Corneal neurotization: a novel surgical procedure to restore sensation and preserve vision in patients with neurotrophic keratopathy

Joseph Catapano MD\textsuperscript{1-3}, Simon SM Fung MA\textsuperscript{4}, Asim Ali MD\textsuperscript{4}, Cecilia Jobst MSc\textsuperscript{1}, Douglas Cheyne PhD\textsuperscript{1}, Ronald M Zuker MD\textsuperscript{3}, Gregory H. Borschel MD\textsuperscript{1-3}

SSTP PhD Candidate: Joseph Catapano

Supervisor: Gregory H. Borschel

\textsuperscript{1}Program in Neurosciences and Mental Health, SickKids Research Institute
\textsuperscript{2}Institute of Medical Science, University of Toronto
\textsuperscript{3}Division of Plastic and Reconstructive Surgery, Department of Surgery, University of Toronto
\textsuperscript{4}Department of Ophthalmology and Vision Science, University of Toronto
INTRODUCTION
Corneal sensation protects the eye from injury and the corneal nerves release neuromediators, such as Substance P (SP), which are necessary for corneal epithelial maintenance and repair. Patients with impaired or absent corneal sensation and innervation develop neurotrophic keratopathy (NK), characterized by occult injury, poor healing, scarring, and vision loss. Even with optimal ophthalmic management, NK progresses to blindness in most patients. Corneal transplant is contraindicated in NK as the graft becomes scarred in the absence of innervation.

Corneal neurotization is a novel surgical procedure, developed at SickKids, that addresses the underlying pathology in patients with NK by reinnervating the cornea using nerve grafts and transfers from normal sensory nerves elsewhere on the face. This current study was designed to document corneal reinnervation after corneal neurotization and assess efficacy in a prospective cohort of patients with advanced NK who had failed other treatments. A rat model of NK and corneal neurotization was also developed to further investigate whether corneal neurotization influences corneal epithelial healing after injury.

METHODS
Study Design and Clinical Outcomes Analysis
This was a prospective study in a consecutive cohort of patients referred to the Hospital for Sick Children. All patients had significant corneal hypoesthesia, clinical evidence of severe NK, and had failed conventional ophthalmic therapy. Patients had corneal neurotization performed by the senior authors (RZ, GHB and AA). The primary endpoints were central corneal sensation (CCS) and best spectacle corrected visual acuity (BSCVA). Corneal sensation was measured with Cochet-Bonnet esthesiometry (range, 0 to 60 mm). At each point of follow-up, corneal sensation, BSCVA, corneal surface integrity, episodes of persistent epithelial defects (PEDs), adjunctive treatments and any side effects or complications from corneal neurotization surgery were documented. Corneal reinnervation was confirmed in eligible patients using immunohistochemistry and magnetoencephalography (MEG).

Documentation of Corneal Reinnervation after Corneal Neurotization
Immunohistochemical analysis was performed on specimens from three patients undergoing corneal transplantation after neurotization. Axons were identified with neurofilament antibody clone 2F11. Comparison was made to one patient with a corneal specimen prior to neurotization and to normal controls. Magnetoencephalography (MEG) was conducted in an adult female (34 years old) with right corneal anesthesia prior to corneal neurotization and then 8 months after. Source localization was carried out on the stimulus-locked averaged evoked responses using an event-related beam-forming algorithm and superimposed on the patient's structural (T1) MRI.

Corneal Healing in an Animal Model of NK and Corneal Neurotization
A novel rat model of neurotrophic keratopathy and corneal neurotization was developed in thy1-GFP+ rat, which expresses green fluorescent protein in all axons. A model of NK was validated by optimizing parameters for stereotactic ablation of the left ophthalmic nerve (V1), which innervates the left cornea. Corneal neurotization was performed with a common peroneal (CP) and sural nerve graft. Each graft was coapted to the right (contralateral) infraorbital nerve and the distal graft was tunneled subcutaneously and sutured directly to the corneal limbus. Four weeks after V1 ablation, whole mount corneal imaging and NeuronJ were used to compare corneal reinnervation in neurotized rats to normal controls and rats receiving only V1 ablation. In a separate group of rats, the left cornea was retrograde labeled with 4% Fluorogold to determine the origin of the left corneal reinnervation after neurotization. Histomorphometric analysis was performed on nerve grafts to quantify the number of regenerated donor axons.

Four weeks after stereotactic V1 ablation, corneal healing was compared by debriding the entire left corneal epithelium. Fluorescein staining and digital imaging were performed every 12
hours until 96 hours after injury to monitor wound size and healing. Wound size was calculated using ImageJ and healing standardized to the initial wound size.

**Statistical Analysis**
Statistical comparisons of pre- and post-operative CCS and BSCVA were performed with the Wilcoxon signed-rank test and categorical data compared using Fisher’s Exact Test. BSCVA was analyzed as logMAR conversion. Laboratory data was analyzed as one-way or two-way ANOVA where appropriate. *p* value < 0.05 was considered statistically significant.

**RESULTS**

**Clinical Response: Corneal Sensation, Visual Acuity and Ocular Surface Health**
Fifteen patients with severe NK and vision loss, having failed previous ophthalmic management, were enrolled into the study from November 2012 to January 2017. Two patients had bilateral NK, resulting in a total of 17 treated eyes. Four eyes had previously undergone emergency corneal transplantation because of corneal perforation, all of which were significantly scarred due to NK. Six months after corneal neurotization, median CCS significantly improved from a median of 0 mm pre-operatively (range, CCS 0-5 mm) to 40 mm (range, CCS 0-60 mm; *p* < 0.001). By final follow-up (range, 6 to 53 months) median CCS improved to 60 mm (range, CCS 10-60 mm; *p* < 0.001). A value of 60 mm is considered indistinguishable from normal sensation. BSCVA was not significantly different prior to surgery compared to the final post-operative visit (0.98 ± 0.69 LogMAR, and 0.95 ± 0.83 LogMAR respectively, *p* = 0.1763).

Ocular surface health appeared to improve after corneal neurotization. Three eyes underwent reversal of pre-operative protective surgical tarsorrhaphy, representing a significant de-escalation of treatment. Thirteen eyes required fewer lubricants to maintain ocular surface integrity. During the entire duration of post-operative follow-up (mean 25.1 months, range 6 to 53 months), 4 eyes (23.5%) experienced PEDs, in comparison to 9 (52.9%) eyes within one year prior to corneal neurotization. To correct pre-operative corneal scarring from NK, 3 eyes underwent corneal transplantation between 24 and 33 months after corneal neurotization. Six months after corneal transplantation, CCS (40-60 mm) had recovered in all 3 eyes, and two of the three grafts remained clear at final follow-up, with one patient experiencing immunologic rejection. Baseline characteristics and outcomes are summarized in Table 1.

**Evidence of Corneal Reinnervation after Corneal Neurotization**
In one patient with corneal transplantation prior to corneal neurotization, the insensate cornea lacked evidence of innervation, with neurofilament (NF) immunoreactivity limited to a single axon profile. The corneal sample from the same patient after corneal neurotization demonstrated abundant NF+ axon profiles in the corneal stroma, consistent with corneal reinnervation after neurotization. Histological examination of the other 2 corneal samples after corneal neurotization also found abundant NF-positive axons (Figure 1 A-G). Normal corneas were used as positive controls.

Prior to corneal neurotization, magnetoencephalography (MEG) identified a clear absence of evoked response in the anesthetic right cornea. Eight months after neurotization, when applying the same stimulus, an evoked response was elicited and localized to the ipsilateral (right) somatosensory cortex. The cornea is normally innervated by the contralateral somatosensory cortex, indicating that the source of corneal reinnervation derived from the contralateral donor nerve (Figure 1 H).

**Healing of the Corneal Epithelium in a Rat Model of NK and Corneal Neurotization**
Stereotactic ablation of the left ophthalmic nerve resulted in complete loss of left corneal axons with minimal corneal reinnervation after 4 weeks and the left cornea developed severe NK,
including extensive corneal scarring, neovascularization and perforation. Corneal neurotization significantly improved corneal reinnervation at 4 weeks with corneal axon density comparable to the uninjured (normal) cornea (Figure 2). Retrograde labeling 4 weeks after corneal neurotization labeled 206 ± 81.9 neurons in the contralateral trigeminal ganglion, confirming reinnervation derived from the contralateral infraorbital donor nerve. While only approximately 206 neurons appeared to reinnervate the cornea, significantly more myelinated fibers were found in the CP (5577 ± 647) and sural nerve grafts (2430 ± 613) used to neurotize the cornea.

Corneal neurotization significantly improved corneal epithelial healing after injury in comparison to denervated controls, and was not statistically different from the uninjured (normal) cornea (Figure 2). Rats with corneal neurotization demonstrated less corneal scarring after epithelial injury, and a far lower incidence of corneal perforation (0 of 10) in comparison to denervated controls (4 of 6; p < 0.01).

DISCUSSION
Even with optimal ophthalmic management including topical lubricants, autologous serum-derived eye drops, and disfiguring surgical tarsorrhaphies to protect the corneal surface from injury, many patients with NK progress to vision loss. Nerve grafts and transfers are an established surgical technique to reconstruct motor deficits and reanimate the face in patients with cranial nerve (CN) VII palsy. Success in facial reanimation provided a strong rationale for the use of nerve grafts and transfers in NK to provide an alternative source of corneal innervation and directly address the underlying neuropathology.

In this prospective cohort study, we found corneal neurotization significantly improved corneal sensation in patients with NK, and reinnervation was confirmed with histology and MEG. Despite the use of fewer lubricants to maintain ocular surface integrity and the reversal of tarsorrhaphy in three patients, patients experienced fewer PEDs, suggesting improved corneal health. Two patients also underwent successful corneal transplantation, providing further evidence of improved ocular surface health after corneal neurotization.

Due to the prospective cohort design of our study, it is important to consider the potential for confounding bias when interpreting our results. While our study definitively documents improved sensation and corneal reinnervation in patients with NK, due to potential confounders it remains less certain whether corneal neurotization restores the neuromediators necessary to improve ocular surface health and promote epithelial healing after injury. NK is a rare and heterogeneous disease, making randomized controlled trials very difficult. Therefore, we designed a novel rat model of NK and corneal neurotization to further investigate whether axons reinnervating the cornea after neurotization influence ocular surface health and improve corneal healing after injury.

We first validated our model, demonstrating that stereotactic ablation of V1 resulted in compete loss of corneal innervation and severe NK. We then developed a method of corneal neurotization, and demonstrated significantly improved corneal nerve density derived from the contralateral donor nerve. Lastly, we used an established corneal healing assay to demonstrate that corneal reinnervation after neurotization significantly improves corneal healing, providing further evidence that axons reinnervating the cornea after neurotization contain the necessary neuromediators to improve ocular surface health.

CONCLUSIONS
Corneal neurotization is a promising surgical procedure that provides patients with severe NK a surgical solution that may rescue vision. Several other centers are now performing corneal neurotization. Coordination of research efforts is required to definitively document improved ocular surface health after corneal neurotization in patients. Our animal model can be used to inform clinical practice and investigate how reinnervation impacts the corneal epithelium and how the cornea regulates nerve regrowth after neurotization.
REFERENCES

3. Alper M. The anesthetic eye: an investigation of changes in the anterior ocular segment of the monkey caused by interrupting the trigeminal nerve at various levels along its course. Trans Am Ophthalmol Soc. 1975;LXXIII.
20. Shimizu T, Izumi K, Fujita S, Koja T. Capsaicin-Induced Corneal Lesions in Mice and the


<table>
<thead>
<tr>
<th>Patient Characteristics</th>
<th>Pre-Operative (n = 18)</th>
<th>Post-operative (n = 18)</th>
<th>Range</th>
<th>p-value</th>
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<tr>
<td>Age (yrs)</td>
<td>Mean 12.2 (± 8.5)</td>
<td>Median 1.9 – 34.3</td>
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<td>Etiology, n (%)</td>
<td>Congenital 12 (77.7)</td>
<td>Traumatic 5 (27.7)</td>
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<td>HSV-keratitis 1 (5.5)</td>
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<td>NK Duration (yrs)</td>
<td>5.6 (± 6.1)</td>
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<td>0.5 – 22.5</td>
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<td>Affected Cornea, n (%)</td>
<td>Left (Unilateral) 7 (46.6)</td>
<td>Right (Unilateral) 5 (33.3)</td>
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<td>Bilateral 3 (20.0)</td>
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<td>Follow-up (mo)</td>
<td>19.1 (± 14.9)</td>
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<td>6 – 53</td>
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<td>Outcomes</td>
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<td>CCS (mm)</td>
<td>Mean 0.83 (± 2.6)</td>
<td>Median 41.21 (± 16.53)</td>
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<td>Median 0 (0 – 0)</td>
<td>Median 60 (40 – 60)</td>
<td>&lt; 0.0001</td>
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<td>BSCVA (logMar) *</td>
<td>Mean 0.98 (± 0.69)</td>
<td>Median 0.95 (± 0.83)</td>
<td>0.7839</td>
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<td>Median 0.7 (0.5 – 1.3)</td>
<td>Median 0.65 (0.3 – 1.5)</td>
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<td>PED, n (%) § ¶</td>
<td>Total 15 (83.3)</td>
<td>4 (22.2)</td>
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<td>Within 1 year of OR 9 (50.0)</td>
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<td>Tarshorrhaphy, n (%) ¶</td>
<td>Present 11 (61.1)</td>
<td>12 (66.6)</td>
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CCS, central corneal sensation; BSCVA, best spectacle corrected visual acuity; PED, persistent epithelial defect; NK, neurotrophic keratopathy

* BCVSA was assessed as logMAR conversion for statistical purposes. Example Snellen conversions are: 20/20 (0 logMAR), 20/200 (1 LogMAR), Light Perception (2.7 logMAR)

§ The incidence of PED in patients was analysed as: i) the total number of patients presenting with a PED prior to and after neurotization, ii) and those presenting with a PED within 1 year prior to surgery and 1 year after surgery
**Central Cornea Axon Density**

![Graph](image)

- **Axon Density** (um/mm²)
  - **Subbasal**
  - **Stromal**

![Bar chart](image)

* p < 0.01

**Corneal Healing Over Time**

![Graph](image)

- **% Healed**
  - **Uninjured Control**
  - **Neurotized**
  - **Denervated**

**F**

- **0 hr**
- **24 hr**
- **48 hr**
- **72 hr**
- **96 hr**

- **Uninjured**
- **Neurotised**
- **Denervated**
Figure 1. Immunohistochemistry of corneal explants and magnetoencephalography (MEG) demonstrate corneal reinnervation after corneal neurotization. Consistent with the patient’s clinical diagnosis of corneal anesthesia, neurofilament staining of corneal tissue prior to corneal neurotization demonstrated no subbasal innervation and only a single linear profile with neurofilament immunoreactivity in the stroma. Chronic inflammation (A) and hemosiderin containing macrophages (A inset, Perl’s iron stain) were distinguished from neurofilament immunoreactivity by their globoid shape and light yellow-brown, granular cytoplasm (B star). A corneal sample in the same patient after corneal neurotization identified subepithelial and stromal discrete clusters of dot and linear profiles (C/D), confirming reinnervation of the cornea after neurotization. Immunoreactive axon profiles were compared with normal controls (E/F). Prior to corneal neurotization, MEG with right corneal stimulation produced no discernable sensory response (SEF), confirming absent corneal sensation prior to corneal neurotization (G, top panel). Eight months after corneal neurotization, repeat MEG with corneal stimulation demonstrated a clearly discernable SEF with corneal stimulation (G, bottom panel, black arrows). When the localization of the SEF was superimposed on the patient’s T1 MRI, the corneal signal post-neurotization was localized to the ipsilateral somatosensory cortex (G, bottom panel, yellow arrow), confirming reinnervation with axons derived from the contralateral face. These findings are consistent with the use of the contralateral supratrochlear nerve as a donor in this patient.

Figure 2. Corneal neurotization significantly increases central corneal axon density and improves corneal healing after injury. Nearly the entire corneal innervation in normal (uninjured) thy1-GFP+ rats (A) is absent 4 weeks after stereotactic V1 ablation (B). Axons regenerating from the contralateral infraorbital nerve after corneal neurotization reinnervate the cornea (C) 4 weeks after V1 ablation and significantly increase both subbasal and stromal axon density (D) (*p < 0.01). Corneal neurotization also significantly improved corneal healing in comparison to rats with corneal denervation (E/F) (*p < 0.01), and statistically were not different from the rate of healing in uninjured controls (E).