Biomarker Identification of Tear Fluid

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Abstract

While some potential biomarkers for ocular diseases have been reported, it is difficult to select a single biomarker that is most important in each ocular disease. After reviewing multiple biomarker studies conducted on small sample subgroups, we concluded that newer analysis techniques may result in different and more specific findings. While progress has been made in developing various omics studies, studies comparing tears of normal and diseased eyes are still lacking. Preliminary omics studies suggest the importance of further studies aimed at identifying potential ocular-associated lipid, non-lipid/protein, and protein biomarkers. In addition, combining potential biomarkers might be a good strategy for the diagnosis and assessment of ocular diseases.

Keywords: Biomarker of tear fluid; Ocular disease; Omics study

Introduction

Tear fluid serves several functions. The primary role of tear fluid is to provide the cornea with a surface of high optical quality and maintain the well-being of the corneal and conjunctival epithelium. The secondary function of tear fluid is to lubricate the eyelids during blinking. Furthermore, tear fluid is known to have bactericidal properties [1]. Performing these functions requires a complex multilayer film containing proteins, enzymes, lipids, and salts produced by more than one glandular system. Clinical evidence suggests that relatively minor changes in concentrations and composition of elements within the tear fluid can profoundly affect tear film stability and ocular health [2-4].

A number of chronic diseases of the ocular surface produce symptoms known collectively as chronic ocular discomfort. Blepharitis, allergic eye disease, hyposecretive dry eye, and conjunctivochalasis are among the most frequent ocular surface disorders, and it has been demonstrated that inflammation plays an essential role in the pathogenesis of these conditions. However, we currently have little definitive information regarding which specific tear fluid components change in response to these diseases; we also do not know the concentrations of these components that can induce physiological changes in the tear film. In this paper, we review the data related to biomarker identification of tear fluid.

Proteomic analysis of tear fluid

The major tear proteins include lysozyme, lactoferrin, secretory immune-globulin A, lipocalin, albumin, and lipophilin; the protein content in tears reportedly varies between 6 and 10mg/mL [5-6]. Up- or down-regulation of tear proteins can be indicative of the underlying pathology [7-11]. Qualitative and quantitative techniques that have been applied to the study of the tear proteome include gel electrophoresis [12], enzyme-linked immunosorbent assay, and high-performance liquid chromatography [13]. More recently, analytical methods with high sensitivity and resolution have been used in detailed studies of changes in tear composition following injury or disease. These methods, which have been used to map tear protein profiles, include several mass spectrometry (MS) technologies, such as matrix assisted laser desorption ionization-time of flight (MALDI-TOF), surface-enhanced laser desorption ionization-TOF (SELDI-TOF), linear ion trap-Fourier transform, and liquid chromatography coupled with electrospray ionization (LC/MS) [10,11,14-16] as well as multiplex bead analysis systems [17]. The wide range in the number of proteins detected and identified in tears results both from differing sensitivities of the MS methods used by various investigators and the high dynamic range of tear fluid, a small group of proteins (lysozyme, lipocalin, and lactoferrin) account for 70%–80% of the protein content [18], which may make detection of less abundant proteins difficult without prefractionation of samples. The results of MS studies have revealed a common set of 30–40 proteins that are consistently identified independent of the sampling and MS detection method. While progress has been made in developing proteomic baseline studies of tear proteins, there have been few studies that compare normal and diseased tears.

While the meibomian gland has been primarily viewed as a lipid factory with a primary purpose of producing and excreting lipids onto the ocular surface, a recent study reported the identification of more than 90 proteins in human meibomian gland secretion [19]. The identified proteins included cellular proteins, such as cytoplasmic, mitochondrial, nuclear, and membrane proteins, which would be expected because of the holocrine nature of the meibomian gland Table 1. Unexpectedly, a number of lipid-binding proteins were also reported, such as potential phospholipid-transporting ATPase 1K, and lipocalin 1, which binds to a number of lipophilic ligands.

Proteins in the tear fluid may play crucial roles in maintaining eye health. Perturbation of proteins in the tear fluid may lead to the development of disease, and thus, tear fluid proteins might be interesting targets for disease diagnosis and further functional characterization.

Lipidomic analysis of tear fluid

The lipid layer of the tear film is a complex mixture of triglycerides, free fatty acids, diesters, cholesterol and wax esters, free cholesterol, hydrocarbons, and polar lipids produced primarily by the meibomian gland [20,21]. A three-layered structure (lipid layer, aqueous-mucin layer, and glycocalyx layer) is the generally accepted model of the
compositional make-up of the tear film [21]. Current tear film models suggest that a chemically stable, lamellar layer of polar phospholipids lies anterior to the aqueous fluid and binds the nonpolar meibomian oil to the aqueous layer [22]. The presence of this polar phospholipid interface is thought to be critical to the spreading of the nonpolar lipid film over the aqueous layer. The polar phosphorylated lipids of the inner layer act as surfactants and as an anchoring layer at the lipid-aqueous interface, whereas the outer, nonpolar layer primarily retards evaporation of the aqueous layer from the tear film. The instability of the outer lipid layer of the tear film, due to changes in the polar lipid concentration, has been identified as a potential factor in the development of dry eye syndrome [23,24].

Analysis of the meibum and tear lipids has also been hampered by a lack of sensitive analytical methods and the need for pooled samples and extensive sample manipulation before analysis (tags or derivatization), similar to tear proteins. Despite the difficulties, the chemical composition of human meibomian gland liquid secretions has been characterized by pico-nuclear magnetic resonance [25] and thin-layer chromatography [26]. Fatty acid fragment profiles have been obtained using LC/MS [27].

**Ocular disease and biomarker research**

An important ongoing and future focus of tear- and meibum film-associated biomolecule research is the measurement and identification of disease state biomarkers [28]. To date, there are a number of tear fluid- and meibum-associated lipids and proteins that have been identified as possible disease-related biomarkers. The new wave of “omics” methods, such as proteomics, metabolomics, glycomics, and lipidomics, have made the identification of disease state markers in meibomian lipid and tear fluid more achievable. Development of bioinformatics databases associated with mass spectrometry and genetic sequencing have further advanced these investigative techniques. Once identified, biomarkers can be used for clinical diagnostics and early detection of dry eye diseases, such as keratoconjunctivitis sicca- associated dry eye (evaporative keratoconjunctivitis sicca), meibomian gland disease, Sjögren syndrome, blepharitis, androgen deficiency, age-related dry eye, and lacrimal gland dysfunctions. Proper and specific diagnosis allows medical intervention that may substantially influence relative success of early treatments and subsequent cures.

**Biomarkers for corneal wounding**

Tears contain a complex mixture of proteins and while changes in some peptides have been found, the rate of most of the more than one hundred tear proteins remains unknown during corneal wound healing. Such potential changes in tear proteins are important because they play important roles in regulating epithelial migration, proliferation and differentiation, cell-to-cell and cell-to-matrix interactions, stroma-extracellular matrix production, inflammation, scar formation, and protection against pathogens. Previous studies have identified some tear proteins that may participate in the corneal wound-healing cascade. For example, fibronectin [29], a glycoprotein present in plasma and the extracellular matrix, has a role in epithelial cell migration and is a temporary substrate for cells migrating on the corneal surface. Other extracellular matrix related proteins released in tear fluids, such as plasminogen activator [30] and matrix metalloproteinases (MMP-8 and MMP-14) [31], have also been suggested to play roles in the wound healing process. Neuropeptides, such as substance P (SP) [32] and calcitonin gene-related peptide (CGRP) [33], are important molecules because of their roles in the neurogenic phase of a wound response, which results in the breakdown of the blood-ocular barrier and allows large numbers of neutrophils to access the tears. A number of growth factors and cytokines, including epidermal growth factor (EGF) [34], transforming growth factor-β [35], vascular endothelial growth factor [36], platelet-derived growth factor-BB [37], hepatocyte growth factor [38], basic fibroblast growth factor [39], tumor necrosis factor-a [39], and interleukin (IL)-6 [40], have been found in tears and may modulate wound healing by stimulating epithelial growth, whereas others may trigger epithelial cell apoptosis. Potential tear protein biomarkers for corneal wounding have been observed in rabbit and human tear protein studies utilizing electrospray ionization (ESI)/MS or SELDI-TOF-MS Protein Chips Table 2 and included 1) an eight-fold increase in lysozyme [14]; 2) upregulation of neutrophil defensins NP-1 and

| Protein | Function |
|---------|----------|
| q2 Macroglobulin receptor | Proteinase and growth factor regulation; lipoprotein metabolism |
| IgA α chain | Mucosal immunity |
| Farnesoid X activated receptor | Cholesterol homeostasis modulation |
| Interferon regulatory factor 3 | Innate immune system’s response to viral infection |
| Lactolin precursor | Secretion and renewal of lacrimal and ocular surface epithelia |
| Lactotransferrin (lactoferrin) | Anti-inflammatory and/or anti-microbial function |
| Lipocalin 1 | Physiological scavenger of potentially harmful lipophilic molecules; the maintenance of tear film stability |
| Lysozyme C precursor | Anti-inflammatory and/or anti-microbial function |
| Potential phospholipid transporting ATPase II | Phospholipid transfer |
| Somatostatin receptor type 4 | Inhibition of adenylate cyclase; activation of both arachidonate release and the mitogen activated protein kinase cascade |
| High affinity IgE receptor β subunit | Binding to the Fc region of the IgE receptor; initiation of the allergic response |
| TrkC tyrosine kinase | The receptor for neurotrophin-3 |

**Table 1: Analysis of proteins in human meibomian gland secretions [19,57,58].**

| Biomarker | Omics | Analytical Method | Sample Source | Marker type | Regulation (ratio) | Ref |
|-----------|-------|-------------------|--------------|------------|------------------|----|
| Lysozyme  | Proteomics | HPLC-ESI/MS | Tear (Rabbit) | Corneal wounding | Up (8) | [14] |
| Neutrophil defensins NP-1 and NP-2 | Proteomic | SELDI-TOF-MS Protein Chip | Tear (Rabbit) | Corneal wounding | Up (6.4-10.2) | [41] |
| A-defensins (HN1-1, HNP-2, and HNP-3) | Proteomic | SELDI-TOF-MS Protein Chip | Tear (Human) | Ocular surface surgery | Up (>10) | [42] |

Ratio: (diseased eye)/(normal eye)

HPLC-ESI/MS: high-performance liquid chromatography-electrospray ionization/mass spectrometry, SELDI-TOF: surface-enhanced laser desorption ionization-time of flight

**Table 2: Tear- and meibum-associated potential protein biomarkers tentatively identified for corneal wounding in animal models and human subpopulations [28].**
**Biomarkers for dry eye**

The number of patients with dry eye is rapidly increasing. Patients with dry eye typically report discomfort, burning, irritation, photophobia, and blurred vision and have an elevated risk of corneal infection and irreversible tissue damage [43]. Several potential tear biomarkers for dry eye syndrome have been identified Table 3 and Table 4. Differential regulation of lactoferrin (down), EGF (down), and aquaporin 5 (up) were found to be associated with a variety of dry eye syndromes (non-Sjögren, Sjögren, and Stevens-Johnson) [9]. Seven tear biomarkers were found to be generally associated with dry eye syndrome: up-regulation of the C-terminal fragment of alpha-1-antitrypsin and calgranulin A, and downregulation of nasopharyngeal carcinoma-associated proline-rich protein, proline-rich protein 4, and proline-rich protein 3 [10]. In another study, up-regulation of 6 proteins, α-enolase; α-1-acid glycoprotein 1; S100 A8 (calgranulin A); S100 A9 (calgranulin B); S100 A4; and S100 A11 (calgizzarin), and down-regulation of 4 proteins, prolactin-inducible protein (PIP); lipocalin-1; lactoferrin; and lysozyme, [44] was associated with dry eye syndrome. In addition, dry eye tear protein exhibited differential expression of proteins as observed in the 25–35 kDa ranges. One of the proteins with significantly reduced levels was proline-rich protein 4 [45]. Calgranulin A and proline-rich protein 4 were identified in the reports of both groups. Ten unidentified proteins were found to be differentially regulated in Sjögren syndrome. These ten proteins were only identifiable by their masses; seven proteins were downregulated (2094, 2743, 14191, 14702, 16429, 17453, and 17792 m/z) and three were upregulated (3483, 4972, and 10860 m/z) [7].

Polar and nonpolar lipid expression changes were observed in the tears of a surgically induced dry eye rabbit model. In this model, the lacrimal and Harderian glands were removed from one eye to produce a dry eye condition, while the contralateral eye was kept normal as a control, and the tear lipids were determined by ESI/QqQ/MS [46] and

| Biomarker | Omics | Analytical Method | Sample Source | Marker type | Regulation (ratio) | Ref |
|-----------|-------|-------------------|---------------|-------------|-------------------|----|
| Lipophilin Cystatin/lysozyme Lipophilin CL Lipocalin Unidentified β-2 Microglobulin Heterodimer of lipophilins A and C Apolipoprotein D monomer | Proteomics | 1-D SDS-PAGE MALDI-TOF/MS ESI-QqQ/MS | Tear (Rabbit) | Surgically induced dry eye | Up (2.1) Up (2.8) Up (1.4) Up (1.1) Up (1.3) Down (0.4) Down (0.5) Down (0.7) | [59] |
| Lactoferrin EGF AQPS | Proteomics | ELISA | Tear (Human) | Dry eye (non-Sjögren, Sjögren & Stevens-Johnson Syndrome) | Down (0.06–0.34) Down (0.06–0.45) Up (4.0, Sjögren only) | [9] |
| α-1-antitrypsin, C-terminal fragment Calgranulin A Unidentified Nasopharyngeal carcinoma-associated proline-rich protein Proline-rich protein 4 Proline-rich protein 3 Unidentified | Proteomics | SELDI-TOF-MS (Shirmer tear collection) | Tear (Human) | Dry eye | Up (1.8) Up (3.0) Up (13.9) Down (0.37) Down (0.32) Down (0.40) Down (0.39) | [10] |
| Ten proteins (m/z data only) | Proteomic | SELDI-TOF-MS (Shirmer tear collection) | Tear (Human) | Sjögren syndrome | 3-Up 7-Down | [7] |
| α-enolase α-1-acid glycoprotein 1 S100 A8 Calgranulin A S100 A9 Calgranulin B S100 A11 Calgizzarin S100 A4 Calcium-Binding protein Prolactin-inducible protein Lipocalin-1 Lactoferrin Lysozyme C | Proteomic | iTRAQ ESI-MS/MS | Tear (Human) | Dry eye | Up (1.7) Up (2.4) Up (1.4) Up (1.7) Up (2.3) Down (0.59) Down (0.76) Down (0.79) Down (0.70) | [44] |
| Metalloproteinase-9 | Proteomic | ELISA | Tear (Human) | Dry eye | Up (4.2–4.54) | [60] |
| EGF Fractalkine/CX3CL1 IL-1Ra IP-10/CXCL10 VEGF | Proteomic | Multiplex bead analysis | Tear (Human) | Dry eye | Up (>10) Up (>10) Up (10) Up (10) Up (10) | [17] |
| Proline-rich 4 protein | Proteomic | 2-D electrophoretic analysis | Tear (Human) | Dry eye | Down (ND) | [46] |

Ratio: (diseased eye)/(normal eye)

ND: not determined, 1-D-SDS-PAGE: one-dimensional-sodium dodecyl sulfate polyacrylamide gel electrophoresis, MALDI-TOF/MS: matrix assisted laser desorption ionization-time of flight/mass spectrometry, SELDI-TOF: surface-enhanced laser desorption ionization-time of flight, ESI-QqQ: electrospray ionization-Quadrupole-hexapole-quadrupole, ELISA: enzyme-linked immunosorbent assay, iTRAQ: isobaric tag for relative and absolute quantitation

**Table 3:** Tear- and meibum-associated potential protein biomarkers tentatively identified for dry eye syndrome in animal models and human subpopulations [28].
Tear- and meibum-associated potential lipid and non-lipid/protein biomarkers tentatively identified for dry eye in animal models and human subpopulations [28].

**Table 4:**

| Biomarker | Omics          | Analytical Method          | Sample Source | Marker type | Regulation (ratio) | Ref. |
|-----------|----------------|----------------------------|---------------|-------------|--------------------|------|
| Serum albumin precursor Ig x chain-VIII | Proteomic | 2-D SDS-PAGE ESI-QTOF/MS | Tear (Human) | Blepharitis | Down (2.0) | [8] |
| Pyruvate kinase α-1-antitrypsin | Lipidic | MALDI-TOF/MS | Tear (Rabbit) | Dry eye | Up (ND) | [48] |
| Prolactin-inducible protein | Lipidic | HPLC/MS | Meibum (Human) | Age-related dry eye | Various changes (ND) | [49] |
| Cystatin SA-III Lacritin precursor Lysozyme Unidentified | Metabonomic | HPLC (Shirmer tear collection) | Tear (Human) | Dry eye | Up (100–345; with low tear secretion subjects) | [50] |
| Tear osmolarity | – | Osmometer | Tear (Human) | Dry eye | Up (ND) | [51] |

**Ratio:** (diseased eye)/(normal eye)

**ND:** not determined, ESI-QTOF/MS: electrospray ionization-quadrupole-hexapole-quadrupole/mass spectrometry, MALDI-TOF: matrix assisted laser desorption ionization-time of flight, HPLC: high-performance liquid chromatography

**Table 5:** Tear- and meibum-associated potential protein and lipid biomarkers tentatively identified for blepharitis and meibomian gland dysfunction in animal models and human subpopulations [28].

| Biomarker | Omics          | Analytical Method | Sample Source | Marker type | Regulation (ratio) | Ref. |
|-----------|----------------|-------------------|---------------|-------------|--------------------|------|
| IL-6 Pro-MMP-9 | Proteomic | ELISA | Tear (Human) | Blepharitis | Up (2.0) | [52] |
| Cholesterol Ceramides | Lipidic | TLC | Meibum (Rabbit) | Meibomian gland deficiency | Up (2) | [53] |
| Phosphoethanolamine Sphingomyelin | Lipidic | TLC/HPLC CI-GC/MS | Meibum (Human) | Chronic blepharitis with KCS | Down (0.28) | [23] |
| Phase transition temperature Hydrocarbon chain order Lipid-lipid interaction enthalpy | Lipidic | IR | Meibum (Human) | Meibomian gland dysfunction | Up (ND) | [54] |

**KCS:** keratoconjunctivitis sicca

**Ratio:** (diseased eye)/(normal eye)

**ND:** not determined, ESI-QTOF/MS: electrospray ionization-quadrupole-time-of-flight mass spectrometry, 2-D-SDS-PAGE: two-dimensional-sodium dodecyl sulfate polyacrylamide gel electrophoresis, ELISA: enzyme-linked immunosorbent assay, TLC/HPLC: thin-layer chromatography/high-performance liquid chromatography, CI-GC: chemical ionization-gas chromatography

There was a significant increase in the concentrations of three types of sphingomyelin lipids and a phosphatidylserine lipid in dry eye tears compared with normal tears. The presence of different sphingomyelin and phosphatidylserine species in dry eye tears suggests that the meibomian gland might compensate to stabilize the tear film in the absence of lacrimal and Harderian gland secretions. In addition, Sullivan et al. [48] described the effect that complete androgen insensitivity syndrome and aging have on the polar and neutral lipid fractions of patient meibum samples. The meibum obtained from women with complete androgen insensitivity syndrome exhibited significant differences in expression levels of nonpolar lipids, including wax and cholesterol esters. The polar lipid fractions also exhibited differences associated with the complete androgen insensitivity syndrome condition that included certain phosphatidylcholine and phosphatidylethanolamine species. Finally, significant differences were observed in the nonpolar and polar lipid profiles associated with aging, suggesting that alterations in the lipid might be related to the age-related increase of observed cases of dry eye syndrome.

In a human tear biomarker study, diadenosine polyphosphates were found to be increased 100–345-fold in patients with reduced tear secretion and symptomatic dry eye [49]. The observed increase in diadenosine polyphosphates could thus be used as an objective biomarker in the initial observance of dry eye condition. In addition, tear osmolarity correlates with dry eye severity, and therefore could provide a biomarker for disease severity [50].

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Biomarkers for blepharitis

Chronic blepharitis is one of the most common eye diseases and is an extremely complex condition that manifests with several different and overlapping signs and symptoms [51]. Symptoms can include crusting of the lid margins, itching and burning eyelids as well as signs of eyelid inflammation, and associated conjunctival and corneal changes. Several investigators have discovered potential tear biomarkers for blepharitis Table 5. Down regulation of nine proteins (serum albumin precursor, α-1 antitrypsin, lacratin precursor, lysozyme, Ig-κ chain VIII, keratin type I cytoskeletal 10, glutathione S-transferase P, Peroxiredoxin-1, Peroxiredoxin-5) have been suggested to play a significant role in maintaining the interfacial movement and a theory that emphasizes ocular surface inflammation and delayed tear clearance as etiological factors. Several investigators have found potential tear biomarkers for conjunctivochalasis Table 6. Twenty-four proteins were significantly upregulated in conjunctivochalasis compared with controls [56]. Eleven proteins were identified, which included proteins belonging to the S100 family (A8, A9, A4; 2.44, 1.71, and 2.82 fold upregulation, respectively), guanosine triphosphate-binding protein 2 (1.95 fold), L-lactate dehydrogenase A-like 6B (2.32 fold), fatty acid-binding protein (2.01 fold), keratin type I cytoskeletal 10 (1.81 fold), glutathione S-transferase P (2.27 fold), peroxiredoxin-1, peroxiredoxin-5 (1.79 and 1.92 fold, respectively), and cullin-4B glyceraldehyde 3-phosphate dehydrogenase (1.96 fold). Moreover, inflammatory cytokines IL-1β and IL-6 were found to be overexpressed in chronic blepharitis [52].

In rabbit lipid biomarker studies, Nicolaides et al. produced a clinically evident meibomian gland deficiency model by daily topical application of 2% epinephrine for a period of 6 months–1 year [53]. Over time, the rabbit model resembled meibomian gland deficiency or chronic posterior blepharitis in humans. In this animal model, the content of both free steroids and ceramides were increased compared with those of normal controls. In a study comparing meibomian gland lipids of patients with chronic blepharitis, Shine and McCulley observed that the expression of polar lipids phosphatidylethanolamine and sphingomyelin was significantly reduced in patients with chronic blepharitis in conjunction with keratoconjunctivitis sicca, compared to individuals with chronic blepharitis and non-keratoconjunctivitis sicca-like symptoms [23]. Polar lipids, such as sphingomyelin and phosphatidylethanolamine are zwitterionic phospholipids that have been suggested to play a significant role in maintaining the interfacial layer between the aqueous and oil layers of the tear film. Foulks et al. [54] suggested that the ordering of lipid molecules is altered in meibomian gland dysfunction and azithromycin can improve the abnormal condition in a manner that correlates with clinical response to therapy.

Biomarkers for conjunctivochalasis

Conjunctivochalasis, defined as a redundant, loose, nonedematous inferior bulbar conjunctiva interposed between the globe and the lower eyelid, has been shown to cause different types of ocular surface irritation [55]. This disorder is a frequent cause of chronic ocular discomfort, such as irritation, epiphora, dryness, and blurred vision, and it generally affects elderly people. Although several reports regarding the etiology of conjunctivochalasis have been published, there continues to be a discrepancy between a theory that emphasizes aging and ocular movement and a theory that emphasizes ocular surface inflammation and delayed tear clearance as etiological factors. While some potential biomarkers for ocular diseases have been reported, it is difficult to select a single most important biomarker for each ocular disease. Some of the reviewed biomarker studies were conducted on small sample subgroups; newer analysis techniques may result in different and more specific findings. While progress has been made in developing various omics studies, studies comparing normal and diseased eye tears are still lacking. Preliminary studies suggest the importance of further research targeted at identifying potential ocular-associated lipid and non-lipid/protein biomarkers and protein biomarkers.

In dry eye and conjunctivochalasis, upregulation of S100 family proteins has been identified. S100 family proteins are markers of inflammation and oxidative processes, and monitoring their levels in tears might be useful for assessing the severity and progression ocular diseases. In dry eye, down-regulation of proline-rich proteins has been identified. Some cytokines may be upregulated in inflammatory eye conditions. Combining several of the potential biomarkers may be a good strategy for diagnosis and assessment of ocular diseases.

References
1. Holly FJ, Lemp MA (1977) Tear physiology and dry eyes. Surv Ophthalmol 22: 69-87.
2. Shine WE, McCulley JP (1998) Keratoconjunctivitis sicca associated with meibomian secretion polar lipid abnormality. Arch Ophthalmol 116: 849-852.
3. Snyder C, Fullard RJ (1991) Clinical profiles of non-dry eye patients and correlations with tear protein levels. Int Ophthalmol 15: 363-389.
4. Prydal JI, Artal P, Woon H, Campbell FW (1992) Study of human preconreal
tumor cell invasion and extracellular matrix degradation. Invest Ophthalmol Vis Sci 34: 1872-1878.

25. Dvir S, Sicher I, Dvir D, Margalit E (2005) Identification of human tear fluid proteins: pro-inflammatory cytokines. Invest Ophthalmol Vis Sci 46: 3843-3848.

26. Dvir S, Dvir D, Sicher I, Margalit E (2004) Identification of tear fluid proteins involved in the immune response to the matrix. Invest Ophthalmol Vis Sci 45: 4220-4225.

27. Dvir S, Sicher I, Dvir D, Margalit E (2003) Identification of tear fluid proteins involved in the immune response to the matrix. Invest Ophthalmol Vis Sci 44: 3843-3848.

28. Dvir S, Sicher I, Dvir D, Margalit E (2002) Identification of tear fluid proteins involved in the immune response to the matrix. Invest Ophthalmol Vis Sci 43: 3843-3848.

29. Dvir S, Sicher I, Dvir D, Margalit E (2001) Identification of tear fluid proteins involved in the immune response to the matrix. Invest Ophthalmol Vis Sci 42: 3843-3848.

30. Dvir S, Sicher I, Dvir D, Margalit E (2000) Identification of tear fluid proteins involved in the immune response to the matrix. Invest Ophthalmol Vis Sci 41: 3843-3848.

31. Dvir S, Sicher I, Dvir D, Margalit E (1999) Identification of tear fluid proteins involved in the immune response to the matrix. Invest Ophthalmol Vis Sci 40: 3843-3848.

32. Dvir S, Sicher I, Dvir D, Margalit E (1998) Identification of tear fluid proteins involved in the immune response to the matrix. Invest Ophthalmol Vis Sci 39: 3843-3848.

33. Dvir S, Sicher I, Dvir D, Margalit E (1997) Identification of tear fluid proteins involved in the immune response to the matrix. Invest Ophthalmol Vis Sci 38: 3843-3848.

34. Dvir S, Sicher I, Dvir D, Margalit E (1996) Identification of tear fluid proteins involved in the immune response to the matrix. Invest Ophthalmol Vis Sci 37: 3843-3848.

35. Dvir S, Sicher I, Dvir D, Margalit E (1995) Identification of tear fluid proteins involved in the immune response to the matrix. Invest Ophthalmol Vis Sci 36: 3843-3848.

36. Dvir S, Sicher I, Dvir D, Margalit E (1994) Identification of tear fluid proteins involved in the immune response to the matrix. Invest Ophthalmol Vis Sci 35: 3843-3848.

37. Dvir S, Sicher I, Dvir D, Margalit E (1993) Identification of tear fluid proteins involved in the immune response to the matrix. Invest Ophthalmol Vis Sci 34: 3843-3848.

38. Dvir S, Sicher I, Dvir D, Margalit E (1992) Identification of tear fluid proteins involved in the immune response to the matrix. Invest Ophthalmol Vis Sci 33: 3843-3848.

39. Dvir S, Sicher I, Dvir D, Margalit E (1991) Identification of tear fluid proteins involved in the immune response to the matrix. Invest Ophthalmol Vis Sci 32: 3843-3848.

40. Dvir S, Sicher I, Dvir D, Margalit E (1990) Identification of tear fluid proteins involved in the immune response to the matrix. Invest Ophthalmol Vis Sci 31: 3843-3848.

41. Dvir S, Sicher I, Dvir D, Margalit E (1989) Identification of tear fluid proteins involved in the immune response to the matrix. Invest Ophthalmol Vis Sci 30: 3843-3848.

42. Dvir S, Sicher I, Dvir D, Margalit E (1988) Identification of tear fluid proteins involved in the immune response to the matrix. Invest Ophthalmol Vis Sci 29: 3843-3848.

43. Dvir S, Sicher I, Dvir D, Margalit E (1987) Identification of tear fluid proteins involved in the immune response to the matrix. Invest Ophthalmol Vis Sci 28: 3843-3848.

44. Dvir S, Sicher I, Dvir D, Margalit E (1986) Identification of tear fluid proteins involved in the immune response to the matrix. Invest Ophthalmol Vis Sci 27: 3843-3848.

45. Dvir S, Sicher I, Dvir D, Margalit E (1985) Identification of tear fluid proteins involved in the immune response to the matrix. Invest Ophthalmol Vis Sci 26: 3843-3848.

46. Dvir S, Sicher I, Dvir D, Margalit E (1984) Identification of tear fluid proteins involved in the immune response to the matrix. Invest Ophthalmol Vis Sci 25: 3843-3848.

47. Dvir S, Sicher I, Dvir D, Margalit E (1983) Identification of tear fluid proteins involved in the immune response to the matrix. Invest Ophthalmol Vis Sci 24: 3843-3848.

48. Dvir S, Sicher I, Dvir D, Margalit E (1982) Identification of tear fluid proteins involved in the immune response to the matrix. Invest Ophthalmol Vis Sci 23: 3843-3848.

49. Dvir S, Sicher I, Dvir D, Margalit E (1981) Identification of tear fluid proteins involved in the immune response to the matrix. Invest Ophthalmol Vis Sci 22: 3843-3848.

50. Dvir S, Sicher I, Dvir D, Margalit E (1980) Identification of tear fluid proteins involved in the immune response to the matrix. Invest Ophthalmol Vis Sci 21: 3843-3848.

51. Dvir S, Sicher I, Dvir D, Margalit E (1979) Identification of tear fluid proteins involved in the immune response to the matrix. Invest Ophthalmol Vis Sci 20: 3843-3848.

52. Dvir S, Sicher I, Dvir D, Margalit E (1978) Identification of tear fluid proteins involved in the immune response to the matrix. Invest Ophthalmol Vis Sci 19: 3843-3848.

53. Dvir S, Sicher I, Dvir D, Margalit E (1977) Identification of tear fluid proteins involved in the immune response to the matrix. Invest Ophthalmol Vis Sci 18: 3843-3848.

54. Dvir S, Sicher I, Dvir D, Margalit E (1976) Identification of tear fluid proteins involved in the immune response to the matrix. Invest Ophthalmol Vis Sci 17: 3843-3848.

55. Dvir S, Sicher I, Dvir D, Margalit E (1975) Identification of tear fluid proteins involved in the immune response to the matrix. Invest Ophthalmol Vis Sci 16: 3843-3848.

56. Dvir S, Sicher I, Dvir D, Margalit E (1974) Identification of tear fluid proteins involved in the immune response to the matrix. Invest Ophthalmol Vis Sci 15: 3843-3848.

57. Dvir S, Sicher I, Dvir D, Margalit E (1973) Identification of tear fluid proteins involved in the immune response to the matrix. Invest Ophthalmol Vis Sci 14: 3843-3848.

58. Dvir S, Sicher I, Dvir D, Margalit E (1972) Identification of tear fluid proteins involved in the immune response to the matrix. Invest Ophthalmol Vis Sci 13: 3843-3848.

59. Dvir S, Sicher I, Dvir D, Margalit E (1971) Identification of tear fluid proteins involved in the immune response to the matrix. Invest Ophthalmol Vis Sci 12: 3843-3848.

60. Dvir S, Sicher I, Dvir D, Margalit E (1970) Identification of tear fluid proteins involved in the immune response to the matrix. Invest Ophthalmol Vis Sci 11: 3843-3848.

61. Dvir S, Sicher I, Dvir D, Margalit E (1969) Identification of tear fluid proteins involved in the immune response to the matrix. Invest Ophthalmol Vis Sci 10: 3843-3848.

62. Dvir S, Sicher I, Dvir D, Margalit E (1968) Identification of tear fluid proteins involved in the immune response to the matrix. Invest Ophthalmol Vis Sci 9: 3843-3848.

63. Dvir S, Sicher I, Dvir D, Margalit E (1967) Identification of tear fluid proteins involved in the immune response to the matrix. Invest Ophthalmol Vis Sci 8: 3843-3848.

64. Dvir S, Sicher I, Dvir D, Margalit E (1966) Identification of tear fluid proteins involved in the immune response to the matrix. Invest Ophthalmol Vis Sci 7: 3843-3848.

65. Dvir S, Sicher I, Dvir D, Margalit E (1965) Identification of tear fluid proteins involved in the immune response to the matrix. Invest Ophthalmol Vis Sci 6: 3843-3848.

66. Dvir S, Sicher I, Dvir D, Margalit E (1964) Identification of tear fluid proteins involved in the immune response to the matrix. Invest Ophthalmol Vis Sci 5: 3843-3848.

67. Dvir S, Sicher I, Dvir D, Margalit E (1963) Identification of tear fluid proteins involved in the immune response to the matrix. Invest Ophthalmol Vis Sci 4: 3843-3848.

68. Dvir S, Sicher I, Dvir D, Margalit E (1962) Identification of tear fluid proteins involved in the immune response to the matrix. Invest Ophthalmol Vis Sci 3: 3843-3848.

69. Dvir S, Sicher I, Dvir D, Margalit E (1961) Identification of tear fluid proteins involved in the immune response to the matrix. Invest Ophthalmol Vis Sci 2: 3843-3848.

70. Dvir S, Sicher I, Dvir D, Margalit E (1960) Identification of tear fluid proteins involved in the immune response to the matrix. Invest Ophthalmol Vis Sci 1: 3843-3848.
49. Peral A, Carracedo G, Acosta MC, Gallar J, Pintor J (2006) Increased levels of diadenosine polyphosphates in dry eye. Invest Ophthalmol Vis Sci 47: 4053-4058.

50. Suzuki M, Massingale ML, Ye F, Godbold J, Elfassy T, et al. (2010) Tear osmolarity as a biomarker for dry eye disease severity. Invest Ophthalmol Vis Sci 51: 4557-4561.

51. Mc Culley JP, Shine WE (2000) Changing concepts in the diagnosis and management of blepharitis. Cornea 19: 650-658.

52. Acera A, Rocha G, Vecino E, Lema I, Durán JA (2008) Inflammatory markers in tears of patients with ocular surface disease. Ophthalmic Res 40: 315-321.

53. Nicolaides N, Santos EC, Smith RE, Jester JV (1989) Meibomian gland dysfunction. III. Meibomian gland lipids. Invest Ophthalmol Vis Sci 30: 946-951.

54. Foulks GN, Borchman D, Yappert M, Kim SH, McKay JW (2010) Topical azithromycin therapy for meibomian gland dysfunction: clinical response and lipid alterations. Cornea 29: 781-788.

55. Meller D, Tseng SC (1998) Conjunctivochalasis: literature review and possible pathophysiology. Surv Ophthalmol 43: 225-232.

56. Acera A, Suárez T, Rodríguez-Agirrebe I, Vecino E, Durán JA (2011) Changes in tear protein profile in patients with conjunctivochalasis. Cornea 30: 42-49.

57. Ma P, Wang N, McKown RL, Raab RW, Laurie GW (2008) Focus on molecules: lacritin. Exp Eye Res 86: 457-458.

58. Wojnar P, Dimholer S, Ladumer P, Berger P, Redl B (2002) Human lipocalin-1, a physiological scavenger of lipophilic compounds, is produced by corticotrophs of the pituitary gland. J Histochem Cytochem 50: 433-435.

59. Ham BM, Jacob JT, Cole RB (2007) Single eye analysis and contralateral eye comparison of tear proteins in normal and dry eye model rabbits by MALDI-ToF mass spectrometry using wax-coated target plates. Anal Bioanal Chem 387: 889-900.

60. Chotikavanich S, de Paiva CS, Li DQ, Chen JJ, Bian F, et al. (2009) Production and activity of matrix metalloproteinase-9 on the ocular surface increase in dysfunctional tear syndrome. Invest Ophthalmol Vis Sci 50: 3203-3209.