Elsevier Editorial System(tm) for American Journal of Ophthalmology

Manuscript Draft

Manuscript Number: AJO-20-478

Title: Direct Versus Indirect Corneal Neurotization for the Treatment of Neurotrophic Keratitis: a Multicenter Prospective Comparative Study

Article Type: Original Article

Keywords: Corneal neurotization; neurotrophic keratitis; corneal ulcer; direct neurotization; indirect neurotization; novel technique

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Abstract (MUST be submitted as a separate file)

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

DESIGN: Multicenter Interventional Prospective Comparative Study.

METHODS: Setting: ASST Santi Paolo e Carlo University Hospital, Milan; S.Orsola-Malpighi University Hospital, Bologna; Santa Maria alle Scotte University Hospital, Siena. *Study Population:* Consecutive patients with NK undergoing corneal neurotization between November 2014 and October 2019; Intervention Procedures: DCN was performed by transferring contralateral supraorbital and supratrochlear nerves; ICN was performed using sural nerve graft. Main Outcome Measures**:** NK healing rate; corneal sensitivity; corneal nerve fiber length (CNFL) measured by in vivo confocal microscopy (IVCM); complication rate.

RESULTS: 26 eyes of 25 patients were included: 16 were treated with DCN and 10 with ICN. After surgery, NK healed in all patients after a mean period of 3.9±1.5 months without differences between patients undergone DCN and ICN. Overall, mean corneal sensitivity improved significantly 1 year after surgery (from 3.07 to 22.11 mm; $p<0.001$) without differences between the two groups. Corneal sub-basal nerve plexus that was absent before surgery in all patients except 3 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: Corneal neurotization allowed the healing of NK and the improvement of corneal sensitivity in all patients thanks to nerve regeneration documented by IVCM. One year postoperatively, DCN and ICN showed comparable outcomes.

Cover Letter

Giuseppe Giannaccare, MD, PhD, FEBOphth
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Catanzaro, March 19th 2020

Dear Richard K Parrish II, MD
Editor in Chief
American Journal of Ophthalmology

Attached please find the electronic version of the manuscript **"Direct Versus Indirect Corneal Neurotization for the Treatment of Neurotrophic Keratitis: a Multicenter Prospective Comparative Study"** by myself and collaborators, which is being submitted for consideration for publication in American Journal of Ophthalmology as "Original Article".
To the best of our knowledge, this study is the largest available in the literature and represents the first attempt to compare the two most-commonly techniques of corneal neurotization, named direct corneal neurotization with the transfer of contralateral supraorbital/supratrochlear nerves and indirect corneal neurotization with sural nerve graft. The data were collected in three Italian 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).
The material represents an original research, has not been previously published and has not been submitted for publication elsewhere while under consideration. I had full access to all the data in the study, and take responsibility for the integrity of the data and the accuracy of the data analysis as well as the decision to submit for publication.
All the authors declare no conflict of interest.

Sincerely,
Giuseppe Giannaccare

Direct Versus Indirect Corneal Neurotization for the Treatment of Neurotrophic Keratitis: a Multicenter Prospective Comparative Study

American Journal of Ophthalmology - Original Article

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Short Title: Direct vs Indirect Corneal Neurotization

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INTRODUCTION. 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 on the ocular surface known as neutrophic keratitis (NK) ranging from superficial punctate keratopathy (stage I) to stromal melting with impending corneal perforation (stage III).[3,4] Neurotrophic keratitis can be caused by several different ocular and systemic diseases, which share the common pathogenic mechanism of the damage of 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, corneal surgery and the chronic use of topical medications.[5] The management of NK is based on a step-ladder approach according to the stage, and raises several challenges for Ophthalmologist, especially in the presence of most severe forms.[6] Medical therapy includes unpreserved tear substitutes at all stages of severity as well as the withdrawn of all preserved therapies in use. Novel topical treatments aiming at stimulating nerve regeneration include nerve growth factor (NGF), regenerating agents and serum-derived products.[7-10] Keratoplasty as well as other surgeries are usually limited to complicated cases since the impaired wound healing along with the frequent eyelid incompetence and the decreased corneal reflex strongly affect their chances of success.[11]

Corneal neurotization (CN) has been recently introduced as a potentially curative surgical procedure in this setting of NK.[12] The technique consists of the transfer of nerve terminations obtained from a healthy district into the insensitive cornea. Two main surgical procedures have been described: the first one involves the transposition of the contralateral or ipsilateral supraorbital/supratrochlear nerves to the anaesthetic cornea ("direct" corneal neurotization, DCN);[12-19] the second one involves the interposition of a nerve graft (mainly sural nerve) between the supraorbital/supratrochlear nerves and the affected cornea ("indirect" corneal neurotization, ICN).[20-27] Each technique offers specific advantages and disadvantages: on one side, DCN mostly requires coronal incision and is therefore longer and more invasive compared to ICN; on the other side, a higher axonal loss likely occurs during ICN due to the end-to-side anastomosis as well as a non-negligible neural deficit is added due to sural nerve harvesting.[28,29]

To best of our knowledge, various recent studies have described the clinical outcomes of patients with NK undergone either DCN and ICN,[12-27] but the direct comparison between the two techniques to assess whether one surgical approach is superior to the other has not yet been performed. Therefore, the aim of this work was to analyze the comparative safety and efficacy of two techniques of corneal CN (namely, DCN and ICN) for the treatment of patients with NK unresponsive to conventional treatment.

METHODS

Study and Patients

This prospective comparative study was conducted between November 2014 and Octo-

ber 2019 in three 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). The study was approved by the local Ethics Committees and adhered to the Helsinki declaration. Written informed consent was obtained from each patient before the enrolment in the study. Consecutive patients with NK who attended the Cornea Service of the three Centers were screened for enrolment. The inclusion criterion was the diagnosis of chronic NK (duration time from the onset > 3 months) owing to central nervous denervation not healed despite conventional treatment. The exclusion criteria were: presence of any active corneal disease other than NK; diagnosis of polyneuropathy or other types of disorder affecting the peripheral nervous system.

In the study protocol, the technique of 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 (impossibility to use contralateral nerves as for DCN), and in patients who underwent previous craniotomy (repeated procedure 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 occurring with ICN.[28,29] However, since DCN is more invasive and requires longer operating time compared to ICN, patients' preferences were also taken into account in the choice of the surgical planning.

Both the surgical procedures were performed under general anaesthesia by one multidisciplinary Equipe for each Center (FB, DR, PF, MD in Milan; FB, FB, EC, GG in Bologna; PG, GG, SB, CM in Siena). Patients were visited by a team composed of Ophthalmologists and Maxillofacial Surgeons before surgery and 1 day, 1 week, 1, 3, 6, 9, 12 months postoperatively, thereafter once per year. Data obtained preoperatively (V0) and postoperatively at 1 year follow-up visit (V1) were used for the main statistical analysis.

Direct Corneal Neurotization

This technique was performed as already described by our Group.[13,18] 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 tunneled 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 retrieved four distal nerve branches in the subconjunctival plane. A tunnel was created under the conjunctiva around the circumference of the limbus using curved scissors in order 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 towards 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.

Indirect Corneal Neutotization

This technique was described for the first time by Elbaz and collaborators,[20] and later

modified by us as described below. Briefly, dissection of donor supratrochlear and/or supraorbital nerves were carried out 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 of 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 neurorraphy 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 that ones described above for DCN.

Combined and Staged Surgical Procedures

When required, CN was combined with other surgeries in order to address concomitant dysfunctions: lagophthalmos was treated by a two-stage sural nerve grafting in a cross-face manner, 2-3 mm lateral canthoplasty and 2 ml lipofilling;[30] tear hyposecretion (Schirmer test < 1 mm/5') by parasympathetic neurotization of the lacrimal gland by a vertical cross-face sural nerve graft; paralytic strabismus with extraocular muscle surgery. In case of healing of the NK but persistence of corneal opacity impairing significantly visual acuity, staged keratoplasty (penetrating keratoplasty [PK] or deep anterior lamellar keratoplasty [DALK]) was performed at least one year after CN.

Corneal Esthesiometry

The sensitivity of the cornea was evaluated using the Cochet-Bonnet esthesiometer (Luneau Ophtalmologie, Chartres, France) which is composed of a 0.12 mm-diameter nylon filament with lengths ranging from 0 to 60 mm. The sensitivity was assessed decreasing the filaments' length of 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.

Neurophysiological Evaluation

The neurophysiological study was conducted with an electromyography equipment (Neurosoft, Neuromep 2 channels EMG, version 2009, Ivanovo, Russia) in order to test the corneal reflex (blink reflex). Evaluation was done in both eyes of each patient in the following chronological order: first in the healthy eye and then in the affected eye. The stimulation was carried out by means of a specially manufactured electrode (cathode), with a sterile dressing on the tip, 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 had a duration of 0.2 milliseconds; the intensity of the stimulation was modulated for each patient on the basis of sensory threshold of the healthy eye.

In Vivo Confocal Microscopy

In vivo confocal microscopy (IVCM) of the central cornea was performed using Rostock Cornea Module of Heidelberg Retina Tomograph, as previously described.[31]

The corneal sub-basal plexus (SNP) is located in supepithelial 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. Three 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 http://www.imagescience.org/meijering /software/neuronj/.meijering.[32] The software was used for the calculation of the corneal nerve fiber length (CNFL) (mm/mm^2).

Statistical Analysis

SPSS statistical software (SPSS Inc, Chicago, IL) was used for data analysis. Values are expressed as mean ± standard deviation. The Wilcoxon test was used to compare the continuous variables at V0 and V1 in both groups. The Mann-Whitney U test was used to compare the changes in continuous variables between DCN group and ICN group. A $P < 0.05$ 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 males, 20 females; mean age 45.44 years) underwent CN in one of the three study Centers and were followed for a mean period of 18.76 month. Sixteen eyes (61.5% of the total) were treated with DCN while the remaining 10 eyes (38.5%) with ICN. Patient #5 underwent two sequential surgeries: DCN as first procedure and 1 year later ICN. Twelve patients (48%) were affected by NK belonged to stage III, 10 (40%) to stage II and 3 (12%) to stage I.
Values of corneal esthesiometry recorded for each patient regardless the type of surgery at V0 and V1 are reported in Table 2. Before surgery, 20 eyes (77%) had complete corneal anaesthesia (esthesiometry null in all corneal regions).

Efficacy Data

After surgery, NK healed in all patients after a mean period of 3.9 ± 1.5 months (range 2 to 6 months) and the healing was maintained throughout the entire follow-up in all cases. No significant differences in the healing time were registered between patients undergone DCN versus ICN (respectively, 3.3 ± 1.4 months vs 4.1 ± 2.0; $P = 0.856$). 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; $p<0.001$). Table 3 shows a comparison of mean corneal sensitivity according to the type of surgery. Although the changes of corneal sensitivity from baseline values were significantly higher in DCN group compared to 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; $P = 0.579$).
At the last follow-up visit, 13/16 (81.2%) patients in the DCN group and 6/9 patients (66,7%) in the ICN group had positive corneal reflex.

In 4 patients, the presence of corneal opacity after the healing of NK impaired significantly visual acuity and required staged corneal transplantation (PK in 3 patients and DALK in 1 patient). In the case undergone DALK 18 months after DCN (patient #15), the corneal button excised at the time of transplantation was analyzed ex vivo using

Hematoxylin-Eosin (H-E) staining, Protein Gene Product (PGP) 9.5 immuno-staining and transmission electronic microscopy (TEM). The H-E staining confirmed that epithelium, Bowman's layer and anterior portion of the stroma showed normal features; the PGP 9.5 staining confirmed the presence of nervous fibers either in the sub-epithelial space and in the stroma; TEM allowed the visualization of unmyelinated nerve axons and nerve endings with a normal ultrastructure. Detailed data from the ex vivo analysis of the neurotized corneal button were previously reported.[13]

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 $\pm$ 4.87 msec in operated eye vs 35.5 $\pm$ 3,31 in contralateral eye and 8.9 $\pm$ 6.02 mAmp in operated eye vs 2.3 $\pm$ 0.84 in contralateral eye).

In Vivo Confocal Microscopy Findings

Corneal SNP was not detectable before surgery in all patients except 3, in whose few thin nerves were visible in the sub-epithelial layer. Mean CNFL was 1.8 ± 0.15 mm/mm^2 (range 1.59 to 1.95). In all patients, as soon as three months postoperatively new nerve fibers appeared forming progressively a regenerated corneal SNP that reached near-normal features one year postoperatively. At V1, corneal SNP was detectable in all patients and the mean value of CNFL was 14.67 $\pm$ 7.92 mm/mm^2 (range 2.69 to 32.70). The change in CNFL from V0 to V1 did not differ significantly between the two groups (P = 0.833).

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 isolated during DCN were very thin and short. This patient required a repeated surgery.

In the immediate postoperative period, all patients undergone DCN had transient, mild face edema including the eyelid and a surgical drainage was maintained for the first two postoperative days. All patients undergone ICN had edema of the upper third while no major complications occurred at the site of harvest 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 during the first postoperative year.

DISCUSSION

The present paper reports the results of CN for the treatment of patients with NK not responding to conventional medications. To the best of our knowledge, our case series is the largest available in the literature and represents the first attempt to compare the two most-commonly used techniques of CN. Neurotrophic keratitis is the clinical consequence of several conditions of genetic, systemic or ocular origin, resulting 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 NGF eye drops (Cenegermin) with proven efficacy in clinical trials and specific target on the root pathology has determined

a paradigm shift in medical management of NK.[7,8] In our current practice, we use routinely Cenegermin for NK cases secondary to peripheral/local diseases (e.g. post-herpetic, dry eye, post-surgical). However, NK recurrence following Cenegermin treatment was reported in some patients and this issue requires further long-term data.[33]

In the present study, the totality of patients had NK owing to central nervous denervation, and the majority of them (all except 3) had a complete damage to the trigeminal ganglion, as well-characterized by Dhillon and co-authors in a previous work.[34] Therefore, we decided to proceed with CN that offers 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) is prior to the approval of Cenegermin in the European Union (July 2017).

Since the first report from Terzis dated about 10 years ago, 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 reported approaches to CN 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 the rest.[35]

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

In our study, the clinical efficacy of CN was demonstrated by the improved sensitive and trophic function of corneal nerves that allowed in all cases the healing of NK that was then maintained during the entire follow-up. The regained corneal sensation was also sufficient to initiate the blinking reflex in the majority of patients. In parallel, IVCM showed the regeneration of corneal nerves that acquired near-normal morphology one year after surgery. However, despite the IVCM metrics of neurotized corneas did not reach normative reference values of an healthy cornea,[36] the regenerated nervous plexus had a trophic function sufficient to heal NK and to maintain over time epithelial integrity. Currently, there is a debate about the exact mechanism of action of CN. Some authors hypothesize that transferred nerves grow progressively towards the central cornea and regenerate a new nervous plexus.[12,20] Others speculate 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 the healing by stimulating pre-existing corneal nerves.[14] However, 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 cannot be ascertained by our analysis, we can neither confirm nor deny these hypotheses. However, a recent animal model of CN confirmed thanks to retrograde labeling the nerve growth through the graft and into the neurotrophic cornea.[37]

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

The comparative analysis between the two techniques suggests that DCN may guarantee higher corneal sensitivity compared to ICN at early postoperative time points (3-6 months). This is an expected finding considering that ICN implies a nerve anastomosis and that it is known that axons progressively populate distal to a neurorrhaphy by about half centimeter per month.[38] However, this difference did not reach statistical significance one year after CN. It should be pointed out that various factors can have influenced this comparison hampering the detection of significant differences. Firstly, unlike the conventional approach that involves an end-to-side neurorraphy,[20,27] we performed in all ICN cases an end-to-end neurorraphy between supraorbital/supratrochlear nerves and sural nerve graft in order to obtain an higher number of growing axons, as demonstrated in another model.[39] However, also other variables may have influenced the regenerative potential of the rerouted nerves, such as patient age, duration of denervation, underlying disease type 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 employed. The data of the comparative analysis between DCN and ICN are not conclusive due to the relatively small sample size and therefore did not allow to establish the technique of choice. It is reasonable to state that DCN could be preferred in patients with severe NK at high risk for corneal perforation due to its earlier re-innervation thanks to the immediate sprouting from the transferred nerves towards the anaesthetic cornea. However, the coronal approach employed in this technique is more invasive compared and this aspect has to be weighted with the higher morbidity of ICN related to the sural nerve sacrifice and the consequent numbness of calcaneus and foot postero-lateral surface. In this regard, the recent preliminary results about minimally invasive DCN 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.[15] Another less invasive approach for DCN that does not require coronal incision has been recently described by our Group in case of isolated damage of the ophthalmic branch and employs the direct transfer of the second division of trigeminal nerve.[40]

In the near furture, 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 and currently this analysis is ongoing at our Institutions. It is also reasonable to hypothesize that CN may benefit from the adjuvant use of NGF eye drops which could synergistically improve postoperative nerve regeneration.

Funding/Support: *This study was supported by a grant from the Italian Society of Ophthalmology (SOI) to Prof. Giuseppe Giannaccare.*

Financial Disclosure: *The authors have no proprietary or commercial interest in any materials discussed in the article.*

Acknowledgments: *None.*

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FIGURE CAPTIONS

Figure 1. Representative slit lamp photographs of the cornea for Patients #16 and #21. Before DCN, the clinical picture showed a neurotrophic keratitis (NK) with active corneal neovascularization **(Parts A and C).** Three months after DCN, NK healed with a marked reduction of corneal neovascularization and significant improvement of corneal transparency **(Parts B and D).**

Figure 2: Representative IVCM images obtained at the level of the corneal sub-basal nerve plexus (SNP) for Patient #16. Before DCN, the corneal SNP is not detectable **(Part A)**. One year after DCN, the regenerated corneal SNP exhibited a near-normal morphology **(Part B).** All images are in the scale of 400 x 400 mm.

Table 1 - Demographic and clinical characteristics of patients included in the study.

Patient (No.)	Age (Year), Gender	Eye	Etiology	Previous Treatment	Onset (Months before Surgery)	Facial Palsy (Y/N)	Clinical Picture	NK Stage (Mackie)	Corneal Reflex (Y/N)	Corneal Neurotization Technique	Follow-up (Months)
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 (1^{st}) 40 (2^{nd})	Y	Corneal ulcer, Nystagmus	III	N	Direct (1^{st}) Indirect (2^{nd})	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 reanima-tion	52	Y	Corneal ulcer with central neovascular leucoma	III	N	Direct	12
16	60, F	RE	Meningioma of pontocerebellar angle	Upper eyelid gold weight, Facial reanimation, stra-bismus surgery	40	Y	Corneal ulcer with active corneal neovascularization; large-angle esotropia	III	N	Direct	12
17	81, F	LE	Bell Palsy + tri-geminal 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)	None	188	N	Keratitis	I	N	Indirect	6

19	73, F	RE	AN with V,VII,VIII cranial nerve palsy	Tarsorraphy	24	Y	Keratitis	II	N	Direct	48
20	42, F	LE	Post-traumatic Bell Palsy + tri-geminal 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 + tri-geminal palsy (unknown origin)	Tarsorraphy	22	Y	Keratitis	II	N	Direct	24
23	63, M	LE	Bell Palsy + tri-geminal 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	None	65	Y	Keratitis	I	N	Indirect	4

*F, Female; M, Male; LE: left eye; RE: right eye; Y/N, Yes/No; HM,Hand Movement; AN, Acoustic Neuroma; AVM, Arteriovenous Malformation; PED Persistent Epithelial Defect. *Patient #5 underwent 2 surgeries: firstly direct corneal neurotization and secondly indirect corneal neurotization.*

Table 2 – Esthesiometry data obtained with Cochet-Bonnet esthesiometer in all the five corneal regions. Values are expressed in mm.

Eyes (n)	V0			V1		
	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; S: superior; I: inferior; N: nasal; T: temporal. N/A, not applicable.

Table 3 – Esthesiometry data according to the type of corneal neurotization. Values are expressed in mm as mean ± SD (range).

Visit	DCN Group	ICN Group	Significance (P)*
Baseline	4.0 ± 8.9 (0-30)	2.5 ± 5.3 (0-15)	0.867
After 1 M	6.5 ± 11.6 (0-40)	5.0 ± 10.1 (0-20)	0.785
After 3 M	15.2 ± 20.3 (0-60)	6.5 ± 7.5 (0-20)	**0.042**
After 6 M	19.8 ± 17.1 (0-60)	9.3 ± 19.1 (0-40)	**0.048**
After 9 M	23.0 ± 25.1 (0-60)	16.2 ± 22.9 (0-45)	0.432
After 12 M	22.3 ± 20.4 (0-60)	17.5 ± 17.3 (0-45)	0.579

DCN = direct corneal neurotization; ICN = indirect corneal neurotization; SD, Standard Deviation.
**Statistical significance of the difference between the two groups of the changes of corneal sensitivity values at each time point compared to baseline values.*

Figure 1

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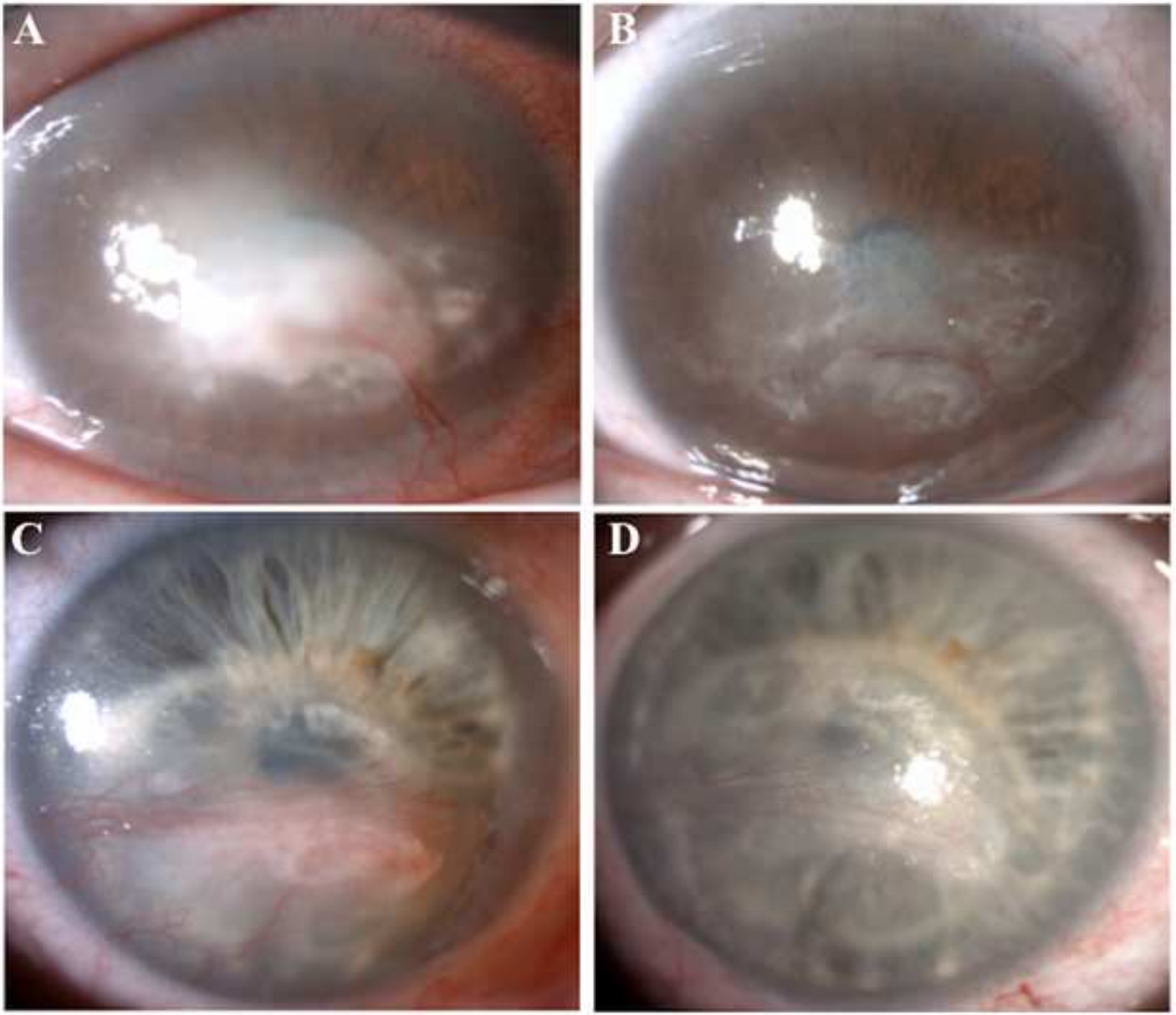

Figure 2

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