In Vivo Evaluation of Corneal Nerves and Epithelial Healing After Treatment With Recombinant Nerve Growth Factor for Neurotrophic Keratopathy

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• PURPOSE: To evaluate the renewal of corneal nerve structure and function in patients with neurotrophic keratopathy (NK) treated with recombinant human nerve growth factor (rhNGF) eye drops.

• DESIGN: Prospective, interventional, before-and-after case series.

• METHODS: This study included 18 patients with NK with a persistent epithelial defect or corneal ulcer, treated with topical rhNGF, and age-matched healthy controls. Patients underwent clinical examination with corneal fluorescein staining, Schirmer 1 tear test, assessment of corneal sensitivity with the Cochet-Bonnet esthesiometer, and morphologic examination of the nerves by in vivo confocal microscopy (IVCM) at baseline and at 4 and 8 weeks of treatment. IVCM analysis was used to assess corneal sub-basal nerve density, number of nerve branches, and the diameter of nerve fibers.

• RESULTS: A complete resolution of the epithelial defect was observed in all patients within 8 weeks. Schirmer 1 test showed a significant improvement of tear film secretion. Change from baseline in corneal sensation was significant ($P < .001$) but did not approach that of healthy controls. After 8 weeks of treatment, there was a significant increase in the mean nerve density in affected eyes as compared to baseline ($P = .007$) as well as in the number of nerve branches ($P = .008$) and nerve fiber diameter ($P = .007$).

• CONCLUSIONS: Topical treatment with rhNGF was effective in promoting complete corneal healing of persistent epithelial defects and corneal ulcers in patients with NK. This was associated with an improvement of corneal sensitivity and an increase of sub-basal nerve density, diameter, and number of nerve branches, indicating improvement in structure and function of corneal nerves. (Am J Ophthalmol 2020;217:278–286. © 2020 Elsevier Inc. All rights reserved.)

NEUROTROPHIC KERATITIS OR NEUROTROPHIC keratopathy (NK) is a corneal disease characterized by epithelial instability and decreased corneal sensitivity owing to impairment of corneal nerves.[1] A recent comprehensive review defines NK as a disease related to alterations in corneal nerves leading to impairment in sensory and trophic function with consequent breakdown of the corneal epithelium, affecting health and integrity of the tear film, epithelium, and stroma.[2] Clinical signs are seen in all these structures depending on the stage of the disease. Traditionally, there are 3 stages of severity according to the Mackie classification.[3,4] Stage 1 NK is characterized by superficial punctate epitheliopathy and tear film alteration, stage 2 is characterized by the presence of a persistent epithelial defect (PED), and in stage 3 the stroma is also affected. In 2018 the Mackie classification was modified and corneal sensitivity was included in the grading scale. Dua and associates proposed 3 stages of NK—mild, moderate, and severe—based on clinical severity and corneal sensitivity. In mild NK, epithelial irregularity is present without a clear epithelial defect and is associated with reduced or absent sensitivity in 1 or more quadrants of the cornea; moderate NK is characterized by PED associated with a variable degree of corneal hypoesthesia or anesthesia; in severe NK, stromal ulceration including frank perforation is present together with corneal hypoesthesia or anesthesia.[2] In vivo confocal microscopy (IVCM) permits detailed en face morphologic evaluation of corneal nerves.[5]

Several studies described the morphology of sub-basal nerve plexus, and, despite differences in the methods of evaluation of nerve fiber density and anatomy, some confocal parameters have been defined to analyze corneal innervation in health and disease.[6–9] In particular, corneal nerve density is considered as the length of nerve fibers visible within a defined area and is expressed in mm/mm^2 or $\mu m/mm^2$; number of branching and thickness of nerve fibers are also considered confocal parameters to evaluate sub-basal nerve plexus. In vivo

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confocal microscopy analysis of sub-basal nerve plexus allows for a direct correlation to be made between corneal innervation and clinical findings.[1,4] Anterior segment optical coherence tomography (AS-OCT) allows assessment of depth and area of cornea affected, permitting accurate grading of NK.[10] These imaging techniques, together with clinical evaluation, have enabled more accurate staging of NK with important therapeutic and prognostic implications.[1] The management of NK is related to its grading: in the mild stage, use of preservative free artificial tears and punctal occlusion are indicated; in moderate NK, the use of therapeutic contact lenses, tarsorrhaphy, or amniotic membrane transplantation are used to promote healing. The management of severe NK is aimed at preventing corneal perforation and promoting healing through the use of amniotic membrane transplantation, tarsorrhaphy, and corneal graft.[2,11]

Biological agents such as IGF-1, substance P, neurotrophins, semaphorins, and vascular endothelial growth factor and serum and plasma derivatives have also been used, with promising results.[11–14] Corneal neurotization is an alternative approach in the management of NK but is an elaborate and invasive procedure that is based on surgical transfer of a healthy nerve segment into corneal tissue to re-establish innervation to restore sensation and trophic function.[15,16]

The introduction of human recombinant nerve growth factor (rhNGF, cenegermin) eye drops, as a licensed-in-Europe specific medication for treatment of moderate and severe NK, has added a new dimension to the management of this condition[17,18] and holds promise in presenting an alternative to the surgical intervention of neurotization in a number of cases. Prior to the introduction of rhNGF, it was demonstrated that topical eye drops of murine-derived nerve growth factor (200 mg/mL) was effective in promoting corneal epithelial healing and increasing corneal sensitivity in mild or moderate NK.[19,20] However, despite the compelling evidence use of murine NGF was not approved for treatment of NK and this therapeutic option was not available for nearly 2 decades.[17,18] Subsequently, an *Escherichia coli*–derived recombinant human nerve growth factor was formulated and a phase I randomized, double-masked, vehicle-controlled study proved its safety in healthy volunteer and NK patients.[17,18] Thereafter, a phase II multicenter randomized double-masked vehicle-controlled trial reported that rhNGF was more effective than vehicle in promoting healing of moderate (stage 2) and severe (stage 3) NK.[17,18] In light of this evidence, the use of cenegermin was recently approved in the European Union for the treatment of neurotrophic keratopathy moderate or severe, and in the United States the Food and Drug Administration authorized the use of cenegermin for all stages of NK,[21] although some European countries, such as the United Kingdom, did not approve the rhNGF as a cost-effective treatment, as there is lack of evidence on its long-term effect.

Therapy with cenegermin is associated with stromal and epithelial healing and return of corneal sensations. In this study we prospectively evaluated corneal healing, restoration of corneal sensations and, by IVCM, the regeneration of corneal nerves, during treatment of NK with cenegermin.

METHODS

THIS PROSPECTIVE OBSERVATIONAL STUDY (ClinicalTrails.gov Identifier: NCT04293549) was approved by the Review Board of the G. d'Annunzio University of Chieti-Pescara. The research adhered to the tenets of the Declaration of Helsinki and written consent was obtained from all patients involved in the study. All subjects were recruited from the Ocular Surface Service of G d'Annunzio University of Chieti-Pescara and from the Division of Clinical Neuroscience, Department of Ophthalmology, University of Nottingham, between March and November 2018. This case series consisted of 18 patients (9 male and 9 female) with documented moderate or severe neurotrophic keratopathy based on a recent classification of NK.[2] We considered a persistent epithelial defect (PED) the presence of an epithelial ulcer in absence of stromal thinning unresponsive to medical treatment for at least 4 weeks. The diagnosis of NK was made on medical and ophthalmologic history, slit-lamp examination, and evaluation of corneal sensitivity with a Cochet-Bonnet esthesiometer. In 8 patients NK was caused by previous episodes of recurrent stromal herpetic keratitis (not active at the time of the study), 4 patients showed NK after keratoplasty, 3 patients underwent chemical trigeminolysis, and in 3 cases a history of uncontrolled diabetes has been reported. Before being enrolled in the study, patients were treated for at least 4 weeks with topical antibiotics, lubricants, and therapeutic contact lens, without clinical success.

Exclusion criteria applied were the presence of infective keratitis, corneal dystrophy, glaucoma, or peripheral ulcerative keratitis. The study also included a control (comparator) group of 20 healthy subjects matched for age and sex.

Patients with moderate or severe NK were treated with cenegermin 20 μg/mL (Dompé Farmaceutici Spa, Milan, Italy) 1 drop 6 times a day in the affected eye for 8 weeks. Therapeutic contact lens use was discontinued during the duration of the study. Patients' medical history, slit-lamp examination, Schirmer I test, corneal esthesiometry, and IVCM examination were acquired at baseline and after 4 and 8 weeks of treatment. Changes in the corneal epithelium and stroma were evaluated by slit-lamp biomicroscopy and photographic documentation of the cornea before and after fluorescein staining. In each study center each examination was performed by the same experienced operator. Tear film secretion was investigated by the Schirmer I test by applying a Schirmer strip (Bio-tech Vision Care, Gujarat, India) in the lower fornix of the affected eye for 5 minutes.

Measurement of corneal sensation was performed with a Cochet-Bonnet esthesiometer (Luneau Ophthalmologie, Chartres, France). The cornea was touched with a 6-cm-length monofilament nylon thread. If the patient did not feel this, the thread was gradually shortened by 0.5 cm until a positive response was recorded. The longest filament length able to induce a positive response was recorded as the corneal sensation threshold. The filament was applied on the corneal defect and on superior, inferior, nasal, and temporal quadrants.

IVCM was performed by using a diode-laser 670 nm (HRT II Rostock Cornea Module; Heidelberg Engineering, Heidelberg, Germany) confocal microscope. Confocal microscopic scanning was focused on the central cornea and superior, inferior, nasal, and temporal quadrants. For each examined zone, at least 5 frames at the level of the epithelium and basal lamina were obtained. Corneal sub-basal nerve morphology and density were traced using NeuronJ, a free semi-automated analysis plug-in program of ImageJ (National Institutes of Health, Bethesda, Maryland, USA). The selected images were the best focused, showing good contrast and the whole image in the same layer (oblique sections were excluded). Images were anonymized before analysis to avoid observer bias. Nerve density was evaluated by tracing all nerve fibers visible in the image; the density of the nerve fibers was calculated in $\mu m/mm^2$. In each frame the number of nerve branches was counted manually and reported as number/frame. The average nerve density and the average number of nerve branches of 5 different IVCM images acquired in each corneal zone examined were calculated. The nerve regeneration rate was calculated using the following formula: nerve density at baseline − nerve density at follow-up/months of follow-up.

• **STATISTICAL ANALYSIS:** Qualitative variables were presented as frequency and percentage. Continuous variables were tested for normal distribution with Shapiro-Wilk test and reported as mean and standard deviation (SD). Nonparametric Friedman test for repeated measures was performed to compare quantitative variables among different follow-up measures, and post hoc analysis was performed with pairwise test.

Mann-Whitney *U* test was used to compare the nerve density, nerve number, nerve branches, and sensation of the cornea between the NK group and the control group.

All tests were 2-sided and a level of statistical significance was set at $P < .05$. All the statistical analyses were performed using R software environment for statistical computing and graphics version 3.5.2 (R Foundation for Statistical Computing, Vienna, Austria; https://www.R-project.org/).

RESULTS

THERE WERE NO SIGNIFICANT DIFFERENCES IN AGE AND SEX between the NK group and the control group ($P > .5$). The age of the patients ranged from 50 to 83 years with an average age of 66.21 ± 9.9 years at baseline. Slit-lamp examination and fluorescein staining showed the presence of epithelial defect in all patients in the NK group.

Complete corneal epithelial healing was achieved in 15 of 18 patients (83.3%) after 4 weeks of rhNGF treatment and in 18 of 18 patients (100%) after 8 weeks (Figure 1). Schirmer I test showed a significant increase of tear film secretion at week 8 compared to baseline ($P = .003$).

At baseline, corneal sensitivity in each quadrant was significantly lower in the NK group vs the healthy comparator group, as seen in Table 1. At the end of 8 weeks, there was a significant increase in corneal sensation in the NK treatment group in the inferotemporal, inferonasal, and superonasal quadrant and in the area of epithelial defect as compared to baseline.

All data regarding the sub-basal nerve fiber density, the number of nerve branches, and the diameter of nerve fibers are presented in Table 2. In the NK group the sub-basal nerve fiber density increased significantly at week 4 and week 8, with a nerve regeneration rate of 1079.129 ± 835 $\mu m/mm^2$ at 4 weeks and 661.898 ± 835 $\mu m/mm^2$ at 8 weeks. The mean density of nerve fibers was significantly higher at week 4 and week 8 in comparison to baseline. The number of nerve branches and the diameter of nerve fibers increased significantly after 4 weeks of treatment (Figure 2).

In relation to the healthy comparator group, the sub-basal nerve fiber density, number of nerve branches, and the diameter of the corneal nerve fibers were statistically significantly less in the NK treatment group. This difference was seen both at baseline and after 8 weeks of treatment despite the documented regeneration of sub-basal nerve plexus in the NK group.

Figures 3 and 4 illustrate 2 representative cases of epithelial healing and increasing of subepithelial nerve fiber in patients affected by NK treated with topical rhNGF.

DISCUSSION

THOUGH NK IS BELIEVED TO RESULT FROM AN ALTERATION in corneal nerves affecting trophic and sensory functions with consequent breakdown of the corneal epithelium, the pathophysiology of NK is not well understood. The clinical picture is often compounded by the manifestations of the underlying condition causing corneal disease or damage. Both trophic and sensory functions are implicated, but the impairment of one them does not sequentially follows the alteration of the other. Sensory and trophic functions can be dissociated, as seen in preganglionic lesions wherein sensations are reduced or lost but trophic function is maintained, probably related to the retention of neurons in the trigeminal ganglion.[22,23] Loss of sensation can be variable,

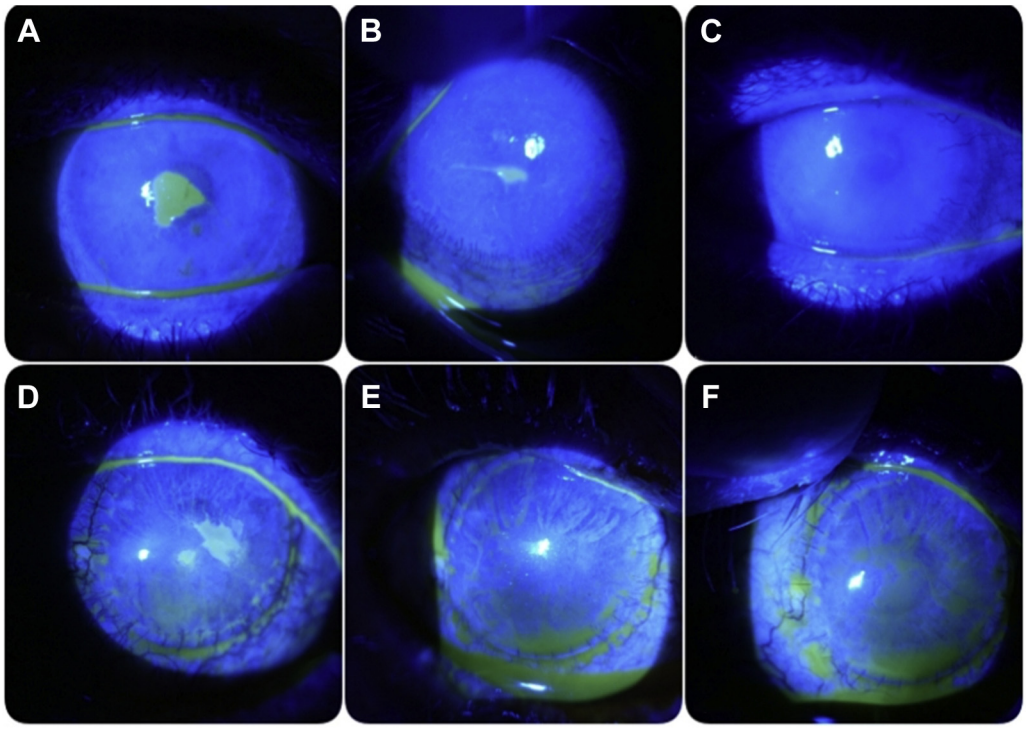

FIGURE 1. Healing of corneal epithelial defects in 2 representative patients affected by neurotrophic keratopathy (NK) on treatment with recombinant human nerve growth factor over time. (A-C) Case 1: Representative images of a case of NK of a female subject, 54 years old, affected by recurrent stromal herpes simplex virus keratitis. (A) Epithelial defect evident at day 0. (B) Epithelial defect at week 4 showing reduction in area of defect at week 4. (C) Complete healing at week 8. (D-F) Case 2: Representative images of a case of NK of a male subject, 62 years old; defect occurred after lamellar keratoplasty. (D) Nonhealing epithelial defect in a full-thickness corneal graft with reduced sensations at day 0. (E) The defect had completely healed at week 4. (F) The epithelium was stable with no defect at week 8.

TABLE 1. Median and Interquartile Range of Cochet-Bonnet Esthesiometry Results

		NK Group			Friedman Test
Quadrant	Control Group	Baseline	4 Weeks	8 Weeks	*P* Value
ST	6.0 (5.6-6.0)	2.0 (0.5-6.0)	2.5 (1.5-4.1)	3.0 (1.6-5.5)	.422
SN	5.0 (4.7-5.2)	1.0 (1.0-5.0)	3.0 (2.0-5.7)	3.7 (2.6-5.5)	.028*
IT	5.3 (4.9-5.7)	0.5 (0-3.7)	2.5 (2.2-5.5)	3.0 (2.2-6.0)	.002*
IN	5.5 (4.7-6.0)	0.5 (0-2.0)	3.5 (2.0-5.1)	4.0 (3.1-6.0)	<.001*
Lesion		0 (0-1.5)	3.0 (1.7-5.5)	3.5 (2.2-6.0)	<.001*

IN = inferonasal; IT = inferotemporal; NK = neurotrophic keratopathy; SN = superonasal; ST = superotemporal.
Asterisk indicates significant increase in corneal sensation, observed at the site of the defect and in 3 of the 4 corneal quadrants examined.

affecting corneal sensations with preservation of sensation in the conjunctiva, as seen typically with corneal pathology such as herpesvirus keratitis; or affecting the entire ocular surface and skin of the face related to the distribution of the ophthalmic division of the trigeminal nerve, as seen in some cases following neurosurgery, tumors, or head injury. When trophic function is preserved, the epithelium shows mild disturbance without frank NK despite complete loss of sensation. In contrast, when both sensory and trophic functions are affected, NK results. Corneal nerves release neuromediators that preserve the health of the corneal epithelial cells and keratocytes. This "nutritional support" is referred to as the trophic function.[24] The cells in turn release growth factors like nerve growth factor

TABLE 2. Median and Interquartile Range of In Vivo Confocal Microscopy Results

		NK Group			
	Control Group	Baseline	4 Weeks	8 Weeks	Friedman Test *P* Value
Density (μm/mm^2)	5312.5 (4972.6-6139.9)	945.0 (413.7-1407.9)	2002.4 (1187.5-2790.7)	2500.5 (2106.6-3339.6)	.007*
Ramification (n)	14.5 (12.2-24.3)	2.5 (0-3.0)	5.0 (3.0-6.5)	6.0 (4.5-9.5)	.008*
Diameter (μm)	8.2 (6.2-8.6)	3.3 (2.8-3.7)	4.7 (3.4-4.9)	6.8 (4.4-8.2)	.007*

NK = neurotrophic keratopathy.

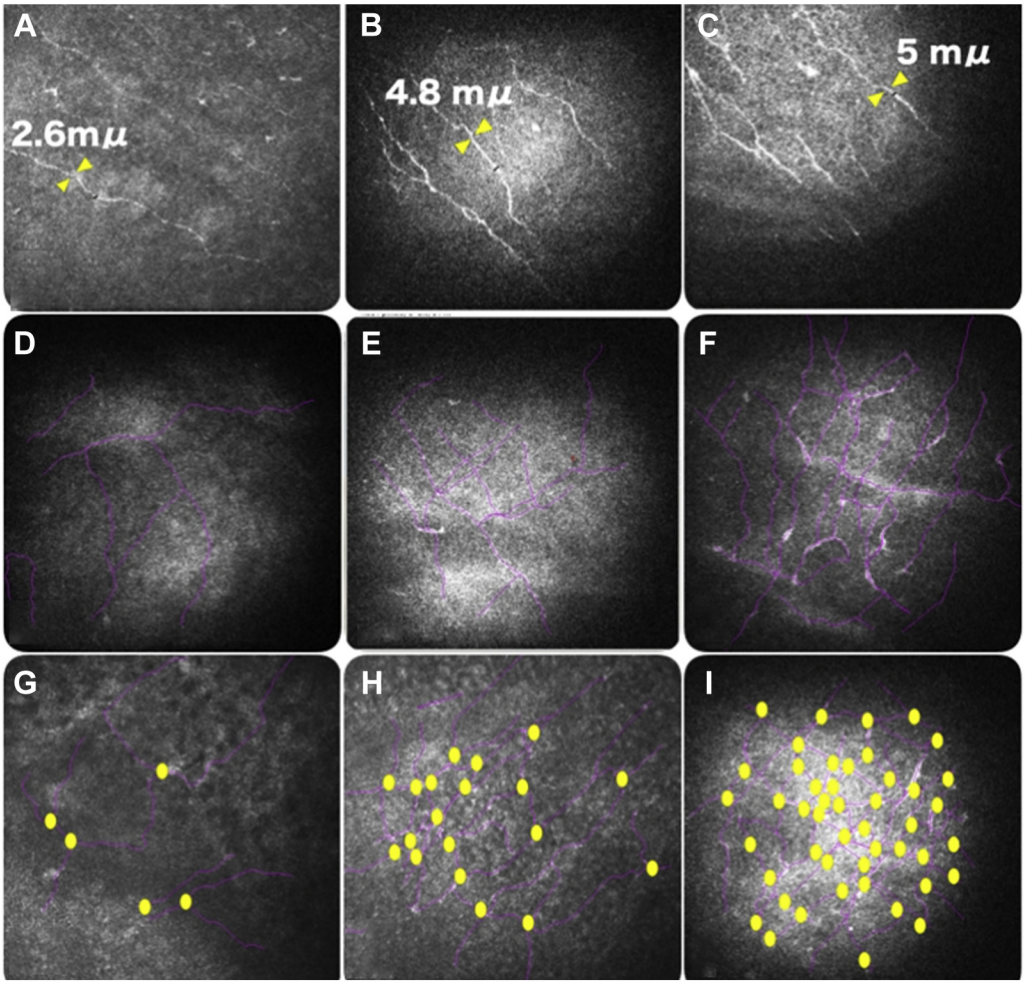

FIGURE 2. In vivo confocal microscopy analysis. (A) The nerve fiber diameter evaluated at day 0. (B) Nerve fiber thickness showed a significant increase at week 4 of treatment with recombinant human nerve growth factor. (C) The improvement was maintained at the 8-week follow-up period. (D-F) The nerve density was automatically traced and showed a progressive significant improvement from baseline (D) compared with week 4 (E) and week 8 (F). G-I. The number of nerve branches was manually counted. (G) Branch tracing at baseline. (H) Nerve branching at 4 weeks. (I) Nerve branching at 8 weeks, which was significantly more than at baseline.

and cytokines that support the health and regenerative turnover of the nerves. The nerves and cells are thus mutually supportive and conversely can induce a vicious cycle of damage when nerve damage causes epithelial loss and reduced nerve growth factor, which affects nerve health. There are several nonsurgical treatments for the

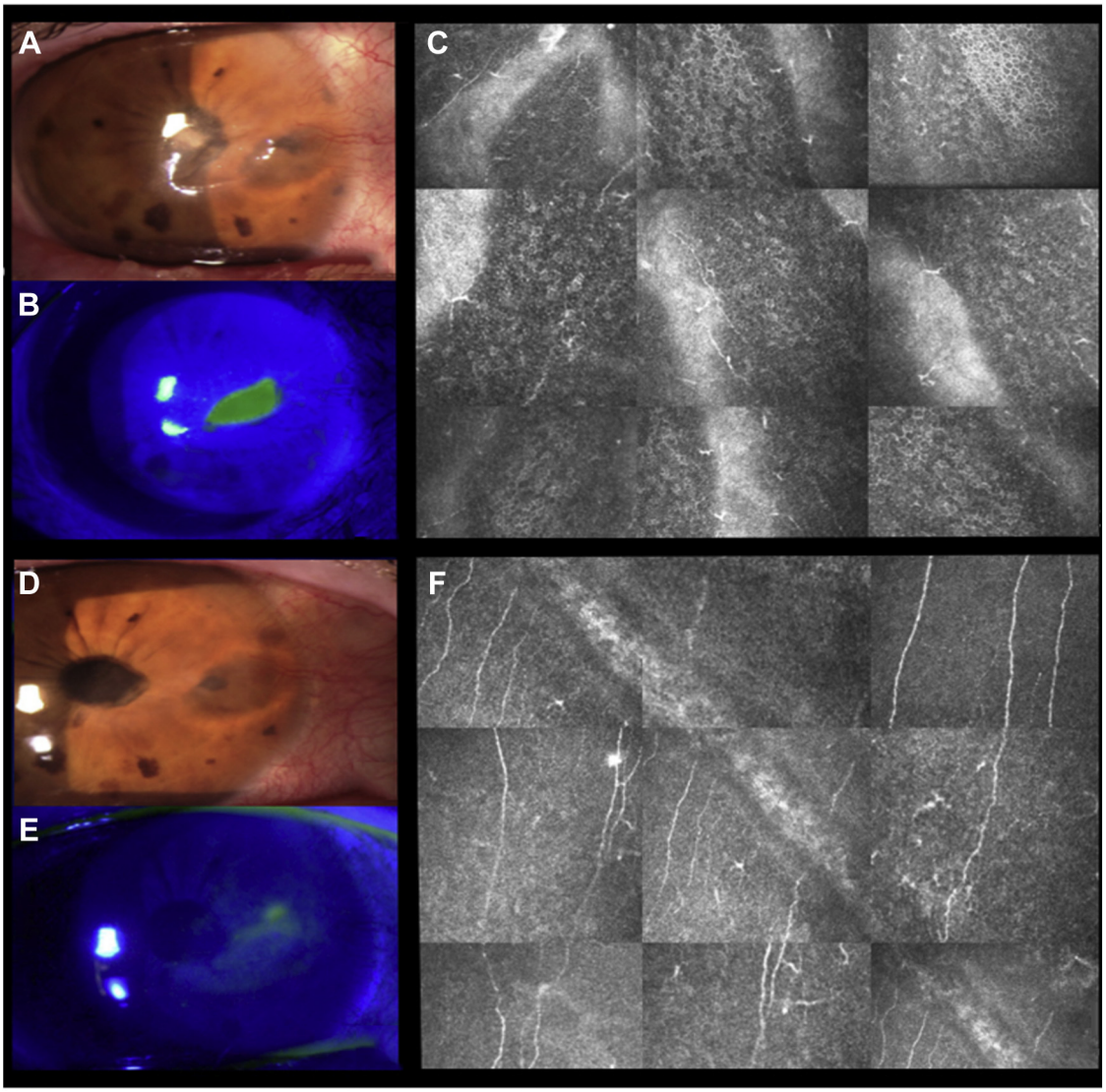

FIGURE 3. Representative case of neurotrophic corneal ulcer in patient affected by recurrent stromal herpetic keratitis. The lesion healed after 8 weeks with topical recombinant human nerve growth factor (rhNGF) treatment. (A,B) Nonhealing neurotrophic corneal ulcer in stromal recurrent herpes simplex virus at stage 3A according to Mastropasqua's classification[1]: persistent epithelial defect with stromal thinning < 50% of the total stromal thickness. (C) Confocal microscopy shows a severely reduced density of subbasal nerve fibers. (D-F) After 8 weeks of treatment with topical rhNGF a complete restoration of corneal epithelium was observed (D,E), along with an evident increase of subepithelial nerve fiber density (F).

management of neurotrophic keratopathy.[2] Most of these are aimed at promoting epithelial healing targeting the epithelial cells or the substrate without any direct effect on the corneal nerves, which are fundamental to the pathophysiology of NK. Several surgical interventions are also invoked for management of NK, the most popular of which are tarsorrhaphy and amniotic membrane transplantation.[2] These, like the nonsurgical approaches, do not directly address the root cause. Recently corneal neurotization was proposed as a surgical approach to obtain corneal reinnervation in neurotrophic keratopathy; with this technique a branch of healthy nerve is transposed directly or via a nerve graft, from the opposite unaffected side to the damaged side. This is a complex procedure with possible surgical complications, though it attempts to address the primary underlying pathology of nerve damage.[15,16]

Recombinant human NGF has emerged as the first medical intervention aimed at restoring nerve health in the treatment of NK by topical administration.[2] Our study reinforces this approach, providing evidence that rhNGF can restore corneal innervation both anatomically and functionally, as demonstrated by IVCM and Cochet-Bonnet esthesiometry. Furthermore, the increase in lacrimation is also indirect evidence of functional recovery. The success

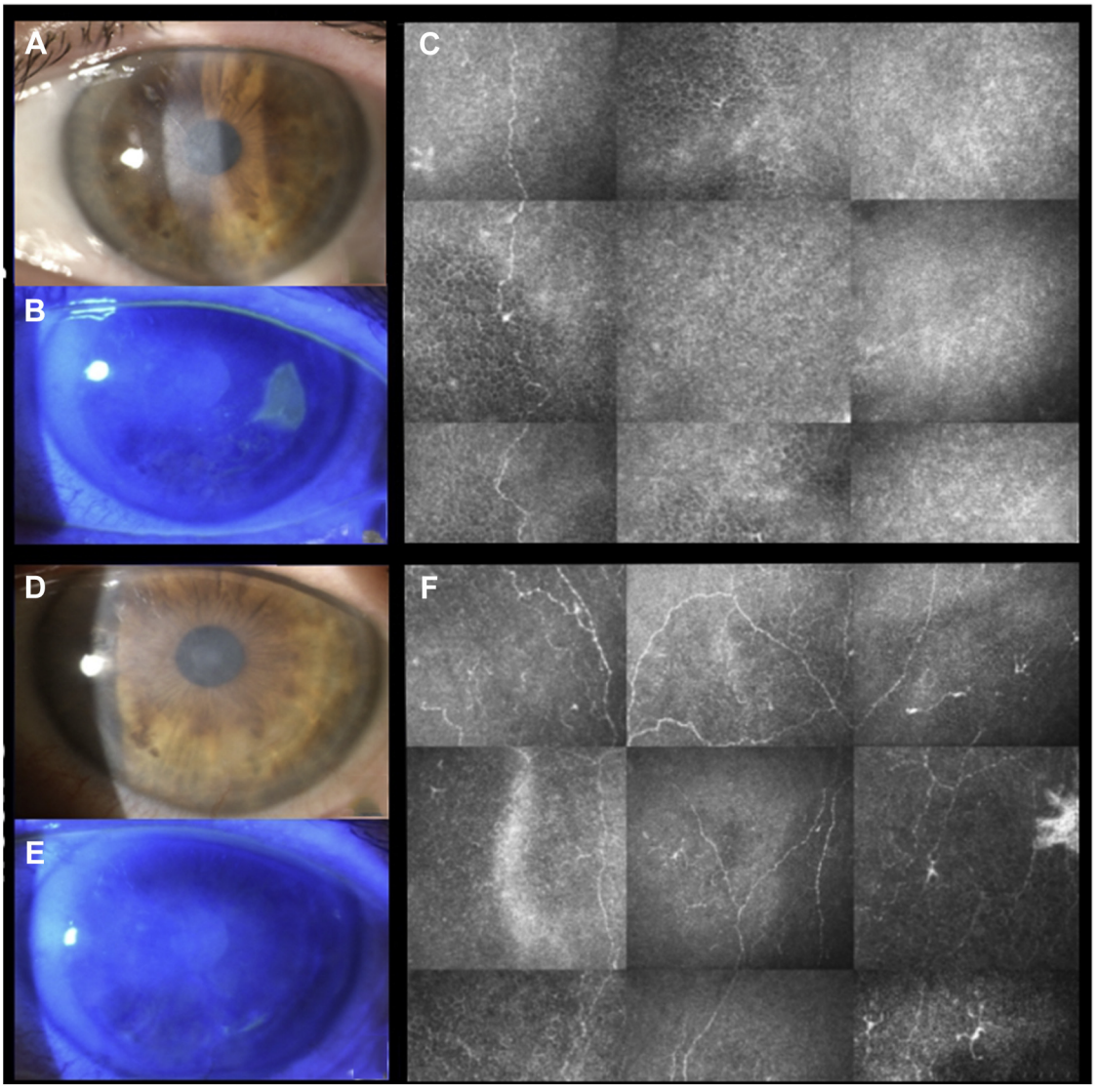

FIGURE 4. Representative case of neurotrophic corneal epithelial defect 2 years after lamellar keratoplasty performed for keratoconus that healed after 8 weeks of topical recombinant human nerve growth factor (rhNGF) treatment. (A,B) Nonhealing neurotrophic epithelial defect in lamellar graft at stage 2B according to Mastropasqua's classification[1]: persistent epithelial defect without stromal thinning and sub-basal nerve density < 5 μm/mm^2. (C) Confocal microscopy showed only 1 nerve fiber in the reconstructed frame. (D-F) After 8 weeks of treatment with topical rhNGF a complete resolution of the epithelial defect was obtained (D,E), along with a significant increase of subepithelial nerve plexus (F).

rate in our study, which included patients with moderate and severe stages of NK, was comparable to that reported in the prospective clinical trial that used identical concentration and dosage of recombinant human NGF eye drops.[17] Moreover, it provides evidence that the healing of corneal epithelial defect or ulceration is associated with corneal reinnervation in the early phases after topical treatment.

The results of the present study showed that corneal ulcer healing was associated with corneal sub-basal nerve regeneration, along with the evidence of improvement of the evaluated quantitative and qualitative nerve parameters (fiber density, branching, and diameter). These morphologic changes were observed as early as 4 weeks after treatment and continued to improve, although at a reduced rate, at the end of the treatment (8 weeks). However, the corneal neural density, ramification, and diameter of sub-basal fibers did not reach the values observed in the healthy control group at all the follow-up time points.

A limitation of the present study is represented by the fact that it was a prospective noncomparative case series; however it has to be considered that treated patients did not respond to conventional therapy for at least 4 weeks before being enrolled, and therefore most of the patients served as their own historic controls. It is conceivable

that the strong association of epithelial healing, objective demonstration of nerve regeneration, and subjective return of corneal sensations would suggest a cause-and-effect relationship between rhNGF and nerve regeneration.

Moreover it is possible that the severe stromal alterations and opacities reducing corneal transparency, in the cases with severe stages of NK, could prevent a clear visualization of eventual residual nerve fibers in the affected areas at the baseline examinations, although the reduced nerve fiber density and the correspondent increase after therapy were documented also in the corneal quadrants in which the stromal melting was not present. In a previous study, spontaneous nerve regeneration has been documented and measured via IVCM in postherpetic keratitis. It did not, however, show a correlation between anatomic increase in nerve fibers and improvement in corneal sensitivity.[25] Our data show that there is significant corneal reinnervation with topical rhNGF treatment, greater than that attributed to the spontaneous reinnervation rate described after herpes simplex virus keratitis,[25] therefore suggesting that the nerve fiber density increase observed is to be related to the treatment effect. Similarly to our observations, other studies reported that IVCM demonstrated a certain degree of corneal reinnervation at the stromal and sub-basal level, starting from 3 months after corneal neurotization.[26,27] In our study the pharmacologically induced corneal reinnervation was associated with a corresponding increase in corneal sensitivity. We observed a significant improvement in corneal sensation in the NK treatment group, in 3 out of 4 quadrants and in the area of epithelial defect, as compared to the pretreatment values. The improvement in corneal sensitivity is likely to be attributable to the increased regeneration of nerve fibers reaching a certain threshold at a much faster rate.

Although the conjunctival sensation has not been tested in this study, we can speculate that the improvement of tear film secretion has been observed because only 3 of the 18 patients enrolled had developed a total trigeminal damage owing to chemical trigeminolysis; in all the other cases conjunctiva and conjunctival sensation were not involved by the pathology causing NK, with consequent preservation of the reflex arc stimulating the lacrimal gland.

Mastropasqua and associates have recently reported that the density of nerve fibers is an important prognostic factor that influences the progression of NK from the mild to the more advanced stage.[1] Consequently we can speculate that topical treatment with rhNGF in a patient affected by NK at stage 1B of Mastropasqua's classification, with a subepithelial nerve fiber density ≤ 5 $\mu m/mm^2$, may regress to stage 1A, with a nerve fiber density ≥ 5 μm and lower risk to develop an epithelial defect; similarly, topical rhNGF treatment in case of moderate NK with low subepithelial nerve fiber density (stage 2B) may lead to the epithelial healing with increasing of subepithelial nerve fiber, with possible reduction of recurrence risk. To investigate and test this hypothesis, prospective randomized clinical trials with long follow-up are required. Long-term clinical observations of the patients treated with topical rhNGF in our center are in agreement with the results of Bonini and associates,[17] suggesting stability of the epithelial integrity over time after treatment; however, specific prospective studies using IVCM to compare the long-term corneal reinnervation effect with the clinical course and possible recurrences are needed to draw better conclusions.

Given the current state of knowledge of the pathogenesis of NK, its response to treatment with conventional medication, and latterly with rhNGF and the advent of corneal neurotization, it is possible to define a rational approach on management of NK. Regeneration of corneal nerves most likely occurs from residual surviving corneal nerves or from perilimbal and conjunctival nerves. Hence when corneal sensations are not completely lost or when the cornea is completely anesthetic, but some conjunctival sensations are preserved, rhNGF is likely to benefit. However, when the damage to the trigeminal nerve is more central, affecting sensations of the entire ocular surface (and facial skin), in theory there will be no nerves present from which reinnervation of the cornea can be induced. In such cases surgical neurotization may be the only option.[28] This surgical intervention should therefore be considered only when the clinical situation indicates complete absence of nerves or when treatment with rhNGF has proven ineffective.

Our study reports for the first time, by means of IVCM, an in vivo documentation of corneal reinnervation induced by rhNGF. Although we report a morphologic evaluation, unable to distinguish the trophic or sensorial function of the nerve fibers visualized at the subepithelial level, the increasing of corneal sensitivity and tear film production, together with the clinical healing of the epithelial ulcer, probably means that the regeneration of both trophic and sensitive fibers is induced by the topical treatment. In conclusion, our data show that IVCM represents an important device for the management of NK, allowing a direct and real-time evaluation of effectiveness of rhNGF treatment; the diffusion of this diagnostic imaging device will be necessary for a rational approach to the diagnosis and treatment of NK.

CRediT AUTHORSHIP CONTRIBUTION STATEMENT

LEONARDO MASTROPASQUA: SUPERVISION. **MANUELA Lanzini:** METHODOLOGY, WRITING - ORIGINAL DRAFT. **HARMINDER SING DUA:** WRITING - REVIEW & EDITING, SUPERVISION. **ALESSANDRO D' UFFIZI:** VALIDATION, INVESTIGATION. **MARTA DI NICOLA:** FORMAL ANALYSIS. **ROBERTA CALIENNO:** INVESTIGATION. **JESSICA BONDÌ:** DATA CURATION. **DALIA G. SAID:** INVESTIGATION, DATA CURATION. **MARIO NUBILE:** CONCEPTUALIZATION, PROJECT ADMINISTRATION, WRITING - REVIEW & EDITING.

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