofilm detection. *BMC Inf Dis* 2013; 13:210–218.