In Vivo Confocal Microscopy Demonstrates the Recovery of the Normal Morphology of Sub-basal Nerve Plexus After Corneal Neurotization for the Treatment of Severe Neurotrophic Keratitis

Introduction

Neurotrophic keratitis is a degenerative disease caused by damage of trigeminal innervation, leading to corneal epithelial breakdown, impairment of healing and, in severe cases, corneal ulceration, melting and eventually perforation. The hallmark of the disease is a decreased or absent corneal sensation.[1] Trigeminal nerve provides not only sensitivity but also trophic factors, thus playing a key role in maintaining the integrity and function of the entire ocular surface. The most common causes include, among others, herpetic keratitis, corneal surgery and diabetes. Medical management consists of supportive measures such as unpreserved tear substitute to moisten the ocular surface, antibiotic to prevent infections, and contact lens to protect the cornea. Novel therapies, including recombinant human nerve growth factor and Regenerating Agent eye drops, are currently under investigation in ongoing Clinical Trials with promising results (respectively NCT01756456 and NCT01242839).

However, to date no satisfactory medical options are available for the treatment of severe neurotrophic keratitis, while surgery is reserved for refractory or complicated cases. Recently a novel surgical technique consisting of the transposition of contralateral supratrochlear and supraorbital nerves to the sclero-corneal limbus of the affected eye with the aim of restoring corneal innervation and sensivitity was introduced.[2-3] To the best of our knowledge, the postoperative recovery of corneal innervation and sensitivity has been investigated and confirmed only indirectly by means of corneal esthesiometry. We describe herein for the first time the process of reinnervation as it progresses over the time after corneal neurotization by means of in vivo confocal microscopy (IVCM).

Case presentation

Patient history

A 46-year-old woman with a 3-year history of recurring chronic neurotrophic keratitis in her right eye due to facial and trigeminal palsies secondary to surgical removal of homolateral statoacoustic nerve neurinoma of pontocerebellar angle was referred to the Cornea Service of our Institution. Lateral tarsorraphy had been performed soon after the palsies' onset to protect the cornea from the exposure. Upon presentation, slit lamp examination revealed a central corneal neurotrophic ulcer (stage III Mackie Classification) with a diameter of 3 x 3 mm, accompanied by stromal melting and tortuous corneal neovessels. The patient was treated with unpreserved tear substitutes and vitamin A ointments, and followed-up for an additional period of 1 year, but the clinical picture remained stable and the corneal ulcer did not heal. Corneal esthesiometry and IVCM were performed three times over the year, and confirmed the complete absence of respectively corneal sensitivity and nerve fibers in the sub-basal plexus. One week prior to corneal neurotization, Schirmer test type I was 1mm/5', break-up time (BUT) was 1 second, ocular surface disease index (OSDI) score was 32. Best-corrected visual acuity was limited to hand motion at 1 foot. Corneal esthesiometety evaluated quantitatively by the Cochet-Bonnet esthesiometer was null in all the 5 corneal regions (Figure 1, part A). IVCM confirmed the complete absence of the corneal sub-basal nerve plexus (Figure 2, part A).

Surgical procedure

The left supraorbital and supratrochlear nerves, along with their main branches which run with different depth and path in the subdermal plane, were identified and dissected through a coronal incision from the undersurface of the frontal skin, in order to harvest a minimal nerves length of 12 cm. The dissected nerves were then tunnelled over the nasal bridge to reach a 10 mm incision in the right superior eyelid. Using a Wright needle, four distal nerve branches were retrieved in the ocular surface (Figure 3). By means of a conjunctivotomy and tenonectomy, the nerves were tunnelled to gain access to the subtenonian area. Each distal branch was passed into the prepared perilimbal space and finally distributed at the four cardinal points. The branches were not fixed to the sclera by sutures in order to avoid possible damage of nerve fibers, but were only positioned in the cardinal points. Four centripetal intra-corneal tunnels (one for each branch) were done in order to facilitate the nerve sprouting towards the central cornea. The conjunctiva was then sutured with 8-0 vicryl suture.

Patient follow-up

After surgery, patient was instructed to instill Tobramycin plus Dexamethasone eye drops four times a day for 4 weeks. Transposed nerves were visible in the desired positions passing around the sclero-corneal limbus under the bulbar conjunctiva by slip lamp examination and anterior segment optical coherence tomography (Figure 4, parts A-B). Three months postoperatively, clinical picture improved significantly with the complete healing of the epithelial defect, the decrease of conjunctival hyperemia and of area and tortuosity of corneal neovessels, and the increase of Schirmer Test type I and BUT to the lower limits of normal range (respectively 8 mm/5' and 5 seconds). IVCM detected few thin and tortuous nerve fibers in the corneal sub-basal plexus (Figure 2, part B), while corneal esthesiometry continued to be null. Six months postoperatively, IVCM detected a higher density of nerve fibers which appeared with a near-normal morphology (Figure 2, part C), while corneal sensitivity was still totally absent. Nine months postoperatively, IVCM detected nerve fibers in the corneal sub-basal plexus with normal density and morphology (Figure 2, part D), and corneal esthesiometry measured 30 to 35 mm in all the 5 corneal regions examined. Currently one year postoperatively, sub-basal plexus continues to be normal in terms of density and morphology, while corneal esthesiometry improved to 40 mm in all the 5 corneal regions (Figure 1, part B).

Figure 1: Slit lamp examination and corneal esthesiometry before (part A) and 1 year after surgery (part B).

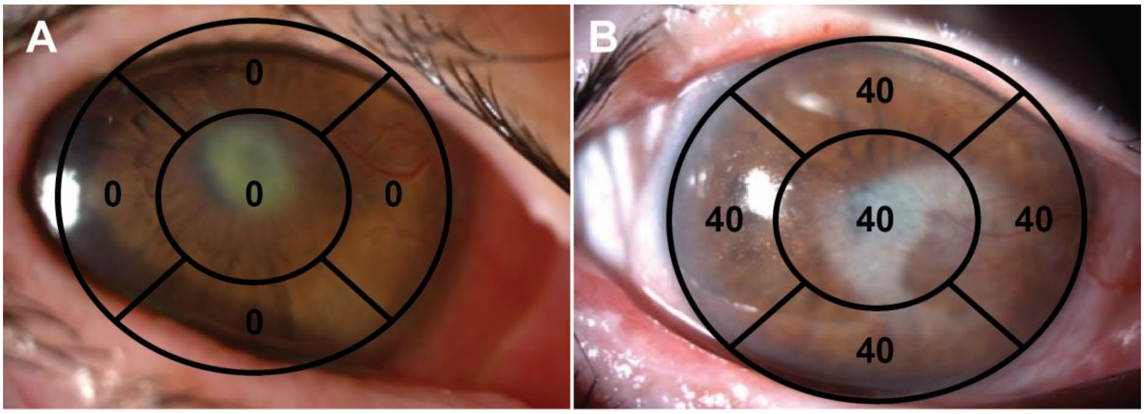


Figure 2: In vivo Confocal Microscopy images preoperatively (part A) and 3 (part B), 6 (part C) and 9 months postoperatively (part D).

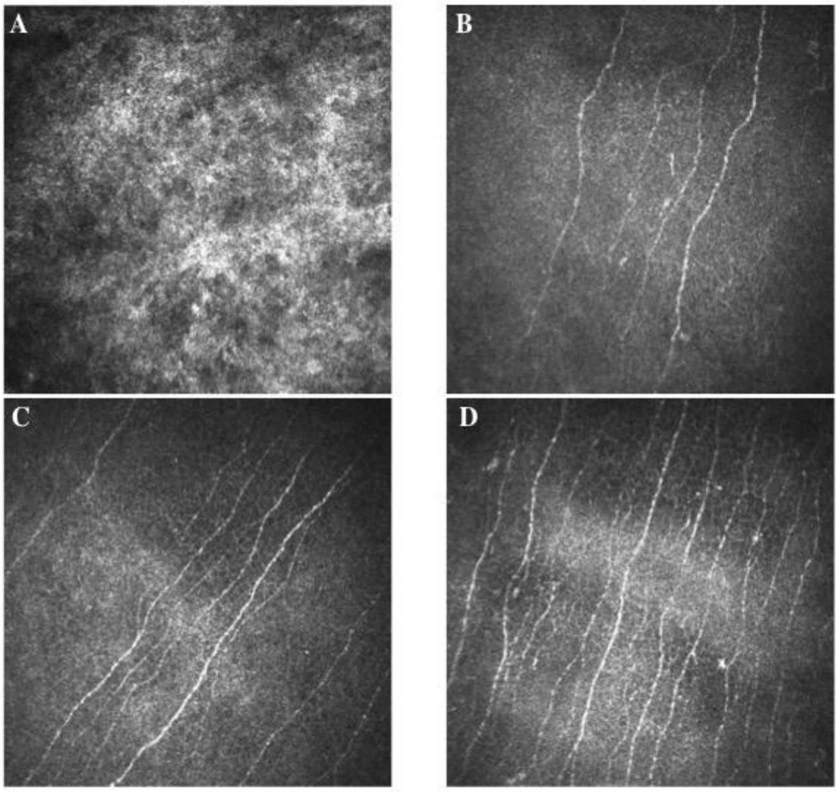


Figure 3: Intraoperative picture of the four distal nerve branches positioned upon the ocular surface.

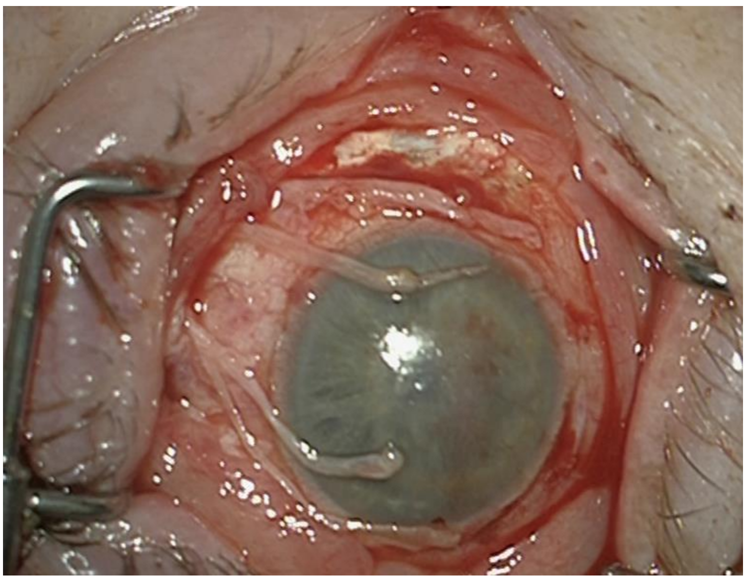
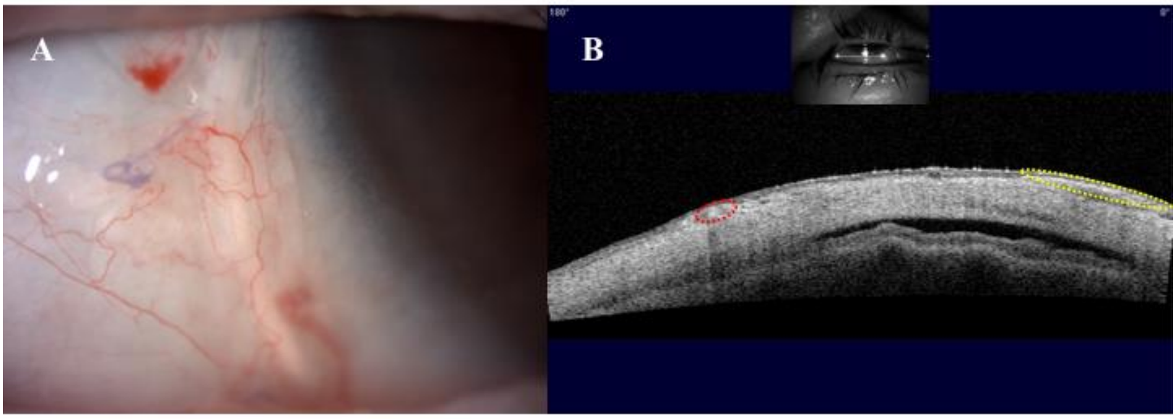


Figure 4: Slit lamp examination showing one branch of the transposed nerves underlying the bulbar conjunctiva and surrouding the temporal sclero-corneal limbus (part A). Anterior-segment Optical Coherence Tomography cross-sectional scan showing the vertical temporal branch (red dashed area) and the horizontal inferior branch (yellow dashed area) (part B).



Discussion

Denervation of the cornea impairs wound healing, leading to chronic ulceration; in addition it represents a contraindication for corneal transplantation, because the new graft would be affected by the same detrimental processes. Recently, novel surgical techniques consisting of different nerves transpositions to the denervated cornea have been proposed in order to restore corneal sensation in severe cases of neurotrophic keratitis.[2-5] Corneal neurotization can be performed through two different surgical techniques, based on the transposition of the contraleteral or ipsilateral supraorbital and/or supratrochlear nerves (direct neurotization),[2-4] or on the sural nerve graft (indirect neurotization).[5] This approach allows to restore corneal sensitivity, and to perform successful subsequent corneal surgery, when required.

To date corneal reinnervation after surgical neurotization has been determined only clinically by

Cochet-Bonnet esthesiometer, while the direct evidence of the ingrowth of the transferred nerves from the sclero-cormeal limbus to the central recipient cornea has not been provided. Thus, it was still not clear if postoperative improvement of ocular surface condition along with the reduction of inflammation occurred after corneal neurotization were related to the action of chemical neuromediators supplied to the ocular surface by the transferred nerve branches anchored to the sclero-corneal limbus, or to an ingrowth of the distal nerve fascicles towards the recipient cornea.

In the case presented herein, we used for the first time in vivo confocal microscopy to objectively observe the process of corneal reinnervation as it progressed over the time after corneal neurotization. We detected new thin corneal nerves in the subepithelial plexus as soon as 3 months postoperatively, which acquired a near-normal morphology and density six months after surgery. However corneal sensitivity measured by Cochet-Bonnet esthesiometer remained null at those time intervals. Different hypotheses can be postulated to explain the structure-function discrepancy between corneal nerves detection by IVCM and the lack of functional response. First of all, although Cochet-Bonnet esthesiometry is considered the gold standard, it is far to be an ideal tool for measuring overall corneal sensitivity; in fact the direct physical pressure of the nylon thread against the cornea results in stimulation of Ad fibers, exploring only the mechanical sensitivity while neglecting chemical and thermal receptors.[6] The second hypothesis concerns the central nervous system remodeling that likely takes place after nerve transfers with a variable time line. Indeed, after corneal neurotization the patient has to shift his/her perception to recognize mechanical stimulation of the cornea as true corneal sensation.

Corneal esthesiometry was partially regained in all regions after nine months, and increased further one year postoperatively. IVCM demonstrated the recovery of the normal morphology of the sub-basal nerve plexus of the cornea as soon as nine months postoperatively. As spontaneous corneal nerve regeneration has been demonstrated in a case of neurotrophic zoster keratopathy after few years of follow-up,[7] we studied over the time the sub-basal nerve plexus by serial IVCM scans and corneal esthesiometries before to proceed with surgical neurotization, which was performed 4 years after the onset of the palsy.

Interestingly, the patient did not report any unpleasant sensation of neuropathic pain, allodynia or disesthesia during the entire follow-up period. However, a stromal leukoma covering completely the visual axis is still present and deep anterior lamellar keratoplasty would be necessary to restore patient’s visual acuity. This surgery would provide not only a significant gain in terms of visual acuity, but also the the histopathologic assessment of nerves in the corneal button excised by using the Karnovsky and Roots modification of nonspecific acetylcholinesterase method, with the ex vivo demonstration of the distribution and spatial arrangement of the nerve bundles that repopulated the cornea after surgical neurotization.[8]

Conclusion

This case report provides new insight supporting the efficacy of corneal neurotization as a definitive treatment option in severe neurotrophic keratitis, representing the first anatomical in vivo validation of reinnervation after corneal neurotization thanks to in vivo confocal microscopy imaging. Corneal sensitivity and normal morphology of the sub-basal nerve plexus were regained within the first postoperative year. In case of concomitant corneal opacity, we advocate a staged approach (corneal transplantation at least 1 year after corneal neurotization) in order to provide an optimal recipient bed for the success of the graft. Prospective randomized clinical trials comparing direct and indirect corneal neurotization are desiderable to establish the “gold standard” technique. Future perspectives of corneal neurotization may include also endoscopic minimally invasive procedure,[10] and the use of either natural or recombinant nerve growth factor-based eye drops to further stimulate nerve fibers growth after surgery.

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