European Review for Medical and Pharmacological Sciences 2024; 28: 1881-1890

Dry eye disease pathogenesis and clinical signs: searching for correspondence in the clinical practice

S. BARABINO[1], J.M. BENÍTEZ-DEL-CASTILLO[2]

[1]Department of Ophthalmology, Ocular Surface and Dry Eye Center, ASST Fatebenefratelli Sacco, Università di Milano, Milan, Italy
[2]Hospital Clínico San Carlos, Universidad Complutense, Clínica Rementería, Madrid, Spain

Abstract. – BACKGROUND: Ocular surface alterations causing dry eye disease (DED) can be described as a vicious circle consisting of different consecutive stages. Among the factors involved, the ocular surface immune-inflammatory response has been established as a key player in the pathogenesis of the vicious circle of DED. Thus, the prompt recognition of the disruption of the immunoregulatory mechanisms is crucial for properly managing the ocular surface alterations. To increase awareness and knowledge of the identification and clinical interpretation of immunological mechanisms of dry eye in clinical practice, we present two clinical cases related to DED patients to provide a practical example of clinical examination application and interpretation of diagnostic parameters in daily practice. Moreover, a literature overview of the available clinical examinations to assess the immunological involvement in DED patients, with a particular focus on the correlation between diagnostic parameters and pathogenesis of clinical signs, is provided with an educational intent.

CASE PRESENTATION: The presented clinical experiences suggested that in ocular surface pathologies, knowledge of the immune-inflammatory pathogenetic mechanisms underlying the observed clinical sign is of great help for understanding what is being observed in the patient and, consequently, for the choice of appropriate therapy. Literature evidence suggests that many different clinical examinations can be used to assess inflammation in DED patients, such as the assessment of hyperemia, staining of the ocular surface and measurement of hyperosmolarity and MMP-9 levels. The combination of impression cytology and flow cytometry to assess for markers of inflammation is considered the best technique to quantify the level of inflammation on the ocular surface, even if not always applicable in clinical practice.

CONCLUSIONS: Literature evidence and clinical experiences suggest that basic diagnostic approaches (assessment of hyperemia, MMP-9 levels, and staining of the ocular surface with Lissamine green or fluorescein) represent useful tools to assess the inflammatory component of DED in everyday practice, providing a guide to establish the correct therapeutic strategy.

Key Words:
Dry eye disease, Ocular surface alterations, Dry eye disease pathogenesis, Dry eye disease symptoms, Clinical examinations.

Background

Dry eye disease (DED) is a complex, multifactorial ocular condition characterized by tear film instability, increased tear film osmolarity, ocular surface inflammation, and damage, as well as neurosensory abnormalities[1-3]. The loss of tear film stability is accompanied by eye symptoms, including dryness, foreign body sensation, burning and itching sensations, photophobia, blurred and fluctuating vision, and visual fatigue with a consequent great impact on the patient's quality of life[2,4,5]. In severe cases, corneal epithelial exfoliation, filamentary keratopathy, and conjunctival lesions may occur[6].

Ocular surface alterations causing DED can be described in the form of a vicious circle consisting of different stages: (1) tear film instability; (2) tear hyperosmolarity; (3) oxidative stress exerted on tear film lipids and epithelial cells; (4) epithelial damage and recruitment of pro-inflammatory cytokines; (5) cell death due to apoptosis and inflammation; (6) nerve malfunction; and (7) anatomical and functional eyelid changes[7,8]. Squamous metaplasia of the conjunctival epithelium and the loss of goblet cells also make a major contribution, as they affect ocular surface lubrication and tolerance of resident saprophytic flora, in which any change increases the

Corresponding Author: Stefano Barabino, MD; e-mail: stebarabi@gmail.com

inflammatory stimulus[9]. In this context, the key role of the ocular surface immune inflammatory response has been established[10,11]. Indeed, the ocular surface is now considered an 'immunological' unit with the ability to respond to external and internal stimuli and modulate the inflammatory, immunological response to avoid possible negative consequences on its components due to an overresponse or chronic activation of the immune system[11]. Otherwise, excessive external stimuli can elicit an adaptive immunological response, with activation of recruited and tissue-resident macrophages, as well as recruitment of leukocytes and plasma proteins, to maintain tissue functionality. This adaptive response is called para-inflammation and shows intermediate characteristics between the basal and inflammatory states[12]. Generally, para-inflammation is switched off as soon as homeostasis is restored. However, if external damaging stimuli persist for a sustained period, para-inflammation can progressively turn from a beneficial and protective response to a detrimental and damaging process, inducing a continuous inflammatory state[10]. Thus, the prompt recognition of the disruption of the immunoregulatory mechanisms is crucial for the proper management of the ocular surface alterations and to avoid perpetuating the vicious circle of dry eye[8]. In particular, the assessment of the presence and grade of inflammation is essential to evaluate the risk of progression and immunologic shift of the disease towards chronic inflammation and, thus, to plan a proper therapeutic strategy.

In clinical practice, even if there is no gold standard test or well-established cutoff values, some indicators of the presence of ocular surface inflammation and other immune factors involved in DED pathogenesis can be assessed during the first-line examination[10]. Thus, awareness and knowledge of the identification of mechanisms and clinical interpretation of indicators related to immunological involvement in patients with DED are fundamental.

In this paper, we present two clinical cases related to DED patients to provide a practical example of applying clinical examinations and interpreting diagnostic parameters in daily practice. Moreover, a literature overview of the available clinical examinations to assess the immunological involvement in DED patients, with a particular focus on the correlation between diagnostic parameters and pathogenesis of clinical signs, is provided with an educational intent.

Case Presentation

The authors selected and reported two clinical cases of patients with signs and symptoms of DED. The study was conducted in accordance with the ethical principles of the revised version of the Declaration of Helsinki. All patients provided written informed consent to treatment and the publication of clinical data and images.

Case 1

A 69-year-old woman was referred by her ophthalmologist because she complained of foreign body sensation and burning in both eyes, with visual disturbance and a need for eye closure. She reported a diagnosis of DED 5 years before being treated with substitute tears and short periods (no more than 10 days) of treatment with topical steroids. Similar treatments were used when symptoms recurred. She also used ointment to improve corneal re-epithelization. OSDI score was high (62.5). The patient also had a dry mouth and painful joints. Some extra examinations were performed. After the visual acuity test, reporting 8/10 [right eye (RE)] and 7/10 [left eye (LE)] and decreased contrast sensitivity, the Schirmer test and slit lamp examination were performed to measure tear secretions (RE: 3 mm/5'; LE: 2 mm/5') and to observe the epithelial cells, respectively. Fluorescein staining was performed and observed with a yellow filter. The staining showed a diffuse corneal pattern with tiny dots in the superior part (Figure 1A), not typical in DED patients; the break-up time (BUT) was very low (RE: 2 s; LE: 1 s). Staining with Lissamine green (Lissafid®, Farmingea S.p.A., Pisa, Italy) was then carried out to look at the condition of the conjunctiva, assess the degree of inflammation, and exclude superior limbic keratoconjunctivitis (Figure 1B). The Lissamine green staining was also observed in the superior part of the eye, suggesting some degree of toxicity in the ocular surface (Figure 1B). Lastly, corneal sensitivity was assessed through a handheld esthesiometer (RE: 6 cm; LE: 5 cm) to correlate signs and symptoms of DED. According to the examination results, this patient was diagnosed with type III DED due to the observed activation of T cells, which is to be considered a chronic form of DED.

The patient was first referred to the rheumatologist to assess for an auto-immune disease, which must be treated as a first step if diagnosed. To manage DED, discontinuation of toxic preserva-

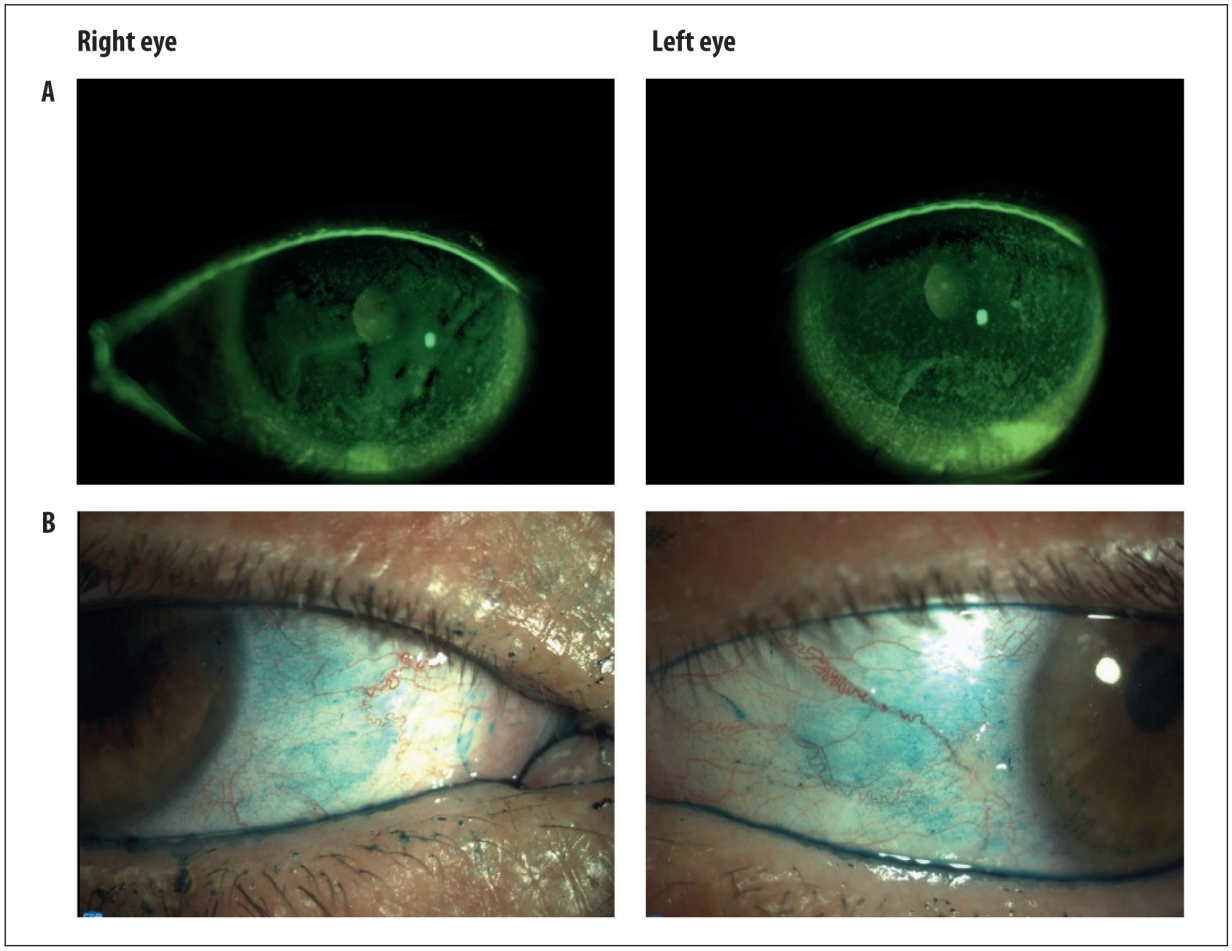

Figure 1. Ocular signs at presentation. **A**, Fluorescein staining observed with a yellow filter showing a diffuse corneal pattern with tiny dots in the superior part. **B**, Lissamine green staining showing diffuse damage of conjunctival epithelial cells in the exposed bulbar conjunctiva.

tive-containing drops was indicated, with a washout period of 10 days. Preservative-free artificial tears and topical steroids for prolonged periods (tapering down the number of instillations according to symptoms and ocular surface changes) were prescribed. Topical cyclosporine once a day and tetracyclines were also prescribed, according to the presence of T-cell infiltration.

This clinical case suggests the importance of taking a medical history of the diagnosis of ocular surface inflammation and systemic anti-inflammatory treatment. Also, the importance of considering preservative-free topical medications and topical anti-inflammatory medications can be suggested.

Case 2

A 69-year-old male patient presented complaining of left eye redness, burning, and poor near vision. DED diagnosis was formulated 5 years before, and the treatments prescribed were lid hygiene, preserved tear substitutes, and ointment at night.

At examinations, the patient reported LE eyelid eczema, conjunctival hyperemia (RE: 0; LE: +2; Figure 2), decreased tear clearance, inflamed lachrymal inferior punctum, ectropion, and keratitis (Figure 2). Near visual acuity was very different between eyes (RE: J2; LE: J5). Due to an inflamed inferior lacrimal punctum, the tears did not come to the nose, and the patient complained about epiphora. Moreover, toxic tears further contribute to eyelid eczema and ectropion. The patient also had rosacea, which was controlled with dermatological treatment.

To manage DED, the patient was advised to stop any type of preserved artificial tears, ointment, and eyelid scrubbing with baby shampoo. Diflucortolone valerate 1 mg/g cream was prescribed for the skin, along with fluorometholone

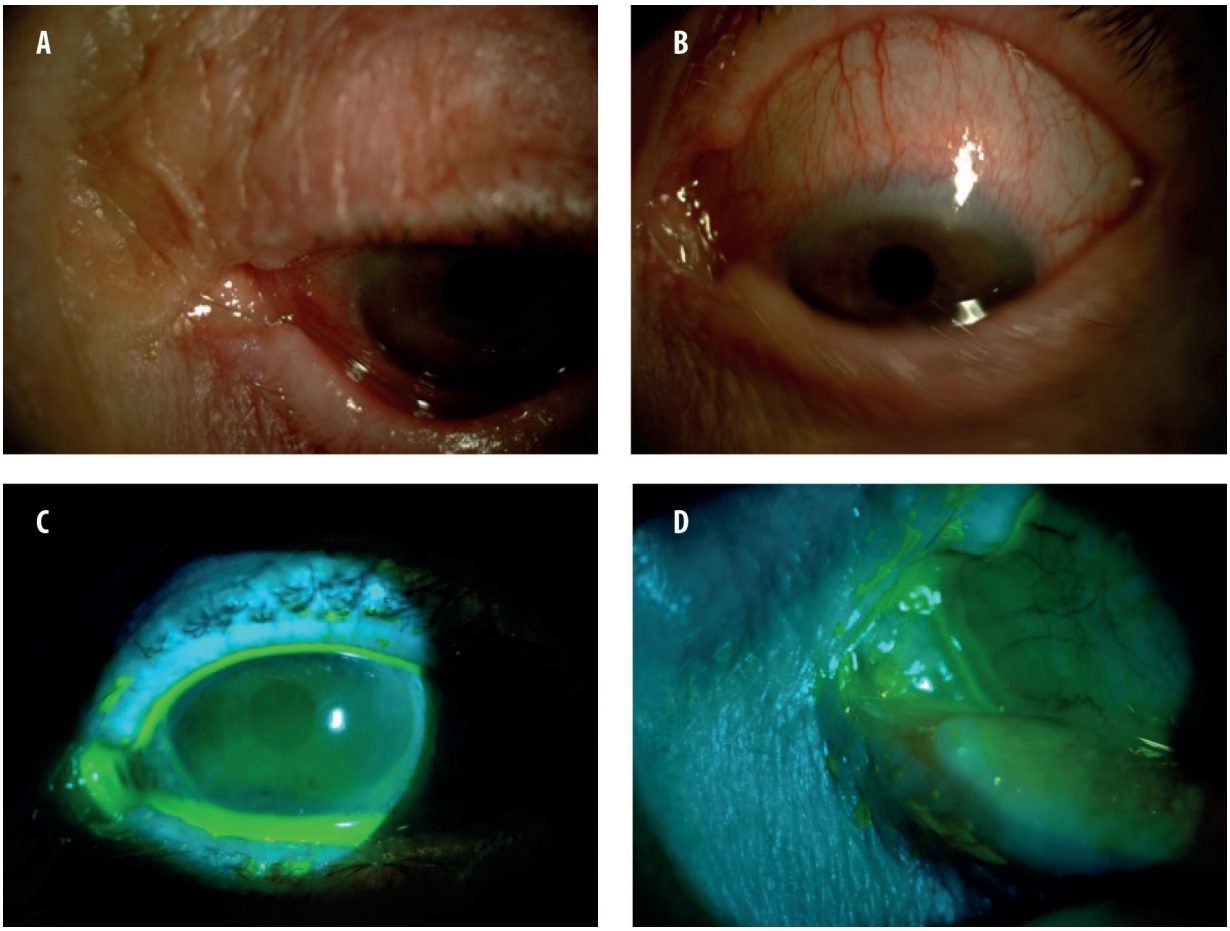

Figure 2. Ocular signs at presentation. **A**, Eczema and ectropion. **B**, Signs of hyperemia. **C**, Fluorescein staining observed with a yellow filter showing decreased tear clearance. **D**, Fluorescein staining observed with a yellow filter showing punctum edema.

0.1% eyedrops (for 1.5 months) and cyclosporin 0.1% eyedrops at night. Lastly, inferior lacrimal punctum dilation was performed to look for the patency of the lacrimal secretory system.

After 2 months, the patient presented with no epiphora, no eyelid eczema, no conjunctival hyperemia or decreased tear clearance, normal lacrymal punctum, no ectropion, and amelioration of keratitis.

This clinical case suggests that DED patients may complain about tearing (epiphora), that burning represents a hallmark of inflammation, which can be caused by preservatives, and that decreased tear clearance means inflammation (or eyelid-blinking problems) and should be treated with anti-inflammatory treatments.

Literature Review

A narrative review was conducted to provide a comprehensive overview of the available examinations useful to detect immunological and inflammatory mechanisms of dry eye in clinical practice, with educational intent, reporting published evidence and the expert opinion of the authors. The literature search was conducted on PubMed up to September 2023, using different combinations of pertinent keywords (e.g., dry eye AND clinical signs; dry eye AND immune-mediated mechanisms; dry eye AND inflammation) without any limitations in time and language. Papers were selected for inclusion according to their relevance to the topic, as judged by the authors.

Assessment of Conjunctival Hyperemia

Conjunctival hyperemia is defined as redness of the bulbar conjunctiva and represents a hallmark of ocular inflammation[13]. The localized inflammatory changes due to DED cause a pathological vasodilatory response of the conjunctival microvasculature and, subsequently, edema and

hyperemia[14]. Moreover, conjunctival hyperemia significantly correlates with conjunctival temperature (calor), another typical sign of inflammation. In particular, a 3-degree change in redness was related to an increase of 0.5°C in temperature[15]. Hyperemia plays a critical role in the efferent component of the immune system, delivering both cellular and humoral immune components to the ocular surface[14]. In this context, the inflammatory process is mediated by physiologically active molecules, such as histamine, cytokines, and associated neuropeptides. Accordingly, hyperemia was correlated with inflammatory cell infiltration, specifically granulocytes, lymphocytes, and monocytes/macrophages, measured by flow cytometry[16].

Hyperemia is the easiest sign to collect among the classic determinants of clinical inflammation in DED and can be evaluated by anterior segment photography and/or with the use of grading scales. When present, bulbar conjunctival hyperemia is usually confined to the area of open-eye exposure. The extent of conjunctival hyperemia is correlated with the severity of ocular inflammation and can be quantified by grading scales, such as the McMonnies/Chapman-Davies scale, the Institute for Eye Research scale, the Efron scale, or other validated bulbar redness scale[10]. Some limitations can be reported for this technique, such as the subjectivity in the interpretation of the degree of ocular redness, the lack of standardization, the influence of external factors, such as lighting conditions, the angle of observation, and patient cooperation, which can all influence the perception of conjunctival hyperemia. To address these limitations, automatic, computer-aided image analysis techniques for conjunctival hyperemia evaluation have been defined[17,18]. Moreover, typical signs of inflammation other than redness and calor, such as pain and loss of function, cannot be assessed by conjunctival hyperemia test, despite the actual presence of inflammation.

Ocular Surface Staining

Corneal and conjunctival staining with topical dyes has been used for a long time to study and characterize ocular surface diseases, including DED, as well as to quantify their severity[19]. In most cases, the staining pattern is useful to diagnose and prognosticate the disease and define the therapeutic management. The bases of dye uptake and staining are based on different anatomical and physiological factors, which are explained in detail in the review by Srinivas and Rao[20]. However, despite common usage, a universally accepted "gold standard" grading scale does not exist for corneal and conjunctival staining, which can impact the ability to diagnose and monitor ocular surface conditions, such as DED[19].

Lissamine green staining

Lissamine green is a vital dye with an excellent safety profile that selectively stains ocular surface areas with disrupted intercellular junctions, detecting damaged or devitalized cells and denatured mucus and thus enlightening epithelial problems of the conjunctiva, one of the first and most reactive structures involved in DED[10,20]. Lissamine green has a peak absorption at the red end of the visible spectrum (630 nm) and is widely used because of its prompt visibility against the white conjunctiva. Up to date, the application parameters for Lissamine green have not been validated nor standardized. However, based on the result of a quality improvement study, a volume of 10 µl can be recommended as the optimal volume for 1% Lissamine green for ocular surface examination in DED patients[21].

A direct correlation between the degree of inflammation and the extent of Lissamine green staining has been proposed by Yang et al[22], who demonstrated that staining scores significantly correlate with the expression of IFN-γ, IL-6, IL-17, and MMP9 in Sjogren's syndrome (SS) and non-SS DED groups. It is worth noting that correlation coefficients of all cytokines were much higher in SS DED compared to non-SS DED. In addition, a pilot study reported a significant correlation between Lissamine green staining and CD45+CD14+ cells infiltration of the conjunctiva only[11]. These data support the correlation between Lissamine green staining and the infiltration of immune cells in the conjunctiva.

In addition, Lissamine green staining patterns can be useful to define the disease etiology based on their location, intensity of staining, and amount of dye uptake. Accordingly, Lissamine green can be used to stain the superior or inferior bulbar conjunctiva, contributing to the differential diagnosis of ocular surface inflammatory diseases, e.g., superior limbic keratoconjunctivitis and conjunctivochalasis[20].

Fluorescein staining

Fluorescein sodium, along with Lissamine green, is a videly used vital dye to view the stability of the tear film and to highlight the integrity of the ocular surface epithelium[19]. In physiologi-

cal conditions, fluorescein is not able to stain the cornea due to the low permeability into the lipid layer of the epithelium. Otherwise, in presence of a disruption of the cell-to-cell junctions of the cornea surface, fluorescein staining is detectable. Although the efficiency of fluorescein in staining the damaged cornea, fluorescein staining is difficult to detect in the conjunctiva because of the poor scleral contrast. To improve the visualization, a cobalt blue or yellow (blue-free) filter can be used, considering that fluorescein absorbs the blue light (490 nm) and emits yellow-green light (530 nm)[20]. Of note, a recent study by Begley et al[23] showed that the time required to reach the maximum grade of corneal staining was highly variable among subjects and significantly longer in patients with Sjögren's Syndrome, as well as the risk of an under-grading of corneal fluorescein staining with early observation, with a consequent impact on diagnosis and treatment assessment.

Similar to what has been reported for Lissamine green staining, the detection of significant fluorescein staining can be related to the presence of active inflammatory components on the ocular surface. Indeed, a correspondence between ocular surface epithelial damage and corneal inflammation has been established and is supported by the alteration of the phenotype and distribution of resident stromal dendritic cells after damage in preclinical models[24,25]. However, today, more stringent data with regard to the presence and the level of inflammation can be collected by the use of Lissamine green.

Tear Film Osmolarity

Normal ocular surface homeostasis requires regulated tear flow, the primary driver of which is osmolarity. Tear hyperosmolarity is caused by an imbalance of water and electrolytes between intracellular and extracellular compartments, which results in a reduction of cell volume and may lead to cell membrane and cytoskeletal integrity changes, as well as denaturation of cytosolic protein[26]. Thus, tear osmolarity and tear film instability have been identified as 'core mechanisms' of DED, regardless of the etiology[27]. Accordingly, tear hyperosmolarity has been found[26] to be the primary cause of discomfort, ocular surface damage, and inflammation in DED, and tests that accurately measure tear osmolarity and tear film instability should be considered for the classification of the severity of DED[28]. Up to date, there are specific instruments designed for measuring tear film osmolarity. The osmolarity 316 mOsm/L threshold is considered to discriminate between mild and moderate/severe DED[29].

Increased osmolarity is responsible for the activation of signaling pathways in the ocular surface. Indeed, exposure to osmotic stress activates mitogen-activated protein kinase (MAPK) pathways and nuclear factor (NF)-κB in the ocular surface[26,30,31]. Desiccating and osmotic stress-mediated MAPK activation, in turn, stimulates corneal epithelial cells to produce proinflammatory mediators, such as cytokines and chemokines (e.g., IL-1β, TNF-α, IFN-γ, IL-8, CXCL10, MMP-1, -3, -9, -10, and -13)[30,32]. For instance, IFN-γ upregulates the adhesion molecule ICAM-1 expression within the epithelium, stromal fibroblasts, and vascular endothelium, increasing vascular permeability[33]. As a consequence, the altered barrier facilitates diffusion of soluble inflammatory factors into the epithelium and stroma and inflammatory cell infiltration into the ocular surface tissues.

MMP-9 immunoassay

As mentioned above, tear hyperosmolarity observed in DED patients has been shown[32] to trigger the expression of inflammatory cytokines and matrix metalloproteinases (MMPs), which in turn activate the MAPK inflammatory cascade.

Among MMPs, MMP-9 can be considered an early marker of inflammation stimulated by increased osmolarity[34]. Its concentration and activity in the tear fluid of DED patients showed a significant correlation with symptom severity scores and ocular signs, such as tear breakup time (TBUT) and corneal and conjunctival fluorescein staining[35,36]. Currently, a widely used point-of-care device for detecting tear MMP-9 is InflammaDry (Rapid Pathogen Screening, Inc., Sarasota, FL, USA), which is based on a lateral flow immunochromatographic assay[37,38]. This test qualitatively determines whether the tear fluid MMP-9 concentration is higher than 40 ng/mL (cut-off value), with 85% sensitivity and 94% specificity for DED diagnosis[37]. In a prospective study, DED patients and control subjects underwent MMP-9 testing of the tear film with InflammaDry, showing that MMP-9 levels identified the presence of ocular surface inflammation in 40% of confirmed DED patients[39].

Laboratory Techniques

In clinical practice, the quantification of levels of inflammation is not feasible during first-line

examinations. Thus, laboratory techniques can be used. In particular, the combination of impression cytology to collect cells, which refers to the application of a cellulose acetate filter to remove the superficial layers of the ocular surface epithelium, with the use of markers of inflammation and flow cytometry, represents a key approach to quantify the level of inflammation on the ocular surface[40]. Unfortunately, the high costs and the need for a laboratory of immunology or a facility with a flow cytometer are important limitations for the routine use of this technique[10].

Impression cytology

Tear film hyperosmolarity, which characterizes DED patients, represents an indirect sign of inflammation. Hyperosmolarity induces HLA-DR (Human Leukocyte Antigen – DR isotype) overexpression in human conjunctival epithelial cells[40,41], and IFN-γ[39] may drive this upregulation[26]. Through impression cytology, it is possible to detect HLA-DR as a biomarker of ocular surface inflammation. This technique is recognized as sensitive, reliable, simple, and non-invasive for investigating DED inflammation. Literature evidence suggests that immune cells isolated from the superficial layer of the conjunctiva may play a pivotal role in the pathogenesis of dry eye[40]. Moreover, quantitative HLA-DR detection by impression cytology has been used in several DED clinical trials, and Epstein et al[42] have published a standard operating procedure for the use of this inflammatory biomarker.

Discussion

Understanding the clinical signs of DED and the related underlying pathogenesis and specific cellular responses is fundamental to improving the diagnosis and favor the development of personalized treatment strategies for long-lasting results. In particular, the evidence implicating inflammation in the pathogenesis of DED has opened up new avenues for the treatment of this complex disorder[8].

The diagnosis and assessment of ocular surface disease have become more technology-dependent in recent times with the advent of newer modalities. However, both clinical experiences and literature evidence suggest that ocular surface staining remains a core component in diagnosing various ocular surface disorders, as they can be considered inexpensive and efficient diagnostic tools to evaluate ocular surface integrity[20]. At the same time, future innovations in this field should be directed toward establishing a standardized system for scoring and grading.

The reported clinical experiences suggested that in ocular surface pathologies, the clinician's awareness of the immune-inflammatory pathogenetic mechanisms underlying the observed clinical sign is of great help for understanding the clinical observations itself and, consequently, for the choice of appropriate therapy. At the same time, the combination of impression cytology to collect cells and the use of markers of inflammation and flow cytometry is considered the best technique to quantify the level of inflammation on the ocular surface, even if not always applicable in clinical practice.

If ocular surface inflammation is diagnosed, the treatment is based on topical steroids and/or topical cyclosporine. At the same time, the use of tear substitutes based on hyaluronic acid (HA) is regularly prescribed in patients with DED, representing the mainstay of treatment[43,44]. In particular, high-molecular-weight HA (HMW-HA) has been shown to promote wound healing and reduce inflammation, thanks to its specific action on CD44 receptors expressed by corneal epithelium[45-48]. Moreover, a linear correlation between the molecular mass of the HA and the mucoadhesive index on the ocular surface model has been reported, suggesting an increased ocular residence time of HMW-HA[49]. Thus, the use of eye drops based on linear HMW-HA should be preferred.

Conclusions

In the first-line evaluation of patients with DED, the extent of the ocular surface inflammation should be carefully assessed, providing a guide to establish the correct therapeutic strategy.

Acknowledgments

Editorial and graphical assistance were provided by Simonetta Papa, Ph.D, Massimiliano Pianta, and Aashni Shah (Polistudium SRL, Milan, Italy). This assistance was supported by Fidia.

Funding

There was no explicit funding for the development of this work.

Conflicts of Interest
JMBdC served as a consultant for Alcon, Brill, Fidia, Santen, and Thea.

Availability of Data and Materials
All data generated or analyzed are included in this article and/or its figures. Further inquiries can be directed to the corresponding author.

Ethics Approval
The review of patient data did not require ethical approval in accordance with local/national guidelines.

Informed Consent
All the participants signed an informed consent form.

Authors' Contributions
Both authors contributed to the definition and contextualization of the paper's contents, critically edited the manuscript, and approved its final version for submission.

References

1) Barabino S. A narrative review of current understanding and classification of dry eye disease with new insights on the impact of dry eye during the COVID-19 pandemic. Ophthalmol Ther 2021; 10: 495-507.
2) Sheppard J, Shen Lee B, Periman LM. Dry eye disease: identification and therapeutic strategies for primary care clinicians and clinical specialists. Ann Med 2023; 55: 241-252.
3) Yu L, Yu C, Dong H, Mu Y, Zhang R, Zhang Q, Liang W, Li W, Wang X, Zhang L. Recent developments about the pathogenesis of dry eye disease: based on immune inflammatory mechanisms. Front Pharmacol 2021; 12: 732887.
4) Bartlett JD, Keith MS, Sudharshan L, Snedecor SJ. Associations between signs and symptoms of dry eye disease: a systematic review. Clin Ophthalmol 2015; 9: 1719-1730.
5) Boboridis KG, Messmer EM, Benítez-Del-Castillo J, Meunier J, Sloesen B, O'Brien P, Quadrado MJ, Rolando M, Labetoulle M. Patient-reported burden and overall impact of dry eye disease across eight European countries: a cross-sectional web-based survey. BMJ Open 2023; 13: e067007.
6) Stapleton F, Alves M, Bunya VY, Jalbert I, Lekhanont K, Malet F, Na KS, Schaumberg D, Uchino M, Vehof J, Viso E, Vitale S, Jones L. TFOS DEWS II Epidemiology Report. Ocul Surf 2017; 15: 334-365.
7) Rolando M, Barabino S, Giannaccare G, Aragona P. Dealing with the persistent pathogenic issues of dry eye disease: the importance of external and internal stimuli and tissue responses. J Clin Med 2023; 12: 2205.
8) Messmer EM, Ahmad S, Benitez Del Castillo JM, Mrukwa-Kominek E, Rolando M, Vitovska O, Baudouin C; a panel of European dry eye disease experts. Management of inflammation in dry eye disease: Recommendations from a European panel of experts. Eur J Ophthalmol 2023; 33: 1294-1307.
9) Aragona P, Baudouin C, Benitez Del Castillo JM, Messmer E, Barabino S, Merayo-Lloves J, Brignole-Baudouin F, Inferrera L, Rolando M, Mencucci R, Rescigno M, Bonini S, Labetoulle M. The ocular microbiome and microbiota and their effects on ocular surface pathophysiology and disorders. Surv Ophthalmol 2021; 66: 907-925.
10) Rolando M, Barabino S. Are there clinical ways to assess inflammation in dry eye disease? Ocul Immunol Inflamm 2021; 29: 1183-1189.
11) Barabino S, Valente C, Montaldo E, Mingari MC, Rolando M, Traverso CE. Does Lissamine green staining of the ocular surface correlate with inflammation in dry eye patients? Invest Ophthalmol Vis Sci 2012; 53: 546.
12) Medzhitov R. Origin and physiological roles of inflammation. Nature 2008; 454: 428-435.
13) Baudouin C, Irkeç M, Messmer EM, Benítez-Del-Castillo JM, Bonini S, Figueiredo FC, Geerling G, Labetoulle M, Lemp M, Rolando M, Van Setten G, Aragona P; ODISSEY European Consensus Group Members. Clinical impact of inflammation in dry eye disease: proceedings of the ODISSEY group meeting. Acta Ophthalmol 2018; 96: 111-119.
14) Singh RB, Liu L, Anchouche S, Yung A, Mittal SK, Blanco T, Dohlman TH, Yin J, Dana R. Ocular redness - I: Etiology, pathogenesis, and assessment of conjunctival hyperemia. Ocul Surf 2021; 21: 134-144.
15) Efron N, Brennan NA, Hore J, Rieper K. Temperature of the hyperemic bulbar conjunctiva. Eur J Ophthalmol 2015; 25: 273-279.
16) Ferrari G, Rabiolo A, Bignami F, Sizzano F, Palini A, Villa C, Rama P. Quantifying ocular surface inflammation and correlating it with inflammatory cell infiltration in vivo: a novel method. Invest Ophthalmol Vis Sci 2015; 56: 7067-7075.
17) Fieguth P, Simpson TL. Automated measurement of bulbar redness. Invest Ophthalmol Vis Sci 2002; 43: 340-347.
18) Yoneda T, Sumi T, Takahashi A, Hoshikawa Y, Kobayashi M, Fukushima A. Automated hyperemia analysis software: reliability and reproducibility in healthy subjects. Jpn J Ophthalmol 2012; 56: 1-7.
19) Begley C, Caffery B, Chalmers R, Situ P, Simpson T, Nelson JD. Review and analysis of grading scales for ocular surface staining. Ocul Surf 2019; 17: 208-220.

20) Srinivas SP, Rao SK. Ocular surface staining: Current concepts and techniques. Indian J Ophthalmol 2023; 71: 1080-1089.

21) Hamrah P, Alipour F, Jiang S, Sohn JH, Foulks GN. Optimizing evaluation of Lissamine Green parameters for ocular surface staining. Eye (Lond) 2011; 25: 1429-1434.

22) Yang S, Lee HJ, Kim DY, Shin S, Barabino S, Chung SH. The use of conjunctival staining to measure ocular surface inflammation in patients with dry eye. Cornea 2019; 38: 698-705.

23) Begley CG, Caffery B, Nelson JD, Situ P. The effect of time on grading corneal fluorescein and conjunctival lissamine green staining. Ocul Surf 2022; 25: 65-70.

24) Hamrah P, Liu Y, Zhang Q, Dana MR. Alterations in corneal stromal dendritic cell phenotype and distribution in inflammation. Arch Ophthalmol 2003; 121: 1132-1140.

25) Rashid S, Jin Y, Ecoiffier T, Barabino S, Schaumberg DA, Dana MR. Topical omega-3 and omega-6 fatty acids for treatment of dry eye. Arch Ophthalmol 2008; 126: 219-225.

26) Baudouin C, Aragona P, Messmer EM, Tomlinson A, Calonge M, Boboridis KG, Akova YA, Geerling G, Labetoulle M, Rolando M. Role of hyperosmolarity in the pathogenesis and management of dry eye disease: proceedings of the OCEAN group meeting. Ocul Surf 2013; 11: 246-258.

27) The definition and classification of dry eye disease: report of the Definition and Classification Subcommittee of the International Dry Eye WorkShop (2007). Ocul Surf 2007; 5: 75-92.

28) Potvin R, Makari S, Rapuano CJ. Tear film osmolarity and dry eye disease: a review of the literature. Clin Ophthalmol 2015; 9: 2039-2047.

29) Lemp MA, Bron AJ, Baudouin C, Benítez Del Castillo JM, Geffen D, Tauber J, Foulks GN, Pepose JS, Sullivan BD. Tear osmolarity in the diagnosis and management of dry eye disease. Am J Ophthalmol 2011; 151: 792-798.e1.

30) Li DQ, Luo L, Chen Z, Kim HS, Song XJ, Pflugfelder SC. JNK and ERK MAP kinases mediate induction of IL-1beta, TNF-alpha and IL-8 following hyperosmolar stress in human limbal epithelial cells. Exp Eye Res 2006; 82: 588-596.

31) Luo L, Li DQ, Pflugfelder SC. Hyperosmolarity-induced apoptosis in human corneal epithelial cells is mediated by cytochrome c and MAPK pathways. Cornea 2007; 26: 452-460.

32) Choi M, Park YM, Ko BY. Comparative evaluation of matrix metalloproteinase-9 immunoassay and tear osmolarity measurement for diagnosing severity of dry eye disease. Korean J Ophthalmol 2023; 37: 409-416.

33) Stern ME, Pflugfelder SC. Dry eye: an immune-based inflammation. in: encyclopedia of the eye [Internet]. Elsevier; 2010; 54-62. Available at: https://linkinghub.elsevier.com/retrieve/pii/B9780123742032000099.

34) Corrales RM, Stern ME, De Paiva CS, Welch J, Li DQ, Pflugfelder SC. Desiccating stress stimulates expression of matrix metalloproteinases by the corneal epithelium. Invest Ophthalmol Vis Sci 2006; 47: 3293-3302.

35) Chotikavanich S, de Paiva CS, Li de Q, Chen JJ, Bian F, Farley WJ, Pflugfelder SC. Production and activity of matrix metalloproteinase-9 on the ocular surface increase in dysfunctional tear syndrome. Invest Ophthalmol Vis Sci 2009; 50: 3203-3209.

36) VanDerMeid KR, Su SP, Ward KW, Zhang JZ. Correlation of tear inflammatory cytokines and matrix metalloproteinases with four dry eye diagnostic tests. Invest Ophthalmol Vis Sci 2012; 53: 1512-1518.

37) Sambursky R, Davitt WF 3rd, Latkany R, Tauber S, Starr C, Friedberg M, Dirks MS, McDonald M. Sensitivity and specificity of a point-of-care matrix metalloproteinase 9 immunoassay for diagnosing inflammation related to dry eye. JAMA Ophthalmol 2013; 131: 24-28.

38) 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.

39) Messmer EM, Von Lindenfels V, Garbe A, Kampik A. Matrix metalloproteinase 9 testing in dry eye disease using a commercially available point-of-care immunoassay. Ophthalmology 2016; 123: 2300-2308.

40) Barabino S, Montaldo E, Solignani F, Valente C, Mingari MC, Rolando M. Immune response in the conjunctival epithelium of patients with dry eye. Exp Eye Res 2010; 91: 524-529.

41) Versura P, Profazio V, Schiavi C, Campos EC. Hyperosmolar stress upregulates HLA-DR expression in human conjunctival epithelium in dry eye patients and in vitro models. Invest Ophthalmol Vis Sci 2011; 52: 5488-5496.

42) Epstein SP, Gadaria-Rathod N, Wei Y, Maguire MG, Asbell PA. HLA-DR expression as a biomarker of inflammation for multicenter clinical trials of ocular surface disease. Exp Eye Res 2013; 111: 95-104.

43) Kathuria A, Shamloo K, Jhanji V, Sharma A. Categorization of marketed artificial tear formulations based on their ingredients: a rational approach for their use. J Clin Med 2021; 10: 1289.

44) Barabino S, Benitez-Del-Castillo JM, Fuchsluger T, Labetoulle M, Malachkova N, Meloni M, Utheim TP, Rolando M. Dry eye disease treatment: the role of tear substitutes, their future, and an updated classification. Eur Rev Med Pharmacol Sci 2020; 24: 8642-8652.

45) Huynh A, Priefer R. Hyaluronic acid applications in ophthalmology, rheumatology, and dermatology. Carbohydrate Res 2020; 489: 107950.

46) Černohlávek M, Brandejsová M, Štěpán P, Vagnerová H, Hermannová M, Kopecká K, Kulhánek J, Nečas D, Vrbka M, Velebný V, Huerta-Angeles G. Insight into the lubrication and adhesion properties of hyaluronan for ocular drug delivery. Biomolecules 2021; 11: 1431.

47) Lardner E, van Setten GB. Detection of TSG-6-like protein in human corneal epithelium. Simultaneous presence with CD44 and hyaluronic acid. J Fr Ophtalmol 2020; 43: 879-883.

48) Labetoulle M, Benitez-Del-Castillo JM, Barabino S, Herrero Vanrell R, Daull P, Garrigue JS, Rolando M. Artificial tears: biological role of their ingredients in the management of dry eye disease. Int J Mol Sci 2022; 23: 2434.

49) Guarise C, Acquasaliente L, Pasut G, Pavan M, Soato M, Garofolin G, Beninatto R, Giacomel E, Sartori E, Galesso D. The role of high molecular weight hyaluronic acid in mucoadhesion on an ocular surface model. J Mech Behav Biomed Mater 2023; 143: 105908.