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# Determinants of Outcomes of Adenoviral Keratoconjunctivitis

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***Purpose:*** To determine host and pathogen factors predictive of outcomes in a large clinical cohort with keratoconjunctivitis.

***Design:*** Retrospective analyses of the clinical and molecular data from a randomized, controlled, masked trial for auricloscene for keratoconjunctivitis (NVC-422 phase IIB, NovaBay; clinicaltrials.gov identifier, NCT01877694).

***Participants:*** Five hundred participants from United States, India, Brazil, and Sri Lanka with clinical diagnosis of keratoconjunctivitis and positive rapid test results for adenovirus.

***Methods:*** Clinical signs and symptoms and bilateral conjunctival swabs were obtained on days 1, 3, 6, 11, and 18. Polymerase chain reaction (PCR) analysis was performed to detect and quantify adenovirus in all samples. Regression models were used to evaluate the association of various variables with keratoconjunctivitis outcomes. Time to resolution of each symptom or sign was assessed by adenoviral species with Cox regression.

***Main Outcome Measures:*** The difference in composite scores of clinical signs between days 1 and 18, mean visual acuity change between days 1 and 18, and time to resolution of each symptom or sign.

***Results:*** Of 500 participants, 390 (78%) showed evidence of adenovirus by PCR. Among adenovirus-positive participants, adenovirus D species was most common (63% of total cases), but a total of 4 species and 21 different types of adenovirus were detected. Adenovirus D was associated with more severe signs and symptoms, a higher rate of subepithelial infiltrate development, and a slower decline in viral load compared with all other adenovirus species. The clinical courses of all patients with non−adenovirus D species infection and adenovirus-negative keratoconjunctivitis were similar. Mean change in visual acuity between days 1 and 18 was a gain of 1.9 letters; worse visual outcome was associated with older age.

***Conclusions:*** A substantial proportion of keratoconjunctivitis is not associated with a detectable adenovirus. The clinical course of those with adenovirus D keratoconjunctivitis is significantly more severe than those with non−adenovirus D species infections or adenovirus-negative keratoconjunctivitis; high viral load at presentation and non-United States origin of participants is associated with poorer clinical outcome. *Ophthalmology 2018;■:1−10 © 2018 by the American Academy of Ophthalmology*

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Conjunctivitis is among the most common conditions in all of medicine, accounting for between 1% and 2% of all visits to primary care providers in the United States.[1] More than 4 million visits were made to physicians in the United States for conjunctivitis in 2005,[2] and more than $430 million in annual costs are associated with diagnosis and treatment of conjunctivitis in the United States.[3] A recent study examining claims from a national managed care provider in the United States identified more than 340 000 cases over a 13-year period, for most of whom antibiotics were (inappropriately) prescribed.[4] Most conjunctivitis is viral, with epidemic keratoconjunctivitis (EKC) being the most severe form; EKC-associated outbreaks in community or healthcare settings last for weeks to months.[5,6] For example, from 2008 through 2010, 6 isolated EKC outbreaks were reported in 4 states (Florida, Illinois, Minnesota, and New Jersey).[7] These outbreaks included more than 400 infected patients and forced a temporary closure of a neonatal intensive-care unit and several clinics. An outbreak at a United States military base in the Philippines in 1989 resulted in 2603 cases and loss of 9038 work days, or almost 36 work years, in addition to incapacitating the base.[8]

Although the vast majority of EKC cases are caused by adenovirus D species (types 8, 37, 53, 54, 56, and 64), several other adenoviral species and types, including adenovirus B (types 3 and 7), adenovirus C (type 2), and adenovirus E (type 4), have been reported.[9] Although most patients have no visual sequela resulting from EKC, a subset will demonstrate subepithelial infiltrates (SEIs). These are considered pathognomonic for EKC[10] and have been associated with long-term vision loss.[11,12] Whether the clinical outcomes for each adenovirus species or type differ has not been studied previously.

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Recently, a large randomized, controlled, masked trial of a candidate therapeutic agent for acute keratoconjunctivitis or EKC (auriclosene; NVC-422 phase IIB, NovaBay) recruited 500 participants with clinical signs and symptoms consistent with keratoconjunctivitis and evidence of adenovirus using an enzyme-linked immunosorbent assay-based rapid screening test (AdenoPlus; Rapid Pathogen Screening, Inc., Saratosa, FL). This was a Food and Drug Administration licensure trial for the aganoside antiviral ophthalmic auriclosene (0.3%) solution/NVC-422.[13] The drug candidate did not meet therapeutic end points; the collected data provided us with a well-characterized keratoconjunctivitis cohort. Using this large dataset, we sought to define the determinants of clinical outcomes in adenoviral keratoconjunctivitis.

## Methods

### Participants and Procedures

Participants were recruited at 58 ophthalmology centers in United States, Brazil, Sri Lanka, and India. Informed consent was obtained, and the work complied with the Health Insurance Portability and Accountability Act. Institutional review board approval for this study was obtained, and the research adhered to the tenets of the Declaration of Helsinki (US: Goodwin IRB (Cincinnati, OH); India: Drug Controller General (DCGI) with local Ethics committees; Sri Lanka: Scientific and Ethical Review, Faculty of Medicine, University of Kelaniya, Colombo, Sri Lanka; Brazil: National Ethics Committee in Research (CONEP) plus local ethic committees). The clinical trial was registered and publically available at clinicaltrials.gov (identifier, NCT01877694).

The inclusion criteria included: (1) 18 years of age or older (except in the United States, where the age criterion was older than 1 year); (2) a recent history of upper respiratory infection, contact with infected person, or recent visit to eye care provider; (3) at least 2 of the 9 clinical signs suggestive of conjunctivitis with onset fewer than 3 days before enrollment; and (4) positive adenovirus screening test results (AdenoPlus). Patients were randomized 1:1 to auriclosene solution or vehicle and were instructed to use 1 drop 8 times daily in both eyes for 10 days.

Subsequent visits were scheduled on days 3, 6, 11, and 18, with an optional visit on day 42. For this analysis, we considered day 18 as the final visit. Bilateral lower conjunctival swabs were collected on day 1 to 18 visits, and full ophthalmologic examinations were performed. Visual acuity (VA) was measured with Early Treatment Diabetic Retinopathy Study (ETDRS) charts. The following 10 clinical signs were assessed at each visit: lymphadenopathy, lid edema, lid erythema, bulbar conjunctival injection, palpebral conjunctival hyperemia, conjunctival discharge or lid crusting, tear meniscus, corneal fluorescein staining, tear film breakup time, and the presence of SEIs. Six subjective ocular symptoms were assessed at every visit: the presence of blurry vision, foreign body sensation, tearing, itching, burning, and photophobia. Signs and symptoms were scored either as 0 or 1 (absent or present) for lymphadenopathy, normal or abnormal tear film breakup time, presence of SEIs, and blurry vision, or as 0, 1, 2, or 3 (absent, mild, moderate, or severe) for the rest of the signs.

### Laboratory Methods

All laboratory procedures, except for whole genome sequencing (WGS), were performed at Viracor Eurofins Clinical Diagnostics (Lee's Summit, MO). No culture study was performed in our study. The adenoviral load (copies per milliliter) was measured by quantitative polymerase chain reaction (PCR) on all specimens. Genomic DNA was isolated from Copan dry swab samples (Copan diagnostics Inc, Murrieta, CA) of the conjunctiva using NUCLISENS easyMAG (bioMerieux, Inc., Durham, NC). TaqMan Fast Advanced Master Mix (Thermo Fisher Scientific, Inc., Waltham, MA) containing 10x Buffer, Taq polymerase, dNTP mix, and the primers were used for the PCR reaction. For each reaction, 10 μl of genomic DNA was used. Primer or probe selection programs using the ABI Primer express software (Applied Biosystems, Foster City, CA) were run and compared with conserved regions from multiple alignments. All primers targeted near the 5′ end adenovirus hexon gene. The following primers were used: 5′-TACATGCATCGCCGG-3′, 5′-TACATGCATATCGCCGG-3′, 5′-GGTCTTACATGCACATCTCGGG-3′, 5′-ACCCYGATGTGACCACSG-3′, 5′-TGCGTTTT-GTCCCGTG-3′, 5′-TGCGTTTTGTGCCCGTG-3′, 5′-CCACCGATACCTACTTCAGCCT-3′, and CACCGAGACGTACTTCAGCCT-3′. The following probes were used: 5′-CCGGGTCTGGTGCAGT-3′, 5′-CAGGACGCCTCGGAGTA-3′, and 5′-CCACGGACACCTACTTCACCCTGGG-3′. Standards were made based on plasmid clones containing the target region of the hexon gene. The quantitative PCR analysis was performed in the ABI 7500 FAST instrument (Applied Biosystems, Foster City, CA) using methods for a single well and stored standard curve. Cycling conditions were 2 minutes at 50°C, 2 cycles of 20 seconds of denaturation at 95°C, followed by 40 cycles of 3-second denaturation at 95°C and 30 seconds of annealing at 60°C.

Specific viral species and types were determined by sequencing of 400 to 500 base pairs of the adenovirus hexon gene. The following primers were used: 5′-CTGATGTACTACAACAGCACTGGCAACATGGG-3′ and 5′-GCGTTGCGGTGGTGGTTAAATGGGTTTACGTTGTCCAT-3′. Cycling conditions for PCR were 2 minutes of denaturation at 95°C, then 40 cycles of 30 seconds at 95°C, 50 seconds at 51°C, and 60 seconds of 72°C, followed by 3 minutes of 72°C.

Shotgun WGS was performed in selected adenovirus-positive and adenovirus-negative samples using Illumina Nextera XT DNA library prep kit (Illumina Inc., San Diego, CA) per manufacturer's protocol. In brief, 1 ng genomic DNA was fragmented by the Nextera NT transposome at 55°C for 5 minutes and kept at 10° C. Polymerase chain reaction amplification was performed using Nextera PCR Master Mix, index primers, and processed DNA. Cycling conditions were 3 minutes at 72°C, 30 seconds of denaturation at 95°C, followed by 12 cycles of 10-second denaturation at 95°C, 30 seconds of annealing at 55°C, and 30 seconds of extension at 72°C and a final step of 5 minutes at 72°C. After the PCR cleanup, the library was normalized. Approximately 2 to 4 μl of normalized library was pooled and loaded. Whole genome sequencing was performed on the MiSeq (Illumina, Inc., San Diego, CA). Sequencing was analyzed by mini Kraken[14] (provided by Illumina) and the Scalable Metagenomics Alignment Research Tool.[15]

### Statistical Analyses

The data from the worse-seeing eye on day 1 were included in the analysis of participants with bilateral involvement. If both eyes had equal VA, then the study eye was chosen by a random number generator. For comparison of categorical variables, we used the Fisher exact test or logistic regressions. Linear regression was used to evaluate continuous variables among 3 adenovirus groups (non−adenovirus D species infection, adenovirus D species infection, adenovirus negative), and analysis of variance was used to compare variables among all 5 groups (adenovirus B, adenovirus C, adenovirus D, adenovirus E, and adenovirus negative). The primary outcome of this analysis was the difference in composite scores of clinical signs between days 1 and 18. Additionally, the

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