An In Vivo Confocal Masked Study on Corneal Epithelium and Subbasal Nerves in Patients with Dry Eye

José M. Benítez del Castillo, Mohamed A. S. Wasfy, Cristina Fernandez, and Julian Garcia-Sanchez

PURPOSE. The objective of the present study was to determine whether dry eye (DE) associated with primary Sjögren’s syndrome (PSDE) and DE not associated with Sjögren’s syndrome (NSDE) are related to an alteration of corneal innervation.

METHODS. Eleven healthy volunteers younger than 60 years (normal [N] < 60 group), 10 healthy volunteers 60 years of age or older (N ≥ 60 group), 11 patients with PSDE, and 10 patients with NSDE were studied. Epithelial and stromal density and subbasal and stromal nerves were investigated by confocal microscopy.

RESULTS. The density of the superficial epithelial cells was 741 ± 306 cells/mm² in the PSDE group; 1022 ± 351 cells/mm² in the NSDE group; 1523 ± 294 cells/mm² in the N ≥ 60 group, and 1529 ± 541 cells/mm² in the N < 60 group (P < 0.0001, ANOVA). The number of subbasal nerves was 2.8 ± 1.2 in the PSDE group, 3.3 ± 0.7 in the NSDE group, 3.1 ± 0.9 in the N ≥ 60 group, and 4.6 ± 0.8 in the N < 60 group (P < 0.0001, ANOVA). The number of beadlike formations observed in the different groups was 387 ± 62/mm in the PSDE group, 325 ± 64/mm in the NSDE group, 182 ± 63/mm in the N ≥ 60 group, and 198 ± 66/mm in the N < 60 group (P < 0.0001, ANOVA). A correlation was found between the number of subbasal nerves and age (P < 0.01) and between the number of subbasal nerves and Schirmer’s test (P < 0.001, Spearman ρ).

CONCLUSIONS. Patients with DE show alteration in the corneal innervations. The demonstration of such alterations introduces new strategies for treatment of this frequent disease. (Invest Ophthalmol Vis Sci. 2004;45:3030–3035) DOI:10.1167/iovs.04-040251

Dry eye is the most frequent cause for which a patient seeks ophthalmic consultation. The prevalence of dry eye is approximately 10% to 20% of the adult population.1

Traditionally, dry eye has been classified into hyposecretory and evaporative.2 Though not as common as dry eye related with age, dry eye associated with Sjögren’s syndrome, is considered as the prototype, for its severity. Recently, dry eye associated with diabetes mellitus,3 use of contact lenses,4 and LASIK5,6 have been related with corneal innervation disturbance.

The ocular surface, the lacrimal gland, and the interconnected reflex arcs constitute a functional unit. In a normal person, the stimulation of afferent nerves produces lid closure and tear secretion. If this functional unit is altered, the tear production is not enough to maintain the necessary homeostasis of the ocular surface.7

The cornea is the tissue most densely innervated in the body and receives sensitive and autonomic (sympathetic and parasympathetic) nerve fibers. The cornea has a nerve density between 20 and 40 times as much as that of the dental pulp and between 300 and 600 times as much as that of the skin.8

The nerve bundles penetrate the corneal periphery in a radial manner, parallel to the superficial corneal surface at the level of the anterior stroma, losing their myelin sheath approximately 1 mm from the limbus. These bundles subdivide into smaller ones and turn 90° (perpendicular to the corneal surface), perforating Bowman’s layer. They then turn another 90° and become situated parallel to the superficial corneal surface, between Bowman’s layer and the basal layer of the corneal epithelium, where they divide again. From there, the individual nerve fibers emerge toward the more superficial layers of the corneal epithelium.9

The initial studies of the arrangement of corneal nerves were based on light and electron microscopy. The main problem with these studies is that corneal nerves degenerate after 13 hours after death.8 The availability of an instrument, the confocal microscope, to obtain images of the human cornea in vivo at a microstructural level, is therefore very useful for studying normal and diseased corneas.

The objective of this study was to know whether dry eye associated with Sjögren’s syndrome and dry eye not associated with Sjögren’s syndrome are also associated with an alteration of corneal innervation.

MATERIALS AND METHODS

Population and Clinical Study

Twenty–one healthy volunteers were studied. These were divided into two groups: one younger than 60 years (normal [N] < 60 group; 11 persons: 10 women and 1 man) whose average age was 30.7 ± 2.6 years (range, 26–34) and one group 60 years of age or older (N ≥ 60; 10 persons: 8 women and 2 men) whose average age was 68.7 ± 7.1 years (range, 61–82). The normal subjects were recruited from the companions of patients who attended the General Consultations in the Ophthalmology Department at San Carlos Clinical Hospital.

The exclusion criteria included the use of contact lenses, the presence of ocular or systemic disease, and drug allergy.

Eleven patients (10 women and 1 man) with hyposecretory dry eye associated with primary Sjögren’s syndrome (PSDE), with an average age of 52.9 ± 8.7 years (range, 35–65) and 10 patients (8 women and 2 men) with hyposecretory dry eye not associated with Sjögren’s syndrome (NSDE), with an average age of 65.8 ± 5.3 years (range, 57–72) were studied. The first group was recruited from the Rheumatology Department at San Carlos Clinical Hospital and the second group from the Unit of Ocular Surface and Inflammation in the Ophthalmology Department at San Carlos Clinical Hospital. The duration of


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study. The exposure time to obtain each microcircuit is therefore only 1/30 × 20 ms = 0.66 ms. To obtain a correct synchronization, the video signal is used to start the synchronization unit.

The objective of the microscope is an immersion lens. Direct contact does not exist between the lens and the cornea, because of the immersion of the lens in a drop of gel. At the end of the examination, the cornea is examined by the slit lamp to verify its integrity. The x–y position of the image and the depth of the section are controlled through the manual movement of the microscope while observing the position of the objective in relation to the cornea and the images in real time that appear in the video monitor. During each examination, the microscope is focused various times from the tear film to the anterior chamber. The total duration of the examination is from 2 to 2.5 minutes. The thickness of the slit remains constant during all the examinations. The lateral optical resolution of the system is 1 to 2 μm, the amplitude of the field (x, y) is 315 × 236 μm (74,340 μm²), and the depth (z) of the resolution of the optical section is 30 μm.15

The images in real time were recorded with a video recorder (Sony Videocassette Recorder SVO-9620; Sony Corp., Tokyo, Japan) through a PAL system (768 × 576 pixels) on super-VHS tapes.

The immersion lens has a numerical aperture of 0.75, magnification ×40, and a working distance of 1.98 mm.

Analysis of the Images

The images were evaluated in a masked manner, in which the investigator (JBC) did not know to which group the images belonged. The corneal layers that we wanted to examine were the epithelium (superficial layer and basal layer), Bowman’s layer, the subbasal layer, and the stroma (anterior and posterior). The best-focused and most representative images were selected. Neither the number of images selected nor the number of images concerning a specific corneal layer was constant throughout the population. Nevertheless, for each patient, all the selected images concerning a specific corneal layer were analyzed and averaged.

Study of the Corneal Epithelium and Stroma. The cellular density was evaluated at the level of the superficial epithelium, the basal epithelial layer, the anterior stroma, and the posterior stroma by counting the cells present in one image (74,340 μm²) using a special grid (0.018 mm²). The results are expressed in cells per square millimeter.

Study of the Corneal Nerves. The following parameters were analyzed:

1. With the image presented at 100% (apparent magnification 1052×):
   a. Number of nerves: defined as the sum of the nerve branches present in one image.
   b. Density: defined as the total length of the nerve fibers observed in one image, expressed in micrometers of nerve fiber within an area of 74,340 μm² (in micrometers per square micrometer).
   c. Number of beadlike formations: defined as the number of such formations present in 100 μm of nerve fiber.
   d. Presence of branching pattern in one image: evaluated as positive if at least one branching pattern was present within an image or negative if not.
   e. Grade of nerve tortuosity (at the subbasal layer): classified in four grades according to a scale.16
   f. Grade of nerve reflectivity: classified in four grades according to a scale.16

2. With the image presented at 200% (apparent magnification ×2064; Photoshop 5.0; Adobe Systems Inc., San Jose, CA):
   a. Thickness: defined as the average diameter of the nerve fiber, after taking three measurements, expressed in micrometers.

Finally, a qualitative description was made of the abnormal findings observed in the images.

The results were collected in a computer spread sheet (Excel 2000; Microsoft Corp., Redmond, WA) and analyzed on computer (SPSS for Windows, ver. 9.0; SPSS Sciences, Chicago, IL).
The reproducibility of the measurements were analyzed, reanalyzing three images of each measurement 1 week after the first measurement. The interobserver variation was calculated, comparing the results obtained by a second investigator (MAW) using the same criteria.

**Statistical Analysis**

The sample size was calculated to detect a significant difference in the average of the number of nerves of 1.80 (comparing the averages with ANOVA) with a corrected probability for multiple contrasts (α = 0.01) and a beta error of 75%.

The normal distribution of the variables was determined with the Kolmogorov-Smirnov test. The quantitative variables are expressed by the average, and the SD and its confidence interval and the qualitative variables by their frequency. The ANOVA of one factor was performed with no statistically significant difference between the two dry-eye groups and the two control groups (P < 0.01, Bonferroni). The density of the basal epithelial cells was PSDE group, 6175 ± 634 cells/mm² versus NSDE group, 5735 ± 432 cells/mm²; and N ≥ 60 group, 5615 ± 624 cells/mm² versus N < 60 group, 5783 ± 841 cells/mm², with no statistically significant difference between the groups. The density of the anterior stromal cells was PSDE group, 1349 ± 220 cells/mm² versus NSDE group, 1184 ± 274 cells/mm²; and N ≥ 60 group, 1075 ± 201 cells/mm² versus N < 60 group, 1107 ± 221 cells/mm² (P < 0.05, ANOVA), with no statistically significant difference between the groups. The density of the posterior stromal cells was PSDE group, 808 ± 118 cells/mm² versus NSDE group, cells/mm² 795 ± 151; and N ≥ 60 group, 768 ± 120 cells/mm² versus N < 60 group, 741 ± 145 cells/mm², with no statistically significant difference between the studied groups.

In relation to the number of subbasal nerves, the results of the analysis were as follows: PSDE group, 2.8 ± 1.2 versus NSDE group, 3.3 ± 0.7; and N ≥ 60 group, 3.1 ± 0.9 versus N < 60 group, 4.6 ± 0.8 (P < 0.0001, ANOVA). A statistically significant difference was observed between the PSDE and the N < 60 groups (P < 0.0001, Bonferroni), the NSDE and the N < 60 groups (P < 0.01, Bonferroni), and the N ≥ 60 and the N < 60 groups (P < 0.05, Bonferroni). The density of the subbasal nerves was PSDE group, 508 ± 12 µm²/mm² versus NSDE group, 593 ± 127 µm²/mm²; and N ≥ 60 group, 624 ± 86 µm²/mm² versus N < 60 group, 769 ± 88 µm²/mm² (P < 0.0001, ANOVA). In the comparison between the different groups, a statistically significant difference was observed between the N < 60 group and the other groups (PSDE P < 0.0001; NSDE P < 0.005; N ≥ 60 P < 0.05, Bonferroni).

Nevertheless, there was no significant difference between the two dry-eye groups eye or between those groups and the N ≥ 60 group. The observed density of the stromal nerves was PSDE group, 358 ± 70 µm/mm² versus NSDE group, 332 ± 44 µm/mm²; and N ≥ 60 group, 313 ± 54 µm/mm² versus N < 60 group, 399 ± 72 µm/mm² (P < 0.05, ANOVA). There was a significant difference only between the N ≥ 60 and the N < 60 groups (P < 0.05, Bonferroni). As regards the thickness of the subbasal nerves, the results of the analysis were as follows: PSDE group, 2.34 ± 0.53 µm versus NSDE group, 2.43 ± 0.47 µm; and N ≥ 60 group, 2.47 ± 0.34 µm versus N < 60 group,

### Table 1. Clinical Data

<table>
<thead>
<tr>
<th></th>
<th>N &lt; 60</th>
<th>N ≥ 60</th>
<th>NSDE</th>
<th>PSDE</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Schirmer’s I</td>
<td>13.3 ± 2.4 (11.6–14.9)</td>
<td>10.4 ± 0.7 (9.9–10.9)</td>
<td>6.3 ± 0.7 (5.8–6.8)</td>
<td>3.9 ± 0.9 (3.4–4.5)</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>Questionnaire</td>
<td>1.9 ± 4.2 (0.9–4.7)</td>
<td>4.4 ± 3.4 (2.6–6.8)</td>
<td>18.9 ± 7.9 (13.2–24.6)</td>
<td>26.6 ± 15.5 (16–36.9)</td>
<td>&lt;0.0001†</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>5.8 ± 0.2 (5.7–6)</td>
<td>5.8 ± 0.2 (5.7–6)</td>
<td>5.1 ± 0.9 (4.4–5.7)</td>
<td>5.3 ± 0.8 (4.8–5.8)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Blink rate</td>
<td>16.4 ± 1.1 (15.7–17.2)</td>
<td>15.8 ± 1.6 (14.6–17)</td>
<td>15.7 ± 2.2 (14.1–17.2)</td>
<td>16.2 ± 1.8 (15.1–17.5)</td>
<td>=0.730</td>
</tr>
<tr>
<td>RB staining</td>
<td>—</td>
<td>—</td>
<td>1.3 ± 0.9 (0.6–1.9)</td>
<td>2.6 ± 0.8 (2.1–3.2)</td>
<td>&lt;0.005</td>
</tr>
</tbody>
</table>

Data in parentheses are 95% confidence interval. P is by ANOVA. RB, rose bengal.

* PSDE vs. NSDE, PSDE vs. N < 60, NSDE vs. N < 60, NSDE vs. N ≥ 60, NSDE vs. N < 60 and N ≥ 60 vs. N < 60; P < 0.005, Bonferroni.
† PSDE vs. N ≥ 60, PSDE vs. N < 60, NSDE vs. N ≥ 60 and NSDE vs. N < 60; P < 0.01, Bonferroni.

**Confocal Microscopy**

A total of 210 images were analyzed, perpendicular to the z-axis on computer (Confo-Commander, ver. 2.7.1; Tomey). The density of the superficial epithelial cells was PSDE group, 741 ± 306 cells/mm² versus NSDE group, 1022 ± 351 cells/mm²; and N ≥ 60 group, 1525 ± 204 cells/mm² versus N < 60 group, 1529 ± 341 cells/mm² (P < 0.0001, ANOVA), demonstrating a statistically significant difference between the two dry-eye groups and the two control groups (P < 0.01, Bonferroni). The density of the basal epithelial cells was PSDE group, 6175 ± 634 cells/mm² versus NSDE group, 5735 ± 432 cells/mm²; and N ≥ 60 group, 5615 ± 624 cells/mm² versus N < 60 group, 5783 ± 841 cells/mm², with no statistically significant difference between the groups.

In relation to the number of subbasal nerves, the results of the analysis were as follows: PSDE group, 2.8 ± 1.2 versus NSDE group, 3.3 ± 0.7; and N ≥ 60 group, 3.1 ± 0.9 versus N < 60 group, 4.6 ± 0.8 (P < 0.0001, ANOVA). A statistically significant difference was observed between the PSDE and the N < 60 groups (P < 0.0001, Bonferroni), the NSDE and the N < 60 groups (P < 0.01, Bonferroni), and the N ≥ 60 and the N < 60 groups (P < 0.05, Bonferroni). The density of the subbasal nerves was PSDE group, 508 ± 12 µm²/mm² versus NSDE group, 593 ± 127 µm²/mm²; and N ≥ 60 group, 624 ± 86 µm²/mm² versus N < 60 group, 769 ± 88 µm²/mm² (P < 0.0001, ANOVA). In the comparison between the different groups, a statistically significant difference was observed between the N < 60 group and the other groups (PSDE P < 0.0001; NSDE P < 0.005; N ≥ 60 P < 0.05, Bonferroni).

Nevertheless, there was no significant difference between the two dry-eye groups eye or between those groups and the N ≥ 60 group. The observed density of the stromal nerves was PSDE group, 358 ± 70 µm/mm² versus NSDE group, 332 ± 44 µm/mm²; and N ≥ 60 group, 313 ± 54 µm/mm² versus N < 60 group, 399 ± 72 µm/mm² (P < 0.05, ANOVA). There was a significant difference only between the N ≥ 60 and the N < 60 groups (P < 0.05, Bonferroni). As regards the thickness of the subbasal nerves, the results of the analysis were as follows: PSDE group, 2.34 ± 0.53 µm versus NSDE group, 2.43 ± 0.47 µm; and N ≥ 60 group, 2.47 ± 0.34 µm versus N < 60 group,
2.14 ± 0.41 μm, with no significant differences. Comparing the thickness of the stromal nerves, the results were as follows: PSDE group, 14.73 ± 2.87 μm versus NSDE group, 12.90 ± 2.92 μm; and N ≥ 60 group, 11.40 ± 2.55 μm versus N < 60 group, 7.64 ± 2.54 μm (P < 0.0001, ANOVA). A statistically significant difference was found between the N < 60 group and the other three groups (PSDE P < 0.0001; NSDE P < 0.0001; N ≥ 60 P < 0.001; Bonferroni). The number of beadlike formations observed in the different groups was PSDE group, 387 ± 62/mm versus NSDE group, 323 ± 64/mm; and N ≥ 60 group, 182 ± 63/mm versus N < 60 group, 198 ± 66/mm (P < 0.0001, ANOVA), with a significant difference between the two dry-eye groups and the two groups of healthy eyes (P < 0.0001, Bonferroni). There were no statistically significant differences between the presence or absence of subbasal or stromal branching or in the nerve reflectivity between the different groups, but there was a significant difference in the grade of nerve tortuosity. The most frequently encountered grades of nerve tortuosity in the different groups were grade 3 in 45.5% of the eyes in the PSDE group, grade 2 in 70% of the eyes in the NSDE group, and grade 1 in 72.7% of the eyes in the N < 60 group (P < 0.0001, Pearson χ², P < 0.0001, ANOVA; Table 2).

Reproducibility of the number of subbasal nerves was 93% and of the number of beadlike formations was 91%. The interobserver variation was 10% for the number of subbasal nerves and 14% for the number of beadlike formations.

No qualitative anomalies were found in neither of both groups of healthy eyes. However, we observed nerve sprouts in 54.5% of the PSDE group and in 30% of the NSDE group and activation of keratocytes in 45.4% of the PSDE group and in 20% of the NSDE group (all eyes with activation of keratocytes also showed nerve sprouts; Figs. 1, 2).

A correlation was found between the number of subbasal nerves and age (P < 0.01) and between the number of subbasal nerves and Schirmer’s test (P < 0.001, Spearman ρ). A close relationship was found between corneal sensitivity and the Schirmer test result (P < 0.05), staining with rose bengal (P < 0.01), and the number of subbasal nerves (P < 0.05, Spearman ρ). We also observed a significant statistical relation between the number of beadlike formations and the density of the superficial epithelial cells (P < 0.0001), basal epithelial cells (P < 0.05), and anterior keratocytes (P < 0.05, Spearman ρ) and between the tortuosity and the density of the superficial epithelial cells (P < 0.001, Spearman ρ). The stepwise multivariable discriminant analysis showed that only rose bengal staining, number of subbasal nerves, and the number of beadlike formations are useful for classifying the diagnostic and control groups adjusted for age.

**DISCUSSION**

The confocal microscope has been used recently for the microstructural clinical investigation of the human cornea. The majority of the studies are qualitative and were directed toward the observation of the corneal structure after local or systemic diseases and after refractive surgery.1,4 The present study is not observational, and a quantitative analysis was performed.

The use of the confocal microscope as an optical dissector has the advantage of being a noninvasive technique in vivo and of making possible dynamic study in real time. Possible limita-

**Table 2. Confocal Data**

<table>
<thead>
<tr>
<th></th>
<th>N &lt; 60</th>
<th>N ≥ 60</th>
<th>NSDE</th>
<th>PSDE</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Density superficial epithelial cells</td>
<td>1528 ± 341</td>
<td>1523 ± 293</td>
<td>1022 ± 330</td>
<td>741 ± 306</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>Density basal epithelial cells</td>
<td>5785 ± 841</td>
<td>5615 ± 624</td>
<td>5735 ± 432</td>
<td>6173 ± 645</td>
<td>0.243</td>
</tr>
<tr>
<td>Density anterior stromal cells</td>
<td>1107 ± 210</td>
<td>1075 ± 201</td>
<td>1183 ± 273</td>
<td>1248 ± 220</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Density posterior stromal cells</td>
<td>741 ± 142</td>
<td>768 ± 119</td>
<td>795 ± 150</td>
<td>808 ± 117</td>
<td>0.659</td>
</tr>
<tr>
<td>Number subbasal nerves</td>
<td>4.64 ± 0.81</td>
<td>3.10 ± 0.87</td>
<td>3.50 ± 0.67</td>
<td>2.82 ± 1.16</td>
<td>&lt;0.0001†</td>
</tr>
<tr>
<td>Number beadings</td>
<td>182 ± 63</td>
<td>323 ± 63</td>
<td>387 ± 62</td>
<td>378 ± 62</td>
<td>&lt;0.0001§</td>
</tr>
<tr>
<td>Tortuosity</td>
<td>1.09 ± 0.54</td>
<td>1.50 ± 0.52</td>
<td>2.30 ± 0.48</td>
<td>3.18 ± 0.75</td>
<td>&lt;0.0001#</td>
</tr>
<tr>
<td>Reflectivity</td>
<td>2.55 ± 0.82</td>
<td>2.60 ± 0.96</td>
<td>2.80 ± 0.63</td>
<td>2.55 ± 0.82</td>
<td>0.879</td>
</tr>
<tr>
<td>Thickness stromal nerves</td>
<td>247 ± 0.54</td>
<td>247 ± 0.54</td>
<td>247 ± 0.54</td>
<td>247 ± 0.54</td>
<td>0.351</td>
</tr>
<tr>
<td>Thickness basal epithelial cells</td>
<td>182 ± 63</td>
<td>323 ± 63</td>
<td>387 ± 62</td>
<td>378 ± 62</td>
<td>&lt;0.0001§</td>
</tr>
</tbody>
</table>

Data in parentheses are 95% confidence interval. P is by ANOVA.
* PSDE vs. N ≥ 60, PSDE vs. N < 60, NSDE vs. N ≥ 60 and NSDE vs. N < 60; P < 0.01, Bonferroni.
† PSDE vs. N < 60 and NSDE vs. N < 60; P < 0.01 and N ≥ 60 vs. N < 60; P < 0.05, Bonferroni.
‡ PSDE vs. N < 60; P < 0.0001, NSDE vs. N < 60; P < 0.005 and N ≥ 60 vs. N < 60; P < 0.05, Bonferroni.
§ N ≥ 60 vs. N < 60; P < 0.05, Bonferroni.
¶ PSDE vs. N < 60 and NSDE vs. N < 60; P < 0.0001 and N ≥ 60 vs. N < 60; P < 0.05, Bonferroni.
# PSDE vs. N ≥ 60, PSDE vs. N < 60, NSDE vs. N ≥ 60 and NSDE vs. N < 60; P < 0.001, Bonferroni.
* PSDE vs. NSDE, PSDE vs. N ≥ 60, PSDE vs. N < 60, NSDE vs. N ≥ 60 and NSDE vs. N < 60; P < 0.05, Bonferroni.
Peptidergic nerves containing neuropeptides have been described in the corneal aged upper layers. Until now, 17 different neuropeptides and neurotransmitters have been demonstrated in the human cornea. The neurotrophic influence of these neuropeptides on corneal epithelial cells has been demonstrated in many experimental studies. In this way, Garcia-Hirschfeld et al. have demonstrated that the mitotic activity in cultures containing corneal cells together with trigeminal neurons is higher than in those containing epithelial cells alone. The initial proliferative peak has been attributed to the neuropeptide SP and the later differentiation to CGRP.

The nerve fibers liberate diffusible factors that stimulate the epithelial growth, proliferation, and differentiation and the production of collagen type VII. The epithelial cells, in their turn, produce the soluble factors neuronal growth factor (NGF) and glial cell-derived neurotrophic factor (GDNF) with a neurotrophic effect.

The lower density and number of nerves at the subbasal level justify the lower corneal sensation observed in the two groups of dry eye. The higher number of beads, the presence of nerve sprouts, and the higher tortuosity are indices of a high metabolic activity, possibly directed to repair the alterations observed at the epithelial level. It has been postulated that the liberation of neuropeptides by the nerve fibers is the origin of the appearance of sprouts in these nerves.

Also, the activated keratocytes express NGF, and it has been observed that the overexpression of NGF induces hypertrophy of the peripheral nervous system. This explains that in corneas of patients with dry eye in whom we observed keratocyte activation, we also found beading and nerve sprouts. The chronic inflammation and the diminished volume and clearance of tears enriched with proinflammatory cytokines, such as IL-1 and -6, lead to the activation of keratocytes, which synthesize NGF and other factors of nerve growth.

It has been demonstrated that diabetic eyes with severe neuropathy have a diminished corneal sensitivity and a smaller number of subbasal nerve fibers. We have observed that eyes with lower corneal sensitivity have a smaller number of subbasal nerves. Moreover, we have found that corneal sensitivity correlates with clinical parameters such as tear production (Schirmer’s test) and the state of the ocular surface (staining with rose bengal).

The lower corneal sensitivity encountered in dry eyes can be explained by the presence of inflammatory cytokines in the

![Figure 1](image_url)

**FIGURE 1.** Young control subject. (A) The ocular surface epithelium was normal. (B) Normal basal epithelial cells. (C) Normal subbasal nerve plexus. (D) Normal anterior keratocytes. The size of each image is $315 \times 236 \, \mu m$.

...
ocular surface. In this way, Xu et al.\textsuperscript{25} have demonstrated that corneal sensitivity in dry eyes, whether due to Sjögren’s syndrome or not, is less than in normal subjects. They discovered the same correlation that we found between corneal sensitivity and the Schirmer test results, as well as between sensitivity and staining with rose bengal. Millidot\textsuperscript{24} has observed that corneal sensitivity diminishes with age, which can justify the nervous alterations demonstrated in older patients and in age-related dry eye.

Tuominen et al.\textsuperscript{22} have observed alterations in the superficial epithelium in patients with Sjögren’s syndrome; however, they did not quantify these alterations. In their study, the eyes of patients with Sjögren’s syndrome showed nerve sprouts, nerve tortuosity, and activation of keratocytes. Our study is the first to describe the appearance of such alterations in patients with non-Sjögren’s dry eye, although to a lesser degree. According to our findings, such anomalies indicated neural regeneration. Moreover, the large number of headlike formations observed in dry eyes indicates an attempt to improve the primary or secondary abnormal epithelial trophism. Primary Sjögren’s syndrome is occasionally associated with different types of neuropathy, of which the most frequent is trigeminal neuralgia.\textsuperscript{23} None of our patients was diagnosed as having trigeminal neuralgia; in all, the blink rate and pupillary movements were normal.

The demonstration of the existence of nervous alterations in patients with dry eye can lead to the use of neuroprotector and/or neurotrophic eye drops for the treatment of this very frequently occurring disease. In this way, Murphy et al.\textsuperscript{21} demonstrated the cure of chronic epithelial defects in dogs with topical treatment with SP and Brown et al.\textsuperscript{26} the cure of a persistent epithelial defect in a child with Riley-Day syndrome using SP and insulin-like growth factor (IGF)-1.

In conclusion, confocal microscopy permits the detection of neuropathy earlier than the determination of corneal sensitivity. Patients with dry eye, whether it is related to Sjögren’s syndrome or not, show alterations in the corneal innervation. By virtue of our results, we cannot determine exactly whether the changes in the pattern of innervation are primary or secondary to the epithelial alterations. The demonstration of such alterations in corneal innervation in patients with dry eye may produce new treatments for this frequently encountered disease.

References


