Corneal Sensory Changes and Nerve Plexus Abnormalities in Chronic Neuropathic Ocular Pain and Dry Eye Postrefractive Surgery

AMANDA VÁZQUEZ, MARTA BLANCO-VÁZQUEZ, ELENA MARTÍNEZ-PLAZA, EVA M. SOBAS, MARÍA J. GONZÁLEZ-GARCÍA, ALBERTO LÓPEZ-MIGUEL, ENRIQUE ORTEGA, AMALIA ENRÍQUEZ-DE-SALAMANCA[1], AND MARGARITA CALONGE[1]

• PURPOSE: **Chronic neuropathic ocular pain (NOP) can develop alongside chronic dry eye (DE) post–laser-assisted in-situ keratomileusis (LASIK), yet its specific characteristics remain poorly understood. This study aims to compare the clinical characteristics of patients who developed both DE and NOP after LASIK to those with only DE and to asymptomatic LASIK patients, to facilitate the diagnosis of NOP.**

• METHODS: **Prospective, cross-sectional "case-control" comparison study. An 89-subject post-LASIK study comprised 3 groups: 34 patients developing NOP and DE (NOP-DE group), 25 patients developing only DE (DE group), and 30 asymptomatic subjects (control group). Assessments included clinical history and symptom questionnaires (OSDI, mSIDEQ, NRS, WFPRS), anxiety and depression evaluation (HADS), tear film stability (osmolarity and TBUT) and production (Schirmer), and ocular surface integrity. Corneal mechanical and thermal sensitivity thresholds were measured using Belmonte's noncontact esthesiometer, whereas tactile sensitivity threshold was assessed pre-/post-topical anesthesia using the Cochet-Bonnet esthesiometer. In vivo confocal microscopy (IVCM) was used to evaluate the sub-basal nerve plexus characteristics and dendritic cell density in the central cornea. Group comparisons and correlations were conducted.**

• RESULTS: **Compared with DE group, patients in the NOP-DE group exhibited significantly more DE symptoms with mSIDEQ ($P = .019$) higher level of pain with NRS and WFPRS, increased use of ocular lubrication ($P = .003$), greater frequency of patients with pathological results on anxiety and depression questionnaires ($P < .001$), and a higher prevalence of central sensitization syndromes ($P < .001$). Additionally, NOP-DE patients demonstrated higher tactile corneal sensitivity post-topical anesthesia ($P = .002$). IVCM revealed lower nerve density ($P = .049$) and higher microneuroma density ($P = .008$) in the sub-basal nerve plexus of NOP-DE patients compared to DE patients without NOP ($P = .008$). Most nerve metrics correlated moderately to strongly with clinical parameters.**

• CONCLUSIONS: **Persistent high corneal tactile sensitivity postanesthesia, reduced nerve density, and increased microneuroma density in the central cornea may serve as diagnostic indicators for confirming NOP in patients experiencing chronic DE post-LASIK. These findings underscore the potential utility of incorporating these measures into clinical assessments to improve diagnostic accuracy and guide management strategies in this patient population.** (Am J Ophthalmol 2025;276: 170–185. © 2025 The Authors. Published by Elsevier Inc. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/))

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From the IOBA (Institute of Applied Ophthalmobiology) (A.V., M.B.V., E.M.P., E.M.S., M.J.G.G., A.L.M., E.O., A.E.S., M.C.), Universidad de Valladolid (UVa), Valladolid, Spain; UDOVA (Pain Unit of Valladolid HURH-HCUV) (A.V., E.O.), National Health System, Castilla y Leon, Spain; CIBER-BBN (Biomedical Research Networking Center Bioengineering, Biomaterials and Nanomedicine) (M.J.G.G., A.E.S., M.C.), Carlos III National Institute of Health, Madrid, Spain

Abbreviations: ANOVA, Analysis of Variance Test; B-NC, Belmonte Esthesiometer noncontact; CB, Cochet-Bonnet esthesiometer; CCLRU, Cornea and contact Lens Research Unit; CELab, Controlled Environment Laboratory; CFS, Corneal Fluorescein Staining; CL, Contact lenses; DC, Dendritic Cells; DE, Dry eye; ETDRS, Early Treatment Diabetic Retinopathy Study; GRC, Global Rating of Change; HADS, Hospital Anxiety and Depression Scale; HRT3, Heidelberg Retina Tomograph III; IASP, International Association for the Study of Pain; IOBA, Institute of Applied Ophthalmobiology; ICC, Intra-class coefficient; IQR, Interquartile range; IVCM, In Vivo Confocal Microscopy; LASIK, Laser in situ keratomileusis; LogMAR, Logarithm of the Minimum Angle of Resolution; MGD, Meibomian Gland Disfunction; mOsm, milliosmole; mSIDEQ, Modified Single Item Dry Eye Questionnaire; NOP, Neuropathic ocular pain; NRS, Numerical Rating Scale; OSDI, Ocular Surface Disease Index; PSE, Preoperative spherical equivalent, RS, Refractive surgery; SD, Standard deviation; SL, Slit Lamp; SPSS, Statistical Package for the Social Sciences; TBUT, Tear Break-up Time; TH, Threshold; VA, Visual acuity; WFPRS, Wong-Baker Faces Pain Rating Scale.

Inquiries to Amanda Vázquez and Margarita Calonge, IOBA (Institute of Applied Ophthalmobiology), Campus Miguel Delibes, Universidad de Valladolid (UVa), Valladolid, Spain; e-mail: amanda.vazquez@uva.es, mcalonge@uva.es

[1] Both senior authors, Margarita Calonge and Amalia Enríquez de Salamanca, contributed equally.

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INTRODUCTION

LASER IN-SITU KERATOMILEUSIS (LASIK) IS ONE OF THE most common corneal refractive surgeries (RS) performed. Proper patient selection usually achieves efficient, predictable, and safe outcomes in more than 90% of cases.[1-4]

The potential side effects of LASIK include dry eye (DE)-related symptoms such as dryness, stinging, burning, photophobia, redness, and visual fatigue. The intensity and duration of these symptoms are highly variable, being more bothersome during the first month, and usually disappearing in the 6-12 months postsurgery. However, persistent symptomatic DE after LASIK has been reported in up to 20% of patients who were photoablated with older laser platforms.[5]

Another potential complication after RS is pain, which often overlaps with DE. It usually starts 2 hours after surgery as acute mild-to-moderate and can last up to 4 days.[6] This pain is nociceptive, meaning that it is the consequence of nociceptors or free nerve endings that respond to surgical intervention, and it is usually associated with tear film abnormalities (eg, tear instability) and corneal epithelial disruption.[5] LASIK surgery also induces corneal nerve damage whose reinnervation does not return to the preoperative state.[7-9] This may result in the development of chronic neuropathic ocular pain (NOP).[10-12] In recent years, the literature reporting NOP after LASIK and other surgeries has increased considerably.[13] Damaged corneal nerve plexus has a high relevance to the dysfunction of the integrated lacrimal functional unit, leading to DE.[14] However, patients with NOP have more intense symptomatology and are discordant with typical signs of DE. We recently reported that among post-RS patients with persistent DE, 78.8% of them also suffered chronic ocular pain, which was neuropathic (NOP) in origin in 63.5% of cases.[13]

The International Association for the Study of Pain defined neuropathic pain as pain that arises as a direct consequence of a lesion or diseases affecting the somatosensory system.[15,16] The DEWS II report classified NOP as another entity differentiated from DE, although it can often be associated.[17] More recently, we published the criteria that chronic ocular pain had to meet to be considered neuropathic in nature (NOP).[13] However, NOP can still be difficult to properly diagnose due to the absence of evident clinical signs and also challenging to treat due to its resistance to conventional analgesic treatments.[10]

The present study aimed to define objective signs that can help diagnose NOP in patients suffering from chronic DE after LASIK, focusing on corneal esthesiometry and the morphologic changes of the sub-basal corneal innervation.

METHODS

This study was designed as a case-control, observational, single-visit, single-center investigation approved by the Ethics Committee of the Valladolid University Clinic Hospital, adhering to the tenets of the Declaration of Helsinki. Written informed consent was obtained from all participants after they had been fully informed, and the privacy rights of human subjects were consistently observed.

• **PATIENTS AND STUDY DESIGN:** Patients with persistent symptoms after undergoing LASIK at external centers were enrolled during their scheduled first visit to the Ocular Pain Unit. A standardized evaluation was performed, and only those with microkeratome-assisted LASIK were included and classified into 3 groups:

1. **NOP-DE group**: Patients with chronic dry eye (DE)-related persistent symptoms[17] and chronic neuropathic ocular pain (NOP) post-LASIK.
2. **DE group**: Patients with chronic DE symptoms post-LASIK but no pain.
3. **Control group**: Asymptomatic patients post-LASIK.

Inclusion criteria:
For all groups:

- Age ≥18 years.
- LASIK performed in both eyes as the only type of ocular surgery, at least 3 months prior to recruitment.
- Absence of ocular symptoms before LASIK (no use of ocular lubricants or a maximum of 2 drops daily for contact lens [CL]-related discomfort.[18]

Additional inclusion criteria for specific groups:

- **NOP-DE group:** Diagnosis of chronic NOP and chronic DE.
- **DE group:** Diagnosis of chronic DE.
- **Control group:** Absence of ocular symptoms.

Exclusion criteria:
Exclusion criteria applied to all groups:

1. Presence of any ocular surface disease other than the one under study (DE or NOP).
2. Concomitant inflammatory ophthalmic diseases.
3. Previous ocular, periocular, or orbital surgeries (except LASIK).
4. Noncompliance with the following study requirements:
   a. Discontinuation of CL use at least 15 days before the study.
   b. Avoidance of topical medications within 7 days before the study (or 4 weeks for special medications such as cyclosporine, tacrolimus, or steroids).
   c. No use of artificial tears within the 12 hours before the study.

The presence of ocular pain was considered when both the Numerical Rating Scale (NRS) and the Wong-Baker Faces Pain Rating Scale (WFPRS) scores were ≥ 2.19 Both, DE and NOP were considered chronic when the duration was at least 3 months.[20]

We considered chronic ocular pain was "neuropathic" (NOP) when at least 3 of the following 5 requirements were present.[13] (1) evidence of damage or injury to the somatosensory nervous system; (2) minimum corneal damage (fluorescein corneal staining ≤ 1 with the Oxford scale); (3) the presence of at least 2 typical descriptors (tingling, pins or needles, stabbing, shooting or electric shock-like pains); (4) abnormal corneal sensitivity including allodynia, hyperalgesia, and/or radiating pain; and (5) persistence of symptoms after topical anesthesia. Additionally, the definite diagnosis of NOP for our patients was corroborated by a medical doctor specializing in oculofacial pain (coauthor EO).[13]

To minimize the variation of a changing external environment, all participants were evaluated under the so-called "simulated normal environment conditions" (23°C and 50% relative humidity), in our Controlled Environment Laboratory (CELab) (www.visionrd.com/celab/) between 9.00 and 13.00 hours. The same investigator performed the clinical assessment to avoid interobserver variability.[21,22]

First, a brief medical history was recorded, including the previous spherical equivalent, the number of surgeries per eye, the onset and duration of symptoms, the comorbidities present, and the treatments used previously and at the time of the study visit.

• **CLINICAL QUESTIONNAIRES:** We used 5 different questionnaires. The *Ocular Surface Disease Index* (OSDI) questionnaire defined the severity of DE-related symptoms according to the following scoring: asymptomatic (score ≤12); mild (score 13-22), moderate (score 23-32), and severe (score 33-100).[23,24] The *Modified Single-Item Dry Eye Questionnaire (mSIDEQ)* assessed the frequency of dryness, foreign body sensation, burning, pain, itching, photophobia, and blurred vision from 0 to 4 scale (0, absence of symptom; 1, rarely felt; 2, sometimes felt; 3, always felt but without affecting daily activities; 4, always felt with affected daily activities) (range, 0-28).[25] The *NRS* scored the intensity of pain on a 0-10 scale: 0-1=no pain, 2-4=mild, 5-7=moderate, 8-10=severe.[19] The WFPRS[26,27] scored the intensity of pain using 6 different faces, with a numerical equivalence, horizontally lined up to express an increasing level of pain intensity from left to right (0 = no pain; 2 = discomfort; 4 = light pain; 6 = moderate pain; 8 = intense pain; 10 = unbearable pain).[28] The level of anxiety and depression were assessed with the *Hospital Anxiety and Depression Scale (HADS)* which consists of a 14-item self-reported scale (range, 0-42), whose overall score is obtained from the sum of its two 7-item subscales (range, 0-21 for each), to assess the existence of anxiety and depression. The subscale cut-off points were 0-7=normal; 8-10=borderline; and >10=existence of a clinical problem.[29] The total HADS score was obtained by summing each subscale.[30,31]

• **VISUAL ASSESSMENT:** The uncorrected and corrected high (100%) and low (10%) contrast visual acuity (VA) was evaluated. The logarithm of the minimum angle of resolution (logMAR) was assessed using a liquid crystal display screen 22" (Topcon CO LDT, Tokyo, Japan) at a distance of 4 m.[32]

• **CLINICAL TESTS:** We performed the tests in both eyes. The starting eye was randomly selected, and the mean was calculated (unless otherwise specified) in the following order:

Tear osmolarity was evaluated using an osmometer (TearLab Corporation, San Diego, CA, USA), and values >308 mOsm/L were considered abnormal.[33]

Bulbar conjunctival hyperemia and Meibomian gland dysfunction (MGD) were assessed using a slit-lamp (SL-D7, Topcon Corporation, Tokyo, Japan) following the Efron scale (range, 0-4).[34]

Tear film stability was evaluated using the fluorescein tear break-up time (TBUT) test (the mean of 3 consecutive measurements), and values ≤ 7 s (s) were considered abnormal.[35,36]

Ocular surface integrity was evaluated at the slit-lamp with fluorescein corneal staining using the Oxford scale (range, 0-5)[37] and the Cornea and Contact Lens Research Unit (CCLRU) grading scale (range, 0-4)[38]; immediately after, conjunctival staining was assessed using Lissamine green strips (I-DEW green, Entod Research Cell UK Ltd, London, UK) with Oxford scale; for both scales, staining ≥ 1 was considered abnormal.

Corneal sensitivity was determined using both noncontact and contact esthesiometry. First, mechanical and thermal (hot and cold) thresholds were registered using a prototype of Belmonte's noncontact gas esthesiometer.[39] Briefly, the device was placed 5 mm from the central cornea and following standard protocols of our research group[40-42] and the level method,[39,43] the mechanical threshold was first determined by triggering 3-seconds with variable airflow (range, 0 to 200 mL/min) pulses at neutral corneal temperature (34 °C). Subsequently, heat or cold thresholds were randomly determined by varying the temperature (range, −4 to 3.6 °C) and flow rate 10 mL/min below the mechanical threshold to avoid mechanical stimulation. Second, after a 30-minute interval to prevent overstimulation, a Cochet-Bonnet esthesiometer (Luneau Ophthalmology, Chartres, Paris, France) was used to estimate tactile sensitivity in the central cornea pre- and post-topical anesthesia following standard protocols (range, 60-0 mm). The longest length detected was recorded as the corneal threshold. After topical anesthetic instillation (1 drop of 0.1% tetracaine and 0.4% oxibuprocaine) (Anestésico Doble Col-

irio; Alcon Cusí, El Masnou, Spain), the assessment was repeated.[45,46]

The anesthetic challenge test was answered between pre and postanesthesia Cochet-Bonnet esthesiometry postanesthesia. Each patient rated the change in intensity of their current ocular symptoms postanesthesia using the *Global Rating of Change* (GRC) scale. It measures enhancement or weakening of symptoms ranging from −5 (completely recovered), through 0 (unchanged) to +5 (very much worse).[44] Nociceptive pain was associated with great improvement (range, −3 to −5), NOP with unchanged or worsening symptoms (range, 0 to +5), and mixed pain with slight enhancement (range, −2 to −1).[13]

Basal tear production was determined using the Schirmer test with topical anesthesia. Values ≤ 5 mm after 5 minutes were considered abnormal.[45]

In vivo confocal microscopy (IVCM) was performed on the central cornea of one randomly selected eye of each patient. Heidelberg Retina Tomograph III (HRT3) and a corneal module (Rostock Cornea Module, Heidelberg Engineering GmbH, Heidelberg, Germany https://www.heidelbergengineering.com/) were used to study the morphology of the sub-basal corneal nerve plexus and the density of inflammatory cells. Previously, 1 drop of the aforementioned anesthetic eye drops was instilled into the inferior conjunctival fornix and a blepharostat was placed to keep the eye open. Viscotears gel (Carbomer 980, 0.2%; Novartis Farmacéutica S.A., Barcelona, Spain) was applied on the outside and inside of a sterile disposable cap (TomoCap) placed over the objective lens. Good quality, nonoverlapping images of the sub-basal nerve plexus in the central cornea were obtained using the Heyex Eye Explorer (HeyexTM) platform, always by the same examiner (AV).

Following previous protocols[9,42] 3 high-quality images of the central cornea from each patient were analyzed by 2 investigators in a masked fashion and the mean value between the 2 observers for each parameter was computed for statistical analysis. Each captured image contained 384 × 384 pixels, covering an area of 400 × 400 µm (0.16 mm^2). For statistical analysis, the mean of each parameter evaluated in the 3 images was calculated, followed by the mean delivered by 2 masked examiners for each parameter evaluated. The Figure 1 shows some examples of images and the parameters analyzed, which are the following:

1. Nerve characteristics: a) number of nerves (n/mm^2 and n/frame): sum of the nerves appearing in the image b) nerve density (mm/mm^2): total length of nerves existing in the image in the determined area; c) nerve length (mm/mm^2): the mean length of the nerves in the image; d) density of nerve branches (n/mm^2) in the image; and e) nerve tortuosity: assessed according to the scale described by Oliveira-Soto and Efron (0 = minimum tortuosity to 4 = maximum tortuosity).[46]
2. Density of microneuromas (n/mm^2): these are terminal enlargements of subbasal corneal nerves, characterized by irregularly-shaped, hyperreflective structures that form at sites of nerve damage or injury[47-49] (Figure 1).
3. Dendritic cell density (n/mm2): these cells are visualized by their distinctive characteristics as bright cell bodies with dendritic structures. Among the different corneal immune cells, only dendritic cells were analyzed; inactive and activated keratocytes were not considered for this study
4. Reflectivity: the histogram of each image was obtained using ImageJ software and used to obtain the mean reflectivity of each image or optical densitometry as an index of corneal transparency.

Nerve number, density and length were measured using the NeuronJ plugin[A] of the ImageJ[B] software. Nerve branching, microneuromas and dendritic were counted manually using the multipoint tool of the ImageJ software, and density was calculated as described in previous studies.[9,42] Two investigators (coautors AV, MB) evaluated the previously masked images and the mean of the values reported by them was calculated.

• **STATISTICAL ANALYSIS:** Data were statistically analyzed using the SPSS software statistical package version 22.0 (SPSS Inc., Chicago, IL, USA) for Mac.

Kolmogorov-Smirnov test was used to check the normality assumption. Qualitative variables presented as frequencies and proportions were compared using the equality of proportions hypothesis or chi-squared test. Quantitative continuous variables were summarized as mean ± standard deviation (SD). Analysis of variance (ANOVA) test was used to compare the 3 groups and Student's t-test for pairs of groups. Levene's test was used to check homogeneity of variance and Welch's test was used when this assumption was not valid.

Variables not following a normal distribution were described using the median [interquartile range (IQR)], unless otherwise specified in the text. In this case, Kruskall-Wallis was used to compare data among groups and Mann-Whitney U test, was performed between pairs or groups.

The Bonferroni correction was applied to adjust the experiment-wise error rate in all group comparisons.

Correlations between quantitative variables was quantified by Pearson or Spearman's correlation coefficient (rho), depending on the normality assumption. The rho correlation coefficient can be interpreted according to the following ranges: from 0.80 to 1.00, very strong; from 0.60 to 0.79, strong; from 0.40 to 0.59, medium; from 0.20 to 0.39, low; and from 0.00 to 0.19, very low.

For the variables obtained with the corneal IVCM, the agreement between the 2 observers was measured using the intraclass correlation coefficient (ICC) (< 0.30 null; 0.31-0.5 mild; 0.51-0.70 moderate; 0.71-0.90 good; and > 0.90 very good).

P-values ≤.05 were considered statistically significant.

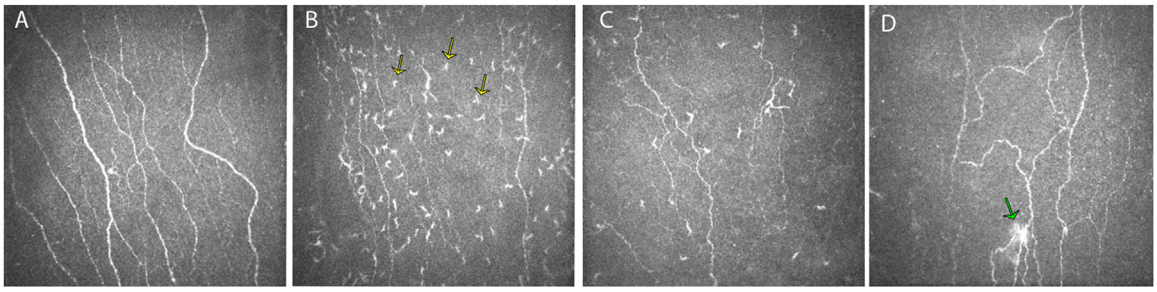

FIGURE 1. Representative in vivo confocal microscopy (IVCM) images of the central corneal nerve plexus of patients and controls. Sub-basal corneal nerve plexus in: (A) an asymptomatic patient after laser in-situ keratomileusis (LASIK) surgery (control group); (B) a patient with dry eye (DE) after LASIK (DE group); and (C) a patient with neuropathic ocular pain (NOP) after LASIK (NOP-DE group). (D) Image showing a microneuroma. Yellow and green arrows indicate dendritic cells and microneuromas, respectively.

RESULTS

A total of 178 eyes of 89 RS LASIK patients were examined, of whom 57 (64.0%) were female; all of them had both eyes operated on. Of these, 34 patients were included in the NOP-DE group, 25 patients in the DE group, and 30 subjects were included in the control (asymptomatic) group. Table 1 summarizes their demographic characteristics, VA, and the main comorbidities. Of note is that the age and sex distribution in the 3 groups was not significantly different, ensuring that these 2 variables are not confounding factors. No significant differences were observed among the groups in either the spherical equivalent or the uncorrected and corrected distance high contrast VA. However, the NOP-DE group exhibited significantly poorer uncorrected and corrected low contrast VA than the control group.

Migraines were diagnosed more frequently in the NOP-DE and DE groups than in the control group. Interestingly, central sensitization-related syndromes[47] were more frequently diagnosed in patients who had the neuropathic type of pain (NOP-DE group) than those without it (DE and control groups). Additionally, the use of artificial tears was significantly higher in the NOP-DE group (100%) than in the DE-group (76%).

Symptomatology and clinical test results are shown in Table 2. The scores of DE- and pain-related questionnaires, as well as the frequency of abnormal values of anxiety and depression were significantly different among the 3 groups, showing more abnormal values in the NOP-DE group than in the DE group.

• **CORNEAL SENSITIVITY:** Corneal sensitivity data are presented in Table 3. Noncontact corneal sensitivity assessed with Belmonte's esthesiometer showed similar results in mechanical threshold for the 3 groups. However, the thermal (both heat and cold) thresholds were significantly lower (sensitivity was higher) in the NOP-DE and DE groups than in the control group.

Tactile corneal sensitivity threshold measured using the Cochet-Bonnet esthesiometer was similar in the 3 groups. However, when it was measured after topical anesthesia, it remained significantly elevated only in the NOP-DE group. The Cochet-Bonnet pain threshold after anesthesia was $\geq$ 10 mm in significantly more patients with NOP than in the DE and control groups.

The GRC scale was significantly higher in the control group. In the NOP-DE group, only 8 (23.5%) patients reported relief of symptoms after topical anesthesia with GRC (−3 to −5) whereas the remaining 26 (76.5%) patients: reported slight improvement (9, 26.4%), no improvement 10 (29.4%) or even increased symptoms (7, 20.6%).

• **IN VIVO CONFOCAL MICROSCOPY:** The data collected using IVCM are shown in Table 4. The agreement between the 2 independent observers when assessing IVCM parameters was very good, except for the analysis of nerve tortuosity, which was moderate. Figure 1 shows representative images of each group.

The nerve density was lower in the 2 symptomatic groups than in the control group, and was still significantly lower in the group with pain (NOP-DE group) than in the one with no pain (DE group). Additionally, the density of microneuromas was significantly higher in the NOP-DE group than in the other 2 groups. The number of nerves and the density of nerve branch points were significantly lower in the NOP-DE and DE groups than in the control group. Dendritic cell density was significantly higher in the NOP-DE group than in the control group, whereas in the DE and control groups, this parameter was similar.

In summary, what differentiated the 2 symptomatic groups was the lower density of nerves and higher density of microneuromas when NOP was present (NOP-DE group) in addition to DE disease (DE group).

• **CORRELATIONS:** The data concerning the correlations between the different parameters measured are shown in Figures 2 and 3. Additionally, Figure 3 shows the graphical representation of the significant and strong/very strong

TABLE 1. Demographics, Clinical Characteristics, and Comorbidities of the 89 Evaluated Subjects Who Had Undergone Laser In-Situ Keratomileusis (LASIK) Surgery

	NOP-DE (Group 1) ($n = 34$)	DE (Group 2) ($n = 25$)	Control (Group 3) ($n = 30$)	*P* Value		
				1 vs 2	1 vs 3	2 vs 3
Age (years)—mean ± SD	39.1 ± 6.5	40.2 ± 8.6	42.2 ± 7.0	.098[a]	.583[a]	.266[a]
Sex: women / men—n (%)	26 (76.5) / 8 (23.5)	16(64.0) / 9 (36.0)	15 (50.0) / 15 (50.0)	.296	.065	.297
Preoperative spherical equivalent refractive error (diopters)—mean±SD	−4.2 ± 3.5	−1.9 ± 3.7	−3.6 ± 2.9	.084	.288	.124
Visual acuity (LogMar)—mean±SD						
High contrast (100%)—without correction	0.2 ± 0.3	0.1 ± 0.2	0.1 ± 0.2	.206	.488	.526
High contrast (100%)—with correction	0.1 ± 0.2	0.0 ± 0.1	0.0 ± 0.1	.094	.671	.114
Low contrast (10%)—without correction	0.6 ± 0.3	0.5 ± 0.3	0.4 ± 0.2	.083	**.036**	.606
Low contrast (10%)—with correction	0.6 ± 0.3	0.4 ± 0.2	0.4 ± 0.2	.065	**.036**	.800
Number of surgeries per eye—mean±SD	2.4 ± 0.9	2.7 ± 1.2	2.2 ± 0.5	.188	.401	.071
Months from surgery to visit—mean±SD	100.5 ± 70.7	114.6 ± 58.4	138.0 ± 42.5	.276	.087	.150
Onset of symptoms (months post-LASIK)—mean±SD	24.5 ± 49.0	33.5 ± 60.1	NA	.773	NA	NA
Months with symptoms—mean±SD	67.7 ± 61.1	75.8 ± 55.6	NA	.407	NA	NA
Comorbidities						
Migraines—n (%)	16 (47.1)	10 (40.0)	0 (0)	.589	**<.001**	**<.001**
Autoimmune hypothyroidism	2	2	0	.749	**.177**	**.115**
Psychiatric disorders—n (%)	5 (14.7)	2 (8)	1 (3.3)	.431	.119	.448
Central sensitization-related syndromes—n (%)	12 (35.2)	0 (0)	0 (0)	**<.001**	**<.001**	1
Arthralgias	2	1	0	**.745**	**.177**	.269
Temporomandibular disorders	1	0	0	**.387**	**.344**	-
Chemical sensitization syndrome	2	0	0	**.217**	**.177**	-
Irritable bowel syndrome	2	0	0	**.217**	**.177**	-
Chronic fatigue syndrome	2	0	0	**.217**	**.177**	-
Fibromyalgia / Cervical dystonia	2 / 1	0 / 0	0 / 0	**.217/.387**	**.177/.344**	-
Sensitivity alterations	1	0	0	**.240**	**.344**	-
Other chronic pain	1	0	0	.387	**.344**	-
Contact hypersensitivity (drug, metals…)	5	3	0	.764	**.029**	.051
Rosacea	1	1	0	.824	**.344**	.269
Topical agents used—n (%)						
Lubricants	34 (100)	19 (76.0)	0 (0.0)	**.003**	**<.001**	**.002**
Topical cyclosporine (>3 months)	8 (23.5)	3 (12.0)	0 (0.0)	.261	**.005**	**.050**
Blood derivates	5 (14.7)	3 (12.0)	0 (0.0)	.747	**.005**	**.050**
Lid hygiene (home-based)	8 (23.5)	5 (20.0)	0 (0.0)	.764	**.029**	**.050**
Analgesics (only those for ocular pain)—n (%)	7 (20.6)	2 (8.0)	0 (0.0)	.431	**.029**	.115

DE: dry eye; LogMar: logarithm of the minimum angle of resolution; NA: Not applicable; LASIK: Laser in-situ keratomileuusis; NOP-DE: Neuropathic ocular pain and dry eye; SD: standard derivation.

Only posthoc pairwise comparisons between groups are shown, not overall group comparisons. Quantitative variables are expressed as mean±SD. For parametric variables, the Analysis of Variance (ANOVA) test was used for groups comparisons and Student's t-test[a] for pairwise comparisons. Levene's test was used to check homogeneity of variance and Welch's test was used when this assumption was not valid. For nonparametric variables, Kruskal-Wallis for groups and Mann-Whitney U test for pairwise comparisons were performed and for qualitative variables, Chi square test. Bonferroni correction was applied to adjust the experiment-wise error rate.

Significant *P*-values are denoted in bold.

correlations between corneal in vivo confocal microscopy (IVCM) and corneal sensitivity variables.

The level of pain, measured with both NRS and WFPRS, was positively correlated with OSDI to a strong degree, with the mSIDEQ to a very strong degree, with the months with symptoms and the anxiety and depression subscales to a medium degree, and with the total HADS in a strong degree. Additionally, the level of pain (NRS and WFPRS) was inversely correlated with the number of nerves, nerve density, and density of nerve branches to a strong degree and inversely correlated with Schirmer test values to a medium degree.

TABLE 2. Symptomatology and Clinical Test Results in the 89 Evaluated Subjects Who Had Undergone Laser In-Situ Keratomileusis (LASIK) Surgery

	NOP-DE (Group 1) ($n = 34$)	DE (Group 2) ($n = 25$)	Control (Group 3) ($n = 30$)	*P* Value		
				1 vs 2	1 vs 3	2 vs 3
OSDI (0-100)—mean±SD	64.2 ± 20.7	51.8 ± 24.7	8.0 ± 3.0	.065	**<.001**	**<.001**
mSIDEQ (0-28)—mean±SD	19.7 ± 3.7	16.5 ± 5.9	5.3 ± 3.0	**.019**	**<.001**	**<.001**
Level of pain, (NRS scale, 0-10)—mean±SD	6.7 ± 2.0	2.2 ± 2.6	0.1 ± 0.3	**<.001**	**<.001**	**<.001**
No pain (0-1)—n (%)	NA	15 (60.0)	30 (100.0)	**<.001**	**<.001**	**<.001**
Mild pain (2-4)—n (%)	8 (23.5)	4 (16.0)	NA	**.02**	**.004**	**.007**
Moderate pain (5-7)—n (%)	10 (29.4)	4 (16.0)	NA	**.006**	**<.001**	**.001**
Severe pain (8-10)—n (%)	16 (47.1)	2 (8.0)	NA	**<.001**	**<.001**	**<.001**
Level of pain, (WFPRS scale, 0-10)—mean±SD	6.7 ± 2.0	2.6 ± 2.7	0.0 ± 0.0	**<.001**	**<.001**	**<.001**
No pain (0)—n (%)	NA	9 (36.0)	30 (100.0)	**<.001**	**<.001**	**<.001**
Discomfort (2)—n (%)	1 (2.9)	8 (32.0)	NA	**.002**	**.003**	.572
Light pain (4)—n (%)	6 (17.6)	3 (12.0)	NA	.061	**.016**	**.021**
Moderate pain (6)—n (%)	11 (32.4)	2 (8.0)	NA	**<.001**	**<.001**	**<.001**
Intense pain (8)—n (%)	12 (35.3)	3 (12.0)	NA	**<.001**	**<.001**	**<.001**
Unbearable pain (10)—n (%)	4 (11.8)	NA	NA	**.034**	**.018**	**.022**
HADS questionnaire (0-42)—mean±SD	18.9 ± 8.0	18.2 ± 11.8	6.1 ± 5.5	.645	**<.001**	**<.001**
Anxiety subscale (0-21)—mean±SD	10.9 ± 4.1	9.8 ± 5.8	4.2 ± 3.2	.419	**<.001**	**<.001**
Anxiety subscale (≥8)—n (%)	25 (73.5)	15 (60.0)	3 (10.0)	**<.001**	**<.001**	**<.001**
Depression subscale (0-21)—mean±SD	8.0 ± 4.3	8.5 ± 6.4	1.9 ± 2.4	.92	**<.001**	**<.001**
Depression subscale (≥8)—n (%)	18 (52.9)	13 (52.0)	1 (3.3)	**<.001**	**<.001**	**<.001**
Tear osmolarity (mOsm/L)—mean±SD	316.2 ± 17.0	314.2 ± 14.1	310.6 ± 13.4	.820	.063	.247
Ocular surface integrity—mean±SD						
Corneal staining (Oxford scale, 0-5)	1.0 ± 0.8	1.5 ± 0.7	0.9 ± 0.8	**.036**	.549	**.018**
Corneal staining (CCLRU, 0-4)	0.9 ± 0.7	1.1 ± 0.6	1.0 ± 0.6	.181	.262	.799
Conjunctival staining (Oxford scale, 0-5)	0.8 ± 0.8	0.8 ± 0.8	0.2 ± 0.4	.981	**.001**	**<.001**
Conjuntival hyperemia (Efron scale, 0-4)	1.4 ± 0.6	1.6 ± 0.7	1.8 ± 0.8	.958	.121	.351
MGD (Efron scale, 0-4)	1.1 ± 0.5	1.2 ± 0.6	0.8 ± 0.6	.56	**.008**	**.005**
Tear stability and production						
TBUT (s)—mean±SD	3.7 ± 2.1	4.1 ± 2.1	5.8 ± 3.7	.334	**.007**	.112
TBUT ≤ 7 s—n (%)	33 (97.1)	21 (84.0)	20 (66.7)	**.029**	**.024**	**.009**
Schirmer test (mm)—mean±SD	6.9 ± 7.4	7.0 ± 4.7	13.9 ± 5.8	.318	**<.001**	**<.001**
Schirmer test ≤ 5 mm—n (%)	19 (55.8)	10 (40.0)	0 (0.0)	**<.001**	**<.001**	**<.001**

CCLRU: Cornea and Contact Lens Research Unit grading scale; HADS: Hospital Anxiety and Depression Scale; MGD: Meibomian gland dysfunction; mSIDEQ: modified single item dry eye questionnaire; LASIK: Laser in-situ keratomileusis; mOsm: milliosmoles; NA: Not applicable; NRS: Numerical Rating Scale; OSDI: Ocular Surface Disease Index;; s: seconds; SD: standard deviation; TBUT: Tear Break-up Time; WFPRS: Wong-Baker Faces Pain Rating Scale.

Only posthoc pairwise comparisons between groups are shown, not overall group comparisons. Quantitative variables are expressed as mean±SD. Since all variables are nonparametric, the Kruskal-Wallis test was used for group comparisons, and the Mann-Whitney U test was used for posthoc pairwise comparisons. For qualitative variables, Chi square test was used. Bonferroni correction was applied to adjust the experiment-wise error rate.

Significant *P*-values are denoted in bold.

OSDI and mSIDEQ questionnaires were also positively correlated to a strong degree with the months with symptoms, anxiety, and depression subscales, and with the global HADS score.

The thermal (heat and cold) corneal sensitivity thresholds correlated inversely to a medium degree. The mechanical threshold showed a low inverse correlation with both types of corneal staining. Heat and cold thresholds correlated with a direct and inverse respectively, with low grade with months with symptoms, symptoms questionnaires, total HADS and MGD. The cold threshold also correlated in a low grade directly with the anxiety subscales, and inversely number of nerves and nerve density. Even so, the heat threshold correlated in a low degree inversely with depression subscale, and directly with nerve density and density of nerve branch points. Cochet-Bonnet threshold cor-

TABLE 3. Corneal Sensitivity Thresholds in the 89 Evaluated Subjects Who Had Undergone Laser In-Situ Keratomileusis (LASIK) Surgery

Characteristics	NOP-DE (Group 1) ($n = 34$)	DE (Group 2) ($n = 25$)	Control (Group 3) ($n = 30$)	*P* Value		
				1 vs 2	1 vs 3	2 vs 3
Corneal esthesiometry (noncontact, Belmonte)—mean±SD						
Mechanical threshold—(mL/min)	119.4 ± 38.2	112.9 ± 28.6	110.2 ± 36.9	.673	.419	.554
Heat threshold (°C)	0.8 ± 0.6	1.1 ± 0.8	1.4 ± 0.7	.243	**<.001**	.076
Cold threshold (°C)	−1.3 ± 1.0	−1.7 ± 1.1	−2.0 ± 0.6	.735[a]	**.048**[a]	.776[a]
Corneal esthesiometry (contact, Cochet Bonnet)—mean±SD						
Without topical anesthesia (mm)	54.8 ± 7.0	57.0 ± 4.6	57.3 ± 2.9	.131	.196	.718
With topical anesthesia (mm)	11.9 ± 15.0	1.5 ± 2.6	1.5 ± 2.8	**.002**	**<.001**	.792
Patients With topical anesthesia ≥ 10 mm—n (%)	17 (50.0)	6 (24.0)	4 (13.3)	**.043**	**.003**	.307
Anesthetic challenge test- GRC scale (−5 to 5)	−0.7 ± 2.0	−0.8 ± 2.6	0.9 ± 2.2	.852	**.017**	**<.001**

DE: dry eye; GRC: Global Rating of Change; LASIK: Laser in-situ keratomileusis; NOP: Neuropathic ocular pain; SD: standard derivation.

Only posthoc pairwise comparisons between groups are shown, not overall group comparisons. Quantitative variables are expressed as mean±SD. For parametric variables, the Analysis of Variance (ANOVA) test was used for groups comparisons and Student's t-test[a] for pairwise comparisons. Levene's test was used to check homogeneity of variance and Welch's test was used when this assumption was not valid. For nonparametric variables, Kruskal-Wallis for groups and Mann-Whitney U test for pairwise comparisons were performed and for qualitative variables, Chi square test. Bonferroni correction was applied to adjust the experiment-wise error rate.

Significant *P*-values are denoted in bold.

TABLE 4. In Vivo Confocal Microscopy of the Corneal Parameters Evaluated in the 89 Evaluated Subjects Who Had Undergone Laser In-Situ Keratomileusis (LASIK) Surgery

Characteristics	Agreement Between Observers ICC [95%CI]	NOP-DE (Group 1) ($n = 34$)	DE (Group 2) ($n = 25$)	Control (Group 3) ($n = 30$)	*P* Value		
					1 vs 2	1 vs 3	2 vs 3
Number of nerves (n/mm^2)	0.97 [0.95-0.98]	30.3 ± 13.7	36.7 ± 18.8	68.1 ± 21.1	.239	**<.001**	**<.001**
Number of nerves (n/frame)	0.97 [0.95-0.98]	4.9 ± 2.3	6.0 ± 2.9	11.2 ± 3.6	.239	**<.001**	**<.001**
Nerve density (mm/mm^2)	0.99 [0.98-0.99]	7.2 ± 3.2	9.6 ± 4.0	15.7 ± 475.7	**.049**[a]	**<.001**[a]	**<.001**[a]
Nerve length (mm/mm^2)	0.95 [0.92-0.97]	1.5 ± 3.3	1.6 ± 3.1	1.5 ± 2.0	.550[a]	.791[a]	.056 [a]
Density of nerve branch points (n/mm^2)	0.97 [0.96-0.98]	11.2 ± 12.4	18.1 ± 15.9	43.4 ± 25.5	.132	**<.001**	**<.001**
Nerve tortuosity (0-4)	0.63 [0.43-0.76]	2.5 ± 0.9	2.6 ± 0.8	2.3 ± 0.6	.958[a]	.958[a]	.282[a]
Density of microneuromas (n/mm^2)	0.97 [0.95-0.98]	1.8 ± 4.6	0.2 ± 0.4	0.1 ± 0.3	**.008**	**.001**	.510
Density of dendritic cells (n/mm^2)	0.98 [0.97-0.99]	73.3 ± 99.1	54.3 ± 57.7	30.1 ± 40.8	.766	**.043**	.132
Reflectivity	1.0 [1.0-1.0]	99.3 ± 14.4	103.3 ± 21.8	101.0 ± 17.2	1[a]	1[a]	1[a]

CI: confidence interval; DE: dry eye; ICC: intraclass correlation coefficient; LASIK. Laser in-situ keratomileusis; NOP: neuropathic ocular pain.

Quantitative variables are expressed as mean±SD. For parametric variables, the Analysis of Variance (ANOVA) test was used for groups comparisons and Student's t-test[a] for pairwise comparisons. Levene's test was used to check homogeneity of variance and Welch's test was used when this assumption was not valid. For nonparametric variables, Kruskal-Wallis for groups and Mann-Whitney U test for pairwise comparisons were performed and for qualitative variables, Chi square test. Bonferroni correction was applied to adjust the experiment-wise error rate.

Significant *P*-values are denoted in bold.

related in a low grade inversely with VA in high contrast, density of microneuromas and reflectivity and directly with TBUT, nerve density and density of nerve branch points. Finally, postanesthetic Cochet-Bonnet correlated in a low grade directly with OSDI, mSIDEQ, pain questionnaires and anxiety subscale and inversely with Schirmer test, number of nerves, nerve density and density of nerve branch points (Figure 2).

Besides the correlations already named above the number of nerves, nerve density and density of nerve branch points

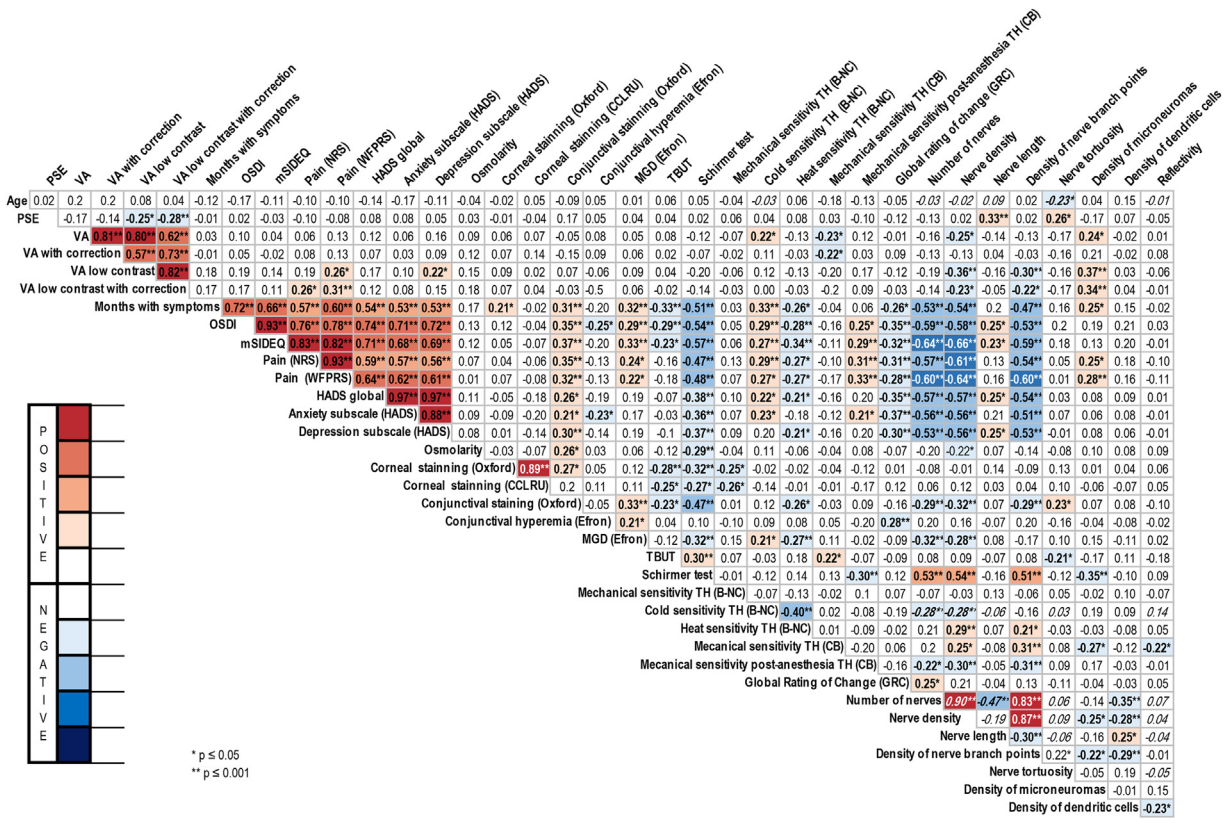

FIGURE 2. Correlation matrix of symptoms, signs, corneal sensitivity thresholds, and corneal *in vivo* confocal microscopy variables. B-NC: Belmonte esthesiometer—noncontact; CB: Cochet–Bonnet aesthesiometer; CCLRU: Cornea and Contact Lens Research Unit grading scale; CFS: corneal fluorescein staining; DC: dendritic cells; GRC: Global rating of change; HADS: Hospital Anxiety and Depression Scale; MGD: Meibomian gland dysfunction; mSIDEQ: Modified Single Item Dry Eye Questionnaire; NRS: Numerical Rating Scale; OSDI: Ocular Surface Disease Index; PSE: preoperative spherical equivalent; RS: refractive surgery; s: seconds; TBUT: Tear Break-up Time; TH: threshold; VA: Visual acuity; WFPRS: Wong-Baker Faces Pain Rating Scale.
Data represent the rho coefficients of Spearman correlation except those in italic font which indicate Pearson correlation. Red color represents direct o positive correlations, and blue color represents inverse correlations. Color intensity represents the degree of correlation according to the scale presented in the image. *$P \leq .05$; **$P \leq .001$.

showed medium and strong inverse correlations with the duration of symptoms, OSDI and symptoms questionnaires, anxiety and depression variables (HADS and its subscales), and Schirmer test results.

In a low degree, there are some nerve correlations. Number of nerves correlated inversely with conjunctival staining, and MGD. Nerve density was inversely correlated with VA, VA low contrast with and without correction, osmolarity, conjunctival staining and MGD. Nerve length was directly correlated with PSE, OSDI, mSIDEQ and total HADS. Density of nerve branch points correlated inversely with VA. VA low contrast with correction and conjunctival staining.

Nerve tortuosity correlated inversely with age and TBUT and directly with PSE, and conjunctival staining. Finally, microneuromas correlated inversely with Schirmer and directly with VA, VA low contrast with and without correction, months with symptoms, and pain with NRS and WFPRS.

DISCUSSION

This study mainly aimed to identify the differences between LASIK patients who developed chronic NOP in addition to DE and those who developed DE but lacked NOP. The addition of NOP to the better-known DE after RS is highly relevant and still underexplored. NOP is a more disabling problem, severely diminishing quality of life, causing biopsychosocial impact, activity limitations, employment affectation, resulting in a sense of disability, and social restrictions.[48] For this purpose, the only relevant control groups were one with only DE and no NOP (DE group) after LASIK and certainly a control group of asymptomatic patients who had also undergone the same kind of surgery so that differential findings could not be attributed to the surgery itself. Because the parameters analyzed in this study have been well documented in ocularly healthy subjects

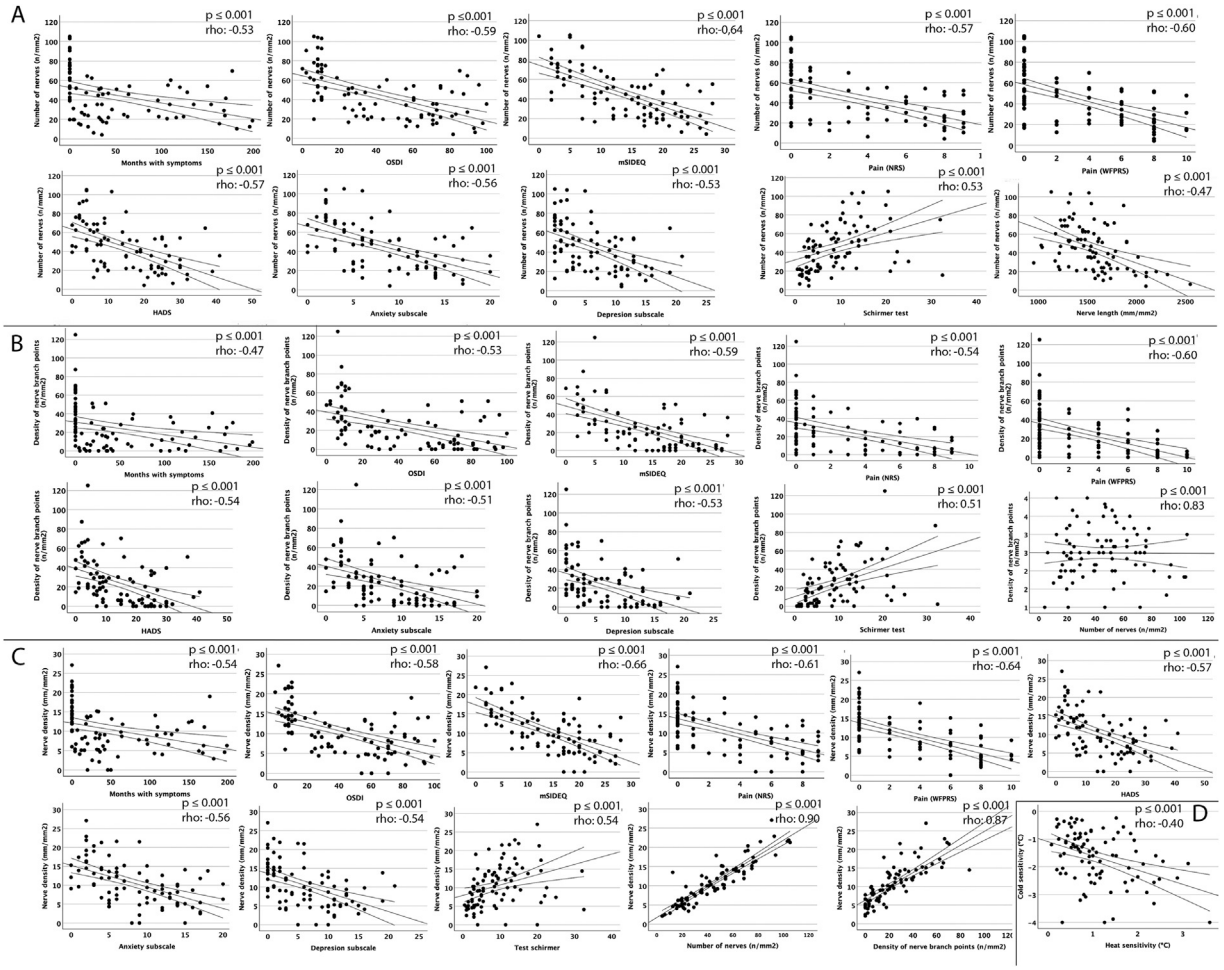

FIGURE 3. Significant and strong/very strong correlations between corneal *in vivo* confocal microscopy (IVCM) and corneal sensitivity variables. (A) Number of corneal nerve-related correlations; (B) Density of nerve branch correlations; (C) Nerve density correlations; (D) Heat and cold thresholds correlation. The central line represents the best-fit line and the area between the lower and upper lines indicates the 95% confidence interval.

with no history of surgery, we understood that this possible control group was not necessary to be recruited.[42,49-56] Additionally, numerous previous studies have compared DE patients with healthy controls and NOP patients with healthy controls independently,[57,58] highlighting the need for their own comparisons.

Our findings revealed that patients with chronic DE after LASIK who additionally developed NOP (NOP-DE group), compared with those who had DE but no NOP (DE group), were more symptomatic, experienced more abnormal levels of anxiety and depression, reported more central sensitization-related syndromes, showed less corneal staining, used more lubrication, and more patients had lower values of tear production and stability. Furthermore, they maintained higher pain levels when measuring corneal sensitivity (with Cochet-Bonnet) after topical anesthesia. Particularly significant were the objective signs found by IVCM in NOP-DE patients: lower nerve density and higher microneuromas density in the corneal sub-basal nerve plexus.

While the severity of pain and other symptoms assessed with NRS, WFPRS and mSIDEQ, respectively was significantly higher in the NOP-DE group than the in the DE group, the severity of most signs was similar between these groups (corneal and conjunctival staining with CCLRU and Oxford scales respectively, MGD with Efron scale, and TBUT or Schirmer test mean values). However, the percentage of patients with impaired tear production and stability was significantly higher in the NOP-DE group. This disproportionality of severe symptoms with mild or no signs at the slit-lamp, often referred to "pain without stain," is typical of NOP patients.[10,59,60] This challenge in diagnosing neurogenic DE and NOP is complicated by the lack of evident clinical signs at the slit-lamp or with commonly used tests, such as the Schirmer test.

NOP patients reported more central sensitization syndromes and neuropsychiatric conditions like anxiety and depression, which have been previously reported by us as more frequently present after RS than before RS and also described as a risk factor for post-RS chronic ocular pain by different authors.[48,59,61-64] Other studies have also included post-traumatic stress disorder,[48,65] and the so-called "chronic overlapping pain conditions,"[48,66,67] meaning that patients suffering from some form of chronic pain often suffer from other types of chronic pain and relate this to central sensitization. Although these conditions are not modifiable factors, it is advisable to study a patient's medical history before recommending RS.

The use of artificial tears was significantly higher in the NOP-DE group than in the DE group, most likely because of their DE-related symptomatology (OSDI and mSIDEQ scores) was also higher. Previously published studies have shown that using artificial tears on an isolated basis in NOP patients was associated with an incomplete response.[68] In addition, typical DE treatment seems less effective in NOP patients,[69] but they are still used as a part of a multimodal approach.[11]

The study of the morphology and function of the corneal nerve plexus can help identify the underlying causes of pain, thus, it is beginning to be considered as an objective biomarker of NOP.[70] IVCM enables visualization of corneal nerve injury and abnormalities in the nerve plexus, whereas corneal esthesiometry quantifies one aspect of nerve fiber functionality.

In our series, patients with NOP-DE reported lower postanesthetic pain thresholds than those with DE and no NOP when tactile sensation was evaluated before and after topical anesthesia with the Cochet-Bonnet esthesiometer. According to previous studies, patients with NOP have central sensitization, which manifests as persistent ocular pain after topical anesthesia that is associated with very severe symptoms and is disproportionate to the signs,[11,61,63,71-75] which is consistent with our findings in these patients. Although peripheral pain is abolished with topical anesthesia, centralized pain can persist despite its application. This test, combined with the lack of signs at the slip-lamp, can help ophthalmologists differentiate between nociceptive and neuropathic pain.

We found distinctive objective differences in IVCM findings in the central cornea between patients with only DE (DE group) and those with additional NOP (NOP-DE group) developed after LASIK. Specifically, NOP-DE patients had significantly lower nerve density and higher microneuromas density than the DE group. A lower density of corneal nerves has been previously reported in DE patients,[64,70,76] in patients with NOP[58] in the centralized pain subtype,[64] and in those with neuropathic-related symptoms (allodynia and photoallodynia).[70,77,78] However, this finding has not been previously demonstrated to differentiate between the presence and absence of NOP in patients with DE post-RS. Nerve density was also significantly lower in the DE group than in the control group formed by asymptomatic patients after LASIK. However, the relevant finding is that the nerve density is even lower, and significantly so, in those DE patients who, after LASIK, also developed NOP. In line with our corneal nerve morphology results, previous studies have shown a decrease in corneal sub-basal nerve number in patients with DE with/without NOP,[58] as well as an increased tortuosity, elevated beadings,[78,79] and raised dendritic cells.[73,74,80] Conversely, Moien et al. did not report differences between these parameters.[70]

A lower density of microneuromas also differentiates patients with DE and NOP from those only experiencing DE after LASIK.[60,64,74,78,81,82] Injured surgical corneal nerves after LASIK, in an attempt at neuroregeneration, can form nerve abnormalities such as microneuromas, irregularly shaped terminal enlargements of sub-basal nerve endings with variable hyperreflectivity. These abnormalities impair functionality and excitability related to hyperalgesia, spontaneous pain, and allodynia.[83] They lead to molecular changes altering nerve excitability, which could explain the presence of pain.[84]

Recently, microneuromas have been considered a biomarker of NOP.[70,85] Artificial intelligence studies have ranked them as the top biomarker of neuropathic pain.[86] Our findings, along with those of other authors,[87] support this conclusion, as DE patients and asymptomatic patients after LASIK did not differ in the presence of microneuromas.[87]

Our findings also showed that patients suffering from chronic DE (with or without NOP), compared with asymptomatic individuals after RS, exhibited poorer low contrast VA, suffered more from migraines, and showed increased heat and cold corneal threshold sensitivity. Additionally, they demonstrated lower nerve density, a lower number of nerves, and lower density of nerve branching in their corneal nerve plexus. These findings align with previous research: migraines appeared to be more prevalent in patients with DE and have been positively correlated with OSDI[88,89]; increased sensitivity or decreased detection thermal thresholds have been previously reported in DE patients than in controls[90]; and DE patients have shown alterations in the corneal nerve plexus.[91-97]

Our outcomes did not show significant differences in mechanical threshold, but thermal hypersensitivity (heat and cold) was detected in DE patients. Previous studies have reported both increased threshold or hyposensitivity[56,98-101] and decreased threshold or hypersensitivity[102-107] for both mechanical and thermal thresholds in DE patients. It is worth noting that while there may not have been statistically significant differences in our patients when NOP was added to DE, there might have been a trend toward significance, which could have been obtained with a larger number of patients. Corneal sensitivity studies using the Belmonte esthesiometer are very limited, and comparisons with other studies are not possible because of the lack of existing research on this specific topic.

The development of NOP after LASIK may be explained by several mechanisms. LASIK-induced damage to the corneal sub-basal nerve plexus disrupts ocular surface homeostasis and sensory feedback. Impaired nerve regeneration postsurgery may lead to persistent abnormalities, such as microneuroma formation and hypersensitivity. Chronic nociceptive input from damaged corneal nerves can result in central sensitization, amplifying pain perception despite minimal peripheral signs. These mechanisms, alongside individual susceptibility factors, likely contribute to the onset and persistence of NOP.[108] The reasons why this phenomenon occurs in some patients but not in the majority remain unknown.

One limitation of our study is the use of manual grading for corneal nerve tortuosity, which, given its subjective nature, resulted in a moderate interclass correlation coefficient (ICC = 0.63). This could introduce some variability in the results. However, our group is actively working on implementing a more objective and automated method for assessing corneal nerve tortuosity, as described in a recent publication, which will help reduce this potential bias in future research.[109] Another limitation of the study is the small sample size, though the prevalence of neuropathic ocular pain (NOP) is estimated to be around 0.1% to 0.2%. Future lines of research include the ongoing development of a long-term longitudinal study with these patients to further expand and validate the findings.

In conclusion, our study revealed that higher post-topical anesthesia corneal tactile sensitivity, lower nerve density and higher microneuromas density in the sub-basal corneal nerve plexus are signs that can help diagnose NOP in patients suffering from DE and pain after RS. Further investigation into concomitant or subsequent cellular and molecular changes associated with nerve damage is warranted. A multidisciplinary team of clinicians and basic scientists is needed to gain deeper knowledge that could lead to better therapeutic options or, ideally, preventive measures.

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CREDIT AUTHORSHIP CONTRIBUTION STATEMENT

Amanda Vázquez: Writing – review & editing, Writing – original draft, Methodology, Investigation, Data curation, Conceptualization. **Marta Blanco-Vázquez:** Software, Data curation. **Elena Martínez-Plaza:** Investigation. **Alberto López-Miguel:** Supervision, Project administration, Formal analysis. **Enrique Ortega:** Supervision, Investigation. **Amalia Enríquez-de-Salamanca:** Supervision, Project administration, Formal analysis, Conceptualization. **Margarita Calonge:** Supervision, Project administration, Conceptualization.

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