Andrew Hartwick, OD PhD
hartwick.4@osu.edu

The Ohio State University
College of Optometry

One eye
two eyes
red eye
pink eye
Conjunctivitis (Cs) is a prevalent condition, comprising a significant proportion of an OD’s practice.

Adenovirus (Ad) is a particularly contagious cause of Cs

- Can remain infectious in a dessicated state for weeks at room temp (Chaberny et al., 2003)

Starts in one eye but moves to the second eye in a majority of cases (Cheung et al, 2003)
Ad-Cs Outbreaks

• Outbreaks can occur wherever people congregate: workplaces, school, military, health care centers, etc

• Nosocomial spread significant public health issue; 3 examples:
  – 17% of 145 cases (Mueller, Klaus 1993)
  – 44% of 192 cases (D’Angelo et al., 1981)
  – 85% of 132 cases (Colon 1991)

• %’s above were # originating at place of eye exam
  – Great practice builder!!

"I DON'T ALWAYS GET PINK EYE,
BUT WHEN I DO, I MAKE SURE THE REST OF MY CLASS GETS IT."
A study in 2008 estimated that Ad-Cs costs the US economy about $670 million in its management, incorporating in work days missed (Udeh et al., 2008)

- Reported average to be 5 days missed (range 2-10)

Specific example (Doyle 1989):
- Outbreak of viral conjunctivitis in microelectronics factory (145 cases among 350 workers)
- Estimated cost close to a million dollars due to work days lost, temporary employee costs and overtime

Can disrupt health care delivery, including optometry
Ad-Cs Morbidity

• Highly symptomatic
  – Bulbar redness, pain, itching, tearing, discharge, marked lid swelling, photophobia, foreign body sensation and decreased vision during the infection

• Typically self-limiting
  – Minority develop corneal infiltrates that coalesce and cause permanent vision loss (Ford et al., 1987)
Ad-Cs Treatment

- Effective treatment approach could decrease the time-course of the condition, limit transmission to other individuals and/or also improve the symptom burden.

- Currently, no FDA-approved treatments for Ad-Cs.

- A treatment that reduces the contagious period by even one or two days could have significant public health and economic impact.

- Current standard of treatment is symptomatic with artificial tears, antihistamines and cold compresses.
Ad-Cs Treatment

- Antibiotics – doesn’t target the underlying etiology
  - Low incidence of co-infection; microbial resistance

- Steroids – can be adjunctive therapy with others
  - Indicated when infiltrates, membranes, uveitis present
  - On its own, linked to increase in viral replication

- Topical anti-virals – some promising results but no compound has established efficacy thru controlled RCT
  - Ganciclovir appears to inhibit viral replication in vitro

- Interferons may be prophylactic to boost immune system
• Povidone-iodine (PVP-I) works by iodination and oxidation of cytoplasmic and membrane compounds

• Broad-spectrum – in addition to adenovirus, there is evidence for effectiveness against:
  – bacteria (no microbial resistance), herpes simplex, Chlamydia and enteroviruses (Reimer et al., 2002)

• Used as a surgical scrub for over 50 years, commonly used to prevent infection during ocular surgeries
• *In vitro*, it is very effective at killing free Ad, less so against intracellular Ad (Monnerat et al., 2006)

• However, in a rabbit model of Ad-Cs, PVP-I (0.4% with 0.1% dex) significantly improved the clinical sign scores and reduced extracellular viral titers (Clement et al., 2011)

| Tx: PVP-I/Dex 7 days | Tx: Cydofovir 7 days | Tx: Tobra/Dex 7 days |
• Ideally, a treatment is not only effective, but it is safe, low-cost and widely accessible

• PVP-I has been used for decades as topical antiseptic on neonates, children, adults

• Ophthalmic PVP-I formulation (Betadine® 5%, Alcon) is FDA- approved for “the prepping of the periocular region and irrigation of the ocular surface”
• The cost per 30 ml single-use package of 5% PVP-I ophthalmic solution is about $21, compared to $140 per 5gm tube of Zirgan™ (gancyclovir).

• PVP-I is widely available in developing countries where it can be prepared from powder or stock solutions meant for other antiseptic purposes.

• Off-label use of PVP-I for Tx of Ad-Cs has gained credence over the last decade in influential reviews and editorials...
• For example…

• In the widely disseminated, annually-published ‘Clinical Guide to Ophthalmic Drugs’, Melton and Thomas reported that “...a one-time application of povidone-iodine should be sufficient for alleviating the condition”

• A few years ago, a group of clinicians/researchers at various optometry schools/practice settings were curious about how wide-spread PVP-I’s use was

• We surveyed OD’s and MD’s at seven clinical conferences using ‘clickers’ or paper surveys
A significant minority of eye care providers report using PVP-I for Ad-Cs.

Therefore, a well-designed randomized controlled trial that tests (validates or disproves) the usefulness of this growing practice was deemed likely to impact clinical practice, regardless of the results.

A positive trial-outcome of a single, in-office treatment of 5% PVP-I could revolutionize management of Ad-Cs, especially in developing countries where external eye infections are endemic.

The absence of effect would spare hundreds of thousands from ineffective treatment. Either result would provide a rational basis for health insurance plans.
• The Reducing Adenoviral Patient-Infected Days (RAPID) Study

Started with 6 clinical sites, finished with 9
• Big decisions regarding study inclusion:
  
  – 1) How far along in the disease for cutoff point?
    • Short cut-off (i.e. 1 day after symptom onset) and recruitment is difficult
    • Long cut-off (i.e. 7 days) and disease may be near resolved; makes it difficult to find treatment effect
  
  – 2) We wanted only Ad-Cs patients randomized to treatment. How to determine whether the pink eye was truly Ad-Cs?
We used a cutoff of 4 or less days since symptom onset.

So, if subject woke up on Sunday with a pink eye, then they could enroll in the study if they came on the Thursday or earlier.

If they presented on the Friday, they were not screened for the study.

A caveat to the study design is that it relied on subject-self-report for symptom onset.
Diagnosis of Ad-Cs

• Timely and accurate diagnosis of Ad-Cs at first visit crucial to the success of this clinical trial

• Only subset of acute Cs cases have adenoviral etiology
  – Proportions vary greatly (ranging from 5 to 62%) based on confirmed Ad-Cs

• Meta-analysis concluded bacterial & viral Cs cannot be reliably distinguished clinically (Reitveld et al., 2003)

So, how do you know it’s pink eye? (and not plaid eye)
Diagnosis of Ad-Cs

- In cases clinically diagnosed as Ad-Cs, concordance with molecular testing is as low as 8% (range 8 to 82%)
  

- Why such a range?

- As a whole, data speaks to the challenge of correctly diagnosing Ad-Cs at 1st visit

So, how do you know it’s pink eye? (and not plaid eye)
The gold standard for confirming Ad-Cs is to test conjunctival swab samples using cell culture or polymerase chain reaction (PCR) techniques.

PCR is probably the most used definitive test currently. An advantage is that it can provide a quantification of the viral titers present in the sample (qPCR).

Disadvantage is that the sample usually has to be sent out to a lab for the testing – takes time
  – Can’t be used to make treatment randomization decisions on the first visit
Adenoviral Immunoassay

- The AdenoPlus™ test was a point-of-care immunoassay.

- Uses monoclonal antibodies raised against the hexon protein that is conserved in all known serotypes, and yields a bivariate “yes/no” result for presence of Ad.

- Major advantage is result obtained within 15 minutes.

- For clinical trials on Ad-Cs, the rapid diagnostic result facilitates treatment randomization at first visit.
Adenoviral Immunoassay
The first publication that evaluated the performance of the AdenoPlus immunoassay reported high positive (94%) and negative (95%) predictive values for the device (Sambursky et al., 2013).

We decided to use Adenoplus as screening tool for eligibility:

- Positive AdenoPlus enable treatment randomization
- Negative AdenoPlus – patient completed baseline visit (including conjunctival swab) but no Tx or F/U

Conjunctival swabs were obtained from screened subjects and PCR was done on samples.
Adenoviral Immunoassay

Eye was anesthetized with proparacaine

After 5 min, sampling fleece was dabbed temporally on the inferior palpebral conjunctiva and dragged nasally

Process was repeated 8 times, with the fleece resting against the nasal palpebral conjunctiva for 5 s at the end

Fleece collector placed in the test cassette body and the absorbent tip was immersed in the supplied vial of buffer for 20 s

Test results were read after 10 min
Conjunctival Swab

- Swab of inferior palpebral conj was obtained
- Immersed in viral transport medium
- Vial placed on ice and
- Frozen at -80°C within 3 h
- Shipped to Wash U, St. Louis for PCR 1-2x/year
Double-Masked Randomization Treatment Trial

• If AdenoPlus came back positive….

• Subject received proparacaine and then either 5 drops of 5% PVP-I or artificial tears. Lid margins were rubbed with Tx-soaked swab. After 1 min, eye was saline lavaged

• Masking of subject to treatment was attempted

• Discharged with PF artificial tears qid for 7 days

• Returned for F/U visits from masked examiner at Days 1-2, 4, 7, 14, 21
PVP-I Application
- What happened?
- We got pink eye.
• Subjects with red/pink eye screened in 9 clinical sites

130/156 Negative AP ≥18 yrs old Sx ≤ 4 days

N = 156 Ineligible

N = 56 Randomized

N = 212 Screened

All 56 had: Positive AP ≥18 yrs old Sx ≤ 4 days

56 Swabs Sent for PCR

N = 26 Artificial Tears

N = 30 5% PVP-I

130 Swabs Sent for PCR

130/156 Negative AP ≥18 yrs old Sx ≤ 4 days
### Predictive Value of the AdenoPlus

<table>
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<th></th>
<th>PCR +</th>
<th>PCR -</th>
<th>Total</th>
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<tbody>
<tr>
<td>AdenoPlus +</td>
<td>28</td>
<td>28</td>
<td>56</td>
</tr>
<tr>
<td>AdenoPlus -</td>
<td>2</td>
<td>128</td>
<td>130</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>30</td>
<td>156</td>
<td>186</td>
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</table>

- **Negative Predictive Power** = \(\frac{128}{130} = 98.5\%\)
- **Positive Predictive Value** = \(\frac{28}{56} = 50\%\)
- With PCR as comparator; **Sensitivity** = 93%, **Specificity** = 81%
Randomized Ad-Cs Subjects

N = 212 Screened

N = 156 Ineligible

N = 56 Randomized

N = 26 Artificial Tears

N = 12 PCR+ for Ad-Cs

N = 14 PCR-

N = 30 5% PVP-I

N = 16 PCR+ for Ad-Cs

N = 14 PCR-

End Result: 12 Ad-Cs in Tears; 16 Ad-Cs in PVP-I
• Most of the AdenoPlus tests were photographed (cell phone)

• Measured ratio of band intensity to compare on PCR positive versus PCR negative results
• Correlation of viral titers to densitometry ratio in samples that were RPS+ & PCR+ (n = 26)

• Interpretation: the brighter the red band, the higher the viral titers on subsequent PCR analysis
• Box plot (median and percentiles)

Note rarity of PCR-ratios greater than ~ 0.9

Clinical pearl:
If red line is brighter than the blue line (ratio >1), you can be more sure it is Ad-Cs

Dunn’s posthoc: 1 vs 2 (p=1.0); 1 vs 3 (p<0.001); 2 vs 3 (p=0.019)
Take-Away Points

- True Ad-Cs is probably more rare than you think
  - There were 212 red/pink eye patients screened for the study – some bias in selection as obvious allergy, trauma, bacterial were not screened
  - Only 30 had PCR-confirmed Ad-Cs

- If the AdenoPlus is negative, you can be about 99% sure that it is not Ad-Cs

- If AdenoPlus positive, flip a coin, 50/50 whether subject was truly Ad-Cs (perhaps darker red line improves odds)

- Recruitment for an Ad-Cs clinical trial is very, very hard
• Perhaps we needed to be more lax on our disinfection protocol.....

YOU GET PINK EYE!

AND YOU GET PINK EYE!

AND YOU GET PINK EYE!

EVERYONE GETS PINK EYE!
• No reported study site nosocomial transmissions in study (other patients getting infected during eye exam)
• We had a strict disinfection regime:
  
  Compliance with CDC Guideline for Disinfection and Sterilization in Healthcare Facilities
  
  – hypochlorite bleach wipes (1:10 dilution) were used to disinfect all surfaces (slit lamp, counter, door knobs) at end of exam
  
  – Patients were asked to wash their hands upon entering the examination room and used paper towels that were subsequently disposed of in red biohazard bags.
  
  – Patient signed consent forms were filed in a red folder indicating biohazard. Pens used were put in biohazard bag
  
  – Clinician changed gloves 3x during exam

• However, one clinical examiner contracted Ad-Cs during study!
Challenge: Treatment

• Big decisions regarding treatment:

  1) Is a single, in-office treatment of PVP-I the best strategy? What about a second treatment on Day 1-2?

  2) Is the standard 5% ophthalmic PVP-I too strong? Would we get better results with a lower dose?

  3) Would adding a steroid to treatment be beneficial?
Treatment and Follow-up

- An advantage of in-office, treatment is that patient compliance is not an issue (compared to drops of low-dose PVP-I)

- A single treatment seemed reasonable for the first study, and it was easier to keep clinicians masked

- While there is actually paradoxical evidence that lower dose PVP-I (1%) releases more free iodine than 5%, we decided to stick with the readily available version

- Similarly, we left steroids out in order to strictly test safety and efficacy of 5% PVP-I as the first study
Challenge: Follow-up Schedule

• Big question:
  • How many follow-up visits necessary? Timing?

• Follow-up schedule was hotly debated, but in the end we wanted to assess natural history of disease

• Five follow-up visits
  – Day 1-2, Day 4, Day 7, Day 14, Day 21
**qPCR-Determined Viral Titers**

- Example from one subject (units are Ad DNA copies/ml)

<table>
<thead>
<tr>
<th></th>
<th>Raw Data</th>
<th>Normalized (%)</th>
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<td><strong>Baseline</strong></td>
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<td>D1-0406 100.0000</td>
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<tr>
<td>1-2 days</td>
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<td>14 days</td>
<td>7814</td>
<td>0.0030</td>
</tr>
<tr>
<td>21 days</td>
<td>0</td>
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</tr>
</tbody>
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Summary of Efficacy Results

• In participants with qPCR-confirmed Ad-Cs, those receiving 5% PVP-I showed significant improvement in certain signs, symptoms and viral titers at day 4 compared to those who received AT.

• There were no significant differences between the groups in viral titers, symptoms or signs at the 1-2, 7, 14 or 21 day F/U visits in participants with confirmed Ad-Cs.

• Data provided great overview of time-course of viral titers. However, future treatment trials should focus strongly on follow-up visits between days 2 and 6.
Efficacy of PVP-I for AdenoPlus+ and PCR- Cs

• In randomized participants who tested negative for Ad-Cs by qPCR, the 5% PVP-I did not significantly improve signs and symptoms at any visit with the exception of clinician-graded eyelid matting on the day 1-2 F/U visit.

• Thus, while PVP-I is supposed to be broad-spectrum, our data does indicate it is more effective against true Ad-Cs

• We are currently pursuing DNA identification approaches to try and find the etiology causing the Cs that was negative for Ad on PCR but positive on the AdenoPlus
Safety of PVP-I

• Only one reported adverse effect in PVP-I group, one subject had mild a.c reaction on the day 1 visit
  – Clinician classified it as ‘not likely related to Tx’

• At start of exam, PVP-I group rated their discomfort as 6.0±3.0 and at end it was 6.2±2.8 (P=0.78)

• At Day 1, the mean discomfort was 4.6±2.6 in PVP-I group and 5.7±2.9 in tears group, so no lingering discomfort caused by treatment the next day

• No significant difference in corneal staining in PVP-I group versus tears
• **Conclusion:** These results indicate that a single, in-office application of ophthalmic 5% PVP-I is a safe treatment, and can improve clinical signs and symptoms in individuals with Ad-Cs, four days after treatment.

• Whether multiple applications of PVP-I across different visits can expand the time-frame of the therapeutic effect beyond 4 days remains a question for future research.