Chapter

Current Diagnostic Tests for Dry Eye Disease in Sjögren's Syndrome

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Abstract

Sjogren's syndrome (Sicca Syndrome) is mainly characterized by the presence of dry eye disease (DED). The diagnosis of DED in patients with Sjogren's syndrome has been limited to tests such as the Schirmer test, tear breakup time (TBUT), and corneal stains; however, currently we can evaluate the functional unit in detail lacrimal, which is affected in patients with dry eye and Sjögren's syndrome; thanks to technology that provides objective details for this difficult diagnostic. The newer evaluations that provide the greatest diagnostic value for Sjogren's syndrome are: noninvasive keratograph tear rupture time (NIKBUT), tear meniscus height (TMH), Schirmer's test, meibography, ocular surface disease index (OSDI), Vital stains of the ocular surface, Matrix Metalloproteinase 9 (MMP-9), Tear osmolarity (TearLab); all of these are important complements to the existing tests, which, although less objective, are not substitutable.

Keywords: NIKBUT (noninvasive keratograph tear rupture time), TMH (tear meniscus height), Schirmer's test, meibography, ocular surface disease index (OSDI), vital stains of the ocular surface, matrix metalloproteinase 9 (MMP-9), tear osmolarity (TearLab)

1. Introduction

Sjögren's syndrome (SS) is a chronic inflammatory disorder characterized by immune-mediated exocrinopathy (inflammation and lymphoplasmacytic infiltration of the lacrimal and salivary glands) with resulting dysfunction. It can occur primarily or in association with a second well-defined rheumatic disease (secondary form). Both ways, they produce inflammation of the exocrine glands with subsequent dry eye and dry mouth [1, 2].

Patients with Sjögren's syndrome (SS) may present with symptoms suggestive of dry eyes. Dry eye typically manifests itself in up to 95% of SS patients; whose complaints are inability to tear, foreign body sensation, conjunctival inflammation, eye fatigue, and decreased visual acuity [3]. Dry eye disease (DED) can be complicated by keratoconjunctivitis sicca, blepharitis, bacterial keratitis, or corneal ulcer [4]. Uveitis, episcleritis, and orbital pseudotumor are systemic manifestations that rarely occur in patients with Sjogren's syndrome [5].

2. Dry eye disease (DED)

"Dry eye disease (DED) is 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." [6]. The description to understand this definition implies the term "multifactorial disease" recognizes DED as a significant and complex functional disorder that cannot be characterized by a single process, sign, or symptom. The term "ocular surface" encompasses structures including the tear film, the lacrimal and meibomian glands, the cornea, the conjunctiva, and the eyelids. "Homeostasis" describes a state of dynamic equilibrium in the body with respect to its various functions and the chemical composition of fluids and tissues. The key elements that contribute to the pathophysiological process, including tear film instability, hyperosmolarity, inflammation, and damage, recognized as etiological triggers of the vicious cycle, were considered important, along with neurosensory abnormalities, which have appeared more and more in recent literature, for inclusion in the definition [6].

2.1 Classification

The DED classification is based on its pathophysiology, acuodeficiency dry eye (ADDE), and evaporative dry eye (EDE). The limit in these categories is extremely diffuse [6]. The Sjogren's syndrome–related ADDE is a secondary phenomenon to immune-mediated exocrinopathy affecting the lacrimal and salivary glands [6]. Findings include decreased tear flow, staining of the ocular surface of injured tissue with vital dyes, and increased tear osmolarity. Meibomian gland dysfunction is also common in SS patients, with a resulting increase in tear evaporation, exacerbating decreased tear production [7, 8].

ADDE not associated with Sjogren's syndrome may be due to primary or secondary deficiency of the lacrimal gland, obstruction of the ducts of the lacrimal gland, and reflex hyposecretion [6]. EDE is due to an excessive loss of water from the exposed ocular surface, in the presence of a normal secretory function, the causes can be extrinsic due to harmful exposure of the ocular surface or intrinsic affecting internal structures or dynamics of the eyelid, may be due to causes related to the eyelid (e.g., Meibomian gland dysfunction [MGD] or decreased blinking) [6].

2.2 Pathophysiology

Tear hyperosmolarity stimulates a cascade of events in ocular surface epithelial cells involving the signaling pathways of MAP and NFkB kinases and the generation of inflammatory cytokines (interleukin 1 [IL-1α; IL-1β]; tumor necrosis factor α [TNF-α]) and proteases, such as MMP-9. This stimulates and readies inflammatory cells on the ocular surface, which become a reservoir for inflammatory mediators. These mediators, in an environment of tear hyperosmolarity, cause a decrease in the expression of mucin in the glycocalyx, the apoptotic death of the superficial epithelial cells, and the loss of goblet cells. However, hyperosmolarity also causes corneal epithelial cell death through nonapoptotic processes. Goblet cell loss is a feature of all types of DED, which is reflected in the reduction of tear levels of MUC5AC. The alteration of mucin expression in the glycocalyx is probably one of the reasons why ocular surface staining occurs in DED and, since it affects the ocular surface wetting, leads to an early

breakdown of the film tear. This amplifies or triggers hyperosmolarity on the ocular surface, with which the vicious circle is closed and the mechanism that perpetuates the disease is established [6]. In ADDE, tear hyperosmolarity occurs when tear secretion is reduced due to lack of production, under normal conditions of evaporation from the eye. In EDE, tear hyperosmolarity is caused by excessive evaporation of the tear film with a normally functioning tear gland [6].

2.3 Eye manifestations

Patients with dry eye present with eye irritation, foreign body sensation, burning, tearing, photophobia, stinging, or sharp intermittent pain. They may also complain of blurred vision that improves with blinking or the instillation of artificial tears; and they may have all, some, or none of these symptoms. A well-conducted medical history contributes greatly to a correct diagnosis and guides a more focused slit lamp examination; therefore, a thorough slit lamp examination should be performed prior to performing any other tests, which may alter or mask relevant examination data, resulting in a misdiagnosis. Signs of dry eye identified on slit lamp examination include superficial corneal erosions, inadequate tear lake volume, early tear film breakdown time, conjunctival hyperemia, conjunctival surface irregularities, and meibomian gland dysfunction [7].

2.4 Diagnosis of DED

The evaluation of the tear function is carried out by means of different tests that translate into the presence or absence of DED; however, there is no gold standard for the diagnosis of dry eye disease; therefore, they must frequently be associated with each other for a reliable diagnosis; The most used in the basic ophthalmology office are mentioned in order to compare them with the new technologies for the diagnosis of dry eye and the sequence of how they should be applied; according to diagnosis report methodology subcommittee of the international dry eye workshop (2007) [8]. This is summarized in **Table 1**.

2.5 Current methods of diagnosis

Symptoms and signs found during history and slit lamp examination may suggest dry eye due to water deficiency, evaporation, or both. Additional tests should be performed because the ophthalmologist may be the one who detects SS in a patient with no previous medical history or who debuts with ocular symptoms. Therefore,

Clinic history
Symptom questionnaire (OSDI)
Tear breakup time (TBUT)
Ocular surface by fluorescein staining
Tear secretion (Schirmer test with or without anesthesia)
Other tests according to your availability

Bron et al. [8].

Table 1.
Test sequence basic.

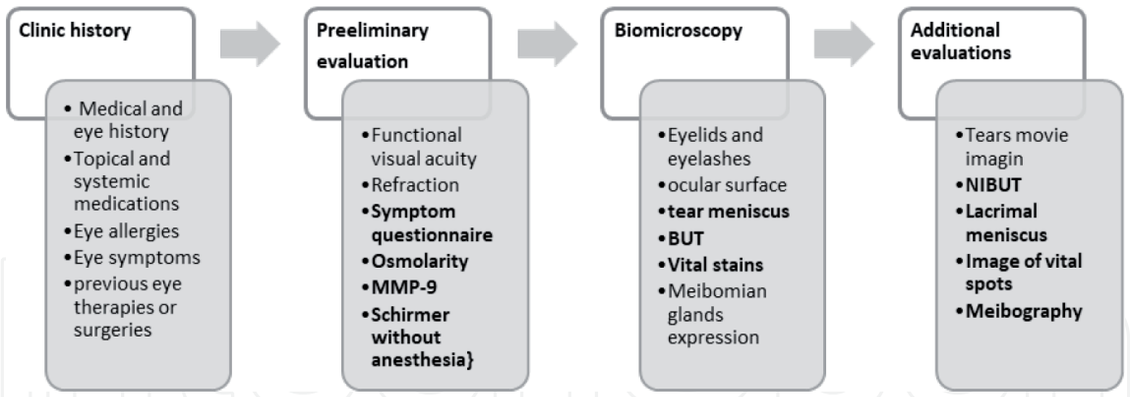

Figure 1.
Sequence of evaluations and tests in dry eye.

the choice of complementary diagnostic methods plays a very important role in the diagnosis, in the same way the sequence to carry out the tests and its correctly applied methodology will be a key point for a successful diagnosis, preventing the tests from overlapping and reporting false negatives or positives [9]. The suggested sequence of evaluations and tests is shown in **Figure 1**.

2.5.1 Symptom questionnaire

The ocular surface disease index (OSDI) was developed to provide a rapid assessment of the symptoms of eye irritation caused by dry eye disease and its impact on vision-related functioning. It is a 12-item questionnaire to evaluate ocular symptoms, functional limitations, and environmental factors related to the environment. Each item has five categories with three subscales with their own type of question; emphasis is placed on symptoms such as photophobia, grit, eye pain, blurred vision, in a period of 2–4 weeks before the visit. The OSDI is a self-administered questionnaire, it contains 12 items to evaluate the symptoms of the ocular surface; that implies little burden and little time for the patient. The OSDI has an overall score and three subscale scores: (A) ocular symptoms [three items], (B) vision-related function [six items], and (C) environmental triggers [three items]. Each OSDI item is scored on a Likert-type scale ranging from 0 to 4 points, where 0 indicates none of the time and 4 all of the time.

The total OSDI score was then calculated based on the following formula;
OSDI = [(Sum of the scores of all answered questions) × 100]/[(Total number of answered questions) × 4].

Overall and subscale OSDI scores range from 0 to 100. Based on their OSDI scores, patients can be classified as normal (0–12 points) or mild (13–22 points), moderate (23–32 points) or severe ocular surface disease (33–100 points) [10]. The reliability indices of the OSDI recently reported indicate that it is adequate, the Pearson correlation was higher than 0.8, and the ICC range was from 0.827 to 0.982; In addition, it was evaluated that it has a parallel reliability between the written and web versions [11].

2.5.2 Tear osmolarity

Tear osmolarity in normal subject ranges from 308 to 312 mOsm/l [12]. In individuals with DED associated with SS, osmolarity is significantly higher than in subjects with DED not associated with SS and even more than in healthy controls [13]. Also some studies have shown positive correlations between tear osmolarity and

disease activity in patients with SS [14]. Dry eye can be due to both increased evaporation, deficiency in its production, and alteration of the composition, in all cases the pathophysiological sequence culminates with an increase in the osmolarity of the tear film (hyperosmolarity) [15].

The evaporation of a smaller volume for the same surface increases osmolarity during the first 24 h from the beginning of the volumetric decrease [16]. Hyperosmolarity causes epithelial damage directly as it causes cell desquamation, complete disappearance of the superficial epithelial cell layers, decrease in cytoplasmic density, and accumulation of rows of mucus product of osmotically altered goblet cells. This phenomenon is generally evident between 15 and 30 days after the osmolar change of the tear film [17]. The epithelium of the cornea and conjunctiva must be completely moistened for complete wettability; the ocular surface conditions warrant that the surface tension of the aqueous layer at the interface with the epithelium is lower than the surface tension of the epithelium that is exposed to the medium [18]. In the mucous layer, mucopolysaccharides are directly responsible for maintaining surface tension stability. Under hyperosmolar conditions, there is accumulation of mucus and destruction of mucin-secreting cells, which causes an increase in surface tension with the consequent decrease in the wettability of the corneoconjunctival epithelium. The principle of osmosis is characterized by the flow of a solvent through a semipermeable membrane, which is generated when there is a difference in concentrations on one side of the membrane; this movement tends to equalize the solute concentrations on both sides, and there the flow stops. The corneoconjunctival epithelium and the mucous layer are a semipermeable barrier on the ocular surface, by increasing osmolarity in the aqueous layer; the aqueous gradient through the water protein channels present in the stroma and toward the aqueous humor; they change in the opposite direction. This directional fluid change produced by hyperosmolarity can cause dehydration of Sulfated Glycosaminoglycan (GAGS) that occupies the spaces between the collagen fibers of the stroma [19, 20]. When these glycoprotein structures are dehydrated, the correct water balance of the stroma will be affected, which will affect the normal maintenance of corneal transparency [21].

The osmolarity of the tear film in dry eye triggers inflammation, immunological processes, and the presence of autoantigens that enhance the inflammatory process. As an example of this, inflammatory markers such as NF-Kβ that migrates from the nucleus to the cytoplasm in the inflammatory process are directly related to the phenomenon of hyperosmolarity of the tear film. Nuclear translocation of NF-Kβ has been shown to be directly proportional to increased tear film osmolarity [22].

Hyperosmolarity is so important in the pathophysiology of dry eye disease that increased osmolarity of the tear film has been suggested to induce functional and structural damage to the corneal nerves and neurotoxicity [23].

The TFOS Dry Eye Workshop II (DEWS II) subcommittee report concluded: "Tear hyperosmolarity is considered to be the trigger for a cascade of signaling events within surface epithelial cells, leading to the release of inflammatory mediators and proteases. These mediators and tear hyperosmolarity cause the loss of goblet cells, epithelial cells and damage to the epithelial glycocalyx. The inflammatory mediators of activated T cells, recruited on the ocular surface, reinforce the damage; resulting in punctiform epitheliopathy characteristic of DED and tear film instability leading to early tear film rupture. This rupture increases the hyperosmolarity of the tear and completes the vicious circle events that cause damage to the ocular surface" [24]. While sophisticated equipment is required to measure tear film osmolarity, we can assess the sodium concentration that we obtain from tears by wetting a Whatman 41 paper strip in the usual

way for the Schirmer test, then colorimetrically measuring the sodium concentration in that. More specifically, the Tear Lab osmolarity system is designed to measure tear osmolarity and facilitate diagnosis in patients with suspected dry eye syndrome. Tear Lab measures osmolarity in 10 s and integrates seamlessly into clinical workflow. The evaluation of tear osmolarity has been shown to be a superior marker to determine the severity of DED, with respect to TBUT, Schirmer I, corneal and conjunctival staining, Meibomian classification and OSDI, whose specificities are (60%, 79%, 85%, 67%, 76%, and 79%) respectively. The test card in conjunction with the Tear Lab Osmolarity System offers a quick and easy method to determine tear osmolarity using nanoliter (nl) volumes of tear fluid obtained directly from the edge of the eyelid. To perform a test, a new test card must be inserted into the collecting pen; then contact the tip of the pen with the tear meniscus, located on the lower eyelid. Once the collection is complete, the pen is placed in the reader's docking station, which will display a quantitative osmolarity test result on the liquid crystal display (LCD) (**Figure 2**). The Tear Lab osmolarity system simplifies the tear collection process by eliminating the need to move tear fluid samples and reducing the risk of evaporation [25].

2.5.3 Matrix metalloproteinase 9 (MMP-9)

The matrix metalloproteinase test (MMP-9) is a useful technique to complement the diagnosis of DED recently developed. Matrix metalloproteinase 9 is an inflammatory biomarker that has been shown to be elevated in the tears of dry eye patients [26]. In tears, MMP-9 expression is normally less than 40 ng/ml and is secreted from the ocular surface epithelium [27]. MMP-9 maintains epithelial barrier function by

Figure 2.
TearLab.

breaking down components of the epithelial basement membrane and tight junction proteins, such as ZO-1 and occludin [28, 29]. But in addition, MMP-9 is related to the pathogenesis of various diseases, such as sterile ulceration, ocular allergy, keratoconus, conjunctivachalasia, and DED [30–32].

In the pathology of DED, since tear hyperosmolarity begins in the early phase of DED, a vicious circle probably induces inflammatory processes and triggers the release of MMP-9 in a relatively late phase of DED [33]. Detection of elevated MMP-9 in tears could be an ideal tool for the diagnosis and treatment of tear dysfunction [34].

The technique for using the MMP-9 device (InflammaDry test) is as follows: the sampling fleece is contacted three times at each location of the lower eyelid conjunctiva (temporal, middle and nasal; from nasal to temporal direction), and it rests against the temporal conjunctiva of the lower eyelid for an additional 5 s. After the sampling fleece is assembled on a sample collector and pressed, the test strip is immersed in a solution for 20 s; 10 min after taking the sample, the result is read (**Figure 3**).

InflammaDry, this type of device has the advantages of low cost, quick analysis, and ease of device preparation. However, it has been studied that a small sample volume, the reliability of the test result can be significantly affected [35].

It has been studied that the elevation of tear MMP-9 in patients with ED appears to have a good diagnostic yield and a correlation with the clinical severity of DED [26, 27]. The reliability for this test was 85% sensitivity and 94% specificity for the diagnosis of DED [36]. But despite these very promising values, two separate reports showed moderate to low positivity in an immunoassay for MMP-9 in study subjects with DED [36–38]; reporting that there were no differences between subjective symptoms and clinical signs of DED between MMP-9 positive and negative groups [37, 38]. In general, although it is a nonspecific marker for patients with DED [39, 40], various immunoassays have reported its usefulness as an aid in the diagnosis of dry eye; however, there are factors that may influence the results of MMP-9 in tears; one of them was demonstrated in an

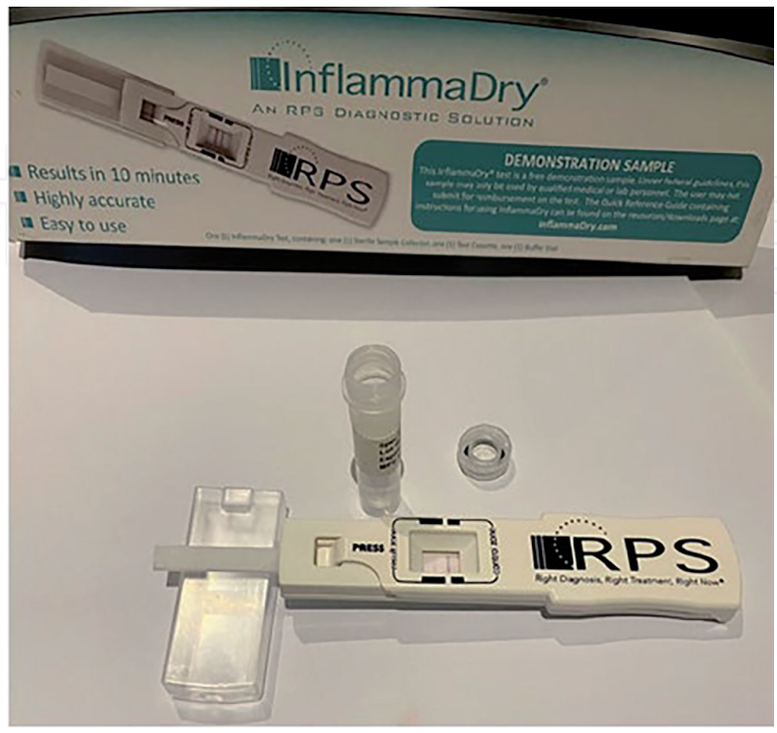

Figure 3.
InflamaDry.

immunoassay where it was concluded that the positivity of the test can depend on the volume of the load and that therefore it can cause false negatives in diseases with aqueous deficiency such as Sjögren's syndrome or graft-versus-host disease [41]. Other useful benefits are the identification of patients with inflammation of the ocular surface and autoimmune disease and can facilitate the decision to establish an anti-inflammatory treatment in these patients [30]. In the same way, its usefulness for the preoperative management of the ocular surface was reported, reducing possible complications when there is an underlying disease. Additionally, this test can also help indicate the need for anti-inflammatory therapies, such as cyclosporine or steroids, and can also predict which patients are most likely to respond [42].

2.5.4 The Schirmer test

The Schirmer test was introduced by Schirmer in 1903, despite the time and disadvantages of performing it, it is still used in most parts of the world, as part of the dry eye test battery. The Schirmer test is a diagnostic method to evaluate the production of watery tears [43, 44]. Topical anesthesia may or may not be used during the procedure, depending on the physician's preference, and may even include nasal stimulation to induce reflex tearing. The Schirmer test allows the study of total tear secretion, that is, it evaluates the sum of the basal secretion plus the reflex secretion if performed without anesthesia; but if performed under anesthesia, it measures the basal secretion produced by the accessory lacrimal glands located in the conjunctiva. The Schirmer test is a diagnostic method to evaluate the production of watery tears [44, 45].

Initially, the method described to perform the Schirmer 1 test is as follows: it should be performed a few minutes after the instillation of topical anesthetic to inhibit the reflex secretion produced by the main lacrimal gland. Whatman No. 1 filter paper 5 mm wide and 35 mm long is used. It is placed in the cul-de-sac at the level of the outer third of the lower eyelid, in a low-light environment, with the patient with his eyes open, and the length of the paper that has absorbed moisture is measured at 5 min [46].

The Schirmer test remains a useful and repeatable test; although over the years several authors have made modifications to their methodology to improve the reliability of the test results; Such studies have been justified by mentioning the lack of practicality for ophthalmologists, especially when performing it routinely due to the 5-min time frame [45]. Therefore, correlations have been evaluated between the different wetting times, the size of the paper strip, or whether it is done with eyes open or closed [45, 47, 48]. The results are controversial. Some authors support the hypothesis that the shorter durations of the 5-min Schirmer test, such as the 1-min test under anesthesia, have a high correlation with the 5-min test and will make this test much more practical for patients [45]. Others affirm that the correlation in this modification was poor and consider it inappropriate to shorten the duration of the Schirmer test [49]. As this controversy has been generated, other tests have been tried with a shorter soak time, such as the Schirmer test of 2 min under anesthesia, and that the 3 mm wide paper strip could be used instead of the standard strip of 5 mm wide; finding results that correlate well with the 5-min test [47]. Then another study was conducted with a 3-min time, the results of which reveal that the 3-min Schirmer test is a reliable alternative for the diagnosis of dry eye and is more useful in daily ophthalmic practice [50]. The position of the eyes (open or closed) when performing the Schirmer test has shown important differences in its results; moisture values in the Schirmer test with eyes open have been reported to show significantly higher results

compared with eyes closed [51, 52]; this may be due to the open eyes moving during the blink, stimulating tearing under the influence of the strip.

Other factors that could influence the results of the Schirmer test are head position, lighting, strip position, corneal condition, humidity, and ambient temperature, so it is important to consider these factors to achieve the standardization of this important diagnostic tool [51].

Therefore, the recommendation to perform the Schirmer test under anesthesia is to perform it with the eyes closed; this could reduce humidity variations, evaporation, and especially reflex tearing [51, 52].

The interpretation of the results of the Schirmer test is different depending on whether anesthesia is used or not. In patients with dry eye, there are clinical differences between the ADDE and EDE types, since the presence of ADDE strongly suggests the suspicion of Sjogren's syndrome; however, although they also present differences in the physiology of tears between these types, no significant differences are found in tear osmolarity, volume, or distribution [53]. In the Schirmer test without anesthesia, total secretion (basal and reflex) is evaluated, the cutoff point is between 10 and 15 mm, and in the Schirmer test with anesthesia, reflex secretion is evaluated, and the cutoff point for this test is ≤5.5 mm/5 min; sensitivity is 85% and specificity is 83% [54]. The Schirmer II test is used on special occasions, as it requires stimulation of the nasal surface with a cotton-tipped applicator and subsequent measurement of reflex tear production. The disadvantages of the Schirmer test, in all its variants, are the time required, patient discomfort, and the low reliability of the test [54, 55].

2.5.5 Tear breakup time (TBUT)

The tear breakup time (TBUT) measures the stability of the tear film. With instilled fluorescein, TBUT is the time interval after a patient blinks until the first appearance of dryness in the tear film [56]. TBUT is measured by inoculating fluorescein into the cul-de-sac, asking the patient to hold the eyelids open after 1–2 blinks, and counting the seconds until a dry spot appears. The appearance of dry spots in less than 10 s is considered abnormal (**Figure 4**) [57]. Subsequently, alternative values

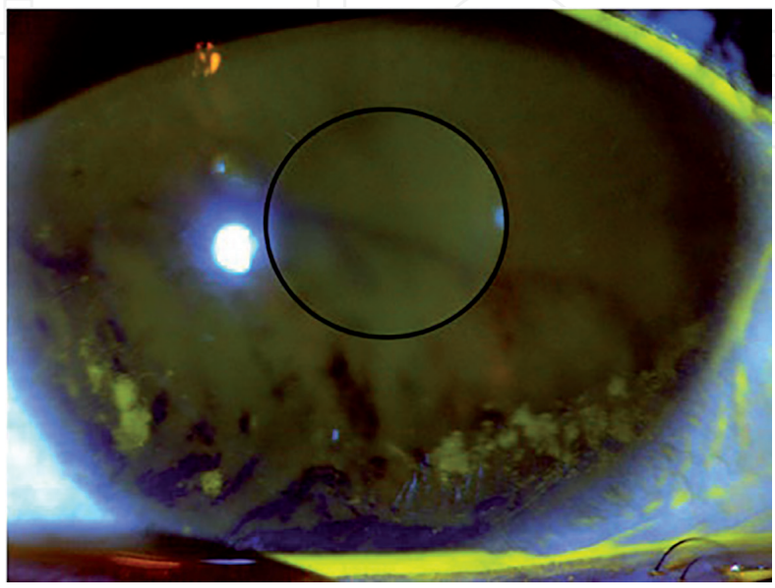

Figure 4.
TBUT.

for BUT are determined by comparing young populations with dry eye and healthy controls, reporting that values less than 5 s occur in subjects with dry eye [58]. The TBUT presents a sensitivity of 72.2% and specificity of 61.6%. The standardization of the method to perform the tear rupture time was carried out by Johnson and Murphy in 2005 [59]. The procedure is summarized in **Table 2**.

Although this test is inexpensive, quick to perform, and uses readily available supplies, it is poorly reproducible and inaccurate [60, 61]. However, the average score of two separate TBUT measurements helps increase their repeatability [62].

2.5.6 Noninvasive keratograph breakup time (NIKBUT)

The tear breakup time (TBUT) was considered one of the most repeatable tear film measurements and although invasive, easy to perform, and interpret [63, 64]. TBUT traditional objective tests are often limited by their invasiveness and low test repeatability and reproducibility [64]. For this reason, new methods of evaluation of the tear film emerged and with the help of the keratograph 5M (K5M) topography, a more objective way of evaluating tear film stability emerged; calling this measurement noninvasive keratograph breakup time (NIKBUT). The time is measured in seconds between the last complete blink and the first alteration of the Placido rings projected on the corneal surface, which the device automatically detects and

1. Instill 1st 5 ul sodium fluorescein 2% in cul-de-sac
2. The patient is asked to blink naturally, loosely so that the fluorescein is naturally distributed.
3. After 10–30 s, the patient is asked to look straight ahead without blinking until instructed.
4. Set the magnification of the biomicroscope to 10×, keep the background illumination constant (cobalt blue light) and use a 12 Wratten yellow filter to improve the observation of the tear film over the entire ocular surface.
5. Use a stopwatch to record the complete blink time and the appearance of a growing micelle.
Tomado de [59].

Table 2.
Evaluation of tear rupture time.

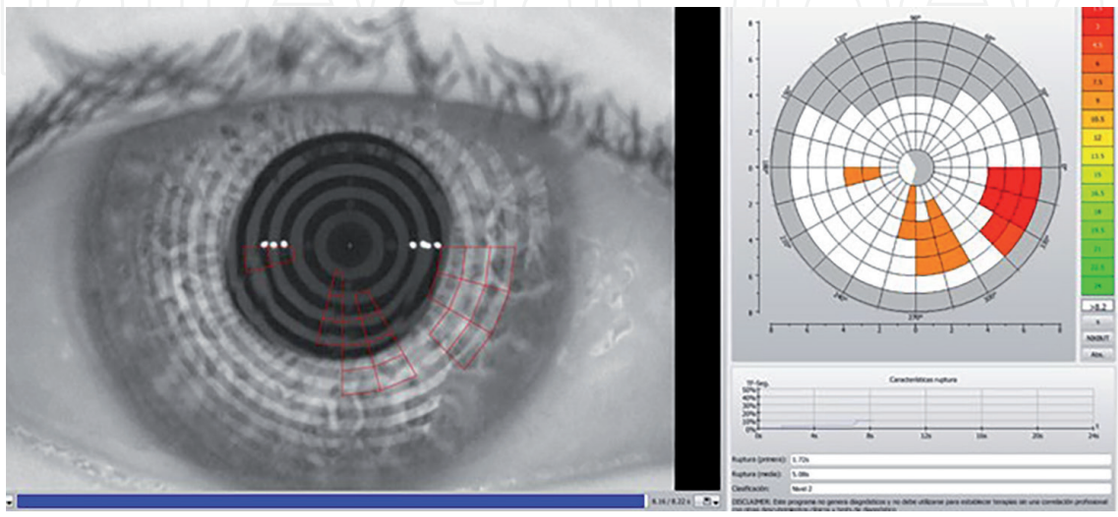

Figure 5.
NIKBUT.

measures. K5M performs two measurements for NIKBUT: the time when the first tear film break occurs (NIKBUT-first) and the average time of all tear incidents (average NIKBUT) (**Figure 5**). Oculus K5M provides noninvasive, objective, and reproducible tear film measurements [65].

2.5.7 Sjögren's international collaborative clinical alliance ocular surface staining (SICA OSS)

Ocular surface staining with various dyes is used to characterize the disease, assess its severity, and monitor the clinical response to therapy. Fluorescein stains macroulcerative and punctate epithelial defects (positive staining) and appears orange under cobalt blue light. Classification of ocular surface staining after instillation of vital dyes is a critical diagnostic component of dry eye. These changes in the ocular surface are now documented with various staining scales such as Oxford, Nei-Clek, and SICA OSS [66]. The evaluation of the ocular surface and the qualification of the three scales are carried out with the methodology described in **Table 3**.

To evaluate the SICCA OSS, it gives the same numerical weight to the corneal and conjunctival changes, the staining must be done in two steps. Fluorescein staining is first performed and the cornea is examined with the slit lamp using the cobalt blue filter. Corneal epithelial staining is a dynamic and time-sensitive process; therefore, to ensure that the test is reproducible, the fluorescein evaluation should begin 4–8 min after instillation of the dye. Punctate epithelial erosions (PEE) that stain with fluorescein are counted and scored as follows. If there is no PEE, the score is 0. If 1–5 PEE are seen, the corneal score is 1 (**Figure 1a**). From 6 to 30 PEE they are scored as 2; and >30 PEE are scored as 3. An additional point is added if: (1) PEE occurred in the central 4 mm diameter part of the cornea (**Figure 1b**); (2) one or more filaments are seen anywhere on the cornea; or (3) one or more confluence patches. The spots, including the linear spots, are found anywhere on the cornea (**Figure 1c**). The fluorescein score for the cornea (the PEE grade plus any extra points for modifiers) is noted in the center square of the SICCA eye staining score form (**Figure 2**). The maximum possible score for each cornea is 6 [67].

The second step is the evaluation of the conjunctiva with lysamine green, it is observed in the slit lamp using a 10× magnification but with reduced illumination and using a neutral density filter. It is important to examine and grade the eyes immediately after applying the lysamine green tint because the intensity and spread of the tint in the eye rapidly diminish after the first 2 min. In addition, the patient must blink several times to prevent the dye from accumulating in the conjunctival folds, which can mimic conjunctival staining. If the dye is not properly instilled, a second drop can be administered and the test is performed immediately afterward.

1. Fluorescein is applied
2. The biomicroscope is configured with 10× magnification and use of cobalt blue light in addition to a yellow filter.
3. Cornea: The upper eyelid is lifted to evaluate the total score of the staining or its absence.
4. Conjunctiva: To determine the degree of staining of the nasal and temporal conjunctiva, the subject is asked to look to the opposite side to evaluate said area.
Tomado de [66].

Table 3.
Evaluation of the ocular surface (Oxford, Nei-cleck and Sica OSS).

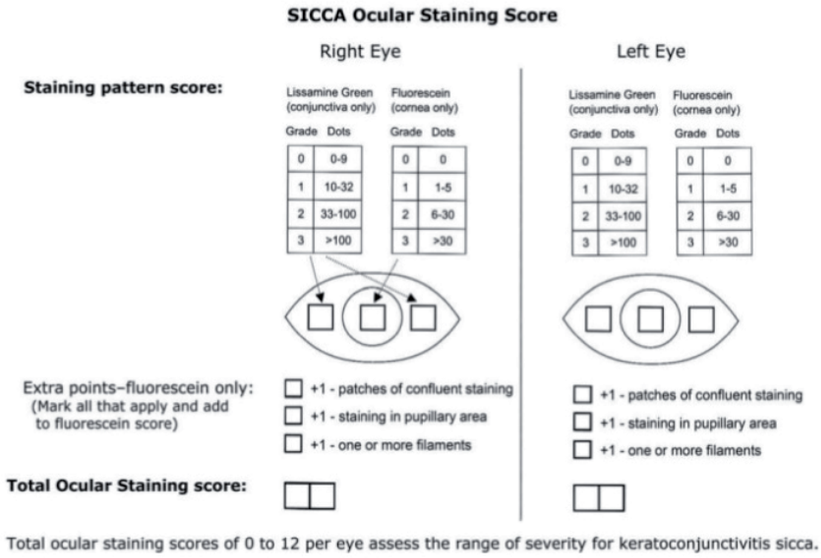

Figure 6.
Sjogren international collaboration clinical Alliance (sicca) ocular staining score form.

The evaluation of this second staining in SICCA OSS, grade 0 (**Figure 3a**) is defined as 0–9 points of lysamine green staining of the interpalpebral bulbar conjunctival (nasal and temporal bulbar conjunctiva classified separately); grade 1 (**Figure 3b**) is defined by the presence of 10–32 points; grade 2 (**Figure 3c**) from 33 to 100; and grade 3 (**Figure 3d**) >100 points. Due to the difficulty of counting individual points in a moving eye in the slit lamp, any area of confluent staining ≥4 mm^2 is considered >100 points. The nasal and temporal areas of the conjunctiva are classified separately with a maximum score of 3 for each area or a maximum total score of 6 for each eye (nasal plus temporal). The total SICCA OSS value for each eye is the sum of the fluorescein score for the cornea and the lysamine green scores for the nasal and temporal bulbar conjunctiva. Therefore, the maximum possible score for each eye is 12. The eyes are classified separately and the scores are recorded on the SICCA OSS Eye Staining Scoring Form at each patient visit. Pinguecula stain, pterygium, and Schirmer's strip artifacts should not be included in the evaluation [67].

A value greater than 0 is considered abnormal and can be a sign of KCS. But scores of 1 or 2 can also represent a late staining artifact, if in the corneal fluorescein interpretation the staining pattern is delayed more than 8 min. Since this could lead to a high level of misclassification, an abnormal OSS is defined as a score of 3 or more (**Figure 6**) [67].

In one study, the relationship between serological markers and dry eye severity was investigated in subjects with primary Sjögren's syndrome (SS). The serum markers anti-Ro/SSA, anti-La/SSB, rheumatoid factor (RF) and antinuclear antibody (ANA), ocular surface disease index (OSDI), Schirmer test I values, time to rupture of the tear film, and SICCA ocular staining score (OSS) were determined. Finding what serum RF and ANA levels are associated with conjunctival staining scores and total OSS based on SICCA OSS in primary SS [68].

2.5.8 The tear meniscus height (TMH)

The meniscus, or lacrimal lake, is the amount of tears that rest at the junction of the bulbar conjunctiva and the margin of the lower eyelid. Measurements of

the height and curvature of the tear meniscus are used to determine the presence or absence of dry eye [65]. The normal height of the tear meniscus is 0.2–0.5 mm; but in patients with dry eye, it is usually less than 0.2 mm [69, 70]. The evaluation of the tear meniscus should be carried out without having instilled artificial tears or other types of drops; since it can be higher and therefore unreliable; although measurement of the tear meniscus is a good parameter for determining tear production, a low tear lake alone does not necessarily indicate dry eye and should be used as an adjunct to other dry eye tests [69]. The tear meniscus can be reliably assessed using a slit lamp that is capable of micrometer measurements, or it can be done by taking a photograph of the tear meniscus after instilling a small amount of fluorescein. Tear meniscus height (THM) is a noninvasive test for tear quantification and is used as a reliable and repeatable adjunct to the diagnosis of DED [71]. Placido's advanced topography, the keratograph 5M (K5M; Oculus Optikgerate GmbH, Wetzlar, Germany), has additional imaging modalities designed to measure TMH [72, 73]. TMH can also be measured using a corneal adapter, in optical coherence tomography, RTVue-100 FD-OCT system (Optovue, Fremont, AC); reliably and noninvasively. Studies comparing these technologies found that although the tear meniscus height measurements measured with keratography are lower than those taken with OCT, they are closely correlated with each other; therefore, either method could be useful to assess the height of the tear meniscus more objectively [74]. Lacrimal meniscus height (TMH) is an important test because it shows a positive correlation with the value of the Schirmer test [75, 76]. Lacrimal point occlusion in patients with dry eye has been shown to induce increases in the volumes of the upper and lower tear meniscus, but no change in lacrimal menisci of control eyes; this may indicate the presence of a self-regulatory mechanism in the lacrimal system that maintains balance in tear volume (**Figure 7**) [77].

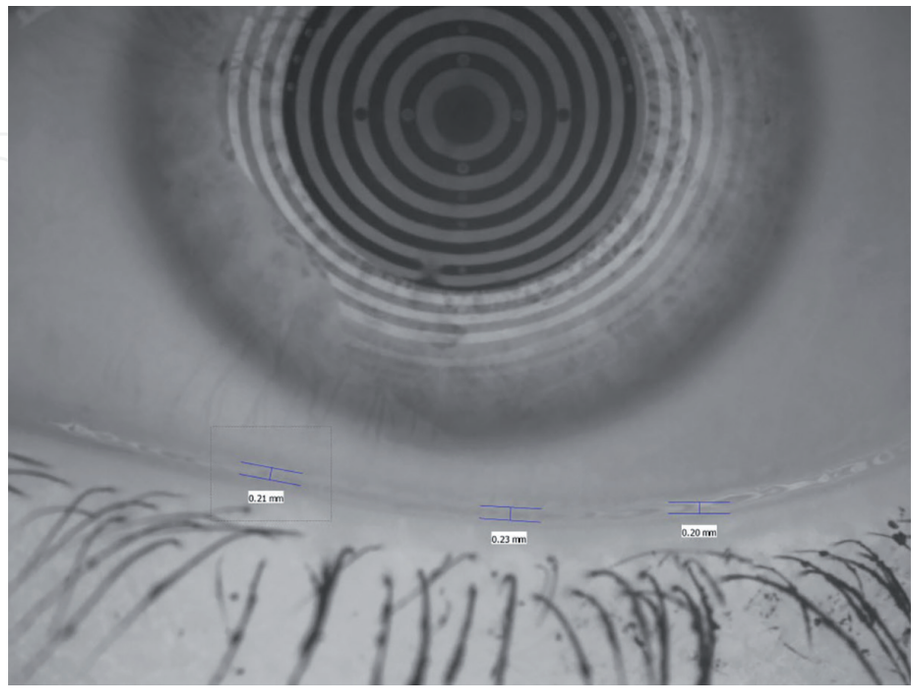

Figure 7.
Tear meniscus height.

2.5.9 Meibography

The meibomian glands are highly specialized sebaceous glands that secrete a lipid compound that is responsible for tear stability. Meibomian gland disease (MGD) is defined as a chronic and diffuse abnormality of the meibomian glands (MG), commonly characterized by terminal duct obstruction and qualitative/quantitative changes in glandular secretion [78]. The prevalence of MGD in published studies ranges from 20% to 70%, depending on diagnostic criteria for evaluating MGD and geographic differences [79, 80]. MGD is considered the main cause of evaporative dry eye [81, 82].

The pathophysiology of MGD is complex and involves several mechanisms and risk factors, including primary obstructive keratinization of the MG orifices, inflammation of the eyelids, abnormal MG secretion, and changes in the microbial flora of the ocular surface or Demodex infestation [24].

Among the main mechanisms of pathophysiology of MGD in patients with SS, the following have been proposed. Women with pSS have been shown to have androgen deficiency similar to that of patients with SLE and RA [83]; in these patients there is a significant decrease in serum levels of androgen precursors (DHEA [dehydroepiandrosterone] and DHEA sulfate), which are the main substrate for androgen synthesis in peripheral tissues. Androgen deficiency in humans has been found to be associated with an increase in eyelid keratinization and the number of occluded and metaplastic orifices of MGs; reduction in the quality of meibum; altered lipid patterns of the meibum; a decrease in the breakup time of the tear film; increased corneal staining with consequent increase in DED symptoms. It has been proposed that the beneficial effect of androgens stimulates the function of this glandular tissue, increasing its lipogenesis and suppressing its keratinization, this may explain why androgen therapy relieves signs and symptoms of DGM and DED in humans [24].

Additionally, it has been proposed that androgen deficiency can lead to a decrease in the influence of insulin-like growth factor 1 (IGF-1) on the meibomian glands. These androgens increase IGF-1 production, positively regulate IGF-1 receptor expression, modulate IGF-1 signaling, and stimulate human meibomian gland epithelial cell function [84]. On the other hand, there is an accumulation of lymphocytes in the conjunctiva of patients with SS and leads to signs of tarsal and periglandular inflammation. Such conjunctival inflammation can create a toxic environment, leading to the release of proinflammatory cytokines that affect the terminal duct of the meibomian gland [85]; as evidence of this, both SSp and SSs subjects and MGD patients showed occluded metaplastic orifices of the MG, as well as decreased quality of the secretions of these glands. These signs are characteristic of obstructive MGD (hyperkeratinization of the terminal duct epithelium, reduced quality of the meibum, and posterior obstruction) [24]. It has been hypothesized that some symptoms in pSS patients, such as sleep disturbances, depressed mood, and fatigue, may also lead to GDM due to decreased blinking, as the above symptoms are already the result of a lower secretion of dopamine, positively with the frequency of blinking. In patients with SS, the lacrimal glands are severely damaged early in the disease; however, the meibomian glands in patients with SSp will undergo further deterioration when the clinical history of dry eye is more than 3 years [85].

Another mechanism contributing to MGD in SS may be that lymphocytic infiltration adjacent to the tarsal conjunctiva in SS could trigger gland destruction.

However, it is possible that conjunctival inflammatory cytokines secreted in the tear film promote hyperkeratinization of the terminal duct epithelium of the meibomian gland [24]. Another process implicated in the pathophysiology of MGD in SSp may be a cytokine-induced alteration in neural-meibomian gland epithelial cell interactions. The meibomian gland is the only sebaceous gland in the human body that has elaborate sensory, sympathetic, and parasympathetic innervation. We found that parasympathetic neurotransmitters and their agonists exert a significant influence on human meibomian gland epithelial cell activity. These cells contain muscarinic acetylcholine and vasoactive intestinal peptide receptors, and the ligands of these receptors stimulate the adenylyl cyclase pathway, enhance intracellular [Ca^{2+}], and can promote cell proliferation [85, 86].

Meibography is a diagnostic technique that allows in vivo evaluation of the morphology of the meibomian glands [87, 88]. For its evaluation, several techniques have been developed, among which are meibography with infrared light. This uses ultraviolet light to produce fluorescence of the gland, the eyelid is everted and through transillumination photographs are obtained to obtain images, which are later graded according to the loss of the meibomian glands. The classification of these images go from 0 to 3; being 0 when there is no loss, 1 when the loss of glands is less than one-third, 2 when the loss is between one-third and two-thirds and 3 when the loss is greater than two-thirds. Meibomian glands are observed differently depending on the method to evaluate them, for example, they appear as dark areas on a lighter background in the contact technique. In contrast, noncontact meibography

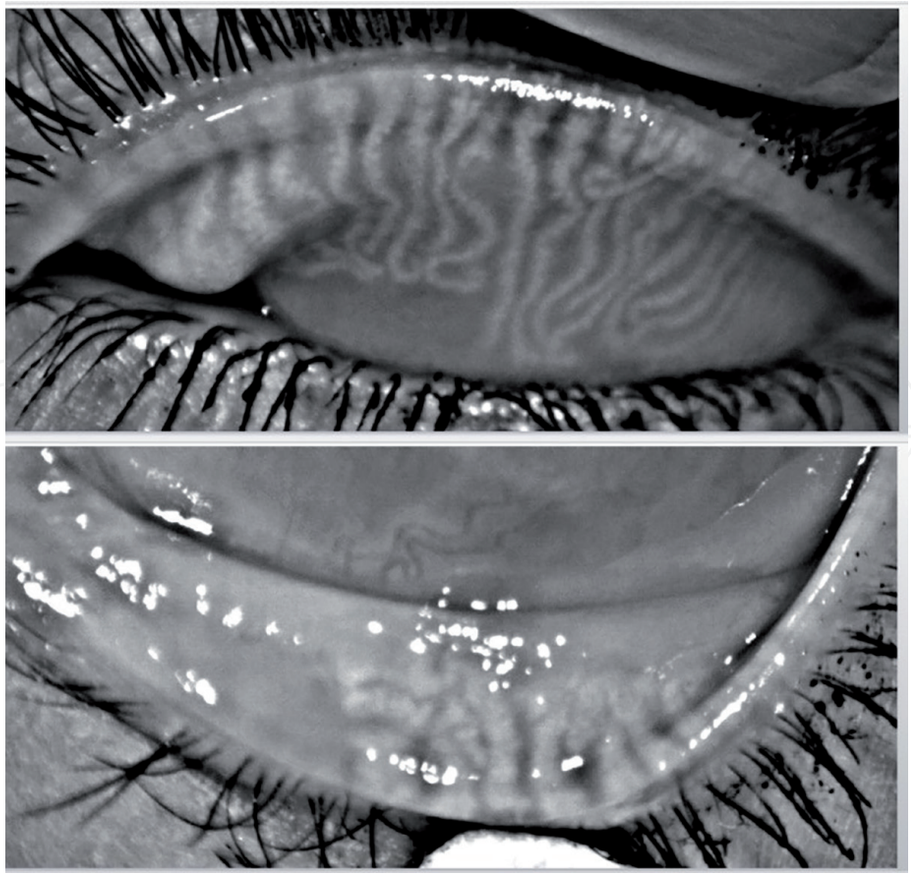

Figure 8.
Meibography.

reveals the meibomian glands as reflected images, and the glands appear as light areas on a darker background [89]. The types of meibography that allow the capture of the morphology of the Meibomian gland in the form of photographs or films are LipiView 2; Tear Science, Cobra, LipiScan, and Tearscope [89]. The meiboscore [87] and the meibo-scale [90] are classification systems to quantify the loss of area of the meibomian gland. The meiboscores of the upper and lower eyelids are summed to obtain a total score of 0–6 for each eye [87]. In contrast, meibo-scale assigns a value from 0 to 4 for each eyelid [90]. The sensitivity and specificity for the diagnosis of MGD by noncontact meibography (cutoff value for meiboscore of +3) as a single test were found to be 49.3% and 64.5%, respectively [91]. The diagnosis of obstructive MGD based on any of the three scores (ocular symptom score, eyelid margin anomaly score, and meiboscore) resulted in a sensitivity of 100% and a specificity of 68.3%; the diagnosis based on two of the three abnormal scores yielded a sensitivity of 84.9% and a specificity of 96.7%; and diagnosis based on the fact that all three scores were abnormal yielded a sensitivity of 66.0% and a specificity of 100% (**Figure 8**) [92].

3. Conclusions

The new emerging technologies for the diagnosis of DED represent a more objective way of talking about this entity that does not have a gold standard for its diagnosis, they facilitate the work and are very useful for research purposes. It is also important to mention that these technologies are of great help to provide an accurate diagnosis and accurate treatment; however, the preexisting tests for the diagnosis of DED are still useful and when applied with adequate methodology and the required sequence, they provide reliable data to make a correct diagnosis of DED; therefore they continue to be valid and must continue to be transmitted to new generations, especially to take advantage of contact with the patient and clinical observation that is not substitutable with anything.

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Conflict of interest

I seriously declare that I have no interest whatsoever, only the academic one, in the elaboration of this chapter.

Notes/thanks/other declarations

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List of abbreviations

AntiRho/SSA and AntiLa/SSB	Antibodies
ANA	Antinuclear antibodies
DED	Dry eye disease
DEWS II	Dry eye workshop II
ADDE	Acuodeficiency dry eye
EDE	Evaporative dry eye
TMH	Tear meniscus height
RF	Rheumatoid factor
GAGS	Sulfated glycosaminoglycan
IL	Interleukin
K5M	Keratograph 5M
KCS	keratoconjunctivitis sicca
MMP-9	Matrix metalloproteinase 9
MGD	Meibomian gland dysfunction
MUC5A	Mucin 5A
OSDI	Ocular surface disease index
OCT	Optical Coherence Tomography
PEE	Punctate Epithelial Erosions
TBUT	Tear breakup time
TNF	Tumor Necrosis factor
NIKBUT	Noninvasive keratograph breakup time
NFkB	Nuclear factor enhancer of the kappa light chains of activated B cells
SS	Sjögren Syndrome
SICA OSS	Sjögren's International Collaborative Clinical Alliance Ocular Surface Staining

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References

[1] Mariette X, Criswell LA. Primary Sjögren's syndrome. The New England Journal of Medicine. 2018;**378**:931

[2] Wang X, Bootsma H, Terpstra J, et al. Progenitor cell niche senescence reflects pathology of the parotid salivary gland in primary Sjögren's syndrome. Rheumatology (Oxford). 2020;**59**:3003

[3] Vivino FB, Bunya VY, Massaro-Giordano G, et al. Sjogren's syndrome: An update on disease pathogenesis, clinical manifestations and treatment. Clinical Immunology. 2019;**203**:81

[4] Kassan SS, Moutsopoulos HM. Clinical manifestations and early diagnosis of Sjögren syndrome. Archives of Internal Medicine. 2004;**164**:1275-1284

[5] Retamozo S, Acar-Denizli N, Rasmussen A, Horváth IF, Baldini C, Priori R, et al. Systemic manifestations of primary Sjögren's syndrome out of the ESSDAI classification: Prevalence and clinical relevance in a large international, multi-ethnic cohort of patients. Clinical and Experimental Rheumatology. 2019;**37**:97-106

[6] Craig J, Nichols K, Akpek E, Caffery B, Dua H, Joo C, et al. TFOS DEWS II definition and classification report. The Ocular Surface. 2017;**15**(3): 276-283

[7] Zeev MS, Miller D, Latkany R. Diagnosis of dry eye disease and emerging technologies. Clinical Ophthalmology. 2014;**8**:581-590

[8] Bron AJ, Abelson MB, Ousler G, Pearce E, Tomlinson A, Yokoi N, et al. Methodologies to diagnose and monitor dry eye disease: Report of the diagnostic methodology subcommittee of the international Dry Eye Workshop (2007). Ocular Surface. 2007;**5**(2):108-152

[9] Milner MS, Beckman KA, Luchs JI, Allen QB, Awdeh RM, Berdahl J, et al. Dysfunctional tear syndrome: Dry eye disease and associated tear film disorders - new strategies for diagnosis and treatment. Current Opinion in Ophthalmology. 2017;**27**(Suppl 1):3-47

[10] Schiffman R. Reliability and validity of the ocular surface disease index. Archives of Ophthalmology. 2000;**118**(5):615

[11] Zhang XM, Yang LT, Zhang Q, Fan QX, Zhang C, You Y, et al. Reliability of Chinese web-based ocular surface disease index questionnaire in dry eye patients: A randomized, crossover study. International Journal of Ophthalmology. 2021;**14**:834-843 10.18240/ijo.2021.06.07

[12] Potvin R, Makari S, Rapuano CJ. Tear film osmolarity and dry eye disease: A review of the literature. Clinical Ophthalmology. 2015;**9**:2039-2047

[13] Méndez GV, Mohamed-Noriega K, Mohamed J, et al. Meibomian gland dysfunction in primary Sjögren's syndrome. ARVO Annual Meeting. 2017;**58**(8)

[14] Schargus M, Wolf F, Tony H-P, Meyer-ter-Vehn T, Geerling G. Correlation between tear film osmolarity, dry eye disease, and rheumatoid arthritis. Cornea. 2014;**33**:1257-1261

[15] Gilbard JP, Farris RL. Tear osmolarity and ocular surface disease in keratoconjunctivitis sicca. Archives of Ophthalmology. 1979;**97**:1642-1646

[16] Holly FJ, Patten JT, Dohlman CH. Surface activity determination of aqueous tear components in dry eye patients and normals. Experimental Eye Research. 1977;**24**:479-491

[17] Gilbard JP, Farris RL, Santamaria J. Osmolarity of tear microvolumes in keratoconjunctivitis sicca. Archives of Ophthalmology. 1978;**96**:677-681

[18] Holly FJ, Lamberts DW. Effect of nonisotonic solutions on tear film osmolarity. Investigative Ophthalmology & Visual Science. 1981;**20**:2336-2345

[19] Fujikawa LS, Foster CS, Gipson IK, Colvin RB. Basement membrane components in healing rabbit corneal epithelial rounds: Immunofluorescence and ultrastructural studies. Journal of Cell Biology. 1984;**98**:128-138

[20] Funderburgh JL, Cintron C, Covington HI, Conrad GW. Immunoanalysis of keratan sulfate proteoglycan from corneal scars. Investigative Ophthalmology & Visual Science. 1988;**29**:116-124

[21] Tseng SCG, Hirst LW, Maumenee AE, Kenyon KR, Sun TT, Green WR. Possible mechanisms for the loss of goblet cells in mucin- deficient disorders. Ophthalmology. 1984;**91**:545-552

[22] Berra A, Berra M. Hyperosmolarity induce nuclear translocation of NF-KB in human conjunctiva epithelial cells. Investigative Ophthalmology & Visual Science. 2005;**46**:4402

[23] Hirata H, Miserska K, Mafurt C, Rosenblatt M. Hyperosmolar tears induce functional and structural alterations of corneal nerves: Electrophysiological and anatomical evidence toward neurotoxicity. Investigative Ophthalmology & Visual Science. 2015;**56**(13):8125-8140

[24] Bron A, de Paiva CS, Chauhan SK, Bonini S, Gabison EE, Jain S, et al. TFOS DEWS II pathophysiology report. The Ocular Surface. 2017;**15**:438-510

[25] Sullivan BD, Whitmer D, Nichols KK, Tomlinson A, Foulks GN, Geerling G, et al. An objective approach to dry eye disease severity. Investigative Ophthalmology & Visual Science. 2010;**51**(12):6125-6130. DOI: 10.1167/iovs.10-5390 Epub 14 July 2010. PMID: 20631232

[26] Yazdani M, Elgstøen KBP, Rootwelt H, Shahdadfar A, Utheim ØA, Utheim TP. Tear metabolomics in dry eye disease: A review. International Journal of Molecular Sciences. 2019;**20**:3755

[27] Korb DR. Survey of preferred tests for diagnosis of the tear film and dry eye. Cornea. 2000;**19**:483-486

[28] Schargus M, Geerling G, Joachim SC. Significance of new methods of examining the tear film in dry eye disease: tear film osmolarity and matrix metalloproteinases (MMP-9). Klinische Monatsblätter für Augenheilkunde. 2018;**235**:597-602

[29] Lemp MA, Bron AJ, Baudouin C, Benítez Del Castillo JM, Geffen D, Tauber J, et al. Tear osmolarity in the diagnosis and management of dry eye disease. American Journal of Ophthalmology. 2011;**151**:792-798

[30] Messmer EM, von Lindenfels V, Garbe A, Kampik A. Matrix metalloproteinase 9 testing in dry eye dis- ease using a commercially available point-of-care immunoassay. Ophthalmology. 2016;**123**:2300-2308

[31] Sambursky R, Davitt WF 3rd, Friedberg M, Tauber S. Prospective, multicenter, clinical evaluation of point-of-care matrix metalloproteinase-9 test for

confirming dry eye disease. Cornea. 2014;**33**:812-818

[32] Asahi M, Wang X, Mori T, Sumii T, Jung JC, Moskowitz MA, et al. Effects of matrix metalloproteinase-9 gene knock-out on the proteolysis of blood-brain barrier and white matter components after cerebral ischemia. The Journal of Neuroscience. 2001;**21**:7724-7732

[33] Sternlicht MD, Werb Z. How matrix metalloproteinases regulate cell behavior. Annual Review of Cell and Developmental Biology. 2001;**17**:463-516

[34] Schargus M, Geerling G, Joachim SC. Significance of new methods of examining the tear film in dry eye disease: tear film osmolarity and matrix metalloproteinases (MMP-9). Klinische Monatsblatter für Augenheilkunde. 2018;**235**:597-602. German. DOI: 10.1055/s-0042-121424 PMID: 28147407

[35] Acera A, Vecino E, Duran JA. Tear MMP-9 levels as a marker of ocular surface inflammation in conjunctivochalasis. Investigative Ophthalmology & Visual Science. 2013;**54**:8285-8291

[36] Sambursky R, Davitt WF, Latkany R, et al. Sensitivity and specificity of a point-of-care matrix metalloproteinase 9 immunoassay for diagnosing inflammation related to dry eye. JAMA Ophthalmology. 2013;**131**(1):24-28

[37] Baudouin C, Messmer EM, Aragona P, Geerling G, Akova YA, Benı'tez-del-Castillo J, et al. Revisiting the vicious circle of dry eye disease: A focus on the pathophysiology of meibomian gland dysfunction. The British Journal of Ophthalmology. 2016;**100**:300-306

[38] Park JY, Kim BG, Kim JS, Hwang JH. Matrix metalloproteinase 9 point-of-care immunoassay result predicts response to topical cyclosporine treatment in dry eye disease. Translational Vision Science & Technology. 2018;**7**:31

[39] Aragona P, Aguennouz M, Rania L, Postorino E, Sommario MS, Roszkowska AM, et al. Matrix metalloproteinase 9 and transglutaminase 2 expression at the ocular surface in patients with different forms of dry eye disease. Ophthalmology. 2015;**122**:62-71

[40] Pinto-Fraga J, Enríquez-de-Salamanca A, Calonge M, González-García MJ, López-Miguel A, López de la Rosa A, et al. Severity, therapeutic, and activity tear biomarkers in dry eye disease: An analysis from a phase III clinical trial. The Ocular Surface. 2018;**16**:368-376

[41] Lanza NL, McClellan AL, Batawi H, Felix ER, Sarantopoulos KD, Levitt RC, et al. Dry eye profiles in patients with a positive elevated surface matrix metalloproteinase 9 point-of-care test versus negative patients. The Ocular Surface. 2016;**14**:216-223

[42] Kaufman HE. The practical detection of mmp-9 diagnoses ocular surface disease and may help prevent its complications. Cornea. 2013;**32**:211-216

[43] Pflugfelder SC, Tseng SC, Sanabria O, et al. Evaluation of subjectiveassessments and objective diagnostic tests for diagnosing tear-film disor-ders known to cause ocular irritation. Cornea. 1998;**17**:38-56

[44] Farris RL, Gilbard JP, Stuchell RN, Mandel ID. Diagnostic tests in kerato-conjunctivitis sicca. The CLAO Journal. 1983;**9**:23-28

[45] Bawazeer AM, Hodge WG. One-minute schirmer test with anesthesia. Cornea. 2003;**22**(4):285-287

[46] Van Bijsterveld OP. Diagnostic tests in the sicca syndrome. Archives of Ophthalmology. 1969;**82**(1):10-14. DOI: 10.1001/archopht.1969.00990020012003

[47] Suphakasem S, Lekskul M, Rangsin R. Assessment of different wetting time and paper strip size of Schirmer test in dry eye patients. Journal of the Medical Association of Thailand. 2012;**95**(Suppl 5):S107-S110 PMID: 22934455

[48] Li N, Deng XG, He MF. Comparison of the Schirmer I test with and without topical anesthesia for diagnosing dry eye. International Journal of Ophthalmology. 2012;**5**(4):478-481. DOI: 10.3980/j.issn.2222-3959.2012.04.14 Epub 18 August 2012. PMID: 22937509; PMCID: PMC3428545

[49] Recep ÖF. Is it possible to shorten the duration of Schirmer test I? Turk Oftalmoloiji Dergisi. 2012;**42**(5):355-358. DOI: 10.4274/tjo.42.73745 2

[50] Özdemir M, Akçay M. Three-minute Schirmer test. Turkiye Klinikleri Journal of Medical Sciences. 2009;**29**(1):146-149

[51] Rieger G. Schirmer-Test in Lokalanästhesie bei geöffneten oder geschlossenen Augen? [The Schirmer test in local anesthesia in open or closed eyelids]. Fortschr Ophthalmol. German. 1986;**83**(2):79-80. PMID: 3710387

[52] Serruya LG, Nogueira DC, Hida RY. Schirmer test performed with open and closed eyes: Variations in normal individuals. Arquivos Brasileiros de Oftalmologia. Jan-Feb 2009;**72**(1):65-67. DOI: 10.1590/S0004-27492009000100013. PMID: 19347125

[53] Khanal S, Tomlinson A, Diaper CJ. Tear physiology of aqueous deficiency and evaporative dry eye. Optometry and Vision Science. 2009;**86**(11):1235-1240

[54] Methodologies to diagnose and monitor dry eye disease: Report of the diagnostic methodology subcommittee of the International Dry Eye WorkShop (2007). The Ocular Surface. Apr 2007;**5**(2):108-152. DOI: 10.1016/s1542-0124(12)70083-6. PMID: 17508118

[55] Damato BE, Allan D, Murray SB, Lee WR. Senile atrophy of the human lacrimal gland: The contribution of chronic inflammatory disease. The British Journal of Ophthalmology. 1984;**68**:674

[56] Lemp MA. Breakup of the tear film. International Ophthalmology Clinics. 1973;**13**(1):97-102

[57] Vitali C, Moutsopoulos HM, Bombardieri S. The Europen Community Study Group on diagnostic criteria for Sjögren's syndrome. Sensitivity and specificity of tests for ocular and oral involvement in Sjögren's syndrome. Annals of the Rheumatic Diseases. 1994;**5310**:637-647

[58] Abelson M, Ousler G, Nally L. Alternative values for tear film breakup time in normal and dry eye populations. Advances in Experimental Medicine and Biology. 2002;**506**(Part B):1121-1125

[59] Johnson M, Murphy P. The effect of instilled fluorescein solution volume on the values and repeatability of TBUT measurements. Cornea. 2005;**24**(7):811-817

[60] Vanley GT, Leopold IH, Gregg TH. Interpretation of tear film breakup. Archives of Ophthalmology. 1977;**95**(3):445-448

[61] Lin YY, Carrel H, Wang IJ, et al. Effect of tear film break-up on higher order aberrations of the anterior cornea

in normal, dry, and post-LASIK eyes. Journal of Refractive Surgery. 2005;**21**(5):S525-S529

[62] Nichols KK, Mitchell GL, Zadnik K. The repeatability of clinical measurements of dry eye. Cornea. 2004;**23**(3):272-285

[63] Phadatare SP, Momin M, Nighojkar P, Askarkar S, Singh KK. A comprehensive review on dry eye disease: diagnosis, medical management, recent developments, and future challenges. Advances in Pharmaceutics. 2015;**2015** Article ID 704946, 12 pages

[64] Mengher LS, Bron AJ, Tonge SR, Gilbert DJ. A non invasive instrument for clinical assessment of the pre-corneal tear film stability. Current Eye Research. 1985;**4**(1):1-7

[65] Tian L, Qu JH, Zhang XY, Sun XG. Repeatability and reproducibility of noninvasive keratograph 5M measurements in patients with dry eye disease. Journal of Ophthalmology. 2016;**2016**:8013621. DOI: 10.1155/2016/8013621 Epub 12 April 2016. PMID: 27190639; PMCID: PMC4844888

[66] Bron A, Evans V, Smith J. Grading of corneal and conjunctival staining in the context of other dry eye tests. Cornea. 2003;**22**(7):640-650

[67] Whitcher J, Shiboski C, Shiboski S, Heidenreich AM, Kitagawa K, Zhang S, et al. A simplified quantitative method for assessing keratoconjunctivitis sicca from the Sjögren's syndrome international registry. American Journal of Ophthalmology. 2010;**149**(3):405-415

[68] Lim SA, Nam S, Kwok SK, Park SH, Chung SH. Serologic markers are associated with ocular staining score in primary Sjögren syndrome. Cornea. 2015;**34**(11):1466-1470

[69] Mainstone JC, Bruce AS, Golding TR. Tear meniscus measurement in the diagnosis of dry eye. Current Eye Research. 1996;**15**(6):653-661

[70] Guillon JP. Non-invasive tearscope plus routine for contact lens fitting. Contact Lens & Anterior Eye. 1998;**21**(Suppl 1):S31-S40

[71] Holly FJ. Physical chemistry of the normal and disordered tear film. Transactions of the Ophthalmological Societies of the United Kingdom. 1985;**104**(4):374-380

[72] Ibrahim OMA, Dogru M, Takano Y, et al. Application of visante optical coherence tomography tear meniscus height measurement in the diagnosis of dry eye disease. Ophthalmology. 2010;**117**(10):1923-1929

[73] Koh S, Ikeda C, Watanabe S, et al. Effect of non-invasive tear stability assessment on tear meniscus height. Acta Ophthalmologica. 2015;**93**(2): e135-e139

[74] Abdelfattah NS, Dastiridou A, Sadda SR, Lee OL. Noninvasive imaging of tear film dynamics in eyes with ocular surface disease. Cornea. 2015;**34**: S48-S52

[75] Savini G, Barboni P, Zanini M. Tear meniscus evaluation by optical coherence tomography. Ophthalmic Surgery, Lasers & Imaging. 2006;**37**(2):112-118

[76] Chen F, Shen M, Chen W, Wang J, Li M, Yuan Y, et al. Tear MENISCUS VOLUME IN DRY EYE AFTER PUNCTAL occlusion. Investigative Ophthalmology & Visual Science. 2010;**51**(4):1965-1969

[77] Lamberts DW, Foster CS, Perry HD. Schirmer test after topical anesthesia and the tear meniscus height in normal eyes.

Archives of Ophthalmology. 1979;**97**(6):1082-1108

[78] Arita R. Meibography: A Japanese perspective. Investigative Ophthalmology and Visual Science. 2018;**59**(14 Special Issue):DES48-DES55

[79] Geerling G, Baudouin C, Aragona P, et al. Emerging strategies for the diagnosis and treatment of meibomian gland dysfunction: Proceedings of the OCEAN group meeting. The Ocular Surface. 2017;**15**:179-192

[80] Eballe' AO, Ellong A, Wang RE, et al. Meibomian glands dysfunction and ocular surface in black people. Journal Français d'Ophtalmologie. 2019;**42**:127-132

[81] Stapleton F, Alves M, Bunya VY, et al. TFOS DEWS II epidemiology report. The Ocular Surface. 2017;**15**:334-365

[82] Schaumberg DA, Nichols JJ, Papas EB, et al. The international workshop on meibomian gland dysfunction: Report of the subcommittee on the epidemiology of, and associated risk factors for, MGD. Investigative Ophthalmology & Visual Science. 2011;**52**:1994-2005

[83] Sullivan DA, Rocha EM, Aragona P, Clayton JA, Ding J, Golebiowski B, et al. TFOS DEWS II sex, gender, and hormones report. The Ocular Surface. 2017;**15**:284-333

[84] Ding J, Liu Y, Sullivan DA. Effects of insulin and high glucose on human meibomian gland epithelial cells. Investigative Ophthalmology & Visual Science. 2015;**56**:7814-7820

[85] Sullivan DA, Dana R, Sullivan RM, Krenzer KL, Sahin A, Arica B, et al. Meibomian gland dysfunction in primary and secondary sjogren syndrome. Ophthalmic Research. 2018;**59**:193-205. DOI: 10.1159/000487487

[86] Hikichi T, Yoshida A, Tsubota K. Lymphocytic infiltration of the conjunctiva and the salivary gland in Sjögren's syndrome. Archives of Ophthalmology. 1993;**111**:21-22

[87] Arita R, Itoh K, Inoue K, Amano S. Noncontact infrared meibography to document age-related changes of the meibomian glands in a normal population. Ophthalmology. 2008;**115**:911-915

[88] Alsuhaibani AH, Carter KD, Abramoff MD, et al. Utility of meibography in the evaluation of meibomian glands morphology in normal and diseased eyelids. Saudi Journal of Ophthalmology. 2011;**25**:61-66

[89] Robin M, Liang H, Rabut G, Augstburger E, Baudouin C, Labbé A. The role of meibography in the diagnosis of meibomian gland dysfunction in ocular surface diseases. Translational Vision Science and Technology. 2019;**8**(6):14-46

[90] Pult H, Riede-Pult BH. Non-contact meibography: Keep it simple but effective. Contact Lens & Anterior Eye. 2012;**35**:77-80

[91] Finis D, Ackermann P, Pischel N, et al. Evaluation of meibomian gland dysfunction and local distribution of meibomian gland atrophy by non-contact infrared meibography. Current Eye Research. 2015;**40**:982-989

[92] Arita R, Itoh K, Maeda S, et al. Proposed diagnostic criteria for obstructive meibomian gland dysfunction. Ophthalmology. 2009;**116**:2058-2063