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REVIEW

How Can We Best Diagnose Severity Levels of Dry Eye Disease: Current Perspectives

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Abstract: Dry eye disease (DED) is a common ocular condition, but the diagnosis relative to other ocular conditions and the evaluation of severity of the condition has often been difficult. This challenge can be due to clinical signs and symptoms not always correlating with each other. An understanding of the various components which create the condition, as well as the diagnostic measures used to evaluate these components, is useful to the clinician working with DED patients. This review paper will discuss traditional diagnostic options, diagnostic imaging, and Advanced Point of Care testing capabilities to determine the severity level of dry eye disease more adequately.

Keywords: dry eye disease, diagnostic imaging, POC testing, biomarkers

Introduction

Dry eye disease (DED) and ocular allergy (OA) are the most common inflammatory disorders of the ocular surface.[1] The prevalence of DED globally is 5–50%, and more than 16 million Americans have been diagnosed with DED while others carry the condition.[2] Approximately one-third of the global population is affected by allergic disease and OA symptoms occur in 40% to 80% of the affected individuals.[3] DED and OA often mimic each other and frequently coexist.[4] An irregular correlation between reported symptoms and observed signs is potentially attributed to the challenge of diagnosing and evaluating the severity of DED.[5]

DED is most often diagnosed in adults over 50 years old but can occur at any age. Risk factors include age, female gender, use of contact lenses, refractive surgeries, smoking, poor health, use of antidepressants or oral steroids, systemic drug effects, autoimmune diseases, thyroid disease, and environmental factors.[6] Up to 46% DED and OA patients impact on their quality of life activities of daily living.[7–10] Significant psychological impact has been reported with DED and even a willingness to shorten their lifespan to be free of DED.[11] Similarly, the most prevalent forms of ocular allergy, seasonal allergic conjunctivitis (SAC) and perennial allergic conjunctivitis (PAC), can cause extreme discomfort and have a significant impact on quality of life and often predispose or exacerbate DED.[7]

Symptoms by themselves are often inadequate for the differential diagnosis of ocular surface disease (OSD), because a wide variety of ocular surface and tear film disorders such as DED, OA, blepharitis, meibomian gland dysfunction (MGD) and bacterial or viral infections can produce the same symptoms.[12,13] Signs and symptoms of DED do not often correlate.[14] Only about two-thirds of patients with dry eye symptoms test positive for DED with traditional confirmatory tests.[15,16] Despite some patients having minimal objective signs such as punctate corneal fluorescein staining, they complain of severe ocular irritation, whereas others present with severe staining and have minimal symptomatology.[16–18] The ability to practically examine tear biomarkers reveals that specific biomarkers correlate to the signs and symptoms in all forms of dry eye.[19]

Typical ocular surface-related symptoms include eye irritation, burning, tearing, stinging, a gritty or foreign body sensation, photophobia, fluctuating vision, redness, intermittent sharp pain, and transient blurred vision. Environments that generate desiccating stress including high temperature, low humidity, air conditioning, and activities that lead to reduced blinking such as driving, computer use, reading, and watching television can worsen dry eye symptoms.[20–22] Ocular itching, frequently

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referenced as the hallmark OA symptom, is not just an allergic issue, just as tear film instability is not just related to DED.[1,23] DED symptoms have features of neuropathic ocular pain, including spontaneous pain, hyperalgesia and dysesthesias.[24,25] Initial ocular surface damage or inflammation may cause central and/or peripheral sensitization, producing hypersensitivity and persistent DED symptoms despite the source of inflammation and/or damage being removed.[26]

The ocular surface is composed of several components working in tandem to create and maintain a healthy refractive surface on the cornea including the conjunctiva, glandular epithelium, nerves that control the blink reflex, main and accessory lacrimal glands, eyelashes and associated glands, meibomian glands, and the nasolacrimal duct.[27,28] A disruption to any component of the ocular surface can lead to an unstable surface, which in turn, can lead to tear film abnormalities and disrupt the refractive capabilities creating visual disturbances and OSD symptoms.

The normal tear film contains a complex mixture of proteins, electrolytes, mucins, cytokines, lysozymes, immunoglobulins, lactoferrin, and growth factors.[29] The tear film also consists of three layers: the mucin layer, the aqueous layer, and the lipid layer. These layers play an important role in maintaining the ocular surface stability. Normal tear film dynamics include distribution, turnover (and drainage), evaporation, and absorption of the tears.[30] DED is a multifactorial disorder of the tear film caused by decreased tear production and/or increased evaporation that can lead to a loss of homeostasis, tear film instability, inflammation, hyperosmolarity, and neurosensory dysfunction.[6] OA has been shown to be associated with changes in the composition of the tear film that leads to tear film dysfunction.[31] Even though DED and OA are different ocular conditions, there is significant clinical overlap between OA and DED and are often thought of as facilitating, or in the least predisposing, each other, or co-existing conditions.[4,32] It is essential to consider the implications of both DED and OA together in the effective management of OSD.

Changes in the quantity and quality of tears will lead to the development of OSD and these parameters are important for making a correct diagnosis. DED is separated into two primary underlying mechanisms: 1) aqueous-deficient dry eye disease (ADDE) and 2) evaporative dry eye disease (EDE). The aqueous layer accounts for the majority of the tear film thickness and is produced primarily by the lacrimal glands. The lipid layer is made up of meibomian glands, and its function is to stabilize the tear film and protect it from evaporation.[33] ADDE reflects a reduction of lacrimal secretion while EDE has normal lacrimal secretion but is due to excessive fluid loss from the exposed ocular surface, both of which lead to tear film instability and increased tear film osmolarity. However, both ADDE and EDE are often present at the same time and may be associated with nerve abnormalities.[34]

Tears in patients with DED have more proinflammatory markers, do not contain as many protective proteins when compared with patients without DED,[35,36] and due to inadequate production of mucins, both soluble and membrane-bound, lack an even distribution of the tear film.[37] A cycle of tear film instability and ocular surface damage result from desiccant stress and a proinflammatory tear film.[38]

Beyond quality-of-life impairment, undiagnosed and untreated OSD prior to ocular surgery leads to less accurate pre-surgical measurements,[39] mild or asymptomatic DED to become severe or chronic DED and leads to worse visual acuity outcomes.[40] Similarly, OA is a significant risk factor for myopic regression and haze after photorefractive keratectomy.

Having standardized methods for diagnosing DED as well as establishing modalities for monitoring the efficacy of different treatments for DED are necessary.[41] Since OSD is multifactorial, one test has been insufficient to diagnose DED,[42] and a panel or battery of tests have been recommended to improve the effectiveness of diagnosis.[12] Moreover, some patients with no significant clinical signs have considerable discomfort, while those with severe dry eye and vision-threatening ocular complications may suffer from only mild symptoms.[43] An ideal OSD diagnostic test should be rapid (less than 15 minutes), objective, quantitative, specific, reproducible and should directly impact clinical management and/or therapeutic decisions. Testing should be simple to allow implementation by ancillary staff and efficient workflow. Advanced tear-based point-of-care quantitative results support early diagnosis and intervention as well as therapeutic monitoring to confirm adequate disease control or progression. The relationship between DED and OA is well described, and any technology that can rapidly quantify biomarkers like MMP-9, immunoglobulin E and lactoferrin in tears will allow for differentiation between DED and OA and more specific interventions.[44]

Traditional Diagnostics

Historically, DED was determined by a combination of a history and physical examination of the lid and meibomian morphology and expression, and questionnaires followed by conventional diagnostic tests for initial assessment that includes corneal staining, tear film assessment, conjunctival staining, tear break-up time (TBUT), and the Schirmer test.[45,46]

Questionnaires

Questionnaires assess and quantify patient's subjective symptoms of DED to create an objective score so that it can be used to better follow patients over time. The most used questionnaires include the Ocular Surface Disease Index (OSDI) and National Eye Institute Visual Function Questionnaire-25 (NEIVFQ-25) due to their multi-dimensionality and the inclusion of patient's quality of life changes.[47–49] Other questionnaires include the Dry Eye Questionnaire 5, McMonnies, and the Standard Patient Evaluation of Eye Dryness (SPEED).[50]

The OSDI is a 12-question assessment developed to grade the symptoms of ocular irritation in DED and vision-related function over the prior week.[51] The OSDI measures symptoms of dry eye symptoms, light sensitivity, grittiness, and pain, but not other common symptoms like foreign body sensation and tearing. The OSDI is reported to have a specificity of (83%) and a sensitivity of (60%) when diagnosing patients with DED.[51] The OSDI was not shown to correlate with Schirmer tear testing[19] (Lam 2013) but correlates with tear break up time (TBUT) in 35% and increases to 42% when the OSDI ≥35.[52] OSDI and TBUT show no correlation to any elevation in cytokines or MMP-9.[53]

The 25-item NEIFQ-25 is a questionnaire derived from a validated 51-question survey. It examines different health-related quality of life aspects including vision, health overall, difficulty with distance and near vision, limitations in social functioning and limitations of role due to vision, dependency on others and mental health symptoms impacted by vision, expectations for vision in the future, difficulties with driving, and discomfort and/or pain around the eyes. This questionnaire may be less sensitive at capturing symptoms associated with milder dry eye disease. Subscales show correlations ranging from 39% to 69%[48] for DED. The NEI-VFQ is only moderately associated with Sjogren's patients when the ocular surface parameters (tear film break-up time, Schirmer score with and without anesthesia, Oxford score, van Bijsterveld score) exist.[49]

The Dry Eye Questionnaire (DEQ) was developed with the intention of diagnosing dry eye disease and quantifying its severity level for the prior week. It quantifies several symptoms using four measurements: level of irritation, frequency of irritation, intensity of the irritation in the morning, and intensity of the irritation late in the day.[54] There is no correlation between DEQ and clinical parameters but instead can differentiate between patient groups such as 1) dry eye vs non-dry eye, 2) Sjogren's syndrome [SS] with keratoconjunctivitis sicca [KCS] vs non-SS-KCS, and 3) control vs SS-KCS and non-SS-KCS.[55] The DEQ shows a correlation coefficient of $r = 0.76$.[56]

Standard Patient Evaluation of Eye Dryness (SPEED) survey is a validated DED symptom survey based on eight questions that assess both the frequency and severity of symptoms over a 3-month period. The patient grades the frequency of their symptoms as well as the severity. The severity is graded on a scale of zero to four with zero being no symptoms and four being intolerable symptoms. The SPEED questionnaire can differentiate symptomatic from asymptomatic patients and is associated with a sensitivity of 90% and specificity of 80%.[50]

Physical Examination

A slit-lamp examination is very informative in the overall assessment of OSD. It is important to look for abnormalities of the lids, conjunctiva, cornea, and tear film. Misalignment of the lids may lead to trichiasis or corneal exposure and can initiate or exacerbate tear film dysfunction.[57,58] Meibomian gland dysfunction (MGD) and blepharitis are common ocular disorders that are frequently associated with DED and with OA, especially with chronic and severe forms (eg AKC).[59] Moreover, DED impacts corneal sensitivity which may be elevated during the early stages and reduced in chronically progressed stages of the disease.[60] Decreased corneal sensation can potentially disrupt the corneal innervation, which can disrupt the homeostasis of the ocular surface, potentially leading to epithelial disruption and cell loss.[61]

Tear Break-Up Time

The quality of meibum may result in tear film instability, creating a desiccating stress on the ocular surface.[62,63] Tear break-up time (TBUT) is an assessment of tear film instability. TBUT is measured after introducing fluorescein dye

instillation into the eye. Once fluorescein is instilled into the eye and under cobalt blue light, the patient is asked to stare without blinking and the cornea/tear film is observed. The time from a blink, to the time a portion of the cornea becomes exposed from under fluorescein-stained tears is measured. A TBUT >8–10 seconds is generally considered normal.[64] Faster TBUT values less than 5–10 seconds are observed in patients with tear instability and indicate mild-to-moderate dry eye disease.[57] TBUT shows a sensitivity of 75% and specificity of 60%.[65]

TBUT is not accurate or reproducible if performed once, but if the average score of 2–3 separate TBUT measurements are used, it increases its utility.[15,28] TBUT measurement does not provide direct information on tear evaporation and the amount of fluorescein that is instilled can potentially alter the TBUT measurements.[66] Patients with MGD frequently present with a rapid TBUT (<10 sec); however, tear film instability and a rapid TBUT can also be observed in patients with mucin deficiency and/or goblet cell loss.[67] The pattern of tear break-up may suggest where the abnormality lies such as surface epithelial layer/membrane-associated mucins or aqueous tear layer/secretory mucins.[68]

Schirmer Tear Test

The Schirmer tear test (STT) is used to quantitatively measure tears produced by the lacrimal gland during fixed time period in patients suspected of having DED.[10] The STT I test measures total tear production, including both the basal and reflex tears.[69] The test is performed by inserting Schirmer tear strips into the lower conjunctival sac without anesthesia.[70] The Schirmer tear test II test (performed with topical anesthesia) measures tear production of lacrimal gland by stimulating the lacrimal reflex for 5 minutes with a wetting result of <10mm considered abnormal.[70]

The amount of tear production correlates with the length of wetting of the strips with values between 10 and 33 mm being normal.[71] Patients identified with aqueous deficiency have a reduced STT score, and scores of less than 5 mm frequently are seen in moderate-to-severe aqueous deficiency.[12]

STT I test demonstrates a sensitivity 42% and specificity 76% for identifying DED in Sjogren's patients.[72] Several factors that may reduce the accuracy of STT results include manipulation of the lid causing reflex tearing,[66] nasal versus temporal lid position during the STT, disruptive tear drainage, and inferior gaze. All these confounders produce a falsely higher result.[73] Finally, a study that compared STT I and STT II determined that results using anesthesia was found to be more reliable and objective in diagnosing DED.[74] In general, in more advanced cases of DED STT is more reproducible.[15]

Similar to the STT, a phenol red thread test is used to measure tear volume by inserting a thread that has the dye implanted with pH sensitive phenol into the lower eyelid. The thread color changes from red to yellow once in contact with tears measured after 15 seconds. Studies showed that phenol red thread tests were equally sensitive in detecting dry eye as Schirmer testing.[75]

Tear Clearance

The fluorescein clearance test analyzes reflex and basal tears and utilizes Schirmer paper, proparacaine and 5 μL of 0.25% fluorescein with 0.4% benoxinate hydrochloride. The disappearance of the dye and wetting of the strip are both measured in 10-minute increments. If both a value of 3 mm or greater dye is seen in the first 10-minute time-frame and if the dye is not detected after 20 minutes, the tear clearance is normal.[76] The clearance test indirectly measures dry eye induced inflammation and may prove valuable in identifying patient's response to anti-inflammatory therapy.[77]

Tear Function Index

Like the STT, the tear function index also evaluates tear production. A 10 μL drop of fluorescein is instilled and after 5 minutes, Schirmer tear strip wetted amount is quantified, and the color intensity is compared to a color standard. The rate at which the color of the fluorescein dye fades is the tear clearance rate, while the tear function index is a calculation achieved by dividing the value of STT1 by the rate of tear clearance. A tear function index score less than 96 is consistent with DED and less than 34 suggests Sjogren's syndrome.[78] Compared to the tear clearance alone or STT1, the function index improves sensitivity and specificity in diagnosing DED.[78] The tear function index is subjective, time-consuming and intraday variations in tear film dynamics limit the clinical value of this test.[79] A higher tear function index numerical value indicates a healthier the ocular surface, whereas values below 96 suggest DED.[68]

Tear Meniscus

The tear meniscus, also known as the tear lake, is the height of tears at the intersection of the lower eyelid margin and the bulbar conjunctiva used to determine whether or not DED is present.[80] A normal tear meniscus height is generally 0.2–0.5 mm while a patient with aqueous-deficient dry eye usually will present with a height less than 0.2 mm as measured by OCT.[79]

Staining

Vital dyes such as Lissamine Green (LG), Rose Bengal (RB), and fluorescein are used to diagnose ocular surface disease, tear film quality, and dryness severity.[81] Healthy epithelium is protected by tight junctions and does not take up dye. Damage to the cells will cause the dye to absorb and cause staining.[82] Unfortunately, no clear relationship exists between ocular surface damage and DED specific staining and a patient's symptoms.[83]

Vital dyes do not detect early DED or differentiate DED from other conditions causing staining,[84] and therefore, vital dyes lack the power to discriminate between mild-to-moderate cases of dry eye.[85] Studies show poor reliability of inferior corneal staining absence or presence in dry eye patients,[17] and in some patients with moderate-to-severe symptoms, no surface staining was present.[86]

Both RB and LG stain dead, devitalized cells and potentially stain healthy cells. RB can be toxic to the cells on the corneal epithelium.[87] and staining occurs in areas that lack membrane-associated mucins on the cornea or conjunctiva.[88] Thus, LG is preferable to RB for DED evaluations as it is associated with less discomfort and corneal toxicity despite being less sensitive at detecting defects.[87,89] Fluorescein stains degenerating or dead cells seen in epithelial erosions and preferentially stains the cornea more than the conjunctiva.[87] If there is an intact epithelium, any pooled fluorescein on the surface will be lifted by a simple cotton wisp applied to the area revealing an unstained are underneath. Mild cases of DED are detected using RB more than fluorescein stain and the cornea shows less staining than the conjunctiva.[90]

Staining tests have been found to have poor repeatability[15] although the level of staining will typically match the level of severity of aqueous deficiency.[91] While mild cases of DED exhibit normal tear production without fluorescein staining, an increase in inflammatory cell mediators may be present in the tears and serve as more accurate measures of DED than just tear production or staining.

Meibomian Gland Expression and Secretion

The diagnosis of MGD can be made by several diagnostic and physical examination tests looking for altered morphology features such as gland atrophy, terminal duct obstruction and gland drop out. Physiologic changes can be examined with diagnostic tests that grade the qualitative and quantitative changes in meibum.[92] Gland expression diagnostic tests are useful to evaluate these physiologic changes in meibum, grading meibomian gland expression and meibomian gland secretion. Meibum quality, quantity, and expressibility are generally thought to represent meibomian gland function.[93] Applying digital pressure to the central lower lid provides gland expression which when followed by assessing the ocular surface damage was recommended by the Diagnostic Subcommittee at the International Workshop on Meibomian Gland Dysfunction.[92]

The expressibility of meibum is commonly evaluated by applying the digital pressure to the glands along the entire length of the eyelid.[94–96] Healthy eyelids secrete clear meibum that is easily expressed with gentle pressure. In patients with MGD, the meibum can become cloudy and opaque with a viscosity like toothpaste and difficult to express.[93] The various qualities of meibum, as well as the ease of expression are rated in different grading schemes. These grading schemes can include counting the number and location of expressible glands, as well as the response of the glands to different levels of digitally applied pressure. Grading and scoring expression provides information related directly to meibomian gland condition.[12,97] However, the value of meibomian gland expressibility and meibomian gland duct appearance has not been fully established in diagnosing DED.[93]

Impression Cytology

Impression cytology is a minimally invasive, rapid, and sensitive method of collecting conjunctival epithelial, goblet, and inflammatory cells[98] that serves as a improved alternative to biopsy of the ocular surface. Ocular surface changes such as goblet cell loss and keratinization are evaluated by examining the collected superficial layers microscopically. It requires

appropriate staining technique and enhanced evaluation microscopically.[98] A recognizable deficiency in goblet cells is the primary characteristic of goblet cell loss. Pathological alterations on the ocular surface can also be used to identify the keratinization of the superficial epithelial layers revealing an unhealthy ocular surface.[8]

Diagnostic Imaging

Digital imaging can be used to support the differential diagnosis of blepharitis and MGD as well as to evaluate tear break-up time and assess tear film stability using corneal topography, aberrometry, functional visual acuity assessment, interferometry, and confocal microscopy.[99] MGD is characterized by posterior lid margin alterations, including prominent blood vessel development, turbid or thickened meibomian gland secretions, and gland orifice plugging which alter the tear film lipid composition and overall tear quality. Specifically, the Keratograph 5M (OCULUS Optikgeraete GmbH, Wetzlar, Germany) and LipiView (Johnson & Johnson Vision, Jacksonville, FL, USA) interferometer devices and LipiScan Imager (Johnson & Johnson Vision, Jacksonville, FL, USA) allow for imaging of the Meibomian glands.[100] Tear protein and lipid analysis may help distinguish dry eye with or without meibomian gland disease.[19,101]

Functional Visual Acuity

Functional visual acuity is assessed monocularly with a device that measures visual over a 30-second blink-free period. In dry eye patients, functional visual acuity was significantly lower than with controls.[22] This may serve as an indicator of tear film instability and underlying ocular surface disease.

Topography

Corneal topography and high-speed videokeratoscopy evaluate indices like surface asymmetry index (SAI), surface regularity index (SRI), and topographic pattern to assess tear film stability and corneal surface regularity. These predict consequent effects on image quality.[102] Alternatively, tear film stability may be assessed automatically using either a grid pattern or Placido disk image by evaluating the distortion change of the reflected ocular surface image.[32,65]

Tear film stability analysis system (TSAS) for the TMS-2N corneal topography instrument (Tomey Technology, Nagoya, Japan) is a sensitive videokeratographer.[103] TSAS uses videokeratography to capture 10 corneal surface topography images at 1-second intervals and analyzes any changes in tear film stability over 10 seconds by quantitatively measuring any areas of corneal irregularity. The more irregularities that are detected, the more severe the underlying tear film instability/DED.

Interferometry

Interferometry is based on interference between light that is reflected from the surface of the lipid layer and light reflected from the interface between the lipid layer and the aqueous layer of the tear film. Colored images of the superficial lipid layer can be created and evaluated over the entire ocular surface. Interference patterns may be used to assess the thickness and quality of the lipid layer.[104]

The lipid layer thickness, blink rate, and images of the meibomian gland structures can be measured using the LipiView (Johnson & Johnson Vision, Jacksonville, FL, USA).[105] It may be used as a non-invasive tear break-up time assessment to determine the interval between the last blink and the appearance of the first lipid layer discontinuity.[106] Thin lipid layers, those less than 60 nm, are correlated with DED while reduced dry eye symptoms are often associated with thick lipid layers, those more than 75 nm.[107] However, since the lipid thickness layer is only measured at one point in time, this may present as a limitation and may not necessarily correlate with TBUT.[108]

Meibography

Meibomian glands make the lipid layer of the tear film. Meibography is a method of evaluating the meibomian gland structure and enables objectivity.[92] Meibography can be used to examine meibomian gland changes such as dilation, truncation and dropout.[109,110]

Wavefront Aberrometry

DED patients show increased optical aberrations relative to normal control eyes because of irregularities to the tear film.[102] Wavefront aberrometry allows for the non-invasive evaluation of higher order aberrations present from tear film instability. Alterations and irregularities in the tear film thickness associated with tear film instability can lead to reduced retinal image quality from higher order aberrations. Moreover, changes in tear volume and quality can create changes in higher order aberrations consistent with DED.[111,112]

OCT

Anterior segment optical coherence tomography (OCT) is a noninvasive diagnostic test that uses emission and reflection of light waves to create a high-resolution, three-dimension, cross-sectional image of biologic structures.[113] OCT devices may also quantify aspects of the tear film including tear volume, tear meniscus height, tear film thickness and cross-sectional.[114] A reduced tear meniscus measurement correlates with symptoms of DED and the Schirmer test.[115]

Confocal Microscopy

Confocal microscopy is another technology providing high-resolution evaluation non-invasively. Confocal microscopy can capture images of all layers of the cornea, including sub-basal corneal nerve density, morphology, and the presence of and inflammatory cells, as well as goblet cells and inflammation of the conjunctiva.[116,117] Confocal microscopy requires interpretation by a skilled clinician.

Advanced Point-of-Care (POC) Testing

Biomarkers

The tear film plays a critical role in supporting the ocular surface health and serves as a reservoir for potential biomarkers. The most desirable biomarkers are rapidly quantifiable, and their assay results directly influence treatment decisions. Easy access to a patient's tears allows for rapid advanced tear-based point-of-care diagnostics to objectively assess these biomarkers. These tests are typically performed by a technician during the patient workup, and since the results are readily available within 10–15 minutes, test results can be used to manage patients during the same office visit. Early detection of objective biomarkers such as tear osmolarity, lactoferrin, total IgE, and matrix metalloproteinase 9 (MMP-9) may not only lead to faster intervention but can also allow for a more targeted approach to therapeutic interventions and disease monitoring over time.

Osmolarity

Increased tear osmolarity is thought to be a hallmark sign of DED and does not differentiate aqueous deficiency from increased evaporation.[6] Hyperosmolarity of the tear film can occur through increased evaporation and instability of the tears and/or decreased flow of the aqueous layer produced from the lacrimal gland. Increased osmolarity of the tear film causes the release of inflammatory cytokines, promotes cell destruction, and produces a decrease in goblet cell number.[30,118] Although the gold standard for tear osmolarity is freezing point depression (FPD), it may be confirmed through vapor pressure, electrical conductivity, or impedance.[119]

TearLab (Trukera, San Diego, CA, USA) osmometer measures tear osmolarity using impedance after collection of a 50 nL tear sample in less than 20 seconds (Table 1). This test must be done before instilling ocular anesthesia drops and other diagnostic tests that create reflex tearing may lead to variable results.[120] This test should be performed bilaterally with the highest number recorded as the determinant value. It is accepted that an osmolarity reading of 308 mOsms/L or higher in one eye or a difference in measurements between the two eyes of greater the 8 mOsms/L indicates an abnormal tear osmolarity.[121]

Hyper osmolarity >308 mOsms/L indicates mild DED and readings above 312 mOsms/L indicate moderate-to-severe DED.[121] Despite a higher tear osmolarity being an indicator of DED, the higher reading may not correlate with other clinical signs or with a patient's symptoms since there is often poor correlation between signs and symptoms in DED patients.[122] There is a modest correlation between staining and symptoms, but none was found between tear osmolarity and symptoms or between tear osmolarity and corneal fluorescein staining.[123]

Table 1 Advanced Point-of-Care Diagnostic Tear Tests

Clinical Condition	Analyte	Result	Sample Size	Time to Results	Variability from Reflex Tearing	Guides Therapeutic Decisions
Dry eye	Osmolarity	Quantitative	50 nanoliters	Less than 30 seconds	Yes	No
Ocular allergy	IgE	Quantitative	1 microliter	Less than 10 minutes	No	Yes
Aqueous deficient dry eye	Lactoferrin	Quantitative	1 microliter	Less than 10 minutes	No	Yes
Inflammatory dry eye	Metalloproteinase-9 (MMP-9)	Qualitative	10 microliters	Less than 15 minutes	No	Yes

Clinical studies have created concern on the utility of tear osmolarity to distinguish healthy controls from DED given the wide variability in tear osmolarity seen in healthy controls and the impact reflex tearing leading to a false lowering of results.[124–128] Tear osmolarity measurements could vary in dry eye patients during the day on average by 21.9 mOsmol/L.[129] A meta-analysis reveals a sensitivity of 67% and specificity 46% for DED diagnosis using a cutoff of 294 mOsmol/L and a sensitivity of 40% and specificity of 100% using a cutoff of 310 mOsmol/L in Sjogren's syndrome patients.[127] Since osmolarity has challenges with clinical reproducibility and does not differentiate between aqueous sufficient dry eye and evaporative dry eye, it cannot be used solely as an indicator of dry eye or to guide clinical management and therapeutic decisions.[130] If other tear-based testing will be performed such as Schirmer testing, lactoferrin, IgE and MMP-9 Inflammatory testing, tear osmolarity must be performed first to reduce the impact of reflex testing.

Lactoferrin

The lacrimal gland secretions that produce most of the tear film aqueous layer contain proteins including lactoferrin and lysozyme enzymes, immunoglobulins, and electrolytes that are involved in maintaining ocular surface health. The lacrimal gland acinar cells produce lactoferrin.[131] A tear film sample used to measure the proteins in lacrimal secretions, such as lactoferrin, has shown discordant levels in patients with DED. This allows lactoferrin to serve as a novel biomarker for DED.[35,119,132–136] Lysozyme, another lacrimal gland protein often associated with lactoferrin has also been found to be reduced in Sjogren's and/or medicated glaucoma patients. Also, despite some studies showing a lack of differentiation between Sjogren's syndrome, non-Sjogren's DED and non-DED patients, lysozyme may still be useful in monitoring damage from beta-adrenergic receptor blocking medications.[137,138]

Lactoferrin is a glycoprotein created by the acinar cells of the main and accessory lacrimal glands[139] and binds iron, providing both an antibacterial and anti-inflammatory effect on the ocular surface.[140] Lactoferrin is a key predictor of the volume and stability of the tear film. The lactoferrin concentration is reduced in the setting of aqueous-deficient dry eye and is shown to be dramatically reduced in tears of both non-Sjogren's and Sjogren's syndrome patients with DED[35] as well as patients with chronic medication induced DED.[141] Tear volumes from the lacrimal gland are noted to positively correlate with the concentration of lactoferrin. Lactoferrin levels have an inverse correlation with corneal staining scores and OSDI scores.[142] Patients with lower tear volume tend to have reduced lactoferrin concentration.[143,144] Further, a direct correlation between lactoferrin concentration and TBUT time[35] as well as a significant negative correlation between Rose Bengal staining and the level of tear lactoferrin in DED with Sjogren's syndrome as well as DED without a diagnosis of Sjogren's syndrome.[140,143,145] In comparison with traditional diagnostics such as the STT, the vital dyes and the tear film break-up-time, the lactoferrin test is reported to be the most reliable, single marker in the diagnosis of KCS.[42] Lactoferrin levels are normal, and not reduced, in the setting of meibomitis related rosacea[146] and OA.[147] A reduction in lactoferrin has been often reported as an indication of contact lens intolerance.[148] Improvement in lactoferrin levels is seen with dry eye disease treatment.[148]

A quantitative point-of-care test to measure tear film lactoferrin in patients suspected of DED is available (Table 1). Lactoferrin can be detected with the use of the T-POC Lactoferrin (Verséa Ophthalmics, Tampa, FL, USA) tear-based testing platform. A glass capillary tube is used to directly collect 1uL of tears from each eye and transferred to an immunoassay test cassette containing a reagent. The test cassette is then inserted into the T-POC digital lateral flow reader which yields a quantitative numeric result within 6–8 minutes. Lactoferrin is an abundant tear protein and has normal tear concentration of 2.2 mg/mL.[140] Since lactoferrin is produced by the accessory and lacrimal gland, it has been seen in similar concentrations in both basal tears and in reflex tears.[149] A cutoff for lactoferrin below 0.9–1.1 mg/mL provides model accuracy for the diagnosis of DED[150] and confirms the presence of ADDE. Lactoferrin has been proposed for the diagnosis of primary Sjogren's syndrome, since the test had a sensitivity of 72% and specificity of 95% while STT I had a sensitivity of 64% and specificity of 85%.[151] Whereas in patients with non-Sjogren's Syndrome DED, lactoferrin showed a sensitivity 79.4% and specificity of 78.3%.[152] A recent meta-analysis confirms lactoferrin concentrations are significantly decreased in tears of DED patients.[153] Lower levels of lactoferrin also correlate with severity of DED,[132] and DED progression is associated with a reduction of lactoferrin.[154]

Lactoferrin concentrations respond to treatment and allow for therapeutic monitoring. Treatment of DED with punctal occlusion[155] and cyclosporine 0.2%[156] was associated with increased tear lactoferrin levels.

MMP-9

Matrix metalloproteinase-9 (MMP-9) is part of a multidomain calcium and zinc ion-dependent enzyme family, produced by epithelial cells, and is involved in wound healing, tissue remodeling, and inflammation.[157] Increased MMP-9 disrupts corneal epithelial barrier function, increases corneal epithelial desquamation, and leads to irregularities of the corneal surface.[157] Corneal cell disruption leads to primary ocular symptoms of dry eye including pain, fluctuating vision, and tearing.[157]

Increased levels of tear MMP-9 are common in patients with ocular surface disease. MMP-9 is involved with rebuilding of the extracellular matrix after a wound to the corneal surface. MMP-9s are linked to sterile corneal ulcers, active pterygia, fungal keratitis, ocular allergy, advanced keratoconus with irregular surface, ocular burns, blepharitis, conjunctivochalasis, and DED.[158–160] Tear MMP-9 is considered a biomarker of damage to the ocular surface and inflammation[27] and is a more sensitive marker of ocular surface disease than other clinical signs.[157]

MMP-9 levels have been observed to be elevated with DED[6,27] and other OSD. A disposable single-use, qualitative immunoassay point-of-care test, InflammaDry® (Quidel Corp) is made to identify MMP-9 levels in tears of patients suspected of DED (Table 1).[161] The acceptable level of tear MMP-9's in healthy patients is between 3 and 40 ng/mL.[16] In a diseased state, MMP-9's can exceed 40 ng/mL in patients with moderate-to-severe DED.[161] The InflammaDry test is performed by dabbing 6–8 times against the lower conjunctiva for a goal of collecting 10μL of tear fluid. The test is activated with a running buffer and results are ready for interpretation after 10–15 minutes.[16,162] Sample volume affects the detection limit. Even if the MMP-9 concentration in the tear film is elevated, if the tear volume is extremely reduced then there is a chance the result will be negative or weak positive. The test is subject to variability because it is qualitative and highly dependent on adequate saturation of the sampling fleece which is influenced by practitioner sampling proficiency. Insufficient tear samples (less than 10 μL) may produce false-negative results, whereas excessive tear samples (more than 20 μL) may produce false-positive results.[163] InflammaDry shows a sensitivity 80–85% and 94–97% specificity in diagnosing dry eye.[16,162]

Inflammatory biomarkers are not unique to DED since many have been identified with conditions such as infection, contact lens use, and ocular allergy.[164]

While inflammation is known to be involved with DED, it is not always recognized in symptomatic patients as even high dose topical steroids or topical cyclosporine only relieve symptoms in 40–80% of the patients.[165–167] Artificial tear eye drops alone failed to demonstrate any reduction in MMP-9 levels.[158] However, confirmation of the presence of clinically significant inflammation can impact clinical decisions. By avoiding punctal occlusion in the presence of high levels of inflammatory mediators and predicting a favorable therapeutic response for immunomodulator or anti-inflammatory treatments such as topical cyclosporine, lifitegrast or steroids treatment failures and unnecessary office visits can be reduced.[168,169]

IgE

OA is an conjunctival inflammatory response to a variety of allergens.[170] Allergens come into contact with the conjunctiva and produce local IgE, which then assimilates into the tears through the blood tear barrier, resulting in higher levels of IgE in the tears.[171] Many ocular surface diseases that contribute to tear film dysfunction, infections, blepharitis, and mechanical and toxic conjunctivitis may mimic the clinical pictures of OA[172] as well as long-term antihistamine usage that has anticholinergic properties due to their high muscarinic receptor binding.[4] Chronic OA is known to create dry eye, which is classified as either reduced tear production or evaporative.[172] Some authors have shown a correlation of total tear IgE to serum IgE,[173–175] but the primary contributor of seasonal allergic conjunctivitis (SAC) severity is the locally produced IgE.[176] When decrease in TBUT is present without the other signs of DED, it may represent a feature of OA.[177]

Patients with forms of OA that are chronic, such as patients with atopic keratoconjunctivitis (AKC) and vernal conjunctivitis (VKC), have reduced tear film break-up times,[178] reduced conjunctival mucins in the tear fluid layer[179] and reduced density of the conjunctival goblet cells.[177]

OA adversely affects tear film stability, which facilitates the ocular allergic process to persist and recur.[177,180,181] Activation of Eosinophils and simultaneous release of inflammatory mediators could damage the conjunctival epithelium and goblet cells. As a result, the deficiency of the mucin layer creates tear film instability. Significantly thicker lipid layer and lipid layer changes that are typical of DED have been found in SAC patients without corneal fluorescein staining pointing to the instability of the tear film caused by changes to the lipid layer.[147] A point-of-care test available commercially measures the IgE level in tears. Patients with IgE levels at least 80 ng/mL point to an allergic conjunctivitis diagnosis, with the tear film level of IgE correlated.[173] Interferometry shows increased lipid layer thickness relative to healthy patients and is negatively correlated with TBUT.[130] Further, DED may create the OA or initiate it. The decreased tear clearance in DED is somewhat responsible for the reduced clearance of allergens and inflammatory mediators, facilitating an antigen that may be insufficient to cause a reaction systemically but may initiate a more limited allergic reaction locally.[44]

Mucins, secreted by conjunctival epithelium goblet cells, serve several functions: they enable the tear film to be spread evenly over the ocular surface, they assist with tear film stability, and they provide a protective layer to the corneal epithelium.[182] Decreased mucins are found in both DED and OA patients and show correlation with Schirmer test, TBUT, and the density of the goblet cells. It has a negative correlation with corneal staining. Dry eye symptoms are greater with lower levels of mucin.[183,184]

The main laboratory tests for allergic conjunctivitis (AC) include skin prick tests, cytological examination, and IgE antibody detection.[185] The tear samples need to be collected from each eye because of potential asymmetrical conditions preferentially collected with a capillary tube as cells and mediators could bind to the strips or sponges. Eosinophil presence with cytology is highly indicative of OA, whereas their lack of presence does not necessarily exclude it.[186] Skin prick tests are a relatively invasive procedure and use many needle pricks containing small volumes of different allergens. Based on the large ocular surface reaction created with the conjunctival provocation test, there is limited clinical use for this test.[187] The identification of conjunctival eosinophils is a difficult and complex process and yielding a low number (approximately 50%) in the conjunctival scrapings of patients with mild allergic conditions.[188] Serum total IgE tests do not differentiate the pathogenesis and severity of the disease and are not diagnostically recommended.[189] There has also been conflicted data between total IgE in tears and serum. For example, one study was unable to demonstrate a correlation between serum total IgE and tear total IgE levels in patients with SAC, while another study found a high correlation in patients with moderate-to-severe SAC.[174,190]

Most tear IgE is produced through the local create of IgE antibodies in the eye.[175,191,192] In OA, IgE may be quantified in tears of patients with perennial allergic conjunctivitis (PAC), SAC, AKC and VKC.[173,174,189] SAC is dominated by local production and symptoms, compared to serum total IgE levels generally revealing the immune response of the whole body with only a modest correlation to serum IgE.[193] Tear IgE varies from 159 IU/mL to less than 1 IU/mL compared with controls of 8 IU/mL to less than 1 IU/mL.[194] Nomura et al sampled tears from patients with AKC, epidemic keratoconjunctivitis (EKC), bacterial conjunctivitis (BC) and normal controls, and determined that their

tear IgE concentrations were significantly increased in allergic conjunctivitis when compared to healthy patients and there was no significant difference found between EKC, BC and the healthy controls.

Following treatment of OA, the tear concentration of total IgE and symptoms improved.[193] The decrease between the measured amount of total tear IgE concentrations at the initial and the primary follow-up visit showed positive correlation with the total clinical scores recorded during both visits. Likewise, at every 2-week follow-up, there was a significant correlation between the change in the clinical score and total tear IgE concentration. Patients at risk for recurrent disease show total IgE concentration significantly higher than those without recurrent disease. Higher initial presenting tear total IgE concentration was also associated with an improvement delay following treatment and may help to gage the course and efficacy of treatment by observing changes that occur in the total IgE concentration of the tears.[193]

Conclusion

DED is a complex multifactorial condition that frequently predisposes or co-exists with OA. Both conditions lead to tear instability and ocular surface inflammation that can lead to chronic symptoms, morbidity, and negatively impact surgical outcomes.

Traditional diagnostics are less reliable than newer advanced tear point-of-care diagnostics that allow improved diagnostic accuracy and therapeutic monitoring (Figures 1A–D). Digital imaging can also serve as a diagnostic adjunct to

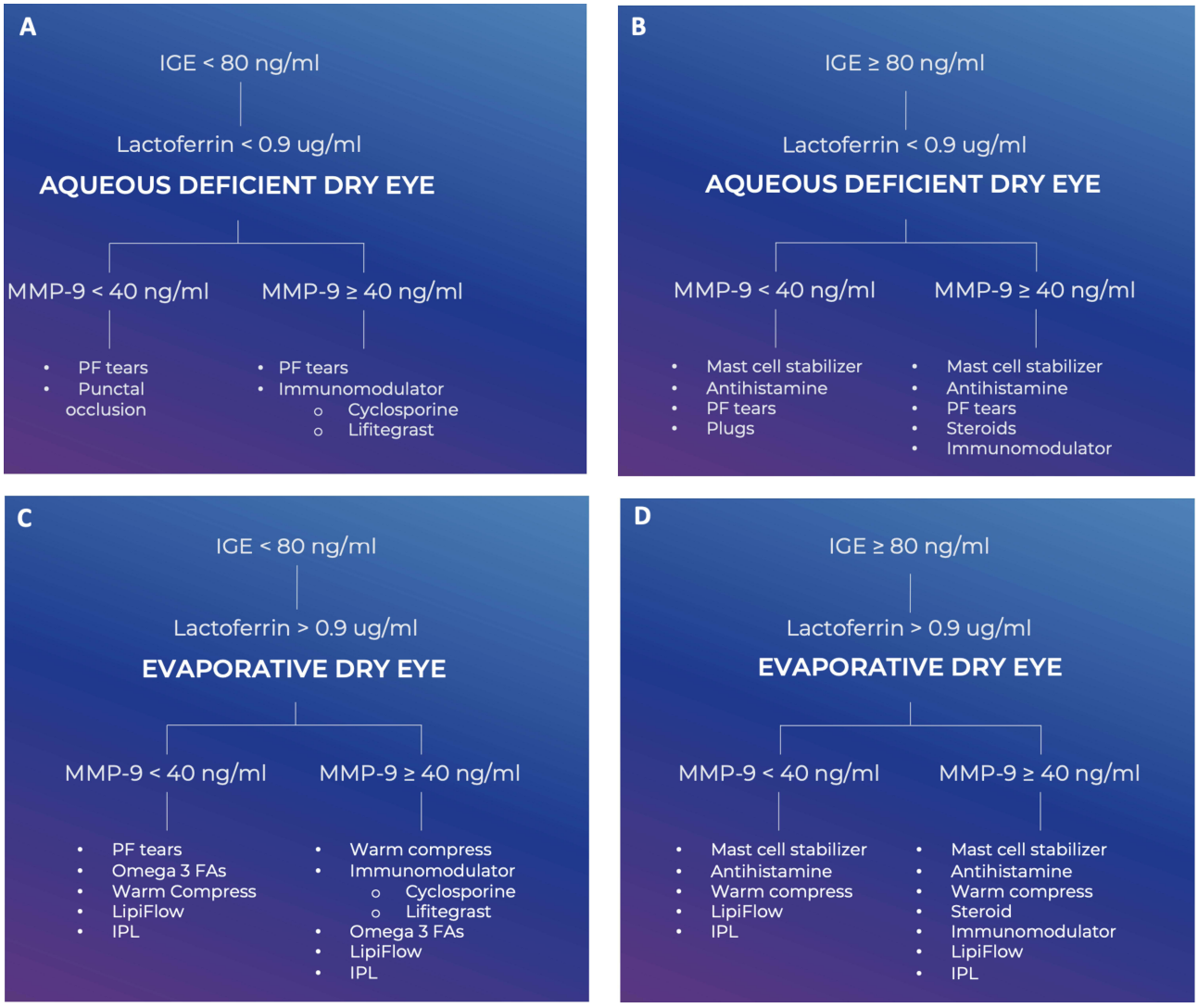

Figure 1 (**A** and **B**): Identification and management of aqueous-deficient dry eye. (**C** and **D**): Identification and management of evaporative dry eye.

tear-based testing by providing visual images which can be used to support a diagnosis and garner patient acceptance and compliance with treatment plans.

Disclosure

Dr Tom Chester reports the following relationships: Allergan – consultant, research; Dompe – consultant; Glaukos – consultant, speaker; Novartis – consultant; Oyster Point – consultant; Sightscience- consultant, speaker, research; Sun – consultant; Tarsus – consultant; Versea – consultant.

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