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**Molecular Testing in a Case of Persistent, Contact Lens-related Acanthamoeba Keratitis**

***Clinical history:*** The patient is a 43-year-old male who sustained a left corneal abrasion while removing his corneal refractive therapy contacts. The abrasion progressed over the following month into a corneal ulcer for which he sought evaluation, and was placed on Tobramycin OS, Besifloxacin OS, Doxycycline PO, and Bacitracin OS for a suspected bacterial infection. Bacterial and fungal culture results were negative. He returned to a follow-up appointment with complaints of increasing ocular pain, headaches, and vision loss, and was placed on prednisolone OS to reduce inflammation.

The patient’s symptoms worsened while traveling to San Diego and he sought further care. In addition to the antibiotic regimen, Polyhexamethylene biguanide, Chlorhexidine, and Valacyclovir were added for *Acanthamoeba* and Herpes coverage, and the prednisolone was stopped. He experienced continued worsening of his vision, developing “unmanageable” pain and pressure sensation in and around the eye with left-sided headache. He presented to the Emergency Room 8 days after starting the PHMB, chlorhexidine and valacyclovir. There was still no growth from the initial bacterial and fungal cultures, and cultures for Acanthamoeba and HSV obtained in San Diego were negative.

***Eye exam:*** Visual acuities were (without correction) 20/50 OD and Hand Motion only OS. Ocular pressures were 13 mmHg OD and 28 mmHg OS. The right eye appeared normal. The left showed lid crusting, 4+ conjunctival injection, and a limbal flush. A 5 x 5 mm corneal epithelial defect with a 6 x 7 (h x w) ringed infiltrate with relative central clearing was present on a diffusely hazy background. There was minimal stromal thinning, and a patchy endothelial plaque superiorly beneath the ring, with 4+ hypopyon. The left pupil was dilated and there was poor visualization of the lens and vitreous.

***Laboratory studies:*** A second eyewash specimen was sent for *Acanthamoeba* molecular testing.

He received treatment to bring down his intraocular pressure and cyclopentolate was added. The next day, real-time polymerase chain reaction testing demonstrated the *Acanthamoeba* 18S ribosomal RNA gene at approximately 16-fold higher levels than the 1,000 copy number standard. The severity of the infection despite ongoing treatment, as suggested by the PCR curves, raised the possibility of a pipetting error. *Acanthamoeba* treament was continued with discontinuation of tobramycin and bacitracin. Although there was improvement in the epithelial defect and hypopyon, the patient continued to experience pain and received a corneal transplant 3 weeks after presentation to the ER.

***Gross pathology:*** The specimen consisted of a soft, transparent, and irregular portion of cornea measuring 0.8 x 0.5 x 0.1 cm.

***Microscopic pathology:***

<http://image.upmc.edu:8080/NeuroPathology/Eye/Eye5/EP5.28.svs/view.apml>?

Histologic sections demonstrated numerous *Acanthamoeba* trophozoites and encysted forms on a background of acute necrotizing keratitis. In contrast to the typical appearance post-treatment, numerous viable *Acanthamoeba* protozoa were observed both superficially and deep with only occasional necrotic forms, consistent with resistance to medical therapy. PAS stain was negative for fungal organisms.

***Pathological diagnosis:*** Acute necrotizing*Acanthamoeba* keratitis.

***Clinical follow-up:*** The culture sent at time of transplant eventually grew *Acanthamoeba.* The patient did well after the transplant, with no signs or symptoms of continued infection. At 4 years after transplant, the graft has remained clear with some mild superior punctate epithelial erosions attributed to dry eye.

***Discussion:***

The prevalence of *Acanthamoeba* keratitis is estimated to be 1.2 per million adults using soft contacts in the United States, and recent studies have suggested as much as a ten-fold higher incidence [1].

Although Acanthamoeba keratitis may be more common than previously suspected, the diagnosis of Acanthamoeba keratitis may be problematic as the clinical features can overlap with herpetic, bacterial, and fungal infections [2]. Furthermore, concurrent infection with more than one agent may occur. A number of methods have been used to identify *Acanthamoeba* including culture, biopsy, molecular testing, and confocal microscopy. Although each testing modality provides useful information, molecular testing for *Acanthamoeba* has proven to be highly sensitive and fast.

Molecular detection of *Acanthamoeba* at our institution involves a PCR-based assay of eyewash specimens and/or contact lens solution. Swabs, scrapings or biopsy is also acceptable. Testing is based on a primer and probe set originally validated by Qvarnstrom et al. at the CDC as part of multiplex assay for several different free-living amoeboid parasites [3]. The primer and probe were adapted and validated for *Acanthamoeba* specificity and detection down to 0.7 cysts/10 microliters and 2.3 trophozoites per 10 microliters [4]. Amplification of the *Acanthamoeba* genome is directed towards a region of the 18s rRNA gene that is shared by 40 *Acanthamoeba* spp. variants. The probe uses a TaqMan strategy requiring the exonuclease activity of Taq polymerase to digest the bound probe, releasing the fluorescent probe signal into the reaction solution and away from its proximity on a quencher molecular on the undigested probe.

As seen in this case, PCR testing of the *Acanthamoeba* 18s ribosomal RNA gene from eye wash specimens provides rapid, non-invasive results that can be used to confirm or guide anti-microbial therapy. In addition to slower turnaround times, cultures may fail to detect the organism as in this case where the initial culture was negative, with the second one reporting positive after the corneal transplant. Boggild et al. demonstrated that from 107 various ophthalmic specimens the sensitivity and specificity of molecular testing was 90% and 90.8%, cultures were 73.7% and 100%, and direct microscopy was 55% and 100%, respectively [5]. Although microscopy is also rapid and non-invasive, it has been noted that confocal microscopy may not discriminate between macrophages and amebicorganisms [6]. In a followup study, sensitivity and specificity of the Qvarnstrom PCR assay were 100% each against culture results [7]. The enhanced sensitivity of PCR testing and the ability to rapidly return results can significantly affect patient care. In addition, the copy number at the time of diagnosis may be predictive of outcome [8].

Although it has been assumed that lack of adaptive immunity to *Acanthamoeba* keratitis may be related to the avascular nature of the cornea or to immune privilege of the eye, studies in a case where immune privilege was already breached due to prior herpetic keratitis suggest alternative mechanisms of immune invasion [9]. Encystation of trophozoites may contribute to treatment resistance, and topical beta adrenoceptor blockers have been suggested for adjunct therapy to reduce the transformation of trophozoites into the highly resistant cyst stage [10]. Although not available for clinical use at this time, speciation by PCR could become a valuable theranostic tool, as non-T4 genotypes are associated with therapeutic resistance [11].

To summarize, this is an unusual case of treatment-resistant *Acanthamoeba* keratitis in which PCR played a key role in establishing the diagnosis, allowing for definitive treatment. The case is also unusual in the extent of PCR and pathological findings. Histologic demonstration of viable trophozoites and cysts in large numbers are not often visualized. Two-thirds of patients are treated without need for transplantation [12], and late stage transplants typically exhibit necrotic organisms and/or empty cysts.

***References:***

1. Page, M.A. and W.D. Mathers, *Acanthamoeba keratitis: a 12-year experience covering a wide spectrum of presentations, diagnoses, and outcomes.* J Ophthalmol, 2013. **2013**: p. 670242.

2. Dart, J.K., V.P. Saw, and S. Kilvington, *Acanthamoeba keratitis: diagnosis and treatment update 2009.* Am J Ophthalmol, 2009. **148**(4): p. 487-499 e2.

3. Qvarnstrom, Y., G.S. Visvesvara, R. Sriram, and A.J. da Silva, *Multiplex real-time PCR assay for simultaneous detection of Acanthamoeba spp., Balamuthia mandrillaris, and Naegleria fowleri.* J Clin Microbiol, 2006. **44**(10): p. 3589-95.

4. Thompson, P.P., R.P. Kowalski, R.M. Shanks, and Y.J. Gordon, *Validation of real-time PCR for laboratory diagnosis of Acanthamoeba keratitis.* J Clin Microbiol, 2008. **46**(10): p. 3232-6.

5. Boggild, A.K., D.S. Martin, T.Y. Lee, B. Yu, and D.E. Low, *Laboratory diagnosis of amoebic keratitis: comparison of four diagnostic methods for different types of clinical specimens.* J Clin Microbiol, 2009. **47**(5): p. 1314-8.

6. Mathers, W.D., S.E. Nelson, J.L. Lane, M.E. Wilson, R.C. Allen, and R. Folberg, *Confirmation of confocal microscopy diagnosis of Acanthamoeba keratitis using polymerase chain reaction analysis.* Arch Ophthalmol, 2000. **118**(2): p. 178-83.

7. Karsenti, N., R. Lau, A. Purssell, A. Chong-Kit, M. Cunanan, J. Gasgas, J. Tian, A. Wang, F. Ralevski, and A.K. Boggild, *Development and validation of a real-time PCR assay for the detection of clinical acanthamoebae.* BMC Res Notes, 2017. **10**(1): p. 355.

8. Ikeda, Y., D. Miyazaki, K. Yakura, A. Kawaguchi, R. Ishikura, Y. Inoue, T. Mito, A. Shiraishi, Y. Ohashi, S. Higaki, M. Itahashi, M. Fukuda, Y. Shimomura, and K. Yagita, *Assessment of real-time polymerase chain reaction detection of Acanthamoeba and prognosis determinants of Acanthamoeba keratitis.* Ophthalmology, 2012. **119**(6): p. 1111-9.

9. Knickelbein, J.E., J. Kovarik, D.K. Dhaliwal, and C.T. Chu, *Acanthamoeba keratitis: a clinicopathologic case report and review of the literature.* Hum Pathol, 2013. **44**(5): p. 918-22.

10. Heaselgrave, W. and S. Kilvington, *The Characterization of an Adrenergic Signalling System Involved in the Encystment of the Ocular Pathogen Acanthamoeba spp.* J Eukaryot Microbiol, 2016. **63**(5): p. 629-34.

11. Arnalich-Montiel, F., B. Lumbreras-Fernandez, C.M. Martin-Navarro, B. Valladares, R. Lopez-Velez, R. Morcillo-Laiz, and J. Lorenzo-Morales, *Influence of Acanthamoeba genotype on clinical course and outcomes for patients with Acanthamoeba keratitis in Spain.* J Clin Microbiol, 2014. **52**(4): p. 1213-6.

12. Ross, J., S.L. Roy, W.D. Mathers, D.C. Ritterband, J.S. Yoder, T. Ayers, R.D. Shah, M.E. Samper, C.Y. Shih, A. Schmitz, and A.C. Brown, *Clinical characteristics of Acanthamoeba keratitis infections in 28 states, 2008 to 2011.* Cornea, 2014. **33**(2): p. 161-8.