The Relationship between Subbasal Nerve Morphology and Corneal Sensation in Ocular Surface Disease

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Purpose. The purpose of this study was to evaluate the relationship between the in vivo confocal microscopic (IVCM) morphology of subbasal corneal nerves and corneal sensitivity in patients with ocular surface disease.

Methods. Ten healthy volunteers (control group), 12 patients with dry eye (dry-eye group), and 14 patients treated with IOP-lowering topical medications (glaucoma group) were included. Central corneal sensation was measured using the contact Cochet-Bonnet esthesiometer. IVCM of the cornea was performed and the following subbasal corneal nerves parameters were analyzed: density, number, width, number of beadings, number of branching, tortuosity, and reflectivity. One eye of each subject was included in the study.

Results. Corneal sensitivity was significantly decreased in dry-eye and glaucoma patients compared with controls. The density and number of subbasal corneal nerves were also significantly decreased in dry eye and glaucoma patients compared with controls. There was no difference in terms of subbasal nerve width, number of beadings, tortuosity, reflectivity, and number of branching between the dry-eye, the glaucoma, and the control groups. In all subjects, corneal sensitivity correlated positively with the density and number of subbasal nerves; however, in the dry-eye group, corneal sensitivity correlated with the density and the number of nerves, whereas in the glaucoma group, corneal sensitivity correlated only with the tortuosity of subbasal nerves.

Conclusions. The relationship between corneal sensation and subbasal nerve morphology, as evaluated with IVCM, depends on the pathophysiological mechanism of ocular surface disease. (*Invest Ophthalmol Vis Sci.* 2012;53:4926–4931) DOI:10.1167/iovs.11-8708

Cornea is the most densely innervated tissue in the human body. Corneal innervation is supplied mainly by the sensory fibers of the ophthalmic branch of the trigeminal nerve, and also by the less numerous sympathetic and parasympathetic nerve fibers.[1] These nerves enter the cornea at the corneoscleral limbus and give rise to a moderately dense midstromal plexus and a dense subepithelial plexus located in the most anterior part of the corneal stroma, immediately beneath Bowman's membrane. After penetrating Bowman's membrane, these nerves form a dense subbasal nerve plexus, the branches of which terminate in all layers of the corneal epithelium.[1]

In addition to their important sensory function, the corneal nerves provide protective and trophic functions and also regulate corneal epithelial integrity, proliferation, and wound healing.[2] Consequently, the anatomy of corneal nerves has been studied for many years by a large variety of methods including light and electron microscopy and, since the mid-1990s, by in vivo confocal microscopy (IVCM).[1,3] IVCM is a noninvasive imaging technique that provides high-resolution images of corneal structures in the living human eye. Although IVCM can visualize the subbasal nerve fibers, the subepithelial plexus as well as the stromal nerves, because of the particular distribution of nerve fibers within the cornea,[4] the majority of IVCM studies on corneal nerves have so far evaluated only the subbasal nerves in the central part of cornea.[3] Subbasal nerve fibers form a plexus between Bowman's membrane and basal epithelial cells easily visible using IVCM as hyper-reflective, well-defined linear structures with dichotomous branches and thin connecting fibers. Several morphologic parameters have been developed,[2] and IVCM has already been used to evaluate corneal subbasal nerves in healthy subjects[2] and in different pathological conditions,[3] such as diabetes mellitus, dry eyes, keratoconus, contact lens wear, glaucoma patients,[5,6] and after penetrating keratoplasty or refractive surgery. Nevertheless, the relationship between the corneal nerve structures evaluated in vivo with IVCM and corneal nerve function remains unclear in ocular surface diseases. In dry-eye patients, corneal sensation has been correlated with subbasal nerve density and number,[7] whereas Martone et al.[6] observed a significant correlation between corneal sensitivity and nerve tortuosity in patients under topical antiglaucoma therapy. Considering the variations in the methods used to evaluate subbasal nerve parameters and the different types and generations of the in vivo confocal microscopes used, it is difficult to compare different studies or different pathologies.[3]

In this study, we analyzed, using the same methodology, the relationship between the subbasal corneal nerves and corneal sensation in two different ocular conditions known to alter the corneal nerves: the dry eyes and eyes chronically treated for glaucoma or ocular hypertension with IOP-lowering eyedrops.

Materials and Methods

Patients

This study was done at the Center of Clinical Investigations (CIC 503) at the Quinze-Vingts National Eye Center, Paris, France, with the

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approval of the Institutional Review Board of Saint-Antoine University Hospital (CPP-Ile de France 5, number 10,793).

Ten healthy volunteers (five women and five men; age 64.28 ± 11.82 years, range 50–85) were recruited (control group). All healthy volunteers had no complaint of ocular surface irritation and no anterior segment abnormality on biomicroscopic examination and ocular surface tests. Twelve patients with dry eye (three Sjögren syndrome and nine non-Sjögren dry-eye patients; nine women and three men; age 66.66 ± 6.46 years, range 58–78) were included (dry-eye group). Dry eyes was defined as Schirmer 1 testing less than or equal to 10 mm, interpalpebral ocular surface fluorescein staining of at least 2 on the Oxford scheme and tear film instability accompanied by complaints of ocular irritation.[8] Fourteen patients treated with IOP-lowering topical medications for glaucoma or ocular hypertension (OHT) for more than 6 months (number of medications: 2.64 ± 1.08, range 1–4; eight women and six men; age 65.76 ± 8.90 years, range 50–80) were also included in the study (glaucoma group). For all groups, the exclusion criteria were the following: the use of contact lenses, previous ocular trauma or surgery, the presence of ocular or systemic disease or the use of topical or systemic medications that may affect the cornea and the ocular surface (except the use of non-preserved tear substitutes in the dry-eye group and antiglaucoma eyedrops in the group treated for glaucoma or OHT). One eye of each subject was included in the study.

Demographic information and medical history were obtained from the medical records. All patients underwent a complete examination of the ocular surface in the following order: tear film breakup time (TBUT), corneal and conjunctival fluorescein staining using the Oxford scheme,[8] Schirmer test without anesthesia, evaluation of corneal sensitivity and IVCM evaluation of the central subbasal corneal nerves.

Central Corneal Sensation

Central corneal sensation was measured using the contact nylon thread Luneau 12/100 mm Cochet-Bonnet esthesiometer (Luneau; Prunay-Le-Gillon, France). The nylon filament, which mechanically stimulates corneal nerves, was applied with a low pressure perpendicularly to the center of the cornea. Starting from 6 cm, the filament length was progressively reduced in 5-mm steps until the first response occurred. The longest filament length (cm) resulting in a positive response was verified twice and recorded as the indicator of the central corneal sensitivity.[9]

In Vivo Confocal Microscopy

In vivo laser scanning confocal microscopy of the cornea was performed using the Rostock Cornea Module of the Heidelberg Retina Tomograph (Heidelberg Engineering GmbH, Heidelberg, Germany). Briefly, the microscope lens is an immersion lens (Olympus, Hamburg, Germany), with a magnification of ×60, and a contact objective covered by a sterile polymethyl methacrylate cap. The images comprised 384 × 384 pixels covering an area of 400 × 400 μm with a transversal optical resolution of 2 μm, an axial optical resolution of 4 μm, and an acquisition time of 0.024 second (Heidelberg Engineering). Before IVCM evaluation, a drop of topical anesthetic (oxybuprocaine 0.4%; MSD-Chibret, Paris, France) and one drop of gel tear substitute (Lacrigel, carbomer 0.2%; Europhta, Monaco) were instilled in the lower conjunctival fornix.[10]

Images of subbasal nerves of the central cornea were acquired using the same illumination intensity (manual mode) and by focusing the microscope beneath the basal epithelium. Approximately 20 images were acquired in the central cornea for each eye and 5 images (400 × 400 μm) were randomly selected for quantitative analysis. Images were analyzed retrospectively using NeuronJ software by a single researcher (A.L.) that was masked regarding patient identity and the results of ocular surface investigations. NeuronJ is a free ImageJ plug in (National Institutes of Health, Bethesda, MD) to facilitate the tracing and quantification of elongated image structures such as nerves

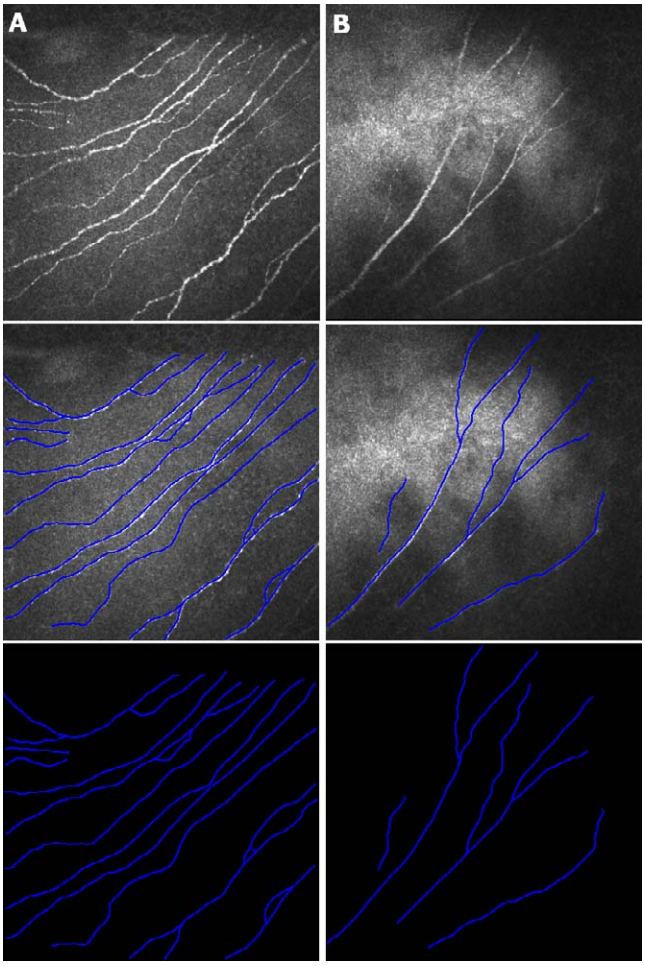

Figure 1. IVCM images (400 × 400 μm) of corneal subbasal nerves. Examples of subbasal nerves density evaluation. The tracing of subbasal nerves was performed using NeuronJ (*blue*), a semi automatic ImageJ plug in to facilitate the tracing and quantification of elongated image structures. Then, the total length/frame of subbasal nerves was measured automatically. (**A**) 24.697 mm/mm^2; (**B**) 8.465 mm/mm^2.

(Fig. 1).[11,12] The different parameters evaluating the subbasal corneal nerves were the following[2]: the density of corneal nerves, defined as the total length of the nerves visible within a frame (expressed in mm/mm^2); the number of corneal nerves, defined as the sum of the long nerve fiber bundles observed within a frame (expressed in number/mm^2); the width of the corneal nerves, defined as the mean of five measures of the width of long nerve fibers within a frame; the number of beadings, defined as the number of beadings in a length of 100 μm of subbasal nerves within a frame; the number of branching, defined as the mean number of fine fibers connecting to long nerve fibers within a frame (expressed in number/mm^2); and tortuosity and reflexivity, classified according to a previously validated scale[2] (Figs. 2, 3). For each eye and each parameter, the results were the mean of the analysis of five images.

Statistical Analysis

Results for descriptive statistics are presented as mean ± SD. Comparisons between groups were done using the Kruskal-Wallis test. The correlations between the different variables were studied using Pearson's correlation coefficient. Probability values less than 0.05 were considered significant. All statistical analyses were performed using XLSTAT 2011 (Addinsoft; Paris, France).

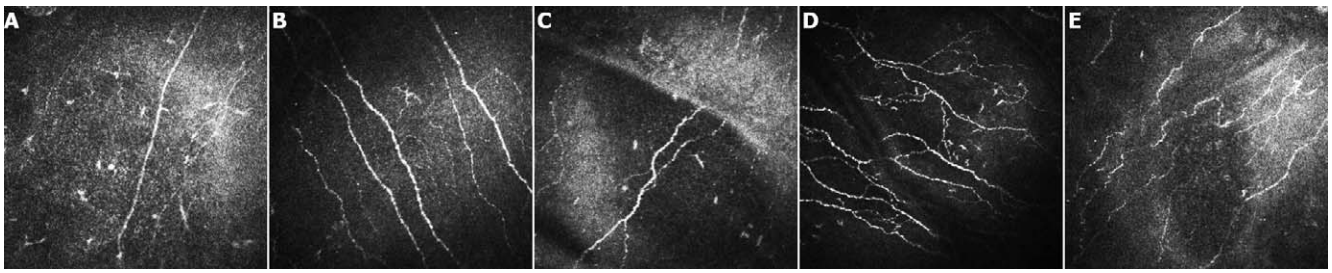

Figure 2. IVCM images (400 × 400 μm) of corneal subbasal nerves. Examples of subbasal nerve tortuosity evaluation: (**A**) tortuosity grade 0, (**B**) tortuosity grade 1, (**C**) tortuosity grade 2, (**D**) tortuosity grade 3, (**E**) tortuosity grade 4.

Results

Corneal sensitivity was significantly decreased in dry eye (5 ± 0.83 mm, $P = 0.006$) and glaucoma patients (5.33 ± 0.57 mm, $P = 0.029$) compared with controls (5.89 ± 0.22 mm); there was no difference between the glaucoma and the dry-eye groups ($P = 0.481$). Compared with the control group, the Schirmer test (9.00 ± 1.63 mm, $P = 0.0001$), the TBUT (5.7 ± 2.49 seconds, $P = 0.001$), and the fluorescein staining test (2.5 ± 0.52, $P = 0.0001$) were altered in the dry-eye group. In addition, the TBUT (7.38 ± 2.91 seconds, $P = 0.016$) and the fluorescein staining tests (1.55 ± 0.51, $P = 0.001$) were abnormal in the glaucoma group. The Schirmer test and the fluorescein staining test were significantly altered in the dry eye group compared to the glaucoma group ($P = 0.001$ and $P = 0.011$, respectively); however, there was no difference for TBUT between these two groups ($P > 0.05$). There was no statistically significant difference among the three groups in terms of age (demographic results and clinical data are summarized in Table 1).

The density of the subbasal nerves was significantly decreased in the dry-eye (9.426 ± 2.640 mm/mm^2) and glaucoma patients (11.765 ± 2.113 mm/mm^2) compared with controls (15.956 ± 2.431 mm/mm^2) ($P < 0.0001$ versus dry eye and $P = 0.009$ versus glaucoma). The number of nerves was also significantly decreased in the dry-eye (24.73 ± 8.01 /mm^2) and glaucoma patients (27 ± 6.26 /mm^2) compared with controls (39.06 ± 7.32 /mm^2) ($P = 0.001$ versus dry eye and $P = 0.005$ versus glaucoma). There was no difference between the dry-eye and glaucoma groups for subbasal nerve density and number ($P > 0.05$). Similarly, no difference in terms of subbasal nerve width, number of beadings, tortuosity, reflectivity and number of branching was observed between the dry eye, the glaucoma and the control groups. The reflectivity of subbasal nerves was increased in the glaucoma group compared with the dry-eye patients ($P = 0.032$) but not with the control group. The IVCM analysis of subbasal nerves is presented in Table 2.

Across all subjects enrolled in the study, there was a weak but significant correlation between corneal sensitivity and the density of nerves ($r = 0.483$; $P = 0.006$), and between corneal sensitivity and the number of nerves ($r = 0.508$; $P < 0.004$). In the dry-eye group, corneal sensitivity correlated highly with the density ($r = 0.644$; $P = 0.045$) and the number of nerves ($r = 0.648$; $P = 0.043$). Conversely, in the glaucoma group, the corneal sensitivity correlated negatively with the tortuosity of the subbasal nerves ($r = -0.623$; $P = 0.03$). (The correlation analysis between corneal sensitivity and the subbasal nerves is presented in Table 3).

Discussion

The well-defined IVCM appearance of the subbasal corneal nerves has facilitated the qualitative and quantitative in vivo analysis in health and disease states or following corneal surgery.[3] Several parameters have been developed for the quantitative analysis of subbasal nerves with IVCM: density, number of nerves, width, branching patterns, number of beads or varicosities, tortuosity, reflectivity, and orientation.[2] Although the morphologic appearances of corneal nerves in ocular surface diseases have been described using these parameters, very few studies have evaluated the relationship between this morphological evaluation and corneal sensation in dry eyes.[7,13–15] In addition, only one study has evaluated this relationship in patients treated for glaucoma or OHT.[6]

In the dry-eye group, we observed a decrease in the number and density of subbasal nerves that correlated well with corneal sensitivity. These results are in accordance with the study of Benitez del Castillo et al.,[7] which evaluated the relationship between the morphologic appearance of the subbasal corneal nerves using IVCM and corneal sensitivity measured with the noncontact Belmonte esthesiometer in 21 patients with dry eyes. In their study, they also observed an increased tortuosity and bead-like formation in patients with dry eyes, which, however, did not correlate with corneal sensitivity. There are conflicting results in the literature

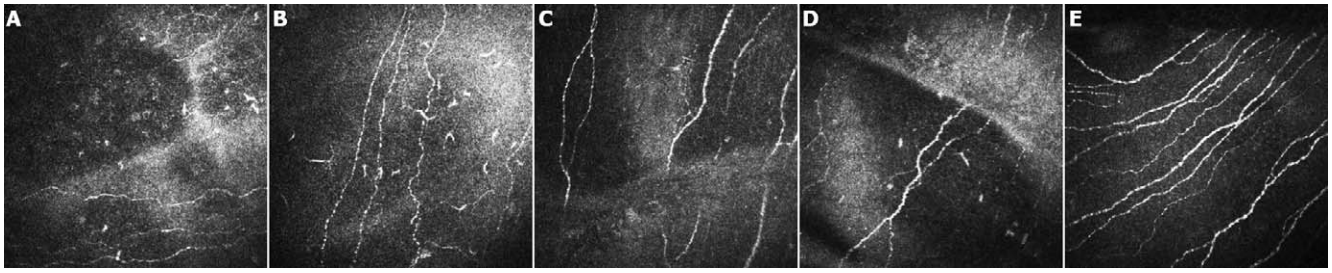

Figure 3. IVCM images (400 × 400 μm) of corneal subbasal nerves. Examples of subbasal nerve reflectivity evaluation: (**A**) reflectivity grade 0, (**B**) reflectivity grade 1, (**C**) reflectivity grade 2, (**D**) reflectivity grade 3, (**E**) reflectivity grade 4.

Table 1. Demographic and Clinical Test Results

	Control	Dry Eye	Glaucoma	*P* Value
Eyes, *n*	10	12	14	NA
Age, y, mean ± SD	64.28 ± 11.82	66.6 ± 6.46	65.76 ± 8.90	>0.05
Sex	5 F, 5 M	9 F, 3 M	8 F, 6 M	NA
Schirmer test, mm, mean ± SD	20.44 ± 5.96	9 ± 1.63	16.27 ± 6.05	0.0001*; >0.05†; 0.001‡
TBUT, s, mean ± SD	11.11 ± 2.67	5.7 ± 2.49	7.38 ± 2.91	0.001*; 0.016†; >0.05‡
Fluorescein staining	0.11 ± 0.33	2.50 ± 0.52	1.55 ± 0.51	0.0001*; 0.001†; 0.011‡
Esthesiometry	5.88 ± 0.22	5.00 ± 0.83	5.33 ± 0.577	0.006*; 0.029†; >0.05‡

F, female; M, male.
* Dry eye versus control.
† Glaucoma versus control.
‡ Dry eye versus glaucoma.

concerning corneal nerves and dry eye. First, considering subbasal nerve density, some authors have observed a decrease,[7,13,16] whereas others found no changes[14,15,17–19] or even an increase in density in patients with Sjögren syndrome.[19] Except for one study,[14] all previous studies have also described an increase in tortuosity of the subbasal corneal nerves in dry-eye patients. The manner of defining the subbasal nerve parameters and the type of confocal microscope used could explain in part these differences. To evaluate the subbasal nerves, some authors selected the images with the higher number of visible nerve fibers[14,16] while others chose the most representative images.[7,13] Moreover, some parameters such as tortuosity or reflectivity, even when compared with a validated scale,[2] remain highly subjective and may be influenced by the automatic acquisition (gain) mode used in some studies.[15,16] We therefore used the same illumination intensity (manual mode) and we randomly selected images in order to decrease image acquisition bias in our analysis.

Second, there are also conflicting results considering corneal sensation in patients with dry eyes. Although increased corneal sensation has been described,[15,20] most studies showed a decreased corneal sensations in patients with dry eyes compared with controls.[7,9,13,14,16,21] Two esthesiometers have so far been used to evaluate corneal sensation: the Cochet-Bonnet esthesiometer and the Belmonte noncontact gas esthesiometer. Although the Cochet-Bonnet esthesiometer explores only the mechanical sensitivity and seems less reproducible and reliable than the noncontact Belmonte esthesiometer,[7] there are variations in the physical characteristics of the stimulus delivered by the noncontact esthesiometer that could also explain these controversial results.[21] However, using the noncontact esthesiometer, two studies also observed a decrease in corneal sensitivity in patients with dry eyes,[7,21] in accordance with our results. Finally, differences between the severity of dry eyes and the proportion of Sjögren syndrome patients also make the comparison between studies difficult.[7]

In dry-eye patients, two studies have found a correlation between IVCM subbasal nerve morphology and corneal sensation,[7] while three studies showed no correlation.[13–15] Similar to our study, in the two studies demonstrating a correlation it was the density and the number of central subbasal corneal nerves that correlated with corneal sensation. These results emphasize the presence of a direct and probably not specific alteration of corneal nerves in patients with dry eyes, leading to a decrease in corneal sensation.[7,21]

The chronic use of topical IOP-lowering drugs and their preservatives is known to cause significant changes in ocular surface[22,23] and the prevalence of signs and symptoms of ocular surface disease in these patients have been demonstrated in several clinical studies.[24] Two studies have evaluated the subbasal nerves with IVCM in patients treated for glaucoma or OHT. Similar to our findings, Baratz et al.[5] showed a decrease in the number and density of subbasal corneal nerves in the medication group of the Ocular Hypertension Treatment Study. Martone et al.[6] observed similar nerve modifications but in association with an increased number of nerve beadings and tortuosity. These authors also demonstrated a direct relationship between nerve tortuosity and corneal sensation, which further supports our results. Although the patients with dry eyes and the patients medically treated for glaucoma experience similar ocular surface symptoms and signs, the different correlations found between corneal sensation and nerve morphology in these two groups suggest that nerve alteration and/or dysfunction could also be different. Decreased corneal sensation has been already described in patients treated for glaucoma[6,25] and particularly with topical beta-blockers.[26,27] Despite the decreased number of subbasal nerves observed in

Table 2. IVCM Analysis of Subbasal Nerve Parameters

IVCM Parameters		Control	Dry Eye	Glaucoma	*P* Value
Density	mm/mm², mean ± SD	15.956 ± 2.431	9.426 ± 2.640	11.765 ± 2.113	<0.0001*; 0.009†; >0.05‡
Number	/mm², mean ± SD	39.06 ± 7.32	24.73 ± 8.01	27.00 ± 6.26	0.001*; 0.005†; >0.05‡
Width	μm, mean ± SD	2.49 ± 0.32	2.58 ± 0.33	2.59 ± 0.34	>0.05*,†,‡
Beads	/100 μm of nerves, mean ± SD	12 ± 7	11.0 ± 8.4	14.7 ± 9.9	>0.05*,†,‡
Tortuosity	grade, mean ± SD	1.64 ± 0.70	1.58 ± 0.27	1.83 ± 0.59	>0.05*,†,‡
Reflectivity	grade, mean ± SD	1.69 ± 0.49	1.26 ± 0.60	1.9 ± 0.87	>0.05*; >0.05†; 0.032‡
Branchings	/mm², mean ± SD	27.91 ± 3.53	26.12 ± 8.70	25.21 ± 11.05	>0.05*,†,‡

* Dry eye versus control.
† Glaucoma versus control.
‡ Dry eye versus glaucoma.

Table 3. Statistical Results of Correlations between Corneal Sensitivity and IVCM Subbasal Nerve Parameters

IVCM Parameters	All Subjects		Dry Eye		Glaucoma	
	r	*P*	*r*	*P*	*r*	*P*
Density	**0.483**	**0.006**	**0.644**	**0.045**	−0.327	>0.05
Number	**0.508**	**0.004**	**0.648**	**0.043**	0.042	>0.05
Width	−0.155	>0.05	0.132	>0.05	−0.463	>0.05
Beads	0.282	>0.05	0.370	>0.05	0.368	>0.05
Tortuosity	−0.067	>0.05	0.317	>0.05	**−0.623**	**0.03**
Reflectivity	0.204	>0.05	0.024	>0.05	0.218	>0.05
Branchings	0.085	>0.05	0.181	>0.05	−0.083	>0.05

Statistically significant values are in bold.

those patients, an additional anesthetic effect of IOP-lowering medications and preservatives could explain these results. In a study evaluating corneal sensitivity after instillation of preserved prostaglandin analogs, a transient reduction in central corneal sensitivity occurred 5 minutes after instillation and lasted less than 30 minutes.[28] The preservatives associated with the active compound in these antiglaucoma drops may also be partly responsible for these effects. We recently showed a direct correlation between decreased corneal sensitivity and the number of benzalkonium chloride (BAC)-preserved antiglaucoma eyedrops instilled every day.[25] In gastroenterology, the chemoneurolytic effect of BAC is used for experimental models of vagotomy.[29] The lack of correlation between the density of corneal nerves and corneal sensation might result from this anesthetic effect of antiglaucoma eyedrops in this group of patients. Unfortunately, considering the sample size of our study we could not differentiate between patients receiving preserved and unpreserved eyedrops or different molecules. In the present study, in those patients treated for glaucoma, corneal sensation correlated directly with subbasal nerve tortuosity. In dry-eye patients, higher tortuosity and number of beadings are considered signs of high metabolic activity in response to epithelial alterations.[7] The relationship between this parameter and corneal sensation may suggest that a higher tortuosity also represents a marker of corneal nerve alterations, or at least dysfunction, in glaucoma patients.

In addition to dry-eye patients and patients treated topically for glaucoma, as previously discussed, the in vivo correlation between corneal nerve structure and function has been analyzed in healthy subjects[30] and other pathological conditions, such as keratoconus,[31] diabetes mellitus,[32] herpes simplex keratitis,[33] after laser refractive surgery,[34] and contact lens wear[35] with different and sometimes contradictory findings. The present study has limitations, particularly the small sample size of patients in each disease type and the use of a contact esthesiometer that evaluates only the mechanical component of corneal sensitivity. However, using a previously described methodology, similar parameters and a similar confocal microscope for the analysis of two different ocular surface diseases, we clearly demonstrate that the relationship between corneal sensation and nerve morphology is directly influenced by the pathology causing corneal nerve damage.

The evaluation of subbasal corneal nerves is made with objective, easily measurable parameters, such as the density and the number of fibers, but also with subjective parameters, such as tortuosity, reflectivity, and the number of beadings, which remain extremely difficult to quantify and thus may be poorly reproducible and unreliable. Nevertheless as previously shown for glaucoma patients, such parameters could also directly correlate with corneal nerve function. Software allowing for a more precise quantification of tortuosity has been developed[30] and could certainly be extremely helpful in the future. The evaluation of nerve reflectivity remains also subjective and variable as this parameter is graded with respect to the background reflectivity. Background reflectivity may be influenced by the confocal microscope type (laser scanning or slit-scanning) and illumination mode (manual or automatic), but also by the underlying disease. Reflectivity has been rarely used for the analysis of subbasal nerves[6,13,14] and was not a discriminate parameter in those studies. One other major limitation of IVCM subbasal nerve studies is the small field of view, although images are usually randomly selected in order to represent at least the central cornea. Mapping reconstruction of corneal subbasal nerves over a large surface already increased our knowledge of corneal innervation by demonstrating a whorl-like pattern of the subbasal nerves[36] and rendering the previous subbasal nerve orientation parameters[2] inappropriate. Future software that could directly reconstruct nerve structures over a large area of cornea during IVCM examination[37] and possibly in three dimensions[38] will certainly improve our analysis of corneal innervation.

The relationship between corneal nerve function and structure remain complex and variable across the different ocular surface diseases. Improvements in IVCM image acquisition and analysis will be an important step to further evaluate this relationship and increase our knowledge of the role of corneal nerves in physiological and pathological conditions.

References

1. Marfurt CF, Cox J, Deek S, Dvorscak L. Anatomy of the human corneal innervations. *Exp Eye Res*. 2010;90:478–492.
2. Oliveira-Soto L, Efron N. Morphology of corneal nerves using confocal microscopy. *Cornea*. 2001;20:374–384.
3. Patel DV, McGhee CN. In vivo confocal microscopy of human corneal nerves in health, in ocular and systemic disease, and following corneal surgery: a review. *Br J Ophthalmol*. 2009; 93:853–860.
4. Auran JD, Koester CJ, Kleiman NJ, et al. Scanning slit confocal microscopic observation of cell morphology and movement within the normal human anterior cornea. *Ophthalmology*. 1995;102:33–41.
5. Baratz KH, Nau CB, Winter EJ, et al. Effects of glaucoma medications on corneal endothelium, keratocytes, and subbasal nerves among participants in the ocular hypertension treatment study. *Cornea*. 2006;25:1046–1052.
6. Martone G, Frezzotti P, Tosi GM, et al. An in vivo confocal microscopy analysis of effects of topical antiglaucoma therapy with preservative on corneal innervation and morphology. *Am J Ophthalmol*. 2009;147:725–735.
7. Benitez-Del-Castillo JM, Acosta MC, Wassfi MA, et al. Relation between corneal innervation with confocal microscopy and

corneal sensitivity with noncontact esthesiometry in patients with dry eye. *Invest Ophthalmol Vis Sci*. 2007;48:173–181.

8. Methodologies to diagnose and monitor dry eye disease: report of the Diagnostic Methodology Subcommittee of the International Dry Eye WorkShop. *Ocul Surf*. 2007;5:108–152.
9. Xu KP, Yagi Y, Tsubota K. Decrease in corneal sensitivity and change in tear function in dry eye. *Cornea*. 1996;15:235–239.
10. Labbe A, Niaudet P, Loirat C, Charbit M, Guest G, Baudouin C. In vivo confocal microscopy and anterior segment optical coherence tomography analysis of the cornea in nephropathic cystinosis. *Ophthalmology.* 2009;116:870–876.
11. Meijering E. Neuron tracing in perspective. *Cytometry Part A*. 2010;77:693–704.
12. Meijering E, Jacob M, Sarria J-CF, Steiner P, Hirling H, Unser M. Design and validation of a tool for neurite tracing and analysis in fluorescence microscopy images. *Cytometry Part A*. 2004; 58:167–176.
13. Benitez del Castillo JM, Wasfy MA, Fernandez C, Garcia-Sanchez J. An in vivo confocal masked study on corneal epithelium and subbasal nerves in patients with dry eye. *Invest Ophthalmol Vis Sci*. 2004;45:3030–3035.
14. Hosal BM, Ornek N, Zilelioglu G, Elhan AH. Morphology of corneal nerves and corneal sensation in dry eye: a preliminary study. *Eye (Lond)*. 2005;19:1276–1279.
15. Tuisku IS, Konttinen YT, Konttinen LM, Tervo TM. Alterations in corneal sensitivity and nerve morphology in patients with primary Sjogren's syndrome. *Exp Eye Res*. 2008;86:879–885.
16. Villani E, Galimberti D, Viola F, Mapelli C, Ratiglia R. The cornea in Sjogren's syndrome: an in vivo confocal study. *Invest Ophthalmol Vis Sci*. 2007;48:2017–2022.
17. Erdelyi B, Kraak R, Zhivov A, Guthoff R, Németh J. In vivo confocal laser scanning microscopy of the cornea in dry eye. *Graefes Arch Clin Exp Ophthalmol*. 2007;245:39–44.
18. Tuominen IS, Konttinen YT, Vesaluoma MH, Moilanen JA, Helintö M, Tervo TM. Corneal innervation and morphology in primary Sjogren's syndrome. *Invest Ophthalmol Vis Sci*. 2003; 44:2545–2549.
19. Zhang M, Chen J, Luo L, Xiao Q, Sun M, Liu Z. Altered corneal nerves in aqueous tear deficiency viewed by in vivo confocal microscopy. *Cornea*. 2005;24:818–824.
20. De Paiva CS, Pflugfelder SC. Corneal epitheliopathy of dry eye induces hyperesthesia to mechanical air jet stimulation. *Am J Ophthalmol*. 2004;137:109–115.
21. Bourcier T, Acosta MC, Borderie V, et al. Decreased corneal sensitivity in patients with dry eye. *Invest Ophthalmol Vis Sci*. 2005;46:2341–2345.
22. Baudouin C. Side effects of antiglaucomatous drugs on the ocular surface. *Curr Opin Ophthalmol*. 1996;7:80–86.
23. Asbell PA, Potapova N. Effects of topical antiglaucoma medications on the ocular surface. *Ocul Surf*. 2005;3:27–40.
24. Leung EW, Medeiros FA, Weinreb RN. Prevalence of ocular surface disease in glaucoma patients. *J Glaucoma*. 2008;17: 350–355.
25. Van Went C, Alalwani H, Brasnu E, Pham J, Baudouin C, Labbé A. Corneal sensitivity in patients treated for glaucoma or ocular hypertension. *J Fr Ophtalmol*. 2011;34:684–690.
26. Kitazawa Y, Tsuchisaka H. Effects of timolol on corneal sensitivity and tear production. *Int Ophthalmol*. 1980;3:25–29.
27. Van Buskirk EM. Corneal anesthesia after timolol maleate therapy. *Am J Ophthalmol*. 1979;88:739–743.
28. Kozobolis VP, Detorakis ET, Maskaleris G, et al. Corneal sensitivity changes following the instillation of latanoprost, bimatoprost, and travoprost eyedrops. *Am J Ophthalmol*. 2005;139:742–743.
29. El-Ghazzawy AG, Gupta N, Swope TJ, et al. Evaluation of benzalkonium chloride chemoneurolytic proximal gastric vagotomy. *Surg Endosc*. 1998;12:207–211.
30. Patel DV, Tavakoli M, Craig JP, Efron N, McGhee CN. Corneal sensitivity and slit scanning in vivo confocal microscopy of the subbasal nerve plexus of the normal central and peripheral human cornea. *Cornea*. 2009;28:735–740.
31. Patel DV, Ku JY, Johnson R, McGhee CN. Laser scanning in vivo confocal microscopy and quantitative aesthesiometry reveal decreased corneal innervation and sensation in keratoconus. *Eye (Lond)*. 2009;23:586–592.
32. Rosenberg ME, Tervo TM, Immonen IJ, Müller LJ, Grönhagen-Riska C, Vesaluoma MH. Corneal structure and sensitivity in type 1 diabetes mellitus. *Invest Ophthalmol Vis Sci*. 2000;41: 2915–2921.
33. Hamrah P, Cruzat A, Dastjerdi MH, et al. Corneal sensation and subbasal nerve alterations in patients with herpes simplex keratitis: an in vivo confocal microscopy study. *Ophthalmology.* 2010;117:1930–1936.
34. Darwish T, Brahma A, O'Donnell C, Efron N. Subbasal nerve fiber regeneration after LASIK and LASEK assessed by noncontact esthesiometry and in vivo confocal microscopy: prospective study. *J Cataract Refract Surg*. 2007;33:1515–1521.
35. Patel SV, McLaren JW, Hodge DO, Bourne WM. Confocal microscopy in vivo in corneas of long-term contact lens wearers. *Invest Ophthalmol Vis Sci*. 2002;43:995–1003.
36. Patel DV, McGhee CN. Mapping of the normal human corneal sub-basal nerve plexus by in vivo laser scanning confocal microscopy. *Invest Ophthalmol Vis Sci*. 2005;46:4485–4488.
37. Zhivov A, Blum M, Guthoff R, Stachs O. Real-time mapping of the subepithelial nerve plexus by in vivo confocal laser scanning microscopy. *Br J Ophthalmol*. 2010;94:1133–1135.
38. Stachs O, Zhivov A, Kraak R, Stave J, Guthoff R. In vivo three-dimensional confocal laser scanning microscopy of the epithelial nerve structure in the human cornea. *Graefes Arch Clin Exp Ophthalmol*. 2007;245:569–575.