Review Article

Taiwan J Ophthalmol 2019;9:150-159

Access this article online

Quick Response Code:

Website:
www.e-tjo.org

DOI:
10.4103/tjo.tjo_56_19

Role of tear film biomarkers in the diagnosis and management of dry eye disease

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Abstract:

In recent years, there has been increasing scientific interest in the use of tear film biomarkers in the diagnosis and management of dry eye disease (DED), owing to their potential important roles in the pathogenesis of ocular surface damage, as well as the technical feasibility of tear sample collection techniques. An Entrez PubMed search was conducted on March 2, 2019, to include papers investigating the use of tear film biomarkers in DED, and the results were classified according to whether the DED is associated with systemic inflammatory disease or not and further classified within each section according to the molecular nature of the biomarker for further discussion. A total of 58 relevant articles were reviewed. Certain cytokines, including interleukin-6 (IL-6), tumor necrosis factor-alpha, IL-17, and IL-8, were found by a number of studies to consistently reflect disease severity well and had strong correlations with tear film metrics and tests for ocular surface damage in dry eye without systemic inflammatory disease. For dry eye with systemic inflammatory disease, IL-17, IL-8, and IL-1 receptor antagonists were shown to be consistently higher in affected eyes and correlated well with ocular surface disease severity in more than one type of inflammatory disease. With the advancement in technology and lowered costs in the future, tear film biomarker counts would allow better diagnosis and monitoring of DED, as well as facilitate personalized treatment strategies.

Keywords:

Diagnosis, dry eye disease, inflammation, systematic review, tear film biomarkers, treatment

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Submission: 31-05-2019
Accepted: 26-07-2019

Introduction

Dry eye disease (DED) is a chronic condition of multifactorial origin, and it can be primarily classified into several major subtypes, including aqueous-deficient dry eye (ADDE) and evaporative dry eye. In patients with evaporative dry eye, lipid-deficient dry eye (LDDE) is the most common cause. For up to 80% of patients with LDDE, there is evidence of dysfunction of the meibomian glands in the eyelids, such as terminal duct obstruction or changes in composition and amount of glandular secretion.[1] The insufficient or poor-quality tear lipid layer causes rapid evaporation of tear film.[2] Therefore, the most common cause of LDDE is meibomian gland dysfunction (MGD).

As for ADDE, a reduced lacrimal secretion causes the high osmolarity gradient. The TearLab (CA, USA) osmolarity system is a commercially available point-of-care device to efficiently measure tear osmolarity. Currently, an osmolarity of 308 mOsm/L is commonly regarded as abnormal and is used across multiple studies.[3] When the ocular surface epithelia is exposed to such an oxidative stress, intracellular signaling pathways are triggered, involving inflammatory mediators.[4-8] These mediators initiate the synthesis and release of pro-inflammatory cytokines, chemokines, and matrix metalloproteinases (MMPs).[3,7-10] The MMPs could promote extracellular

How to cite this article: Fong PY, Shih KC, Lam PY, Chan TC, Jhanji V, Tong L. Role of tear film biomarkers in the diagnosis and management of dry eye disease. Taiwan J Ophthalmol 2019;9:150-9.

matrix degradation, causing loss of the epithelial barrier.[7] Such cell death, together with a poorly lubricated corneal surface, would expose the nociceptors to environmental insults, damage them, and cause pain in DED.[10] The inflammatory cytokines would also recruit T-cell populations to raise the level of inflammation.[7,11] Goblet cell loss is another important factor to considering the pathogenesis of DED, triggered by the large amount of pro-inflammatory cytokines on the ocular surface.[12] It is one of the features in ADDE, especially those caused by severe diseases, such as Stevens–Johnson syndrome (SJS) and ocular graft-versus-host disease (ocular GVHD).[10] Loss of goblet cells decreases the amount of mucus present on the ocular surface, worsening the hyperosmolarity. It is also said to lower the immune tolerance in mucosal tissues.[10]

A biomarker is measured objectively and often represents the pathogenic and biological responses in the body. The use of a biomarker will lead to better diagnosis, categorization, and more effective management of DED.[13,14] Using biomarkers in tear film is very desirable owing to the fact that we can obtain them in a noninvasive and accessible manner. In addition, it is anatomically close to the disease site, increasing the specificity for ocular surface inflammatory disease.[3,14-16]

This paper aims to systematically review the use of tear film biomarkers in the diagnosis and management of DED.

Methodology

This paper systematically reviews relevant published original articles in the past 5 years to investigate the use of tear film biomarkers in the diagnosis and management of DED. An Entrez PubMed Search was conducted on March 2, 2019, using the key words "tear film," "proteomic," "lipidomic," "cytokines," "matrix metalloproteinase,"

"dry eye disease," and "ocular surface disease." The search results were limited to the studies written in English, conducted on human subjects, and published within the past 5 years. The initial search resulted in 83 papers. We further manually curated the papers for clinical relevance. For example, a paper showing investigating significant differences in tear levels of a particular biomarker between DED patients and healthy controls is deemed a relevant paper. Conversely, a paper investigating tear levels of a certain biomarker in ocular surface disease without comparison with controls is excluded from our review. After curation, we finally ended with 58 relevant papers for review [Table 1].

Use of Tear Film Biomarkers in Dry Eye without Systemic Inflammatory Disease

Pro-inflammatory cytokines

Interleukin-6

Due to DED having an inflammatory origin, pro-inflammatory cytokines are well known for being possible biomarkers in DED. Among which, interleukin-6 (IL-6) has been supported as the best probable indicator for the disease. IL-6 secretion is induced by infectious and allergic processes systemically,[4,17] whereas on the ocular surface, it is secreted in the wake of desiccation stress.[1,18] A pleiotropic pro-inflammatory cytokine is known to cause multiple processes, such as the synthesis and release of acute-phase proteins and MMPs, decrease tear production, and cause cell death.[1,17,19-21] It also induces T-helper 17 cell (Th-17) differentiation, which leads to further release of other pro-inflammatory cytokines.[22,23] Across multiple studies, IL-6 was found to be raised in DED patients compared to controls, for both the cytokine itself and its messenger ribonucleic acid (mRNA) levels,[17,21,24-26] and across different patient groups, both with and without MGD.[4] Further investigation has revealed that IL-6 levels correlate well with ocular surface parameters, notably a negative relationship with Schirmer's test score and tear film break-up time (TBUT),[2,8,19,24,27] and a positive relationship with the ocular surface disease index (OSDI) score.[4,18,21] Moreover, some studies have found that treatment of dry eye with both artificial tears, steroids, and warm compression would concomitantly lower the level of IL-6.[1,5,25]

Tumor necrosis factor-alpha

Tumor necrosis factor alpha (TNF-α) is another pro-inflammatory cytokine that can stimulate MMP activation and limit fibrosis.[10,28] It could reduce tear production.[21] Multiple studies have pointed out its correlation with disease severity, being raised significantly in DED patients,[4,21,24,27] and having correlations with OSDI score and Schirmer's test score.[8,18,21]

Interleukin-1 beta

The pro-inflammatory cytokine IL-1β is an important mediator of inflammation. It is well known for inducing the secretion of other pro-inflammatory cytokines, including IL-6 and TNF-α, chemokine IL-8, and similar to IL-6, acute-phase proteins, and MMPs.[1,10,17] On the ocular surface, it can cause metaplasia of epithelial cells and keratinization of the ocular surface.[1,7,28,29] While a number of studies have revealed a difference in IL-1β levels between patient and control populations,[17,21,26] some other studies did not find any significant relationship between its levels and disease severity.[4,19,22,27] However,

Table 1: Comparison of tear biomarkers between dry eye disease with or without systemic inflammatory disease

Cytokine	Use in DED without systemic inflammatory disease	Use in DED with systemic inflammatory disease
IL-6	Raised in DED compared to controls, and correlates well with Schirmer's test, TBUT, and OSDI scores	Raised and correlated well with ocular surface parameters in SS patients
TNF-α	Raised in DED compared to controls, correlates well with Schirmer's test and OSDI scores	Raised and correlated with Schirmer's test score in SS patients but decreased in SJS patients
IL-1β	Raised in DED compared to controls though some did not find any significant relationship	Raised in SJS patients
IL-12	Lowered in MGD-related DED compared to those without controls; negative correlation with Schirmer's test scores	Decreased in SJS patients but increased in ocular GVHD patients compared to controls
IFN-γ	Correlates well with tear osmolarity, ocular surface staining, and Schirmer's test scores, but diversified findings of its relationship with disease severity	Decreased and correlates with the severity of SJS patients
IL-17	Raised in DED compared to controls, negative correlation with Schirmer's test score	Raised and correlates very well with ocular surface parameters in SS, ocular GVHD, and SJS patients
IL-4 and IL -5	Roughly correlated to disease symptom severity	Raised compared to controls and correlates well with Schirmer's test and TBUT score in SS patients
IL-10, IL-13, TGF-β	Did not show good correlation with symptom severity	Raised in ocular GVHD patients, and decreased in SJS patients, for IL-10 and IL-13; for TGF-β, increased levels in SJS patients
IL-1RA	Raised in DED compared to controls	Raised and correlates well with ocular surface parameters in ocular GVHD and SS patients
IL 8/CXCL8	Raised in DED compared to controls, correlates well with ocular surface parameters and pain sensation	Raised in SS, SJS, and ocular GVHD patients, correlated well with ocular surface parameters in ocular GVHD and SS patients
IP-10/CXCL10	Correlation with disease severity supported by few literature	Decreased in ocular GVHD and SJS patients compared to controls
MMP-9	Diversified findings with respect to its correlation with disease severity	Findings supported by few literature
VEGF	Diversified findings with respect to its correlation with disease severity	Decreased in SJS patients compared to controls
EGF	Decreased in DED compared to controls	Decreased in ocular GVHD and SS patients compared to controls

DED=Dry eye disease, IL=Interleukin, TNF-α=Tumor necrosis factor α, IFN gamma=Interferon-γ, TGF-β=Transforming growth factor-β, MMP=Matrix metalloproteinase, VEGF=Vascular endothelial growth factor, EGF=Epithelial growth factor, OSDI=Ocular surface disease index, MGD=Meibomian gland dysfunction, SS=Sjogren's syndrome, SJS=Steven–Johnson syndrome, GVHD=Graft-versus-host disease

administration of dry eye treatment was found to significantly decrease tear IL-1β levels.[1,5]

Interleukin-12

Another pro-inflammatory cytokine, IL-12, induces naïve T-cells to differentiate into Th-1 helper cells.[4] It is found to be significantly lowered only in patients with MGD-related DED,[4] compared to those without MGD. However, another study has shown that its levels did have a negative correlation with Schirmer's test scores and a slight correlation with temporal corneal staining.[8] On application of artificial tears or steroid medication, its levels are slightly reduced.[1,5]

Interferon gamma

Interferon gamma (IFN-γ) is secreted by Th-1 helper T-cells, cytotoxic T-lymphocytes, and natural killer cells,[2,4,10,12] playing multiple roles in the adaptive and innate responses, such as defense against bacterial infections.[12] On the ocular surface, it secrets the following desiccation stress[2] and is well known for inducing conjunctival goblet cell loss, thus reducing mucin production and apoptosis of the lacrimal gland acini as well.[2,4,10,12] Several studies have shown that IFN-γ levels correlate very well with tear osmolarity, ocular surface staining, and Schirmer's test scores.[2,8,30] This signifies the participation of Th-1 helper cells on the ocular surface inflammation in DED and that IFN-γ reflects the dryness of the ocular surface. However, diversified findings were found with respect to the relationship of IFN-γ levels and symptom severity.[8,21]

Interleukin-17

IL-17 is a unique pro-inflammatory cytokine in the sense that it is produced solely by Th-17 helper cells, a population of lymphocytes that mainly act against extracellular bacteria and fungi.[31] On the epithelial surface, it acts on epithelial, stromal, and immune cells, inducing them to secrete other pro-inflammatory cytokines, such as IL-1, IL-6, and TNF-α, as well as MMPs 3 and 9, which in turn disrupt the epithelial basement membrane.[32,33] In the majority of studies, IL-17 was found to be raised significantly compared to control patients,[23,24,27,32,34] particularly in patients with an underlying systemic inflammatory disorder (which mostly leads to ADDE). In addition, its negative correlation with Schirmer's test

scores was noted.[8,34] However, in Grosskreutz *et al*., inhibition of IL-17 production by systemic administration of secukinumab could not influence the severity of dry eye.[35] Together with IL-6, its role in causing DED and as a biomarker for DED is well supported by a lot of literature.

Other pro-inflammatory cytokines

Apart from the abovementioned, differences were also spotted in the levels of other pro-inflammatory cytokines between DED patients and healthy controls, including those of IL-2[21] and IL-33[36] those of and IL-22[34] and IL-23[23] (both of which lie on the Th-17 axis). Yet, the findings for these cytokines are only supported by a very small amount of research papers and thus are yet to have an agreeable result as a biomarker compared to other cytokines.

Anti-inflammatory cytokines

Anti-inflammatory cytokines are predominantly related to the action of Th-2 helper T-cells, which in general lowers the inflammation in tissues and stimulates B-cell differentiation. The levels of such cytokines also differed slightly between DED patients and healthy controls. This includes IL-4, which stimulates Th-2 differentiation,[17,21,36] and IL-5, which is secreted by Th-2 helper cells to act on other cells.[21,26,36] Their levels were roughly correlated to the disease symptom severity. On the other hand, three other anti-inflammatory cytokines, IL-10, IL-13, and TGF-β, did not show much relationship with any parameter of DED or the symptom severity. Even though they were said to resolve inflammation well by suppressing T-cell immunity, stimulating goblet cell production (thus increase mucus production), and generate regulatory T-cells, respectively,[10,16] the majority of studies that measured their levels across different patient populations did not find any significant results.[4,18,19,22,27,36]

Interleukin-1 receptor antagonist

IL-1 receptor antagonist (IL1RA) is an endogenous inhibitor of IL-1 action, by binding to the IL-1 receptor. It is produced by corneal epithelial cells and fibroblasts.[19,20] The balance of IL-1β and IL-1RA is important in the resolution of inflammation and infectious diseases.[37] It is significantly increased in the tears of DED patients compared to controls and correlated well with Schirmer's test scores and TBUT values.[19]

Chemokines

Interleukin-8 (IL-8/CXCL8)

Chemokines are another group of proteins that play an important role in mediating inflammation, mainly by recruitment of leukocytes at the sites of inflammation. The most well known of these is IL-8/CXCL8, a small chemokine.[21] It is secreted by corneal epithelial cells upon inflammation or prolonged desiccation stress,[1,20] to attract neutrophils to migrate through avascular tissues such as the cornea.[7,21] Its levels were found to be increased in DED patients[4,21,27] and generally had a correlation with ocular surface parameters and pain sensation.[8,19,21]

Other chemokines

Other chemokines were less supported as indicators for DED, also due to a small size of literature supporting their use. IFN-γ-inducible protein 10 (IP-10/CXCL10) is described as a potent chemoattractant for Th-1 and natural killer cells,[16,19,38] released by corneal epithelial cells after IFN-γ stimulation. Yet, only Enríquez-de-Salamanca *et al*. found a significant difference of IP-10/CXCL10 levels between DED populations and controls,[19] and Tong *et al*. found a negative correlation between its levels and Schirmer's test scores.[8] Similarly, significant findings for monocyte chemoattractant protein-1 (MCP-1/CCL2),[1,8] macrophage inflammatory protein 1alpha (MIP-1a/CCL3), regulated on activation, normal T-cell expressed, and secreted (RANTES)/CCL5,[4,8] eotaxin/CCL11, and fractalkine/CX3CL1[19] were only noted by a small number of studies.

Degradative enzymes

Matrix metalloproteinase 9

MMP-9, as aforementioned, acts on the proteins on the corneal epithelial basement membrane and tights junction proteins such as occludin, resulting in an irregular corneal surface and epithelial cell loss.[9,10,30,39,40] With tissue degradation, it facilitates leukocyte migration, to further heighten the severity of inflammation. In addition, it can activate cytokines. At the same time, MMP-9 is involved in normal tissue modeling, accelerating a healing response.[3,9,10,39] It is described as the most important proteolytic enzyme participating in ocular surface inflammatory diseases. In fact, the InflammaDry test, which measures the levels of MMP-9 in tears using a cutoff level of 40 ng/mL, is one of the United States Food and Drug Administration-approved office tests in the management of dry eye. A hand-held immunoassay device is used to measure the amount of MMP-9 in a tear sample at the lower palpebral conjunctiva. The sample is put in a test cassette with buffer solution, and the result is shown as "positive" or "negative" by the presence of two lines, one red and one blue, in the result window.[40] It is important to note that this facilitates only a "binary" read-out and may not be useful in patient monitoring in the long term.[3,14] While some studies have pointed out elevated levels of MMP-9 in DED,[3,8] many others did not find any relevance between the MMP-9 concentration and the clinical signs and symptoms of DED.[30,40-42] Lanza *et al*. stated that different levels of MMP-9 were the reason why some DED patients did not respond well to anti-inflammatory agents,[40] while Schargus *et al*.

mentioned that MMP-9 is only useful in the diagnosis of medium to severe DED[42] and that MMP-9 can also be raised in the event of other ocular surface inflammatory diseases.[3] Clearly, further studies to clarify the role of MMP-9 in dry eye and ocular surface inflammatory disease are needed.

Growth factors

Vascular endothelial growth factor

Growth factors are a group of proteins that have the potential to be used as biomarkers as well. VEGF is released after IL-1β and IL-6 stimulation[17] and promotes the growth of vascular endothelial cells, triggering vasodilation. It is important in the remodeling of severe allergic conjunctival disorders, such as vernal keratoconjunctivitis and atopic keratoconjunctivitis.[19] Yet, studies have diversified results on its correlation with DED patients: In Benitez-Del-Castillo Sánchez *et al*., it is significantly decreased in DED patients compared to the controls;[17] yet, in Enríquez-de-Salamanca *et al*., it is significantly increased in MGD patients compared to the controls.[19] Epithelial growth factor (EGF) is produced by the human lacrimal gland, and participates in wound healing and production of keratocytes.[16,43] Therefore, it is a good indicator of lacrimal gland health and function. In the event of DED, EGF levels are decreased.[4] Insulin-like growth factor 1 induces proliferation, migration, and survival of epithelial cells, and it was found to correlate with Schirmer's test score and TBUT.[44] No significant results were found for the fibroblast growth factor and IL-7 (a hematopoietic growth factor).

Novel proteins

Some studies have discussed the use of novel proteins as biomarkers for DED. These proteins participate in the dry eye pathophysiological process but may or may not inflict direct damage to epithelial cells or cause inflammation. Prostaglandin E2 (PGE2), produced by damaged corneal cells during inflammation to elicit the inflammatory pain state, was found to correlate well with ocular surface parameters. Furthermore, its levels decreased after intense pulsed light therapy.[25] Serotonin sensitizes and amplifies the pain at peripheral nerves via second messenger signaling pathways in nerves. This neurotransmitter was found by Chhadva *et al*. to be higher in patients with ADDE and had a negative correlation with Schirmer's score.[45] Palate lung nasal clone is a glycoprotein that maintains the fluid balance on mucosal surfaces (including the ocular surface) and also has antibacterial effects. Schicht *et al*. detected higher levels of palate lung nasal clone in DED patients, especially ADDE patients.[46] Malate dehydrogenase 2 (MDH2) is the mitochondrial isoform of malate dehydrogenase, an essential enzyme in the citric acid cycle. It participates in intracellular signal transduction.[47] MDH2 levels were significantly increased in DED subjects compared to controls. Its levels further correlated with subject pain, Schirmer's test results and TBUT. With artificial tear treatment, MDH2 levels and activities were also greatly decreased. It is described to be a marker of corneal injury.[47]

Steroids

Endogenous steroid levels were also investigated as to whether they are appropriate markers for DED. Pieragostino *et al*. discussed adrenal steroids affecting the amount of lipids secreted by the meibomian glands, thus affecting tear film stability. They found a significant decrease in cortisol, androstenedione, and 17-hydroxyprogesterone in DED patients.[48] Choi *et al*. looked into the use of lipid peroxidation markers, which would be present at a high amount on the ocular surface owing to the light-induced oxygen free radicals. Among the few substances examined, 4-hydroxy-2-nonenal and malondialdehyde were found to be significantly raised in patient populations and correlated with TBUT, Schirmer's test, and symptom scores.[49]

Use of Tear Film Biomarkers in Dry Eye with Systemic Inflammatory Disease

Sjogren's syndrome (SS) is an autoimmune disease attacking the lacrimal and salivary glands.[50] Different T-cell populations, such as Th-1, Th-2, and Th-17, infiltrate the exocrine glands and subsequently the ocular and mucosal surfaces, leading to patients showing dry mouth and dry eyes.[29,33,36,51] Depending on whether the patient has another autoimmune disease, SS can be classified as primary SS or secondary SS.[31]

GVHD is a disease that chronically affects a patient after receiving hematological stem cell transplantation. Such a disease has ocular complications, by inflicting inflammation-related fibrosis on the conjunctiva and lacrimal glands (thus, drastically reducing tear production). Ocular GVHD mimics severe ADDE, damaging the patient's quality of life.[13,16] Unlike SS where the histological picture is characterized by lymphocyte infiltrate of lacrimal glands, GVHD is characterized by lacrimal gland fibrosis. SJS, and its severe form, toxic epidermal necrolysis, are acute exfoliative blistering disorders of the skin, following adverse drug reactions. The ocular surface is the most common mucosal surface affected, with SJS characteristically involving the eyelid margin.[28]

Pro-inflammatory cytokines

Interleukin-6

IL-6, which could decrease tear production and raise inflammatory levels in any kind of stress, was found to be significantly increased in SS patients and also correlated well with ocular surface parameters for them.[20,24,25] IL-1β,

which decreases tear production and induces synthesis of acute-phase proteins such as IL-6, was increased in SJS patients.[28] After mucous membrane grafting in SJS patients, the levels of IL-1 decreased together with the inflammation. IL-2, a Th-1 cytokine that acts in an autocrine fashion for Th-1 proliferation and response, was found to be significantly increased in SJS patients and correlated well with the disease severity.[28] Meadows *et al*. also stated that its levels were much higher in ADDE than in LDDE.[33]

Tumor necrosis factor-alpha

TNF-α levels were raised in SS patients and showed a negative relationship with Schirmer's test scores.[24] Yet, in SJS, TNF-α levels were decreased, showing a resistance of myofibroblasts to apoptosis and fibrosis.[28] On the other hand, IFN-γ levels were only correlated with severity of SJS. Similar to TNF-α, patients had a lowered expression of IFN-γ, showing the cells' resistance to the apoptotic and fibrotic effects of the cytokine. Furthermore, IFN-γ is able to suppress differentiation of monocytes and macrophages, as well as suppress IL-17. A lowered IFN-γ level is able to sustain the inflammation in SJS.[28] IL-12 is another cytokine that can limit fibrosis. It is under-expressed in SJS patients;[28] however, due to its inflammatory effects, its levels are increased significantly in ocular GVHD patients.[16] IL-15 is a cytokine primarily involved in activating natural killer cells to combat viral infections. Differences in expression of IL-15 were found in the SJS patients' tears.[28] IL-33 is a cytokine belonging to the IL-1 family that induces Th-2 cytokine production.[36] It is found to be significantly increased in SS patients' tears and correlated well with symptom and fluorescein staining scores. Luo *et al*. pointed out its unique role in balancing different populations of helper T-cells in DED.[36]

Interleukin-17, -22, and-13

The Th-17 axis, which involves the cytokines IL-17, IL-22 and IL-23, plays a crucial role in the pathogenesis of DED in patients with underlying inflammatory disorders. Previous studies have pointed out the fact that Th-17 cells are involved in systemic inflammatory diseases such as systemic lupus erythematosus, rheumatoid arthritis, inflammatory bowel disease, and psoriasis, as well as ocular inflammatory diseases such as uveitis and scleritis.[32,38] Indeed, IL-17 was spotted by numerous studies to be raised in DED patients, especially patients with a systemic inflammatory disorder. Such patients predominantly have ADDE rather than LDDE.[32-34] Zhang *et al*. also compared the IL-17 levels in both tears and serum between primary SS patients and nonsicca controls and found the IL-17 levels to be significantly raised.[31] Other studies have indicated that IL-17 levels related well with questionnaire and clinical parameter scores.[23,32,34] Therefore, IL-17 is frequently described to be strongly correlated to SS. Apart from SS, the cytokine was also found to correlate well with clinical parameters in SJS and ocular GVHD patients. IL-22 and IL-23 were found to be increased in SS patients compared to controls in two different studies. IL-22 had good positive correlations with questionnaire and keratography scores and negative correlations with TBUT and Schirmer's test scores,[34] whereas no correlation was drawn between IL-23 levels and ocular surface parameters.[23]

Anti-inflammatory cytokines

Interleukin-4 and -5

Studies also drew correlations between the levels of anti-inflammatory cytokines with the disease severity. In SS patients, IL-4 was significantly increased compared to non-SS patients and controls and they correlated well with the ocular surface parameters such as Schirmer's test and TBUT.[24,36] IL-5 was found by Luo *et al*. to be significantly raised in SS patients too.[36]

Interleukin-10

Meanwhile, IL-10, which suppresses T-cell immunity, was found to be significantly increased in ocular GVHD patients and was commensurate with OSDI and TBUT scores, as well as corneal staining results.[16] It was decreased in SJS patients. A similar result was found for IL-13 in SJS patients.[28] As for TGF-β, which is well known for suppressing inflammation, it was found to be significantly increased in SJS patients' tears.[28]

Interleukin-21

Lim *et al*. found an increase in IL-21 mRNA and protein expression in SS patients compared to non-SS patients and controls and correlated with Schirmer's and fluorescein staining scores, but they also mentioned that this regulatory cytokine might reflect severe diseases, rather than being specific to primary SS.[29]

Interleukin-1 receptor antagonist

The endogenous inhibitor of IL-1β and IL-1RA was found to be a potent biomarker among many systemic diseases. It was found to increase significantly in ocular GVHD and SS patients and varied well with ocular surface parameters in ocular GVHD and SS patients.[16,20] Due to these diseases being chronic diseases, the results may suggest that IL-1RA is important in mediating and resolving chronic diseases by modulation of immune response.

Chemokines

Interleukin-8 (IL-8/CXCL8)

Apart from IL-17 and IL-1RA, the chemokine IL-8/CXCL8 is another cytokine found to be raised in the tears of patients having various systemic inflammatory diseases. The tears of SJS, ocular GVHD, and SS patients all had significantly increased IL-8/CXCL8 levels, with

correlations with clinical parameters drawn in ocular GVHD and SS patients.[16,20,28] López-Miguel *et al*. also stated that IL-8/CXCL8 expression was further heightened after exposure to desiccation.[20] These may suggest the crucial role of IL-8/CXCL8 in chemoattraction and activation of neutrophils on the ocular surface in the event of such inflammatory diseases.

Interferon gamma-inducible protein 10 (IP-10/CXCL10)

IP-10/CXCL10, which helps attract activated Th-1 and natural killer cells, is another important molecule to consider as a potential biomarker. The expression of this chemokine was downregulated significantly in both ocular GVHD and SJS patients[16,28] and also correlated well with signs and symptoms in ocular GVHD patients' tears.[16] Cocho *et al*. and Gurumurthy *et al*. have pointed out that low levels of IP-10/CXCL10 are important in sustaining the chronic inflammation, severe dry eye, and tissue damage.

Other chemokines

Other chemokines also showed significant results in systemic inflammatory results. RANTES/CCL5 expression was decreased in SJS patients;[28] MCP-1/CCL2 showed differences in the tears of SJS patients compared to their controls.[28]

Degradative enzymes

Matrix metalloproteinase-9

While MMP-9 has a significant role in DED without an underlying cause, it has limited potential in DED due to an underlying cause. Only López-Miguel *et al*. found a significant increase in MMP-9 after exposing SS patients to desiccation and an inverse correlation with Schirmer's test values.[20] This may show that MMP-9 does not have a significant role in most systemic inflammatory diseases.

Growth factors

Among the growth factors, EGF has the largest potential to be a biomarker, found to be decreased in patients with ocular GVHD and SS patients.[4,16] Its active role in wound healing and tear production means that a decreased amount of tears is one of the key features in these systemic inflammatory diseases. As for VEGF, studies have found decreases in SJS patients.[28] Differences in expression were also detected, among SJS patients' tears, for fibroblast growth factor 2, granulocyte-monocyte colony stimulation factor, and IL-7 (a hematopoietic stem cell growth factor).[28]

Novel proteins

Sjogren's syndrome

The use of novel, specific proteins in tears as biomarkers for DED associated with a particular disease has been under extensive research. Many of these proteins are either enzymes or factors that participate in intracellular signaling, which possibly results in the inflammations in these diseases. The research has been the most comprehensive for tears of SS patients, with the following proteins found to be increased in SS [Table 2]:

1. Cathepsin S, a cysteine endopeptidase that helps in major histocompatibility complex class II processing in antigen-presenting cells[50]
2. DNA (apurinic or apyrimidinic site) lyase (APEX1), an enzyme that regulates transcription factors and DNA repair during oxidative stress
3. Thioredoxin-dependent peroxidase reductase (PRDX3), an enzyme that regulates the transcription factor NFκB and helps in B-cell survival
4. Copine (CPNE1), a protein involved in TNF-α receptor signaling, thus inflammation and apoptosis
5. LIM-domain-only protein 7, a multifunctional protein important for cell signaling and protein degradation
6. Aconitate hydratase, an enzyme participating in the Krebs cycle[52]
7. Fatty acid binding protein, an intracellular chaperone for insoluble long-chain unsaturated fatty acids (thus solubilizing them) that influences oxidative stress in many diseases[53]
8. Anti-salivary gland protein 1, an autoantibody[54]
9. Anti-carbonic anhydrase 6, an autoantibody that was increased much more in SS patients' tears than that in non-SS DED patients' tears and in non-SS DED patients' tears than control patients' tears, known also to cause dry mouth symptoms[55]
10. Anti-parotid secretory protein, another autoantibody[54]
11. Many of these proteins were specific for SS-associated DED alone and were not detectable in DED due to other inflammatory diseases. Aqrawi *et al*. noted the use of liquid chromatography-mass spectrometry in obtaining the proteins, which is an accurate and precise method to detect the level of such proteins in tear fluid.[52]

Table 2: Potential molecules used as tear film biomarkers, classified by underlying inflammatory disease

Disease	Potential molecules
SS	IL-6, TNF-α, IL-33, IL-17, IL-22, IL-23, IL-4, IL-5, IL-21, IL-1RA, IL-8/CXCL8, MMP-9, EGF
	Novel proteins CTSS, APEX1, PRDX3, CPNE1, LMO7, ACO_2, FABP, SP1, CA6, PSP
Ocular GVHD	IL-12, IL-17, IL-10, IL-1RA, IL-8/CXCL8, IP-10/CXCL10, EGF
	proteins histones, lipocalin-1, IGHG1, PPL
SJS	IL-1β, IL-2, TNF-α, IFN-γ, IL-12, IL-15, IL-17, IL-10, IL-13, TGF-β, IL-8/CXCL8, IP-10/CXCL10, RANTES/CCL5, MCP-1/CCL2, VEGF, FGF2, GM-CSF, IL-7

SS=Sjogren's syndrome, SJS=Steven-Johnson syndrome, IL=Interleukin, TNF-α=Tumor necrosis factor-alpha, IFN-γ=Interferon-γ, TGF-β=Transforming growth factor-β, MMP=Matrix metalloproteinase, VEGF=Vascular endothelial growth factor, EGF=Epithelial growth factor, RANTES=Regulated on activation, normal T-cell expressed, and secreted, PPL=Periplakin, FABP=Fatty acid-binding protein, PSP=Parotid secretory protein, CA6=Carbonic anhydrase 6, SP1=Salivary gland protein 1, ACO_2=Aconitate hydratase, IGHG1=Immunoglobulin gamma-1 C chain, GM-CSF=Granulocyte-monocyte colony stimulation factor

Ocular graft-versus-host disease

For ocular GVHD, a different spectrum of proteins was detected to be either upregulated or downregulated in patients' tears.

1. Histones: It is not only a structural protein in nucleosome assembly but also participates in TLR-mediated pro-inflammatory signaling. Neutrophils release them together with antimicrobial enzymes to form neutrophil extracellular traps, but their clearance is dysregulated in systemic inflammatory diseases. It increased in diseased patients
2. Lipocalin-1: It is a lipid binder and scavenger in normal tears and downregulated in diseased patients
3. Periplakin: It involved in cornification of dermal epithelial cells to form the epidermal barrier. It downregulated in diseased patients
4. Immunoglobulin gamma-1 C chain: It involved in antigen binding and complement activation and downregulated in diseased patients.[13]

Discussion

Analysis of tear film cytokines, chemokines, and particles can enable us to know much more on the pathophysiology of DED. The cytokine/chemokine assays provide evidence that inflammation is a key feature of the pathophysiology.[4,17,18,20,21,27] In particular, the presence of pro-inflammatory cytokines such as IL-1β and IL-6 represent ocular surface stress and damage,[4,19] whereas the amounts and compositions of proteins and growth factors such as EGF enable us to assess the lacrimal gland function.[43,50] Moreover, certain "signature cytokines" can indicate the parent cells, which secrete these signature cytokines that participate in the disease process. The involvement of pro-inflammatory cytokines, anti-inflammatory cytokines, and cytokines on the Th-17 axis in DED due to an underlying systemic inflammatory disorder shows that Th-1, Th-2, and Th-17 all participate in the ADDE.[23,33] However, dry eye without an underlying systemic inflammatory disorder has predominantly Th-1 involvement, suggesting that ADDE may have a larger immunologic component.[33]

As a diagnostic marker for DED, tear film biomarkers are described to be more sensitive than tests such as Schirmer's test and can provide additional diagnostic accuracy.[4,52] They are also described to be better indicators of disease severity than corneal fluorescein staining and Schirmer's test.[4] In particular, IL-6, IL-8, and IL-17 have been mentioned across multiple studies to be potential biomarkers in DED. They have been shown to be the most consistently increased in DED patients. In addition, since inflammation is a key component in both ADDE and LDDE, we can target inflammatory cytokines to limit the damage in DED. Although systemic inhibition of IL-1β and IL-17 was found to be ineffective in Grosskreutz *et al*.,[35] it has been advised that both pro-inflammatory and anti-inflammatory cytokines be used in monitoring other anti-inflammatory treatments in DED patients.[1,2]

According to Roy *et al*., an ideal biomarker should be relevant to the clinical setting and disease severity, should be reproducible, should have rigorous tests and clinical trials on the potential biases that influence it, should be easy and economical to use, and should have a definite use in the clinical setting.[14] Yet, at present, the collection of tear film biomarkers has presented numerous errors. Elution and bead-based multiplex immunoassays involve a high cost, preventing tear film biomarkers from being used widely in the clinical setting.[50] Differences in collection techniques can greatly influence the amount and composition of proteins and molecules collected, with researches showing that samples obtained from Schirmer's strips have higher protein counts than samples in capillary tubes.[44] More importantly, both methods of collection easily result in collection of reflex tears, which may affect the concentration of proteins in the samples.[44] Apart from the above, the use of different instruments, or different settings for the collection of tears, may further increase the variability of the samples collected.[14] Added to the biological variation, absolute concentrations may present with too high a variability to be used as a biomarker at present. Its reproducibility is not robust and biases are present. Roy *et al*. and Willcox *et al*. have suggested reporting a percentage change for a particular cytokine, or the proportion of a particular cytokine among a panel of others, as an alternative to using absolute concentrations for severity measurement.[14,56] We can also develop a composite score involving the more potent biomarkers as mentioned above, which would be more sensitive in detecting differences between DED patients and controls.[14]

Despite the above limitations, the use of tear film biomarkers still has potential to be further developed. Rigorous procedures can be developed to standardize the collection of tear fluids in patients and to minimize the variability in tear molecule levels due to systematic differences. At the same time, we can also further investigate the underlying cell-mediated mechanism with the use of tear film biomarkers. We can also try to apply tear film biomarkers in prolonged monitoring of DED, testing the changes of different cytokines together with different existing parameters used to evaluate DED.

Conclusion

The identification of molecules in tear fluid as biomarkers or indicators of DED is advancement in finding a

noninvasive method to investigate the pathogenesis and severity of different types of dry eye, with potential in diagnostics and monitoring of the disease. Further investigation is warranted to have a better understanding of the disease processes, as well as make it a more robust parameter by improving the correlation of particular cytokines with the clinical findings in DED. In the near future, we can anticipate more tear cytokines being used as reliable and valid markers for DED in clinical practice

Financial support and sponsorship
Nil.

Conflicts of interest
The authors declare that there are no conflicts of interests of this paper.

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