

SJOGREN'S SYNDROME AND OCULAR SURFACE CHANGES BY LASER-SCANNING IN VIVO CONFOCAL MICROSCOPY

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ABSTRACT

INTRODUCTION: Dry eye disease (DED) and ocular allergy are common conditions in the eye practice. They have more than 20% of prevalence and that fact increases healthcare problems and affects life quality. Rheumatologic diseases such as Sjogren's syndrome (SS) are often associated with ophthalmic findings, and belong to the dry eye but with decreased tear film production.

MATERIAL AND METHODS: The laser-scanning confocal microscopy (LSCM) was performed to 16 eyes of 8 patients with subjective complains and objective clinical signs of DED and SS. The results of examinations were compared with control group of 28 eyes of 14 healthy patients. The qualitative analysis was performed to calculate the cell density in all cornea layers. The presence and distribution of Langerhans cells (LCs) in cornea was estimated. The quantitative survey understood monitoring of inflammation signs and corneal changes such as a presence of leukocytes. Changes in subbasal nerve plexus (SNP) and Meibomian glands (MGs) were evaluated over time.

RESULTS: The quantitative analysis presented calculations of cell density of all cornea layers. The most important finding was the reduction of epithelium cell density. In healthy patients it was 5048.6 ± 56 cells/mm² compared to 4603 ± 70 cells/mm² in DED-SS patients, but also the keratocyte number was significantly lower. The density of corneal LCs was compared to the healthy patients: 71 ± 8 cells/mm² and 19 ± 3 cells/mm² respectively. The quantitative observation showed visible changes in SNP especially in tortuosity and destiny of sub-basal nerves.

CONCLUSION: The ability of LSCM technology to examine different components of the ocular surface has opened new doors for studying the physiology and pathology. LSCM allows understanding the pathological changes of DED and SS. Our study proved that these diseases affect all structures of the anterior ocular surface.

Keywords: dry eye disease, Sjogren's syndrome, laser-scanning confocal microscopy, cornea

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INTRODUCTION

Dry eye disease (DED) and ocular allergy are common problem often underdiagnosed in the eye care practice. They are ones of the leading reasons for patient's visits to ophthalmologists. DED and allergy have more than 20% of prevalence and that fact increases healthcare problems and affects life quality (1-4). DED includes a broad spectrum of signs and symptoms that make classification very complex.

Rheumatologic diseases are often associated with ophthalmic findings, particularly diseases re-

lated with dry eye and the ocular surface. Sjögren's syndrome (SS) is a chronic autoimmune disease in which white blood cells destroy the exocrine glands, especially salivary and lacrimal glands. It is a group of diseases overseen by rheumatologists but its management requires more specialty areas: rheumatology, ophthalmology and oral medicine (5,6,7).

The DED and SS have different pathogenic mechanisms. Their clinical differences are not always as clear-cut as expected. The cause is a common inflammatory involvement of the same ocular surface structure (epithelium of cornea and conjunctiva) (1). The high-tech diagnostic instruments are rapidly becoming important additional tools for correct management of these diseases.

In vivo laser-scanning confocal microscopy (LSCM) is an emerging noninvasive technology that allows exploring eye surface structures at the cellular level. The provided images are comparable to known methods of histology without affecting the steady state of observed tissues (1,7). The most suitable ocular surface issues to be studied by LSCM are cell net, innervation, Meibomian glands and inflammation changes (1,8).

The purpose of this work is to evaluate the changes of ocular surface and to present the distribution of Langerhans cells (LCs) in cornea of patients with DED and SS by in vivo laser-scanning confocal microscopy and to define the markers of inflammatory reaction.

MATERIALS AND METHODS

The LSCM was performed to 16 eyes of 8 patients (all subjects were female, mean age 48.2 ± 8.9 years) with subjective complains and objective clinical signs of DED and laboratory proved SS. The results of the examinations were compared with the results of the control group of 28 eyes of 14 healthy patients (all female, mean age 30.5 ± 5.4 years). All subjects underwent slit-lamp examination to determine their clinical status. The qualitative analysis was performed to calculate the cell density in all cornea layers. The presence and distribution of Langerhans cells (LCs) in cornea was estimated. The quantitative survey understood monitoring of inflammation signs and corneal changes such as a presence of leukocytes. Changes in subbasal nerve plexus (SNP) and

Meibomian glands (MGs) were also followed in this study.

LSCM application to the cornea provided new important information about changes in this avascular, thin and transparent, and multilayered tissue (1). LSCM was performed on all subjects with a new generation confocal microscope, the Rostock Corneal Software Version 1.2 of the HRTIII-RCM (Heidelberg Retina Tomograph III - Rostock Cornea Module, Heidelberg Engineering GmbH, Germany), based on a diode laser with a 670nm wavelength. Gel (Corneregel, Bausch&Lomb GmbH, Berlin, Germany) was used as a coupling agent between the applanating lens cap and objective lens.

After the eye was anaesthetized (Alcain, 0,5% collyr, Alcon) the Tomo-Cap was positioned onto the center of the cornea or onto the palpebral conjunctiva of the averted eyelid by adjusting the controller, and the digital images of the corneal layers or underlying Meibomian glands (MGs) were observed on the computer screen.

The examination started when the first superficial cells were visualized. The digital micrometer gauge was set at zero, and then by pressing on the foot pedal, sequence images were recorded by a charge-coupled device camera (maximum 30 frames/s) while gradually moving the focal plane into the explored tissue until the structures were visualized. The length of a single confocal microscopy examination session was approximately 10 min. None of the subjects complained of discomfort nor any adverse effects were observed as a consequence of the examination.

RESULTS

The quantitative analysis presented calculations of cell density of all cornea layers. The most important finding was the reduction of epithelium cell density. In healthy patients it was 5048.6 ± 56 cells/mm² compared to 4603 ± 70 cells/mm² in DED-SS patients, but also the keratocyte number was significantly lower. The results are presented in number of cells in mm² (Table 1). Using LSCM there was a visible structural difference between healthy and DED-SS corneas (Fig. 1).

The presence and distribution of Langerhans cells (LCs) in cornea was estimated in order to demonstrate an inflammatory reaction. The most sig-

Table 1. Quantitative data for all cornea layers in healthy and DED-SS patients

| | Healthy patients (cells/mm ²) | DED and Sjogren syndrome patients (cells/mm ²) |
|-------------------|--|---|
| Epithelium | 5048.6±56 | 4603±70 |
| Anterior stroma | 288.40±43 | 112±12 |
| Posterior stroma | 251.20±39 | 150±13 |
| Endothelium | 2848.20±108 | 2532±102 |

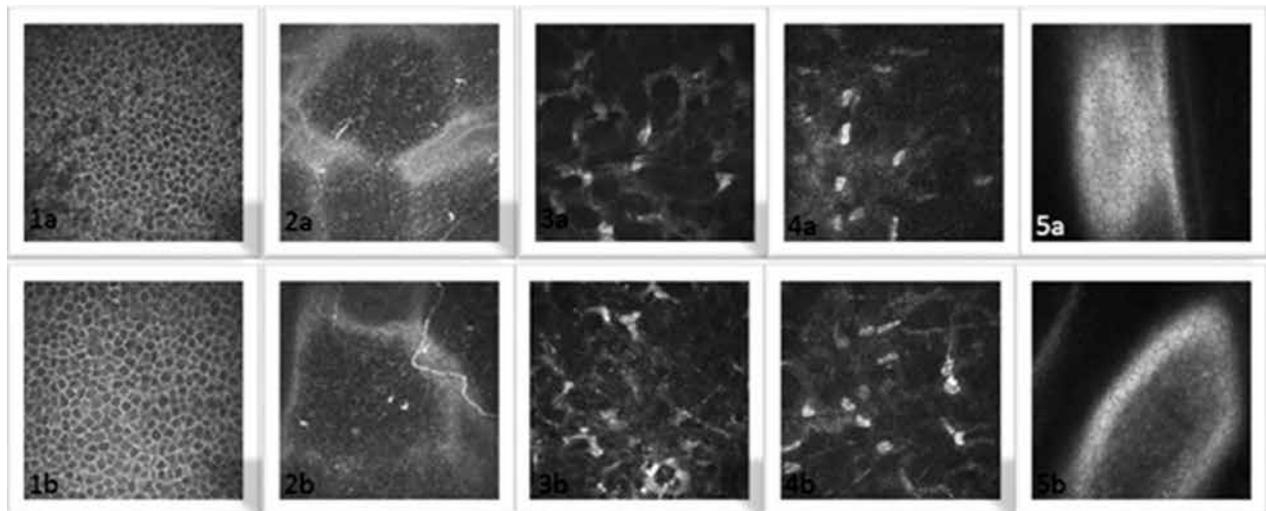


Fig. 1. Quantitative imaging by LSCM of healthy and DED-SS corneas

1-healthy cornea and 2-cornea of dry eye disease and Sjogren syndrome patient:

a) epithelium; b) subbasal nerve plexus; c) anterior stroma; d) posterior stroma; e) endothelium;

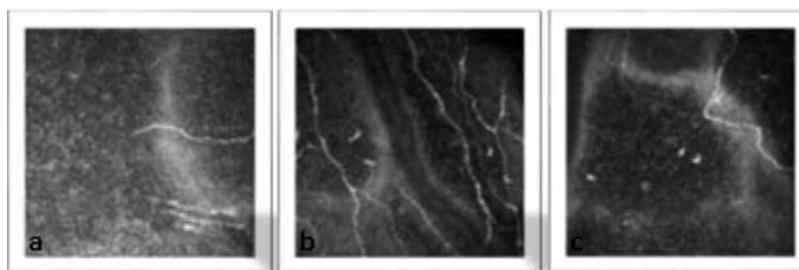


Fig. 2. LSCM demonstrating Langerhans cells in healthy and DED-SS patients

a) In healthy patients: normal structure

b) and c) DED-SS patients: decreased subbasal nerve density and increased tortuosity, high level of Langerhans cells

nificant change in DED and SS patients is the increased density of corneal LCs or dendric cells compared to the healthy patients group: 71±8 cells/mm² compared to 19±3cells/mm². On attentive quantitative observation a difference on SNP is visible. The

most frequent findings are in tortuosity and destiny of sub-basal nerves (Fig. 2). In vivo confocal assessment of inflammatory cells in stroma presented among small hyper-reflective cells were probably leukocytes. These cells were also found around the

Meibomian glands (MGs). The later showed changes in their normal anatomic structure such as fully or partly obstruction of glands' lumens and accumulation of Meibum into gland lumen (Fig. 3). The acinar diameter and orifice diameter were increased.

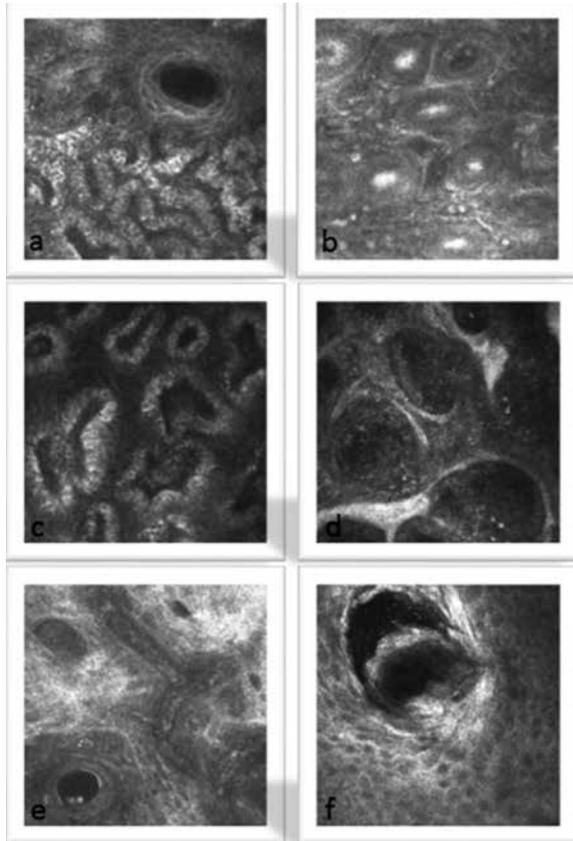


Fig. 3. LSCM demonstrating Meibomian glands

- a) normal MGs*
- b) MGs and inflammatory cells around*
- c) normal healthy MGs' structure*
- d) lack of connective tissue*
- e) normal gland orifice*
- f) increased gland diameter and partly obstructed lumen*

DISCUSSION

The disease of dry eye is complex, multifactorial ocular surface disease, involving the eye like a morphofunctional unit (1). This system integrated the tear film, lacrimal glands, cornea, conjunctiva, Meibomian glands, lids, and the sensory and motor nerves (10).

Recently, comparison of *in vivo* LSCM data for patients with primary SS, non-Sjogren dry eye disease and control individuals showed significant changes related with DED clinical signs regarding densities of corneal epithelias (1,9). Villani et al. in an elegant study demonstrated that all cornea in range of DED values vs. controls, as it is shown 941–1506 vs. 1902 cells/mm² presented a decreased superficial epithelial cell density. The densities of basal epithelial were also decreased (3616–4875 vs. 5164 cells/mm²) respectively (1,11).

Previous other confocal findings partly confirmed and led the hypothesis that changes on the epithelium and especially difference in cell density are consequential indicators for tissue damage (1,12). Other *in vivo* LSCM studies provided several partially validated parameters which included dendric cells (DCs), presumed leukocytes and activated keratocytes (9,11,12). In our study we encounter a significantly decreased number of basal epithelial cells, as well as a decreased number of stromal keratocytes. This is another confirmation of the destructive effect of the dry eye on the anterior ocular surface.

Originally described by Engelmann in 1867 (7,13), DCs are antigen-presenting cells of bone marrow origin: the current concept is that corneal epithelial DCs are identical to the immunologically active LCs of the epidermis. The presence and distribution of LCs in the central cornea is an issue of ongoing discussion: some investigators have already shown the presence of LCs in patients with herpes keratitis (7,14) and in contact lens wearers and in healthy volunteers (15,16) by using confocal microscopy.

An Italian research group, Macheda et al., gave also the mean values of LCs density calculated in central corneas and peripheral corneas of three groups: SS, non-SS DED and healthy patients. There was a difference in LCs distribution: an increasing cell number from the center to the periphery of the cornea, but this trend difference between LCs density in these two corneal areas was significant only in normal subjects. LCs density in central cornea of DED-SS patients was calculated to 55.55 cells/mm² and 29.89 cells/mm² in healthy subjects. The density in peripheral cornea was 66.66 cells/mm² and 35.29 cells/mm² in healthy ones (5). In our studies we had a significantly increased number of DCs in patients

with SS in comparison to normal subjects. This advanced difference might be a result of the severity of the disease in all included patients.

The conjunctival densities of cells presumed to be leukocytes have elevated in all SS, non-SS DED and DED patients (87 vs. 177-498 cells/mm²) compared to healthy subjects (1,9,11). *In vivo* LSCM assessment of "presumed leukocytes" has recently been partially validated in tarsal and bulbar conjunctivas (1,12,17). Ibrahim et al. quantified presumed MG leukocyte density. They reported that as a MG and DED disease diagnostic tool (1). The density of leukocytes was 100% for both sensitivity and specificity with a cut-off at 300 cells/mm² (18).

The hyper-reflective stromal cells were identified in confocal reports (1). They were presumed to be activated keratocytes. They were not histologically described, and could be interpreted in more than one way (1,9,12,19). These cells could be understood as normal stromal cells in which the light from the confocal microscope ray strikes at an unusual angle. These changes were associated to cornea inflammation in DED patients (1,9,12) or Graves' orbitopathy. But they could be also normal stromal cells. However, they might be cells that disappear during the tissue processing, or change their shape. Future clinical and pathological correlations are definitely required to improve our understanding.

Corneal nerves are involved in the maintenance of ocular surface homeostasis playing a key trophic role and also regulating secretion and distribution of tears (1,11). The cornea is the most densely innervated tissue in the body, and is a main player in DED. LSCM allows the description of the morphology, density, and disease-induced alterations of corneal nerves, particularly the sub-basal nerve plexus (1,8).

Villani et al., comparing SS, non-SS DED, DED, and healthy individuals, found that DED patients had decreased numbers of nerves (2.9-4.3 vs. 5.8 n/frame), and increased tortuosity (1.5-2.5 vs. 1.2, grading 1-4) and bead-like formations (218-304 vs. 116 n/100 mm) (1,9). More recently, Labbe et al. (20) reported decreased nerve density (9.426 vs. 15.956 mm/mm²) and number (24.73 vs. 39.06 n/mm²), which is supposed to be related with DED. They did not describe significant nerve tortuosity and beading changes (1,20). Recently, there are several small stud-

ies that quantitated and analyzed morphologic SNP changes (1,12). They found partially conflicting data (1,12). As visible from our results we confirm these observations.

About MGs in DED and SS patients recent confocal studies of team of Milan confirmed increased acinar diameters (94-106 vs. 49-53mm) and orifice diameters (48-50 vs. 32-35mm), increased secretion reflectivity, and decreased acinar unit density (57-64 vs. 110-121n/mm²) in MG Disease (MGD) (1, 11). The results were compared with these of healthy patients.

Ibrahim et al. first validated the confocal parameters of MG acinar longest diameter, acinar shortest diameter, and acinar unit density for the diagnosis of MGD (1,18). The primary SS patients have less obvious interpretation; they showed persistence of inflammation signs, but without morphologic changes. That led to the hypothesis of an inflammatory-atrophic nonobstructive MGD (1,12,21). We encountered bright, round cells presumed to be inflammatory cells in all tested eyes and believe that this is firm evidence that although primary affecting lacrimal secretory glands SS has a severe impact on the MGs as well. That explains the severity of symptoms and signs confirming the advance of the disease.

CONCLUSION

The ability of LSCM technology to examine different components of the ocular surface has opened new doors for studying the physiology and pathology. LSCM allows the understanding of the pathological changes of DED and SS corneas and the assessment of prognosis and treatment as well. Our study proved that DED and SS affect all structures of the anterior ocular surface.

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