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Multiple Cranial Neuropathies Due to a Mixed Infection in Skull Base Osteomyelitis: A Nanopore Sequencing Study

Keun Tae Kim[a]
Yu Hun Jeong[a]
Seon-Jae Ahn[b]
Jangsup Moon[b,c]
Yong Won Cho[a]
Kon Chu[b,d]

[a]Department of Neurology, Keimyung University School of Medicine, Daegu, Korea
[b]Department of Neurology, Seoul National University Hospital, Seoul, Korea
[c]Department of Genomic Medicine, Seoul National University Hospital, Seoul, Korea
[d]Comprehensive Epilepsy Center, Biomedical Research Institute, Seoul National University Hospital, Seoul, Korea

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Correspondence
Kon Chu, MD, PhD
Department of Neurology, Comprehensive Epilepsy Center, Biomedical Research Institute, Seoul National University Hospital, 101 Daehak-ro, Jongno-gu, Seoul 03080, Korea
Tel +82-2-2072-1878
Fax +82-2-3672-7553
E-mail stemcell.snu@gmail.com

Dear Editor,

Skull base osteomyelitis (SBO) has substantial mortality and its incidence is increasing due to the growing prevalence of immunodeficiency diseases such as AIDS, diabetes mellitus, chemotherapy, and immune suppressants.[1,2] *Staphylococcus aureus* and *Pseudomonas aeruginosa* are reported as the two most common causative pathogens in SBO.[2] However, identifying the offending pathogen in SBO is challenging due to the vagueness of its clinical symptoms, and tissue cultures and imaging modalities may only reveal the tip of the iceberg.[2-4] Nanopore technology is a new technique for sequencing that involves measuring the current flow through nanoscale molecular holes. Nanopore sequencing provides many advantages for metagenomics research, including simple and rapid library preparation, and real-time analysis of the reads.[5] Here we report a case of multiple cranial neuropathies caused by SBO of mixed infection in order to raise awareness of this condition.

A 70-year-old male presented to the neurology clinic with right ocular pain and facial palsy. He had been taking cephalosporins since visiting the otolaryngology clinic 5 days previously, but facial pain and diplopia had developed over the preceding 2 days. In addition, total right-eye blindness occurred, which prompted him to visit the neurology clinic. He had no previous illness other than hypertension. The examination at the neurology clinic revealed multiple unilateral cranial nerve (CN) palsy, including of CNs 2, 3, 4, 5, 6, and 7. He had right facial pain and headache, but was alert without fever or neck stiffness. The initial laboratory test revealed leukocytosis at 16,070/mm^3 with left shifting (neutrophils at 79.6%) and elevated C-reactive protein at 11.2 mg/dL. Serum glucose and HbA1C were 262 mg/dL and 14.9%, respectively. Paranasal sinus computed tomography (PNS-CT) revealed fatty infiltrations in the inferomedial extraconal space of the right orbit, and retrobulbar and retromaxillary fat, suggesting inflammation around the right sinus (Fig. 1A-C). Since endoscopic sinus surgery (ESS) led to methicillin-resistant *Staphylococcus aureus* (MRSA) being identified in tissue cultures, we administered vancomycin. After 2 weeks the CN palsy remained unchanged, but the facial pain had gradually improved. Follow-up PNS-CT showed progression of inflammation involving the pterygoid muscle, sinus wall erosion, and soft-tissue invasion into the right middle ear (Fig. 1D-F). With clinical suspicion of invasive fungal infection, the patient underwent a second ESS with debridement and tissue culture. We performed 16S rDNA PCR for bacterial identification (Fig. 1G) and PCR of the D1/D2/D3 region of the large-subunit rDNA (Fig. 1H) for fungal detection in tissue specimens. Nanopore sequencing (Oxford Nanopore Technologies, Oxford, UK) was performed from the PCR products to identify *Acinetobacter bereziniae* and multiple fungal strains, including *Malassezia globosa*, *Yarrowia lipolytica*, *Aspergillus clavatus*, *Fusarium verticillioides*, and *Candida albicans*.

The facial edema and headache improved after initiating liposomal amphotericin B at 5.0 mg/kg. The extents of soft tissue swelling and necrosis were decreased in follow-up images.

Based on the clinical response and diagnostic evaluations, the neuromicrobiological manifestations were compatible with SBO involving a mixed infection of bacteria and fungi. *Candida albicans*, *Fusarium proliferatum*, and *Acinetobacter baumannii* were identified in the tissue culture at 1 week after initiating the antifungal agent.

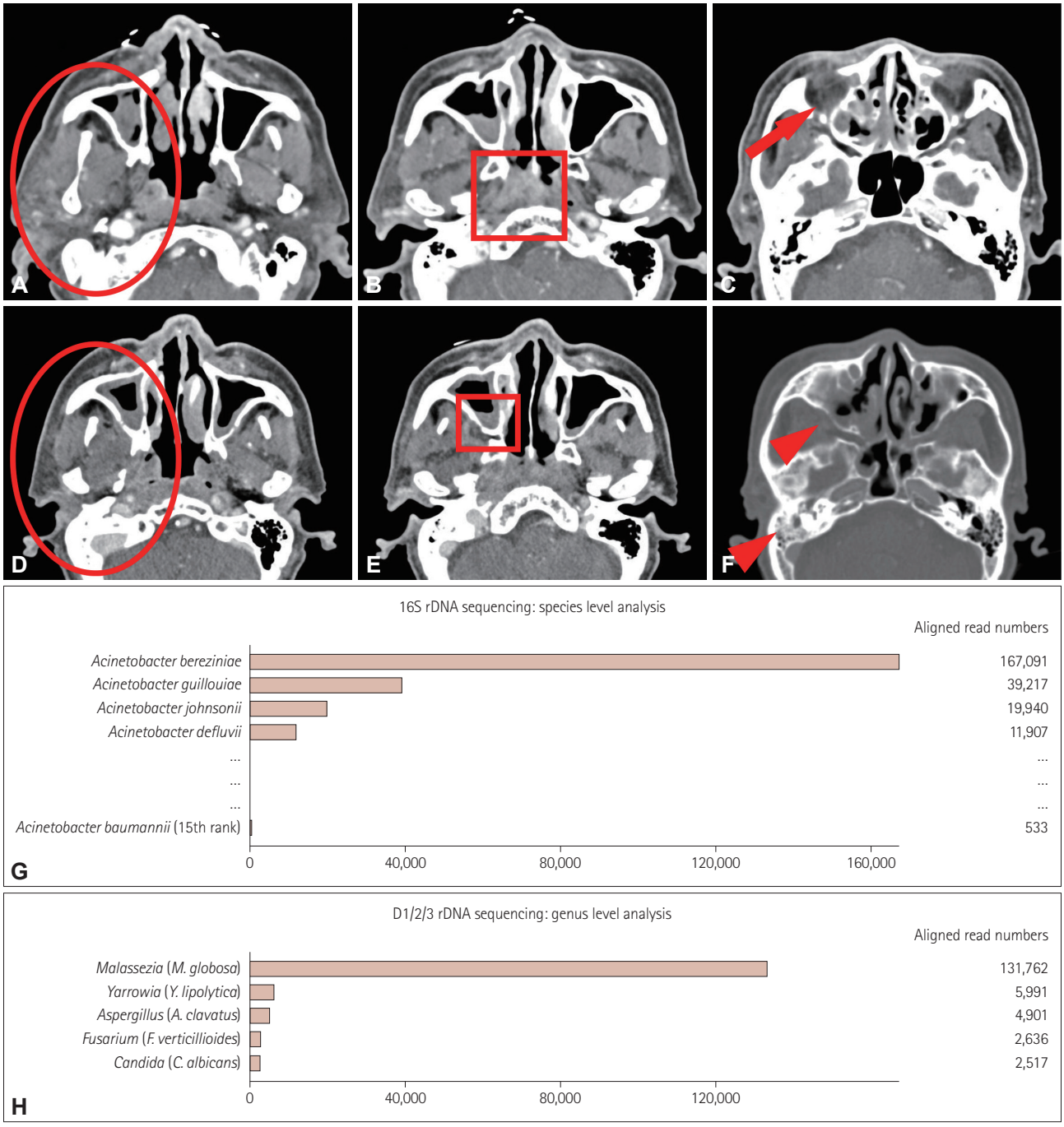

Fig. 1. Enhanced paranasal sinus computed tomography before (A–C) and after (D–F) antibacterial treatment, and nanopore sequencing data (G and H). A: Fat infiltration, and loss of fat plain of pterygoid space and fat infiltration with swelling and enhancement (ellipse). B: Swelling and enhancement of the nasopharynx (square). C: Fat infiltration in the inferior portion of the orbit (arrow). D: Nondifferentiation of the pterygoid space due to the progression of inflammation (ellipse). E: Penetration of the posterior wall of the maxilla by fat infiltration (square). F: Bone erosion in the posterior wall of the maxilla (upper arrowhead), and soft tissue invasion into the right middle ear (lower arrowhead). G: 16S rDNA sequencing for species-level analysis. The most-closely aligned species are considered pathogen species. The result indicates that *Acinetobacter baumannii* is not the causative agent. H: D1/D2/D3 rDNA sequencing for genus-level analysis. The species in parentheses is the most-closely aligned for the corresponding genus.

Tissue culture is usually considered to confirm the causative strain; however, it has a low sensitivity and may miss a mixed infection.[1,4] In our case, antifungal treatment was delayed since only MRSA was identified in the first tissue culture and as the causative agent. However, a reassessment was needed since clinical discrepancy was suspected after 2 weeks of the appropriate antibiotic treatment. MRSA is believed to be a cofactor rather than coexisting independently, because *Staphylococcus aureus* has the potential to cause inflammation, tissue barrier disruption, and mucociliary dysfunction.[6]

A mixed infection of bacteria and fungi should be considered in SBO, since approximately 50% of cases might be negative in tissue cultures.[7] Clinicians should be aware of this protean condition to differentiate it from treatment failure or an insufficient treatment period. Nanopore technology can contribute to the early identification of multiple pathogens. However, it should still be considered as a complementary diagnostic approach rather than as providing a definitive diagnosis, since the possibility of sequencing errors and misalignments are limitations of sequencing-based diagnostics.

Author Contributions

Conceptualization: Keun Tae Kim, Kon Chu. Data curation: Yu Hun Jeong, Seon-Jae Ahn, Jangsup Moon. Writing—original draft: Keun Tae Kim. Writing—review & editing: all authors.

ORCID iDs

Keun Tae Kim https://orcid.org/0000-0002-7124-0736
Yu Hun Jeong https://orcid.org/0000-0001-6220-7953
Seon-Jae Ahn https://orcid.org/0000-0003-0520-7812
Jangsup Moon https://orcid.org/0000-0003-1282-4528
Yong Won Cho https://orcid.org/0000-0002-6127-1045
Kon Chu https://orcid.org/0000-0001-5863-0302

Conflicts of Interest

The authors have no potential conflicts of interest to disclose.

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