Direct Versus Indirect Corneal Neurotization for the Treatment of Neurotrophic KeratopathyA Multicenter Prospective Comparative Study

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• PURPOSE: To analyze the comparative safety and efficacy of two techniques of corneal neurotization (CN) (direct corneal neurotization [DCN] vs indirect corneal neurotization [ICN]) for the treatment of neurotrophic keratopathy (NK).

• DESIGN: Multicenter interventional prospective comparative case series.

• METHODS: This study took place at ASST Santi Paolo e Carlo University Hospital, Milan; S.Orsola-Malpighi University Hospital, Bologna; and Santa Maria alle Scotte University Hospital, Siena, Italy. The study population consisted of consecutive patients with NK who underwent CN between November 2014 and October 2019. The intervention procedures included DCN, which was was performed by transferring contralateral supraorbital and supratrochlear nerves. ICN was performed using a sural nerve graft. The main outcome measures included NK healing, corneal sensitivity, corneal nerve fiber length (CNFL) measured by *in vivo* confocal microscopy (IVCM), and complication rates.

• RESULTS: A total of 26 eyes in 25 patients were included: 16 eyes were treated with DCN and 10 with ICN. After surgery, NK was healed in all patients after a mean period of 3.9 months without differences between DCN and ICN. Mean corneal sensitivity improved significantly 1 year after surgery (from 3.07 to 22.11 mm; $P <$ .001) without differences between the 2 groups. The corneal sub-basal nerve plexus that was absent before surgery in all patients, except 4, become detectable in all cases (mean CNFL: 14.67 ± 7.92 mm/mm^2 1 year postoperatively). No major complications were recorded in both groups.

• CONCLUSIONS: CN allowed the healing of NK in all patients as well as improvement of corneal sensitivity in most of them thanks to nerve regeneration documented by IVCM. One year postoperatively, DCN and ICN showed comparable outcomes. (Am J Ophthalmol 2020;220:203–214. © 2020 Elsevier Inc. All rights reserved.)

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CORNEAL SENSORY NERVES PLAY A KEY ROLE IN maintaining the anatomic integrity and function of the corneal epithelium. Their action is critical for blinking reflex, wound healing, and tear production.[1,2] The lack of the trophic effect provided by sensory nerves leads to impairment in corneal healing, with a broad spectrum of changes at the level of the ocular surface (known as neutrophic keratopathy [NK]), which ranges from superficial punctate keratopathy (stage I) to stromal melting with impending corneal perforation (stage III).[3,4] NK can be caused by several different ocular and systemic conditions, which share the common pathogenic mechanism of damage to the trigeminal nerve (fifth cranial nerve) at any level, from the nucleus to the corneal nerve terminations. The most common causes include herpetic keratitis, intracranial space-occupying lesions, and neurosurgical procedures. Other ocular causes are chemical and physical injuries, dry eye disease, diabetes, corneal surgery, and long-term use of topical medications.[5] The management of NK is based on a step-ladder approach according to the severity stage, and raises several challenges for ophthalmologists, especially in the presence of the most severe forms.[6] Medical therapy includes unpreserved tear substitutes at all stages of severity, as well as withdrawal of all preserved therapies in use. Novel topical treatments aimed at stimulating nerve regeneration include nerve growth factor, regenerating agents, and serum-derived products.[7–11]

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Keratoplasty and other surgeries are usually limited to complicated cases because the impaired wound healing, along with the frequent eyelid incompetence and decreased corneal reflex, strongly affect the chances of long-term success.[12]

Corneal neurotization (CN) has been recently introduced as a potentially curative surgical procedure in the setting of NK.[13] The technique consists of the transfer of normally functioning nerves obtained from a healthy district into the insensitive cornea. Two main surgical approaches have been described: the first involves the transposition of the contralateral or ipsilateral supraorbital/supratrochlear nerves to the anaesthetic cornea (direct corneal neurotization [DCN]).[13–20] The second involves the interposition of a nerve graft (mainly, the sural nerve) between the supraorbital and/or

supratrochlear nerves and the affected cornea (indirect corneal neurotization [ICN]).[21–28] Each technique offers specific advantages and disadvantages. On one hand, DCN mostly requires coronal incision and is therefore longer and more invasive; on the other hand, a higher axonal loss due to the end-to-side anastomosis and a non-negligible neural deficit because of sural nerve harvesting occur after ICN.[29,30]

To best of our knowledge, recent studies have described the clinical outcomes of patients with NK who underwent either DCN and ICN,[13–28] but a direct comparison between the 2 techniques to assess whether one surgical approach is superior over the other has not yet been performed. Therefore, the aim of this work was to analyze the comparative safety and efficacy of DCN and ICN for the treatment of patients with NK who are unresponsive to conventional treatment.

METHODS

• **STUDY AND PATIENTS:** This prospective comparative case series was conducted between November 2014 and October 2019 in 3 Italian tertiary cornea centers (ASST Santi Paolo e Carlo Hospital, University of Milan; S.Orsola-Malpighi Hospital, University of Bologna; Santa Maria alle Scotte Hospital, University of Siena, Italy). The study was approved by the local ethics committee of each center and adhered to the Declaration of Helsinki. Written informed consent was obtained from each patient before enrollment in the study. Consecutive patients with NK who attended the cornea service of the 3 centers were screened for enrollment. The inclusion criterion was the diagnosis of chronic NK (reduced or abolished corneal sensation measured by Cochet-Bonnet esthesiometer with a duration time from the onset of >3 months) because of nonhealed central nervous denervation despite conventional treatment. The classification system proposed by Mackie was used for the stratification of patients according to NK severity stage.[4] The exclusion criteria were presence of any active corneal disease other than NK and diagnosis of polyneuropathy or other types of disorder affecting the peripheral nervous system.

In the study protocol, CN (DCN vs ICN) was chosen according to patient's clinical characteristics and preferences. ICN was preferred in children (due to low invasiveness), in cases of bilateral NK (it is impossible to use contralateral nerves for DCN), and in patients who underwent previous craniotomy (repeated procedures may increase the risk of complications, like encephalitis). In all the other cases, DCN was chosen as first-line procedure due to the higher axonal loss secondary to the end-to-side anastomosis that occurs with ICN.[29,30] However, because DCN is more invasive and requires longer operating time compared with ICN, patients' preferences were also taken into account in the choice of surgical planning.

Both surgical procedures were performed under general anaesthesia by 1 multidisciplinary clinician from each center (F.B., D.R., P.F., M.D. in Milan; F.B., F.B., E.C., G.G. in Bologna; P.G., G.G., S.B., C.M. in Siena). Patients were visited by a team composed of ophthalmologists and maxillofacial surgeons before surgery and at 1 day, 1 week, 1, 3, 6, 9, and 12 months postoperatively, and thereafter once per year. Data obtained preoperatively (V0) and postoperatively at the 1-year follow-up visit (V1) were used for the main statistical analysis.

• **DCN:** This technique was performed as already described by our group.[14,19] Briefly, through a coronal incision at the vertex, the supratrochlear and supraorbital nerves were identified and carefully dissected under high magnification proximally to the supraorbital margin, up to at least 10 cm in length. Then, the dissected nerves were tunnelled over the nasal bridge through a small incision along the lid crease of the upper eyelid of the affected side. A Wright needle inserted through a tiny incision under the upper lid from the superior fornix was used to carefully retrieve 4 distal nerve branches in the subconjunctival plane. A tunnel was created under the conjunctiva around the circumference of the limbus using curved scissors to distribute the nerves in the cardinal points of planned insertion, where a scleral-corneal tunnel for each fascicle was made into the anterior corneal stroma to help nerve growth toward the center of the cornea. The nerves were then fixed in the desired position with fibrin glue, and the conjunctiva was repaired with 8-0 vicryl suture.

• **ICN:** This technique was described for the first time by Elbaz et al.[21] and later modified by us as described in the following. Briefly, dissection of donor supratrochlear and/or supraorbital nerves was performed through a 2-cm incision over the medial upper eyelid just inferior to the brow. This step was simultaneous to harvesting of the sural nerve graft, which was approximately 15 cm in length. The graft was reversed and tunnelled subcutaneously over the nasal

bridge through a small incision in the upper eyelid of the affected side and an end-to-end neurorrhaphy was performed. Distally, the nerve graft was tunneled subconjunctivally to the perilimbal area of the cornea using a Wright needle. Interfascicular dissection was performed to separate 4 nerve fascicles. The subsequent steps coincided with those previously described for DCN.

• **COMBINED AND STAGED SURGICAL PROCEDURES:** When required, CN was combined with other surgeries to address concomitant dysfunctions: lagophthalmos was treated by a 2-stage sural nerve graft in a cross-face manner, 2-3 mm lateral canthoplasty, and 2 ml lipofilling[31]; tear hyposecretion (Schirmer test <1 mm/5 min) was treated by parasympathetic neurotization of the lacrimal gland by a vertical cross-face sural nerve graft; and paralytic strabismus was treated with extraocular muscle surgery. In case of healing of the NK but persistence of corneal opacity that significantly impaired visual acuity, staged keratoplasty (penetrating keratoplasty or deep anterior lamellar keratoplasty [DALK]) was performed at least 1 year after CN.

• **OPHTHALMOLOGICAL EXAMINATION:** During each visit, patients underwent a detailed ophthalmological examination, including best-corrected visual acuity test (BCVA) (decimal fraction), slit-lamp examination, corneal fluorescein staining using a cobalt blue light and a 7503 Boston yellow filter, and slit-lamp photography. The area of corneal epithelial defect was calculated in mm^2 using ImageJ analysis software (National Institute of Health, Bethesda, Maryland, USA). Corneal healing was defined as <0.5 mm of fluorescein staining in the greatest dimension of the lesion area. The sensitivity of the cornea was evaluated using the Cochet-Bonnet esthesiometer (Luneau Ophtalmologie, Chartres, France), which consists of a 0.12-mm-diameter nylon filament with lengths ranging from 0 to 60 mm. Sensitivity was assessed by decreasing the lengths of the filaments in 5 mm steps until the patient felt the touch. If a positive answer was not detected, the fiber length was shortened in steps of 5 mm each, and the procedure was repeated. Three consecutive measurements were conducted in 5 different regions of the cornea (central, inferior, superior, nasal, and temporal). The maximum value of sensitivity recorded within the 5 areas for all patients at each visit was used for the analysis. During each esthesiometry evaluation, patients were also asked about the site of the perception of the corneal tactile stimulation.

• **NEUROPHYSIOLOGICAL EVALUATION:** The neurophysiological study was conducted with electromyography equipment (Neurosoft, Neuromep 2 channels EMG, version 2009, Ivanovo, Russia) to test the corneal reflex (or blink reflex). Evaluation was done in both eyes of each patient in chronological order, first in the healthy eye and then in the affected eye. The stimulation was performed using a specially manufactured electrode (cathode), with a sterile dressing on the tip, which was applied in the peripheral temporal cornea. The anode was positioned temporally on the orbital region, in the projection of the orbicularis oculi muscle. Electrical stimulation lasted 0.2 ms; the intensity of the stimulation was modulated for each patient on the basis of the sensory threshold of the healthy eye. Threshold and latency of the reflex were analyzed and compared between operated eyes and contralateral ones.

• **IN VIVO CONFOCAL MICROSCOPY:** *In vivo* confocal microscopy (IVCM) of the central cornea was performed using the Rostock Cornea Module of Heidelberg Retina Tomograph, as previously described.[32] The corneal sub-basal plexus (SNP) is located in subepithelial area, immediately at or posterior to the basal epithelial layer and anterior to the Bowman's layer, typically at a depth of 50 to 80 μm. The 3 most representative scans of the corneal SNP obtained in all patients before and after CN were selected based on technical quality and analyzed with "Neuron J". This is a semi-automated nerve-tracing plugin that can be freely downloaded from the public domain at https://imagescience.org/meijering/software/neuronj/.[33] The software was used for the calculation of the corneal nerve fiber length (CNFL) (mm/mm2).

• **STATISTICAL ANALYSIS:** SPSS statistical software version 22.0 (SPSS Inc, Chicago, Illinois) was used for data analysis. Values are expressed as mean ± SD. The Wilcoxon test was used to compare the continuous variables at V0 and V1 in overall patients and separately in the 2 groups. The $\chi 2$ test was used to compare the proportion of patients with NK in severity stages I, II, and III in the DCN and ICN groups. The Mann-Whitney U test was used to compare the changes in continuous variables between the DCN and ICN groups. Spearman's correlation analysis was used to evaluate the correlation between postoperative corneal sensitivity and corneal reflex measured by latency and threshold sensitivity. A P value <0.05 was considered statistically significant.

RESULTS

• **DEMOGRAPHIC AND BASELINE DATA:** Demographic and clinical characteristics of each patient included in the study are reported in Table 1. Overall, 26 eyes of 25 patients (5 men, 20 women; mean age 45.44 years) underwent CN and were followed for a mean period of 18.76 months. Twelve (48%) patients affected by NK were in Mackie stage III, 10 (40%) patients were in stage II, and 3 (12%) patients were in stage I.

Sixteen eyes (61.5%) were treated with DCN, whereas the remaining 10 eyes (38.5%) were treated with ICN.

TABLE 1. Demographic and Clinical Characteristics of Patients Included in the Study

Patient (No.)	Age (y), Sex	Eye	Etiology	Previous Treatment	Onset (mos Before Surgery)	Facial Palsy (Y/N)	Clinical Picture	NK Stage (Mackie)	Corneal Reflex (Y/N)	Corneal Neurotization Technique	Follow-Up (mos)
1	42, F	RE	AN	Facial reanimation	29	Y	Sequelae of corneal perforation with central leucoma and PED	III	N	Direct	49
2	25, M	RE	Brain AVM	Tarsorrhaphy	46	Y	Corneal neovascularization, nystagmus	III	Y	Direct	18
3	21, F	RE	Congenital V-VII cranial nerves atrophy	Tarsorrhaphy	252	Y	Central neovascular leucoma, PED	II	N	Direct	16
4	24, M	RE	Brain AVM		14	Y	Corneal ulcer with neovascularization, nystagmus	III	N	Direct	12
5*	19, F	LE	Cerebellar AVM	Lateral and medial rectus muscle recession in LE; tarsorrhaphy; facial reanimation	28 (first) 40 (second)	Y	Corneal ulcer, nystagmus	III	N	Direct (first) Indirect (second)	12
6	50, F	RE	AN	Facial reanimation	12	Y	PED	II	N	Direct	26
7	64, M	LE	AN	Tarsorrhaphy; facial reanimation	23	Y	PED	II	N	Direct	24
8	21, F	LE	Trigeminal neuroma		16	N	PED	II	Y	Direct	10
9	47, F	RE	AN	Tarsorrhaphy; facial reanimation	31	Y	Corneal ulcer	III	N	Indirect	20
10	35, F	RE	AN	Tarsorrhaphy; facial reanimation	34	Y	PED	II	N	Indirect	21
11	30, M	RE	AN	Tarsorrhaphy; facial reanimation	108	Y	Corneal neovascularization, PED	II	N	Indirect	16
12	27, F	RE	Cerebellar AVM	Medial rectus muscle recession in RE	48	Y	Corneal neovascularization, nystagmus, esotropia	II	N	Indirect	15
13	22, F	RE	Traumatic V,VI,VII,VIII cranial nerves palsy		240	Y	Corneal neovascularization	III	N	Direct	5
14	46, F	RE	AN	Tarsorrhaphy	48	Y	Corneal ulcer with central neovascular leucoma	III	N	Direct	24
15	68, F	RE	Condrosarcoma in pontocerebellar region	Tarsorrhaphy, facial reanimation	52	Y	Corneal ulcer with central neovascular leucoma	III	N	Direct	12

Continued on next page

TABLE 1. Demographic and Clinical Characteristics of Patients Included in the Study (*Continued*)

Patient (No.)	Age (y), Sex	Eye	Etiology	Previous Treatment	Onset (mos Before Surgery)	Facial Palsy (Y/N)	Clinical Picture	NK Stage (Mackie)	Corneal Reflex (Y/N)	Corneal Neurotization Technique	Follow-Up (mos)
16	60, F	RE	Meningioma of pontocerebellar angle	Upper eyelid gold weight, facial reanimation, strabismus surgery	40	Y	Corneal ulcer with active corneal neovascularization; large-angle esotropia	III	N	Direct	12
17	81, F	LE	Bell palsy + trigeminal palsy (unknown origin)	Tarsorrhaphy	48	Y	Corneal ulcer with central neovascular leucoma	III	N	Direct	12
18	37, M	LE	Clinoid meninigioma (II,V,IV cranial nerves palsy)		188	N	Keratitis	I	N	Indirect	6
19	73, F	RE	AN with V,VII,VIII cranial nerves palsy	Tarsorraphy	24	Y	Keratitis	II	N	Direct	48
20	42, F	LE	Post-traumatic Bell palsy + trigeminal palsy (unknown origin)	Tarsorraphy	20	Y	Keratitis	I	N	Indirect	12
21	64, F	RE	AN	Tarsorraphy	22	Y	Corneal ulcer with active corneal neovascularization	III	N	Direct	36
22	54, F	RE	Bell palsy + trigeminal palsy (unknown origin)	Tarsorraphy	22	Y	Keratitis	II	N	Direct	24
23	63, M	LE	Bell palsy + trigeminal palsy (unknown origin)	Tarsorraphy	24	Y	Keratitis	II	N	Indirect	18
24	57, M	LE	Prostatic bone methastatis	Tarsorraphy	18	Y	Keratitis	I	Y	Indirect	12
25	64, F	LE	AN		65	Y	Keratitis	I	N	Indirect	4

AN = acoustic neuroma; AVM = arteriovenous malformation; HM = hand movement; LE = left eye; PED = persistent epithelial defect; RE = right eye.
*Patient #5 underwent 2 surgeries, first, direct corneal neurotization and second, indirect corneal neurotization.

TABLE 2. Esthesiometry Data Obtained With Cochet-Bonnet Esthesiometer in All Five Corneal Regions

	V0			V1		
Eyes (n)	Central Value	Mean Value	Maximum Value	Central Value	Mean Value	Maximum Value
1	0	0	0	20	8	20 (C/S)
2	20	20	20 (C)	30	27.5	30 (C/S/T)
3	0	4	5 (S/I/N/T)	0	22	30 (S/N/T)
4	30	12	30 (C)	25	28	30 (I/N/T)
5	0	0	0	0	0	0
6	0	0	0	0	1.7	5 (T)
7	0	0	0	5	3	5 (C/S/I)
8	0	0	0	5	6	15 (N)
9	0	0	0	10	3	10 (C)
10	0	3	15 (N)	0	3.4	15 (S)
11	0	0	0	0	0	0
12	0	0	0	10	8	10 (C/S/I/N)
13	0	0	0	35	33	40 (S)
14	0	0	0	N/A	N/A	N/A
15	0	0	0	40	44	50
16	0	0	0	40	36	40
17	0	0	0	0	0	0
18	0	0	0	10	5	10
19	0	0	0	N/A	N/A	N/A
20	0	0	0	40	8	60 (T)
21	0	0	0	35	27.5	45 (N/C)
22	0	0	5	35	22	50 (S)
23	0	0	0	45	28	45 (C)
24	0	0	0	30	1.7	20 (T)
25	0	0	5	35	3	45 (S)
26	0	0	0	N/A	N/A	N/A

Corneal quadrant: C = central; I = inferior; N = nasal; S = superior; T = temporal.
Values are expressed in mm.

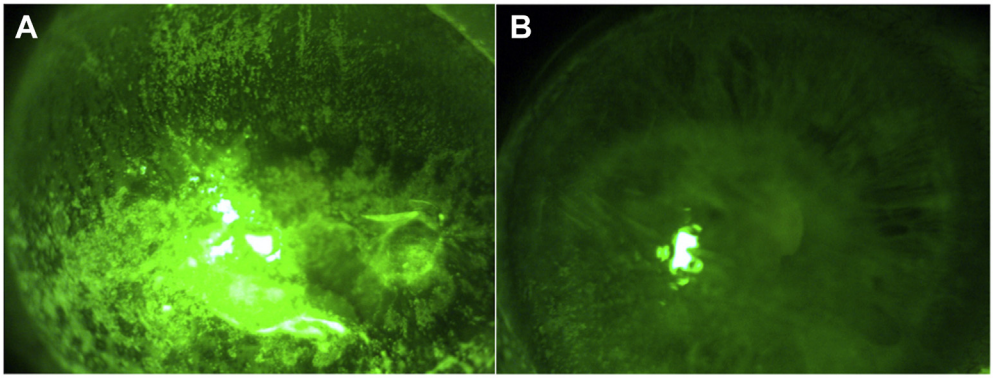

FIGURE 1. Representative slit-lamp photographs of the cornea (patient #16) before and after surgery. A. Before direct corneal neurotization (DCN), the clinical picture showed a neurotrophic keratopathy (NK) with a large epithelial defect. B. Three months after DCN, NK healed with a complete closure of the epithelial defect.

Patient #5 underwent 2 subsequent surgeries: DCN as the first procedure and ICN 1 year later. Values of corneal esthesiometry recorded at V0 for each patient, regardless of the type of surgery, are reported in Table 2. Before surgery, 20 eyes (77%) had complete corneal anaesthesia (esthesiometry null in all corneal regions).

TABLE 3. Esthesiometry Data According to the Type of Corneal Neurotization

Visit (mos)	DCN Group	ICN Group	Significance (*P* value)*
Baseline	4.0 ± 8.9 (0-30)	2.5 ± 5.3 (0-15)	.867
After 1	6.5 ± 11.6 (0-40)	5.0 ± 10.1 (0-20)	.785
After 3	15.2 ± 20.3 (0-60)	6.5 ± 7.5 (0-20)	**.042**
After 6	19.8 ± 17.1 (0-60)	9.3 ± 19.1 (0-40)	**.048**
After 9	23.0 ± 25.1 (0-60)	16.2 ± 22.9 (0-45)	.432
After 12	22.3 ± 20.4 (0-60)	17.5 ± 17.3 (0-45)	.579

Bold values denote statistical significance at the $P < 0.05$ level.
DCN = direct corneal neurotization; ICN = indirect corneal neurotization.
Values are expressed in mm as mean ± SD (range).
*Statistical significance of the difference between the 2 groups of the changes of corneal sensitivity values at each time point compared with baseline values.

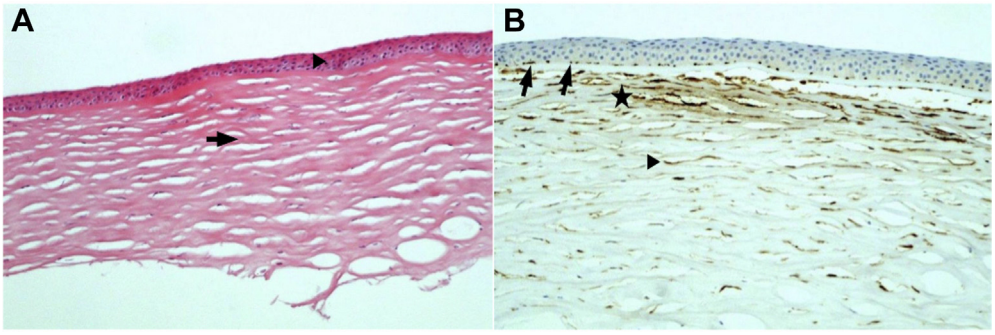

FIGURE 2. ***Ex vivo*** **histopathological examination of the neurotized corneal button (patient #15): hematoxylin eosin staining and protein gene product 9.5 immunostaining. A. Conventional histopathological staining with hematoxylin eosin of the anterior corneal lamella showed a normal layered epithelium (arrowhead) and stroma (arrow). B. Protein gene product 9.5 immunostaining of corneal nerve fibers (brown is positive staining), showed the sub-basal nerve plexus (arrows), the subepithelial nerve fibers (star), and the stromal nerves (arrowhead).**

Baseline characteristics did not differ between the DCN and ICN groups for age (45.9 ±21.4 years vs 42.1 ± 15.4 years), denervation time (57.2 ± 74.8 months vs 57.6 ± 53.1 months), corneal sensitivity (4.0 ± 8.9 mm vs 2.5 ± 5.3 mm), and area of the epithelial defect (24.9 ± 20.1 mm vs 12.4 ± 12.2 mm2) (all $P > .133$). Conversely, baseline values of BCVA and NK severity stage differed significantly between the 2 groups. In particular, decimal BCVA was significantly lower in the DCN group (0.19 ± 0.23 vs 0.42 ± 0.28; $P = .044$), whereas NK severity stage was significantly higher in the DCN group (0% vs 40% for stage I, 31.25% vs 40% for stage II, and 68.75% vs 20% for stage III; $P = .009$).

• **EFFICACY DATA:** After surgery, NK healed in all patients after a mean period of 3.9 ± 1.5 months (range: 2-6 months) (healing rate 100%). Furthermore, healing was maintained throughout the entire follow-up in all cases. Slit-lamp photographs taken before and 3 months after CN in a representative case (patient #16) are shown in Figure 1.

Overall, the area of the epithelial defect significantly decreased from V0 to V1 (from 19.70 ± 18.10 to 0.11 ± 0.13 mm2; $P < .001$). When the DCN and ICN groups were analyzed separately, this statistical significance was confirmed in both groups (from 24.90 ± 20.10 to 0.12 ± 0.14 mm2; $P = .001$ and from 12.40 ± 12.20 to 0.10 ± 0.13 mm2; $P = .006$, respectively). The postoperative decrease of the area of the epithelial defect did not significantly differ between the 2 groups (24.81 ± 20.08 mm2 for DCN vs 12.31 ± 12.09 mm2 for ICN; $P = .120$). No significant differences in the healing time were registered between patients who underwent DCN versus patients who underwent ICN (3.3 ± 1.4 months vs 4.1 ± 2.0 months; $P = .856$).

One year after CN, corneal sensitivity improved in 12/15 patients (80%) of the DCN group and in 5/6 patients (83.3%) of the ICN group. Overall, mean corneal sensitivity improved significantly 1 year after CN (from 3.07 at V0 to 22.11 mm at V1; $P < .001$). Table 3 shows a comparison of mean corneal sensitivity according to the type of surgery. When separately analyzing the DCN and ICN

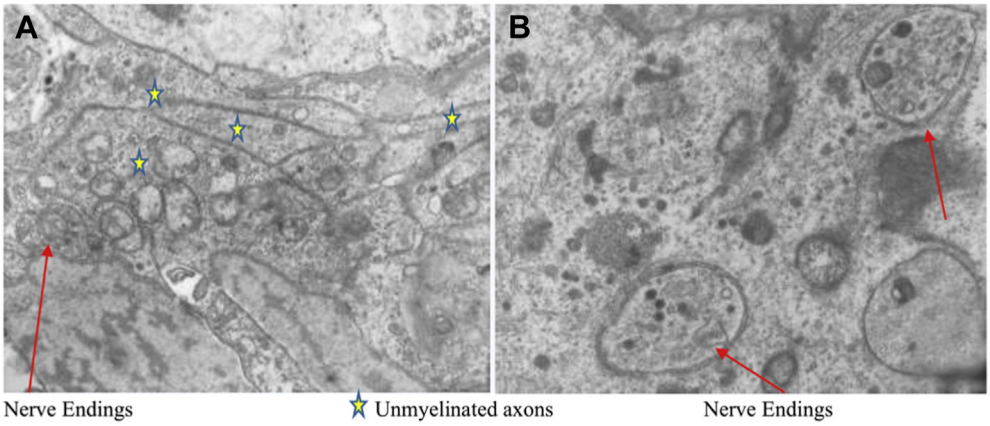

FIGURE 3. Transmission electron microscopy (TEM) of the neurotized corneal button (patient #15). A-B. TEM Images (Hitachi H-300, Hitachi, Ltd, Tokyo, Japan) of ultrathin (60 nm) sections of the neurotized corneal button, showing unmyelinated axons and nerve endings with normal ultrastructure.

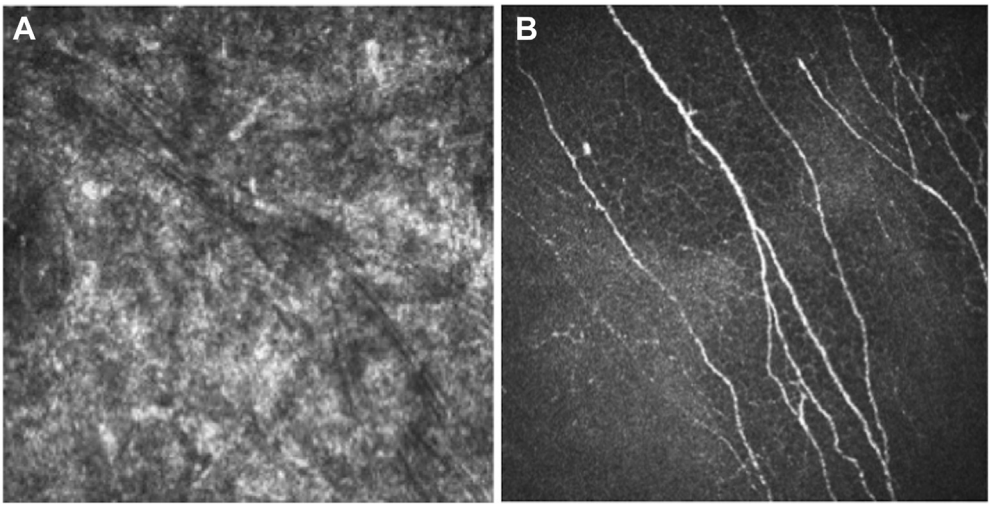

FIGURE 4. Representative *in vivo* confocal microscopy images obtained at the level of the corneal sub-basal nerve plexus (SNP) (patient #16). A. Before direct corneal neurotization (DCN), the corneal SNP was not detectable. B. One year after DCN, the regenerated corneal SNP exhibited a near-normal morphology. All images are on the scale of 400 × 400 mm.

groups, this statistical significance was confirmed in both groups (from 4.0 ± 8.9 to 26.3 ± 19.6 mm; $P = .036$ and from 2.5 ± 5.4 to 22.5 ± 18.3 mm; $P = .002$, respectively). However, although the changes of corneal sensitivity from baseline values were significantly higher in the DCN group compared with the ICN group in the intermediate time points of 3 and 6 months postoperatively, the difference did not reach statistical significance at V1 (17.5 ± 17.3 for ICN vs 22.3 ± 20.4 mm for DCN; $P = .579$). At the last follow-up visit, 13/16 (81.2%) patients in the DCN group and 6/9 (66.7%) patients in the ICN group had positive corneal reflex measured in a subjective fashion. All the patients without corneal reflex had an absent corneal sensitivity.

Overall, decimal BCVA improved significantly 1 year after CN (from 0.29 ± 0.27 to 0.46 ± 0.28; $P < .001$). When analyzed separately in the DCN and ICN groups, BCVA values improved significantly in the former group (from 0.19 ± 0.23 to 0.45 ± 0.30; $P = .004$) but not in the latter group (from 0.42 ± 0.28 to 0.48 ± 0.27; $P = .054$). However, the postoperative improvement of decimal BCVA did not significantly differ in the DCN group compared with the ICN group (0.25 ± 0.33 vs 0.06 ± 0.07; $P = 0.089$).

In 4 patients, the presence of corneal opacity after healing of NK significantly impaired visual acuity and required staged corneal transplantation (penetrating keratoplasty in 3 patients and DALK in 1 patient). In the case who

underwent DALK 18 months after DCN (patient #15), the corneal button excised at the time of transplantation was analyzed *ex vivo* using hematoxylin and eosin staining, protein gene product (PGP) 9.5 immuno-staining, and transmission electronic microscopy (TEM). The hematoxylin and eosin staining confirmed that epithelium, Bowman's layer, and anterior portion of the stroma showed normal features (Figure 2A); the protein gene product 9.5 staining confirmed the presence of nervous fibers either in the sub-epithelial space and in the stroma (Figure 2B). TEM allowed visualization of unmyelinated nerve axons and nerve endings with a normal ultrastructure (Figure 3). Further data from the *ex vivo* analysis of the neurotized corneal button were reported in a previous paper.[14]

At 1 year, neurophysiological examination showed a partial recovery of the electrical activity of the neurotized cornea in terms of both latency and threshold sensitivity (respectively, 50.2 ± 4.87 ms in the operated eye vs 35.5 ± 3.31 ms in the contralateral eye and 8.9 ± 6.02 mA in the operated eye vs 2.3 ± 0.84 mA in the contralateral eye). No significant correlation was found between postoperative values of corneal sensitivity and corneal reflex parameters (Rs = −0.414, P = .206 for threshold sensitivity; Rs = 0.109, P = .780 for latency).

• **IVCM FINDINGS:** Corneal SNP was not detectable before surgery in all patients except 4, in whom few thin nerves were visible in the sub-epithelial layer. Mean CNFL was 1.8 ± 0.15 mm/mm2 (range: 1.59-1.95 mm/mm2). In all patients, new nerve fibers appeared as soon as 3 months postoperatively, progressively forming a regenerated corneal SNP that reached near-normal features 1 year postoperatively. At V1, corneal SNP was detectable in all patients and the mean value of CNFL was 14.67 ± 7.92 mm/mm2 (range: 2.69-32.70 mm/mm2). The change in CNFL from V0 to V1 did not differ significantly between the 2 groups (P = .833). Representative IVCM images obtained at the level of the corneal SNP for patient #16 are shown in Figure 4.

• **SAFETY DATA:** CN was completed in all cases without major complications. Adequate nerve isolation was possible in all patients except patient #5, whose branches of supraorbital and supratrochlear nerves, which were isolated during DCN, were very thin and short. This patient required a repeated surgery. In the immediate postoperative period, all patients who underwent DCN had transient, mild face edema, including in the eyelid; surgical drainage was maintained for the first 2 postoperative days. All patients who underwent ICN had edema of the upper third of the face, whereas no major complications occurred at the site of harvesting of the sural nerve. All patients reported partial numbness of the frontal region on the harvesting side immediately after surgery. This deficit of sensitivity gradually reduced in size and intensity within the first postoperative year. A typical side effect experienced by all patients who regained corneal sensitivity was the misperception of the corneal tactile stimulation in the contralateral forehead. This complication occurred in the first 3-6 postoperative months, regardless the technique employed. Then, the sensation shifted from the forehead to the cornea about 6-9 months after surgery. This phenomenon reveals the adaptation changes that occur due to the cerebral plasticity.

DISCUSSION

THE PRESENT PAPER REPORTS THE RESULTS OF CN FOR THE treatment of patients with NK who did not respond to conventional medications. To the best of our knowledge, our case series is the largest available in the literature and represents the first attempt at comparing the 2 most commonly used techniques of CN. NK is the clinical consequence of several conditions of genetic, systemic or ocular origin that result in epithelial erosion and defects, which in most severe cases may proceed to ulceration, stromal melting, and perforation. Until recently, conventional medical treatment was palliative and mainly based on lubrication and protection of the ocular surface. The recent welcomed advent of recombinant human nerve growth factor eye drops (Cenegermin, Dompé Farmaceutici, Milan, Italy) with proven efficacy in clinical trials and specific target on the root pathology has determined a paradigm shift in medical management of NK.7-9 In our current practice, we routinely use Cenegermin for NK cases secondary to peripheral and/lor local diseases (eg, postherpetic, dry eye, postsurgical). However, NK recurrence following Cenegermin treatment was reported in some cases, and this issue requires further long-term data.[34]

In the present study, all of the patients presented with NK because of central nervous denervation, and most of them (all except 4) had complete damage to the trigeminal ganglion, as well-characterized by Dhillon et al. in a previous work.[35] Therefore, we decided to proceed with CN, which offered the chance to restore nerve function, even if there has been an irreparable damage to the original location of innervation. Furthermore, the date of initiation of this prospective study (November 2014) was before the approval of Cenegermin in the European Union (July 2017).

Since the first report from Terzis et al. dated about 10 years ago,13 different techniques and refinements have been proposed for the surgical re-innervation of the insensate cornea based on either the transfer of contralateral or ipsilateral supratrochlear and supraorbital nerves (DCN) and the use of an interpositional graft (sural, great auricular or lateral antebrachial cutaneous nerves) as a connection to the anaesthetic cornea (ICN). All approaches have proved clinically efficacious in terms of both improvement of corneal sensitivity and NK healing, but it is unclear

whether one of these is more reliable and effective than other procedures.[36]

In our study, we prospectively compared the 2 most used techniques: DCN with the transfer of the contralateral supraorbital and/or supratrochlear nerves, and ICN with the interpositional use of a sural nerve graft. A randomized design was not applicable because the2 techniques are not fully interchangeable. For instance, DCN is not feasible in cases with bilateral impairment of ophthalmic division of the trigeminal nerve.

In our study, the clinical efficacy of CN was demonstrated by the improved sensitive and trophic function of corneal nerves that allowed the healing of NK in all cases, which was then maintained during the entire follow-up. In most patients, the regained corneal sensation was also sufficient to initiate the blinking reflex. In parallel, IVCM showed the regeneration of corneal nerves that acquired near-normal morphology 1 year after surgery. However, despite IVCM, metrics of neurotized corneas did not reach normative reference values of a healthy cornea,[37] and corneal sensitivity remained absent after surgery in a few patients (n = 3). The regenerated nervous plexus had a trophic function sufficient to heal NK and to maintain epithelial integrity in all cases over time. Currently, there is a debate about the exact mechanism of action of CN. Some authors have hypothesized that transferred nerves grow progressively towards the central cornea and regenerate a new nervous plexus.13,21 Others have speculated that the improvement following CN is related to the paracrine action of the transferred nerve fascicles thanks to the release of neurotrophic factors that assist healing by stimulating preexisting corneal nerves.15 In our study, the *ex vivo* analysis of the neurotized corneal button excised at the time of staged DALK confirmed the presence of nerve fibers with normal ultrastructure. Because the continuity between perilimbal transferred nerves and graft nerve fibers could not be ascertained by our analysis, we can neither confirm nor deny these hypotheses. However, a recent animal model of CN confirmed the nerve growth through the graft and into the neurotrophic cornea thanks to retrograde labeling.[38]

The goal of NK treatment is not only the healing of the keratopathy but also the restoration of the ocular surface homeostasis necessary for the success of staged corneal surgery when visual rehabilitation is further required. In our study, all the cases who underwent keratoplasty after CN (n = 4) had successful outcomes with clear and epithelialized corneal grafts.

The comparative analysis between the 2 techniques suggested that DCN might guarantee higher corneal sensitivity compared with ICN at early postoperative time points (3-6 months). This was an expected finding considering that ICN implied a nerve anastomosis, and it is known that axons progressively populate distal to a neurorrhaphy by about one-half centimeter per month.[39] However, this difference did not reach statistical significance 1 year after CN. Furthermore, unlike the ICN group, the DCN group showed significant improvement of BCVA after surgery. However, this significance should be interpreted with caution because both groups differed significantly for baseline BCVA, and the postoperative improvement of visual acuity did not significantly change in the 2 groups.

Various factors could have influenced this comparison, hampering the detection of significant differences. First, unlike the conventional approach that involves an "end-to-side" neurorrhaphy,21,28 we performed an "end-to-end" neurorrhaphy in all ICN cases between the supraorbital/supratrochlear nerves and sural nerve graft to obtain a higher number of growing axons, as demonstrated in another model.[40] However, other variables might have also influenced the regenerative potential of the rerouted nerves, such as NK severity and combined surgical procedures.

In conclusion, our results confirm that CN is a safe and effective procedure for NK, regardless the type of surgical technique used. The data of the comparative analysis between DCN and ICN did not provide conclusive evidence about the technique of choice, likely due to the relatively small sample size and the inhomogeneous baseline characteristics of the patients (main limitations of the study).

The recent preliminary results about minimally invasive DCN being feasible by a single surgeon through an upper eyelid crease incision using either a combination of endoscopic and direct visualization or direct visualization alone are promising but need more robust evidence.16 Another less invasive approach for DCN that does not require coronal incision has been recently described by our group in cases of isolated damage of the ophthalmic branch and uses the direct transfer of the second division of trigeminal nerve.[41]

In the near future, a deeper comprehension of the mechanisms underlying the effects of CN will derive from the evaluation of tear expression of cytokines and growth factors after each CN procedure; currently, this analysis is ongoing at our centers. It is also reasonable to hypothesize that CN may further benefit from the adjuvant use of nerve growth factor eye drops that could synergistically improve postoperative nerve regeneration.

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