Conjunctival In Vivo Confocal Scanning Laser Microscopy in Patients with Sjögren Syndrome

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PURPOSE. To demonstrate the conjunctival alterations in patients with Sjögren’s (SSDE) and non-Sjögren’s syndrome dry eye (NSSDE) using a new generation confocal microscope (HRTIII/RCM; Heidelberg Engineering, Heidelberg, Germany), in a prospective controlled study.

METHODS. Twenty-eight right eyes of 28 patients with SSDE (28 women; mean age, 58.2 ± 14.3 years), 7 right eyes of patients with NSSDE (7 women; mean age, 66.1 ± 14.4 years), and 14 right eyes of 14 age- and sex-matched control subjects were studied. All subjects underwent the Schirmer test, tear film breakdown time (BUT), vital staining, and confocal microscopy of the temporal bulbar conjunctiva. The density of conjunctival inflammatory infiltrates were also assessed.

RESULTS. The tear quantity, stability, and vital staining scores were significantly worse in patients with SSDE or NSSDE than in control subjects (P < 0.001 and P < 0.05, respectively). Eyes of the patients with SSDE or NSSDE had a significantly higher density of conjunctival and corneal inflammatory infiltrates than did the control eyes (P < 0.001). Conjunctival inflammatory cell densities showed a negative correlation with tear stability and tear quantity and a positive correlation with the vital staining scores. Conjunctival epithelial cell densities were significantly lower in SSDE and NSSDE compared with control subjects (P < 0.05). The density of epithelial cysts was significantly higher in SS than in healthy control eyes (P < 0.001).

CONCLUSIONS. Confocal scanning laser microscopy was an efficient and a noninvasive tool for the quantitative assessment of the conjunctival inflammation and epithelial cell densities as well as evaluation of conjunctival morphologic alterations, such as microcysts in patients with SSDE and NSSDE dry eye disease. (Invest Ophthalmol Vis Sci. 2010;51:144–150) DOI: 10.1167/iovs.08-2722

Sjögren’s syndrome (SS) is a multifactorial autoimmune disorder, mainly affecting the salivary and lacrimal glands, which is influenced by genetic as well as environmental factors that are not yet completely understood. SS occurs worldwide and in people of all ages. The peak incidence has been reported to be in the fourth and fifth decades of life, with a female-to-male ratio of 9:1.1 The incidence of primary SS reported in the literature varies between 1/100 and 1/1000.2

The diagnosis of primary SS in Japan is based on the presence of any of the following five criteria: ocular symptoms; oral symptoms; at least one of the following ocular signs: Schirmer’s test result less than 5 mm, rose bengal or fluorescein score greater than 4 points (in the Van Bijsterfeld scoring); evidence of inflammation in the lacrimal or minor salivary gland; autoantibodies in the serum to Ro (SSA) or La (SSB) antigens or both.3

Inflammation of the ocular surface has been reported as the pivotal event in SS-related dry eye disease,4 the diagnosis of which requires an algorithm of multiple tests including Schirmer test, BUT, and corneal surface evaluation with fluorescein, rose bengal, or lisamine green staining. The ocular surface dryness in SS has been linked to lacrimal hyposecretion, and the accompanying inflammatory reactions of the ocular surface are believed to result in gradual ocular surface epithelial damage.4

The study of the conjunctiva at the cellular level by relatively invasive methods such as impression or brush cytology5 with incorporation of immunohistochemistry staining or flow cytometry6 for inflammatory markers revealed elevated conjunctival inflammation in patients with SS.

Confocal microscopy is a newly emerging, noninvasive technology that is useful as a supplementary diagnostic tool for the in vivo assessment of the histopathology of many ocular surface diseases and anterior-segment disorders, including the in vivo examination of the cornea, bulbar and palpebral conjunctiva, and meibomian glands (MGs).7,15

In this study, we used laser scanning confocal microscopy to evaluate the morphologic changes in the conjunctiva in patients with Sjögren syndrome (SSDE) or non-Sjögren syndrome dry eye (NSSDE) and compared the results with those in healthy control subjects. We also investigated the relation of conjunctival and corneal inflammation as assessed by confocal microscopy to clinical ocular surface findings and tear functions.

METHODS

Subjects and Examinations

Twenty-eight right eyes from 28 patients with SSDE (28 women; mean age: 58.2 ± 14.5 years), seven right eyes from patients with NSSDE (7...
women; mean age: 66.1 ± 14.4 years), and 14 right eyes of 14 age-and sex-matched control subjects (mean age 60.6 ± 17.5) were studied. The diagnosis of the aqueous tear deficiency was made by the following criteria: symptoms of dry eye, abnormality of tear production as determined by the Schirmer test (<5 mm after 5 minutes), tear film instability (<5 seconds), and positive ocular surface rose bengal and fluorescein vital staining. The SS diagnosis was made according to the Japanese consensus criteria. Informed consent was obtained from all subjects. Examination procedures were board reviewed, and the study was conducted in accordance with the tenets of the Declaration of Helsinki. None of the patients had a history of Stevens-Johnson syndrome; chemical, thermal, or radiation injury; or any other systemic/ocular disorder or had undergone any ocular surgery or had contact lens use that might create an ocular surface problem. All patients were using nonpreserved artificial tears for 8 weeks at the time of initial examination and underwent tear function and ocular surface examinations including confocal microscopy before institution of additional dry eye treatment. The results were compared with the same examination parameters performed on the healthy control subjects. Two patients with SSDE who consented to the same tear function and ocular surface examinations including confocal microscopy underwent re-examinations 1 month after initiation of additional therapy. One patient received 0.1% fluorometholone eye drops three times daily, and a viscous agent (0.3% nonpreserved topical hyaluronate eye drops) five times a day, as well as upper and lower punctual plug insertion (Superflex Plugs 0.9 mm; Eagle Vision, Memphis, TN) as additional treatment, whereas the other patient received a viscous agent (0.5% nonpreserved topical hyaluronate eye drops) five times a day in addition to nonpreserved artificial tears containing 0.1% potassium and 0.4% sodium.

Tear Function Tests and Ocular Surface Vital Staining

The standard tear film breakup time measurement was performed. The ocular surface was examined by the double vital staining method. Two microliters of a preservative-free combination of 1% rose bengal and 1% fluorescein dye were instilled into the conjunctival sac, according to a published method.16,17 The interval between the last complete blink and the appearance of the first corneal black spot in the stained tear film was measured three times, and the mean value of the measurements was calculated. Fluorescein and rose bengal staining of the ocular surface were also noted and scored. Both fluorescein and rose bengal staining scores ranged between 0 and 9 points. Any score above 5 points was regarded as abnormal. For further evaluation of tear film instability, the Schirmer test was performed without anesthesia. The standardized strips of filter paper (Alcon Inc., Fort Worth, TX) were placed in the lateral canthus away from the cornea and left in place for 5 minutes with the eyes closed.18 Readings were recorded in millimeters of wetting for 5 minutes. A reading of less than 5 mm was referred as an aqueous deficiency.

Confocal Scanning Laser Microscopy

In vivo confocal scanning laser microscopy (CSLM) was performed on all subjects with a new generation confocal microscope (Rostock Corneal Software Version 1.2 of the Heidelberg Retina Tomograph II; RCM/HRT II; Heidelberg Engineering GmbH, Dossenheim, Germany). After the administration of topical anesthesia with 0.4% oxybuprocaaine, the subject’s chin was placed in a chin rest. The objective of the microscope was an immersion lens covered by a polymethylmethacrylate cap (Tomo Cap; Heidelberg Engineering GmbH). Comfort gel (Bausch&Lomb, GmbH, Berlin, Germany) was used as a coupling agent between theapol planating lens cap and the conjunctiva. The center of the cap was applanated onto the center of the temporal interpapibular bulbar conjunctiva by adjusting the controller, and in vivo digital images of the conjunctiva were visualized directly on the computer screen. When the first superficial cells were seen, the digital micrometer gauge was set at 0. Sequence images were recorded by a charge-coupled device (CCD) color camera (five frames/s), by pressing on the foot pedal while gradually moving the focal plane forward into the conjunctival stroma. The laser source used in the HRTII/RCM is a diode laser with a wavelength of 670 nm. Two-dimensional images consisted of 384 × 384 picture element, covering an area of 400 μm². The transverse field of view was captured with a 400-FOV field lens.

Conjunctival and Corneal Image Analysis

At least five sequences (100 images per sequence) of conjunctival CSLM images were taken for each eye. The morphologic characteristics of the conjunctiva were observed in patients with SSDE or NSSDE and compared with those of normal control subjects. Three images with high resolution and contrast were selected from the basal epithelial layer for inflammatory cell counting. We chose the basal epithelial cell layer to analyze the inflammatory cell densities, because inflammatory cells appear like round hyperreflective bodies with the basal epithelial area providing an excellent contrast for inflammatory cell counts. Each epithelial cell at three different depths (superficial cells, <10 μm; intermediate cells, 10–30 μm; deeper cells, >30 μm) as well as each inflammatory cell was manually marked with a blue dot inside each 400 × 400-μm frame, and the cell counts were performed automatically (Cell Count software; Heidelberg Engineering GmbH). The results were expressed as cell density ±SD (cells per square millimeter). Epithelial microcysts appearing as dark round bodies were also evaluated for their density. The images were selected by a co-researcher who did not know whether the subject was a patient with SSDE or NSSDE or a control subject.

At least three nonoverlapping images out of 100 scans with high resolution and quality from a single sequence were selected for density calculation from each eye. The inflammatory cell densities of the central cornea were observed in patients with SSDE or NSSDE and were compared with those of normal control subjects. Three images with high resolution and contrast were selected for inflammatory cell counting from the basal epithelium and analyzed as just described.

Statistical Analysis

The Kruskal-Wallis test was used to compare the examination parameters between the SSDE or NSSDE subjects and the normal control subjects (Instat software, version 3.0; GraphPad, San Diego, CA). The Spearman rank correlation analysis was used to determine the correlation between the conjunctival inflammatory cell densities and the tear quantity, tear stability, and ocular surface vital staining. A probability of less than 1% was considered statistically significant.

RESULTS

There were no significant differences in distribution of age and sex between the normal control subjects and the patients with SSDE or NSSDE (P = 0.32).

Tear Functions and Ocular Surface Staining Scores

Comparisons of tear functions and ocular surface findings between normal control subjects and patients with NSSDE or SSDE are shown in Table 1. The mean Schirmer test result was significantly higher in the normal control subjects (15.5 ± 10 mm) than in the patients with SSDE (2.5 ± 1.6 mm; P < 0.001). Although the mean Schirmer test result in the eyes with NSSDE (5.5 ± 2.2 mm) tended to be higher than in the SSDE dry eyes, there were no statistically significant differences. The mean BUTs were significantly lower in the SSDE eyes (3.2 ± 1.0 seconds; P < 0.001) and in the NSSDE eyes (3.9 ± 1.0 seconds; P < 0.05) than in the normal control eyes (9.1 ± 4.7 seconds). The mean fluorescein and rose bengal staining scores were significantly higher in the SSDE eyes (3.4 ± 2.2 and 3.1 ± 1.8
In Vivo Confocal Microscopy Conjunctival Inflammatory Cell Densities

The mean conjunctival inflammatory cell densities in the SSDE, NSSDE, and healthy control eyes were 433 ± 435.8, 134.8 ± 124.2, and 10 ± 17.9 cells/mm², respectively. The differences were statistically significant, as shown in Figure 1 (P < 0.001). The mean corneal inflammatory cell densities in the SSDE, NSSDE, and healthy control eyes were 45.5 ± 38.5, 22.2 ± 25.1, and 2.23 ± 8.5 cells/mm², respectively (Fig. 1). The statistical significances are shown in Figure 1. In all the SSDE eyes, in vivo confocal microscopy consistently displayed inflammatory infiltrates within the epithelium, mainly consisting of polymorphs, dendritic cells, and/or lymphocytes. Figures 2A and 2B display representative in vivo confocal scans from a patient with SSDE and an age- and sex-matched healthy control subject, respectively. Confocal microscopy images from the patients with SSDE who consented to additional examinations after 1 month of follow-up demonstrated extensive inflammation consisting of polymorphs, dendritic cells, and/or lymphocytes within the conjunctival epithelium before treatment. Figures 3A-C show representative confocal scans from a 52-year-old woman with SSDE. After 1 month of dry eye treatment, considerable reduction of conjunctival inflammatory infiltrates at two conjunctival epithelial depths (65 and 75 μm) were observed (Figs. 3B-D). Pretreatment inflammatory infiltrates mainly consisted of polymorphs and dendritic cells. Figures 4A and 4B show representative in vivo confocal microscopy scans and anterior segment photographs of the ocular surface in a 54-year-old woman with SSDE. After 1 month of treatment, a considerable decrease in the inflammatory cell density was observed (Figs. 4A1, 4B1). Fluorescein staining scores decreased from 5 points to 1 point (Figs. 4A2, 4B2) and rose bengal staining scores changed from 5 to 3 points (Figs. 4A3, 4B3) The pretreatment BUT value was 3 seconds and increased to 5 seconds after 1 month of treatment.

The Correlation between Inflammatory Cell Density, Tear Stability, Tear Quantity, and Vital Staining Scores

Spearman correlation analysis showed significant positive linear correlation between inflammatory cell densities and vital staining scores. The inflammatory cell density had a significant negative linear correlation with the Schirmer test scores and tear film breakup times. The Spearman correlation coefficients and the probabilities are shown in Table 2.

Conjunctival Epithelial Cell Density Comparisons

The mean conjunctival epithelial cell densities at all three depth settings, namely (<10 μm, 10–30 μm, and >30 μm) were significantly lower in the patients with SSDE compared with the healthy control subjects (P < 0.05). The mean conjunctival superficial epithelial cell densities at <10 μm were significantly lower in the SSDE eyes than in the NSSDE eyes (P < 0.01). The mean conjunctival epithelial cell densities at

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TABLE 1. Comparison of Tear Functions and Ocular Surface Vital Staining

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<thead>
<tr>
<th></th>
<th>Schirmer Test 1 (mm)</th>
<th>BUT (s)</th>
<th>Fluorescein Score (points)</th>
<th>Rose Bengal Score (points)</th>
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<tr>
<td>Dry Eye SS</td>
<td>2.5 ± 1.6</td>
<td>3.2 ± 1.0</td>
<td>3.4 ± 2.2</td>
<td>3.1 ± 1.8</td>
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<td>(n = 28 eyes)</td>
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<tr>
<td>Dry Eye Non SS</td>
<td>5.5 ± 2.2</td>
<td>3.9 ± 1.0</td>
<td>2.1 ± 2.1</td>
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<td>(n = 7 eyes)</td>
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<tr>
<td>Healthy control</td>
<td>13.5 ± 10</td>
<td>9.1 ± 4.7</td>
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<td>(n = 14 eyes)</td>
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Kruskal-Wallis test (nonparametric ANOVA).
two depths—10 to 30 μm, and >50 μm—were significantly lower in the NSSDE eyes than in compared with healthy control eyes (P < 0.05; Fig. 5).

Comparison of Conjunctival Epithelial Microcysts

The mean conjunctival epithelial microcyst density was significantly higher in the SSDE eyes than in the healthy control eyes (Fig. 6; P < 0.001). Figures 7A and 7B show the representative confocal scans of the epithelial cysts from a patient with SSDE and an age- and sex-matched healthy control subject. The difference in conjunctival epithelial microcyst density was not statistically significant between the NSSDE group and the SSDE group, and the healthy normal control subjects.

DISCUSSION

The ocular surface inflammation and mechanical influences in aqueous-deficiency dry eye disease take a toll on the ocular surface health with conjunctival and corneal epithelial damage. To delineate the status of the ocular surface epithelium and any coexisting inflammation in SS and other ocular surface disorders, cytomorphic findings of conventional ocular surface examination techniques such as vital staining, conjunctival impression, and brush cytology have been reported to be important components of clinical diagnosis.

Indeed, conjunctival impression and brush cytology in patients with SSDE have shown increased grades of squamous metaplasia, decreased goblet cell density, and the presence of inflammatory cell infiltrates.19–21

In evaluating the conjunctival epithelium, minimally invasive techniques are most desirable. Confocal microscopy is a new emerging noninvasive technology that is useful as a supplementary diagnostic tool for the in vivo assessment of the histopathology of many ocular surface diseases and anterior segment disorders, including the in vivo examination of the cornea, bulbar and palpebral conjunctiva, eyelids, and MGs.7–15

In vivo confocal microscopic observations of the cornea in patients with SS revealed a patchy and an irregular corneal epithelium, decreased density of the superficial corneal epithelium, decreased or normal subbasal nerve numbers, increased nerve tortuosity, increased sprouting or bead-like formations in the subbasal nerves, activated anterior keratocytes, and a decrease in corneal thickness.7,10,12 Subbasal nerve density assessed by in vivo confocal microscopy has been observed to correlate with vital staining scores and/or Schirmer test results in patients with SS.12

Although corneal morphologic alterations have been extensively studied by in vivo confocal microscopy in SS, a PubMed and Medline search with the key words “Sjögren’s syndrome,” “in vivo confocal microscopy,” “dry eye,” and “conjunctiva" revealed no studies in the literature, to our surprise. To be able to provide a better understanding of the conjunctival morphologic alterations and the relevant inflammatory processes occurring in SS, we evaluated the ocular surface and the tear functions by performing a tear film BUT test, Schirmer test, ocular surface vital staining, and bulbar conjunctival confocal scanning laser microscopy and compared the results with those in healthy normal control subjects.

In accordance with previous studies, we observed a significantly lower tear stability and ocular surface epithelial damage in patients with SSDE or NSSDE when compared with control subjects.22–24 The tear quantity was significantly lower in patients with SSDE than in control subjects. Our in vivo confocal microscopy observations disclosed the presence of considerable inflammation in the conjunctiva and cornea of patients with SSDE and NSSDE, as evidenced by the significant increase of the inflammatory cell density in the patients. In addition, corneas of the patients with dry eye seemed to harbor fewer inflammatory cells than in conjunctival scans, perhaps owing to the avascular nature of the corneal tissue. Corneal and conjunctival inflammatory cells were significantly higher in SSDE and NSSDE eyes when compared with control eyes; likewise, corneal and conjunctival inflammatory cell density was significantly higher in SSDE compared with NSSDE eyes. The inflammatory infiltrates consisted mainly of polymorphs, dendritic cells, and/or lymphocytes. Dendritic cells near the limbus or conjunctiva can be seen in inflammatory ocular surface diseases such as infectious keratoconjunctivitis, ocular trauma, contact lens use, SSDE, NSSDE, atopic keratoconjunctivitis, herpetic keratitis. It is also possible to observe dendritic cells in pigmented ocular lesions which might be melanocytes. Melanocytes may have a rounded polyhedral shape or dendritic

**Figure 2.** In vivo confocal microscopy of the bulbar interpalpebral temporal conjunctiva. (A) Image from patient with SS shows extensive inflammatory cells near the basal epithelium (round and hyperreflective cells; scan depth, 50 μm). (B) A healthy control eye shows an absence of inflammatory cells at the basal epithelial layer.

**Figure 3.** Representative confocal images from a 52-year-old woman with SS. A reduction of conjunctival inflammatory infiltrates occurred at two conjunctival epithelial depths, (A, B) 65 and (C, D) 75 μm, after 1 month of treatment with 0.1% fluorometholone eye drops, a viscous agent (0.3% nonpreserved sodium hyaluronate eye drops), or nonpreserved artificial tears, and the insertion of upper and lower lacrimal punctal plugs. Pretreatment inflammatory infiltrates mainly consisted of polymorphic and dendritic cells.
architecture both in histopathology and in in vivo confocal scans. Patel et al. reported melanocytes in the normal human corneal limbus rete pegs as round hyperreflective bodies. Mastropasqua et al. showed that in eyes affected by immune-mediated inflammation, epithelial dendritic cells were considerably higher, both in the limbus and in the central cornea than that observed in the control group. Thus, we believe that cells with dendrites in our scans are likely to be dendritic cells, as the ocular surface disease in both SSDE and NSSDE is associated with inflammation, and none of our subjects had atypical ocular melanocytosis and/or nevi. Conversely, the round hyperreflective bodies observed in our scans most possibly were polymorphs (inflammatory cells) and not melanocytes, because the background in our scans lacked a view of the rete peg architecture, a finding also reported by Patel et al.

The inflammatory cell density assessed by in vivo confocal microscopy showed a negative linear correlation with tear quantity and stability as well as positive linear correlations with the vital staining scores. We have reported that conjunctival inflammatory cell density shows correlations with ocular surface health parameters in patients with atopic keratoconjunctivitis.

Conjunctival inflammatory cell density seems to be a promising new confocal microscopy parameter that we believe will aid in the diagnosis and evaluation of the severity of ocular surface disease in SSDE and NSSDE. Indeed, confocal microscopy could effectively disclose the reduction of inflammatory cell infiltrates in the two patients who consented to further examinations after a 1-month treatment for ocular surface disease. In vivo confocal microscopy examinations may prove useful in the assessment of the efficacy of different treatment modalities for dry eye disease. The assessment of the differential effect of anti-inflammatory treatment on dendritic or polymorphic cell infiltrates by in vivo confocal microscopy may provide invaluable information that remains to be determined in future prospective trials. Another important observation was the significant decrease in the density of the superficial, intermediate, and deeper conjunctival epithelial cells, which may be due to the elevation of the ocular surface inflammatory status and disturbances in the overall turnover of epithelial cells. It should be noted that our findings in relation to conjunctival epithelial cell density in the different layers were different from the cell sizes reported by Messmer et al. who did not note differences in cell size at different layers, perhaps because they used a different calculation methodology. When we focused on areas of positive conjunctival rose bengal staining, we came across round, dark spots with linings that we

<table>
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<th>Spearman Correlation Coefficient</th>
<th>P</th>
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<td>Schirmer 1 (mm)</td>
<td>-0.4201</td>
</tr>
<tr>
<td>BUT (s)</td>
<td>-0.7380</td>
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<tr>
<td>Fluorescein score (points)</td>
<td>0.5551</td>
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<tr>
<td>Rose bengal score (points)</td>
<td>0.5416</td>
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There is a positive correlation with the vital staining scores and significant negative correlations with the Schirmer test and BUT values.

**TABLE 2. The Correlation between Inflammatory Cell Densities and Tear Quantity, Tear Stability, and Ocular Surface Vital Staining**

**FIGURE 4.** Representative in vivo confocal microscopy scans and anterior segment photographs of the ocular surface in a 54-year-old woman with SS, before and after 1 month of treatment with 0.3% sodium hyaluronate eye drops and nonpreserved artificial tears. Before treatment, there were (A1) inflammatory cells within the epithelium (arrowbeads). (A2) extensive central and lower corneal punctate staining, and (A3) positive rose bengal staining. After treatment, confocal scanning showed (B1) clearance of the conjunctival inflammatory cells and concomitant improvements in (B2) fluorescein and (B3) rose bengal staining.

**FIGURE 5.** Comparison of conjunctival epithelial cell densities of different planes of depth in SSDE, NSSDE, and normal control eyes. Note the significantly lower densities of conjunctival epithelial cells in all layers in SSDE eyes compared with healthy control eyes (P < 0.0001). Conjunctival epithelial cell density in NSSDE tended to be lower than that in the healthy control.
believe corresponded to areas of epithelial microcysts. The density of these epithelial microcysts which was significantly increased in the conjunctiva of patients with SS may be another useful parameter in the assessment of conjunctival disease and the response to treatment. In vivo confocal microscopy evaluation of conjunctival microcysts has been shown to provide valuable insight into the analysis of filtering blebs after trabeculectomy. It is well known that conjunctival epithelium takes on a cystic appearance when changes or disturbances in epithelial maturation create debris field microcystic spaces.

Mechanical abrasion secondary to tear deficiency in dry eye disease has been reported to create an inflammatory environment in which conjunctival epithelial cells and lymphocytes are stimulated to produce and secrete various cytokines including IL-1α and β, IL-6, IL-8, TGF-β1, and TNF-α into the tear film. Elevated cytokine levels within the tear film, combined with the inflammation in the lacrimal gland and reduced concentrations of essential lacrimal-gland–derived factors such as epidermal growth factor (EGF) and retinol can create an environment in which terminal differentiation of the ocular surface epithelium is impaired. As a consequence, the epithelium may lose the ability to express surface-protecting molecules including membrane bound mucins like MUC-1 and with deterioration of the ocular surface protective mechanisms.

Solomon et al. have reported elevation of inflammatory cytokines and an increased number of S-phase cells in the conjunctival epithelium of patients with SS compared with those of control subjects, which creates an environment where the terminal differentiation of the ocular surface epithelium is impaired. Indeed, cytokines have been reported to be involved in signal proliferation, cell differentiation, or even the death of a cell. Tear fluid epidermal growth factor (EGF) has been found to be markedly depressed in eyes with severe aqueous tear deficiency and with loss of the ability to produce reflex tears. EGF concentration in tears of patients with aqueous deficiency has been shown to be correlated inversely with corneal fluorescein staining scores and with conjunctival goblet cell densities, suggesting that EGF may be important for promoting normal cellular differentiation and maintaining ocular surface homeostasis.

Transforming growth factor (TGF)-β1 is another immune cytokine that is elevated in the conjunctiva of patients with SSDE. It has been shown to downregulate inflammation and promote epithelial differentiation, effects that may restore ocular surface homeostasis. We strongly believe that our findings obtained by in vivo confocal microscopy reflect those observations by Solomon et al. and Pflugfelder et al.

Although not reported in this study, the preliminary observations of an ongoing prospective trial by us suggest that in vivo confocal microscopy can find another potential application as a tool of noninvasive impression cytology in dry eye syndromes. In vivo confocal microscopy can disclose epithelial cellular architecture by allowing observation of the nucleus, cytoplasm, and cellular borders, thus enabling calculation of cell size and the nucleocytoplasmic ratio, as well as visualization of goblet cells and calculation of their densities (Dogru M, et al. IOVS 2009;50:ARVO E-Abstract 519).

In summary, we reported the first in vivo confocal scanning laser microscope study to elucidate the alterations of conjunctival morphology in SS by describing new confocal microscopy parameters such as conjunctival epithelial cell and microcyst densities and levels of inflammatory infiltrate. Although the findings of this study may be limited by the number of subjects, it provides interesting new data regarding the conjunctival morphologic alterations in SS and provides additional strong evidence of the role of inflammation in the ocular surface disease process. It is our sincere wish that our observations and suggestions from the current work will stimulate new and collaborative lanes of research among dry eye specialists all over the world.

**Figure 6.** A comparison of conjunctival epithelial microcyst densities in SSDE, NSSDE, and healthy control eyes showed significantly higher densities in SS eyes than in healthy control eyes (P < 0.001).

**Figure 7.** In vivo confocal microscopy images from the temporal interpalpebral bulbar conjunctiva of a patient with SS and a healthy control subject. (A) Image from a 52-year-old woman with SS shows microcysts in the superficial conjunctival epithelium (depth, 5 μm). The mean density was 128.3 cysts/mm². (B) Image from a 50-year-old female control subject shows a relatively smaller number and size of epithelial microcysts. The mean density was 13 cysts/mm².

**References**


