

# Pathogenesis and management of adenoviral keratoconjunctivitis

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**Abstract:** Human adenovirus (HAdV) is a ubiquitous virus that infects the mucosa of the eye. It is the most common cause of infectious conjunctivitis worldwide, affecting people of all ages and demographics. Pharyngoconjunctival fever outbreak is due to HAdV types 3, 4, and 7, whereas outbreaks of epidemic keratoconjunctivitis are usually caused by HAdV types 8, 19, 37, and 54. Primary cellular receptors, such as CAR, CD46, and sialic acid interact with fiber-knob protein to mediate adenoviral attachment to the host cell, whereas adenoviral penton base–integrin interaction mediates internalization of adenovirus. Type 1 immunoresponse to adenoviral ocular infection involves both innate immunity mediated by natural killer cells and type 1 interferon, as well as adaptive immunity mediated mainly by CD8 T cells. The resulting ocular manifestations are widely variable, with pharyngoconjunctival fever being the most common, manifesting clinically with fever, pharyngitis, and follicular conjunctivitis. Epidemic keratoconjunctivitis, however, is the severest form, with additional involvement of the cornea leading to development of subepithelial infiltrates. Because there is currently no US Food and Drug Administration-approved treatment for adenoviral ocular infection, current management is palliative. The presence of sight-threatening complications following ocular adenoviral infection warrants the necessity for developing antiadenoviral therapy with enhanced therapeutic index. Future trends that focus on adenoviral pathogenesis, including adenoviral protein, which utilize host receptors to promote infection, could be potential therapeutic targets, yielding shorter active disease duration and reduced disease burden.

**Keywords:** adenoviral pathogenesis, antiviral therapy, keratoconjunctivitis, tropism

## Introduction

Human adenovirus (HAdV) is a ubiquitous virus that infects various mucoepithelial cells of the body. Primary targets of HAdV infection are eyes, the genitourinary tract, respiratory tract, and gastrointestinal tract.<sup>1</sup> In fact, HAdV is the most common cause of infectious conjunctivitis and red eye worldwide, accounting for up to 75% of all conjunctivitis cases and affecting people of all ages and demographics.<sup>2</sup> Due to its high frequency and many infected patients not seeking medical attention, the incidence is difficult to ascertain.<sup>3</sup> The majority of adenoviral ocular infections are subclinical, with some being mildly symptomatic and self-limiting.<sup>4,5</sup> HAdV infection in immunocompetent individuals is usually subclinical in nature; however, there is likely to be increased ocular morbidity and disease burden in individuals with compromised immune systems.<sup>3</sup> Morbidity is higher in the pediatric population than the adult population with immunocompromised status.<sup>3,6</sup> Risk factors for invasive

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and disseminated HAdV include solid-organ or stem-cell transplant and severe immunosuppression. T-cell depletion can result in development of inadequate or lack of cellular immunoresponse to AdV. Depletion of T cells associated with stem-cell transplant, use of long-term immunosuppressants, and absence of T cells associated with congenital immunodeficiency constitute a significant risk for a persistent and severe course of HAdV infection.<sup>6</sup>

The mode of transmission of AdV-associated ocular infection include direct contact with infected fomites, water, and fecal oral contamination.<sup>7</sup> It is noteworthy that AdV can survive on a fomite, with the contaminated fomite acting as a medium for transmission.<sup>5</sup> Pharyngoconjunctival fever (PCF) outbreak is due to HAdV types 3, 4, and 7.<sup>8–10</sup> Outbreaks of epidemic keratoconjunctivitis (EKC) are usually caused by HAdV types 8, 19, 37, and 54.<sup>11–14</sup> Periodic outbreaks of AdV ocular infections occur in crowded communities, with children playing a prominent role in maintaining and transmitting the virus to others in their immediate environment.<sup>15</sup>

Its highly contagious nature, long incubation period, and lack of gold standard testing or treatment cause adenoviral conjunctivitis to be a significant global economic burden.<sup>16</sup> Besides medical costs secondary to nosocomial infections, the failure to diagnose this condition in a timely and accurate fashion leads to an increased number of affected individuals, resulting in excess time away from work and future loss of earning potential due to disability. Misdiagnosis additionally increases health-care spending, due to failure to initiate proper prevention methods, the need for more frequent physician follow-up, and inaccurate prescription of therapeutic agents.<sup>16</sup> The focus of this review is on the viral pathogenesis of HAdV, clinical ocular manifestations that develop in response to host-defense mechanisms, current laboratory diagnostic methods, and therapeutic approaches to manage these ocular infections.

## Adenoviral pathogenesis

AdV is a lytic, nonenveloped double-stranded DNA virus, with a 36 kb genome encoding more than 40 structural and nonstructural proteins.<sup>17</sup> AdV is 90 nm in diameter with an icosahedral capsid that contains 240 trimers of hexon protein and 12 pentamers of penton base proteins. Fiber protein protrudes from the penton base and consists of an N-terminal tail in contact with penton base protein and a C-terminal knob domain that serves as a binding site for host cellular receptors.<sup>1</sup> Classification of AdVs into seven groups (A–G) is on the basis of their hemagglutination properties, tissue tropism, serology, DNA homology, and host-receptor usage.<sup>7,18–22</sup> The

Human Adenovirus Working Group proposed the characterization and typing of HAdVs on the basis of whole-genome sequence analysis.<sup>23</sup> To date, there have been 86 types of HAdV reported by this group.<sup>24</sup> HAdV belongs to the genus *Mastadenovirus* and family adenoviridae.<sup>18</sup>

Major structural proteins of AdV include a homopentameric penton base that mediates AdV internalization into the host,<sup>25</sup> a homotrimeric fiber protein that plays a role in viral attachment to host cellular receptors,<sup>26</sup> and hexons that are recognized by neutralizing antibodies.<sup>5,7,18</sup> Minor capsid proteins include proteins VI, III, VIII, and IX. Core proteins of HAdV include IVa2, V, VII, MU and terminal protein.<sup>18,27</sup> Primary cellular receptors, such as CAR,<sup>28</sup> CD46 (membrane cofactor protein),<sup>29</sup> sialic acid,<sup>30</sup> desmoglein 2,<sup>31</sup> heparin sulfate proteoglycans,<sup>32</sup> CD80, CD86,<sup>33</sup> and GD1a,<sup>34</sup> interact with fiber-knob protein to mediate adenoviral attachment to host cells.<sup>1,35</sup>

The presence of AdV on the ocular surface is associated with activation of ocular surface epithelial cells secreting interleukin 8 (IL8), which binds to its chemokine receptors CXCR1 and CXCR2. The subsequent phosphorylation of downstream molecules culminates in the translocation of CAR and  $\alpha_v\beta_3$  integrin from the basolateral surface of the polarized epithelial cell to the apical surface to enable interaction of AdV fiber knob with CAR on the apical surface of the epithelial cell.<sup>19,35,36</sup> Because CD46 is located above the tight junction, it is considered a suitable receptor for mediating adenoviral entry.<sup>37</sup> It is noteworthy that each of the RGD (arginine–glycine–aspartate) loops on the penton protein serves as a binding site for integrins.<sup>37</sup> RGD-loop spatial arrangement promotes the signaling process involved in internalization of AdV into the host cell; however, it does not provide efficient binding site for neutralizing antibodies.<sup>18</sup> Many epithelial cells express vitronectin-binding integrins ( $\alpha_v\beta_3$  and  $\alpha_v\beta_5$ ) that promote internalization of AdV, depending on the RGD sequence in the penton base protein.<sup>25,35,38–40</sup> Most HAdVs have the RGD peptide in their penton base, except HAdV-F40, HAdV-F41, and HAdV-D60.<sup>4,35</sup>

Following adenoviral attachment, internalization of AdV into an endosome is mediated by the interaction of the homopentameric penton base and vitronectin-binding integrin.<sup>18,25,37</sup> Penton base–integrin interaction activates an intracellular signaling cascade that culminates in the internalization of AdV into clathrin-coated vesicles.<sup>18,37,39,41–43</sup> Following internalization of the adenoviral nucleocapsid into the endosome, acidification of the endosome is required to initiate several steps involved in the uncoating of AdV. It is of note that the acidic pH of the endosome triggers the

AdV-uncoating process and penetration of the endosome.<sup>37,44</sup> These steps facilitate the release of the viral DNA genome into the nucleus. The first step is vertex dissociation from the adenoviral capsid, a temperature-dependent process that occurs at or near the plasma membrane. Removal of the vertex is followed by further uncoating of the virus in the endosomal pathway, which is linked to endosomal penetration. There is loss of peripentonal hexons, pIIIa, pVIII, and pIX, as well as the release of pVI, a minor capsid protein required for disruption of the endosomal membrane<sup>18,37,45–47</sup> and facilitation of nuclear importation through movement in a microtubule- and dynein-dependent mechanism and dock at the nuclear pore complex, a proteinaceous channel that mediates movement of the nucleocapsid viral genome into the nucleus, where viral replication takes place.<sup>18,37,48,49</sup>

The replication cycle of AdV consists of an early stage and a late stage.<sup>50</sup> The early stage of adenoviral replication is characterized by the generation of adenoviral E1A protein (encoded by the *E1A* gene) that transactivates other early genes *E1B*, *E2*, *E3*, and *E4*, which play important roles in the early stage of the AdV replication cycle.<sup>1,50–54</sup> Adenoviral DNA replication is dependent on viral DNA polymerase, preterminal protein, and DNA-binding protein.<sup>55–58</sup> The *E2* gene encodes adenoviral DNA polymerase, precursor terminal protein, and DNA-binding protein, which play an important role in viral DNA replication.<sup>52,59,60</sup> The *E3* gene encodes the E3 gp19K protein (E3 Mr 19k glycoprotein), which prevents the display of viral antigen complexed to MHC class I molecules on the surface of virally infected cells.<sup>61,62</sup> The *E4* gene encodes E4 proteins, which shut off of host-cell protein synthesis.<sup>1,63,64</sup> Additionally, this gene is required for the expression of late genes during adenoviral DNA replication.<sup>65</sup> The late stage of adenoviral replication ensues when late genes encode the adenoviral structural proteins.<sup>65–67</sup> Adenain (23k cysteine protease), encoded by the *L3* gene, promotes maturation and generation of progeny DNA that is packaged in virions.<sup>1,37,59</sup> Newly generated genome duplexes of AdV may either enter further replication rounds or undergo processing and encapsidation into virions.<sup>59</sup>

## Host response to adenovirus

The human immune system consists of many levels of defense against invasion by pathogens, including viruses. The epithelium of the ocular surface provides a physical barrier to invasion by viruses and constitutes the first level of defense against pathogens, including AdVs. The chemical barrier, along with the physical barrier, prevents viruses from gaining access to the subepithelial layers of

the conjunctiva and cornea. Antimicrobial proteins found in the tear film and expressed by the ocular surface epithelia are innate immune molecules that provide an effective chemical barrier to invading microbes. Defensin is a major antimicrobial protein that possesses antiviral activity.<sup>68</sup> Nguyen et al<sup>69</sup> demonstrated that human  $\alpha$ -defensin blocks the uncoating of AdVs, thereby inhibiting adenoviral infection. In addition to the partial disassembly of the virus, it has been shown that human  $\alpha$ -defensin inhibits HAdV-mediated endosomal lysis.<sup>70</sup> However, a breach of the physical and chemical barrier results in the initiation of cellular innate immune response and subsequent generation of antiviral immune response.<sup>68</sup>

Innate immune response to adenoviral ocular infection is mediated by natural killer (NK) cells, monocytes, and type I interferon (IFN). Adaptive immunity is mediated by IgA, CD8 T cells, and T-helper 1 ( $T_H1$ ) cells. Type I IFNs are innate antiviral cytokines, whereas IFN $\gamma$  plays a major role in antiadenoviral adaptive immune response.<sup>7,71</sup> Additionally, plasmacytoid and conventional dendritic cells participate in immune response to AdV.<sup>38</sup> Viral proteins and nucleic acid are virus-associated molecular patterns that bind to pattern-recognition receptors on macrophages and dendritic cells, which in turn results in the activation of macrophages to release proinflammatory cytokines, such as IL1 $\beta$ , IL6, IL8, IL12, and TNF $\alpha$ .<sup>19</sup> The activated dendritic cell traffic to regional secondary lymphoid tissue to activate naïve T cells to generate effector T cells, such as CD8 cytotoxic T cells and  $T_H1$  cells.<sup>71–73</sup> Adenoviral infection of plasmacytoid dendritic cells result in adenoviral DNA interacting with TLR9, which results in the production of type I IFN.<sup>74</sup> Additionally, NK cells respond to viral infection by promoting and regulating the adaptive immune response via cross talk with dendritic cells and T cells, as well as via production of IFN $\gamma$  and TNF $\alpha$ .<sup>75</sup> It is of note that IFN $\gamma$  secreted by NK cells promotes the upregulation of costimulatory molecules on conventional dendritic cells, priming them for efficient T-cell activation. NK cells are activated by IL12 and type I IFN to release IFN $\gamma$ , TNF $\alpha$ , perforin, and granzyme.<sup>75</sup>

Antibody-mediated response to adenoviral ocular infection is mediated by neutralizing antibodies directed against hexon to prevent hexon–host cell interaction, complement-mediated lysis of AdV-infected cells, and antibody-dependent cell-mediated cytotoxicity via NK cells.<sup>76</sup> Sumida et al<sup>77</sup> demonstrated that neutralizing antibodies are directed against the hexon protein of AdV. Perforin and granzyme released by cytotoxic T lymphocytes (CTLs) and NK cells are responsible for cell-mediated cytotoxicity of AdV-infected cells, whereas

IFN $\gamma$  and TNF $\alpha$  released by T<sub>H</sub>1 cells and CTLs also play a pivotal role in clearance of adenoviral ocular infection.<sup>72,78,79</sup>

Although cell-mediated immunity is necessary for limiting viral growth, AdV is able to persist in the host via the use of immunoevasive strategies, such as inhibiting antiviral response mediated by IFNs, preventing cytolysis of AdV-infected cells by TNF $\alpha$ , promoting intracellular survival by blocking apoptosis of infected cells and evading CTL-mediated cytolysis of infected cells by preventing the display of viral peptide complexed to major histocompatibility complex (MHC) class I molecules on the surface of virally infected cells.<sup>80–84</sup> Leonard and Sen<sup>83</sup> demonstrated that adenoviral E1A protein inhibits expression of IFN $\gamma$ -inducible genes, which translates to E1A protein-mediated inhibition of IFN $\gamma$  expression. Additionally, E1A protein has an inhibitory effect on IFN $\alpha$ , a potent innate antiviral cytokine.<sup>83</sup> Lesokhin et al<sup>85</sup> demonstrated that adenoviral E3 protein can inhibit the ability of TNF $\alpha$  to induce the secretion of chemokines, such as IL8 and MCP1. Adenoviral E3 protein (E3-14.7K) blocks the ability of TNF $\alpha$  to induce the apoptosis of AdV-infected cells.<sup>86,87</sup> Adenoviral virus-associated-inducible RNA antagonizes the activation of IFN-induced dsRNA-activated protein kinase, a protein that plays an important role in mediating the antiviral action of IFN.<sup>88</sup>

NK cells are one of the first responding lymphocytes of the innate immune system in cases of viral infection of mucosal surfaces, such as the conjunctiva, which are responsible for cytokine production, apoptosis, and engaging the cell-mediated immunoresponse.<sup>75,89</sup> Studies have reported that AdVs retain the ability, through the use of multiple pathways, to inhibit the NK response in the early and acute stages of the conjunctivitis. This is able to occur through the upregulation of inhibitory ligand human leukocyte antigen E, in addition to the downregulation of several ligands that are responsible for the initiation of NK-cell-activating receptors.<sup>90</sup> Additionally, concomitant downregulation of human leukocyte antigen A/B/C expression is a strategy used by the virus to modulate MHC class I expression in infected conjunctival epithelium. Group D HAdV evades immunomediated cytolysis by downregulating expression of ligands (CD112 and CD155) for activating NK cell receptor DNAM1/CD226, which in turn translates to impairment of NK-cell-mediated immunity against AdV. This allows the virus to remain active on the conjunctival surface and give rise to persistent infection.<sup>75</sup>

## Viral and tissue tropism

Adenoviral tropism is related to affinity of host cellular receptors for fiber knob for viral entry and penton base for viral

internalization.<sup>5</sup> Binding affinity for host cellular receptors plays a role in viral tropism, with broadening of viral tropism linked to degree of binding affinity.<sup>35</sup> As such, affinity between virus and cellular receptors is likely to be high if few receptor molecules are adequate to produce an infection. If binding sites on cellular receptors are masked or many receptor molecules required to cause infection, such affinity of the interaction between virus and cellular receptor is low.<sup>35</sup> HAdVA, -C, -D, -E, and -F species use CAR,<sup>91</sup> whereas HAdVB species use CD46<sup>92–94</sup> or membrane glycoprotein desmoglein 2<sup>31</sup> as cellular receptor for viral attachment. Species HAdVD37 utilizes sialic acid<sup>30</sup> and GD1a<sup>34</sup> as cellular receptors to infect the eye, whereas species HAdVD8 and -D19 utilize CAR and sialic acid as cellular receptors to cause ocular infection.<sup>20,91</sup> Tissue tropism of AdV is dependent on the affinity between viral attachment proteins and host cellular receptors, as well as adenoviral grouping. It is of note that HAdVD species have affinity for ocular tissues, and as such they are implicated in adenoviral ocular surface infections.<sup>5</sup>

## Clinical manifestations

Clinical manifestation of adenoviral ocular infection is usually lytic in nature, with persistent infection attributed to latent AdV infection.<sup>5,95</sup> Lytic infection occurs upon completion of the viral replicative cycle in permissive cells, with release of up to 1 million progeny viruses per cell, of which up to 5% of virions are capable of causing infection.<sup>5,7</sup> Chronic or latent AdV infection is characterized by production of small amounts of virus, which serves as a source for nosocomial spread of AdV in hospitals, chronic-care facilities, and day-care centers.<sup>7</sup> PCF is caused by HAdV types 1, 2, 3, 4, 5, and 7 and is associated with swimming in insufficiently chlorinated pools and lakes (Table 1).<sup>5,76,96</sup> In contrast, EKC is a highly contagious ocular infection caused by AdV types 8, 19, 37, 53 and 54. EKC usually occurs as an outbreak emanating from eye clinics with contaminated instruments and solutions (Table 1).<sup>5,7,97</sup>

**Table 1** Overview of ocular involvement and clinical manifestations with specific adenoviral serotypes<sup>2,8–10,12–14,37,41,99,100,106,110</sup>

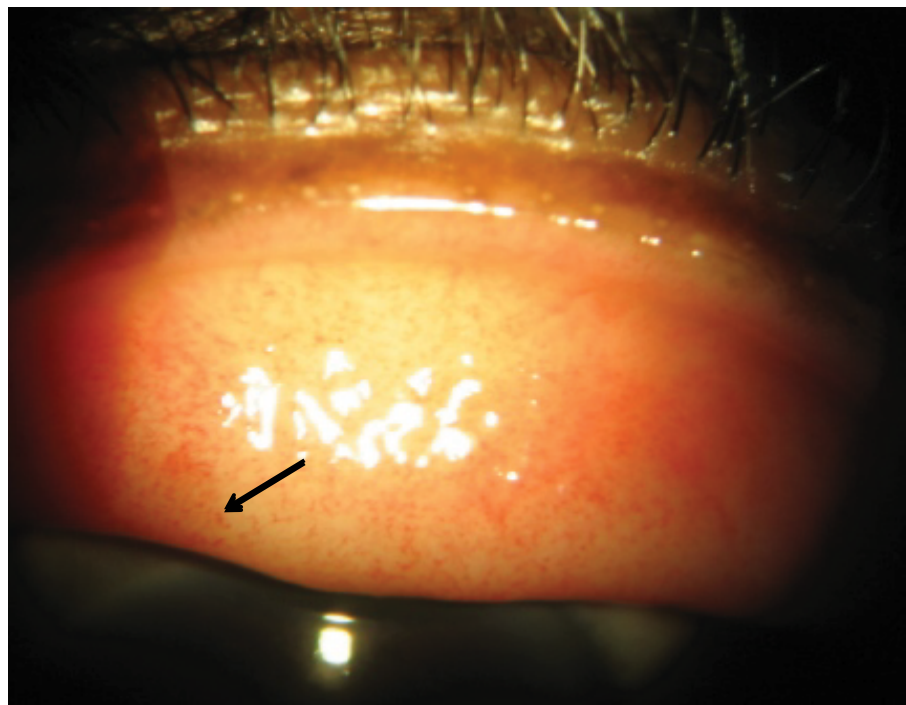
Ocular structure	Clinical manifestations	Subtypes involved
<b>Adnexa</b>	Eyelid edema, lacrimal gland enlargement, nasolacrimal duct inflammation	1–5, 7, 8, 19, 37, 53, 54
<b>Conjunctiva</b>	Follicles, hyperemia, edema, petechial hemorrhages, pseudomembranes	1–5, 7, 8, 19, 37, 53, 54
<b>Cornea</b>	Multifocal punctate keratitis, subepithelial infiltrates	8, 19, 37, 53, 54

The ocular manifestations of HAdV infections are widely variable. PCF is the most common presentation, primarily affecting children.<sup>98</sup> Conjunctival entry of AdV has been investigated using microarray technology, which demonstrated that nuclear localization of the virus occurred about 1.5 hours following infection, inducing early transcription followed by late transcription hours later.<sup>80</sup> PCF manifests clinically with fever, pharyngitis, rhinitis, follicular conjunctivitis, and regional lymphoid hyperplasia. Replication of AdV in the conjunctiva is associated with follicular hypertrophy, edema, hyperemia, and petechial hemorrhages.<sup>5,8,10,99,100</sup> Conjunctival hyperemia and edema are attributed to acute inflammation induced by innate immunoresponse to AdV in the conjunctiva. Proinflammatory cytokines, such as TNF $\alpha$  released during the innate immunoresponse, induce conjunctival vasodilation and capillary leakage, which results in conjunctival hyperemia and edema, respectively. Petechial hemorrhages are due to AdV-induced vasculitis, which is associated with persistent AdV-induced conjunctival inflammation, in which dilated conjunctival capillaries become compromised and fragile with consequential increased capillary permeability and rupture of conjunctival capillaries (Figure 1).<sup>72,101</sup> The appearance of follicular response, which appears as avascular, round, or oval clusters of lymphocytes, depends on the patient's immunoresponse to the AdV

infection.<sup>72</sup> Pseudomembranes are likely due to exudation of serum, fibrin, and leukocytes from dilated capillaries in the conjunctiva and follicular reaction in the inflamed palpebral conjunctiva.<sup>2,101</sup> Pseudomembranes consist of leukocytes, necrotic epithelial tissue, and fibrin on the inflamed surface of the palpebral conjunctiva. Pseudomembranes could potentially form a conjunctival scar and symblepharon if they are not removed.<sup>2,102</sup> Pseudomembranes are always present in the more symptomatic eye (Figure 2).<sup>103</sup> PCF is typically self-limiting and will resolve in 2–3 weeks.<sup>2</sup>

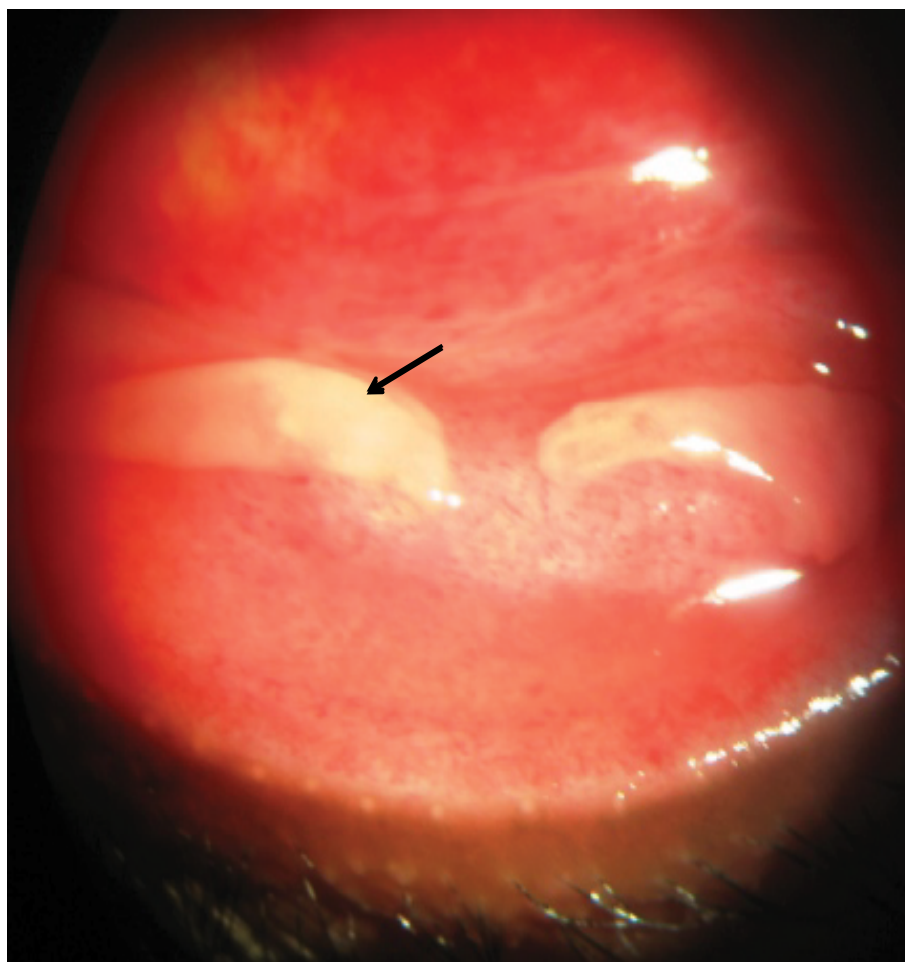
EKC is the most severe form of ocular HAdV infection.<sup>2,104,105</sup> Patients will often report redness, tearing, foreign-body sensation, and photophobia. They may also have flu-like symptoms, such as generalized fever, malaise, and myalgia.<sup>3,100</sup> Magnetic resonance imaging in EKC reveals that there is even deeper penetration of ocular tissue, with evidence of associated eyelid edema and lacrimal gland enlargement. It is also often accompanied by nasolacrimal duct inflammation, giving rise to excessive symptomatic tearing in these patients.<sup>100,106</sup> It is noteworthy that AdV infection of the ocular surface primarily affects the conjunctival surface, but in EKC, AdV may also gain entry into the cornea, inducing innate and cell-mediated immunoresponses.<sup>75,80</sup>

The most unique characteristic of this type of conjunctivitis is its ability to gain entry into the cornea, resulting in a



**Figure 1** Slit-lamp photography of superior palpebral conjunctiva with petechial hemorrhages (black arrow) in a patient with adenoviral keratoconjunctivitis.  
**Note:** Courtesy of DeGaulle | Chigbu.





**Figure 2** Slit-lamp photography of inferior palpebral conjunctiva with pseudomembrane (black arrow) in a patient with adenoviral keratoconjunctivitis.  
**Note:** Courtesy of DeGaulle I Chigbu.

severe inflammatory response that often leads to detrimental visual disturbance.<sup>106</sup> This inflammatory response is as a result of several factors. It is well documented that the corneal stroma houses a significant number of cells, including fibroblasts, dendritic cells, and macrophages. As such, these cells respond greatly in cases of infection or insult, resulting in the production of chemokines that facilitate leukocytic infiltration.<sup>74</sup> The entry of AdV into the cornea begins with its entry to the cell membrane following initial infection. Next, cell binding occurs as the virus makes its way to the cytoplasm, yielding intracytoplasmic accumulation and subsequent clinically evident stromal inflammation shortly thereafter.<sup>107,108</sup> It is of note that the process through which the virus enters the host is clathrin-mediated endocytosis. Caveolae, lipid rafts that allow entry into the plasma membrane, contain the membrane protein caveolin 1, a ubiquitous protein in several cell types, mainly fibroblasts. AdVs use this pathway to gain access into the cornea.<sup>41</sup>

Corneal involvement may begin as early as 2–3 days following initial clinical presentation. Changes to the corneal

tissue that occur following the immunoresponse are seen clinically as keratitis, often associated with adenoviral keratoconjunctivitis. Multifocal punctate keratitis is usually due to replication of the AdV in the corneal epithelium, and this gradually develops into focal areas of epithelial opacities.<sup>109</sup> Approximately 7–10 days following the initial clinical manifestation of EKC, multiple, focal subepithelial infiltrates (SEIs) arise in the anterior stroma of the cornea.<sup>13</sup> Interaction of adenoviral virus-associated molecular patterns with pattern recognition receptors on superficial keratocytes is associated with infection and activation of keratocytes in the anterior corneal stroma, with consequential upregulation of proinflammatory mediators, such as chemokines. IL8 and MCP1 are chemokines released by AdV-infected keratocytes that mediate leukocyte infiltration in AdV-infected corneal stroma. IL8 recruits neutrophils, whereas MCP1 recruits monocytes and T cells. Accumulation of lymphocytes, macrophages/monocytes, and activated fibroblasts in response to AdV-infected corneal keratocytes is a hallmark histopathological feature of subepithelial (stromal) corneal

infiltrates.<sup>110–113</sup> These opacities may persist for up to several weeks to years and have the tendency to result in visual decrease, glare, photophobia, and irregular astigmatism.<sup>3,110</sup> Kaye et al<sup>114</sup> used primers for AdV hexon genes to amplify extracted adenoviral DNA from ocular samples of patients who had a history of AdV. These researchers were able to demonstrate the presence of amplifiable adenoviral DNA in ocular samples, such as tears, indicating that the presence of adenoviral DNA was associated with chronic papillary conjunctivitis. As such, chronic or recurrent papillary conjunctivitis in patients with previous AdV-related keratoconjunctivitis is associated with the persistence of adenoviral DNA in the tear film and conjunctiva.<sup>114</sup>

## Laboratory diagnostic methods

The diagnosis of ocular HAdV infection is mainly based on clinical examination. However, some laboratory tests are available to aid in the confirmation of the diagnosis. The first method of identification is the isolation of the virus in cell cultures, using HEK cell cultures, or through the use of complement fixation test.<sup>115,116</sup> In HEK cell cultures, samples are obtained from the conjunctiva of affected patients and inoculated into HEK cells to analyze structural changes to host cells from viral invasion. Complement fixation test methods involve blood samples tested for the detection of antibodies related to AdV infection. Side-by-side comparison of these two methods indicates superior sensitivity with HEK cell culture testing.<sup>115</sup> The limitation is the timing of laboratory testing, in that it takes ~1–2 weeks for results to be made available.<sup>116</sup>

Polymerase chain reaction assays offer a timely and more sensitive approach to laboratory diagnosis of HAdV when compared with cell cultures. This method utilizes the extraction of conjunctival samples with polymerase chain reaction amplification and full characterization with gel electrophoresis to identify the presence of HAdV. The drawback of this procedure, however, is that it is not usually able to be performed on-site and transport of cell samples are necessary.<sup>2,22</sup>

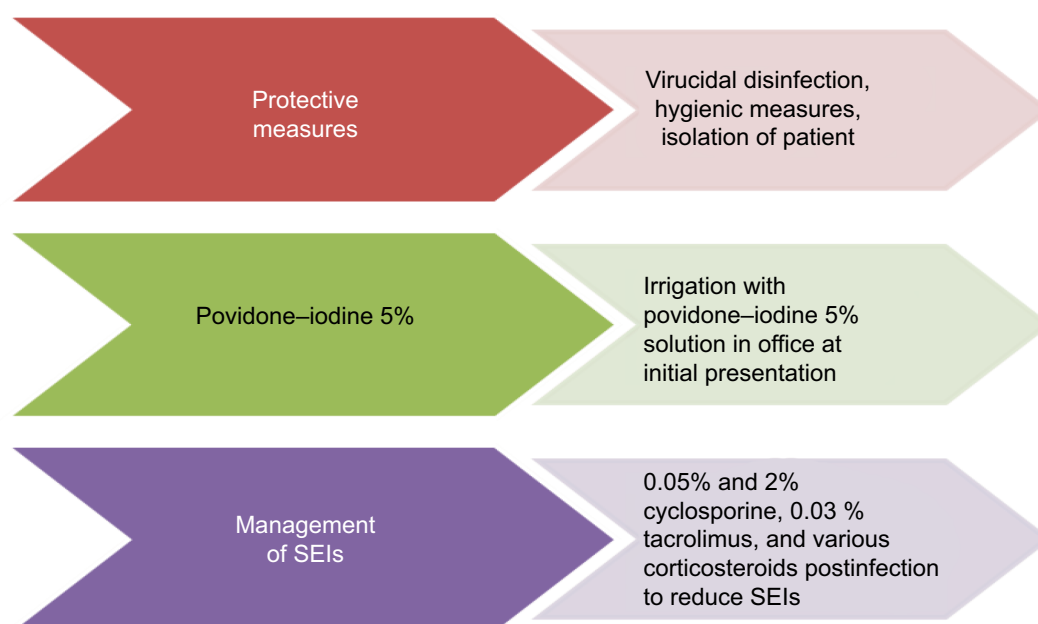
Most recently, the US Food and Drug Administration (FDA) approved rapid antigen-detecting kits that can be used chairside as a point-of-care test to diagnose adenoviral conjunctivitis.<sup>117</sup> The Rapid Pathogen Screening AdenoDetector utilizes lateral flow immunochromatography, which identifies the hexon protein portion of HAdV to diagnose quickly and accurately the presence of AdV on the ocular surface.<sup>118,119</sup> The procedure for this test involves collecting a tear sample from multiple areas of a patient's conjunctiva during his or her active disease. The sample is then placed in a

buffer solution, where it sits for 10 minutes, finally indicating a positive test denoted by one blue control line and one red test line or a negative test denoted by only the blue control line.<sup>120</sup> It is able to detect 53 types of HAdV with an 88% sensitivity and 91% specificity.<sup>2,118</sup>

## Clinical management

Clinical management of ocular AdV infections is targeted to provide relief of symptoms, as well as shorten the duration of infection. Although adenoviral conjunctivitis is a self-limiting condition typically lasting 2–3 weeks, treatment options have been investigated to alleviate symptoms or shorten time of infection. Patients with adenoviral ocular infection are highly contagious for the first 2 weeks after onset of symptoms. Because AdV is a nonenveloped virus, it can survive on inanimate objects for several weeks after contamination.<sup>121,122</sup> Outbreaks of adenoviral ocular infection have been linked to transmission of AdV from contaminated ophthalmic equipment, environmental surfaces, multipurpose eyedrops, and contaminated hands of health-care workers.<sup>121</sup> As such, it is important to institute appropriate levels of infectious disease control measures to reduce the ability of contaminated instruments in the eye clinic to serve as a source of transmitting the viral infection to other patients.<sup>121,122</sup> Infection control measures include reeducating health-care workers on the importance of personal hygiene via handwashing before and after patient contact, high-level disinfection of shared or reusable equipment that come into contact with patient eyes or bodily fluids being immersed in 3% hydrogen peroxide for a minimum of 10 minutes, designating an examination room for triaging patients with suspected adenoviral ocular infections, and use of disposable tonometer tips and single-dose eyedrops in the eye clinic (Figure 3).<sup>121,123</sup> Surfaces within the examination room should be cleaned and disinfected as a means of reducing transmission of HAdV to other patients.<sup>124</sup> Tonometry and other procedures requiring contact with ocular surfaces should be postponed. Patients should be instructed to reduce transmission risk via avoidance of eye touching and sharing such items as towels and pillowcases.<sup>125</sup> Patients should also be advised to stay isolated during the infective phase, as well as thoroughly educated on the normal course of the disease to prevent the need for frequent office follow-ups.<sup>123</sup>

There is currently no FDA-approved treatment for ocular HAdV. However, antiviral drugs, such as ganciclovir and cidofovir, have demonstrated promise in the treatment of various HAdV types with *in vitro* studies.<sup>53</sup> Cidofovir is a nucleoside analog that was developed to fight against DNA



**Figure 3** Flowchart highlighting management efforts for EKC patients.<sup>2,121–123,127,141,143</sup>  
**Abbreviations:** EKC, epidemic keratoconjunctivitis; SEIs, subepithelial infiltrates.

viruses, as it directly passes the cell membrane and targets the DNA polymerase of the virus to halt replication.<sup>126</sup> Topical application to the ocular surface reduces time and exposure of viral shedding. The utilization of cidofovir for the treatment of HAdV is controversial, as studies have not exhibited a statistically significant improvement in the amelioration of symptoms or course of the disease.<sup>2,53</sup> It has also been demonstrated to cause substantial ocular toxicity, even at low doses, making it of little clinical value.<sup>127,128</sup>

Ganciclovir is also a synthetic nucleoside analog inhibiting DNA polymerase and effective against several DNA viruses, most notably the herpes family.<sup>53,129</sup> The marginal efficacy of ganciclovir in treating adenoviral keratoconjunctivitis could be attributed to the lack of viral thymidine kinase in AdV.<sup>6,129,130</sup> It has been suggested that valganciclovir, a prodrug of ganciclovir, inhibits DNA replication of AdV via blockade of adenoviral DNA polymerase and induction of intracellular modification of purine–pyrimidine nucleotide balance.<sup>131,132</sup> In an *in vitro*-based study, Huang et al demonstrated the dose-dependent antiadenoviral effect of ganciclovir on HAdV3, -4, -8, -19, and -37 species associated with ocular adenoviral keratoconjunctivitis. They suggested that commercially available ganciclovir ophthalmic gel 0.15% may be therapeutically beneficial.<sup>129</sup> A small-scale study has indicated the potential of ganciclovir in the inhibition of the formation of SEIs; however, larger scale data are lacking.<sup>119,133</sup>

Topical immunoglobulin (Ig) also has exhibited antiviral activity, specifically with HAdV, in both *in vitro* and *in vivo*

studies by successfully neutralizing the virus on the ocular surface. Ig shows promise in that it can act in the early phases of infection, aiding in viral clearance for earlier resolution of clinical symptoms and prophylaxis against future infection, as it is known also to demonstrate broad-spectrum antimicrobial characteristics. Though studies have displayed success with Ig as a potential treatment for EKC and AdV-related conjunctivitis, the availability of it from serum donors as a topical solution is questionable, due to issues with product consistency.<sup>134</sup> Also, despite success with animal *in vivo* studies, human data are lacking.<sup>2</sup>

Because the course of HAdV infection in immunocompromised patients is usually severe and persistent, antiadenoviral chemotherapy and cellular immunotherapy would be therapeutically beneficial. Antiviral chemotherapy have shown promise in treating immunocompromised individuals with adenoviral infection. Cidofovir and ganciclovir have been used as antiviral pharmacotherapy in immunocompromised individuals with adenoviral infection. Brincidofovir is a lipid conjugate of cidofovir with reduced propensity to cause nephrotoxicity usually associated with cidofovir. It is more orally bioavailable than cidofovir. Brincidofovir has demonstrated significant antiadenoviral activity.<sup>6,135</sup> Grimley et al<sup>136</sup> demonstrated the antiadenoviral activity of brincidofovir in immunocompromised pediatric and adult patients. Toth et al<sup>137</sup> demonstrated the antiadenoviral efficacy of combination therapy of brincidofovir and valganciclovir, which translated to reduced dose and duration of therapy. This could be a very



beneficial therapeutic strategy for immunocompromised patients. Adoptive transfer of HAdV-specific T cells is a beneficial cellular immunotherapeutic strategy for treating adenoviral infections in immunocompromised individuals, such as recipients of hematopoietic stem cell transplants.<sup>6,138</sup> The adoptive transfer of HAdV-specific T cells boosts antiadenoviral cellular immunity. Zandvliet et al<sup>139</sup> demonstrated that HAdV hexon-specific T cell-adoptive immunotherapy resulted in the generation of CD8<sup>+</sup> and CD4<sup>+</sup> T-cell immunoresponse that cleared adenoviral infection in immunocompromised patients. The use of adoptive HAdV-specific T-cell transfer is an effective immunotherapeutic protocol and is an alternative to the use of antiviral chemotherapy to treat adenoviral infection in immunocompromised individuals.<sup>140</sup>

Povidone–iodine, an antiseptic often used for sterilization prior to general and ocular surgery, has broad-spectrum antimicrobial properties to inhibit various bacteria, viruses, fungi, and other organisms.<sup>127</sup> As such, its potential use in the treatment of HAdV has been and is currently still under investigation. Povidone–iodine 5% solution alone is often used in clinical practice as in-office irrigation to decrease viral spread. Potential efficacy with povidone–iodine in conjunction with a topical corticosteroid is also favorable, in that the steroidal component provides symptomatic relief while the povidone–iodine destroys the virus.<sup>2,127,141</sup> Phase II clinical trials are under way for a combination povidone–iodine 0.4%–dexamethasone 0.1% ophthalmic suspension, showing promise as a future treatment approach to ocular AdV.<sup>141</sup> Most recently, a controlled randomized study utilizing povidone–iodine 1.0% and dexamethasone 0.1% eyedrops concluded that patients placed on this regimen had nearly complete recovery of signs and symptoms of adenoviral infection in as little as 5 days.<sup>142</sup> Using this combination four times per day was superior to patients using artificial tears only or those using dexamethasone 0.1% only. This was likely due to the fact that the povidone–iodine inhibited viral replication, exhibiting antiviral effects, while the dexamethasone component served as an anti-inflammatory component and alleviated patient symptoms. It was also highlighted that patients treated too early with a corticosteroid alone, such as the dexamethasone-only control group, may actually show increased viral replication and prolonged viral shedding, a mechanism that is counteracted with the adjunct use of povidone–iodine (Figure 3).<sup>142</sup>

Despite the self-limiting nature of ocular AdV, SEIs may remain well after the infection subsides. Topical corticosteroids alone are implicated in their treatment in the capacity that they may aid in clearance of these often visually significant opacities. The drawbacks of corticosteroid

use in these cases are many, in that the SEIs often return following discontinuation.<sup>2</sup> Treating with corticosteroids in the active phase of the infection may also be detrimental in that it has been shown actually to prolong viral shedding and inhibit immunoclearance.<sup>119,127</sup> This is apart from the other known negative side effects of prolonged corticosteroid use: glaucoma and cataract formation. Due to the negative effects of corticosteroids, 0.05% and 2% cyclosporine, as well as tacrolimus 0.03% agents, may instead be beneficial in clearance of SEIs without the damaging potential outcomes (Figure 3).<sup>127,143</sup>

## Conclusion

The presence of sight-threatening complications postocular adenoviral disease warrants the necessity of developing antiadenoviral therapy with enhanced therapeutic index and safety profile.<sup>21</sup> As such, adenoviral genes and proteins involved in the process of adenoviral pathogenesis could serve as potential therapeutic targets for developing antiviral therapy. Currently, investigations are under way targeting genes that are important in initiating adenoviral replication. Furthermore, caveolin is a known important mediator for adenoviral infection of corneal cells. It has been reported that HAdVD37 can enter corneal fibroblasts via the caveolin 1-associated lipid-raft-specific pathway, in which, there is triggering of caveosomal signaling and expression of proinflammatory mediators, and it is possible that lipid-raft-mediated endocytosis could be a target for antiviral therapy in adenoviral corneal infection.<sup>41</sup> There is also ongoing research on the use of RNA interference to mediate the silencing of viral genes required for viral replication via siRNA, short-hairpin RNA, or artificial miRNA.<sup>144</sup> RNAi-mediated silencing of early and late genes of AdVs, specifically or particularly DNA polymerase and preterminal proteins, can inhibit viral replication.<sup>51</sup> Using primary human limbic cells, Nikitenko et al<sup>145</sup> demonstrated the ability of siRNA to downregulate replication of viral DNA in HAdVD species (D8 and D37) via silencing of *E1A* and *E2B* mRNA. They demonstrated that downregulation of *E1A* gene expression in HAdV species D8, D19, and D37 using siRNA inhibited AdV DNA replication with consequential blockade of AdV multiplication.<sup>145</sup> Therefore, it is imperative to develop antiviral therapy that targets specific proteins required for HAdV survival, with the intent of reducing AdV-associated ocular complications and disease burden.

## Disclosure

The authors report no conflicts of interest in this work.

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