TFOS DEWS III Diagnostic Methodology

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ABSTRACT

A standard approach to the diagnosis of dry eye disease across eye care practitioners is critical to reassuring the patient, providing consistency between practitioners and informing governments as to the true prevalence and resulting healthcare needs. The Tear Film & Ocular Surface Society (TFOS) Dry Eye Workshop (DEWS) III has reviewed the evidence-base since their previous reports published in 2017 and revised the definition to "Dry eye is a multifactorial, symptomatic disease characterized by a loss of homeostasis of the tear film and/or ocular surface, in which tear film instability and hyperosmolarity, ocular surface inflammation and damage, and neurosensory abnormalities are etiological factors." Key features from the definition include that dry eye disease is multifactorial, is a disease and not a syndrome and is always symptomatic. Differential diagnosis and ocular examination guidance is given along with the risk factors that should be discussed with the patient. The recommended screening questionnaire is the OSDI-6 with a cut-off score ≥4. A positive result together with a non-invasive breakup time <10s or alternatively tear film hyperosmolarity (≥308mOsm/L in higher eye or an interocular difference >8mOsm/L) gives a diagnosis of dry eye. In addition, the ocular surface should be stained and positive symptomology together with >5 corneal fluorescein and/or >9 conjunctival lissamine green punctate spots and/or lid margin lissamine green staining of ≥2mm length & ≥25 %width also gives a diagnosis of dry eye. Subclassification was separated into tear film (lipid, aqueous and mucin/glycocalyx) and ocular surface and adnexa (anatomical misalignment, blink/lid closure, lid margin, neural dysfunction, ocular surface cell damage/disruption and primary inflammation/oxidative stress) components, with appropriate clinical tests and cut-offs provided to identify these etiological drivers in an individual, to inform appropriate management and therapy.

Keyword: dry eye disease (DED); definition; diagnosis; differential diagnosis; subtypes; subclassification; blepharitis, meibomian gland dysfunction (MGD)

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Abbreviations:

DED	Dry eye disease
DEWS	Dry eye workshop
IL	Interleukin
LIPCOF	Lid-parallel conjunctival folds
MGD	Meibomian gland dysfunction
MMP	Matrix metalloproteinase(s)
OCT	Optical coherence tomography imaging
OR	Odds ratio
OSDI	Ocular Surface Disease Index
TFOS	Tear Film & Ocular Surface Society

1 Report Aims

The Tear Film & Ocular Surface Society (TFOS) second dry eye workshop (DEWS II) Diagnostic Methodology report [1] provided practical, clinical diagnostic recommendations for dry eye disease (DED), based on the evidence available for tests with diagnostic potential to align with the revised definition of DED.[2] It also provided a clear rationale for the framework which informed the number and characteristics of the selected tests. Questions to inform a differential diagnosis were proposed. The need for subclassification post-diagnosis was emphasised, to inform management approaches. The purpose of the TFOS DEWS III Diagnostic Methodology report was to:

- Revisit the current definition [2] to ensure it aligns with current understanding of DED
    - Provide a rationale for the components of the definition
    - Highlight considerations when a patient only has symptoms or signs associated with DED
    - Define associated conditions
- Draw on risk / associated factors for DED (from the TFOS DEWS III Digest report) and masquerading diseases to guide appropriate history and symptom-taking
- Identify any updates required to the 2017 diagnosis of DED, reiterating the rationale for change and perceived challenges with regard to the available evidence
- Propose a new etiological driver-based approach to subclassification, identifying the tests that indicate the driver involved in an individual's dry eye, which can then be linked to management approaches by the TFOS DEWS III Management and Therapy report
- Discuss possible future directions that could help to inform further advances in DED diagnosis and subclassification

2 Definitions

2.1 Dry eye disease (DED)

In 2017, after careful consideration of the terminology including diction, word order, emphasis, and accepted meaning, DED was defined by a multidisciplinary and transnational committee as “a multifactorial disease of the ocular surface characterized by a loss of homeostasis of the tear film, and accompanied by ocular symptoms, in which tear film instability and hyperosmolarity, ocular surface inflammation and damage, and neurosensory abnormalities play etiological roles.”. This was ratified by the full TFOS DEWS II authorship consisting of 150 clinical and basic science research experts from around the world, who utilized an evidence-based approach and a process of open communication, dialogue and transparency to consolidate the understanding of DED [3].

It was the consensus of TFOS DEWS III that the definition did not require radical change based on our updated understanding of the disease pathology and the tear film [4], but noted the intrinsic role of the ocular surface tissues as well as the tear film in homeostasis leading to repositioning of this aspect in the revised definition to read “Dry eye is a multifactorial, symptomatic disease characterized by a loss of homeostasis of the tear film and/or ocular surface, in which tear film instability and hyperosmolarity, ocular surface inflammation and damage, and neurosensory abnormalities are etiological factors.” Key aspects of the definition include:

2.1.1 Multifactorial

There are many aspects of our lifestyle that can impact the ocular surface including digital environments [5], environmental conditions [6], nutrition [7], use of cosmetics [8], elective medication and procedures [9], contact lenses [10], societal factors [11] and general lifestyle [12]. All of these, therefore, need to be considered in the management of the disease and appropriate treatment or therapy is likely to include more than one approach or treatment. In addition, genetic factors have been found to play a role in DED. [13-15]

2.1.2 Disease, not a syndrome

When a combination of symptoms and physical findings are common to a group of patients, but the direct cause is not yet understood, it is referred to as a syndrome (from the Greek roots meaning ‘running together’) [16]. However, once causative agents or processes have been identified that have a moderately high degree of certainty, then it is appropriate to use the term ‘disease’ rather than a ‘syndrome’.[16] Dry eye was once considered a syndrome due to insufficient understanding of its etiology. However, advances in medical knowledge have revealed its clearly identifiable diagnostic features [1] and disease progression [4] and response to specific treatments [17]. These insights support the current recognition of dry eye as a disease entity rather than a syndrome [2]. Defining dry eye as a disease is an important issue for patients (as this can affect reimbursement for clinical care and treatment as well as their understanding of their symptoms that are impacting their quality of life), and for eye care practitioners, since in some countries, certain professions are not permitted to treat ‘disease’. The TFOS DEWS III consensus is that all eye care practitioners play an important role in managing patients with DED within the limits of their clinical competency, by providing evidence-based advice on lifestyle factors [18] and over-the counter treatments, as a minimum.

2.1.3 Dry eye is a subset of ocular surface disease and can co-exist with other ocular surface disease

DED is defined as a symptomatic disease and thus, by definition, must always be accompanied by ocular symptoms. Recognition of DED as a symptomatic disease is not new; the National Eye Institute/Industry Workshop in 1995 defined dry eye as “a disorder of the tear film … associated with symptoms of ocular discomfort” [19]. The first TFOS DEWS report identified dry eye as a “disease of the tears and ocular surface that results in symptoms of discomfort …” [20] and TFOS DEWS II as a “disease of the ocular surface

characterised by a loss of homeostasis of the tear film and accompanied by ocular symptoms …." [2]. Hence, ocular surface signs in the absence of symptoms reflects the presence of ocular surface disease (Figure 1), but does not signify the existence of DED, specifically.

2.1.3.1 Signs vs symptoms

It is acknowledged that there are pathological conditions that result in symptoms without clinically significant signs (see Section 4.2), that present with signs but without symptoms (see Section 4.1), or that exhibit both dryness symptoms and signs but are not DED as they have a different pathophysiology (see Section 3.1). Such conditions can co-exist with dry eye disease; for example a patient with severe symptoms, but only mild signs, may have neuropathic pain in conjunction with mild DED. This is important to recognise from a management perspective as it may indicate the need for a multimodal approach [17].

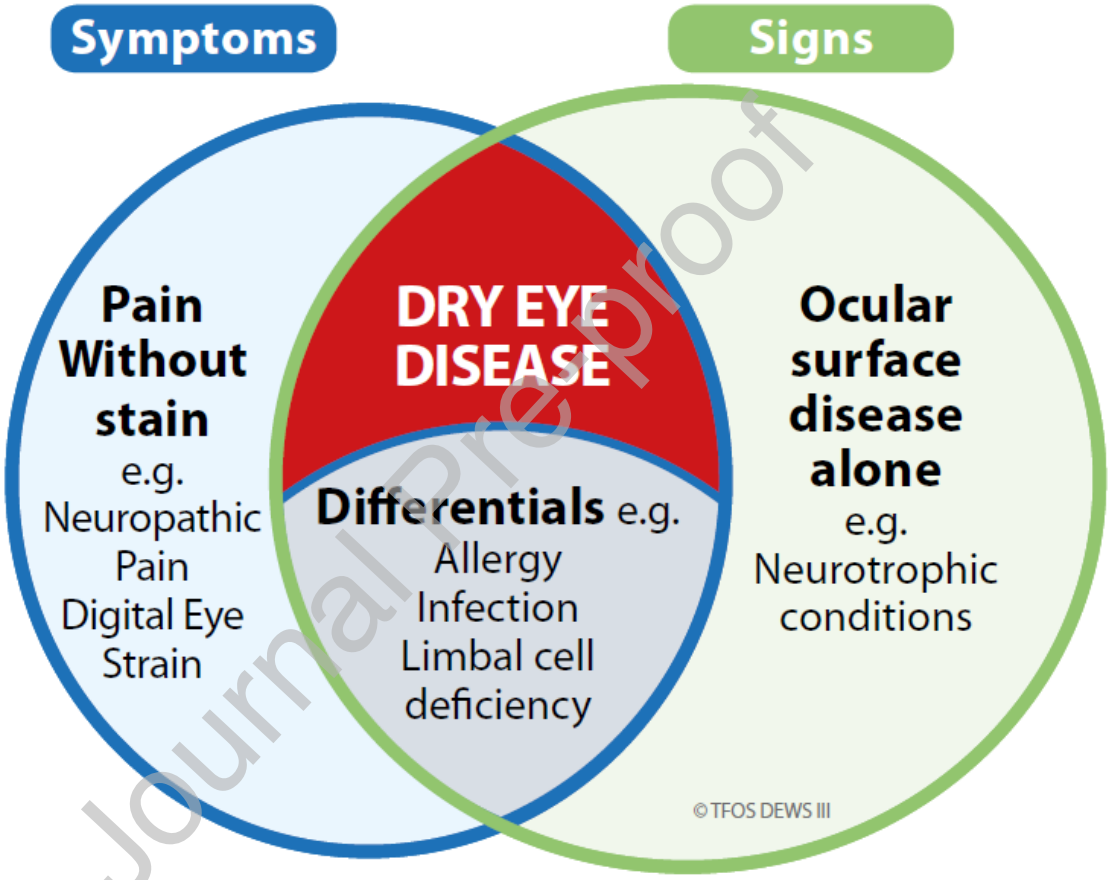

Figure 1: Venn diagram illustrating that dry eye disease requires the presence of both signs and symptoms, and exclusion of differential diagnoses. Dry eye disease can co-exist with other forms of ocular surface disease and symptomatic conditions.

2.1.4 Ocular symptoms include vision

Vision and vision-specific tasks can be significantly affected by DED due to destabilisation of the tear film disrupting the smooth air-tear interface between blinks or damage to the ocular surface affecting corneal transparency [21-25]. An unstable tear film results in light scatter and visual fluctuations between blinks [26-28]. Sensitivity to light and "spots in vision" are more commonly reported accompanying DED than glaucoma and/or cataract [29]. Hence, while vision is not specifically mentioned in the definition of DED (as there are many associated symptoms), visual disturbance is acknowledged to be encompassed within the terminology of "ocular symptoms". As also previously noted by TFOS DEWS II, the term "symptoms" is

considered to cover a wide range of possible patient-reported sensations associated with DED, which can include discomfort and / or visual disturbance [3].

2.1.5 Pathophysiology elements

The component of the definition describing the disease pathology (see TFOS DEWS III Digest Pathophysiology section [4]) needs to exclude other conditions with both signs and symptoms, but with different pathophysiology and therefore appropriate management therapies. Clinically, these can be excluded through a process of differential diagnosis (See Section 3.1).

3 History and Symptoms

3.1 Differential Diagnosis

Both symptoms and signs of DED are heterogeneous. Therefore, the differential diagnosis of DED is extensive and encompasses most ocular surface disease (Figure 2). Several of the ocular surface changes caused by conditions identified as differential diagnoses of DED may increase the risk of DED, and DED may sometimes exacerbate other ocular surface diseases with similar symptoms (see section 3.2). Thus, many of the differential diagnoses are often comorbid with DED. There are also non-ocular surface disease diagnoses that may mimic dry eye symptoms, that are particularly important to consider when no ocular surface disease signs are present. Table X gives an overview of differential diagnoses of DED and their key differential features. The following sections focus on the most common differential diagnoses of DED.

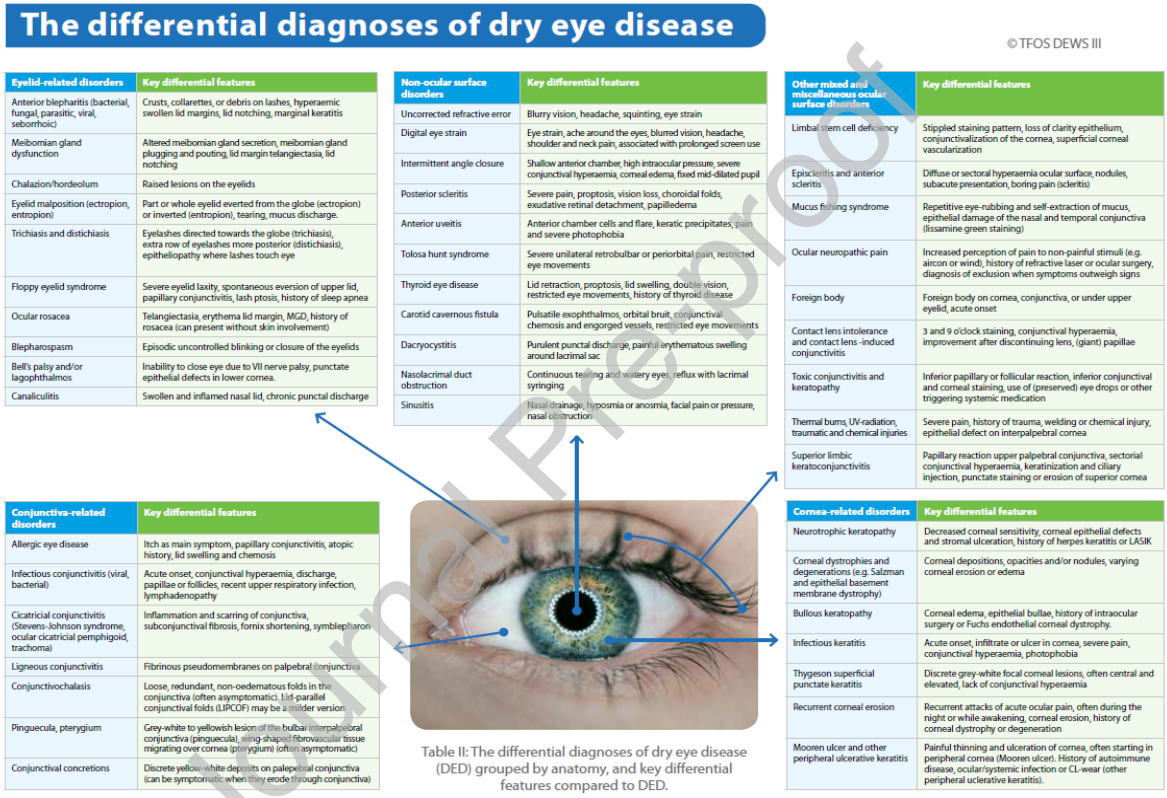

Figure 2: The key differential diagnoses of dry eye disease (DED) grouped by anatomy, and main differential features compared to DED.

3.1.1 Eyelid-related disorders

While patients with anterior blepharitis or MGD do not always have DED, as these conditions can be asymptomatic, they are commonly a driver of DED (see section 3.5.3.2) Diseases affecting the eyelids, such as chalazion, hordeolum, entropion, ectropion, trichiasis/distichiasis, floppy eyelid syndrome, blepharospasm, ocular rosacea, Bell's palsy and canaliculitis may result in symptoms similar to DED including watery eyes as a neuroregulated response to dry or irritated ocular surface [30], and so a thorough eyelid examination should be performed in every patient suspected of having DED [31].

3.1.2 Conjunctival and corneal abnormalities

To distinguish between DED and other etiologies, a thorough patient history is vital; this is especially important in patients with a history of contact lens wear [32], use of multiple eye drops, or exposure to toxic chemicals [33, 34]. Observation of the ocular surface provides further useful information. For example, a "curl" pattern of fluorescein staining (which may indicate epithelial stress for example due to medication toxicity) [33] or corneal conjunctivalization (which may be indicative of limbal stem cell deficiency) [32, 35]. Fluorescein staining in the superior cornea may point to superior limbic keratoconjunctivitis [36], floppy eyelid syndrome [37] or can result from contact lens wear issues [38-40]. Non-responsiveness to standard DED therapies warrants consideration of concurrent systemic conditions such as mucus membrane pemphigoid (ocular cicatricial pemphigoid) or Stevens-Johnson syndrome [41, 42]. Even though DED can co-exist in these conditions, early diagnosis is vital as advanced therapies including systemic immunomodulatory therapy are often required.

Superficial punctate keratitis can commonly be observed in a variety of corneal disorders. If it is primarily the superior cornea that is affected, possible causes include vernal keratoconjunctivitis, superior limbic keratoconjunctivitis, trachoma, poorly fitting contact lenses, floppy eyelid syndrome, trichiasis or distichiasis [38]. If it is primarily inferior in location, it might, more likely be attributable to ocular rosacea, atopic keratoconjunctivitis, allergic keratitis, blepharitis, exposure keratopathy, lower eyelid margin lesions, topical medication toxicity, entropion or ectropion, trichiasis or distichiasis [38]. If the superficial punctate keratitis is primarily interpalpebral, it may be contact lens-related (chemical toxicity, tight lens syndrome, overwear syndrome), or due to exposure to ultraviolet light, neurotrophic keratopathy or DED [38].

Filamentary keratitis is a persistent corneal condition, recognizable by the presence of fine strands (from mucin bound degenerated epithelial cells) and mucus which adhere to the corneal surface [43-45]. It may arise secondary to DED, causing discomfort symptoms and photophobia; blepharospasm and increased blinking are also common [46].

3.1.2.1 Conjunctival-related disorders

3.1.2.1.1 Allergic conjunctivitis

The signs and symptoms of dry eye overlap with those of allergic conjunctivitis, and the conditions can coexist [47, 48]. In a study in of 689 randomly sampled patients from an ambulatory optometric practice in California, United States, 57% of those reporting itching had clinically significant dryness and 45% of those with dry eye reported itching [49]. Immunoglobulin E (IgE) antibodies against seasonal or perennial allergens are commonly evaluated by a blood test [50] and diagnostic tests are available to detect the presence of IgE in the tear film. Additionally, typical ocular signs of allergy, such as eyelid edema, and conjunctival papillae as well as conjunctival chemosis, help differentiate allergic conjunctivitis from DED [51, 52]. A strong family history of allergy, atopic dermatitis, and/or the presence of asthma is common in those patients [53]. Atopic and vernal keratoconjunctivitis, chronic, bilateral, inflammatory and visually threatening forms of the disease also have signs and symptoms similar to DED and can serve as a trigger for DED. Signs of inflammation can be found in the cornea, conjunctiva, and eyelids. Typically, symptoms are photophobia, burning, tearing, itching, mucous discharge, and hyperemia and papillary hypertrophy of the eyelids.

Some of the most common signs found in both atopic keratoconjunctivitis and DED (although generally less severely in DED) include superficial punctate keratitis, conjunctival injection or hyperemia, anterior blepharitis, meibomian gland dysfunction (MGD) and tear instability [39, 54-57]. Atopic keratoconjunctivitis should be considered if there are signs such as conjunctivitis (possibly with scarring) and periorbital eczema [58], corneal neovascularization, symblepharon, keratoconus, and sometimes anterior polar cataracts [59, 60]. Again, a family history of allergy, atopic dermatitis (occurring in 95%), asthma (occurring in 87%) and periorbital eczema are common [39, 61, 62].

3.1.2.1.2 Viral conjunctivitis

While viruses cause approximately 80% of cases of acute infectious conjunctivitis in adults, they may be responsible for only around 20% of pediatric cases [63, 64]. Even though watery discharge is typical of viral conjunctivitis this may also be seen in about 25% of bacterial cases [63, 64]. Most viral conjunctivitis involves the highly contagious adenovirus (65–90%),[65] which has an incubation period of 4 to 10 days before being clinically observable [66] and may have associated pharyngoconjunctival fever and epidemic keratoconjunctivitis. Other causes of viral conjunctivitis include herpes viruses, picornaviruses, and several systemic viral infections [39, 64]. Unilateral herpetic keratitis can affect the tear film of both eyes [67, 68]. Even though viral conjunctivitis shares a number of findings with DED such as tearing, burning, redness, irritation, photophobia and blurred vision, the following signs and symptoms may help to differentiate a viral etiology [39, 69]:

- Acute onset of signs and symptoms
- Redness and irritation initially in one eye, which often spreads to the other eye within a few days.
- Recent upper respiratory tract infection or close contact with someone with a red eye.
- Crusting around the eyes in the morning.
- Examination findings are watery, mucoid discharge and red, edematous eyelids.
- Preauricular lymphadenopathy (swelling of the lymph nodes in front of the ears which drain lymph fluid from the area around the eyes, cheeks and surrounding scalp).

Epstein-Barr virus infects more than 90% of the adult population [70]. Even though the infection of ocular structures with the Epstein-Barr virus results most commonly in transient follicular conjunctivitis [71], it can also present with signs and symptoms similar to DED, as well as with keratitis, uveitis, choroiditis, retinitis, ocular glandular syndrome, papillitis and ophthalmoplegia [72]. Several systemic viruses, including measles, rubella (German measles), mumps, and influenza, are also frequently associated with conjunctivitis [69].

3.1.2.1.3 Bacterial conjunctivitis

Bacteria as a causative agent of conjunctivitis occurs more often in children than in adults (70% vs 20% of cases) [63 73]. Purulent conjunctival discharge and morning eyelash crusting may suggest a bacterial involvement, but this does not rule out a viral cause [63]. Patients with bacterial conjunctivitis may complain of similar symptoms as DED, such as burning, stinging, irritation, foreign body sensation and photophobia. In contrast to dry eye, there is typically significant conjunctival hyperemia (often more than with viral conjunctivitis or DED) and discharge (typically moist and mucopurulent). Affected patients often complain about matting or clumping of the eyelashes, mostly in the morning. Bacterial conjunctivitis can occur in one or both eyes and systemic findings may be present, especially in children, such as purulent rhinorrhoea and respiratory infection, fever and malaise [74]. Chlamydial conjunctivitis should be considered in sexually active persons who present with a chronic follicular conjunctivitis, that is more prominent in the lower palpebral conjunctiva, and with mucopurulent discharge [75].

3.1.2.1.4 Cicatrizing conjunctivitis

Cicatrizing conjunctivitis is characterized by inflammation and scarring of the conjunctiva. Cicatrization can range from mild and subtle, with only subconjunctival fibrosis, often seen as fine white lines at the palpebral conjunctiva, to extensive, with fornix shortening, multiple symblepharon and ankyloblepharon. It can induce ocular dryness and can lead to gross distortion of the anatomy of the eyelid and ocular surface, including entropion, trichiasis, limbal stem cell deficiency and keratinization of the ocular surface. Causes of cicatrizing conjunctivitis are many and include autoimmune disease such as ocular pemphigoid, epidermolysis bullosa, sarcoidosis, systemic sclerosis, Sjögren disease and lichen planus, bacterial and viral conjunctivitis such as trachoma and adenoviral conjunctivitis, thermal and chemical burns, graft versus host disease, Stevens-Johnson syndrome, ocular rosacea and atopic keratoconjunctivitis. Conjunctival biopsy is important in confirming a diagnosis and to identify the cause. [76-78]

3.1.2.1.5 Conjunctivochalasis

Conjunctivochalasis is characterized by loose, redundant, non-oedematous folds in the conjunctiva, the more mild expression of which is lid-parallel conjunctival folds (LIPCOF). It is most often seen in the inferior bulbar conjunctiva, overlying the inferior lid margin. It is often asymptomatic, but can lead to tear film instability and symptoms consistent with DED, including irritation, dryness, foreign body sensation, mucus discharge, and tearing. The etiology of conjunctivochalasis is still unclear, but it is more common with increasing age, in patients with DED, and contact lens wearers. Symptoms may be increased during downward gaze or with vigorous blinking [79, 80].

3.1.2.1.6 Pinguecula and pterygium

A pinguecula is a benign degeneration of the conjunctiva, that presents as a gray-white or yellowish lesion on the bulbar interpalpebral conjunctiva, adjacent to the limbus, which may cause localized disruption of the tear film due to a change in lid-globe juxtaposition. Wind, dust, and UV exposure are factors associated with pinguecula and pterygium development. Pinguecula are usually asymptomatic, but may present with mild foreign body sensation or itch [81]. A pterygium is a growth of epithelial and fibrovascular tissue from the conjunctiva migrating over the corneal limbus in a wing-shaped way. Pterygia have similar associated factors as pingueculae, and a pterygium is often preceded by, or is comorbid with, a pinguecula. Irregular astigmatism with a pterygium can lead to visual symptoms, and irritation may be present. Inflammation of pterygia and pingueculae is also possible, leading to conjunctival hyperemia and edema, and increased chance of irritation [82, 83].

3.1.2.1.7 Conjunctival concretions

Conjunctival concretions are benign discrete yellow-white deposits, most commonly found in the palpebral conjunctiva or fornices. Most are idiopathic and the result of degenerative changes with ageing, but they may be secondary to conjunctival inflammation, such as that associated with allergic keratoconjunctivitis, trachoma and DED. They are normally asymptomatic, but when they erode through the conjunctiva they may irritate the cornea and bulbar conjunctiva [84].

3.1.2.2 Corneal-related disorders

3.1.2.2.1 Neurotrophic keratitis

Neurotrophic keratitis is characterized by a decrease in corneal sensitivity and stems from dysfunction in the ophthalmic division of the trigeminal nerve, which can be triggered by

conditions such as diabetes mellitus, ocular herpes simplex/zoster, non-ocular surface neoplasia or ophthalmic surgery. This dysfunction ultimately leads to a decrease in aqueous tear production [39].

3.1.2.2.2 Corneal dystrophies and degenerations

Corneal dystrophies and degenerations present with similar symptoms as DED. They can also lead to recurrent corneal erosions. The more common cases seen in clinical practice are Salzmann's nodular degeneration, epithelial basement membrane dystrophy, and Fuchs endothelial corneal dystrophy. Salzmann's nodular degeneration is a non-inflammatory degeneration of the anterior cornea with blue-white-grayish sub-epithelial nodules of the cornea, usually mid-peripheral. It can lead to symptoms of decreased visual acuity and irritation, pain, foreign body sensation, and blepharospasm. [85, 86] epithelial basement membrane dystrophy, previously known as map-dot-fingerprint dystrophy affects around 42% of the general population and is characterized by abnormal epithelial turnover, maturation and production of basement membrane. Typical signs are gray patches, microcysts and fine lines in the corneal epithelium, and typical symptoms are blurred vision and pain. [87, 88] Fuchs' endothelial cornea dystrophy is a hereditary, progressive disease of the posterior cornea, with progressive decline of corneal endothelial cells, and the formation of extracellular matrix excrescences in Descemet's membrane. This can eventually lead to corneal edema, bullous keratopathy (see Section 3.1.2.2.3), loss of vision, photophobia, epiphora, and pain. [89]

3.1.2.2.3 Bullous keratopathy

Bullous keratopathy forms small vesicles or bullae in the cornea due to endothelial dysfunction. These blister-like formations can rupture painfully and disrupt vision. Treatment options may include 5% sodium chloride or other hyperosmotic eye drops to reduce swelling, amniotic membranes, bandage contact lenses for comfort, antiglaucoma medications (when associated with glaucoma) to decrease fluid flow into the cornea and corneal transplants to replace damaged tissue [90].

3.1.2.2.4 Infectious keratitis

Infection of the cornea can be microbial (bacteria, fungal or parasitics), or viral (herpes simplex or zoster), and is a sight-threatening condition, that always needs to be ruled out in patients with ocular surface symptoms. Contact lens wear, topical steroid use, ocular trauma, previous keratitis and previous ocular surgery are important risk factors. Blurred vision, bulbar hyperaemia, pain, photophobia, tearing and discharge, and an acute, unilateral clinical picture should particularly raise suspicion. [91].

3.1.2.2.5 Thygeson's superficial punctate keratitis

Thygeson's superficial punctate keratitis is a chronic and recurrent corneal epitheliopathy with episodes of foreign body sensation, tearing, photophobia and visual symptoms. Clinical signs are multiple, slightly elevated, round or oval, white-gray intraepithelial corneal lesions, usually with little or no conjunctival involvement. Etiology and optimal treatment is unknown as yet, but mild topical corticosteroids and immunomodulatory agents are often effective in causing regression of the keratitis. [92]

3.1.3 Other mixed and miscellaneous ocular surface disorders

3.1.3.1 Limbal stem cell deficiency

Limbal stem cell deficiency is characterized by loss or deficiency of the limbal epithelial stem cells, that are essential for the maintenance of the corneal surface and its physiological functioning. Diagnosis is primarily clinical, with varying signs depending on the stage, such as a stippled staining pattern of the epithelium, late fluorescein staining, loss of corneal

transparency, conjunctivalization of the cornea and superficial corneal vascularization. Symptoms include ocular redness, discomfort, pain, tearing, photophobia and decreased vision, the latter occurring particularly when the visual axis is involved. [93]

3.1.3.2 Episcleritis

Episcleritis is a usually benign and self-limiting disease of the episcleral tissue that presents as superficial bulbar redness, most commonly in the interpalpebral area. In the majority of patients, the inflammation is limited to a single sector, but may involve the entire episclera, and it may present with a semi-mobile nodule. Symptoms are often not present, but may include irritation and tenderness. [94]

3.1.3.3 Mucus fishing syndrome

Mucus fishing syndrome is caused by repetitive eye-rubbing and self-extraction of mucus discharge (for example that arises as a result of DED or allergic conjunctivitis) out of the eye, leading to chronic inflammation of the ocular surface, and even more mucus production, sparking a vicious cycle. An important clinical sign is epithelial damage (as seen by lissamine green staining) of the nasal and temporal conjunctiva. [95, 96]

3.1.3.4 Ocular neuropathic pain

Ocular neuropathic pain, or corneal neuropathic pain, is characterized by increased perception of pain in response to stimuli that are normally not painful. It is usually a diagnosis of exclusion, when symptoms outweigh clinical signs. Questionnaires that potentially could be used for the diagnosis of ocular neuropathic pain have been identified, although no gold standard questionnaire has been established. [97] Neuropathic pain can result from injury or disease of peripheral corneal nerves, but may also have a central nervous system origin. The use of an esthesiometer may help in determining hypersensitivity of the cornea [98]. A proparacaine challenge test may help distinguish between neuropathic pain of peripheral or central origin [99]. Reported symptoms may be very similar to those of DED, albeit often very severe, and treatment is often difficult and encompasses both regular ocular surface treatments including anti-inflammatory options, and systemic analgesics, tricyclic antidepressants, anticonvulsants (for example gabapentin and pregabalin), low dose naltrexone, and electrical neurostimulation to reduce pain. [100-102].

3.1.4 Non-ocular surface disease disorders

Finally, the early stage of several non-ocular surface disease disorders of the eye, orbit and surrounding tissues may mimic symptoms of DED. These should be considered particularly when no ocular surface signs are visible. Ocular disorders include refractive error (such as latent hypermetropia), digital eye strain, intermittent angle closure, anterior scleritis and uveitis. Orbital disorders include Tolosa-Hunt-syndrome, thyroid eye disease (which may ultimately lead to severe ocular surface disease secondary to chronic exposure), and carotid cavernous fistula and disorders of the nasolacrimal duct that include dacryocystitis and nasolacrimal duct obstruction. In addition, sinusitis and headache disorders may also present with referred pain around the eye. [103].

3.1.5 Summary

Many conditions can mimic symptoms and/or signs of DED. Failing to investigate possible comorbidities may lead to delayed diagnoses of source conditions and may impact the optimal treatment. To aid in the differential diagnoses, specific questions are listed below. For patients in whom the differential diagnostic history and symptoms suggest it may not be DED, a detailed anterior eye examination using a slit-lamp biomicroscope is warranted [39], including assessment of:

- Eyelashes for blepharitis, trichiasis, distichiasis, milphosis, and/or poliosis
- Eyelids, including palpebral conjunctiva for irregularities or the presence of follicles, papillae or swelling, and eyelashes for crusting or cylindrical dandruff, and meibomian gland orifices for blockage, pouting or presence of Demodex tails
- Ocular surface for conjunctivochalasis, pinguecula, pterygium and any post treatment/surgery signs of corneal disruption (see section 3.5.4.1)
- Bulbar conjunctiva for redness and/or swelling
- Cornea, including staining, for signs of ulceration, marginal keratitis, erosion, lesions and possible trauma
- Anterior chamber for the presence of cells or keratic precipitates indicating intraocular inflammation.

Medications which can cause DED and the use of cosmetics should be considered (as reviewed by the TFOS Lifestyle reports [8, 9] as well as risk / associated factors (as reviewed in the TFOS DEWS III Digest [4]) including general health conditions.

Key clinical differential diagnosis questions, alongside asking about a patient's general health and medication, are:

- Do you feel eye pain rather than discomfort?
    - DED commonly presents with symptoms of discomfort, light sensitivity or blurred vision, rather than pain, especially in mild to moderate dry eye. If pain is present, investigate for signs of trauma / erosion / infection / ulceration / acute glaucoma and consider administering a pain questionnaire (see section 3.1.2.2).
- Do you have any facial flushing/redness, mouth dryness or enlarged salivary glands?
    - Trigger for rosacea, sarcoidosis or Sjögren disease investigation.
- When did your symptoms start and can you recall any triggering event?
    - DED is a chronic condition. If there is increased dryness when waking up or dryness symptoms at night, consider incomplete lid closure, for example.
    - If the onset was sudden or linked with an event, examine the eyes for trauma / infection / ulceration.
    - If the symptoms are linked with contact lens use, refer to their contact lens practitioner to consider alternative approaches that might improve symptoms (such as changing lens material, fit or modality) [104]
- Is your vision affected and if so, does it improve on blinking?
    - A reduction in vision which does not improve with blinking, especially with sudden onset, requires an urgent complete ophthalmic examination to rule out conditions such as vascular occlusions.
- Are the symptoms or any redness much worse in one eye than the other?
    - DED is commonly bilateral. If signs and/or symptoms are significantly greater in one eye, investigate for signs of trauma / infection / ulceration.
- Do the eyes itch, are they swollen or crusty, or is there any discharge?
    - Ocular itching is more likely associated with allergies/history of atopy, while a mucopurulent discharge is associated with ocular infection. Reported itching, specifically along the lash line, warrants assessment for Demodex related anterior blepharitis.[105]

3.2 Risk factors

Identifying risk / associated factors may help in finding causes and pathophysiological mechanisms of DED, in determining subtypes, in setting a differential diagnosis, in identifying comorbidities, and in explaining possible discordance between symptoms and signs (see section 3.3.3). In addition, it may help in patient education, and many associated factors may be modifiable, offering the potential to improve symptoms and signs and help prevent/slow progression of disease. In clinical practice, administering a questionnaire to the patient (such as via paper or a digital application) for completion before the consultation is

recommended to save time and to ensure that all commonly associated factors have been addressed.

DED is a multifactorial and heterogeneous disease, and over 200 associated factors have been proposed in the literature. Not all these factors necessarily play a causative role, and some associated factors are linked more strongly with symptoms, while others are linked with signs. Figure 3 shows consistent and probable associated factors of DED as described in the TFOS DEWS III Digest Epidemiology section.[4] The following section briefly highlights the most important risk / associated factors by category. Irrespective of a possible causal mechanism, all associated factors described may be useful in elucidating the etiology of DED and seeking to identify the possible disease driver(s) in an individual. Table 1 presents frequently described risk factors that have inconclusive evidence (either directly conflicting information in peer-reviewed publications, or inconclusive information but with some basis for a biological rationale).

Figure 3: Consistent and probable associated factors of DED described in the TFOS DEWS III Digest Epidemiology section [4].

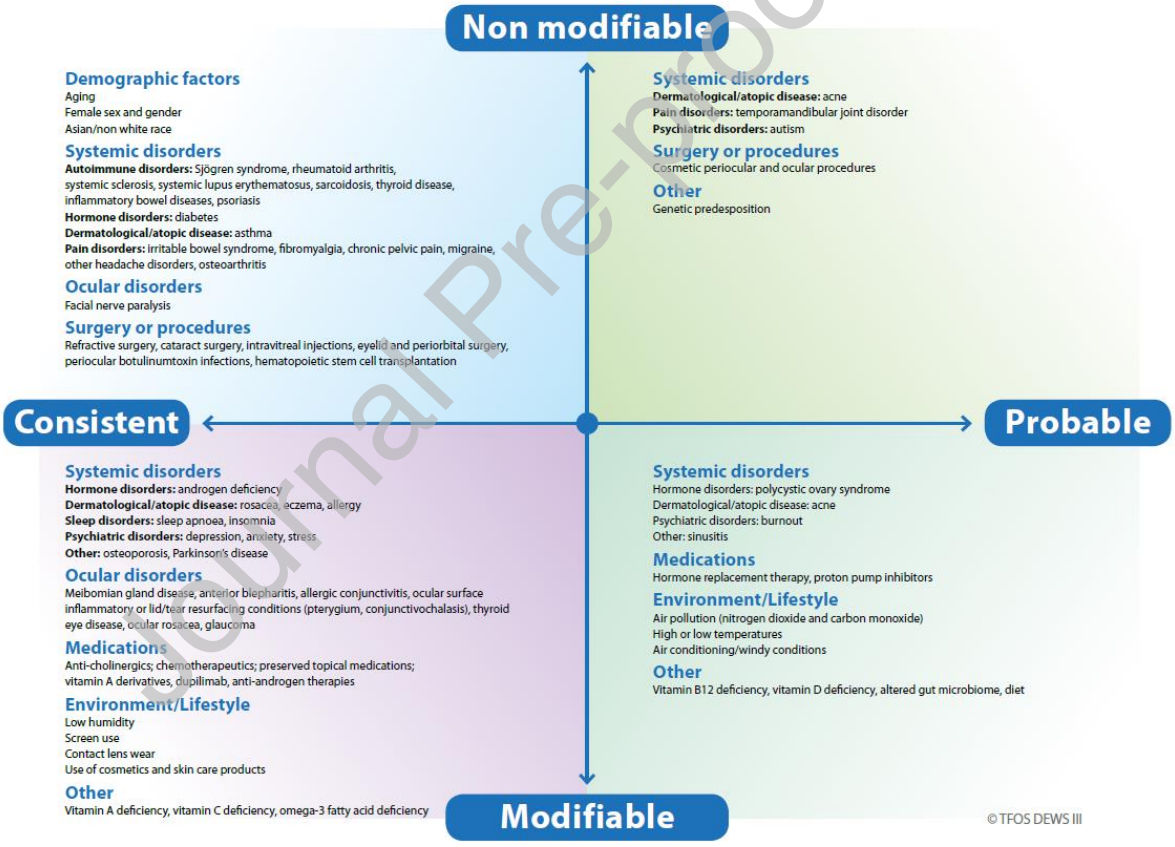

Table 1: Frequently described risk factors that have inconclusive evidence (either directly conflicting information in peer-reviewed publications, or inconclusive information but with some biological rationale):

• Smoking [106-108]
• Caffeine [7, 109-112]
• Alcohol [7, 113, 114]
• Water intake [7, 115, 116]
• Food restriction [7]
• Mediterranean diet (possible positive effect) [117-121]
• Menopause [122, 123]
• Air pollution from particulate matter of < 10µm [6]
• Oral contraceptives [124-126]

3.3 Symptomology

3.3.1 Routine questions based on variability of symptoms

Patients with DED often report sensations of grittiness and burning alongside dryness, while contact lens wearers frequently complain of dryness, along with sensations of scratchiness and watery eyes, driving likelihood of failure for long term successful wear [127-129]. Individuals diagnosed with DED present with variable symptom severity, ranging from mild to severe, throughout the day.[39] Dryness symptoms are typically worse upon waking than later in the morning and tend to increase towards the end of the day in contact lens wearers [130-133]. Ocular allergies, exposure to air conditioning, and climatic factors that change between seasons may be associated with seasonal variations in symptoms and signs. However, a cross-sectional, retrospective cohort study in two Japanese clinics found that none of the symptoms examined (dryness, irritation, pain, fatigue, blurring and photophobia) showed significant seasonal variation [134]. This was confirmed by another study, which failed to show seasonal or weather-related variation in the severity of presenting signs or symptoms of DED over a period of 3 years in 652 people in Oslo, Norway [135]. Hence seasonality of DED symptoms, doesn't seem to warrant specific questioning beyond the differential diagnosis if the patient reports the eyes generally itch. However, itching along the lash line may indicate a Demodex blepharitis issue [105]

3.3.2 Standardised questionnaires

Around 25 questionnaires can be identified from a literature search using terms "dry eye" and "questionnaire" as well as by research articles cited in the identified publications. Each questionnaire warrants evaluation to assess its clinical relevance, known population validity, concurrent validity, internal consistency and reproducibility [136, 137]. Additionally, uni-dimensional evaluation validity should be confirmed using Rasch analysis and it should be confirmed whether "health-related quality of life" has been assessed.

Questionnaires such as the Ocular Surface Disease Index (OSDI), Impact of Dry Eye in Everyday Life (IDEEL), Dry Eye-Related Quality-Of-Life Score (DEQS), University of North Carolina Dry Eye Management Scale (UNC DEMS) and the 25-Item National Eye Institute Visual Function Questionnaire (NEI VFQ-25) are validated to a greater or lesser extent in regard to their utility in assessing the impact of DED on health-related quality of life [137]. These questionnaires are not interchangeable, underscoring the necessity of recommending a single, comprehensive, standardized tool in establishing robust diagnostic criteria [137]. A diagnostic questionnaire should have a low response burden on the patient, be simple to score for the clinician and contribute to the assessment of severity and monitoring treatment efficacy. TFOS DEWS II recommended the use of either the OSDI or the 5-item Dry Eye Questionnaire [138]. However, subsequent comparisons of the results from these questionnaires have shown poor comparability. [139-141]

The OSDI-6 was introduced in 2018 to reduce the response burden on patients. Researchers conducted a study that included Rasch analysis, to determine if the effectiveness of the 12 item OSDI questionnaire, could be maintained with a shortened version (Figure 4). The questionnaire was further adapted such that questions would reflect a recall period of one month versus only one week in the original OSDI. The resulting abbreviated version, OSDI-6, consisting of six questions, was found consistently to be more repeatable than either the full OSDI and DEQ-5 [142]. Moreover, the scoring method that involves simply summing the item scores and using a diagnostic cutoff of ≥4 to indicate symptoms of DED, allows for a quicker evaluation by clinicians [142]. It is possible that this abbreviated format might also be more suitable for children, who are known to better tolerate questionnaires that require less time and assistance to complete. [143] Similarly to the original OSDI, the OSDI-6 results can be indexed against severity, as normal (0-3 points), mild-to-moderate DED (4-8 points), severe DED (>8)[144]. A Chinese translation of the OSDI-6 found it to be repeatable, valid and psychometrically responsive to DED in a Chinese population [145]. The OSDI-6 thus yields comparable outcomes to the full OSDI, requires less time to complete, and maintains similar variability and improved sensitivity to treatment effects [146]. Consequently, the OSDI-6 merits consideration as a suitable screening questionnaire for DED diagnostic purposes while other questionnaires may offer supplementary aid for risk factor identification and treatment selection, after confirming the initial diagnosis.

	Constantly	Mostly	Often	Sometimes	Never
Have you experienced any of the following *during a typical day within the last month?*					
1. Eyes that are sensitive to light?	4	3	2	1	0
2. *Vision blurring between blinks, with your refractive correction*?	4	3	2	1	0
	Symptoms and visual disturbance subscale ⇨				
Have problems with your eyes limited you in performing any of the following *during a typical day within the last month?*					
3. Driving or being driven at night?	4	3	2	1	0
4. Watching TV, or a similar task?	4	3	2	1	0
	Visual function / tasks subscale ⇨				
Have your eyes felt uncomfortable in any of the following situations *during a typical day within the last month?*					
5. Windy conditions?	4	3	2	1	0
6. Places or areas with low humidity?	4	3	2	1	0
	Environmental subscale ⇨				

Figure 4: Questions of the OSDI-6 [146, 147]. The diagnostic cut-off is a summed score of ≥4.

3.3.3 Discordance between signs and symptoms

It is well established that there is a poor correlation between symptoms and signs in DED. A systematic review including 33 population-based and cohort studies, with a total of 175 individual sign-symptoms associations, found the vast majority of correlations to range between +0.4 and –0.4 [148]. This means that dry eye signs generally explain only up to 15% of the variation in symptoms within a population.

There are numerous methodological, statistical, but also physiological reasons why the correlation between symptoms and signs in studies is low.

- Firstly, measurement error is a common problem for many of the DED tests, and the order in which tests are performed or the instillation of fluorescein, for example, can impact test findings [39]. Outcomes are often subjectively scored, not only symptoms, but also purportedly objective signs such as fluorescein break-up time, staining of the ocular surface, and MGD.

- Secondly, DED is recognised to be a dynamic disease, in which symptoms can fluctuate over time, and where test outcomes may be affected by environmental factors, time of the day, and recent artificial tear use [39]. Furthermore, symptoms are often scored as an average over a defined period (such as 'during the last week' in the OSDI), leading to differences in time period over which signs are measured and symptoms are scored.
- Thirdly, DED is multifactorial, with potentially more than one etiology being identified in clinical practice [47, 149]. Studies that include different subgroups of DED in one analysis risk a dramatic fall in correlation values, such as when patients with neuropathic pain are included in studies focusing on more objective DED subtypes [150]. Indeed, when certain subgroups of patients with DED have been analysed independently, higher correlations between symptoms and signs have been found [148].
- Fourth, neurosensory abnormalities and inflammation of the ocular surface, which are considered two core mechanisms of dry eye, are not (directly) measured in common clinical dry eye tests. With time, changes in nerve status may effect changes in reported symptomology in DED. For example, a small study of patients with Sjögren disease found that those with advanced corneal staining counterintuitively reported fewer symptoms than patients with more mild corneal staining [151]. Increasing age can also lead to a reduction in corneal sensitivity [152, 153]. A study in the United States of America found correlations between a number of tear film measures and symptoms scores on the 5-item Dry Eye Questionnaire and OSDI to have coefficients all lower than r=0.18, while symptoms were much more strongly correlated with non-ocular pain, depression and post-traumatic stress disorder scores [154]. A large single-centre study in the Netherlands also found poor correlation between common dry eye signs and OSDI symptom scores (all r<0.30), and found correlations to be significantly lower in women than in men, indicating sex differences in symptom reporting in DED [150]. A wide variety of factors, including depression, stress, age, sex and gender can affect symptomology scores.
- Fifth, other comorbid ocular surface disease (that are often associated with DED) may also lead to symptoms (see section 3.1), risking obscuration of true correlations between dry eye signs and symptoms in analyses.
- Sixth, signs of DED may not necessarily lead to symptoms. For example, MGD is a common finding in asymptomatic persons [155]. Seventh, treatment may alter the correlation between symptoms and signs, with certain treatments influencing one variable more than another [156]. Frequently, clinical trials show a positive effect of a DED treatment on symptoms only, but not signs, or vice versa [157-162]. In addition, some longer duration trials have found signs to improve at a later stage than symptoms (for example with artificial tears), or vice versa (with cyclosporine)[163, 164].
- Finally, despite the longitudinal nature of these clinical trials, there remains a lack of natural history studies that attempt to correlate dry eye signs and symptoms *within* the individual patient rather than *between* patients; there is therefore a need for *within* patient correlation studies across different time points, where it might be expected that relationships between signs and symptoms may be stronger.

In recent years, several studies have explored predictors of discordance between symptoms and signs in DED. In a study in the Netherlands, 648 patients were ranked based on a composite dry eye signs' severity score, and also ranked according to symptoms using the OSDI score. Next, a risk factor association analysis was performed with the difference between these two rank scores being the dependent variable. Factors associated with a finding of symptoms exceeding signs were chronic pain syndromes, a history of atopic diseases, allergies, use of antihistamines, depression, the use of antidepressants, and osteoarthritis. Predictors of fewer symptoms than signs were increased age, Sjögren disease and graft-versus-host disease [165]. A similar study in the United States of America with 326 patients replicated many of these factors, finding associations between increasing age and

fewer reported symptoms, while mental health and chronic pain disorders associated with more symptoms. In addition, the study found quantitative sensory testing scores that indicate hyperalgesia to be associated with more symptoms than signs [166]. A recent Taiwanese study with 1229 patients using a similar approach found female sex, and a history of cataract, pterygium and conjunctivochalasis surgery to be associated more with symptoms than signs, and people of older age and those using artificial tears to be relatively less symptomatic than their signs might suggest [167].

Other studies looking at discordance between signs and symptoms in DED have found corneal microneuroma-like structures and increased corneal dendritic cell density [168], but also decreased corneal nerve density [169], and tear and conjunctival cytokines [168, 170] in patients where symptoms outweighed signs. Table 2 lists all the factors across studies that were associated with symptoms outweighing signs, and conversely, those with significantly fewer symptoms than signs. These factors may help in understanding outcomes in clinical practice, aid in patient education (such as in explaining about central sensitisation mechanisms or altered nerve status after surgery) and offer clues for differential diagnoses and comorbid disorders (for example atopy and allergic conjunctivitis) in patients with DED exhibiting discordance between signs and symptoms. The findings also emphasise the need for clinicians not to rely solely on symptom-reporting in older patients, or those with Sjögren disease or graft-versus-host disease, as symptoms may be understated in relation to the severity of effects on the ocular surface.

Predictors of more symptoms than signs	**Predictors of fewer symptoms than signs**
Demographics	
Female sex [150, 167]	Older age [165, 167, 171]
Black race [166]	
Pathophysiological Factors	
Tear and conjunctival cytokines (IL-10, IL-2, IL-6, IL-17a, tumor necrosis factor alpha) [168, 170]	Decreased corneal nerve density [169]
Increased corneal dendritic cell density [168]	
Hyperalgesia as demonstrated with quantitative sensory testing [166]	
Non-ocular pain intensity [166]	
Comorbidity Factors	
Post traumatic stress disorder [166]	Sjögren disease [165, 172]
Allergy [165]	Graft-versus-host disease [165]
Atopic disorders [165]	Benign prostatic hyperplasia [166]
Osteoarthritis [165]	Hypertension [166]
Depression [165, 166]	
Anxiety [166]	
Chronic pain syndromes [165, 166, 173-175]	
Pharmaceuticals	
Use of analgesics [166]	Use of artificial tears [167]
Use of anxiolytics [166]	
Use of antidepressants [165, 166]	
Use of antihistamines [165]	
Lifestyle Choices	
Current smoking [166]	

Surgery
Cataract surgery [167]
Pterygium surgery [167]
Conjunctivochalasis surgery [167]

Table 2: Factors associated with a discordance between symptoms and signs in dry eye disease. A higher number of studies showing an association is reflected by placement of the factor higher on the list.

3.3.4 Paediatric considerations

While DED is common in children [176] (see TFOS DEWS III Digest epidemiology section),[4] diagnostic tests have largely been validated only in adults. One study examined the quantification of dry eye symptoms in children (6 to 15 years of age) using standardized questionnaires, identifying the completion time was ≤2 min for each individual questionnaire and while younger participants took longer to complete and required more assistance, especially with longer visual analog scales, repeatability was noted to remain high across the age range [177].

3.4 Ocular Examination

3.4.1 Diagnostic Homeostasis Test Battery

3.4.1.1 What is a diagnosis

As previously described, the ability to receive a diagnosis is critical for patients to acknowledge their symptoms and / or signs as real and that they have the attention of health care practitioners, as well as being required for healthcare insurance coverage, where available. For practitioners, diagnostic criteria inform which evidence-based clinical practice guidelines to follow and provide confidence in making patient diagnoses that are consistent with their peers. A standardised diagnosis is essential for industry and researchers to be able to target and validate the efficacy and safety of new products and to obtain regulatory approvals. Appropriate health resource allocation requires robust epidemiological and economic data that are based on consistent diagnoses. Hence diagnosis of a disease must be characterized by standardised, universally-adopted criteria, based on widely available and inexpensive tests with validated cut-off values. There are advantages in having a simple screening element that can rapidly identify those individuals who would benefit from further testing by a health care practitioner.

3.4.1.2 Need for standardisation

The definition of DED (see Section 2.1) dictates that symptoms must be present, and a loss of homeostasis of the tear film and / or ocular surface must be established. Identifying that the expected symptoms are present in a standardized way requires a validated questionnaire as discussed in Section 3.3.2. While TFOS DEWS II recommended two possible questionnaires that could be used, these questionnaires have since been shown to not be equivalent, risking variability in diagnosis depending on the instrument chosen, so it is appropriate to select a single questionnaire to be used in this setting. Based on evidence published since TFOS DEWS II, this has been identified as the OSDI-6 with a summed cut-off score of ≥4 to be positive for dry eye symptoms (see section 3.3.2).

3.4.1.3 Other approaches since TFOS DEWS II

The Asian Dry Eye Society [178, 179] supported by the Japanese Dry Eye Society [180] suggested that DED should be diagnosed by confirming the presence of ocular symptoms (using any one of four possible questionnaires) along with identification of an unstable tear film (assessed with fluorescein tear breakup time of <5s). This approach shows similarities to

TFOS DEWS II, but lacks the standardisation that can be offered by a single questionnaire as proposed by TFOS DEWS III. Furthermore, changing the chemical composition and volume of the tear film by instilling fluorescein prior to assessment of its stability is not ideal. The Korean Dry Eye Society defined dry eye as “a disease of the ocular surface characterized by tear film abnormalities and ocular symptoms” without referring to the pathophysiology, or specifying the means by which to diagnose ocular symptoms (no questionnaire proposed). The diagnostic criteria included an unstable tear film in the form of fluorescein breakup time <7s (a test which is invasive in itself, and recommended without scientific justification for the cut-off), with a Schirmer test (<10 mm) and ocular surface staining considered “adjunctive criteria” [181].

It has been suggested that diagnostic certainty (in the form of sensitivity and specificity) can be increased by requiring multiple discriminatory tests to be positive [182]; leading to the recommendation that both corneal AND conjunctival staining needed to be present to match a Bayesian-informed global prevalence of DED [183]. However, the reported global prevalence used had been generated from existing studies that relied on these various diagnostic criteria which introduces bias into the approach. Further, using the sensitivity and specificity of tests from multiple studies is also susceptible to bias [184] as previously identified in the TFOS DEWS II report [1]. There is increasing chance of misdiagnosing or under-diagnosing a disease if standardised diagnostic criteria are not used because it can require excessive time, consumables costs or equipment which is expensive or has limited availability. Corneal staining often occurs in later and in more severe stages of DED, so while requiring its presence will increase diagnostic specificity, it will exclude appropriate treatment for many individuals whom clinicians recognise to have a marked loss of quality of life as a result of their symptomatic ocular surface disease and would currently be identified as having DED [185].

Attempts have been made to use dry eye tests to differentiate diseases that affect the ocular surface such as Sjögren disease, graft-versus-host-disease, Graves’ orbitopathy, facial nerve palsy, non-proliferative diabetes mellitus and glaucoma treated with preserved topical drops, with mixed results [186-188].

3.4.1.4 Use of sensitivity and specificity to select diagnostic tests

Without there being a recognised gold standard, and due to the well-established low correlation between signs and symptoms in DED [189-191], the level of certainty in derived sensitivity and specificity values of new DED ‘diagnostic’ tests is low. Sensitivity and specificity depend on the inclusion and exclusion criteria for the ‘healthy’ and ‘disease’ groups, increasing with the severity of signs or symptoms required to be allocated to the DED group (spectrum bias). Individuals who do not meet the criteria of either group prevents generalisability of the results across the broad population (sampling bias). Selection bias occurs when the patient groups are selected with test(s) with a similar focus to the test being evaluated [192]. Parallel testing (requiring multiple tests to be positive) will increase the sensitivity and specificity of differentiating a selected ‘healthy’ and ‘disease’ group, but this approach is just as susceptible to the bias in group selection. Detailed discussion and examples of these issues are reported in the TFOS DEWS II Diagnostic Methodologies report [1].

3.4.1.5 Tests to establish a loss of homeostasis of the tear film

While the diagnostic or screening potential of tests such as thermography [193-195], interferometry [196], lipid layer pattern/thickness [197] and tear evaporation rate [198] have been evaluated, they are not widely used in clinical or research settings. Artificial intelligence has been used to show that multiple factors including age, ocular surface staining and symptoms are the most important predictors of an unstable tear film, followed by meibomian gland drop-out and expressibility, blink frequency, osmolarity and meibum quality [199], but it would not be practical to assess all of these in making a diagnosis of DED.

Tear film stability, as usually assessed by the tear film breakup time, is defined as the measured time interval between a blink and the appearance of the first discontinuity in the

tear film [200, 201]. While it is commonly assessed by instilling fluorescein, illuminating the ocular surface with blue light and observing the fluorescence through a yellow filter [202], the application of fluorescein itself reduces the stability of the tear film and increases its volume so the tear film generally breaks up much more quickly [203-205] and the measurement may not be an accurate reflection of the natural tear film status [206-208]. Fluorescein breakup time is also limited as a measure of tear film stability due to the requirement for subjective assessment by the observer and, while an attempt has been reported to automate this measurement [209], the required equipment is not available in clinical settings. Instead of the time taken for the first break in the tear film to be detected, a metric to describe overall disruption in tear film surface quality (assessed from a placido disc reflection) has been used to assess tear film instability [210-212], but this has not been widely adopted. Non-invasive objective assessment, usually with a Placido disc reflected from the tear film surface, determines the time to the first detected break as well as mapping the locations of the tear film disruption and the velocity of the destabilised area. Clinically insignificant differences occur between the objective measurements by many automated devices to measure non-invasive breakup [213, 214], but not all. [215-217] The detection algorithm results can be adjusted in the validation stage of new instruments (such as adjusting the threshold of discontinuity in the mires) to benchmark more closely to existing devices as there is no 'gold standard'. Controlling environmental factors such as temperature, humidity and air circulation during and immediately before the measurement is important, as well as instructing the patient to blink naturally several times and then to cease blinking until instructed to blink again. [218]. The Ocular Protection Index is the ratio of the tear film breakup time divided by the blink interval, but as with other signs of a loss of homeostasis of the tear film and/or ocular surface, levels deemed 'pathological' are poorly associated with patient reported DED symptoms [219].

Increased osmolarity of the tear film occurs within the pathophysiology of dry eye [4] with estimated localized levels up of up to around 900 mOsm/kg predicted across the ocular surface at points of tear film destabilisation [220]. Point-of-care devices that measure osmolality are currently limited to sampling from the tear meniscus, as the volume of tears is too small to sample from the ocular surface. Osmolarity has also been reported to differ according to the location along the lid margin from which it is sampled [221]. While some studies have advocated for its diagnostic role [222, 223], other studies suggest current instrumentation yields results that are not repeatable from a single *in vivo* measurement [224] and values vary with the device used [225]. Tear meniscus osmolarity may account for <5% of the variability in other tear film and ocular surface homeostasis signs [226] and is therefore unreliable in identifying individuals where DED is diagnosed [227], or the DED severity is determined [228] with other dry eye metrics. The daily variation of osmolarity has been suggested to be a better marker of DED [229], but this is impractical for diagnosic purposes in a clinical setting. Tear osmolarity was able to predict poor surgical outcomes and patient dissatisfaction in a cataract surgery population with mild ocular surface disease, again suggesting it provides additional information to tear stability and ocular surface damage [230] and hence is an important metric in diagnosing DED [4].The inclusion of osmolarity amongst the possible signs of a loss of homeostasis of the tear film in the TFOS DEWS II diagnostic algorithm made less than 5% difference in the prevalence of DED [231, 232]. Similarly to osmolarity, tear composition alters where there is loss of homeostasis of the tear film, but not in a measurably consistent manner, and current point-of-care tests for other tear film biomarkers are of limited value (see Section 3.5.4.4.2) [233].

3.4.1.6 Tests to establish a loss of ocular surface homeostasis

Loss of homeostasis of the ocular surface is most widely established by topically applying ophthalmic dyes such as fluorescein and lissamine green, and the resulting staining of the tissues is considered to be a diagnostic sign of DED [234-236]. Staining is one of the clinical signs most strongly associated with dryness symptoms in moderate-to-severe DED [150, 237]. Punctate staining, while not pathognomonic of DED, is commonly associated with desiccation stress, particularly when present in the inferior quadrant of the cornea [238, 239].

Fluorescein staining occurs due to a loss of corneal epithelial cell integrity such as a disruption in superficial cell tight junctions or defective glycocalyx [238, 240]. Lissamine green has largely replaced the use of rose bengal as it is less toxic to the ocular surface [241], staining epithelial cells if the cell membrane is damaged, irrespective of the presence of mucin [238, 242-244]. Staining of the eyelid margin conjunctiva that wipes the ocular surface during blinking (termed lid wiper epitheliopathy) possibly due to mechanical stress resulting from insufficient lubrication [245] is common in patients with DED and associated with poor lipid spread, poor tear film stability, abnormal lid anatomy and blink speed [246, 247]. It is also an earlier diagnostic sign than corneal and conjunctival staining in the natural history of DED pathophysiology [248-250]. A clinically detectable poor seal between the eyelids has also been identified as factor associated with symptoms of ocular discomfort [251].
The diagnostic potential of point-of-care inflammation testing (MMP-9) has been reviewed, but while inflammation was common in severe DED, it was not sensitive to more mild to moderate DED which is more common in the general population.[252] Several studies have investigated the potential of epithelial thickness as a diagnostic test for DED; while the central epithelial thickness has largely to be found to be similar in DED compared to health controls, the superior epithelium is generally thinner, especially in more severe disease,[253, 254] but this finding is not consistent.[255, 256]
Squamous metaplasia and goblet cell density of the conjunctiva can be assessed using impression cytology and, as goblet cell density reduces in patients with dry eye, it has been suggested as a DED diagnostic technique [257, 258]. Impression cytology removes cells from the three most superficial layers of the epithelium, typically by applying cellulose acetate filters or biopore membranes; the cells can then be analyzed by techniques such as microscopy, flow cytometry, immunoblotting analysis, immunocytochemistry and polymerase chain reaction to meet the aims of the investigation [259]. It is a useful alternative to a biopsy, but the changes observed are not pathognomonic of DED [260, 261].

3.4.1.7 Practical diagnostic criteria considerations
Sections 3.4.1.5.and 3.4.1.6 highlight that the key tests to assess the homeostatic status of the tear film remain unchanged from those derived in TFOS DEWS II. The key homeostatic markers are thus non-invasive tear film breakup time, osmolarity and ocular surface staining. The sequence of diagnostic assessment can affect the results as restricting blinking and bright lights can stimulate reflex tearing. It is therefore recommended that tear film assessment tests are carefully ordered, from least to most invasive [262].
The OSDI-6 is a short questionnaire, ideal for screening and is recommended to be conducted as the first component within routine eye examinations to identify those patients who would benefit from a fuller diagnostic evaluation to determine the likely drivers of disease.
As identified in section 3.4.1, it is critical that diagnosis follows a standard protocol. While combining more tests can improve sensitivity, this may be at the expense of clinical utility.

TFOS DEWS II recommended that one of three signs of a loss of homeostasis needs to be present:

- non-invasive breakup time (first break) <10s: as highlighted in Section 3.4.1.5, this test establishes a loss of homeostasis of the tear film There are now a range of affordable instruments available to the practitioner to avoid the adverse impacts of fluorescein dye on the robustness of the test result as is well documented (see Section 3.4.1.5). Where there is no access to such a device to allow non-invasive measurement of breakup time, fluorescein can be applied, but the volume instilled should be minimised and a cut-off of <5s applied as a positive sign of instability. [204, 205]
- osmolarity ≥308 mOsm/L in either eye or interocular difference > 8 mOsm/L (cut-offs established with the TearLab device only). This test serves as a marker of loss of homeostasis for both the tear film and ocular surface (see Section 3.4.1.5). Consideration of the inter-eye difference alone was found to be valid in one study [263]

but not in another (at least in relating to dryness symptoms) [264] and interocular osmolarity was found to have modest, but superior discriminative ability than absolute osmolarity (higher value of the two eyes) [265].

- ocular surface staining of >5 corneal or >9 conjunctival punctate spots or lid margin staining of ≥2 mm length and ≥25 % width following the instillation of both fluorescein and lissamine green dyes [238, 266]. It is more common to observe corneal and conjunctival staining in severe DED (see section 3.5.4.3)
  Corneal observation: The corneal surface, following the installation of fluorescein drops or application of a moistened fluorescein strip, should be observed using a blue light of 495 nm, as this is the peak excitation wavelength for fluorescein (rather than the 450 nm 'cobalt blue' peak of light filters historically used in slit-lamp biomicroscopes) [202]. An observation filter with a band pass at 500 nm limits visibility to the wavelength of the excited fluorescence molecules (emittance around 515 nm) while excluding those from the applied blue light [202]. Consensus on the ideal time after instillation for assessment is 1-4 minutes [238, 262, 267].

Conjunctival and lid margin observation: Lissamine green staining is critical for observation of conjunctival and lid margin staining assessment. A lissamine green strip (note, not all brands are equivalent)[268, 269] should be moistened with sterile saline with the whole drop applied to the eye after having been placed on the strip for at least 5s to allow the dye to be eluted for maximal concentration [269, 270]. The staining should be observed between 1 and 5 min post-instillation of lissamine green,[269-271] potentially through a red filter to aid visualization [241, 272]. Everting the eyelids multiple times should be avoided as this can stress the tissues and affect the degree of staining observed, whereas exposure time seems to be less impactful on the outcomes.[273] Assessment of lid wiper staining generally involves subjective estimation of the length and sagittal depth of staining, but may be optimised using semi-objective imaging techniques [274].

Grading: It should be noted that if using a grading scheme such as the Oxford scale, applying half unit increments improves sensitivity and repeatability, while summing regional grading doesn't give a comparable score to global ocular surface staining and increases variability [275]. For diagnosis, counting punctate spots should result in higher consistency. Despite claims that objective assessment of corneal staining can be used as a highly successful automated dry eye diagnostic system [276], a review of objective techniques suggest this still has limited reliability [275]. While there are challenges to grading staining of the lid margin [277], a diagnostic criterion of 2mm or more length over at least 25% of the sagittal lid width still seems appropriate.

A study found that a substantial proportion of patients 'diagnosed' with DED by a consultant ophthalmologist without following any set criteria, reported symptoms and exhibited hyperosmolarity, but no other obvious signs of DED; however, tear film stability was assessed invasively and staining of only the corneal (not bulbar or lid margin conjunctiva) was included [191].
In a UK population study, among the TFOS DEWS II diagnostic signs for DED, conjunctival staining (with lissamine green dye) occurred in most people diagnosed with DED, followed by reduced non-invasive tear breakup time, lower or upper lid wiper epitheliopathy staining, corneal staining and signs of tear hyperosmolarity. [231] The prevalence of DED was notably consistent if any one of the three markers indicating a loss of homeostasis was omitted from the TFOS DEWS II diagnostic algorithm, indicating its robustness.[231] In a larger study [232], it was found that evaluating just one of the three TFOS DEWS II homeostatic signs resulted in between 12.3% and 36.2% of patients who would otherwise have met the DED diagnostic criteria, not being assigned this diagnosis; hence at least two signs need to be assessed, even although only one needs to be positive. While comprehensive ocular surface staining evaluation in combination with symptoms had the highest sensitivity (87.7%) of the three markers, the sensitivity dropped to 44.6% if corneal staining only was evaluated; hence

conjunctival and lid margin staining assessment (with lissamine green) is critical to diagnosing DED [182, 232]. Omitting either non-invasive tear breakup time or osmolarity each dropped the sensitivity by less than 5%. The prevalence of DED within the population was substantially reduced if diagnosis required symptoms plus two of the three signs to be positive (by between 43.7% to 61.2%) and by 65.9% if all three signs indicating a loss of tear film homeostasis were required [232]. The outcomes of this analysis did not change significantly across differing severities of DED symptoms, confirming the robustness of the DED diagnostic approach (Figure 5).

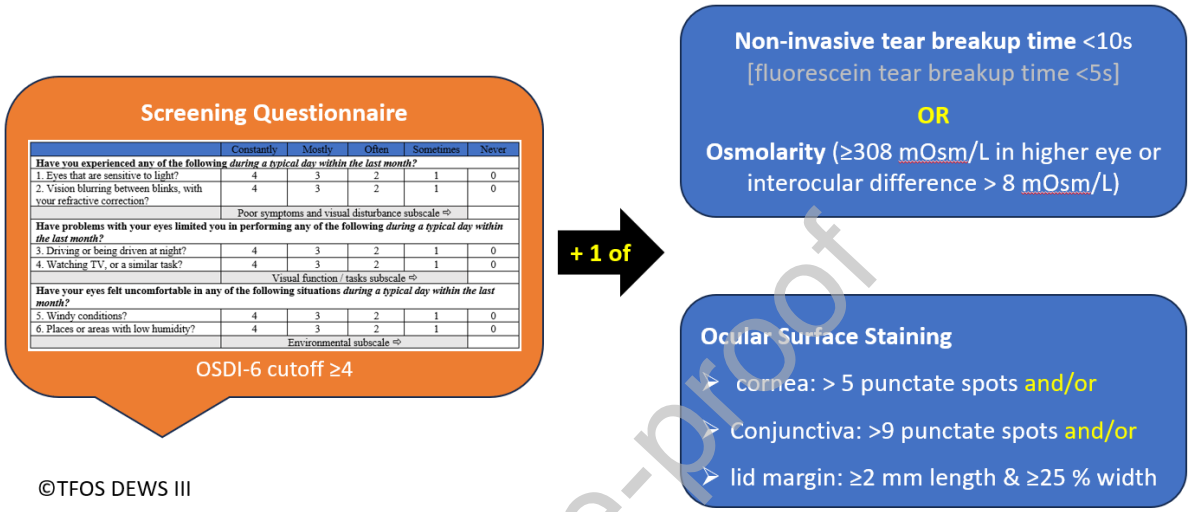

Figure 5: TFOS DEWS III diagnostic algorithm

3.4.2 Advanced screening

Diagnosing and monitoring DED often relies on specialized equipment (such as a slit-lamp biomicroscope) and dyes, which are not readily available in non-eye care setting. A non-invasive and simplified test for DED could enable earlier diagnosis and intervention to help prevent disease onset or exacerbations [278]. Additionally, improved accessibility to DED screening could raise public awareness and encourage high-risk or undiagnosed individuals to seek attention by an eye care practitioner.

Blinking is a natural process that refreshes tear film, removes ocular surface debris, and maintains vision quality [279]. Altered blinking physiology is a common feature in DED and is implicated in its pathogenesis [280-282]. As such, DED-associated blinking patterns could potentially serve as a non-invasive biomarker. Blink rate, interblink interval and maximum blink interval (defined as the length of time that the participants can comfortably keep the eye open before blinking), have been reported to be useful in distinguishing between healthy participants and patients with DED [283-288].

Maximum blink interval (cut-off 12.4 seconds) has demonstrated a sensitivity of 82.5% and specificity of 51.0% for a DED diagnosis based on OSDI symptoms and fluorescein tear breakup time) [289]. A smartphone application has been tested [278, 287, 290, 291], showing comparable results with conventional paper-based OSDI and subjectively observed maximum blink interval [292, 293]. The app-based maximum blink interval had an area under the curve of 0.649, with a cut-off of 10.5 seconds on a digital device with an iOS, but a much shorter 7.0 seconds on an Android operating system, (compared to 12.4 seconds subjectively assessed) for a symptoms (perhaps due to differences in refresh rate and software algorithms) and fluorescein breakup time DED based diagnosis [294]. Similarly, interblink interval, with a cut-off of 3.1 seconds, has shown an approximately 80% sensitivity and 70% specificity for a DED diagnosis based on general symptoms and corneal staining [283]. Furthermore, patients with DED are reported to have higher blinking rates [295], with a

greater proportion of incomplete blinks [296]. By analyzing such blinking characteristics, clinicians may be able to gain valuable insights into a patient's ocular surface status.

As maximum blink interval is dependent on an individual's tolerance to eye pain, an alternative approach is asking an individual to report when their eyes become uncomfortable after a blink while keeping their eyes open, which demonstrated a sensitivity of 66%, specificity of 88%, and an area under the curve of 0.77 (cut-off 10 seconds) compared to a full TFOS DEWS II diagnosis of DED, improving to 71%, 90%, and 0.81 respectively when considered in combination with the OSDI score [288].

3.4.3 Severity rating

The severity of a disease is rated for a diverse range of purposes, but primarily for evaluation and communication [297]:

- to predict prognosis
- to characterize the impact of the disease on the person's well-being at a given point in time
- to establish the basis for treatment decisions
- to evaluate disease activity and monitor response to treatment

Some classifications are based on pathological or physiological status, while others have used impairments or specific symptoms (such as pain) and still others have characterized severity based on exercise tolerance or functional status [297]. Symptoms and outcomes tend to be used in systems that are designed to reflect the patient experience. Pathological or physiological measures have been incorporated in systems used to predict prognosis and both tend to be used to guide treatment, or measure response to treatment [297]. Some severity classification systems also take into account the presence of other conditions, diseases, demographics or behaviors in their staging, if these are considered risk factors for a poorer prognosis.[297]

In other eye diseases, severity ratings vary from those based on the risk of advanced disease progression, for example in age-related macular degeneration [298], to those variably combining signs and symptoms which aligns with clinician consensus, for example in keratoconus [299-301]. The former approach requires a large sample of natural history data [302] and the latter, a reasonably high association between subjective and objective clinical metrics [303], neither of which are currently available for DED.

Severity grading in DED has been based on factors such as inflammatory cytokine concentrations [304], corneal fluorescein staining scores [305] and doctor (clinical judgement) rating [306]. The ODISSEY group proposed a severity rating based on the expert opinion of 10 ophthalmologists [307] where the DED diagnosis relied on poor tear stability alone, and severe dry eye was considered to be those with an OSDI score ≥33 and corneal fluorescein staining grade ≥3 on the Oxford scale, or with additional criteria if there was less staining. An objective composite index of disease severity has been proposed using an independent component analysis approach [308], however key non-invasive tests of the tear film and ocular surface were missing such as non-invasive tear breakup time, interferometry, tear meniscus height and lid wiper epitheliopathy and the cut-off for each test's severity grade was based on an "expert panel" of five; the amount of independent information provided by each test (eigenvalue) was used as its weighting for each component's contribution to a composite score, which was based on the sum of the squared measures, divided by the square-root of the sum of the weighting coefficients. A survey approach involving 37 corneal specialists in a hospital setting (non-representative of dry eye practitioners) of unknown location identified clinical tests and cut-offs they felt represented a diagnosis and reflected severity (mild, moderate, severe and very severe) of DED [309]; seven tests were identified and overall severity was based on an algorithm of combining the scores with equal weighting (identified as a limitation) which was based on clinician-ratings (not defined) of 50 patients. A recent study suggested observing corneal cell morphology and density with *in vivo* confocal microscopy in areas which stain with fluorescein and lissamine green dyes may be a reliable

basis for clinical grading of DED severity, but the cohort of 24 participants was classified into severity grades based on the Chinese Cornea Society criteria which also involved staining, as well as fluorescein tear breakup time, so the observation was not surprising.[310]

A recent review of how to "best diagnose severity levels of DED" erroneously described 'asymptomatic DED', only mentioned non-invasive tear breakup time as a form of interferometry and concluded, without proposing a clinical algorithm, that the evaluation of severity of the condition has often been difficult.[311] While the original TFOS DEWS report proposed a severity matrix [312] based on a prior Delphi panel of 17 DED specialists,[313] this was not adopted by TFOS DEWS II (2017) due to the limited association between the characteristics included and the lack of evidence for the tests included to inform their weighting in a composite algorithm. Likewise, the Asian Dry Eye Society did not propose a severity algorithm for DED [179]. However, the Korean Dry Eye Society proposed a severity matrix, but without scientific justification for the proposed tests, and the severity levels provided [181].The revised American Academy of Ophthalmology Preferred Practice Pattern guidance [314] mentions the role of DED severity in informing management, but without guidance on how to rate severity. In a recent survey in the UK, patients with DED rated symptom frequency and severity along with tear film stability as the most desired aspects of their DED to improve with treatment, although other factors such as ocular surface, corneal nerve and tear gland damage followed by tear volume and constituents were rated only slightly less important.[315]

3.5 Subclassification to identify DED etiological drivers

3.5.1 Purpose of a DED subclassification

Diagnostic subcategories (for diseases and most syndromes) are simply concepts. Their purpose is to segregate multifactorial aspects to allow a better characterization of patient outcomes and to guide decision-making regarding treatment [316]. TFOS DEWS and DEWS II confirmed the importance of subclassifying DED into aqueous deficient or evaporative forms, or a combination of the two [2, 312]. However, several studies have confirmed that at least two-thirds of those with DED exhibit the evaporative form [317-321] which is recognised to have a number of etiologies including lid-related and ocular surface-related,[322] which, without distinction, limits the ability to target treatment to the appropriate etiology. The selection of tests used to differentiate evaporative from aqueous deficient forms of DED varies between studies and a Delphi panel approach has attempted to establish agreement [323]. In addition, it has been noted that between 18 % and 29 % have no obvious signs of either a reduction in tear volume or disruption to meibomian gland structure and function, suggesting the need to acknowledge other subtypes of the disease [191, 319-321, 324]. DED is accepted as a multifactorial disease [2, 20] so addressing the different mechanisms leading to an individual's DED could impact treatment outcomes. Disease heterogeneity will reflect differences in the underlying pathophysiology, genetic risk and environmental contributors of affected individuals [325]. Clinical tests that identify the possible drivers of an individual's disease (which are not mutually exclusive), in turn, inform the appropriate treatment approach(es) [326]. The Asian Dry Eye Society [179] proposed four targets for therapy (lipid, aqueous and mucin layers along with ocular surface inflammation), but with a target of the epithelium consisting of membrane-associated mucins and epithelial (goblet) cells. Expanding on this approach, the following section outlines those clinical tests that inform the clinician about the contribution to DED from:

3.5.2 Tear Film Deficiencies

The latest understanding on the tear film is reported in the TFOS DEWS III Digest.[4]. It has been proposed that differences observed in the fluorescein tear breakup patterns can inform the clinician about which tear film layer has been disrupted. Area break is thought to be associated with severe aqueous-deficient DED; spot, dimple, and line breaks with rapid expansion are associated with decreased wettability DED, with a line break thought to be associated with mild-to-moderate aqueous-deficient DED; and a random break appearance

is associated with increased evaporation DED [327-329]. However, to date, there has been only limited published evidence to support these hypotheses [329].

3.5.2.1 Lipid component

3.5.2.1.1 Interferometry

Tear interferometry allows the tear film lipid layer thickness to be estimated, noninvasively [330, 331]. Due to nature of the thin lipid layer overlying a body of aqueous with different refractive index, reflections from the air-lipid and the lipid-aqueous interfaces create interference patterns, which can be analyzed quantitatively or semi-quantitatively [332]. Lipid layer interference pattern grades correlate with corneal staining and tear film breakup time [333]. Lipid layer thickness should be increased in hypersecretory MGD and decreased in obstructive meibomian MGD, but a direct association between the thickness of the lipid layer and the rate of tear evaporation has not been proven [334, 335]. There are various non-invasive diagnostic devices for assessing the tear film lipid layer [336-342]. Some devices attach to a slit-lamp biomicroscope base while others are stand-alone instruments. Most require subjective grading of the lipid pattern [343], which equates the pattern observed to an estimated thickness [337]. A dynamic lipid layer interference patterns test has been proposed, reporting the optimal number of blinks to observe a significant change in lipid pattern as being up to five forced followed by 10 natural blinks at 2s intervals; in patients with DED, the number of blinks required to change the lipid pattern (2.4 $\pm$3.1 blinks) was statistically lower than in healthy subjects (18.1 $\pm$ 5.9 blinks)[344]. The LipiView interferometer has a sensitivity of around 68% and specificity of 64% if a cut-off value of 75 nm is used for MGD diagnosis [332, 345]; the coefficient of variability for inter-observer repeatability was 13 nm and the intra-observer repeatability 16 nm in healthy individuals, with values less than 60 nm considered pathological [346]. In a study of 221 participants, optimal diagnostic cut-offs for DED based on the TFOS DEWS II criteria were <72nm with the LipiView and a grade of ≤3 subjectively, based on interferometric patterns (modified Guillon scale).[197] Lipid layer thickness values obtained with the LipiView instrument have been reported to correlate well with meibomian gland loss [347]. Another technique, using a spectrophotometer, claims an ability to directly image tear muco-aqueous and lipid layer thicknesses *in vivo* with nanometer axial resolution [348, 349].

3.5.2.1.2 Lipid turnover

The turnover of the lipids of the tears assessed by fluorophotometry 0.9 ± 0.4 % / min) is slower than the aqueous turnover (10.3 ± 3.7 % / min).[350] Contrast-enhanced optical coherence tomography (OCT) imaging has also been used to evaluate the clearance of lipids in human tears [351]. A system that combines simultaneous OCT and thickness-dependent fringes interferometry can be used for *in vivo* assessment, simultaneously imaging both the lipid layer thickness and overall tear film thickness [352, 353]. The analysis of the OCT's *en face* maps of the tear lipid layer provides complementary information to interferometry [354]. The tear film imager uses spectral interference to allow real-time evaluation of the rate of lipid thickness change and discontinuations over a large field of view at nanometer axial resolution [330]. The mucoaqueous thickness correlates with the Schirmer score and lipid or fluorescein tear breakup time [349].

3.5.2.1.3 Evaporimetry

A meta-analysis of studies measuring evaporation found raised levels in patients with DED, particularly evaporative DED (normal, 13.57 $\pm$6.52 x 10^{-7} g/cm^2/s; aqueous deficient dry eye, 17.91 $\pm$ 10.49 x 10^{-7} g/cm^2/s; evaporative dry eye, 25.34 $\pm$ 13.08 x 10^{-7} g/cm^2/s)[355]. Different instruments have been used for assessing evaporation, although only one is currently commercially available [356-359]. Tear evaporation has been shown to be reduced by eyelid warming therapy [360]. Confounding factors are the sampling response rate to blinking, perspiration within the sampling area, palpebral aperture, variation in blink speeds and patterns, and in the level of chamber ventilation, and differences in resistance to evaporation caused by humid, and still air [361, 362].

3.5.2.1.4 Thermography

Thermography uses a specialized camera to detect infrared radiation emitted from the ocular surface, mapping changes in the ocular surface temperature that are presumed to be caused by tear fluid evaporation [363]. The technique seems repeatable [364]. Thermal cooling of the ocular surface is a predictor of soft contact lens induced dryness symptoms [365]. Ocular surface temperature decreases more rapidly following a blink in individuals with adequate tear volume, but unstable tear films.[366-368] The temperature differential between the central cornea and limbus is higher in DED than in normal,[369] with evaporative DED associated with higher ocular surface temperature than aqueous deficient DED and patients with MGD having higher ocular surface temperature than those with healthy eyes.[367, 370]

3.5.2.1.5 Meibum expressibility and quality (meibometry)

Meibomian gland functionality is assessed by testing the expressibility of meibum and the quality of expressed meibum [332, 371]. Meibum quality is typically graded as 0 (clear fluid), 1 (cloudy fluid), 2 (cloudy particulate) or 3 (toothpaste-like). The expressibility of meibum from the meibomian glands of the upper and lower eyelids is graded after 10-15 seconds of applying pressure as 0 (all glands expressible), 1 (3 to 4 glands expressible), 2 (1 to 2 glands expressible) or 3 (no glands expressible). Meibomian gland expressor devices have been developed as a means of standardizing the pressure of 'diagnostic' expression, which aims to be equivalent to that of a natural blink [371]. Meibum quality and expressibility are correlated with gland loss and lipid layer thickness [372-375].

Meibometry is a technique applied to assess lipid volume [376], involving sampling lipid from the lower lid margin with a loop of translucent plastic tape. The tape is air-dried for 3 min to allow evaporation of any contaminating tear fluid and the optical density of the oil retained on the tape is assessed using a diode laser. A correlation has been reported between lower lid meibometry readings and expressibility of meibomian lipid from the central upper lid, as well as a reduction in volume in patients with MGD and an improvement after meibomian gland orifice probing [377].

A test strip made of hemp oil-absorbing material, designed specifically to absorb tear lipid, has been developed. The folded end is placed in the conjunctival sac of lower eyelid and the length of infiltration (over 1 minute seemed optimal) measured and recorded, similar to the Schirmer test [378].

Tear lipidomics are reviewed in Section 3.5.4.4.2.

3.5.2.2 Aqueous

As the aqueous component represents the majority of the tear film volume, techniques assessing tear volume are often used to quantify this tear component.

3.5.2.2.1. Meniscometry or tear meniscus assessment

Meniscometry involves non-invasive biometry of the lower eyelid tear meniscus, usually in the form of a central height in primary gaze. Subjective methods of tear meniscus height measurement, such as estimating the meniscus height relative to a height-adjusted slit-lamp beam scale, has shown poor inter-visit reproducibility [379]. Slit-image photography has also been used to quantify tear meniscus height, radius, width, as well as cross-sectional area and radius of meniscus curvature [376]. The TFOS DEWS II Diagnostic Subcommittee proposed tear meniscus height assessment as a differential factor for the subclassification of DED, describing a cut-off value of 0.20 mm or lower as an indicator of aqueous-deficient DED, which has subsequently been confirmed [39, 320, 380]. Tear meniscus height should be measured directly below the pupil midline (±1mm) as it is affected by varying lid morphologies more peripherally.[381] The timing of the assessment after a blink should be controlled, with 1.0 to 2.5s after two blinks found to be most robust; a single measure of tear meniscus height is sufficient, using either with infrared or visible white light (although these are not interchangeable).[382] Alternative meniscometry systems have been developed in

research settings, projecting a target to dynamically visualise the tear meniscus curvature, without the need for fluorescein instillation [379, 383, 384].

OCT allows the cross-sectional area of the tear prism or even the volume along the lower lid to be quantified [385, 386]. Spectral-domain OCT has enabled higher resolution, greater imaging depth and faster acquisition (facilitating three-dimensional volume imaging) compared to time-domain OCT, improving image quality and measurement repeatability [330, 387-389]. The high repeatability allows changes in tear meniscus morphology after fluid instillation to be tracked, to determine tear clearance rate [390]. Meniscus measurements are instrument-dependent [391] and can be distorted by anatomical features such as lid parallel conjunctival folds, conjunctivochalasis, or other disruption to the shape of the lid margin or ocular surface [392]. Furthermore, analysis of the image may be complex, time-consuming and operator-dependent [393].

3.5.2.2.2 Phenol red thread test

The slight alkalinity of the tear film (between pH 7 and 8)[39] allows the length of a thin cotton thread, with the folded end hooked over the temporal end of the eyelid, moistened by tear absorption over a 15 second period to be observed from a color change of yellow to red. Compared to the Schirmer test (see Section 3.5.2.2.3), the small profile and limited amount of pH indicator in the thread is expected to limit the chance of triggering substantial reflex tearing and as a result, intersession repeatability is good.[394] While the phenol red test is thought to indirectly measure the tear volume, it is only weakly correlated with other established methods such as fluorophotometry or tear meniscus height.[394, 395] In addition, it is weakly correlated with dry eye symptoms[396] Reported agreement with the Schirmer test is variable between studies.[397, 398] An arbitrary aqueous deficient DED cut-off value of 20 mm has been adopted in clinical practice [399] and values of <9mm in 15 seconds suggest more severe cases of aqueous deficient DED[400]. Due to issues with accessibility of a commercialized product, techniques to develop an equivalent test have been described [401].

3.5.2.2.3 Schirmer test

The Schirmer test is an invasive test of tear volume, that involves assessing the length of a (Whatman 41) filter paper strip that becomes saturated by tears, 5 minutes after hooking the end of the strip, folded at the notch, over the lower lid margin, within the temporal one-third [332]. Technique variations, such as the use of anaesthetic which aims to differentiate basal from reflex tearing [402], indicate poor repeatability, sensitivity and specificity [1]. Using the wetting of the Schirmer strip over the final 4 minutes out of the 5, seems to be more robust than other time intervals including assessment over the full 5 minutes, but not surprisingly, accounts for <3% of the variance in fluorescein breakup time, a key homeostatic marker for all DED subtypes, or meibomian gland secretion, a recognised marker of evaporative DED.[403]

3.5.2.2.4 Strip meniscometry

Strip meniscometry involves placing a strip with a 0.4mm diameter central duct into the lower lid tear meniscus for 5 seconds [404-406]. A cut-off of ≤ 2.5 mm has been adopted, with the results correlating with other tear film assessments and the values are moderately repeatable [407, 408].The combination of strip meniscometry and fluorescein breakup time have been proposed as a more sensitive approach for assessing DED than either test alone.[386]

3.5.2.2.5 Tear clearance

Measuring the fluorescence of instilled quantities of fluorescein across the ocular surface (termed fluorophotometry) can be used to quantify tear turnover, reported as the decrease (in percent) per minute [409]. Alternative approaches to measuring tear flow include conducting a Schirmer test 5 minutes after instilling fluorescein with anesthetic, with both the length of wetting and the intensity of strip staining compared to a standard color plate after 5 minutes recorded [410]. Due to the various factors that can affect Schirmer test results, the tear function

index, which is the value obtained by dividing the value of the Schirmer test by the tear clearance rate, is a measure that has been used by a limited number of authors to assess patients with DED [411-413]. Other methods of tear clearance assessment include anterior segment OCT, which has been used to evaluate the early phase of tear clearance [414]. Lacrimal scintigraphy (tracking a small amount of radioactive material instilled into the conjunctival cul-de-sac) has also been used to measure tear clearance [415, 416].

3.5.2.2.6 Lacrimal gland patency

A clinical test for examining the patency of the palpebral lobe of the lacrimal gland has been described, which involves having the patient look inferonasally while the upper eyelid is retracted in the superotemporal direction. Dry 2% fluorescein ophthalmic strips are applied onto the exposed palpebral lobe multiple times over 20s. This allows the number and location of ductules per lobe as well as the tear flow rate to be assessed [417]. Alternatively the patency of the lacrimal gland can be assessed by stimulating the ocular surface with a pure CO_2 gas jet at 200 ml·min^{-1} for 3s, delivered 5 mm from the cornea and measuring the increase in reflex tearing volume with a Schirmer strip [418].

3.5.2.2.7 Tear proteins and other components

The presence of the antibacterial and anti-inflammatory lacrimal gland proteins, lipocalin, lactoferrin, and lysozyme, which possess anti-inflammatory and anti-bacterial properties [419] can be assessed as an indirect measure of lacrimal gland function. Tear protein concentration has generally been found to decrease with age [420]. Although lactoferrin has been proposed as a biomarker of DED [421-423], low tear lactoferrin levels are also found in giant papillary conjunctivitis, vernal keratoconjunctivitis, and chronic meibomitis associated with acne rosacea [424-426]. Tear film urea levels are linearly related to Schirmer's test values; for diagnosing DED, a cutoff of $\leq$ 37.2 mg/dL has been reported to provide a sensitivity of 96% and a specificity of 76% [427].Tear fluid proteomics are reviewed in section 3.5.4.4.2.7.

3.5.2.3 Mucin / glycocalyx

3.5.2.3.1 Mucins

Human conjunctival goblet cells synthesize and secrete the largest type of gel-forming, non-surface-bound mucin in the eye, MUC5AC, which acts to protect and lubricate the ocular surface, mitigating friction during blinking [4, 428]. Patients with DED typically show reduced concentrations of soluble MUC5AC in the tear film [429]. Together with lipids, a concomitant increase in MUC5AC protein expression in tears in infants may contribute to their greater tear film stability [430]. Goblet cell count and tear MUC5AC protein are decreased in Graves´ ophthalmopathy, thought to be possibly due to ocular surface inflammation secondary to ocular surface exposure [431].

Immunohistochemistry and immunoelectron microscopy have been used to examine binding of the HI85 antibody, which recognizes carbohydrate epitopes on the MUC16 mucin on the surfaces of apical conjunctival cells [432, 433].

Other membrane-associated mucins, MUC1, MUC4, as well as MUC16 (glycocalyx) and gel-forming mucin MUC5AC, have been studied using different techniques [244, 434, 435]. In other research large decreases in sialic acid (almost 7-fold) [436] and increases in galectin-3 (proxies of the glycocalyx/mucin) [437] have been observed in the tear film and shown to strongly correlate with clinically assessed disease severity [438].

3.5.2.3.2 Rose bengal and lissamine green (see section 3.4.1.7)

Rose bengal has been shown to stain ocular surface epithelial cells that are unprotected by the mucin rich glycocalyx [439], but it has been shown to suppress human corneal epithelial cell viability *in vitro*, indicating toxicity [240].. Lissamine green is less toxic to the ocular surface and has largely replaced the use of rose bengal in clinical care and research. Lissamine

green is a vital dye that stains epithelial cells only if the cell membrane or intracell junctions are damaged, irrespective of the presence of mucin [39, 440].

3.5.2.3.3 Conjunctival impression cytology

After the instillation of topical anesthetic, a filter strip is pressed against the bulbar conjunctival surface, usually upper, for 5-10 seconds using forceps. The samples are then fixed using 95% ethanol, stained with periodic acid-Schiff reagent and fixed on a slide to be viewed with a light microscope [261]. The Nelson classification system is used most frequently to grade the density, morphology, cytoplasmic staining affinity and nucleus/cytoplasm ratio of conjunctival epithelial and goblet cells across the ocular surface [39]. There is variation in goblet cell distribution across the conjunctival surface, with the lower forniceal conjunctiva goblet cell density higher than in the bulbar conjunctiva [441]. Recently, moxifloxacin-based fluorescence microscopy has emerged as a novel technique that enables efficient, non-invasive and *in vivo* animal imaging of goblet cells [442]. Confocal imaging, on the other hand, can be used to assess corneal cell morphology [443], goblet cell density [444] and conjunctival squamous metaplasia (from nucleocytoplasmic ratios) [445] *in vivo* in humans.

3.5.2.3.4 Ferning test

Whole tears collected by one of many possible techniques [446] are transferred immediately to a small plastic centrifuge tube (0.5 ml or less); a sample (1–2 µl) is then pipetted onto a clean glass microscope slide and allowed to dry for 7–10 minutes under normal room temperature (20–26°C) and room humidity (up to 50 %) [447]. The slide then can be observed under a light or digital microscope with various magnifications [446]. Depending on the tear film composition, a variety of ferning patterns can be observed. The classification is typically: type I: uniform large arborization; type II: ferning abundant but of smaller size; type III: partially present incomplete ferning; and type IV: no ferning [448]. Healthy tear samples typically produce full dense ferning patterns (types I and II), while the ferning pattern are often fragmented or absent in patients with DED [446].

The exact nature of what determines the ferning pattern is still not fully understood [1, 449], though a causal link has been proposed between tear ferning pattern and the ocular mucins [449]. Hyperosmolarity affects the appearance of ferning patterns [446] as does introducing electrolytes into the tear film [450]. Tear ferning patterns have been found to be repeatable [451]. A correlation has been found between the ferning test grade, non-invasive breakup time [452] and low Schirmer test values, but no correlation has been reported between ferning pattern and various tear protein levels. [453]

3.5.3 Eyelid anomalies

3.5.3.1 Blink and lid closure anomalies

Blinking is a complex neuromuscular process that plays a vital role in maintaining ocular surface homeostasis and proper functioning of the tear film. Specifically, it facilitates the even distribution of tears, mucin, and lipids, essential for lubrication, protection from eye irritation, and removal of debris and foreign bodies [289, 454-456]. Blinking is primarily controlled by the orbicularis oculi muscle, innervated by the facial nerve (cranial nerve VII) [457]. The levator palpebrae superioris muscle, innervated by the oculomotor nerve (cranial nerve III), and Müller's muscle, innervated by sympathetic fibres, are also contributory [458]. Sensory input from the cornea and conjunctiva, relayed via the trigeminal nerve (cranial nerve V), modulates the blink reflex [459].

Blinking can be categorized into three types [460]. The first is spontaneous blinking, which is the unconscious and coordinated closure of both upper eyelids occurring briefly and symmetrically without any evident stimulus. The second is reflex blinking, which is triggered by trigeminal, visual, and acoustic stimuli. Lastly, voluntary blinking is defined as the closure of eyelids consciously initiated by the individual. The normal rate for spontaneous blinking ranges from 10-15 blinks per minute [295, 461]. This rate can be influenced by multiple factors,

such as age, cognitive load, social activity, neurological and psychiatric diseases, fatigue, eye injury, medication, contact lens wear and dryness [295, 460].
Abnormalities in blinking patterns have been implicated in DED development [279, 282, 289]. Blink rates, interblink interval and maximum blink interval can differentiate patients who are considered healthy from those with DED [285, 291], likely due to the changes in ocular surface exposure and failure to restore tear film structure between blinks. Patients with DED often exhibit increased blink rates [295] relative to normal. However, reduced blink rates have also been observed both in individuals with and without DED, particularly during activities requiring prolonged visual attention (e.g., screen use), leading to insufficient tear film distribution and increased tear evaporation rates [462]. Similarly, studies have shown that patients with DED had shorter mean and maximum interblink intervals compared to healthy controls [283, 289, 463]. Notably, people with DED also demonstrated higher rates of incomplete blinking [296], which in itself is associated with inadequate expression of meibomian gland secretions into the lipid layer on the ocular surface, exacerbating tear film instability [464-466].
Given the multifactorial nature of DED, a comprehensive evaluation of blinking physiology is essential for understanding its pathophysiology and employing effective treatment strategies. Clinical monitoring is usually subjective and surreptitious to avoid a change in blink pattern from the patient [467], but objective image analysis is becoming more widely available. Improving blink quality and frequency through behavioral modifications and therapeutic interventions can significantly benefit ocular surface health and patient comfort. Poor lid seal is also linked to symptomology in DED and can be detected by placing a pen torch or transilluminator against the relaxed, closed, outer upper eyelids of semi-reclined patients and observing visible light emanating from the lid area between the lashes [251].

3.5.3.2 Lid margin health

3.5.3.2.1 Anterior blepharitis

Anterior blepharitis has been defined as “an inflammation of the lid margin anterior to the gray line and concentrated around the lashes” which may be accompanied by squamous debris or cylindrical dandruff around the lashes (Figure 6), and inflammation may spill onto the posterior lid margin”[47, 468, 469]. The term ‘blepharitis’ is often used by clinicians to describe anterior blepharitis, with posterior cases referred to by the more specific etiology, such as meibomian gland dysfunction. The pathophysiology of anterior blepharitis is multi-staged and relates to microbial changes that can culminate in inflammation: periocular bacteria build a defensive structure known as a biofilm, which predisposes to forming on the eyelid margin due to its moisture, nutrients and warmth. Biofilms are composed of a polysaccharide/protein matrix, that adhere strongly to surfaces due to proteins like adhesin produced by bacteria such as *Staphylococcus epidermidis* and *Staphylococcus aureus*. Biofilms allow bacteria to evade the impacts of desiccation and host defense mechanisms, facilitating gene activation and inflammatory virulence factors [470]. Dry eye symptoms [471] and signs,[472] as well as contact lens discomfort,[473, 474] are reduced on treatment anterior blepharitis,[475] implicating anterior blepharitis as one of the key triggers of the multifactorial disease.
Common clinical manifestations of anterior blepharitis include the presence of squamous debris or cylindrical dandruff around the base of the lashes, accompanied by vascular changes in the lid skin [468, 476] Anterior blepharitis is associated with ocular rosacea, seborrhea and hypersensitivity caused by staphylococcal toxins, infectious processes (bacterial or viral) or infestation by phthiriasis or *Demodex, or c*ombinations of these triggers [477].
The most frequently identified species are *Staphylococcus epidermidis (in about one third of cases)*, followed by *Pseudomonas* (approximately 20%), *Staphylococcus aureus*, *Propionibacterium*, *Corynebacteria*, and *Moraxella* [478, 479]. Immunologic mechanisms have been documented, with enhanced cell-mediated immunity to *Staphylococcus aureus* detected in 40% of patients with chronic blepharitis. Seborrheic blepharitis, in contrast, presents with greasy deposits and is commonly associated with seborrheic dermatitis of the eyebrows and scalp [476, 480].

3.5.3.2.1.1 Demodex associated blepharitis

Anterior blepharitis can also result from the activity of *Demodex folliculorum* or *brevis*, parasites identified in 14% to 89% of the population (especially in older patients) [105, 481-483]. There are two types identified in the human eyelids: Demodex folliculorum and Demodex brevis [484]. Demodex folliculorum is typically found in the eyelash follicles of the eyelids and observed with high magnification slit-lamp microscopy [485].

Characteristic features are apparent at the base of the lashes, and these can be present in asymptomatic individuals. *Demodex* mite presence is associated with changes in the anterior lid margin, such as increased scale intensity and cylindrical dandruff or sleeves. Cylindrical dandruff are considered pathognomonic for the presence of *Demodex* mites [485, 486]. Demodex can be detected by examining epilated eyelashes under white light microscopy at high magnification [487, 488] or on a slit-lamp at high magnification. Manipulating an eyelash with cylindrical dandruff around its axis in a circular motion [489, 490] or by applying lateral traction [491] using fine-tipped metal forceps, can reveal mite tails at the insertion point of the lashes. Detection of Demodex using *in vivo* confocal microscopy has also been described, but this technique is cumbersome, time-consuming and costly, limiting its diagnostic utility [491, 492].

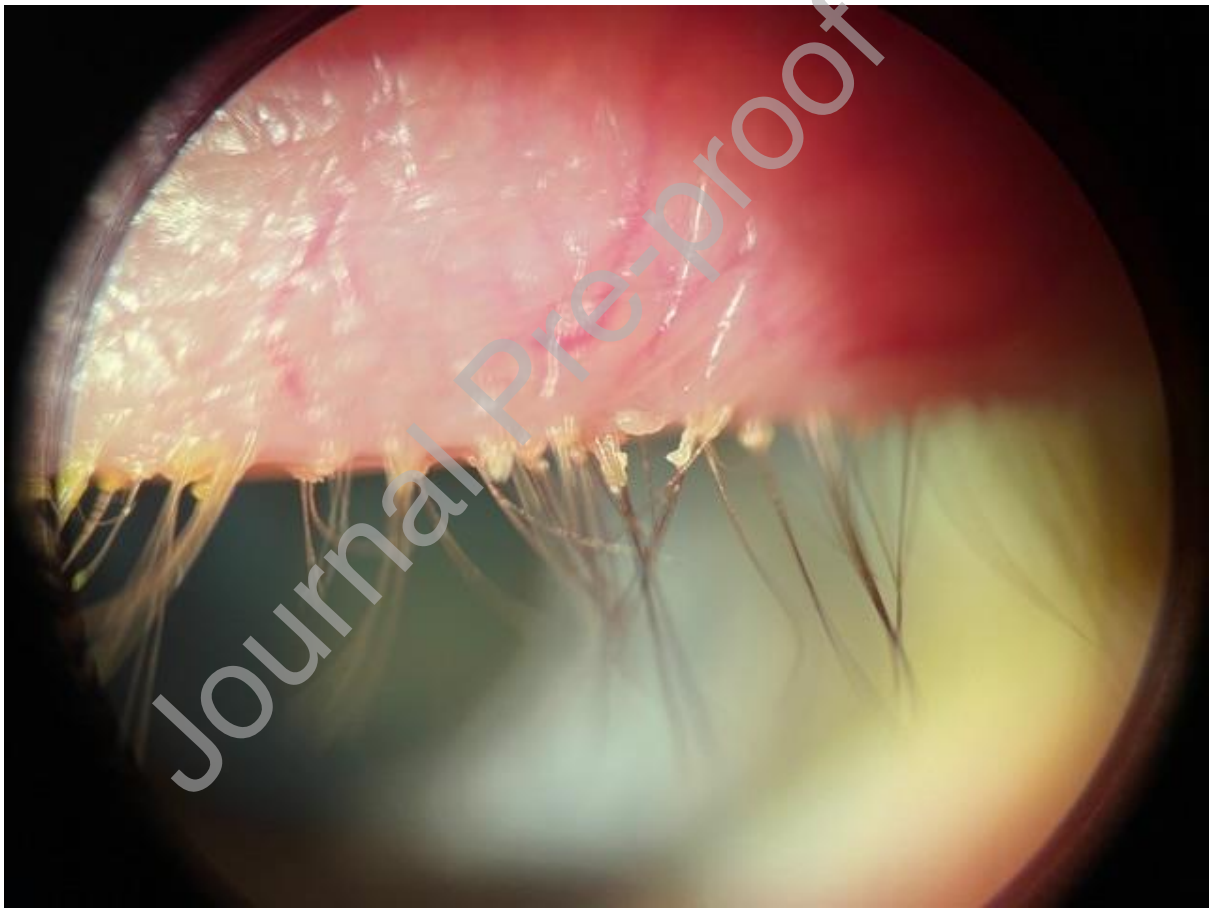

Figure 6: Anterior blepharitis.

3.5.3.2.2 Meibomian gland dysfunction (MGD)

MGD, a major contributing factor to DED, is described as “a chronic, diffuse abnormality of the meibomian glands, commonly characterized by terminal duct obstruction and/or qualitative/quantitative changes in the glandular secretion” in the TFOS International Report on Meibomian Gland Dysfunction Definition and Classification report published in 2011. [468]. The diagnosis of MGD is primarily clinically-based, focusing on the detection of signs indicative of altered meibomian gland secretions, lid margin changes, and meibomian gland dropout [372]. The severity of MGD is classified based on subjective symptoms, abnormal signs at the orifices (plugging, pouting and capping), vascularization or reddening of the

eyelid margin, anterior or posterior displacement of the mucocutaneous junction, eyelid margin irregularity and rounding, gland drop-out observed by meibography (where imaging both upper and lower lids is important [493]), corneal and tear film abnormalities (such as superficial punctate keratitis) and the quality of the expressed meibum [494-496]. Various clinical grading criteria for gland appearance have been proposed [495, 497-499]. Additionally, the term "plus disease" has been used to refer to co-existing or accompanying disorders of the ocular surface and/or eyelids [331]. Another scoring system combines subjective symptoms, slit-lamp microscopy findings, and tear test results to classify MGD severity into stages 1 to 4 and describes the relationship between meibomian gland dysfunction severity and serum lipids [500]. An alternative scoring system integrates subjective symptoms, *in vivo* confocal microscopy findings, slit-lamp microscopy, meibography, tear film breakup time and corneal staining scores, classifying MGD severity into three stages [494, 500]. Yet another scoring system elected to combine scores for secretion appearance and the digital pressure needed to express the meibum [501].

The number of lower eyelid meibomian glands yielding liquid secretion has been assessed as a metric of MGD [371]. Assessing meibum expressibility, color and quantity is crucial [502], either as the highest grade from eight expressed glands (total score range: 0 to 3) or summing all eight gland expressibility scores (total score range: 0 to 24) [467, 468]. Anatomically, there are more meibomian glands in the upper eyelid, with a median count of 31, compared to a median count of 26 in the lower eyelid [469]. The glands in the upper eyelid are notably longer and slimmer than those in the lower eyelid. Studies on lower and upper eyelids separately highlight secretion differences between the nasal and temporal sides. The lower eyelid reportedly has higher secretion rates and poorer meibum quality than the upper eyelid [467]. Additionally, nasal glands appear more active, with activity diminishing toward the temporal margin [503]. Upper eyelid meibography has been proposed to be an integral component of comprehensive meibomian gland evaluation.[493]

Non-invasive infrared or transillumination photography of meibomian glands has advanced the assessment of two-dimensional gland silhouettes [498, 504]. Meibography enables direct visualization of meibomian gland morphology, highlighting partial or total non-visible meibomian tissue as dropout or loss [372, 505]. This can be assessed through subjective grading [468, 506-508], or semi-quantitative or quantitative analyses [509]. Asymptomatic individuals have been found to have meibomian gland loss <16.9% for the upper and <28.7% for the lower eyelid [498].Various gland morphological characteristics have been identified, including truncation, dilation and tortuosity [510, 511], but none in isolation seems to be a good predictor of DED [512].

Tortuosity of the meibomian glands in the upper eyelid has been observed to correlate negatively with tear film stability, while tortuosity in the lower eyelid correlated with dry eye symptoms [508]. However, its variability makes it less reliable as a standalone diagnostic parameter [503, 508] and it is only weakly correlated with meibomian gland expression [372]. Meibomian gland thickness increases with overall loss, potentially as a compensatory response, although this does not improve expressibility [372, 503]. On the other hand, distorted and thinned glands appear to be transitional phases before dropout [468, 469, 502]. However, meibomian gland length has been identified as the key morphological metric for function in terms of expressibility. [372].

OCT has been used to image meibomian glands and may be more sensitive than traditional meibography techniques [513]. *In vivo* confocal microscopy can observe meibomian gland orifice tissues at a cellular level, enabling evaluation of acinar density, acinar diameter, enlarged meibomian gland orifice and conjunctival inflammatory cell density [514]; it can also allow assessment of glandular atrophy and peri-glandular fibrosis. However, the equipment is expensive, requires a learning curve to obtain good images, and the technique requires contact with the epithelium, posing disadvantages with respect to invasiveness and

prolonged examination burden. The technique is unable to permit visualization of the glands themselves in the human eyelid margin due to light attenuation at that tissue depth; the structures imaged are rete ridges located at the dermal–epidermal junction, with alterations believed to indicate a shift of the mucocutaneous junction [515]. Clinical use remains limited [331, 494, 514, 516]. Lipid quantification at the eyelid margins and biochemical analysis of gland secretions for lipid components and markers are still being researched (see Section 3.5.4.4.2).[494]

The inner border of the eyelid margin plays a crucial role in helping maintain ocular surface integrity by ensuring even spread of the thin tear film with each blink. Normally, the eyelid margin features a convex inner border with a keratinized epidermis, which ends abruptly behind the posterior margin of the meibomian orifices. This is followed by the mucocutaneous junction, creating a transition zone between the wet, non-keratinized conjunctival tissue of the ocular surface and the dry, keratinized tissue of the eyelid margin [517]. This area, known as the lid wiper, comes into contact with the ocular surface to distribute the tear film and any morphological changes at this site may be associated with tear film instability and early signs of DED [266, 518]. The lid wiper extends from the mucocutaneous junction to the sub tarsal fold sagitally and from the medial punctum to the lateral canthus horizontally. Posterior migration of the mucocutaneous junction leads to lid-margin keratinization [519]. Mechanical factors between the eyelid and the ocular surface, contribute to diseases perceived to be friction-related (in the form of sheer forces), including superior limbic keratoconjunctivitis, lid wiper epitheliopathy and conjunctivochalasis [520]. Damage to the epithelium of the marginal conjunctiva at the lid wiper zone is a clinical sign indicative of DED [271] and its staining and grading is covered in Section 3.5.4.3.

Factors such as aging, inflammation, hormonal imbalance, bacterial growth, eye drops, and oral medications can induce hyperkeratinization of the meibomian gland ductal epithelium, altering meibum transparency and viscosity [494]. These changes are hypothesized to lead to gland obstruction and reduced secretion.
Keratinization of the lid margin can also result from long-term rigid contact lens use or severe systemic conditions such as Stevens-Johnson syndrome/toxic epidermal necrolysis. These conditions lead to loss of the mucocutaneous junction barrier, epidermalization, whitish keratin deposits over the lid wiper zone, dyskeratosis, T-cell and neutrophil infiltration and altered local microbiome.(Muntz et al., 2020, Singh et al., 2021)[521, 522]. Keratinization can also occur with androgen insensitivity [523] and androgen deficiency [47, 123], *Staphylococcus aureus* over-colonization [524], estradiol increases of cornulin [525], stearoyl-CoA desaturase-1 deficiency and related upregulation of ceramides due to altered fatty acid metabolism [526], hyperlipidemia [527] and isotretinoin [528].

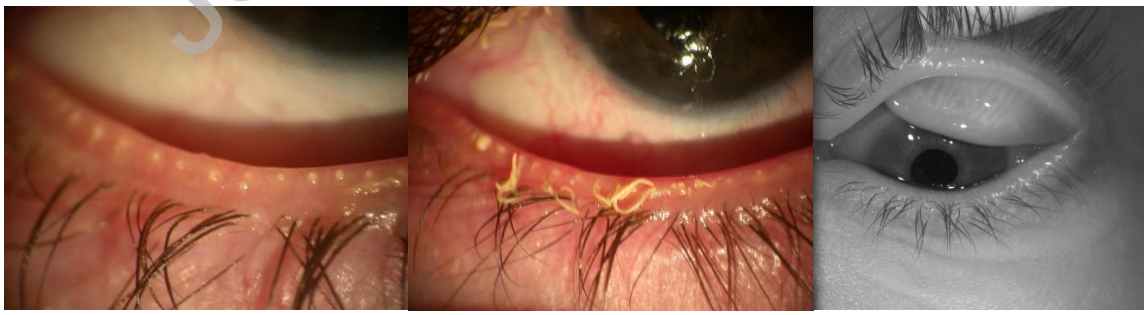

Figure 7: A) blocked meibomian glands; B) ‘toothpaste’ like meibum; C) upper lid meibography of an eye with meibomian gland truncation.

3.5.3.2.3 Ocular rosacea

Acne rosacea has long been recognized as an inflammatory disease resulting from a complex interaction of abnormalities of the innate and adaptive immune system,

accompanied by mast cell dysfunction and / or neurovascular compromise. However, the exact mechanisms and roles of these different components of the pathophysiology remain incompletely elucidated [529]. Acne rosacea is currently diagnosed [530] based on the presence of at least one 'diagnostic phenotype' (centro-facial erythema with periodic intensification or phymatous changes) or at least two 'major phenotypes' (papules and pustules, flushing, telangiectasia, or ocular rosacea); however, ocular rosacea is not well defined, with a list of features proposed from blepharitis and conjunctival injection as indicative of mild to moderate disease, to punctate keratitis, infiltrates, vascularization and scleritis indicating moderate to severe disease [530]. A recent review identified ten typical ocular signs and nine diagnostic steps for recognising ocular rosacea, but many of these overlap with other posterior blepharitic conditions, with the main differentiating features being concurrent signs of rosacea on the skin, recurrent hordeola/chalazia, corneal vascularization, corneal infiltrates/ulcers and anterior uveitis [531].

3.5.4 Ocular Surface Abnormalities

3.5.4.1 Anatomical misalignment

Ultraviolet radiation and other chronic environmental exposures can cause changes in the corneal and conjunctival cells, leading to disruption of the smooth ocular surface, for example pterygia,[532, 533] pinguecula,[534]. LIPCOF (see section 3.1.4.6) [392, 535] and conjunctivochalasis [535]. Any such raised structures can affect the flow of the tear film, the position/function of the glands and the conformity between the eyelids and the ocular surface, reducing tear film stability and altering tear volume distribution [536].

LIPCOF are undulations in the inferior bulbar conjunctiva, parallel to the margin of the lower lid. LIPCOF may be observed as the initial signs of conjunctivochalasis (possibly having the same aetiology),[537] but exhibit a smaller cross-sectional area [392, 538], do not occur centrally [539-541] and have no apparent relationship with age.[539] LIPCOF have a moderately high predictive ability for differentiating symptomatic eyes with poor tear stability [540, 542, 543], but not in all studies [544]. Independent groups have shown that OCT can clearly resolve LIPCOF morphology, such as LIPCOF area, that correlates well with subjective grading [545, 546]. While other signs are associated with DED, such as ocular/conjunctival redness, epithelial thickness [547], corneal nerve damage, inflammatory cell migration into the cornea, a loss of corneal sensitivity, changes to the meibomian glands (morphology and expressibility) and blinking, there is not strong evidence for the role of each in the overall diagnosis of DED. These other tests are covered with respect to their role in determining the etiological drivers of DED (section 3.5).

3.5.4.2 Neural dysfunction

Corneal neuropathic pain is complex with a pragmatic and systemised approach needed for management [548]. Abnormal corneal sensitivity has been associated with signs and symptoms in individuals with ocular surface disease [549, 550]. The corneal nerves serve both sensing and nutritional functions. The sensing function is not only linked to the blink reflex, but also to tear secretion by the lacrimal gland. Corneal sensitivity is a measure of corneal nerve function and an indicator of the integrity of the protective mechanisms of the ocular surface [551]. Morphological and anatomical features can be directly observed by *in vivo* confocal imaging and objectively graded using software [552 553] , whereas function is assessed with the aid of a contact or non-contact corneal esthesiometer to assess corneal sensitivity. Lid margin sensitivity determined by non-contact esthesiometry has been demonstrated to be strongly correlated to corneal sensitivity; [550] lid margin sensitivity thresholds exhibited marginally higher predictive performance than corneal sensitivity for clinical signs of DED, as defined by the TFOS DEWS II criteria, and were significantly correlated with non-invasive tear film breakup time, corneal, conjunctival and lid wiper staining.[550] Liquid jet esthesiometers have also been developed and corneal sensitivity to cold found to be related to digital eye strain.[554] The corneal sub-basal nerves may be evaluated in detail using non-invasive *in vivo* confocal microscopy [555]. Corneal sub-basal

nerve plexus density and length tend to decrease and the tortuosity increase, whereas the loss of corneal endothelium is accelerated in patients with DED, indicating damaged nerve fibres (Figure 8)[556-558]. Moreover, the damage to the nerves in DED may prevent the nervous system from exerting its immunomodulatory role, leading to changes in corneal sensitivity [559]. Although many studies have shown that the number and density of the sub-basal nerves in patients with DED decreases significantly and strongly correlates with the decrease in corneal sensitivity [560-563], some studies show no relationship, perhaps due to variations in DED subtypes and severity of disease being examined [564]. Some studies have also found that different clinical presentations of DED show corresponding corneal sensitivity changes.[565]

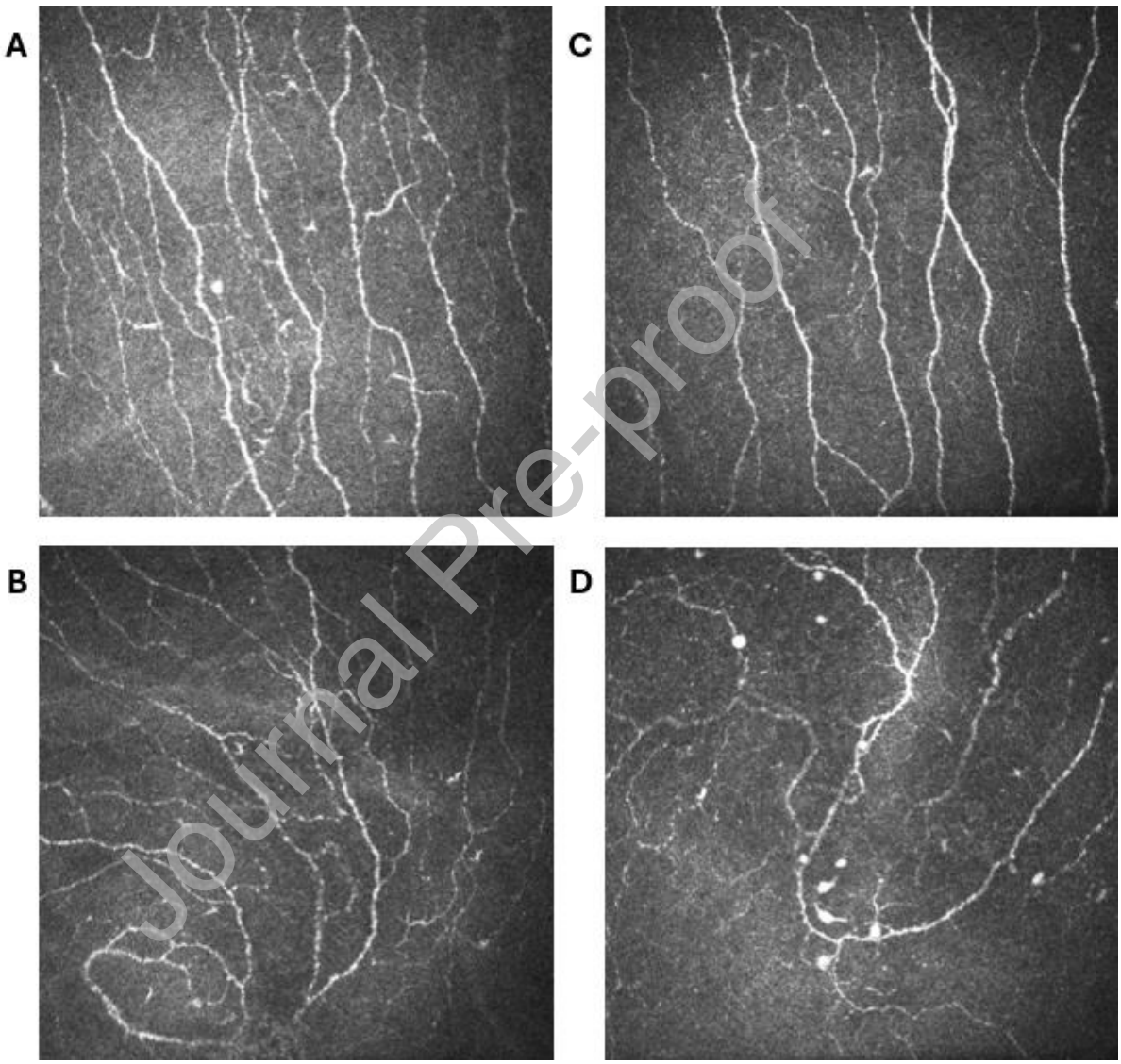

Figure 8: Corneal nerves observed by in vivo confocal microscopy (IVCM).
(A) central corneal and (B) inferior whorl nerves in a health eye.
(C) central corneal and (D) inferior whorl nerves in an eye with dry eye disease.

Severe neuropathic corneal pain, as an abnormality of corneal sensitivity with extreme effects, has attracted significant attention from researchers aiming to more fully understand its underlying nerve abnormalities (see TFOS DEWS III Digest pain and sensation section).[4] The decrease in corneal nerve density in neuropathic corneal pain is consistent with other types of DED. However, the relationship between microneuromas and nerve beading (Figure 9) with corneal nerve pain is still unclear, and further studies are required to confirm [566, 567].

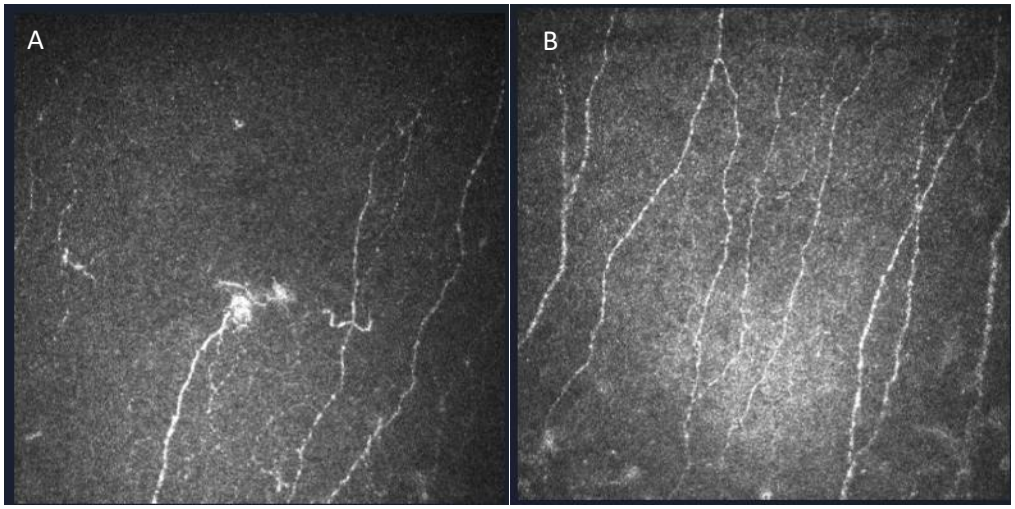

Figure 9 : (A) microneuromas in dry eye. (B) nerve beading in dry eye

Study	Cohort	Design	Results
Kaltenieceet al. 2017 [555]	N=35 with obesity	cross-sectional	• high intra and inter-observer reproducibility for all corneal nerve parameters • 6 images sufficient for analysis
Tepelus et al. 2017 [557]	N=24 DED, N=44 Sjögren N=10 control	cross-sectional	• density of nerve fibres decreased, primarily in Sjögren disease • nerve tortuosity and reflectivity decrease in all DED • density of dendritic cells higher in DED groups • nerve and cell changes correlated with symptomology
Kheirkhah et al. 2017 [558]	N=20 DED N=13 control	retrospective longitudinal	• endothelial cell density and nerves lower in DED • loss is greater than literature reported normal ageing
Levy et al. 2017 [559]	N=30 Sjögren [N=15 control]	prospective, non-randomized treatment study	• 6 months topical cyclosporin A 0.05% increased corneal sub-basal nerve density (only) • decreased dendritic cell numbers, more so in those with more severe baseline symptoms and staining • corneal sensitivity increased
Cardigos et al. 2019 [556]	N=116 with non-Sjögren DED N=20 control	cross-sectional	• corneal sub-basal nerve plexus density and length lower, and tortuosity higher in DED • corneal sub-basal nerve plexus strongly associated with Schirmer test score and tear breakup time
Ross et al. 2020	N=14 with severe	cross-sectional	• sub-basal nerve density reduced

[566]	ocular pain >1year, 4 with neuropathic pain		compared with controls • more activated keratocytes and spindle, lateral and stump microneuromas in stroma in those with neuropathic pain
Moein et al. 2020 [567]	N=30 DED N=25 neuropathic pain N=16 controls	retrospective, case-controlled	• similar lower nerve density and higher dendritic cell numbers in DED and neuropathic groups • no difference in nerve beading • microneuromas present only in neuropathic pain group
Maity et al. 2024 [568]	N=28 Sjögren disease N=25 meibomian gland dysfunction	Cross-sectional	• similar dendritic cell density • nerve fibre length, density and branching lower with Sjögren disease • tear osmolarity weakly negatively correlated with corneal nerve parameters

Table 3: Confocal studies of corneal nerve abnormalities in DED.

Systemic diseases that are associated with DED can cause corneal hypoesthesia such as in patients with diabetes [569-571]. In addition to nerve reduction, a significant reduction in nerve beading frequency has also been reported, possibly due to reduced metabolomic activity in patients with diabetes [572-574]. Corneal nerve changes have also been observed in patients with Graves' ophthalmopathy (often associated with DED), perhaps due to nerve degeneration [575]. However, there appears to be no research on corneal sensitivity related to Graves' ophthalmopathy, and its correlation with structural alterations in nerves.

3.5.4.3 Ocular surface cellular damage / disruption

Punctate staining of the cornea and bulbar conjunctiva observed following the application of dyes, such as sodium fluorescein (Figure 10), rose bengal, and lissamine green, is a key diagnostic marker of numerous anterior segment conditions, including DED [238]. The distribution of punctate staining may provide an indication of potential aetiology [238, 576], and DED is traditionally thought to be predominantly associated with interpalpebral or inferior staining [238]. In recent years, there has been growing interest in the utility of eyelid marginal conjunctival staining or lid wiper epitheliopathy in the diagnosis of DED [1, 577].

To facilitate standardised recording of the severity of ocular surface staining and lid wiper epitheliopathy (Section 3.5.4.3), several grading systems have been devised, and the most commonly used are summarised in Table 4 [578]. Corneal and conjunctival staining grading systems include the van Bijsterveld system [579], the National Eye Institute (NEI) Industry Workshop guidelines [19], the Collaborative Longitudinal Evaluation of Keratoconus (CLEK) schema [580], the Oxford scheme [581], the Lexitas grading system [582], the area–density combination index [583], and the Sjögren's International Collaborative Clinical Alliance (SICCA) ocular staining score [584]. A recent study modified the Oxford scheme to incorporate half-unit increments for the assessment of corneal staining and reported improved sensitivity and repeatability [275]. The corneal and conjunctival staining component of the global consensus TFOS DEWS II diagnostic criteria was based on the SICCA grading system [1]. Lid wiper epitheliopathy is most commonly assessed relative to Korb's grading system, which combines the horizontal length of staining in mm, as well as the sagittal width relative to the eyelid margin [266]. The eyelid margin staining component of the global consensus TFOS DEWS II diagnostic criteria was based on this grading system.[1]

Key diagnostic accuracy studies evaluating the discriminative performance of ocular surface staining and lid wiper epitheliopathy in the detection of DED are summarised in Table 5. Overall, lid wiper epitheliopathy (C-statistic range, 0.69 to 0.80) demonstrated superior discriminative ability relative to corneal (C-statistic range, 0.52 to 0.57) and conjunctival staining (C-statistic range, 0.51 to 0.63) [222, 248, 250, 540, 585], which would support the incorporation of all three staining parameters in the routine diagnostic workup of DED [1]. The reasons underlying the greater diagnostic performance of lid wiper epitheliopathy are not completely understood. Previous studies have reported that corneal and conjunctival staining more commonly present in patients with severe DED [248, 250] and demonstrate poorer correlation with other dry eye signs and symptoms in mild-to-moderate disease [248, 250]. Moreover, a number of epidemiological and diagnostic studies have suggested that lid wiper epitheliopathy might be an earlier clinical sign than corneal and conjunctival staining in the natural history of DED [249, 586] and it remains uncertain whether the greater exposure to shearing and viscosity-induced hydrodynamic forces during the blink cycle might predispose the lid wiper region to earlier damage [537].

Table 4: Commonly used grading systems for ocular surface staining and lid wiper epitheliopathy.

Grading system	Details
van Bijsterveld staining score [579]	Corneal staining scoring: 1: sparsely scattered spots 2: densely scattered spots 3: confluent spots Conjunctival staining scoring: Divided into nasal and temporal zones. 1: few separated spots 2: many separated spots 3: confluent spots
NEI staining score [19]	Corneal staining scoring: Divided into five sectors (central, superior, inferior, nasal, and temporal), each scored from 0 to 3. Conjunctival staining scoring: Divided into superior paralimbal, inferior paralimbal, and peripheral area, both nasally and temporally, each scored 0 to 3.
CLEK staining score [580]	Cornea staining scoring: Divided into five sectors (central, superior, inferior, nasal, and temporal), each scored 0 to 4 in 0.5 increments. Conjunctival scoring: Divided into four sectors (superior, inferior, nasal, and temporal), each scored 0 to 4 in 0.5 increments. Intra-class correlation coefficient [587]: Fluorescein: 0.76 Rose bengal: 0.40
Oxford staining score	Corneal staining scoring:

[581]	Whole corneal area scored from 0 to 5 dependent on the intensity of punctate staining displayed pictorially, with the intensity of dots increasing on a logarithmic scale between grades. Conjunctival staining scoring: Whole conjunctival area scored from 0 to 5 dependent on the intensity of punctate staining displayed pictorially, with the intensity of dots increasing on a logarithmic scale between grades.
Korb grade [266]	Lid wiper epitheliopathy horizontal length grading: 0: <2 mm 1: 2 to 4 mm 2: 5 to 9 mm 3: ≥10mm Lid wiper epitheliopathy sagittal width grading: 0: <25% of the lid wiper 1: 25% to <50% of the lid wiper 2: 50% to <75% of the lid wiper 3: ≥75% of the lid wiper
Area–density combination index [583]	Corneal staining area scoring: A0: no punctate staining A1: >⅓ A2: ⅓ to ⅔ A3: >⅔ Corneal staining density scoring: D0: no punctate staining D1: sparse D2: moderate D3: high with lesion overlap
SICCA staining score [584]	Corneal fluorescein staining scoring: 0: 0 dots 1: 1 to 5 dots 2: 6 to 30 dots 3: >30 dots Extra points for confluent patches, staining within the pupil or filaments Conjunctival lissamine green staining scoring: Divided into nasal and temporal zones 0: 0 to 9 dots 1: 10 to 32 dots 2: 33 to 100 dots 3: >100 dots Intra-class correlation coefficient: 0.90 to 0.91 [588]

Table 5: Key diagnostic accuracy studies assessing the discriminatory performance of ocular surface staining and lid wiper epitheliopathy in detecting dry eye disease.

Study	Methods	Outcomes
Lemp et al. 2011 [222]	Sample size: 314 Index tests: Corneal staining (NEI score) Conjunctival staining (NEI score) Reference standard: Composite disease severity index, derived from the TFOS DEWS severity scale (clinical signs only). Incorporation bias: Ocular surface staining formed part of both index testing and reference standard.	Corneal staining: C-statistic: 0.77 Sensitivity: 54% Specificity: 89% Conjunctival staining: C-statistic: 0.88 Sensitivity: 60% Specificity: 91%
Pult et al. [540]	Sample size: 47 Index tests: Corneal staining with fluorescein (CCLRU scale) Conjunctival staining with lissamine green (CCLRU scale) Lid wiper epitheliopathy with lissamine green (Korb grading) Reference standard: OSDI score (clinical symptoms only). Incorporation bias: None.	Corneal staining: C-statistic: 0.52 Sensitivity: not reported Specificity: not reported Conjunctival staining: C-statistic: 0.51 Sensitivity: not reported Specificity: not reported Lid wiper epitheliopathy: C-statistic: 0.75 Sensitivity: 48% Specificity: 96%
Wang et al. 2019 [248]	Sample size: 552 Index tests: Corneal fluorescein staining (Oxford score) Conjunctival lissamine green staining (Oxford score) Superior lid wiper epitheliopathy (lissamine green) (Korb grading) Inferior lid wiper epitheliopathy (lissamine green) (Korb grading) Reference standard: TFOS DEWS II criteria (excluding staining parameters) Incorporation bias: None.	Corneal fluorescein staining: C-statistic: 0.56 Sensitivity: 25% Specificity: 86% Conjunctival lissamine green staining: C-statistic: 0.52 Sensitivity: 11% Specificity: 94% Superior lid wiper epitheliopathy: C-statistic: 0.71 Sensitivity: 65% Specificity: 73%

		Inferior lid wiper epitheliopathy: C-statistic: 0.69 Sensitivity: 72% Specificity: 66%
Wang et al. 2024 [250]	Sample size: 2,066 Index texts: Corneal fluorescein staining (SICCA score) Conjunctival lissamine green staining (SICCA score) Superior lissamine green lid wiper epitheliopathy (Korb grading) Inferior lissamine green lid wiper epitheliopathy (Korb grading) Reference standard: TFOS DEWS II criteria (excluding staining parameters) Incorporation bias: None.	Corneal fluorescein staining: C-statistic: 0.57 Sensitivity: 38% Specificity: 76% Conjunctival lissamine green staining: C-statistic: 0.63 Sensitivity: 58% Specificity: 64% Superior lid wiper epitheliopathy: C-statistic: 0.72 Sensitivity: 72% Specificity: 66% Inferior lid wiper epitheliopathy: C-statistic: 0.71 Sensitivity: 77% Specificity: 60%
Yeniad et al. 2010 [585]	Sample size: 86 Index texts: Fluorescein lid wiper epitheliopathy (Korb grading) Rose bengal lid wiper epitheliopathy (Korb grading) Lissamine green lid wiper epitheliopathy (Korb grading) Reference standard: OSDI score (clinical symptoms only). Incorporation bias: None.	Fluorescein lid wiper epitheliopathy: C-statistic: 0.80 Sensitivity: 44% Specificity: 93% Rose bengal lid wiper epitheliopathy: C-statistic: 0.78 Sensitivity: 43% Specificity: 90% Lissamine green lid wiper epitheliopathy: C-statistic: 0.76 Sensitivity: 39% Specificity: 90%

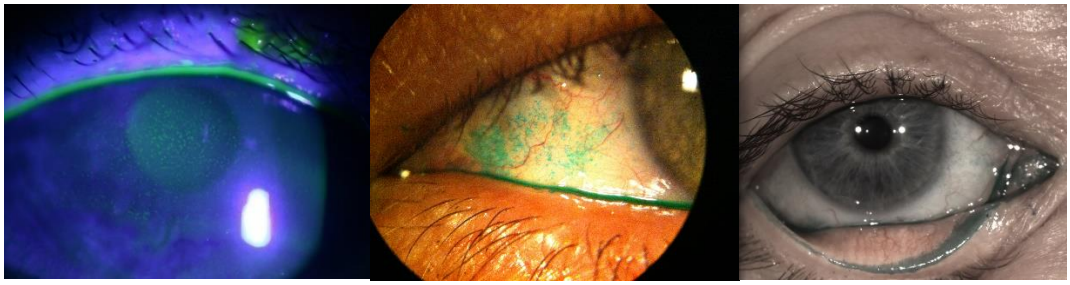

Figure 10: A) Fluorescein corneal staining illuminated with a blue light; B) Lissamine green bulbar conjunctival staining under white light; C) Lissamine green lower lid wiper staining under white light

3.5.4.4 Primary inflammation / oxidative stress

Inflammation plays an etiological role in the pathophysiology of DED [149]. Assessing the presence and intensity of inflammation is essential to determine the severity of the disease [589], the risk of progression [590] and to inform its management. Inflammation at the ocular surface can be both a cause and a consequence of DED [591]. Ocular surface inflammation can occur due to ocular surface damage [592], however autoimmune diseases are intrinsically significant contributors to DED [593]. In systemic immune-mediated conditions such as Sjögren disease, lymphocyte infiltration in the lacrimal gland can result in damage and fibrosis [594, 595], resulting in reduced tear secretion and elevated inflammatory cytokine levels in tears [596]. Therefore, the assessment of inflammation in DED frequently includes both local and systemic investigations to help elucidate the source of inflammation. As an example, it has been reported that MMP-9–positive patients respond more favorably to topical cyclosporine than MMP-9–negative patients [597], and the use of topical anti-inflammatory therapy has been associated with a reduction in HLA-DR [598] and MMP-9 tear levels [599, 600]. Treatments targeting novel inflammatory pathways in DED are continuously being explored and developed [601, 602], although many to date have failed. Current diagnostic inflammatory tests have some limitations [603, 604]. While image-based comparative scales and noncomparative methods based purely on clinical observation remain valid assessment tools for use in the clinic, objective conjunctival redness quantification is substantially more sensitive and reliable than subjective grading [605, 606] and can be performed using a smartphone.[607] Moreover, enhancing the user-friendliness of confocal microscopy by developing a non-contact imaging technique with adequate resolution or providing a wider field of imaging would probably facilitate its adoption. In terms of molecular-based diagnostic tests, the lack of standardized methods for tear fluid collection and biomarker quantification [608, 609], as well as the absence of normal reference values [610], are key aspects that may limit the accuracy, reproducibility and overall implementation of these techniques. The cost and technical complexity of some of these diagnostic tests may also be an important limitation to their implementation. However, improving clinical outcomes, avoiding unnecessary therapies, and accelerating patients' recovery not only stands to benefit patients but also to save costs.

3.5.4.4.1 Imaging-based diagnostic tests

3.5.4.4.1.1 Ocular conjunctival redness

Conjunctival ocular hyperemia occurs with dilation of the microvasculature arising from a multitude of etiologies [606, 611, 612]. The vasodilatation of conjunctival microvessels plays a critical role in the efferent component of the immune system, providing both soluble mediators and cellular elements to the site of inflammation [613]. Accurate assessment of the underlying causes of conjunctival hyperemia is key in differentiating systemic causes from a localized inflammatory response [614]. Both descriptive [615] and reference image-based [616-618] subjective grading scales can facilitate the detection and monitoring of changes in the conjunctival microvasculature during follow-up, supporting decision-making in modifying the treatment plan. Multiple studies have shown that these scales have limited inter- and intra-observer repeatability [605, 619], which prompted the development of computer-based

photograph-analysis techniques to allow objective grading of conjunctival redness allowing higher precision and repeatability [605, 606, 620-627].

3.5.4.4.1.2 In vivo confocal imaging

In vivo confocal imaging can aid in identifying characteristic structural changes in the cornea, conjunctiva, meibomian glands and lacrimal gland in patients with DED [330, 628]. Conflicting findings in corneal and conjunctival epithelial cell changes in DED studies may result from DED disrupting cell renewal, but simultaneously promoting cell repair at the same time, affecting the apoptosis-proliferation balance [629].

Previous studies have reported characteristic changes in the corneal stroma of patients with DED such as a significant increase in anterior corneal stromal keratocyte density [630] and abnormal stromal hyper-reflectivity indicating increased activity [631]. An increase in dendritic cell density has been reported in patients with DED [630, 632, 633]. Mature and immature dendritic cells have been found in the corneal stroma of these patients [634]. Interestingly, increased dendritic cell density has been correlated with severe symptoms [557] as well as with aqueous deficient DED due to immune disease [635]. Dendritic cell and activated keratocyte density, as well as reduced corneal sub-basal nerve fibre length have shown an indirect association with inflammation on the ocular surface, through a significant reduction following treatment with topical corticosteroids [636, 637]. Similarly, long-term therapy with topical cyclosporine for more than 6 months has shown a positive impact on corneal epithelial, stromal, dendritic, and nerve confocal imaging parameters [559, 638] (see TFOS DEWS III Digest Pathophysiology section).[4]

3.5.4.4.2 Tear Biomarker diagnostic tests

3.5.4.4.2.1 Matrix metalloproteinases

Matrix metalloproteinases (MMPs) are a family of enzymes that are core to several ocular and systemic inflammatory processes [639, 640]. MMPs are generated by connective tissues and pro-inflammatory cells [641], and can be detected in tears of patients with DED [642, 643]. In DED, corneal epithelial damage can result in a local inflammatory reaction that leads to increased secretion of MMPs [591]. MMP-1, -3, -9, -10, and -13 are the MMPs most notably elevated at the corneal surface, splitting epithelial basement membrane components and tight junction proteins (such as ZO-1 and occludin) that maintain corneal epithelial barrier function [643, 644]. Studies have reported a significant correlation between MMP-9 degree of positivity and ocular surface fluorescein staining [228, 597]. However, tear volume has an impact on the assay indicator, and therefore, a MMP-9 test degree of positivity may not correlate as strongly with MMP-9 tear concentration in cases of either low tear volume or reflex tearing [645]

3.5.4.4.2.2 Cytokines and chemokines

Measurement of cytokines and chemokines may enable differentiation of ocular surface inflammation caused through the innate immune response, and adaptive immune response (see TFOS DEWS III Digest Pathophysiology section).[4] Further, within the adaptive immune response, these markers may separate into Th1, Th2, Th17, and Treg-mediated responses [646]. IFN-γ, the dominant cytokine associated with the Th1 response, has been associated with goblet cell loss and squamous epithelial hyperplasia. Consequently, some clinical studies demonstrated correlation between higher IFN-γ and tear deficiency, though other studies found contradictory results [647-649], possibly due to inherent differences in assay methods, or population characteristics. IFN-γ may be specifically associated with an increase in osmolarity [650]. Th17-mediated ocular inflammation may be induced through IL-17 signaling. IL-17 activates MMP-9 which contributes to damage to the corneal epithelial barrier [646, 651]. A significant correlation between both corneal and conjunctival staining scores and presence of Th cells has been reported, although Th subtypes such as Th1 and Th17, were not detectable at high enough levels for establishing correlations with tear film stability and volume in patients with DED [652]; in addition, no correlation was found between DED and the detection of IL-1β, Il-6, IL-8, IL-10, IL-17A, IFNγ, and tumor necrosis factor alpha in tears [653], although cytokine upregulation has been detected in patients with Sjögren disease [654]. However higher MMP levels are found in patients with DED [655], likely from episodic flares [656] and MMPs have been found to correlate with osmolarity and tear volume, more strongly than

with tear stability and symptoms [657]. The variable nature of cytokine levels and DED corresponds with the heterogeneous nature of ocular surface inflammation [658-660], changes over time [661] and the location of sampling [662]. This confirms that inflammation is more often downstream (a consequence) rather than intrinsic (a driver) in DED (see TFOS DEWS III Digest Pathophysiology section).[4]

3.5.4.4.2.3 Neurotrophic Factors and Neuropeptides

Neuropeptides and neurotrophic factors have a role in mediating sensory information and in regulating aspects of neuronal function and cell survival. [649].

Serotonin, which is a peripheral nerve sensitizer, is found at a higher concentration in tears of patients with DED than in those of normal eyes and correlates with symptoms [663]. Serotonin is activated by inflammation and sensitizes peripheral nerves, perhaps playing a role in the development of corneal hypersensitization in DED [664]. Increased nerve growth factor has a protective role in DED, improving the integrity of the epithelial cell layer and tear secretion [665, 666]. Lacrimal gland dysfunction has been associated with decreased calcitonin gene-related peptide [665, 666]. Substance P has been found to be raised in tears of DED patients after refractive surgery [667] and nerve growth factor has also been found to be raised in tear fluid of patients with neuropathic pain [668]. Hence these neurotrophic factors and neuropeptides appear to regulate tear aqueous production such that deficiencies in an individual with DED may indicate a strategy for improving tear secretion.

3.5.4.4.2.4 Ocular surface immune markers

Major histocompatibility complex based markers have long been identified as a risk factor for DED, particularly with Sjögren disease [669, 670]. Antigen presentation through the major histocompatibility process seems to play an intermediary role in T-cell activation and the cytokine-based inflammatory cascade [646]. While both major histocompatibility complex class I and class II have been connected to inflammation in DED, HLA-DR, a member of the class II family, has been more thoroughly investigated [671, 672]. A gene expression analysis of mRNA transcripts observed from conjunctival impression cytology sampling discovered a correlation between HLA-DR, CD40, and IFN. This connection further links inflammatory DED with T cell activation [671]. Conjunctival impression cytology samples collected on a heterogenous group of patients with DED revealed that the percentage total cells expressing HLA-DR was positively correlated with conjunctival and corneal staining scores [672] and weakly with tear volume [673]; although HLA-DR percentage analysis was not a sensitive diagnostic marker for DED in itself, it may represent a means of helping identifying specific dry eye subtypes based on the lymphocytic response responsible for the ocular surface inflammation in a particular patient, and guiding therapeutic decisions. Neutrophils, macrophages, mast cells, T-cells and dendritic cells have been found to increase in DED across several studies, particularly in more severe levels of dry eye found in autoimmune disease such as Sjögren disease and graft versus-host disease. [674],

3.5.4.4.2.5 Inflammasome markers

The inflammasomes are innate immune system sensors that induce an inflammatory form of cell death, known as pyroptosis, in response to harmful stimuli such as pathogens or oxidative stress, among others [675, 676] (see TFOS DEWS III Digest Pathophysiology section).[4] Reactive oxygen species are involved in the pathogenesis of DED [677], and have been suggested as a priming signal for inflammasome activation [602]. NOD-like receptor protein-3 (NLRP3) inflammasome, a key driver in the innate immune system, has a role in DED pathogenesis [677, 678], is upregulated in the tear film of people with Sjögren disease [678] and is activated by hyperosmolarity [677, 679]. Tear levels of caspase-1, a molecule involved in the inflammasome cascade, and various clinical signs of ocular surface damage in patients with DED and patients using topical glaucoma medications have been found to be correlated [680]. Moreover, tear levels of Gasdermin-D, a pyroptosis executor, are also elevated in patients with DED [681]

3.5.4.4.2.6 MicroRNAs

MicroRNAs are non-coding RNAs that serve as significant regulators in a variety of molecular pathways [682] (see TFOS DEWS III Digest pain and sensation section).[4] Several

studies have identified tear microRNAs as potential biomarkers for ocular diseases, including Sjögren disease [683] and DED [684-686], among others [608, 687]. Nine tear microRNAs (miR-127-5p, miR-1273h-3p, miR-1288-5p, miR-130b-5p, miR-139-3p, miR-1910-5p, miR-203b-5p, miR-22-5p, and miR-4632-3p) associated with inflammation have been found to be upregulated in the tears of patients with DED.

3.5.4.4.2.7 Oxidative stress markers

Oxidative stress, an imbalance of free radicals and antioxidants that leads to cell damage, may play a role in the pathogenesis of DED [688]. Proteomic analysis of tears from patients with DED shows an upregulation of proteins associated with oxidative stress injury [689]. It is well established that oxidative damage triggers an inflammatory response, resulting in ocular surface dysfunction [690-692]. Moreover, oxidative stress may cause the progression of DED by exacerbating inflammation by triggering the vicious circle of DED. Oxidative stress biomarkers, which indicate the degree of oxidative stress, have been found elevated in the tears and conjunctiva of patients with DED [693]. The detection of oxidative stress biomarkers through tear film or conjunctival impression cytology samples may be undertaken to evaluate DED status, monitor the efficacy of drugs or evaluate disease progression. Oxidative markers such as lactoferrin (tears), peroxiredoxin 2 (tears), SOD (tears), CAT (tears), and GSH-Px (tears) are downregulated in DED [689, 694]. In contrast, markers such as S100A8 (tears), S100A9 (tears), reactive oxygen species (conjunctiva), LPO (conjunctiva), 4-HNE (conjunctiva), MDA (conjunctiva) and HEL (tears) are upregulated in these patients [692, 695-698]. A correlation between ocular surface oxidative stress markers and topical treatments has been described by several authors [696, 699, 700], indicating the utility of these markers in monitoring response to anti-oxidant therapies.

3.5.4.4.2.8 Serum markers

DED is associated with chronic inflammatory systemic conditions including, collagen vascular diseases [701], rheumatoid arthritis [702], or Sjögren disease [703], among others [704]. Acute phase reactants such as erythrocyte sedimentation rate and C-reactive protein indicate active systemic inflammation [705]. However, previous studies found that levels of these reactants do not correlate with ocular surface symptoms or tear parameters in DED [701, 706], although serum inflammatory markers PM-Scl100 and Sm were associated with more severe DED symptoms, while inflammatory markers U2SnRNP A', Ro52, La, DNA, and Ro60 were associated with more severe ocular surface disease signs [707, 708]. Other serum inflammatory-related markers such as antinuclear antibody and IL-2 receptor(sIL-2R), or anti-double-strand DNA antibody, have been associated with DED in primary Sjögren disease [709, 710] or systemic lupus erythematosus [701]. Similarly, serum levels of IL-17, a proinflammatory cytokine [711], are significantly increased in patients with DED and high fluorescein staining scores [712]. S100A8/A9 and granulysin have been found to be higher in more severe Stevens-Johnson Syndrome [713]. In addition, in a large population-based study, decreased serum androgens were found to be highly associated with DED diagnosis and symptoms [714].

Table 6: Subclassification of DED etiological drivers recommended tests and cut-offs [where available]

		Standard testing		Advanced testing
Tear Film Deficiencies	**Lipid**	Interferometry – grade ≤ 3 (non-amorphous or colored pattern) or <72nm on LipiView [197, 320, 343]		
		Meibum expressibility/quality – meibum not clear or limited expressibility [332, 371, 715].		
	Aqueous	Meniscometry - tear meniscus height ≤0.20mm [39, 320, 380].		Strip meniscometry - ≤ 2.5mm wetting length [404-406].
				Tear proteins and other chemical components testing
	Mucin / glycocalyx	Rose bengal or lissamine green staining - >9 punctate spots [581]		Immunohistochemistry and immunoelectron microscopy of tear film
				Impression cytology – goblet cell density and epithelial cell morphology
Eyelid Anomalies	**Blink / lid closure**	Partial blinking observation - >40% occurrence [463] Lagophthalmos / inadequate lid seal - observed		
	Lid margin	Anterior blepharitis observation		
		MGD	Meibography – gland length <75% [320, 372, 509] Gland plugging - observed [715] Telangiectasia - observed [715] Gland expressibility	
		Keratinization	Slit-lamp biomicroscopy	
		Ocular rosacea	Slit-lamp biomicroscopy	
Ocular Surface Abnormalities	**Anatomical misalignment**	Slit-lamp biomicroscopy		Corneal topography
	Neural dysfunction	Puff or physical sensation - corneal and lid margin sensitivity thresholds ≥0.8 mbar [550] although instruments are not comparable [716]		*In vivo* confocal microscopy – normative values available for nerve length, branch and density metrics [717]
	Ocular surface cellular damage / disruption	Corneal fluorescein staining - >5 punctate spots [250, 581] Conjunctival lissamine green staining - >9 punctate spots [250, 581] Lid wiper staining - >2mm length and 25% width [250]		
	Primary inflammation / oxidative stress	Bulbar conjunctival hyperaemia – >1.5 Efron scale or >0.95 objective JENVIS [604]		In vivo confocal microscopy Tear film and ocular surface molecular testing
Systemic Drivers	Check for systemic conditions			

3.5.5 Systemic diseases leading to dry eye

Many systemic diseases significantly contribute to DED through inflammation, autoimmunity, metabolic dysregulation and ocular surface exposure. Interdisciplinary collaboration in the management of DED patients with underlying systemic conditions is important, such as coordinating care with rheumatologists, endocrinologists, or other relevant specialists to optimize both ocular and systemic outcomes.

3.5.5.1 Autoimmune conditions

Systemic inflammatory diseases such as rheumatoid arthritis, systemic lupus erythematosus, Stevens-Johnson syndrome and Sjögren disease are closely associated with DED. Chronic inflammation leads to the infiltration of immune cells in the lacrimal gland, reducing tear production and altering tear film composition [718]. Key inflammatory cytokines include IL-1, tumor necrosis factor-alpha and MMPs [719]. Patients with ankylosing spondylitis treated with tumor necrosis factor inhibitors showed improvement in clinical and laboratory disease parameters, tear production, DED severity, and impression cytology scores, suggesting tumor necrosis factor inhibitors may restore lacrimal gland acinar cells affected by proinflammatory cytokines [720]. In rheumatoid arthritis, the activation of the NF-κB pathway and overexpression of tumor necrosis factor alpha and IL-6 lead to systemic and ocular inflammation [721]. The full potential of tumor necrosis factor alpha inhibitors to reduce ocular surface inflammation and improve tear production in rheumatoid arthritis remains uncertain [722, 723].

Autoimmune diseases, particularly Sjögren disease, are closely associated with DED. Sjögren disease involves lymphocytic infiltration of exocrine glands, affecting the production of aqueous tears and saliva, respectively, and leading to dry eye and dry mouth. Detection of antibodies, such as anti-Ro/SSA and anti-La/SSB, support its autoimmune nature [724]. Gene analysis of conjunctiva imprints revealed 53 differentially expressed genes in Sjögren disease compared to healthy controls, that indicated immune activation in patients with Sjögren disease [725]. Higher percentages of antigen-presenting cells and mature dendritic cells in the conjunctiva are associated with more severe keratoconjunctivitis sicca in Sjögren disease, which may contribute to goblet cell loss.[726]

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Multidisciplinary treatment for both the underlying systemic disease as well as managing the residual ocular symptoms of DED is generally appropriate. In rheumatoid arthritis, for example, disease-modifying antirheumatic drugs such as methotrexate and biologics like tumor necrosis factor inhibitors help control systemic inflammation and improve ocular symptoms.[721]

3.5.5.2 Hormonal imbalance

Hormonal changes can trigger DED by affecting tear production and quality, especially in women, who experience hormonal fluctuations throughout their lives [727]. Hormonal imbalance and its management in patients with DED is covered in the TFOS DEWS III Digest.[4]

3.5.5.3 Metabolic disease

Metabolic diseases such as diabetes mellitus significantly impact the ocular surface and can lead to DED [4, 7]. Hyperglycemia and advanced glycation end-products contribute to microvascular damage and neuropathy, affecting the lacrimal glands and corneal nerves [728]. Patients with diabetes often exhibit reduced tear secretion, increased tear osmolarity, and altered corneal sensitivity [729]. Antioxidant therapy, along with strict glycemic control, can mitigate the effects of diabetes on the body [730] and the ocular surface [731, 732]. The impact of medications and procedures related to managing metabolic disease on the ocular surface are covered in the TFOS Lifestyle reports.[7, 9]

3.5.5.4 Exposure

A multitude of diseases as well as trauma can cause intermittent (for example nocturnal) or constant lagophthalmos (such as facial nerve palsy), leading to exposure of the ocular surface and DED [733]. A number of these conditions and the impact of medications and procedures related to ocular exposure on the ocular surface are covered in the TFOS Lifestyle reports [9, 12].

3.6 Tests for monitoring treatment

In monitoring treatment effects over time, it is important to consider indicators that may lag (such as corneal staining [249]), those that can change more rapidly (such as symptomology) and the treatment's mechanism of action [734]. It is therefore recommended that practices adopt a standardised, reproducible and repeatable DED protocol that involves validated questionnaires, diagnostic tests and clinical exam that remains consistent from visit to visit. While every real-life patient encounter will undoubtedly not be as clear cut as this hypothetical example, clinicians are encouraged to make diagnostic and treatment decisions based on scientific evidence and on tracking subjective and objective data over time, and with the aid of clinical judgment, experience and acumen as outlined in this report.

As the number of testing options increases, the volume of clinical data that is required to be processed at each patient visit also increases and can be, at times, overwhelming. This becomes increasingly challenging as physician time becomes progressively constrained and face-to-face consultation times are shortened. With handwritten paper records, processing diagnostic data trends over long periods of time encompassing multiple office visits was challenging, time consuming and rarely done thoroughly. However, in the current electronic medical record era, the ability to seamlessly and instantaneously process, summarize, plot and chart large volumes of disparate diagnostic data over long periods of time is much easier to facilitate. DED/ocular surface disease-specific electronic medical record platforms are now available and enhance the ability to track and monitor DED symptoms, objective tests, exam findings and treatment outcomes [735] as well as real-world registries which also provide the facility to monitor patient progress and benchmark against peers [736]. Additionally, with the ubiquity of smartphones and the burgeoning emergence of smart glasses and wearable health monitors/sensors, there is unprecedented potential for gathering and analyzing real-time continuous data in-between office visits data [292, 737-739]. Machine learning and artificial intelligence (see section 5.1), when integrated into electronic medical record platforms and smart devices, with access to process reliable data from disparate physicians, practices and geographic locations, may possibly provide novel insights into DED that are as yet unknown, facilitating more accurate diagnoses, better treatments and more strategic clinical trial designs [740]. With the assistance of machine learning and artificial intelligence, historically-challenging diagnoses such as neurotrophic keratopathy and neuropathic corneal pain will likely be made earlier, with the potential to improve patient outcomes and reduce late-stage sequelae.

4 Patients with only symptoms or ocular surface signs

There are numerous explanations highlighted in section 3.3.3 for the, often vexing, clinical scenario of discordant signs and symptoms. Significant corneal staining in a pain-free patient, colloquially referred to as 'stain without pain', may indicate neurotrophic keratopathy. [741] Conversely, when the clinical signs of ocular surface dysfunction are mild or subtle in the absence of patient-reported symptoms it might indicate an early, preclinical or situational DED [155]. In the extreme, a clinical scenario involving significant symptomology in the absence of, or out of proportion to, clinical signs, colloquially referred to as 'pain without stain' might be indicative of neuropathic corneal pain [175].

4.1 Ocular surface disease in the absence of symptoms

It is recommended to treat any significant ocular surface disease prior to a patient undergoing any type of eye surgery (such as laser vision correction or cataract removal), initiating contact lens wear, starting any high risk topical or systemic medications and/or any other intervention known to cause or exacerbate DED (see TFOS DEWS III Digest Iatrogenic section).[4] The surgical informed consent process should include a discussion of the risk of worsening signs and/or symptoms of DED, including visual symptoms, which may require intensive long-term treatment to control. The importance of identifying, offering education about and treating early preclinical DED/ocular surface disease is particularly critical in the setting of refractive, cataract or laser vision correction surgery where patient expectations tend to be particularly high.[742]

There is a high prevalence of ocular surface disease in presurgical cataract patients, many of whom have few or no reported symptoms [743, 744]. It is speculated that the stark disconnect between signs and symptoms in this older population is due to a combination of generational stoicism, inherent bias to focus on the perceived 'bigger' problem (for example their poor vision due to cataract) and/or age-related reductions in corneal nerve density and sensation [152, 153, 165, 167, 171, 745]. In the younger patient population, contact lens intolerance from DED is a common reason for seeking laser vision correction surgery,[746] but can also place the patient at higher risk of postsurgical complications if not identified, discussed and managed preoperatively.

Many studies have shown that DED, especially when significant corneal staining is present, can adversely affect the accuracy of preoperative measurements (such as keratometry, topography, optical pachymetry and aberrometry) potentially leading to post-surgical refractive error misses, poor and/or fluctuating visual quality (especially when multifocal or extended-depth of focus intraocular lenses are implanted) and overall lower patient satisfaction [28, 747-751]. Due to the high prevalence of ocular surface disease in this patient population, an algorithm has been designed by the American Society of Cataract and Refractive Society to identify and treat visually significant ocular surface disease preoperatively [752].

4.1.1 Diagnosing Ocular Neurosensory Abnormalities

A medical history questionnaire or intake form should include often missed non-ophthalmic risk factors for neurotrophic keratopathy (diabetes, herpes, Parkinson's, multiple sclerosis, prior brain surgery such as acoustic neuroma, cerebrovascular accidents and congenital dysautonomia) and for neuropathic corneal pain (such as small fibre peripheral neuropathies, fibromyalgia, migraine, irritable bowel, anxiety, depression and post-traumatic stress disorder) [753]. Clinical suspicion for neurosensory abnormalities should be raised in any patient with marked discordance of signs and symptoms, risk factors and/or positive answers to targeted triaging questions. Corneal surgical incisions, laser corneal ablation, suction prior to flap creation by microkeratome or femtosecond laser, phototoxicity from the operating microscope, benzalkonium chloride-preserved postoperative drops amongst other factors can exacerbate preexisting or induce new corneal neurosensory abnormalities, potentially leading to visually significant post-operative ocular surface disease [9, 33, 107, 754-761].

4.1.2 Diagnosing Neurotrophic Keratopathy

Neurotrophic keratopathy is considered a rare disease, but is likely underdiagnosed [762]. It is caused by a unilateral or bilateral abnormality of the trigeminal nerve, resulting in decreased or absent corneal sensation, and leading to diffuse corneal punctate epithelial defects (Mackie stage 1), persistent epithelial defects with characteristic smooth rolled edges (stage 2), and stromal melting with the potential for corneal perforation (stage 3) [763, 764]. A more nuanced and detailed 6-stage grading system has recently been proposed by a Neurotrophic Keratopathy Study Group to better refine the stages of progression and to allow practitioners

to identify NK at earlier stages [741]. Because early stages of neurotrophic keratopathy can involve corneal epitheliopathy with staining, increased mucous viscosity and decreased tear film stability, it is often misdiagnosed and treated as moderate-to-severe DED. The pattern of corneal staining in neurotrophic keratopathy is often diffuse involving the entire cornea in contrast to the inferior or interpalpebral staining typically seen in DED [551, 765]. Patients with neurotrophic keratopathy will also typically have lower blink rates and poorer blink quality [762] whereas patients with DED and poor blink quality, tend to blink more frequently [766]. Patients with neurotrophic keratopathy typically don't self-report symptoms of pain or discomfort, inconsistent with the level of corneal staining, but they may complain about reduced visual acuity, quality, stability and performance. This clinical scenario of 'stain without pain' should alert clinicians to suspect the possibility of NK as early as possible, prior to the patient failing long-term DED treatment, and, ideally well before potentially blinding stromal breakdown occurs in later stages.

Reduced or absent corneal sensitivity is suggestive of neurotrophic keratopathy and therefore corneal sensation should be assessed as soon as there is a clinical suspicion and prior to instillation of anaesthetic drops. Various methods are available for assessing corneal sensitivity. The Cochet-Bonnet esthesiometer is quantitative and makes contact with the ocular surface while the gas esthesiometers are quantitative, non-contact, and able to assess chemical, thermal and/or mechanical corneal sensitivity [716, 767-770]. Both are commonly used in research and in specialty referral centres but historically have been costly and impractical for most general eye care practitioners. Cheaper and simpler methods involving contact with the eye's surface that are commonly used in practice include a wisp from a cotton-tip applicator, a corner of a disposable facial tissue and/or unwaxed and unflavored dental floss and can provide a quick qualitative assessment of corneal sensation in a primary eye care setting [741]. A patient with normal sensation will blink and report discomfort when the central cornea is touched, a patient with a hypoesthetic cornea may blink but not report sensation and one with an anaesthetic cornea typically will not register a blink or any sensation to the stimulus [741]. A modified-Delphi expert panel on neurotrophic keratopathy strongly recommended corneal sensitivity testing for persistent epithelial defects after 14 days, new painless epithelial defects, a history of herpetic eye disease or procedures that may injure the trigeminal nerve, and pain in an eye with multiple concurrent risk factors for neurotrophic keratopathy such as poorly controlled diabetes and either reduced blink rate or a history of corneal surgery [771]. As more modalities for non-contact, reproducible, quantifiable and inexpensive esthesiometry become available,[769, 772-774] corneal sensation can be performed earlier in the diagnostic subtyping process and ideally be incorporated into office-based routine DED/ocular surface disease protocols (see section 6.1).

Once clinical suspicion for neurotrophic keratopathy and reduced corneal sensation are identified, further investigation with corneal *in vivo* confocal microscopy can be diagnostically confirmatory (see sections 3.5.3.6.1.2 and 3.5.3.4). Studies have consistently demonstrated significant alterations in the corneal nerves, epithelial cells and corneal stroma in patients with neurotrophic keratopathy [775-778]. Of note, corneal sensation may remain relatively normal despite significant reductions (of 50 to 80%) in sub-basal nerve density accompanied by morphological changes such as increased tortuosity and beading [779], and may be clinically detectable only when the nerve density drops below 1000 µm nerve length /frame [780, 781]. Epithelial abnormalities include enlarged and irregular cell shapes, decreased cell density and squamous metaplasia correlating with disease severity [780, 781]. Severe disorganization, altered keratocyte morphology and presence of hyperreflective cells can be observed in stage 3 along with increased dendritiform cell density, particularly in the central cornea, suggesting a possible inflammatory component [782]. Substantial reductions in sub-basal nerve density are commonly seen in patients with neurotrophic keratopathy secondary to herpetic eye disease [777, 778, 781]. Interestingly, patients with unilateral herpes simplex and zoster ophthalmicus may exhibit contralateral reductions in sub-basal nerve density [67, 780].

4.2 Symptoms in the absence of ocular surface disease

Unlike nociceptive pain that involves the triggering of nociceptors from local tissue damage, neuropathic pain, which can be peripheral or central in origin, is caused by an abnormality in the somatosensory nervous system [783]. Diagnosing neuropathic corneal pain is primarily clinical and exclusionary and based heavily on clinical history, risk factors and the discordance in terms of the level of symptoms in the absence of corresponding clinical signs, colloquially termed 'pain without stain' [784]. While DED is commonly the initial diagnosis and treatment target for many of these patients, neuropathic pain does not respond to conventional treatments in the same way, and artificial tears do not provide the same temporary symptom relief [785]. DED and neuropathic corneal pain can coexist, requiring treatment for both. Indeed, DED may have been the trigger, leading to neuropathic corneal pain, often in patients with comorbid chronic pain (such as migraine and fibromyalgia), psychiatric and/or mental health disorders [175, 786, 787]. Patients with Sjögren disease also have a higher risk of chronic ocular pain with neuropathic features [788]. Other risk factors and associated comorbidities of neuropathic ocular pain include DED, diabetes, sarcoidosis, small fibre neuropathies, herpetic eye disease, prior eye surgery, infection, trauma, contact lens wear and radiation keratopathy, and many of these overlap with risk factors for neuropathic keratopathy [789]. An association with long-COVID has also been identified [790, 791]. To confound the inherent diagnostic challenges even further, a subset of patients with stage 1 neurotrophic keratopathy and concomitant neuropathic corneal pain has also been reported [792]. If neuropathic corneal pain is suspected on the basis of clinical history, symptoms and a lack of signs on examination, a pain-specific validated questionnaire such as the Ocular Pain Assessment Survey or Neuropathic Pain Symptom Inventory, which has been modified for the eye, can be utilized to score symptoms [793, 794].

4.2.1 Diagnosing corneal neuropathic pain

If the patient's eyes are painful despite lubricating drops and the pain is incited by light, wind or other triggers, neuropathic corneal pain should be suspected. In such a situation, a proparacaine challenge test can be performed to aid in both diagnosis and differentiation between peripheral and central etiologies [789, 795, 796]. If the pain is completely ameliorated by an anaesthetic drop the patient likely suffers from peripheral or nociceptive corneal pain whereas, if the pain persists unchanged afterwards, then a central neuropathic pain mechanism is likely; if only partial relief is achieved then a mixed mechanism of peripheral and central neuropathic corneal pain is likely [795]. Differentiating peripheral from central neuropathic corneal pain is important as the treatment strategies differ significantly between the two [797, 798]. While corneal aesthesiometry is a critical test for neurotrophic keratitis it is less useful in the workup of neuropathic corneal pain as studies have revealed both higher and lower sensitivities in these patients and overall poor diagnostic correlation [152, 796, 799, 800] although more objective, non-contact techniques offer promise in identifying neuropathic pain [769]. Reduced density of sub-basal nerves is a finding common to both neurotrophic keratitis and neuropathic corneal pain, but the increased presence of sub-basal and stromal microneuromas, detectable by confocal microscopy, is more common in neuropathic corneal pain [168, 169, 566, 567, 801]. Further studies are needed to establish whether confocal microscopy can be used reliably for differentiating the etiological drivers in a patient with DED.

5 Future advances

5.1 Artificial intelligence

Artificial Intelligence, a term coined by emeritus Stanford Professor John McCarthy in 1955, was defined by him as "the science and engineering of making intelligent machines." A wide range of definitions have now been proposed, making it difficult to assess claims on its use. It has been suggested that it is already widely used in DED clinical tests and research [802], but it could be argued whether an algorithm to detect a change in pixel contrast or an 'edge'

of a placido ring is truly “intelligence”. Machine learning thorough training has been used to try and predict video frames that a specialist identified as showing breakup, with a sensitivity of 78% and specificity of 86% [803]. There is the potential that machine learning could make a clinical test used in the diagnosis of dry eye more objective, but as such a diagnosis would require a gold standard disease group against which to compare the individual, and a consensus of which tests are most suitable to identify this group based on available evidence, along with practical considerations such as cost and equipment availability, as artificial intelligence cannot in itself drive development or change in the diagnostic algorithm for dry eye. It certainly can aid in image analysis however, and particularly structural segmentation such as in meibography [804-809]. Rating of corneal fluorescein staining, on the other hand, remains more challenging [810]. Potential barriers to widespread adoption of artificial intelligence include cost, accessibility, regulatory and ethical considerations, training requirements and integration with existing diagnostic protocols [811].

5.2 Tear biomarker testing of tears

MMPs are one of many classes of proteases secreted into the tears in DED. Since MMPs can destroy tight junctions in the ocular surface epithelium, increased levels of MMPs reflects loss of ocular surface barrier function, [812-814]. MMPs are produced as inactive proenzymes and can be cleaved to become active enzymes [815]. It is therefore important for future MMP tests to detect enzyme activity levels and not simply total tear protein levels. MMP-9 is detected more commonly in severe DED and has been proposed as a potential means of monitoring the success of DED management; patients with a positive MMP-9 test showed a greater benefit from topical cyclosporine than those who were MMP-9-negative [597]. A silicon nanowire-based field-effect transistor MMP-9 tear film biosensor was found to have a sensitivity of 87% and specificity of 90% for DED [816]. More advanced point-of-care tear proteomic test kits for identifying DED subtype drivers are needed along with independent validation / replication studies.

Lymphotoxin-alpha (LT-α), a member of the tumor necrosis factor superfamily, is expressed by T cells, B cells, and natural killer cells, playing a crucial role in immune system development and function [817-819]; this includes the formation of lymphoid organs, maintenance of lymphatic microenvironments, host defense, and modulation of inflammation. Despite its established association with inflammation, emerging research has identified a negative correlation between LT-α levels in the blood and fatigue symptoms (often linked to proinflammatory processes) in patients with primary Sjögren disease [820, 821]. This suggests that LT-α's role in inflammation might be more complex than previously thought. Levels of multiple tear protein markers (TNF-α, IL-10, IL-1β, IL-1Ra, IL-17A, and IL-12/23 p40) were elevated in patients with DED with high LT-α (>700 pg/mL) compared to those with lower LT-α (≤700 pg/mL), indicating possible differences in pathogenesis [822].

5.3 Sustainability

To date, there has been a significant paucity of literature examining the sustainability implications of diagnostic testing and practices for DED [823]. Future research is required to characterise the potential sustainability implications, environmental effects, and carbon footprint of the production, use, and disposal of different types of diagnostic instruments, dyes, consumables, treatments and packaging.

5.4 Need for experience-informed approach to un/under-researched areas

Where research on best practice is either limited, conflicting or logistically or ethically difficult to obtain, a group process using collective intelligence may help [824]. This can for example be applied to achieve consensus on the best clinical criteria for diagnosis or to initiate treatment [825]. The Delphi technique is a systematic process designed to establish consensus in a group of experts. A series of questionnaires is distributed, and controlled feedback with group statistical responses is given each time, until answers are converged and a predefined criterion is reached to bring the process to a close. Important aspects, in order to obtain valid

outcomes, include systematic identification of the problem area, the selection of panel members based on objective and predefined criteria, anonymity of panelists and responses, controlled feedback, and stability of results including *a priori* defined closing criteria [824].

A Delphi approach has been used to define ocular surface disease activity and damage indices [826]. Several Delphi or other group process approaches have been conducted in the past two decades to establish a best practice on a diagnostic aspect of DED and blepharitis. The ODISSEY European Consensus group defined a two-step scoring algorithm for diagnosing DED, but only at a severe level. Symptom-based assessment and corneal fluorescein staining were considered to be the two most important criteria. In case of discordance between these two tests, identification of additional criteria was recommended.[827] Separately, the DIDACTIC study used a Delphi approach to categorize signs and symptoms to identify DED pathophysiology. A total of 19 items were deemed indicative of evaporative dry eye, and 12 items of aqueous deficient DED.[323]. Using nominal group and Delphi techniques, a group of Italian ophthalmologists reached consensus on criteria for classification of DED. Three types were classified: a transient and reversible form, a recurrent form, and a chronic form, each with its own clinical characteristics [828]. Recent Delphi panels on Demodex-associated blepharitis have achieved consensus on it being chronic and recurrent [486], with the presence of cylindrical dandruff at the base of the eyelashes, visible Demodex mites, lid margin telangiectasia, and a previous history of anterior blepharitis not responding to treatment, being proposed as the most indicative independent signs [485]. Cylindrical dandruff is considered pathognomonic for Demodex blepharitis, with the suggestion that patients with >10 collarettes should be treated even in the absence of symptoms and that treatment efficacy can be tracked by the extent of cylindrical dandruff resolution [829].

Examples of areas in the field of diagnostic methodology of DED that lack scientific evidence and could benefit from a future Delphi approach include:

- how should DED severity be graded
- is site-specific itch useful in differentiating dry eye from allergy
- what cut-offs should be used for etiological drivers without current evidence-based diagnostic thresholds
- the best sequence for instilling fluorescein and lissamine green to assess ocular surface staining

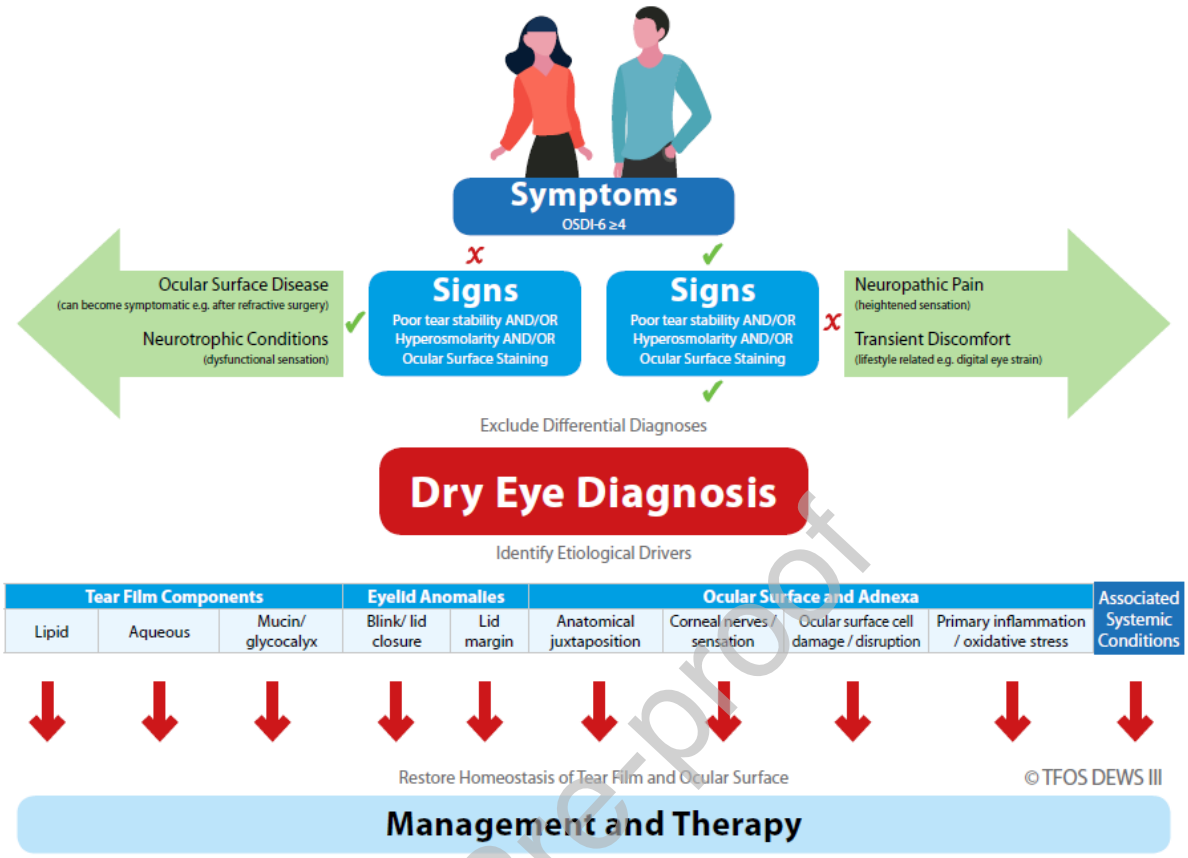
6 Summary

A standardised approach is critical to providing robust epidemiological information on DED in future.[4] It is critical that all practitioners and researchers adopt the same approach for the field to move forward for the benefit of patients.

6.1 Workflow and enhanced link to individualised management

In those patients that identify dryness type symptoms, the OSDI-6 screening questionnaire should be used to quantify these (see Section 3.3.2; Figure 4) and a score of ≥4 used as a prompt for further investigation. Risk factors should be explored (see Section 3.2; Figure 3; Table 1) and a differential diagnosis conducted (see Section 3.1; Figure 2). If the practitioner lacks the expertise and access to instrumentation to facilitate a detailed examination of the eye, the triaging questions (see Section 3.1.5) will help to identify patients for whom referral is appropriate. Diagnosis of DED requires evaluation of ocular surface staining (including the cornea, conjunctiva and lid margin) along with tear film instability and/or hyperosmolarity (Figure 5), and the criteria met by demonstrating a positive score in at least one of these three indices of tear film and ocular surface homeostasis. In those diagnosed with DED, it is important to identify the etiological driver(s) of the individual's disease (see Section 3.5; Table 6) to inform the most appropriate management and therapy option(s) (as described in the TFOS DEWS III Management and Therapy report;[17] Figure 11)

Figure 11: Classification of dry eye disease



An example of a test sequence to diagnose and identify the drivers (subtypes) of dry eye disease based on key tests / observations is presented in Table 7. Tests should be ordered from least to most invasive, to best maintain the integrity of the assessments and minimise impact on subsequent test results. Slight variations in test order may be expected where tests applied differ, but, in any case, the same test order should be utilised consistently on each patient at any one site, to enhance consistency in diagnosis and subclassification. Diagnosis and assessment for subtype drivers may be conducted in a single visit (if time allows) or may be separated into two visits to include a rapid diagnosis as part of routine testing, with follow up for subtype driver analysis at a separate visit. Test order will be adjusted accordingly, always from least to most invasive.

Table 7: An example of a test sequence (from least to most invasive) to diagnose (second column) and identify the drivers (subtypes – third column) of dry eye disease based on key tests / observations in the same appointment.

Sequence	Diagnosis	Subtype Drivers
1	Symptoms Screening - OSDI-6	
2		Blink / lid closure - rate / completeness / lid seal
3		Aqueous – tear meniscus height (using infrared illumination)
4	Non-invasive tear breakup time	
5		Anatomical lid/globe misalignment –

		features such as pterygia
6		Inflammation – redness
7		Lipid – interferometry
8	Osmolarity	
9		Lid margin – eyelashes, lid margin, diagnostic expression
[10]	[Fluorescein tear breakup time]	
11	Ocular surface staining	Ocular surface damage- corneal, conjunctival and lid wiper staining Mucin – conjunctival staining Lid margin – keratinisation staining
12		Lid margin – meibography
13		Neural dysfunction - corneal nerves / sensation (if contact methods are used)

6.2 Patient communication

When communicating with a patient experiencing dry eye, active, two-way communication should be prioritized by openly discussing their symptoms, addressing concerns, educating them on lifestyle modifications to manage the condition, and empowering them to actively participate in their treatment plan. Key aspects include: explaining the chronic nature of DED, setting realistic expectations, discussing environmental triggers, promoting proper eye hygiene practices and recommending appropriate artificial tears or other treatments based on their individual needs and as indicated by their individual identified etiological drivers. Patients are usually highly engaged in their desired outcomes[315] and if DED is suspected, a follow up appointment is likely to be needed to perform a differential diagnosis, conduct the diagnostic algorithm, identify the etiological drivers (with the potential for a technician to conduct these measurements) and to discuss these with the patient and ideally to show them the images capture, to make shared decisions on appropriate management and therapy.[17]

6.3 Key diagnostic methodological changes from TFOS DEWS II

The revised definition: "Dry eye is a multifactorial, symptomatic disease characterized by a loss of homeostasis of the tear film and/or ocular surface, in which tear film instability and hyperosmolarity, ocular surface inflammation and damage, and neurosensory abnormalities are etiological factors." Key features of DED from the definition that have been reemphasised include that DED is multifactorial, a disease not a syndrome and always symptomatic.

The recommended screening questionnaire is the OSDI-6 with a cut-off of a score ≥4 (Figure 4). Other questionnaires can be used as desired to gain further understanding of the environmental risk factors, but the standardised diagnostic questionnaire is necessary to achieve diagnostic consistency for all patients.

Key clinical differential diagnosis questions, alongside asking about a patient's general health and medication, are:

- Do you feel eye pain rather than discomfort?
- Do you have any facial flushing/redness, mouth dryness or enlarged salivary glands?
- When did your symptoms start and can you recall any triggering event?
- Is your vision affected and if so, does it improve on blinking?
- Are the symptoms or any redness much worse in one eye than the other?
- Do the eyes itch, are swollen, crusty or have given off any discharge?

A detailed examination of the ocular surface is recommended where the responses to these questions suggest possible presence of eye conditions that might masquerade as DED, with a guide to the ocular examination presented in Figure 2.

Known DED risk / associated factors (Figure 3) have been updated based on the scientific evidence[4] and factors commonly assumed to be associated with, or even cause, DED for which the evidence is equivocal, are reported (Table 1).

The diagnostic algorithm for DED has been refined (Figure 5) and in mitigating the established variability between questionnaires, only one (the OSDI-6) is recommended for the diagnostic algorithm. The impact on the diagnosis of each individual signs of a loss of homeostasis of the tear film and ocular surface recommended in TFOS DEWS II has been examined demonstrating that the lack of non-invasive breakup time or osmolarity only has a minor effect. Hence the revised, TFOS DEWS III approach has been shown to be robust[232] and further simplifies the procedure for application in clinical practice.

In aligning treatment strategies that possess different mechanisms of action with the multiple established drivers of dry eye disease, it has become clear that a greater number of distinct subtypes than simply aqueous and evaporative need to be acknowledged to ensure optimal patient care. This report has compiled the evidence on a more detailed subclassification of the disease based on the etiological drivers, so that these can be identified for an individual patient for the purpose of informing appropriate management and therapy. Cut-offs for the identified clinical tests have been provided where available (Table 6). Many of these clinical tests are already part of a standard clinical DED routine (Table 7), with the TFOS DEWS III approach offering structure and links to management and therapy (Figure 11).[17]

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Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.