



# Molecular and cellular biomarkers in dry eye disease and ocular allergy

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## Purpose of review

To summarize recent studies in biomarkers in dry eye disease (DED) and ocular allergy in the last 18 months.

## Recent findings

New significant correlations with clinical symptoms and signs have been described for some tear molecules (proteins, cytokines/chemokines, metalloproteinases, mucins and lipids). Correlation with disease severity has been shown for some of them. New proposed DED biomarkers include some proteins, such as S100A8, S100A9, lipocalin-1, secretory phospholipase A2 and some cytokines and chemokines; mucin-1 mRNA conjunctival expression levels are shown to be a very sensitive and specific DED diagnosis biomarker; metalloproteinase-9 tear level is confirmed as a good DED biomarker; cell parameters such as conjunctival cell viability and proliferative capacity have been also proposed as DED biomarkers. In ocular allergy, protein activated receptor-2, heat shock protein-70, eosinophil cationic protein and hemopexin have been pointed as potential biomarkers. Characterization of neuromediators tear levels confirmed the involvement of these molecules in both DED and ocular allergy.

## Summary

Biomarkers are essential to monitor health status. Several studies have proposed different molecules and/or cellular parameters as potential biomarkers for DED and ocular allergy. The knowledge presented in these studies will further add support to the discovery and development of new drugs and the establishment of personalized medicine.

## Keywords

biomarkers, cytokines/chemokines, dry eye disease, inflammation, ocular allergy

## INTRODUCTION

Biomarkers are essential to monitor health status. A biomarker can be a molecule or another parameter specifically involved in disease pathogenesis, that could be used to detect disease (diagnostic biomarker) or which expression, either alone and/or in combination with others, is associated to severity of the disease (activity biomarkers), or can function as an indicator of treatment outcomes (prognostic, therapeutic biomarker).

DED is an inflammatory disorder of the lacrimal functional unit of multifactorial origin leading to chronic ocular surface disease [1]. It is an increasing healthcare problem, due to both its high prevalence and to its capacity of affecting patients' quality-of-life, work-related issues, as well as healthcare resources [2]. It is universally recognized that symptoms and clinical signs do not correlate well in DED and thus the development of new pharmacological therapies is hampered by scarcity of reliable evaluation endpoints.

Allergy is also a health-care problem, as at least an estimated 20% people in the industrialized countries suffer from this disease. Ocular allergy-related disorders include several clinical subtypes, such as allergic conjunctivitis (acute seasonal and chronic perennial), the controversial giant papillary conjunctivitis (GPC), vernal keratoconjunctivitis, (VKC) and atopic keratoconjunctivitis (AKC) [3]. Of all of these, both VKC and AKC are the most relevant allergic-related diseases in ophthalmology, as they can eventually involve the cornea and

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## KEY POINTS

- Biomarkers can provide new tools for disease diagnosis, activity, and treatment both in DED and ocular allergy.
- The fact that samples can be easily obtained from the ocular surface makes biomarker search an attractive field in these diseases.
- Biomarker studies require proper patient phenotyping as well as agreement in patient classification.

produce permanent visual loss. The release of allergic mediators onto the ocular surface and tear film, is thought to be responsible for many of the corneal and conjunctival manifestations. However, the exact mechanism leading to a sight-threatening outcome is not completely understood. In order to elucidate this, several studies have been carried out in an attempt to identify the specific molecules present in the different types of ocular allergies and the source of them.

For the reasons above, there is growing interest in the identification of biomarkers for these two ocular surface diseases. Up-to-date, several different molecules have already been proposed as potential biomarkers for DED and ocular allergy. These include different proteins, cytokines/chemokines, lipids, and clinical-based, image-based or laboratory-based tests. Some previous reviews about this subject have been already published [4,5].

The aim of this review was to gather recent data (for the last 18 months) on new studies about molecules whose expression in tears and/or conjunctival/corneal tissues or meibum is significantly different between patients and controls and/or correlates with clinical parameters in DED and ocular allergy, and that have been proposed or suggested as potential biomarkers.

## DRY EYE DISEASE

Several types of molecules, including proteins, lipids, neuromediators, and cellular markers have been proposed as potential biomarkers in DED.

### Tear proteomics

Different studies have analyzed DED patients' tear protein profiles by means of proteomic techniques, such as liquid chromatography-tandem mass spectrometry (LC-MS/MS), iTRAQ technology combined with LC and MS, SELDI TOF protein chip arrays. Differences in some protein levels (such as S100A4,

S100A8/calgranulin A, S100A11/calgizzarin, proline-rich protein 3, proline-rich protein 4,  $\alpha$ -enolase, prolactin-inducible protein (PIP), mammaglobin B, lysozyme C, or lactoferrin) between DED patients and controls were found [6,7]. For instance, Tong *et al.* [8<sup>■</sup>], by iTRAQ technology, found that S100A8/calgranulin A and S100A9/calgranulin B correlated to meibomian gland disease (MGD) level and redness, and lipocalin-1 correlated with tearing in DED-non-Sjögren Syndrome.

The secretory phospholipase A2 (sPLA2)-IIa has been suggested as also an important biomarker as elevated tear levels seem to be associated with several inflammatory diseases, including DED [9,10<sup>■</sup>].

Cytokines/chemokines, growth factors and/or their receptors are the most studied in either tears or corneal/conjunctival cells, as immune processes have been demonstrated to play a key role in DED. TNF- $\alpha$ , IL-1, and IL-6 are consistently increased in DED samples, showing correlations with some clinical parameters [11,12].

A cytokine profile of DED patient's tears has been recently published by Boehm *et al.* [13<sup>■</sup>]. By means of antibody microarrays, they found increased tear levels of IL-1 $\beta$ , IL-6, IL-8, TNF- $\alpha$ , and IFN- $\gamma$  and increased expression of lipocalin, cystatin SN, and  $\alpha$ -1 antitrypsin in DED patients, with interesting correlations with some clinical tests. When considering the whole DED group, their analysis confirmed an increase in all these molecules in the tear film of DED patients. However, these elevations were minimal in DED because of lipid layer deficiency, in disagreement with previous reports that found increased levels of IFN- $\gamma$ , IL-1 $\beta$ , and IL-8/CXCL8 by [11] or EGF, fractalkine/CXCL1, IL-1RA and IP-10 [12] in patients with MGD-induced DED. These differences and some other issues pointed out by Tong *et al.* [14] were explained by authors as based on the different way of classifying patients and defining controls [15]. They can also be explained by the different tear sample collection and cytokine measure method, as elution from Schirmer strips [13<sup>■</sup>] collects tears, mainly reflex, but also cells, whereas only tear, mainly basal, are collected by a capillary tube [11,12].

VanDerMeid *et al.* [16<sup>■</sup>] have also examined by Luminex multiplex bead assays the tear levels of IL-1 $\alpha$ , IL-1 $\beta$ , IL-6, IL-8 and TNF- $\alpha$ , as well as metalloproteinases (MMPs) 1, 2, 7, 9 and 10 (see MMPs subheading below) extracted from Schirmer strips in healthy individuals clinically evaluated for DED. All five cytokines correlated inversely with Schirmer values; IL-1 $\alpha$ , IL-6, and TNF- $\alpha$  also correlated directly with tear osmolarity, although as explained by authors, the fact that the individuals

included in this study were healthy and only in five of them tear osmolarity readings were more than 308 mOsm/L, any conclusion associating osmolarity or cytokine concentrations to DED from their findings would be circumspect.

Recently, Choi *et al.* [17<sup>¶¶</sup>] reported elevated tear concentration of CCL3/MIP-1 $\alpha$ , CCL4/MIP-1 $\beta$  and CCL5/RANTES and CCR5 expression in ocular surface in Sjögren syndrome patients compared to non-Sjögren syndrome or controls and higher levels in non-Sjögren syndrome than controls. CCL3/MIP-1 $\alpha$  and CCL4/MIP-1 $\beta$  in tears of patients with DED showed positive correlation with tear clearance rate and goblet cell density. CCL5/RANTES tear levels significantly correlated positively with tear break-up time (TBUT), basal tear secretion, tear clearance rate, keratoepitheliopathy score and goblet cell density. These results are in agreement with previous ones from the same senior author (KC Yoon) in C57BL/6 mice in a desiccating stress model [18]. CCR5<sup>+</sup>CD4<sup>+</sup> and CCR6<sup>+</sup>CD4<sup>+</sup> cells were increased in DED patient's conjunctiva, pointing out that both Th1 and Th17 pathways are involved in DED pathogenesis; in contrast, Th2 CCR4<sup>+</sup>CD4<sup>+</sup> cells were not increased [17<sup>¶¶</sup>], as previously suggested in animal models by other authors.

MMPs have also been involved in ocular surface damage in ocular surface inflammatory diseases. MMP-9 has been paid particular attention in the case of DED, in which its gene transcript has been described to be significantly increased in DED patients' conjunctival cells as well as in corneal and conjunctival epithelial cells from C57BL/6 dry-eye mice [19]; Additionally, MMP-9 protein levels have been described to be increased in tears of aqueous-deficient DED patients vs. controls [20]. MMP-9 was proposed as a useful biomarker for diagnosis, classifying and monitoring DED by Chotikavanich *et al.* in 2009, [21] as they showed that tear MMP-9 activity was significantly higher in DED patients, being this activity associated with increased MMP-9 mRNA expression in conjunctival epithelial cells. Furthermore, Chotikavanich *et al.* [21] data further supported MMP-9 as a good candidate for DED biomarker because tear MMP-9 activity correlated strongly with clinical parameters, with positive correlation with symptom severity score, topographic surface regularity index (SRI) scores, corneal and conjunctival fluorescein staining scores, decreased low-contrast visual acuity and percentage area of abnormal superficial corneal epithelia, and negative correlation with fluorescein TBUT.

VanDerMeid *et al.* [16<sup>¶</sup>] have recently studied the correlations of tear MMP-9 (and also MMP-1, MMP-2, MMP-7, and MMP-10 levels) along with

some cytokine levels (see above) with four DED diagnostic tests: Schirmer's test, TBUT, tear osmolarity and ocular surface disease index (OSDI) in a healthy volunteer group. They found that all MMPs correlated inversely with Schirmer test score, whereas MMPs 2, 9 and 10 correlated positively with tear osmolarity. However, if MMP-9 is going to be included as a biomarker molecule, in the same way that when including some cytokines/chemokines as explained above, the time of the day when tears are obtained should be carefully taken into consideration, because tear MMP-9 levels present a diurnal variation in humans [22<sup>¶¶</sup>]. A diurnal variation in cytokine/chemokine tear levels has also been shown for some other molecules [23]. Also, Tong and Petznick [24], pointed out the fact that when doing these correlation studies, age effect should always be adjusted so results will not be confounded by a potential age effect.

Several previous studies have already demonstrated alterations of ocular mucin (MUC) levels in DED [25–27]. Recently, two separate studies by Corrales *et al.* [28<sup>¶</sup>] and Gipson *et al.* [29<sup>¶</sup>] have further focused on these molecule levels in DED patients, and have shown differences in MUC-1 [28<sup>¶</sup>,29<sup>¶</sup>], MUC-2 [28<sup>¶</sup>], MUC-4 [28<sup>¶</sup>], MUC-5AC [28<sup>¶</sup>], and MUC-16 [29<sup>¶</sup>] conjunctival levels in DED patients compared with controls. However, although the Gipson study shows increased levels of MUC-1 mRNA and protein in DED, in agreement with previous results by Caffery *et al.* [30], the study by Corrales *et al.* [28<sup>¶</sup>] shows the opposite, with significantly reduced mRNA levels of MUC-1 in DED patients' conjunctival epithelium, in agreement with another previous study by Imbert *et al.* [31]. As explained by Corrales *et al.* [29<sup>¶</sup>], differences in the primers used in each study, or the fact that conjunctival impression cytologies (CIC) from different patients were pooled in Caffery's study whereas individual samples were analyzed in Corrales' study may account for this discrepancy in results. MUC-1 genotypic differences have been proposed by Imbert-Fernandez *et al.* [32] as being related to susceptibility to ocular surface damage in DED, based upon the fact that MUC-1/A and MUC-1/B splice variants have different inflammatory activities [32].

Corrales *et al.* also showed reduced mRNA levels of MUC-2, MUC-4 and MUC-16 in DED patients' conjunctival epithelium. They also calculated the sensitivity and specificity of mucin gene expression for DED diagnosis, which revealed that MUC-1 expression levels had the greatest sensitivity and specificity, higher than 80%; this was further validated by using an external dataset of patients and controls. These authors therefore propose using

MUC-1 expression levels as a diagnostic marker of DED. Furthermore, MUC-4 model, which was the second best after MUC-1 in identifying DED patients, was revealed as the one that better classified healthy controls [28<sup>¶</sup>].

The study by Gipson *et al.* [29<sup>¶</sup>] besides increased levels of MUC-1 mRNA (in CIC) and protein (in tears) and MUC-16 protein levels in postmenopausal DED women also shows that mucin levels were correlated with clinical characterization of the patients, including staining and symptoms, which further reinforces the role of these molecules as potential biomarkers.

### Tear lipidomics

Several studies have addressed the determination of composition and nature of lipids in the tear film secreted by the meibomian gland, and some studies have already shown differences in the meibomian fatty acid composition in patients with MGD and aqueous-deficient DED [33]. The group of Lam *et al.* has recently published about the meibum lipid composition in DED Asian patients [34<sup>¶¶</sup>], in which they examined the meibum lipidome, including 256 lipid species of DED patients, compared with that of controls. Sphingomyelin and phosphatidylcholine species were significantly increased in DED patients. Additionally, moderate-DED patients had a significant lower level of triacylglyceride compared with mild-DED patients, and that TAG/54:7, TAG/54:5, TAG/56:7 and TAG/56:6 highly unsaturated TAG species, and a number of phosphatidylcholine species were significantly higher in patients than in controls. Furthermore, the 16:1/32:2, 16:1/31:1, 18:2/32:2 and 16:1/34:2 O-acyl- $\omega$ -hydroxy-fatty acids (OAHFA) species levels were significantly different among the mild, moderate and severe DED patients, being 16:1/32:2 and 16:1/34:2 levels in mild-DED patients significantly higher as compared to moderate-DED and severe-DED patients, with a consistently decreasing trend in all the OAHFA species studied as DED severity level increased. Thus, authors suggest OAHFAs levels to function as indicators of disease progression. However, that study had some limitations stated by the authors, including the lack of age-matched and sex-matched controls.

### Tear neuromediators

Ocular surface innervation, as integrating part as of the lacrimal functional unit [35], has been proposed to play a role in the pathogenesis of DED, through neuromodulators' release in neurogenic inflammation process.

Tear levels of some neuromediators have been proposed as potential useful markers of DED severity. Lambiase *et al.* [36<sup>¶</sup>] correlated substance P, calcitonin gene-related peptide (CGRP), neuropeptide Y (NPY), vasoactive intestinal peptide (VIP) and nerve growth factor (NGF) tear levels in DED patients with clinical findings. NGF tear levels were significantly increased in DED patients, of which authors relate to corneal epithelial damage. CGRP and NPY were significantly decreased in tears from DED patients, of which authors relate to impaired lacrimal function. When DED patients were divided in three subgroups [10 non-Sjögren syndrome, five Sjögren syndrome, and four ocular cicatricial pemphigoid (OCP)] a different neuromediator pattern for each group was observed: NPY was decreased in Sjögren syndrome group; CGRP was increased and NGF was decreased in non-Sjögren syndrome group, and CGRP was decreased in OCP group [36<sup>¶</sup>].

Correlation studies of tear levels of these molecules with clinical parameters revealed that NGF correlated directly with DED severity score, whereas CGRP and NPY showed an inverse correlation. NGF tear levels as well correlated positively with conjunctival hyperemia and fluorescein staining, CGRP directly correlated with Schirmer test and negatively with fluorescein staining, and NPY inversely correlated with TBUT and fluorescein staining. No correlations of neuromediators tear levels with clinical severity or clinical signs were found when patients were subdivided, neither Schirmer test nor TBUT values were significantly different between subgroups.

### Cellular markers

Some cell parameters, such as conjunctival cell viability and their proliferative capacity and also, the number and relative expression of intraepithelial lymphocyte subsets have been recently suggested as potential disease biomarkers in DED by some authors.

In a study published in 2011, Reinoso *et al.* [37<sup>¶</sup>] evaluated cell viability and cell cycle kinetics of the conjunctival epithelium of 23 patients with evaporative-type DED caused by MGD, as compared to 17 healthy controls. Conjunctival cells were collected from inferior fornix and tarsal conjunctiva by brush cytology before and after 2 months of treatment. Conjunctival cell viability and proliferative capacity, in addition to the number of intraepithelial lymphocytes were significantly decreased in DED patients [37<sup>¶</sup>]. DED treatment caused a significant decrease in lymphocytes and in the CD4/CD8 ratio. Interestingly, some of these cell

**Table 1. Potential biomarkers in dry eye disease**

| Potential biomarker | Sample type             | Patient groups                                      | Technique                       | Comparison vs. control (C)                                                               | Correlations with clinical parameters                                                                                                                    | References                                                                                     |
|---------------------|-------------------------|-----------------------------------------------------|---------------------------------|------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------|
| S100A8/CalgranulinA | Tears (Schirmer strips) | DED: NS                                             | iTRAQ                           | NA                                                                                       | (+) MGD level, (+) redness                                                                                                                               | Tong <i>et al.</i> , 2011 [8 <sup>***</sup> ]                                                  |
| S100A9/CalgranulinB | Tears (Schirmer strips) | DED: NS                                             | iTRAQ                           | NA                                                                                       | (+) MGD level, (+) redness                                                                                                                               | Tong <i>et al.</i> , 2011 [8 <sup>***</sup> ]                                                  |
| Lipocalin-1         | Tears (Schirmer strips) | DED: NS<br>DED: DRYaq; DRYlip;<br>DRYaqlip; control | iTRAQ<br>Antibody<br>microarray | NA<br>↑ DRYaq vs. control<br>and vs. DRYlip;<br>↑ DRYaqlip vs. control<br>and vs. DRYlip | (+) Tearing<br>(-) BST (DRYaq, DRYaqlip);<br>(-) fluorescein (DRYaq);<br>(-) Lissamine green (DRYaq,<br>DRYlip, DRYaqlip)                                | Tong <i>et al.</i> , 2011 [8 <sup>***</sup> ]<br>Boehm <i>et al.</i> , 2011 [13 <sup>■</sup> ] |
| IL-1α               | Tears (Schirmer strips) | Control                                             | Luminex                         | NA                                                                                       | (-) Schirmer score;<br>(+) Tear Osm                                                                                                                      | VanDerMeid <i>et al.</i> ,<br>2012 [16 <sup>■</sup> ]                                          |
| IL-1β               | Tears (Schirmer strips) | DED: DRYaq; DRYlip;<br>DRYaqlip; control            | Antibody<br>microarray          | ↑ DRYaq vs. control<br>and vs. DRYlip;<br>↑ DRYaqlip vs. control<br>and vs. DRYlip       | (-) BST (DRYaq, DRYaqlip);<br>(-) fluorescein (DRYaq,<br>DRYlip, DRYaqlip);<br>(-) TBUT (DRYaqlip);<br>(-) Lissamine green (DRYaq,<br>DRYlip, DRYaqlip); | Boehm <i>et al.</i> ,<br>2011 [8 <sup>***</sup> ]                                              |
|                     |                         | Control                                             | Luminex                         | NA                                                                                       | (-) Schirmer score                                                                                                                                       | VanDerMeid <i>et al.</i> ,<br>2012 [16 <sup>■</sup> ]                                          |
| IL-6                | Tears (Schirmer strips) | DED: DRYaq; DRYlip;<br>DRYaqlip; control            | Antibody<br>microarray          | ↑↑ DRYaq vs. control<br>and vs. DRYlip;<br>↑↑ DRYaqlip vs. control<br>and vs. DRYlip     | (-) BST (DRYaq, DRYaqlip);<br>(-) fluorescein (DRYaq,<br>DRYlip);<br>(-) TBUT (DRYaqlip);<br>(-) Lissamine green<br>(DRYaq, DRYlip)                      | Boehm <i>et al.</i> , 2011 [13 <sup>■</sup> ]                                                  |
|                     |                         | Control                                             | Luminex                         | NA                                                                                       | (-) Schirmer score;<br>(+) Tear osmolarity                                                                                                               | VanDerMeid <i>et al.</i> ,<br>2012 [16 <sup>■</sup> ]                                          |
| IL-8                | Tears (Schirmer strips) | DED: DRYaq; DRYlip;<br>DRYaqlip; control            | Antibody<br>microarray          | ↑ DRYaq vs. control<br>and vs. DRYlip;<br>↑ DRYaqlip vs. Control<br>and vs. DRYlip       | (-) BST (DRYaq, DRYaqlip);<br>(-) fluorescein (DRYaq,<br>DRYlip, DRYaqlip);<br>(-) TBUT (DRYaqlip);<br>(-) Lissamine green<br>(DRYaq, DRYlip, DRYaqlip)  | Boehm <i>et al.</i> , 2011 [13 <sup>■</sup> ]                                                  |
|                     |                         | Control                                             | Luminex                         | NA                                                                                       | (-) Schirmer score                                                                                                                                       | VanDerMeid <i>et al.</i> ,<br>2012 [16 <sup>■</sup> ]                                          |
| TNF-α               | Tears (Schirmer strips) | DED: DRYaq; DRYlip;<br>DRYaqlip; control            | Antibody<br>microarray          | ↑ DRYaq vs. control<br>and vs. DRYlip;<br>↑ DRYaqlip vs. control<br>and vs. DRYlip       | (-) BST (DRYaq, DRYaqlip);<br>(-) fluorescein<br>(DRYaq, DRYlip);<br>(-) TBUT (DRYaqlip);<br>(-) Lissamine green<br>(DRYaq, DRYlip)                      | Boehm <i>et al.</i> , 2011 [13 <sup>■</sup> ]                                                  |
|                     |                         | Control                                             | Luminex                         | NA                                                                                       | (-) Schirmer score;<br>(+) Tear osmolarity                                                                                                               | VanDerMeid <i>et al.</i> ,<br>2012 [16 <sup>■</sup> ]                                          |

(Continued)

**Table 1 (Continued)**

| Potential biomarker   | Sample type               | Patient groups                                                          | Technique                       | Comparison vs. control (C)                                                                                     | Correlations with clinical parameters                                                                                                   | References                                                                                         |
|-----------------------|---------------------------|-------------------------------------------------------------------------|---------------------------------|----------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------|
| IFN- $\gamma$         | Tears (Schirmer strips)   | DED: DRYaq; DRYlip; DRYaqlip; control                                   | Antibody microarray             | $\uparrow$ DRYaq vs. control and vs. DRYlip;<br>$\uparrow$ DRYaqlip vs. control and vs. DRYlip                 | (-) BST (DRYaq, DRYaqlip);<br>(-) fluorescein (DRYaq, DRYlip, DRYaqlip);<br>(-) TBUT (DRYaqlip);<br>(-) Lissamine green (DRYaq, DRYlip) | Boehm <i>et al.</i> , 2011 [13 <sup>■</sup> ]                                                      |
| Cystatin-SN           | Tears (Schirmer strips)   | DED: DRYaq; DRYlip; DRYaqlip; control                                   | Antibody microarray             | $\uparrow\uparrow$ DRYaq vs. control and vs. DRYlip;<br>$\uparrow\uparrow$ DRYaqlip vs. control and vs. DRYlip | (-) BST (DRYaq, DRYaqlip);<br>(-) fluorescein (DRYaq);<br>(-) TBUT (DRYaq, DRYaqlip);<br>(-) Lissamine green (DRYaqlip)                 | Boehm <i>et al.</i> , 2011 [13 <sup>■</sup> ]                                                      |
| $\alpha$ -antitrypsin | Tears (Schirmer strips)   | DED: DRYaq; DRYlip; DRYaqlip; control                                   | Antibody microarray             | $\uparrow$ DRYaq vs. control and vs. DRYlip;<br>$\uparrow$ DRYaqlip vs. control and vs. DRYlip                 | (-) BST (DRYaq, DRYaqlip);<br>(-) fluorescein (DRYlip, DRYaqlip);<br>(-) BUT (DRYaq);<br>(-) Lissamine green (DRYaq, DRYlip, DRYaqlip)  | Boehm <i>et al.</i> , 2011 [13 <sup>■</sup> ]                                                      |
| CCL3/MIP1 $\alpha$    | Tears; Conj. Biopsy       | DED: SS and NS; control                                                 | ELISA/IHC                       | $\uparrow$ SS vs. NS and vs. C (tears); $\uparrow$ DED vs. C (tissue)                                          | (+) TCR; (+) GCD                                                                                                                        | Choi <i>et al.</i> , 2012 [17 <sup>■</sup> ]                                                       |
| CCL4/MIP1 $\beta$     | Tears; Conj. Biopsy       | DED: SS and NS; control                                                 | ELISA/IHC                       | $\uparrow$ SS vs. NS and vs. C (tears); $\uparrow$ DED vs. C (tissue)                                          | (+) TCR; (+) GCD                                                                                                                        | Choi <i>et al.</i> , 2012 [17 <sup>■</sup> ]                                                       |
| CCL5/RANTES           | Tears; Conj biopsy        | DED: SS and NS; control                                                 | ELISA/IHC                       | $\uparrow$ SS vs. NS and vs. C (tears); $\uparrow$ DED vs. C (tissue)                                          | (+) TBUT; (+) BTS; (+) TCR; (+) KS; (+) GCD                                                                                             | Choi <i>et al.</i> , 2012 [17 <sup>■</sup> ]                                                       |
| CCR5                  | Conj. Biopsy              | DED: SS and NS; control                                                 | IHC                             | $\uparrow$ DED vs. control                                                                                     | ND                                                                                                                                      | Choi <i>et al.</i> , 2012 [17 <sup>■</sup> ]                                                       |
| MMP-1                 | Tears (Schirmer strips)   | Control                                                                 | Luminex                         | NA                                                                                                             | (-) Schirmer score                                                                                                                      | VanDerMeid <i>et al.</i> , 2012 [16 <sup>■</sup> ]                                                 |
| MMP-2                 | Tears (Schirmer strips)   | Control                                                                 | Luminex                         | NA                                                                                                             | (-) Schirmer score;<br>(+) Tear osmolarity                                                                                              | VanDerMeid <i>et al.</i> , 2012 [16 <sup>■</sup> ]                                                 |
| MMP-7                 | Tears (Schirmer strips)   | Control                                                                 | Luminex                         | NA                                                                                                             | (-) Schirmer score                                                                                                                      | VanDerMeid <i>et al.</i> , 2012 [16 <sup>■</sup> ]                                                 |
| MMP-9                 | Tears (Schirmer strips)   | Control                                                                 | Luminex                         | NA                                                                                                             | (-) Schirmer score;<br>(+) Tear osmolarity                                                                                              | VanDerMeid <i>et al.</i> , 2012 [16 <sup>■</sup> ]                                                 |
| MMP-10                | Tears (Schirmer strips)   | Control                                                                 | Luminex                         | NA                                                                                                             | (-) Schirmer score;<br>(+) Tear osmolarity                                                                                              | VanDerMeid <i>et al.</i> , 2012 [16 <sup>■</sup> ]                                                 |
| MUC-1                 | Conjunctival cells. (CIC) | DED: moderate-severe; control<br>DED: NS- postmenopausal women; control | qRT-PCR<br>qRT-PCR/WB/<br>ELISA | $\downarrow$ mRNA DED vs. control<br>$\uparrow$ mRNA and prot DED vs. control                                  | >80% sensitivity and specificity for DED diagnosis<br>ND                                                                                | Corrales <i>et al.</i> , 2011 [28 <sup>■</sup> ]<br>Gipson <i>et al.</i> , 2011 [29 <sup>■</sup> ] |
| MUC-2                 | Conjunctival cells (CIC)  | DED: moderate-severe; control                                           | qRT-PCR                         | $\downarrow$ mRNA DED vs. control                                                                              | ND                                                                                                                                      | Corrales <i>et al.</i> , 2011 [28 <sup>■</sup> ]                                                   |
| MUC-4                 | Conjunctival cells (CIC)  | DED: moderate-severe; control                                           | qRT-PCR                         | $\downarrow$ mRNA DED vs. control                                                                              | ND                                                                                                                                      | Corrales <i>et al.</i> , 2011 [28 <sup>■</sup> ]                                                   |

|                                                    |                          |                                                                               |                  |                                                |                                                                           |                                                  |
|----------------------------------------------------|--------------------------|-------------------------------------------------------------------------------|------------------|------------------------------------------------|---------------------------------------------------------------------------|--------------------------------------------------|
| MUC-5AC                                            | Conjunctival cells (CIC) | DED: moderate-severe; control                                                 | qRT-PCR          | ↓ mRNA DED vs control                          | ND                                                                        | Corrales <i>et al.</i> , 2011 [28 <sup>■</sup> ] |
| MUC-16                                             | Conjunctival cells (CIC) | DED: NS- postmenopausal women; control                                        | qRT-PCR/WB/ELISA | ↑ mRNA and prot DED vs. control                | (+) staining and symptoms                                                 | Gipson <i>et al.</i> , 2011 [29 <sup>■</sup> ]   |
| Sphingomyelin (SM)                                 | Meibum                   | DED: mild, moderate, severe; control                                          | HPLC/MS MS/MS    | ↑ DED vs. control                              | ND                                                                        | Lam <i>et al.</i> , 2011 [34 <sup>■</sup> ]      |
| Phosphatidylcholine (PC)                           | Meibum                   | DED: mild, moderate, severe; control                                          | HPLC/MS MS/MS    | ↑ DED vs. control                              | ND                                                                        | Lam <i>et al.</i> , 2011 [34 <sup>■</sup> ]      |
| Triacylglycerides (TAGs)                           | Meibum                   | DED: mild, moderate, severe; control                                          | HPLC/MS MS/MS    | ↓ Moderate DED vs. mild DED                    | ND                                                                        | Lam <i>et al.</i> , 2011 [34 <sup>■</sup> ]      |
| OAHA                                               | Meibum                   | DED: mild, moderate, severe; control                                          | HPLC/MS MS/MS    | ↓ Severe DED vs. moderate DED and vs. mild DED | (-) disease severity                                                      | Lam <i>et al.</i> , 2011 [34 <sup>■</sup> ]      |
| Nerve growth factor (NGF)                          | Tears (microsponges)     | DED: SS, NS and OCP; control                                                  | ELISA            | ↑ DED vs. control; ↓ NS                        | (+) DED severity score; (+) conjunct hyperemia; (+) fluorescein staining  | Lambiase <i>et al.</i> , 2011 [36 <sup>■</sup> ] |
| calcitonin gene-related peptide (CGRP)             | Tears (microsponges)     | DED: SS, NS and OCP; control                                                  | ELISA            | ↓ DED vs. control; ↑ NS                        | (-) DED severity score; (+) Schirmer test score; (-) fluorescein staining | Lambiase <i>et al.</i> , 2011 [36 <sup>■</sup> ] |
| Neuropeptide Y (NPY)                               | Tears (microsponges)     | DED: SS, NS and OCP; control                                                  | ELISA            | ↓ DED vs. control; ↓ SS                        | (-) DED severity score; (-) TBUT; (-) fluorescein staining                | Lambiase <i>et al.</i> , 2011 [36 <sup>■</sup> ] |
| Conjunctival cell viability                        | Conjunctival cells (BC)  | DED: Evaporative-type -MGD: Pretreatment and 2 months post-treatment; control | Flow cytometry   | ↓ DED vs. control                              | NSig                                                                      | Reinoso <i>et al.</i> , 2011 [37 <sup>■</sup> ]  |
| Conjunctival cell proliferative capacity           | Conjunctival cells (BC)  | DED: Evaporative-type -MGD: Pretreatment and 2 months post-treatment; control | Flow cytometry   | ↓ DED vs. C control                            | NSig                                                                      | Reinoso <i>et al.</i> , 2011 [37 <sup>■</sup> ]  |
| Conjunctival intraepithelial T cells CD4/CD8 ratio | Conjunctival cells (BC)  | DED: Evaporative-type -MGD: Pretreatment and 2 months post-treatment; control | Flow cytometry   | ↓ Post-treatment-DED vs. pretreatment DED      | (-) DED dryness score pre-treat DED patients                              | Reinoso <i>et al.</i> , 2011 [37 <sup>■</sup> ]  |
| Conjunctival intraepithelial CD3+ T cells          | Conjunctival cells (BC)  | DED: Evaporative-type -MGD: Pretreatment and 2 months post-treatment; control | Flow cytometry   | NSig                                           | (-) DED intensity score post-treat DED patients                           | Reinoso <i>et al.</i> , 2011 [37 <sup>■</sup> ]  |
| Conjunctival intraepithelial CD19+ B cells         | Conjunctival cells (BC)  | DED: Evaporative-type -MGD: Pretreatment and 2 months post-treatment; control | Flow cytometry   | NSig                                           | (+) DED intensity score post-treat DED patients                           | Reinoso <i>et al.</i> , 2011 [37 <sup>■</sup> ]  |

BTS, basal tear secretion (Schirmer test with anesthesia); BS, brush cytology; C, control; CIC, conjunctival impression cytology; DED, dry eye disease; DRYaq, aqueous-deficient DED; DRYlip, patients with changes in lipid layer DED; DRYaqlip, patients with a combination of both aqueous-deficient DED and changes in lipid layer; GCD, goblet cell density; HPLC, high-pressure liquid chromatography; IHC, immunohistochemistry; IL, interleukin; KS, keratoepitheliopathy score; MGD, meibomian gland disease; MMP, metalloproteinase; MS, mass spectrometry; MS/MS, tandem mass spectrometry; MUC, mucin; NS, non-Sjögren; OAHA, O-acyl- $\omega$ -hydroxy-fatty acids; OCP, ocular cicatricial pemphigoid; qRT-PCR, quantitative real-time polymerase chain reaction; SS, Sjögren syndrome; TCR, tear clearance rate; TBUT, tear break-up time; WB, Western blotting; (-): negatively correlated; (+): positively correlated. NA, not applicable; ND, not done; NSig, not significant.

**Table 2. Potential biomarkers in ocular allergy**

| Potential biomarker                    | Sample type                                                   | Patient groups                                                                  | Technique               | Comparison vs. controls                                                                    | Correlations with clinical parameters                                        | References                                         |
|----------------------------------------|---------------------------------------------------------------|---------------------------------------------------------------------------------|-------------------------|--------------------------------------------------------------------------------------------|------------------------------------------------------------------------------|----------------------------------------------------|
| Protein activated receptor-2 (PAR-2)   | Conjunctival epithelial biopsy                                | SAC: in season, out season; controls                                            | IHC                     | ↑ SAC-in season vs. SAC-out season and vs. control                                         | ND                                                                           | Yeoh <i>et al.</i> , 2011 [38 <sup>■</sup> ]       |
| HSP-70                                 | Conjunctival epithelial biopsy                                | SAC: in season, out season; controls                                            | IHC                     | ↑ SAC exposed to pollen vs. control                                                        | ND                                                                           | Yeoh <i>et al.</i> , 2011 [38 <sup>■</sup> ]       |
| Eosinophil cationic protein (ECP)      | Tears (capillary tube)                                        | AKC: moderate or severe, before and after tacrolimus                            | ELISA                   | ↓ Post-treatment vs. pre-treatment                                                         | (+) Conjunctival injection; (+) conjunctival edema; (+) fluorescein staining | Watkamatsu <i>et al.</i> , 2011 [39 <sup>■</sup> ] |
| Hemopexin                              | Tears (capillary tube)<br>Conjunctival epithelial cells (CIC) | VKC: mild, moderate, severe; controls                                           | 2D-PAGE<br>ELISA<br>ICC | ↑VKC vs. control<br>↑In VKC dekeratinized conjunctival epithelium and necrotic macrophages | (+) Disease severity                                                         | Pong <i>et al.</i> , 2011 [41 <sup>■</sup> ]       |
| Substance P (SP)                       | Tears (microsponges)                                          | SAC: out of season, baseline and after conjunctival provocation test; controls. | ELISA                   | ↑ allergic patients post-conjunctival provocation test vs. baseline                        | ND                                                                           | Sacchetti <i>et al.</i> , 2011 [43 <sup>■</sup> ]  |
| Calcitonin gene-related peptide (CGRP) | Tears (microsponges)                                          | SAC: out of season, baseline and after conjunctival provocation test; controls. | ELISA                   | ↑ Allergic patients postconjunctival provocation test vs. baseline                         | ND                                                                           | Sacchetti <i>et al.</i> , 2011 [43 <sup>■</sup> ]  |
| Vasoactive intestinal peptide (VIP)    | Tears (microsponges)                                          | SAC: out of season, baseline and after conjunctival provocation test; controls. | ELISA                   | ↑ Allergic patients postconjunctival provocation test vs. baseline                         | ND                                                                           | Sacchetti <i>et al.</i> , 2011 [43 <sup>■</sup> ]  |

(+), positively correlated; AKC, atopic keratoconjunctivitis; CIC, conjunctival impression cytology; ICC, Immunocytochemistry; IHC, immunohistochemistry; ND, not done; PAC, perennial allergic conjunctivitis; PAGE, polyacrylamide gel electrophoresis; SAC, seasonal allergic conjunctivitis; VKC, vernal keratoconjunctivitis.

parameters showed significant correlations with clinical symptoms and signs [37].

A summary of all the mentioned articles about biomarkers in DED can be found in Table 1.

## OCULAR ALLERGY

Regarding ocular allergy, some tear proteins and neuromediators have been described as potential biomarkers in recent studies.

### Tear proteomics

Mucosal epithelia in some atopic conditions, such as asthma and allergic dermatitis, show permeability changes that have been related to an increased expression of protein activated receptor-2 (PAR-2), which is an important regulator of epithelial barrier permeability. In order to investigate whether similar mechanisms are involved in conjunctival epithelium in ocular allergy, Yeoh *et al.* [38] evaluated the expression of PAR-2 and that of heat shock protein (HSP)-70 HSP-90 and ICAM in conjunctival epithelial biopsies obtained from six patients with seasonal allergic conjunctivitis (SAC)-in-season, and compared it with eight control nonatopic patients or those with out-of-season allergic conjunctivitis ( $n=4$ ) finding that mean percentage staining of PAR-2 was significantly greater in SAC-in-season compared with controls or out-of-season allergic conjunctivitis patients. Authors also showed the over-expression of the HSP-70 in the conjunctival epithelium of SAC patients when exposed to pollen [38], suggesting that this protein also plays an important role in the regulation of the allergic response in allergic eye disease.

Eosinophil cationic protein (ECP) has been proposed as a possible marker in the treatment of AKC by Watkamatsu *et al.* [39] in a study in which tear ECP concentration in nine AKC patients, before and after tacrolimus ophthalmic solution, was analyzed and correlated with clinical signs. ECP tear levels were found to be significantly decreased after treatment. A strong and significant positive linear correlation between ECP levels and some clinical signs, such as conjunctival injection, conjunctival edema and fluorescein staining, was found. Therefore, the measurement of tear ECP levels was proposed by these authors as a potent marker to do the follow-up of allergic conjunctivitis and to evaluate the responsiveness to a specific treatment.

Hemopexin, an inflammatory protein, was found for the first time to be present in tear film by Pong *et al.* in 2010 [40] in a proteomic study in tears from normal individuals and VKC patients. In 2011

Pong *et al.* [41] further quantified and correlated tear hemopexin concentrations in 14 VKC patients with clinical severity and attempted to characterize its causative role in the development of VKC in animal models. Hemopexin was found to be elevated in the tear film of patients with VKC compared with controls, and significantly associated with disease severity, suggesting that it could be a potential biomarker for disease diagnosis and monitoring. Authors also point out that increased concentration of hemopexin in tears could be attributed to further breakdown of the ocular surface, increased vascular permeability from the circulation, or both of these mechanisms.

### Neuromediators

The involvement of neurogenic inflammation in ocular allergy has been described, as well as in the case of DED, in some articles. These were reviewed in 2010 by Mantelli *et al.* [42]. In 2011, with the aim to further characterize the role of these molecules in ocular allergy processes, the group of Sacchetti *et al.* [43] evaluated tear levels of substance P, CGRP, NPY, and VIP in 10 healthy and 15 allergic individuals both at baseline and after conjunctival allergen provocation test. Tear samples were collected with microsponges, and neuromediator levels were assayed by ELISA. Their results showed that after conjunctival allergen provocation test, SP, CGRP, and VIP were significantly increased in allergic tears as compared to baseline. As pointed out by Sacchetti, imbalance of tear neuropeptides during allergic reactions of the ocular surface showed by their data requires further studies to clarify the specific role of individual neuropeptides in healthy conditions and in the mucosal response at different organ sites.

A summary of all the mentioned articles about biomarkers in ocular allergy can be found in Table 2.

## CONCLUSION

Several molecules, including proteins, lipids, neuromediators as well as some cellular parameters, such as cell viability and proliferative capacity, that showed significant differences and/or correlated significantly with clinical symptoms or signs, have been pointed out as potential biomarkers for either DED or ocular allergy. Some studies were done in relatively small samples, and a consensus in classification and phenotyping of patients among different studies should be reached for proper biomarker designation. Nonetheless, all this work adds highly valuable new data to the knowledge in the field of biomarkers in these ocular diseases. This will clearly help us in the future to develop new diagnosis tests

and find new therapeutic alternatives, including the development of targeted drug therapy and personalized medicine for ocular surface chronic diseases.

Although each of these studies shows particular molecule(s) as potential biomarker(s) (either of disease, activity, therapeutic...), a panel including a combination of several of them rather than a single molecule, will be more likely required for specific and accurate biomarker development.

## Acknowledgements

None.

## Conflicts of interest

There are no conflicts of interest.

## REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 000–000).

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5. Enriquez-de-Salamanca A, Calonge M. Cytokines and chemokines in immune-based ocular surface inflammation. *Expert Rev Clin Immunol* 2008; 4:457–467.
6. Grus FH, Podust VN, Bruns K, et al. SELDI-TOF-MS ProteinChip array profiling of tears from patients with dry eye. *Invest Ophthalmol Vis Sci* 2005; 46:863–876.
7. Zhou L, Beuerman RW, Chan CM, et al. Identification of tear fluid biomarkers in dry eye syndrome using iTRAQ quantitative proteomics. *J Proteome Res* 2009; 8:4889–4905.
8. Tong L, Zhou L, Beuerman RW, et al. Association of tear proteins with ■ Meibomian gland disease and dry eye symptoms. *Br J Ophthalmol* 2011; 95:848–852.

This is an interesting article in which by means of iTRAQ technology several tear proteins in DED and Meibomian gland disease patients were analyzed and in which correlation of S100A8 and S100A9 with MGD level and of lipocalin-1 with tearing is shown, further supporting the involvement and the potential of these proteins as biomarkers in DED.

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10. Wei Y, Epstein SP, Fukuoka S, et al. sPLA2-IIa amplifies ocular surface ■ inflammation in the experimental dry eye (DE) BALB/c mouse model. *Invest Ophthalmol Vis Sci* 2011; 52:4780–4788.

In this article Wei et al. [10] by using an experimental DED animal model analyze the role of the sPLA2-IIa in ocular surface inflammation, and demonstrate its implication in it, especially when ocular surface is presentized or compromised.

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This is a study in which five cytokines (IL-1 $\beta$ , IL-6, IL-8, TNF- $\alpha$  and IFN- $\gamma$ ) and lipocalin, cystatin and a1-antitrypsin tear levels were studied in aqueous-deficient DED patients, patients with changes in the lipid layer, or patients with a combination of these two, as compared with controls, showing that increased levels of those molecules were found in aqueous-deficient DED patients, or patients with a combination of aqueous-deficient DED and with changes in the lipid layer, but not in those that only had changes in the lipid layer, excluding an inflammatory contribution in this type of DED in discrepancy with other studies.

14. Tong L, Lee SY, Petznick A. Clinical considerations in proinflammatory cytokine profiling of tears from patients with dry eye by means of antibody microarrays. *Invest Ophthalmol Vis Sci* 2011; 52:9610.
15. Grus F, Boehm N. Author response: clinical considerations in proinflammatory cytokine profiling of tears from patients with dry eye by means of antibody microarrays. *Invest Ophthalmol Vis Sci* 2011; 52:9610–9611.
16. 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.

The authors in this study analysed the correlations of IL-1 $\alpha$ , IL-1 $\beta$ , IL-6, IL-8, TNF- $\alpha$ , MMP-1, MMP-2, MMP-7, and MMP-9 tear levels with clinical scores of Schirmer's test, OSDI, TBUT and tear osmolality in a healthy group of patients that were clinically evaluated for DED, finding significant negative correlation for cytokines and MMPs levels with Schirmer's test and significant positive correlation for IL-1 $\alpha$ , IL-6, TNF- $\alpha$ , MMP-2, MMP-9 and MMP-10 with tear osmolality.

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This is an interesting study showing increased MIP-1 $\alpha$ , MIP-1 $\beta$  and CCL5/RANTES tear levels as well of CCR5 expression in the ocular surface of Sjögren syndrome, and nonSjögren syndrome DED patients. MIP-1 $\alpha$ , MIP-1 $\beta$  and CCL5/RANTES showed as well significant correlations with some clinical parameters. In this study also the involvement of Th1 and Th17 pathways in DED pathogenesis, but not of the Th2, is shown.

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20. Acera A, Rocha G, Vecino E, et al. Inflammatory markers in the tears of patients with ocular surface disease. *Ophthalmic Res* 2008; 40:315–321.
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This is an interesting study that shows of the diurnal variation of MMP-9 levels in tears, highlighting the importance of taken into consideration the time of tear sample obtention.

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28. Corrales RM, Narayanan S, Fernandez I, et al. Ocular mucin gene expression ■ levels as biomarkers for the diagnosis of dry eye syndrome. *Invest Ophthalmol Vis Sci* 2011; 52:8363–8369.

This is an interesting study in which authors demonstrate that among other mucins, conjunctival MUC-1 mRNA expression levels had the highest sensitivity and specificity (>80%) for DED diagnosis, proposing MUC-1 levels as a diagnostic marker of DED.

29. Gipson IK, Spurr-Michaud SJ, Senchyna M, et al. Comparison of mucin levels ■ at the ocular surface of postmenopausal women with and without a history of dry eye. *Cornea* 2011; 30:1346–1352.

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31. Imbert Y, Darling DS, Jumblatt MM, et al. MUC1 splice variants in human ocular surface tissues: possible differences between dry eye patients and normal controls. *Exp Eye Res* 2006; 83:493–501.
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33. Joffre C, Souchier M, Gregoire S, et al. Differences in meibomian fatty acid composition in patients with meibomian gland dysfunction and aqueous-deficient dry eye. *Br J Ophthalmol* 2008; 92:116–119.
34. Lam SM, Tong L, Yong SS, et al. Meibum lipid composition in Asians with dry ■ eye disease. *PLoS One* 2011; 6:e24339.

In this interesting article, authors analyze the Meibum lipid composition in Asians with dry eye disease showing differences in some types of lipids as compared to controls, as well as some lipids species (OAHFA) significantly decreased as DED severity level increased, and thus proposed as biomarkers of disease progression.

35. Stern ME, Beuerman RW, Fox RI, et al. The pathology of dry eye: the interaction between the ocular surface and lacrimal glands. *Cornea* 1998; 17:584–589.

**36.** Lambiase A, Micera A, Sacchetti M, *et al.* Alterations of tear neuromediators in dry eye disease. *Arch Ophthalmol* 2011; 129:981–986.

The authors address in this study the analysis of several neuromediator levels in tears of DED patients, and correlated them with clinical findings, showing alterations in these molecule levels as well as specific correlations with some clinical parameters. Also, a specific pattern of neuromediator expression was found when patients were classified as Sjögren syndrome NS and OCP.

**37.** Reinoso R, Calonge M, Castellanos E, *et al.* Differential cell proliferation, apoptosis, and immune response in healthy and evaporative-type dry eye conjunctival epithelia. *Invest Ophthalmol Vis Sci* 2011; 52:4819–4828.

In this article, two cellular parameters, cell viability and cellular proliferative capacity in conjunctival epithelium of evaporative-type DED patients, are shown to be significantly decreased compared with controls. Also, some other cells parameters, as well as ratio of subpopulations of intraepithelial lymphocytes showed significant correlations with clinical parameters.

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PAR-2 increased tear expression in SAC-in season patients is shown by the authors and proposed to have a role regulating conjunctival epithelial barrier permeability in ocular allergy.

**39.** Wakamatsu TH, Tanaka M, Satake Y, *et al.* Eosinophil cationic protein as a marker for assessing the efficacy of tacrolimus ophthalmic solution in the treatment of atopic keratoconjunctivitis. *Mol Vis* 2011; 17:932–938.

ECP tear levels are analyzed as a potential marker for the follow-up of allergic conjunctivitis and for treatment responsiveness evaluation in this article by the authors.

**40.** Pong JC, Chu CY, Chu KO, *et al.* Identification of hemopexin in tear film. *Anal Biochem* 2010; 404:82–85.

**41.** Pong JC, Chu CY, Li WY, *et al.* Association of hemopexin in tear film and conjunctival macrophages with vernal keratoconjunctivitis. *Arch Ophthalmol* 2011; 129:453–461.

This is an interesting study showing correlation of Hemopexin tear levels with disease severity in VKC patients.

**42.** Mantelli F, Micera A, Sacchetti M, Bonini S. Neurogenic inflammation of the ocular surface. *Curr Opin Allergy Clin Immunol* 2010; 10:498–504.

**43.** Sacchetti M, Micera A, Lambiase A, *et al.* Tear levels of neuropeptides increase after specific allergen challenge in allergic conjunctivitis. *Mol Vis* 2011; 17:47–52.

The authors determine in this study the tear levels of several neuromediators in allergic subjects both baseline and after conjunctival allergen provocation test, showing that after provocation test substance P, CGRP and VIP levels were significantly increased in these patients.