Ocular Pharmacology of Tear Film, Dry Eye, and Allergic Conjunctivitis

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Contents

Abstract

Dry Eye Disease (DED) is "a multifactorial disease of the tears and ocular surface that results in symptoms of discomfort, visual disturbance, and tear-film instability with potential damage to the ocular surface." DED comprises two

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etiologic categories: aqueous-deficient dry eye (ADDE) and evaporative dry eye (EDE). Diagnostic workup of DED should include clinical history, symptom questionnaire, fluorescein TBUT, ocular surface staining grading, Schirmer I/II, lid and meibomian pathology, meibomian expression, followed by other available tests. New diagnostic tests employ the Oculus Keratograph, which performs non-invasive tear-film analysis and a bulbar redness (BR). The TearLab Osmolarity Test enables rapid clinical evaluation of tear osmolarity. Lipiview is a recently developed diagnostic tool that uses interferometry to quantitatively evaluate tear-film thickness. In DED, epithelial and inflammatory cells produce a variety of inflammatory mediators. A stagnant tear film and decreased concentration of mucin result in the accumulation of inflammatory factors that can penetrate tight junctions and cause epithelial cell death. DED treatment algorithms are based on severity of clinical signs and symptoms, and disease etiology. Therapeutic approaches include lubricating artificial tears and immunomodulatory agents.

Keywords
Conjunctivitis • Diagnostics • Dry eye • Ocular surface • Tear film • Therapy

1 Tear Film Structure and Physiology

The tear film forms a layer approximately 3 μm thick and 3 μL in volume on the anterior conjunctival surface, and serves multiple functions including lubrication, antimicrobial protection, nutrition, maintenance of corneal transparency and surface stem cell population, removal of debris, and preservation of the quality of image projected to the retina. Estimates of tear turnover rate range between 0.12 and 1.47 μL/min (5–22.2%/min) (King-Smith et al. 2000; Dartt and Willcox 2013).

Tear film composition is dynamic, responding to environmental conditions in order to maintain ocular surface homeostasis. The film itself is an emulsion of three components: an outer lipid layer secreted by the meibomian, Zeis, and Moll glands; an intermediate aqueous layer secreted by the main and accessory lacrimal glands; and an inner mucin layer secreted by conjunctival goblet cells. The lipid layer is composed of a combination of low polarity lipids, such as wax and cholesterol esters, and high polarity lipids, such as triglycerides, free fatty acids, and phospholipids. The aqueous layer is composed of inorganic salts, bicarbonate ions, glucose, urea, enzymes, proteins, and glycoproteins. While traditionally understood as three separate and distinct layers, new studies suggest that the mucin and aqueous layers integrate to create a gradient of decreasing mucin concentration outwards to the aqueous layer (Dartt and Willcox 2013).

2 Dry Eye Disease

Dry eye disease (DED) is a complex symptomatic syndrome with myriad clinical variations, defined by the International Dry Eye WorkShop (DEWS) as "a multifactorial disease of the tears and ocular surface that results in symptoms of discomfort, visual disturbance, and tear-film instability with potential damage to the ocular surface." (International Dry Eye Workshop (DEWS) Definition and Classification 2007) DED, synonymous with keratoconjunctivitis sicca (KCS), was subdivided by DEWS into two etiologic categories: aqueous-deficient dry eye (ADDE) and evaporative dry eye (EDE).

The pathophysiology of DED involves numerous pathways leading to a final common denominator of lacrimal functional unit (LFU) dysfunction. The LFU consists of the ocular surface (cornea, limbus, conjunctiva, conjunctival blood vessels), tears and their associated machinery (lacrimal glands, meibomian glands, goblet cells, epithelial cells, nasolacrimal duct), and relevant components of the nervous, endocrine, immune, and vascular systems. These elements preserve corneal clarity by maintaining lubrication, nutrition, and the surface stem cell population, while minimizing inflammation and microbial overgrowth.

2.1 Aqueous-Deficient Dry Eye

ADDE is caused by reduced lacrimal tear secretion, and can be further divided into two subgroups: Sjogren syndrome (SSDE) and non-Sjogren syndrome (non-SS) conditions. Sjogren syndrome is an autoimmune exocrinopathy in which activated T lymphocytes infiltrate lacrimal and salivary glands, causing apoptosis of acinar and ductular cells and subsequent dysfunction. Dry eye caused by gland hyposecretion is further worsened by a neurosecretory block, which may be caused by antibodies directed against muscarinic receptors of the glands, or inflammatory cytokines in tear film. Clinically, patients present with symptoms of both dry eye and dry mouth (xerostomia); diagnosis can be aided by lab tests for autoantigens that are expressed by surface epithelial cells (anti-Ro and anti-La). Sjogren syndrome may occur as primary disease, but more often is secondary to a known autoimmune condition, most commonly systemic lupus erythematosus (SLE), polyarteritis nodosa, granulomatosis with polyangiomatosis, systemic sclerosis, primary biliary cirrhosis, or mixed connective tissue disease.

Non-SS dry eye can be divided into four categories of conditions: primary lacrimal gland deficiencies, secondary lacrimal gland deficiencies, obstruction of the lacrimal gland ducts, and reflex hyposecretion. Primary lacrimal gland deficiency is most commonly attributable to age-related dry eye (ARDE). As normal individuals age, glands are obstructed by the accumulation of ductal changes, including periductal fibrosis, interacinar fibrosis, paraductal blood vessel loss, and acinar cell atrophy. Other uncommon forms of primary lacrimal gland deficiency are: congenital alacrima, a rare cause of childhood DED; and familial dysautonomia (Riley Day syndrome), a progressive, autosomal recessive, neuronal

developmental abnormality characterized by insensitivity to pain. In the latter condition, impaired sympathetic and parasympathetic lacrimal gland innervation and poor ocular surface sensation impede both emotional and reflex tearing (International Dry Eye Workshop (DEWS) Definition and Classification 2007).

Secondary lacrimal gland deficiencies may be associated with a number of systemic conditions in which the lacrimal gland is infiltrated by cells causing dysfunction: sarcoidosis (invasion by non-caseating granulomas); lymphoma (lymphomatous tissue); and AIDS (CD-8T lymphocytes). In graft vs. host disease (GVHD), fibrosis occurs ~6 months after transplantation with the invasion of periductal CD-4 and CD-8T cells, and antigen-presenting fibroblasts. Ablation or denervation of the lacrimal gland secondary to trauma or surgery may also cause DED.

Cicatrizing disorders that lead to lacrimal gland duct obstruction include: trachoma, which causes trichiasis, tarsal and conjunctival scarring, and meibomian gland dysfunction; cicatricial or mucous membrane pemphigoid, which causes severe conjunctival blistering; erythema multiforme, which is an acute and self-limited cutaneous disorder of variable etiology (drug, infection, malignancy) that may cause conjunctival scarring; Stevens–Johnson syndrome; and chemical and thermal burns.

Finally, any impairment of reflex hyposecretion can cause non-SS ADDE. Physiologic tearing occurs in response to a variety of stimuli: the cornea and lid margins are densely innervated by sensory branches of the trigeminal nerve, lacrimal and meibomian glands receive both parasympathetic and sympathetic innervation, and goblet cells have parasympathetic innervation. These pathways form the reflex arcs that control reflex tear secretion. However, surface sensory loss may lead to decreased reflex hyposecretion and blink rate (which causes dry eye through evaporative tear loss). Impaired corneal sensitivity is found in a multitude of common conditions including chronic contact lens wear, diabetes, refractive surgery, or neurotrophic keratitis (caused by HSV or HZV infection, or CN V damage); it can also occur secondary to systemic beta blockers, atropine, keratoplasty, or the limbal incision of extracapsular cataract surgery. Reflex motor block, or damage to CN VII, also leads to reflex hyposecretion since damage to postganglionic, parasympathetic fibers to the lacrimal gland decreases secretomotor function, and lagophthalmos due to incomplete lid closure increases evaporative loss of tears. Trauma may cause damage to these pathways, as well as systemic medications including antihistamines, beta blockers, antispasmodics, diuretics, tricyclic antidepressants, and selective serotonin reuptake inhibitors.

2.2 Evaporative Dry Eye

EDE is characterized by a pathologically high level of tear evaporation and can be caused by internal conditions that affect lid structures or dynamics, or environmental factors and exposures. An example of an intrinsic cause is the reduced blink rate that accompanies driving, watching TV, reading, and computer work, leading to rapid evaporation. In contrast, environmental factors act directly on the external

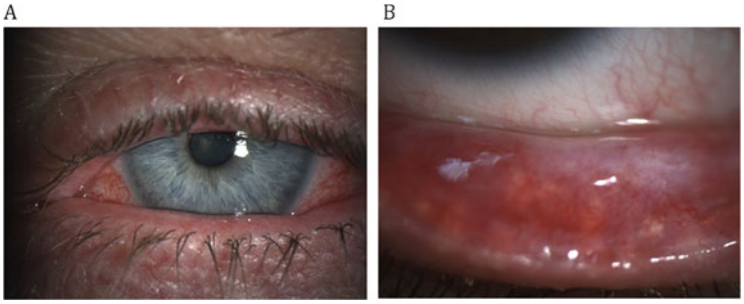

Fig. 1 (**a**) Lid margin telangiectasias, madarosis, and margin thickening and irregularity are characteristic of inflammatory conditions such as ocular rosacea. (**b**) Chronic severe inflammation can lead to conjunctival fibrosis

surface; common culprits include central heating, dry climate, air pollution, wind, chemical burns, and contact lens wear.

The most common cause of EDE is meibomian gland dysfunction (MGD), which is chronic inflammation of the eyelid margin posterior to the gray line that may be accompanied by squamous debris, terminal gland obstruction, and qualitative or quantitative changes in glandular secretion. MGD can be identified at slit lamp by morphologic features of duct orifice plugging, increased viscosity of excreta, or inability to express oil from the glands (Fig. 1). It can be evaluated with qualitative grading, meibography to measure the degree of gland dropout, or meibometry to quantify the amount of oil in the lid margin reservoir. Causes may be local (posterior blepharitis); systemic (such as acne rosacea, seborrhoeic dermatitis, atopic dermatitis); or syndromal (anhidrotic ectodermal dysplasia, ectrodactyly syndrome, Turner syndrome). Cicatricial MGD may occur secondary to local tissue damage such as with trauma, burn, pemphigoid, erythema multiforme, or vernal keratoconjunctivitis. Other causes of MGD include meibomian gland replacement, which occurs in distichiasis; gland deficiency, which may be congenital or acquired; or reversible gland atrophy, which is caused by isotretinoin acne treatment.

Intrinsic EDE causes include conditions that compromise lid apposition or decrease blink rate. For example, dry eye is common and often severe in thyroid eye disease, which causes lid retraction and proptosis leading to an increased palpebral fissure and lagophthalmos. The decline in blink rate that may accompany Parkinson's disease results from a decrease in the quantity of dopaminergic neurons in the substantia nigra, and is proportional to disease severity. In addition to increased tear evaporation time, infrequent blinking impairs clearance of lipid-contaminated mucin.

Tear film instability may be caused by indoor environmental factors, such as high temperature and low relative humidity (RH), as found in air-conditioned cars, offices, and airplane cabins. "Cool and dry" conditions are ideal, with recommended RH of about 40%. Likewise, outdoor exposure to sun, dust, and wind has

been shown to worsen DED (Gayton 2009). These environmental exposures and trauma may also lead to corneal abnormalities, such as pterygium, which can disrupt the tear film and lead to symptoms of dry eye.

While women overall are more likely to have dry eye symptoms, hormonal studies suggest that sex hormone changes influence ocular surface conditions through both aqueous production and evaporative mechanisms: by impacting tear secretion, meibomian gland function, and conjunctival goblet cell density. This relationship is not fully understood but, in clinical studies, women taking oral contraceptives have been found to have significantly higher goblet cell density. Chronic reduction in androgen levels has been associated with meibomian gland dysfunction, the absence of anti-inflammatory cytokines such as transforming growth factor-beta (TGF-β), and the release of proinflammatory cytokines such as interleukins (IL-1β, IL-2), interferon γ (IFN-γ), and tumor necrosis factor α (TNF-α). Accordingly, DED is more common in low-androgen conditions: postmenopause, primary ovarian failure, and autoimmune conditions. Furthermore, postmenopausal women on hormone-replacement therapy have a higher prevalence of DED, especially in women who are on estrogen only regimens (Gayton 2009; Peters and Colby 2013).

3 Epidemiology

Prevalence of dry eye increases with age, affecting 10% of adults age 30–60 and 15% of adults over age 65, and is more common among females (Weisenthal et al. 2015; Schaumberg et al. 2003). Based on data from large population based studies, the Women's Health Study (WHS) and the Physicians' Health Study (PHS), an estimated 3.23 million women and 1.68 million men in America over the age of 50 suffer from DED (International Dry Eye Workshop (DEWS) Epidemiology 2007).

The epidemiology of DED is limited by the different definitions employed by various studies. However, consistent evidence has been found to implicate several risk factors, including female sex, older age, postmenopausal estrogen therapy, a diet that low in omega 3 essential fatty acids, a diet with a high ratio of omega 6 to omega 3 fatty acids, antihistamines, connective tissue disease, history of refractive surgery, vitamin A deficiency, androgen deficiency, hepatitis C infection, radiation therapy, and bone marrow transplantation (International Dry Eye Workshop (DEWS) Epidemiology 2007).

4 Symptom Analysis

Patients with DED present with a diversity of symptoms that include pain, dryness, grittiness, itching, redness, burning or stinging, foreign body sensation, and light sensitivity. As symptoms may persist or worsen over time, DED has been shown to negatively impact patients' quality of life, both in general and vision-related. Given

the variability of clinical tests, assessing DED symptoms in their entirety becomes fundamentally important to guide treatment decisions.

Hallek et al. at the University of Illinois at Chicago developed a four-domain symptom burden tool for comprehensive clinical evaluation of DED impact. Symptoms are classified into two main dimensions, sensory and reactive, and further subdivided into four domains: the sensory dimension is divided into symptom persistence and symptom intensity, and the reactive dimension is divided into activity interference and affective interference. A combination of visual analog, numerical, verbal descriptive, and verbal rating scales were then employed to calculate a numeric score for a patient's experience (Hallak et al. 2013).

In a cross-sectional 48-patient pilot study of this symptom burden assessment tool, the authors found that persistence of symptoms, and not intensity, was correlated with affective interference (or the "mood" of individuals). Because DED has been shown to correlate with anxiety and depression, the study concluded the need for an affective component to be added to standardized DED questionnaires, such as the Ocular Surface Disease Index (OSDI) (Li et al. 2011; Galor et al. 2012; Fernandez et al. 2013).

Authors also found that irrespective of clinical signs, the majority of patients reporting low symptom intensity received less aggressive treatments; management is governed by perceived severity. However, there is a well-established disconnect between signs and symptoms of DED (Mertzanis et al. 2005; Nichols et al. 2004; Johnson 2009). Traditional therapies for DED replace or conserve a patient's tears without correcting the underlying disease process. As a result the study concluded that clinicians need to objectively assess the type and severity of DED in order to effectively address the disease pathophysiology.

5 Diagnosis

Diagnostic tests to assess tear stability, ocular staining, and reflex tear flow, should be chosen based on patients' report of symptoms. Per DEWS the recommended order of tests is as follows: Clinical history, symptom questionnaire, fluorescein TBUT, ocular surface staining grading, Schirmer I/II, lid and meibomian pathology, meibomian expression, followed by other available tests. The DEWS Diagnostic Methodology Subcommittee recommends the administration of structured symptomatology questionnaires to patients presenting with potential DED in order to use clinic time most efficiently. Several questionnaires have been validated and clinicians may choose one based on practical factors such as time, staff available to implement, and end use (International Dry Eye Workshop (DEWS) 2007).

Tear turnover may be evaluated by measuring tearfilm breakup time (TFBUT) in seconds. A standard amount of fluorescein is applied to the eye (as a drop, or by placing a fluorescein-impregnated strip that is wet with saline) initial instruction for the patient to blink in order to distribute the fluorescein. The patient should then be asked to open the eyes without blinking. Viewing under cobalt blue or yellow barrier light at the slit lamp, the clinician measures TFBUT: the interval between

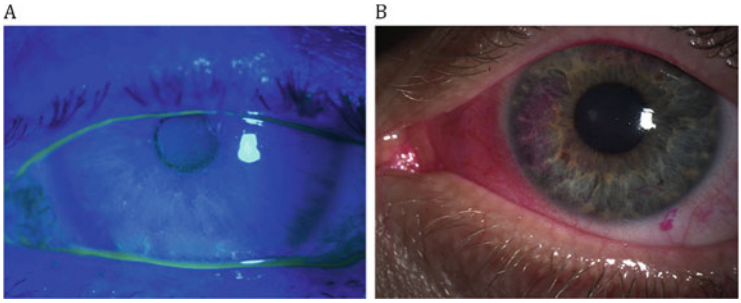

Fig. 2 (**a**) Sodium fluorescein dye stains devitalized epithelial cells, and highlights a narrow tear lake. (**b**) Rose bengal dye stains devitalized epithelial cells, those unprotected by mucin or glycocalyx, and proliferating cells; however, it carries the disadvantage of ocular surface toxicity

the last complete blink and the appearance of micelle, or disruption in the tear film. TFBUT cut-off for dry eye diagnosis is less than 10 s. While this test does not require precision to identify extreme cases, it is subject to operator error; dye must be instilled delicately so that it doesn't elicit reflex tearing, and a standard amount of fluorescein should be placed in the eye (International Dry Eye Workshop (DEWS) 2007).

Epitheliopathy is a characteristic feature of DED, and surface integrity is quantified by grading of ocular surface staining with vital dyes. Most commonly used is hydrophilic sodium fluorescein dye, which diffuses into the corneal stroma to highlight areas of epithelial loss when viewed under cobalt blue light. In contrast, lissamine green (LG) adheres to epithelial cells that are devitalized or unprotected by mucin or glycocalyx; rose bengal (RB) adheres to these in addition to proliferating cells (Fig. 2). LG and RB dyes bear a number of advantages: both are poorly visible within the tear film so the dye in the tear film does not obscure the staining pattern (as with fluorescein, Fig. 2); and since these dyes do not diffuse into the substantia propria of the conjunctiva, there staining pattern lasts longer. While both are well visualized with the backdrop of a light colored iris, they are difficult to see against a darkly pigmented background. RB staining also carries the disadvantage of ocular toxicity, causing stinging and pain that are worse with photoactivation. The degree of staining is dose dependent, however, so instilling a smaller amount or concentration of dye will modify the result. Therefore this test is best performed after instillation of topical anesthetic, and should be followed with saline irrigation (International Dry Eye Workshop (DEWS) 2007).

For each ocular surface staining test, a saline moistened dye-impregnated strip is first used to instill dye on the inferior palpebral conjunctiva. After 15 s, corneal and conjunctival staining are graded by a slit lamp examination (cobalt blue filter is used for fluorescein dye, and rose filter is used for RB and LG). The 1995 National Eye Institute/Industry Workshop scale divides the cornea into 5 zones and the conjunctiva into 6 zones, and each zone is graded from 0 to 3 based on the density of punctate staining. The final staining score is the sum of the individual scores

from all 11 zones. While a greatly simplified Oxford system has since been developed to evaluate ocular surface staining, the NEI scale is preferable because it isolates the visual axis in its own corneal zone (Lemp 1995).

Aqueous tear deficiency is best assessed with the Schirmer test, in which standardized Schirmer strips are bent at the notch and placed carefully over the lower lid margin near the temporal angle of the lids. Strips remain in place for 5 min while the patient keeps both eyes closed, and afterwards the wetting length is measured. The Schirmer I test may be conducted with or without the application of topical anesthetic; the diagnostic cut-off for severe dry eye is generally considered 5 mm or less tear production. Schirmer II is preceded by stimulation of nasal mucosa. Intrasubject variation invalidates comparison of results between individual patients, but same subject comparison can prove valuable despite day-to-day variation of results for a given patient (Whitcher et al. 2010).

6 New Diagnostics

A number of new diagnostic tests employ the Oculus Keratograph, which performs non-invasive tear film analysis (Fig. 3). The keratograph uses a Placido bowl with a camera aperture that has a fixation mark in the center. The device provides consistent illumination, allowing scanning of the exposed bulbar conjunctiva. The system generates a bulbar redness (BR) score automatically, which is based on the area percentage ratio between the vessels and the rest of the analyzed area. The BR range between 0.0 (0%) and 4.0 (40%, the maximum ratio) objectively evaluates ocular surface redness.

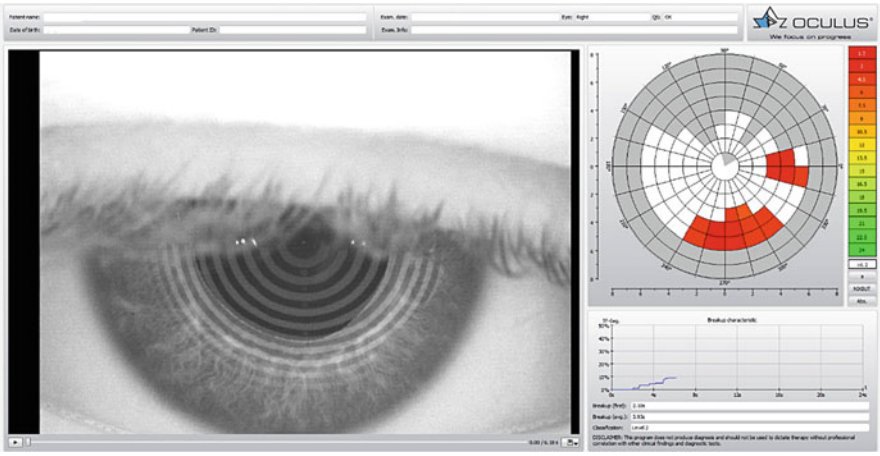

Fig. 3 The Oculus Keratograph calculates first TFBUT (time at first break up of tears) and average TFBUT (average time of all breakup incidents), as well as tear meniscus height (TMH) throughout the cornea

The same machine is also used to calculate non-invasive keratograph tear film breakup time (NIKBUT); this is a more objective measure of tear film stability than a slit lamp evaluation of TFBUT, and does not require application of fluorescein. The keratograph measures tear breakup time twice for each eye using infrared (IR) video and automatically generates two measures of output: NIKBUT-first (time at first break up of tears) and NIKBUT-average (average time of all breakup incidents).

IR images are also used to evaluate tear meniscus height (TMH), an element of tear film quality. The Oculus TMH tool uses an integrated ruler to measure TMH, which is graded perpendicular to the lid margin at the central point relative to the pupil center.

The failure of lacrimal tear secretion involved in ADDE causes tear film hyperosmolarity, and subsequent epithelial cell hyperosmolarity, which in turn initiates a cascade of inflammatory events involving MAP kinases, NFkB signaling pathways, cytokines IL-1 and TNF-alpha, and matrix metalloproteinase 9 (MMP-9, an endopeptidase involved in tissue remodeling). The presence of tear hyperosmolarity and MMP-9 in tear film are therefore valuable tools as they implicate an aqueous deficient etiology of DED (though it is not possible to differentiate between dysfunction of the lacrimal gland itself or other elements of the tear-production pathway).

The TearLab Osmolarity Test, FDA approved in 2009, enables rapid clinical evaluation of tear osmolarity. An abnormal salinity reflects a failure of homeostatic regulation, a key feature of DED; when left unchecked, hyperosmolar tears in early stage DED will lead to damage of the cornea and conjunctiva characteristic of late stage disease. The outcome is continuous: the higher the osmolarity, the more severe the dry eye. To perform the test, a Test Card is touched to the inferior tear meniscus to collect ~50 nL of tear fluid by passive capillary action. The machine then utilizes a temperature-corrected impedance measurement to provide an indirect assessment of osmolarity. One prospective clinical study evaluated the relationship between clinical metrics of DED (OSDI, TFBUT, surface staining, Schirmer, meibomian scoring, tear osmolarity) with a composite of these scores; tear osmolarity was the only marker to demonstrate a linear relationship without significant scatter. This test is also benefitted by its objectivity, quantitative nature, and operator independence (Sullivan et al. 2010).

Lipiview is a recently developed diagnostic tool that uses interferometry to quantitatively evaluate tear film thickness (Fig. 4). Infrared and transillumination images created through dynamic illumination and adaptive transillumination allow clinicians to visualize eyelid morphology and detect structural changes suggesting gland dilation, atrophy, or drop out in severe disease (Hosaka et al. 2011).

Inflammatory markers in tears are also a new focus of diagnostic tests. For example, corneal endothelial cells produce endopeptidases, such as MMP-9, after desiccating stress; this promotes corneal extracellular matrix degradation and epithelial cell loss. InflammaDry is an FDA-approved clinical tool that measures MMP-9 protein in human tears. The test must be performed prior to instilling ocular anesthetic or performing Schirmer testing. Tear fluid sample is collected by dabbing the sampling fleece on the inside of the patient's palpebral conjunctiva

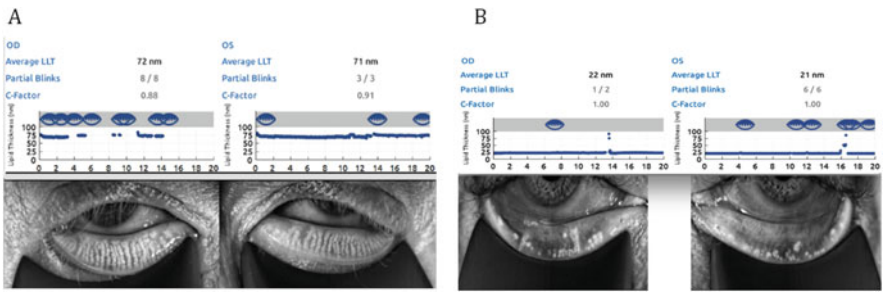

Fig. 4 Lipiview is a new diagnostic tool that uses dynamic infrared transillumination to visualize structural changes suggesting meibomian gland dilation, atrophy, or dropout. Images (**a**) and (**b**) demonstrate a visible contrast between normal gland structure and gland dropout, and a dramatic difference in lipid layer thickness (LLT)

at least 6–8 times, and then rest against the conjunctiva for 5 s. Once this sample is immersed in a buffer solution for at least 20 s, the test cassette is laid flat for 10 min. The result, represented by indicator lines, is binary: a positive result reflects a concentration of MMP-9 $>=40$ ng/mL, and a negative result reflects a concentration of MMP-9 <40 ng/mL.

7 Pathophysiology

7.1 The Innate and Adaptive Immune Systems

The innate immune system is the first line of defense in preventing microorganism invasion of the ocular surface. This system is composed of several nonspecific mechanical and chemical elements, including epithelial tight junctions and epithelial cell sloughing, reflex tearing, the barrier of closed eyelids, the conjunctival mucous membrane, mucins (glycosylated proteins produced by epithelial cells), anti-inflammatory factors (such as lactoferrin), proteolytic enzymes, pattern recognition receptors (PRPs), toll like receptors (TLRs), antimicrobial peptides (such as lysozyme, defensins, cathelicidins, and lipocalin), secreted phospholipase A2 (sPLA2), and secretory Immunoglobulin A (sIgA).

In the case of LFU dysfunction, epithelial and inflammatory cells produce a variety of inflammatory mediators that suppress T cell activation and inhibit complement-mediated tissue damage. Blinking, tear secretion, and tear drainage are all essential to flush away these inflammatory mediators from the ocular surface.

When they accumulate on the ocular surface, the proinflammatory cytokines IL-1 and IFN-γ cause squamous metaplasia of epithelial cells; IFN-γ inhibits goblet cell differentiation; intrinsic (stress-associated mitogen-activated protein kinase) and extrinsic (TNF and Fas/Fas ligand) pathways cause apoptosis of epithelial cells; and MMPs (such as MMP-9) promote corneal extracellular matrix degradation (Stevenson et al. 2012). Therefore, in the context of a stagnant tear film and

decreased concentration of mucin, the resultant accumulation of inflammatory factors can penetrate tight junctions and cause epithelial cell death (Narayanan et al. 2013).

Once these primary protective mechanisms are infiltrated, the adaptive immune system is activated. Adaptive immunity is acquired through specific antigen exposures and is later triggered through re-exposure. Antigen-presenting cells (APCs), such as dendritic cells, elicit a response of T lymphocytes and antibody-producing B lymphocytes, to attack a recognized pathogen.

For example, exposure of corneal epithelial cells to elevated tear osmolarity activates apoptosis of the epithelial surface cells and stress-associated mitogen-activated protein kinases. These in turn stimulate transcription factors (such as nuclear factor kB and activator protein 1) and the production of proinflammatory cytokines, chemokines, and MMPs. Cytokines and chemokines facilitate the maturation of APCs, which migrate to lymphoid tissue to expand the population of CD4+ helper T cell subtypes 1 and 17 (TH1 and TH17). These T cells travel to the ocular surface where TH 1 secretes IFN γ, and TH 17 secretes IL 17, which stimulates the production of MMPs. IFN γ and MMP-9 cause further damage to epithelial cells as noted above. The key to this proinflammatory cycle is the snowball effect (Stevenson et al. 2012).

7.2 External Stressors and Hyperosmolarity

Factors that disturb the homeostasis of the LFU ecosystem increase tear osmolarity. In a healthy state, the osmolarity of tear film is 296–302 mOsm/L; however, in patients with DED, this value rises to 316–360 mOsm/L. The hyperosmolar environment is caused by aqueous tear deficiency and/or increased evaporation of tears, and it stimulates a cascade of osmotic, mechanical, and inflammatory damage, as described above. It also stimulates formation of neutrophil extracellular traps (NET). Numerous neutrophils egress from circulation into tear film during ocular surface inflammation, and NETS on the ocular surface of patients with severe tear deficiency are associated with expression of type I interferon, plus inflammatory cytokines like interleukin-6 and tumor necrosis factor-alpha in ocular surface cells.

Tibrewal et al. recently reported that the amount of NETs released by neutrophils increased exponentially as hyperosmolarity increased, suggesting that NETs likely play a larger role in severe DED with greater hyperosmolarity (>350 mOsm/L) than mild dry eye with minimally elevated osmolarity. Furthermore, neutrophils were found to continue to release NETs, albeit in reduced amounts, even if the iso-osmolar milieu was restored (Tibrewal et al. 2014). The clinical implication of this finding is that although pulsed application of iso-osmolar or hypotonic artificial tear eye drops will intermittently reduce osmolarity, neutrophils will continue to release NETs once exposure to hyperosmolarity recurs.

8 Management

The development of pharmacological therapies for DED has been limited by our incomplete understanding of the mechanism, pathogenesis, and clinical manifestation of DED. Classification of DED by etiology is valuable in choosing a therapeutic approach because while ADDE and EDE often coexist and most treatments are effective for both types (such as artificial tears, cyclosporine, and steroid drops), some therapies are harmful if inappropriately used. For example, punctual plugs therapeutically increase tear retention time in ADDE, but in the presence of MGD they also increase ocular surface exposure to toxic inflammatory factors (Whitcher et al. 2010).

8.1 Stepwise Treatment

The International Task Force at Delphi in 2006 developed stepwise treatment algorithms based on severity of clinical signs and symptoms, and disease etiology. This was modified by the International DEWS in 2007, which published a dry eye grading scheme that assigns a severity score of 1–4+ based on each of 9 diagnostic metrics: discomfort, severity and frequency; visual symptoms; conjunctival injection; conjunctival staining; corneal staining (severity/location); corneal/tear signs; lid/meibomian glands; TFBUT; and Schirmer score.

The DEWS treatment scheme is based on severity. For level 1 it recommends education and counseling, environmental management, elimination of offending systemic medications, and preserved tear substitutes or allergy eye drops. If these are inadequate, level 2 treatment involves preservative-free tears, gels and ointments, steroids, cyclosporine A, secretagogues such as pilocarpine (now rarely used); and nutritional supplements. Level 3 treatment entails tetracycline, autologous serum tears, and punctal plugs (after control of inflammation). For refractory level 4 DED, they recommended topical vitamin A, contact lenses, acetylcysteine, and moisture goggles, or surgical treatment (such as tarsorrhaphy) (International Dry Eye Workshop (DEWS) Management and Therapy 2007).

Prior to pharmacologic therapies, clinicians should consider risk factor modification, such as: smoking cessation, home humidifier use, diet modification to increase consumption of omega three fatty acids, and discontinuation of systemic medications associated with dry eye (diuretics, antihistamines, anticholinergics, and psychotropics are most common).

8.2 Therapeutic Approaches

Lubricating artificial tears are hypotonic or isotonic buffered solutions, of neutral to slightly alkaline pH, containing electrolytes (including potassium to maintain corneal thickness, and bicarbonate to promote recovery of epithelial barrier function), a high colloidal osmolality (such as in glycerin or erythritol, which counteract

hyperosmolar tear film), and viscosity agents to enhance retention time. Viscosity in drop and ointment formulations is achieved with macromolecular complexes: short-acting preparations are often based on carboxymethyl cellulose, polyvinyl alcohol, polyethylene glycol, or hydroxymethyl cellulose; longer-acting ointments are based on carbomer gels or paraffin. Lubricant agents are distributed both over-the-counter and by prescription; though there have been no large, randomized controlled clinical trials to compare the many ocular lubricants on the market, this class of agents remains the mainstay of DED therapy.

While multi-dose artificial tears are mandated by the FDA to contain preservatives such as benzalkonium chloride (BAK) in order to inhibit microbial growth, these are toxic in high quantities (instillation more than 4–6 times/day). The cytotoxic damage they inflict on epithelial cell shape, junctions, and microvilli can cause epithelial cell necrosis; the effect increases with decreased tear secretion and turnover (as found in DED), high concentration of preservatives, and frequency of exposure. For more severe dry eye characterized by lacrimal hyposecretion requiring frequent administration of lubricants, punctual occlusion, or use of multiple drops that have preservative elements, preservative-free preparations in single-use vials are preferable.

Tears may also be substituted with biologically compatible drops, autologous serum tears, which are made from serum that is isolated from the cellular components of blood. To prepare, peripheral blood (20 mL) is taken from a patient, centrifuged to separate the serum, which is then diluted to 20% with sterile saline. The tears are stored in a bottle coated with protection from UV light and must be stored in a freezer for 1 month only, to maintain the desired composition. Serum has an osmotic pressure (300 mOsm) and pH (7.2–7.5) nearly matching that of natural tears (302 mOsm and 7.4, respectively). While the exact mechanism of action is unknown, serum tears contain key ingredients of epidermal growth factor (EGF), vitamin A, TGF-beta, and fibronectin, which promote epithelial healing and are also found in natural tears (Tsubota and Yamada 1992). Similarly, serum has been shown to upregulate mucin production, and contains serum antiprotease which inhibits collagenases. The addition of these trophic components to the water and electrolytes found in traditional artificial tears has been demonstrated to effectively treat DED and persistent epithelial defects (PEDs), and improve TFBUT and vital dye staining when compared to artificial tears.

Immunomodulatory agents also play a significant therapeutic role for DED. The most commonly used anti-inflammatory drop is topical cyclosporine A (CsA) 0.05%, as it has been shown to alleviate the symptoms of DED in about 50% of patients. CsA is a lipophilic peptide that binds with a group of proteins known as cyclophilins. By binding with cyclophilin A, which is found in the cytosol, CsA inhibits calcineurin, a protein phosphatase that dephosphorylates regulatory sites on transcription factors such as nuclear factor of activated T-lymphocytes (NFATs). Through this mechanism CsA selectively inhibits interleukin-2 (IL-2), which is required for the transcription of T cells, thereby suppressing a cell-mediated immune response and interrupting inflammatory cytokine production. However, since the T cell life span can last 110–180 days, CsA may take several months to

take effect and a short course of topical steroids may be prescribed at the outset of treatment. CsA also binds to cyclophilin D to block the opening of the mitochondrial permeability transition pore (MPTP), thereby inhibiting epithelial cell apoptosis. With long-term use topical CsA increases tear production and goblet cell density. Commercially distributed as Restasis, CsA 0.05% is packaged without preservatives in single-use vials and twice daily dosing (Hessen and Akpek 2014).

Clinically, pulsed use of corticosteroid drops are often used off label for DED as they have been shown to improve the efficacy of artificial tears or punctal plugs alone. Corticosteroids have multiple mechanisms of action. They increase synthesis of lipocortin A, which suppresses phospholipase A2, an early step in the inflammatory cascade. Prostaglandin synthesis is halted at the levels of phospholipase A2 and cyclooxygenase (COX-1 and COX-2), thereby inhibiting local leukocyte adhesion and chemotaxis, as well as systemic inflammatory responses such as vasodilation and vascular permeability. Steroids also inhibit nuclear factor-kB (NF-kb), a transcription factor that promotes synthesis of proinflammatory molecules, thereby stimulating lymphocyte apoptosis; likewise, they decrease the production of inflammatory mediators on a genomic level (Hessen and Akpek 2014). In murine models topical steroids protect the integrity of corneal epithelial tight junctions, prevent desquamation of epithelial cells, and decrease MMP-9 levels, thereby preserving barrier function (International Dry Eye Workshop (DEWS) Management and Therapy 2007). In humans, pulsed dosing of loteprednol 0.5% (an ester steroid) starting with use 4 times daily for 1 week, followed by a slow taper, has been shown to improve bulbar conjunctival hyperemia and central corneal fluorescein staining scores by over 25% (Pflugfelder et al. 2004). Loteprednol and fluorometholone, a ketone steroid, have also been found to convey lower risk of elevated intraocular pressure when compared to other ketone steroid drops, prednisolone and dexamethasone.

In the case of artificial tears, cyclosporine and steroid drops, innate and active immune protection against microbial invasion may be compromised. For example, the antimicrobial peptides of the innate immune system lose their ability to kill Pseudomonas aeruginosa in the presence of carboxymethyl cellulose solutions in vitro. Cyclosporine has been found, in vitro, to inhibit the production of cytokines involved in wound healing, and increase susceptibility of epithelial cells to viral infection by reducing interleukin production; in human corneal epithelial cells, it has been shown to inhibit cell proliferation (Narayanan et al. 2013). Despite the potential drawbacks these agents remain the mainstay of treating DED.

In cases of meibomian gland dysfunction, the goal of all treatments is to improve the flow of meibom secretions, and a stepwise approach to treatment is employed to minimize antibiotic exposure. Warm compresses are used to dilate meibomian gland orifices, lid scrubs exfoliate debris from the lid margin, and lid massages coax secretions from inspissated glands, all in order to clear the pathway for oil flow. Washing the lid margin with dilute soap also decreases bacterial colonization, which has been shown to inhibit conjunctival goblet cell proliferation and increase the breakdown of meibomian lipid (International Dry Eye Workshop (DEWS) Management and Therapy 2007; Gilbard 2005). While these are valuable and

proven tools for mild to moderate DED, more severe disease that is resistant to treatment may merit antibiotic therapy. Oral tetracyclines are commonly used to treat DED given their dual impact of broad spectrum antibacterial prophylaxis (though only minocycline and doxycycline are able to reach an effective concentration on the ocular surface), and anti-inflammatory effect, achieved through reduction of MMPs, IL-1α and TNF-α (Narayanan et al. 2013). A new approach to treatment of MGD is LipiFlow, in-office thermodynamic treatment for obstructed meibomian glands. The device is a disposable apparatus that is inserted under a patient's upper and lower eyelids; it transfers heat to the palpebral conjunctiva while applying graded, pulsatile pressure to the outer eyelid to express meibom from glands (Finis et al. 2014; Lane et al. 2012).

For both ADDE and EDE, non-pharmacologic tear preservation can be achieved through a longer-acting approach, such as punctual occlusion, or a physical barrier, such as moisture chamber spectacles and contact lenses. Punctal plugs act by increasing longevity of tears in the conjunctival sac. Plugs are dumbbell shaped, with a wide collar that rests at the puncta, a narrow segment that extends into the canaliculus, and a bulb at the end to anchor the plug internally. Temporary plugs are absorbable, made of collagen and lasting a couple months; these are often used to determine if a more permanent plug would be an effective treatment. In contrast, semi-permanent plugs are made of silicone or polymers. The complications of the procedure are few: excessive tearing (usually only if the upper and lower punctum are both occluded), development of a pyogenic granuloma, canaliculitis, or dacryocystitis. Tear retention is also the goal of moisture chamber spectacles, or contact lenses (such as silicone rubber lenses and rigid gas permeable scleral lenses) (International Dry Eye Workshop (DEWS) Management and Therapy 2007).

9 Allergic Conjunctivitis

As atopic diseases became more prevalent in the latter half of the twentieth century, so has allergic conjunctivitis (AC), which is estimated to impact up to 40% of the US population (Singh et al. 2010). AC encompasses several distinct conditions: seasonal allergic conjunctivitis (SAC), perennial allergic conjunctivitis (PAC), atopic keratoconjunctivitis (AKC), vernal keratoconjunctivitis (VKC), and giant papillary conjunctivitis.

Diagnosis of DES requires differentiation from other common ocular surface inflammatory conditions. Because patients often find it difficult to characterize their discomfort, a nuanced history of symptoms can help elucidate whether the quality of pain is "burning" (more typical of DES) or "itching" (more specific for AC).

In SAC and PAC, allergens interact with IgE bound to sensitized mast cells, causing cross-linking of IgE at the mast cell membrane with subsequent degranulation and release of histamine, tryptase, prostaglandins, and leukotrienes. This early phase reaction lasts 20–30 min.

Mast cell degranulation also induces a late phase reaction by activating vascular endothelial cells that promote expression of chemokine and adhesion molecules,

including monocytes chemotactic protein-1 (MCP-1), intracellular adhesion molecule (ICAM-1), vascular cell adhesion molecule (VCAM), p-Selectin, and chemotactic factors IL-8 and eotaxin. These mediators recruit activated inflammatory cells (eosinophils, neutrophils, and T lymphocytes) to the conjunctiva. This late phase reaction is prolonged and plays a role in more severe forms of AC, with clinical manifestations of conjunctival injection, itching, chemosis, and conjunctival papillae found on exam (Bonini et al. 2009).

Because the conjunctiva has direct exposure to the environment and an abundant vascular supply to deliver immune mediators, SAC and PAC are common conditions that affect people of all ages. Seasonal allergies account for nearly 90% of all AC, and the most common allergens are airborne pollens that reach peak concentration during the spring and summer. Perennial exposures such as dust mites and mold can also stimulate the same ocular surface response.

AKC and VKC, in contrast, do not involve sensitization of immune mediators to specific environmental exposures and often involve the cornea as well (keratoconjunctivitis). TH2 lymphocytes are thought to play a role in the pathophysiology of these conditions by producing inflammatory cytokines IL-4 and IL-13, which are found in abundance in patients with AKC and VKC; this is a common pathway among allergic disorders.

VKC is a chronic inflammatory condition, most commonly affecting males (2:1 ratio) in tropical climates, that is characterized by broad "cobblestone" papillae on the upper tarsal conjunctival surface, mucus discharge, severe itching, and photophobia. Eosinophilic infiltration of the cornea may lead to the development of a well-circumscribed sterile epithelial "shield ulcer" with underlying stromal opacification, that can cause scarring even after resolution. "Tranta's dots" are collections of necrotic eosinophils, neutrophils, and epithelial cells that are found in crypts along the limbus in active disease. Epithelial cells may also release toxic mediators that compound this injury with macroerosions and plaques.

An estimated 15–20% of the US population has atopy, a genetic predisposition to developing a heightened immune response to common allergens. AKC is the ocular corollary of the atopic conditions of asthma and eczema, which are present in 95% and 87% of patients with AKC, respectively (Guglielmetti et al. 2010). A family history of atopy is often positive. The pathophysiology involves IgE-mediated chronic mast cell degranulation and TH1 and TH2 lymphocyte derived cytokines, which cause severe itching, chemosis, and redness, leading to conjunctival scarring and atopic cataracts if uncontrolled. Like VKC, AKC patients may also have giant papillae and Tranta's dots, but AKC more commonly affects patients in their late teens through the 5th decade of life, while VKC usually resolves by age 20.

9.1 Diagnosis

Allergic conjunctivitis is a clinical diagnosis. Referral to an allergist is essential for systemic workup, including allergen skin testing (scratch test or intradermal injections) and in-vitro IgE antibody tests.

9.2 Treatment

Primary treatment of PAC involves allergen identification and avoidance. Cold compresses may provide symptomatic relief. The primary topical therapy for all types of AC is artificial tears, which mechanically protect and flush the ocular surface of immune mediators.

Traditional topical pharmacologic therapy for allergic conjunctivitis includes mast cell stabilizers, H-1 receptor antagonists, and combination mast cell stabilizers with H-1 receptor antagonists, nonsteroidal anti-inflammatories (NSAIDs), and steroids. Mast cell stabilizers prevent degranulation (via an unclear mechanism) but require a loading dose before reaching effective concentration and therefore have a delayed effect. The pharmacology of antihistamines is correspondingly insufficient in resolving symptoms when used as monotherapy. Because these agents reversibly block only H-1 receptors, leaving other inflammatory mediators uninhibited, they provide rapid but only temporary symptomatic relief.

In the past decade multimodal agents have become the mainstay of therapy because they couple the effect of H1 antagonists and mast cell stabilizers with other anti-inflammatory mechanisms. For example, a broader anti-inflammatory effect is achieved with Azelastine, a selective second generation H1 receptor antagonist that blocks intercellular adhesion molecules (ICAMs), and Epinastine, which blocks H2 receptors and thereby reduces eyelid swelling.

If symptoms persist despite first line treatment supplemental agents may be added. NSAIDs inhibit cyclooxygenase, reducing conjunctival redness and itching mediated by prostaglandins D2 and E2. Corticosteroid drops more directly treat AC by antagonizing NFκB, TGF-β, and activating T-lymphocytes into TH2.

Severe cases require systemic therapy, such as oral antihistamines and less commonly intranasal corticosteroids, in complement to local treatment. Allergen-specific immunotherapy by subcutaneous injection (or sublingual administration) may benefit patients with detectable IgE antibodies to known allergens.

10 Bacterial Conjunctivitis

Conjunctivitis is a nonspecific term that describes inflammation of the conjunctiva which may be caused by a wide range of conditions. AC is the most common etiology, but bacterial, viral, chemical, and toxic conjunctivitis also occur frequently and present with overlapping symptoms. Viral conjunctivitis occurs more frequently in the summer and is the most common cause of infectious conjunctivitis overall and among adults. Bacterial conjunctivitis (BC), which occurs more frequently in the winter, is the most common form among children (50–75% of cases) and the second most common cause in adults (Høvding 2008).

The nonspecific symptoms of chronic BC allow it to masquerade as DES or AC. In a cohort study of 184 culture-positive cases, 58% of patients reported itching, 65% burning, and 35% serous or no discharge. Three signs were found to be strongly predictive of bacterial etiology: bilateral mattering of the eyelids, lack of

itching, and no prior history of conjunctivitis. Notably the type of discharge did not correlate with etiology (Rietveld et al. 2004).

BC may be secondary to a systemic condition, such as in gonorrhea, chlamydia, graft-versus-host disease, and Reiter syndrome. Local BC may be transmitted oculogenitally, contaminated fingers or fomites.

BC may be subdivided into hyperacute, acute, and chronic forms depending on severity and speed of onset. Hyperacute BC is distinguished by rapid onset, profuse purulent discharge, lid swelling, and decreased vision. It is most commonly caused by Neisseria gonorrhoeae, which is treated with intramuscular ceftriaxone. Testing for coexisting genital chlamydial infection is requisite as it is positive in 54% of men and 74% of women (Azari and Barney 2013).

In contrast, acute BC lasts a little over a week and is characterized by conjunctival injection, mucopurulent discharge, and ocular discomfort. In adults the most common isolated pathogens are Staph aureus, Streptococcus pneumoniae, and Haemophilus influenzae, while in children the most common culprits are H. influenzae, S. pneumoniae, and Moraxella catarrhalis.

Chronic BC presents similarly but lasts more than 4 weeks. Its most common pathogens are S. aureus, Moraxella lacunata, and enterics (Azari and Barney 2013).

10.1 Diagnosis

Practitioners should maintain a high degree of suspicion when contact lens wearers present with symptoms of conjunctivitis because this population is more likely to develop conjunctivitis caused by gram negative pathogens. Slit lamp exam should include a thorough evaluation of corneal integrity and the tarsal conjunctiva. Papillary and membranous conjunctivitis suggest a bacterial cause. If there is mucus discharge, it should be cultured to test for both viral and bacterial growth.

10.2 Treatment

A Cochrane meta-analysis that reviewed the treatment of suspected acute BC in 3,673 patients from 11 randomized clinical trials demonstrated that topical antibiotics improve the 5-day remission rate by only 31% compared with placebo. Many cases are self-limited, as clinical remission occurred by days 2–5 in 64% of those treated with placebo. Treatment with antibiotics was, however, associated with significantly better rates of clinical remission by days 2–5 (RR = 1.31), with possible benefit for late clinical remission (by days 6–10 RR = 1.27, with 95% CI = 1.00–1.61). These data suggest a high degree of overtreatment of acute infectious conjunctivitis with antibiotics. Notably, there were no serious adverse sight-threatening outcomes in any placebo group (Sheikh and Hurwitz 2001).

Clinically, topical antibiotics are indicated in patients with contact lens history, ocular surface diseases, corneal trauma, use of immunosuppressive medications, or history of ocular surgery. One large systematic review of 40 studies found that topical antibiotics had higher rates of clinical and microbiological remission in patients with positive bacterial culture, while only microbiological remission was significantly improved in patients with clinically suspected BC (Epling 2010). Patients with culture-positive results should be treated similarly, as well as those with suspicion for more aggressive pathogens that can penetrate an intact cornea (e.g., N gonorrhoeae, H influenzae, Corynebacterium diptheriae, and Listeria monocytogenes).

For adult patients with suspected but uncultured BC, who do not fall into a high risk group or have evidence of ulcerative keratitis, empiric treatment with broad spectrum topical antibiotics may be beneficial. Because the benefit of topical antibiotics is short lived and decreases the duration of symptoms without altering the outcome, some practitioners recommend antibiotics only if symptoms persist beyond 1–2 days. When conjunctivits is suspected to be bacterial but does not respond to appropriate therapy, chlamydial conjunctivitis should be tested for and empirically treated with a single dose of azithromycin, given its varied but often smoldering presentation and potential for scarring.

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