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## Editorial

### The tear biomarkers as source of information for the ocular surface. The tears as mirror of the eyes?☆

### Biomarcadores de la lágrima como fuente de información de la superficie ocular. ¿Las lágrimas como espejo del ojo?

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The lachrymal film is the first element of the ocular optic system and fulfills important functions comprising mechanical and antimicrobial protection, ocular surface nutrition and reflective functions, among others. The formation and stability of the lachrymal film both in healthy and diseased eyes depend on the composition and physical properties of the tear. One of the difficulties exhibited by ocular surface diseases is an overlap in clinic and exploration, partly due to the frequent coexistence of both factors in the same subject, which makes its diagnosis and treatment more difficult. Numerous examples of this can be found in pathologies such as blepharitis, dry eye syndrome or ocular allergy. We cannot forget the multiplicity of situations in which the ocular surface is altered for iatrogenic causes including glaucoma, cataract postop period, etc.

In recent years the search of biomarkers for human diseases has been highly successful due to the development of increasingly sensitive techniques in the field of genomics and proteomics. The description of these biomarkers will allow the scientific community to develop more reliable diagnostic methods, to determine the intimate mechanisms of a disease, forecast its progression and get closer to therapeutic optimization. Due to these advances in analysis techniques,

increasingly smaller sample sizes are required to obtain complete analysis. The identification of the physiopathology of ocular surface diseases through tear analysis requires a comprehensive knowledge of all its components. This knowledge cannot be obtained with isolated analyses as is common practice. However, as sample sizes are very small, in some cases the analysis has to be divided even though it is best to analyze all tear components together. With the assistance of precise techniques that require very small amounts of tears such as profile composition techniques, high-performance liquid chromatography or protein arrays which enable the analysis of up to 500 proteins in a single tear sample, it is easier to analyze an integrated analysis of tear components.

Many articles endeavor to describe the best method for collecting the largest possible volume of tears without irritating the ocular surface. The first tests carried out in the early 1980s analyzed the lachrymal film directly by means of breakup time measurements. Subsequently, techniques were developed to collect tears and analyze their composition with conventional biochemical analysis. The most usual methods for collecting tears have been the Schirmer strips, plus capillaries and polyester rods or surgical sponges.<sup>1</sup> The shortcomings of all these methods become evident in analyzing tears for

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pathologies such as dry eye syndrome because the volume that can be obtained is very small.

In the current model, the lachrymal film comprises 2 layers in which the mucoaqueous layer is covered by 2 layers of lipids having the main function of preventing evaporation and improving lachrymal film stability. Polar lipids form a hydrophilic layer adjacent to the mucoaqueous layer and the non-polar lipids form a hydrophobic layer in the tear-air interface.

The analysis of mucines in tears is crucial. Mucines are the glycoproteic component of mucus and their main characteristic is the ability to adhere to hydrophobic as well as hydrophilic molecules. The mucosa layer maintains the lachrymal film adhered to the corneal epithelium, thus avoiding its separation. In addition, it constitutes a barrier against bacterial invasion and inflammatory cells. Recently, Corrales et al. have described the use of a mucine, specifically MUC1 by means of expression analysis as a diagnostic test for dry eye pathology.<sup>2</sup>

Lipids are another component to be taken into account. By means of lipidomics, which allow the analysis of lipids in tears, or metabolomics, it is possible to identify lipids that are altered or lipidic profiles in various ocular surface pathologies. Lipids become highly relevant in pathologies such as dry eye or blepharitis.

Finally, most of the articles published in recent years about tears refer to proteic biomarkers. These are easily approachable therapeutic targets, either inhibiting the synthesis in the lachrymal glands or in the corneal or conjunctival epithelium by means of counter-direction oligonucleotides, RNA silencers or intracellular signal inhibitors, including selective blockage of receptors by means of monoclonal antibodies.

Our results in the analysis of tear proteome in various ocular surface pathologies have differentiated up to 250 proteins. In the tears of patients with conjunctivochalasis we found 24 proteins that were significantly overexpressed against controls. Three of these proteins belonged to the S100, S100-A8, S100-A9 and S100-A4 family. These proteins are involved in pro-inflammatory processes of the ocular surface and in the etiopathogeny of the disease.<sup>3</sup> In addition, we also found that protein is involved in the syntheses and degradation of the cytoskeleton such as the GTP-binding protein 2 and the tubulin-specific chaperone A, as well as proteins involved in oxidative stress processes such as peroxiredoxin-1, -5, glutathione S-transferase P (GSTP) and the fatty acid-binding protein epidermal, the expression of which was greater in the tears of patients with conjunctivochalasis than in the tears of healthy patients. In summary, these results evidenced that inflammation, keratinization and oxidative stress processes appear to intervene in conjunctivochalasis.

In keratocone, traditionally classified as a non-inflammatory disease, recent tear analyses of patients with this pathology have uncovered the presence of an inflammatory component in the tear in direct contact with the altered corneal surface.<sup>4</sup>

In analyses of the tear proteome of patients with keratocone we observed an alteration in the tear composition vis-à-vis healthy patients utilized as controls.<sup>5</sup> On the one hand, the overall concentration of protein in the tear of keratocone patients was lower, perhaps due to the increase observed in the concentration of proteases compared to the control tear, and on the other hand the differential expression of proteins such as cistatin, lipocalin-1, lipofilin and phospholipase A2 were found to be altered in keratocone patients, suggesting that these proteins could play a relevant role in the pathology of keratocone.

There is a long way to go yet, although developments in recent years give rise to hopes in the diagnostic field through the integration of biotechnology and clinic. Once the molecular markers that characterize each ocular pathology have been detected, the next step would be to study the mechanisms by means of which those markers become altered in order to restore lachrymal homeostasis.

Tears are a very important source of information about what is happening on the ocular surface, and knowledge about its alterations by means of biomarker analysis in various pathologies will show the way to cure them.

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