CASE REPORT

# Ex vivo photoactivated chromophore for keratitis-corneal crosslinking on inverted graft: new approach to manage interface infectious keratitis after deep anterior lamellar keratoplasty

*Alessandro Mularoni, MD, Laura Rania, MD, Aurelio Imburgia, MD, Vincenzo Scorcia, MD, Giuseppe Giannaccare, MD, PhD, FEBO*

A 44-year-old man affected by keratoconus underwent deep anterior lamellar keratoplasty (DALK). Two weeks postoperatively, slitlamp examination showed grayish-white lesions in the donor–host interface. Corneal scraping and donor broth culture tested negative for both bacteria and fungi. In vivo confocal microscopy showed elongated particles resembling *Candida* pseudofilaments. Despite antimicrobial therapy, the clinical picture worsened, and surgical approach was chosen: the graft was peeled off and inverted (epithelium facing down), and ex vivo photoactivated chromophore for infectious keratitis-corneal crosslinking (PACK-CXL) was performed; the recipient bed was washed with antibiotics/antifungals and the graft resutured. Microbiological and histological evaluations of biopsy specimen tested positive for *Candida albicans*. Postoperative slitlamp examination revealed a progressive recovery of graft transparency with disappearance of the infiltrates. To the authors' knowledge, this is the first report of ex vivo PACK-CXL on inverted DALK graft for the treatment of interface infectious keratitis. This technique allowed the resolution of the infection while sparing the use of new donor tissue.

Interface infectious keratitis is a recently described condition that might develop after any type of lamellar keratoplasty.[1] In fact, the infection develops in the virtual space existing between the graft and the host, where the hypoxic sequestered environment stimulates the proliferation of the microorganism, largely protected from the host immune response. The inadvertent donor to host transmission of infection at the time of keratoplasty represents the most common cause of interface infectious keratitis, and fungi in the form of *Candida* species account for most microorganisms isolated.[2]

Medical treatment is largely ineffective on halting the progression of the infection because the site of infection is not reachable by sufficient concentration of both topical and systemic drugs, particularly in case of intact epithelium of the cornea.[3] Intrastromal injections of drugs allow to deliver higher concentrations at the site of the infection and can be effective in early-onset interface infectious keratitis confined to interface and immediately adjacent stroma.[4] In most severe cases, therapeutic penetrating keratoplasty is instrumental in eradicating the infection, allowing also histological and cultural examinations that are needed to reach a definite diagnosis and to plan targeted therapy.[5]

In the recent years, corneal crosslinking (CXL) has been increasingly used for infectious keratitis, and the new terminology photoactivated chromophore for infectious keratitis-CXL (PACK-CXL) was coined to help distinguish its use from conventional CXL for keratoconus.[6] We report in this study the outcomes of a modified technique of PACK-CXL for the management of a case of interface infectious keratitis occurring after deep anterior lamellar keratoplasty (DALK).

## CASE REPORT

A 44-year-old man affected by keratoconus in both eyes was referred for corneal transplantation because of the progressive deterioration of visual acuity in his right eye. On examination, Snellen corrected distance visual acuity was limited to 20/63 in the right eye, and slitlamp

From the Department of Ophthalmology, Ospedale di Stato della Repubblica di San Marino (Mularoni, Rania, Imburgia), San Marino; and the Department of Ophthalmology, University of Magna Græcia (Scorcia, Giannaccare), Catanzaro, Italy.

Corresponding author: Giuseppe Giannaccare, MD, PhD, FEBO, Viale Europa, 88100, Germaneto, Catanzaro, Italy. Email: giuseppe.giannaccare@gmail.com.

examination showed severe ectasia with apical corneal scarring. The patient underwent manual DALK, and the donor button was prepared by the surgeon (A.M.) removing Descemet membrane and endothelium by gently swabbing the posterior surface of the corneoscleral rim with dry cellulose sponge. Surgery was uneventful except for the occurrence of a microperforation during dissection that did not prevent the completion of the intended DALK. One week postoperatively, a double anterior chamber was diagnosed, and the complication was managed with intracameral air tamponade. Two weeks postoperatively, Descemet membrane was completely reattached, but slitlamp examination showed grayish-white lesions located in the donor–host interface, which were suspected for infection (Figure 1, A). Corneal scraping and in vivo confocal microscopy (IVCM) were performed to try to identify the microorganism possibly involved. IVCM showed the presence of high-contrast elongated particles resembling *Candida* pseudofilaments in the posterior stroma (Figure 1, B). The eye bank was contacted for a feedback about microbiological testing of the donor broth that was confirmed to be negative. Empirical broad-spectrum antimicrobial therapy with vancomycin (50 mg/mL), ceftazidime (50 mg/mL), gentamycin (14 mg/mL), voriconazole (10 mg/mL), and amphotericin B (5 mg/mL) eyedrops every hour was commenced without waiting for culture results that 1 week later tested negative for both bacteria and fungi. The clinical picture progressively worsened in the following 2 weeks, the infiltrates became denser, and the graft lost transparency (Figure 1, C). Because the medical therapy was being ineffective, a surgical approach was chosen. After removing all stitches and peeling off the DALK graft, the recipient bed appeared to be clear and not infiltrated. A small biopsy consisting of a triangular piece of the posterior side of the graft in the stroma adjacent to the lesions was obtained. Then, the graft was inverted (epithelium facing down), soaked with 0.1% hypotonic riboflavin (Ricrolin) 1 drop every 2 minutes for 15 minutes, and accelerated CXL (VEGA CBM-X-Linker, CSO) (10 mW/cm$^2$ for 9 min; total fluence 5.4 mJ/cm$^2$) was performed ex vivo. Finally, the recipient bed was washed with antibiotics and antifungals, and the graft was resutured.

Postoperatively, the antimicrobial treatment was continued according to the preoperative regimen. Because both microbiological and histological evaluations of the biopsy specimen tested positive for *Candida albicans*, voriconazole and amphotericin B were continued, and the remaining therapy was interrupted. One month later, slitlamp examination revealed a marked improvement of graft transparency, and previous infiltrates were no longer detectable (Figure 1, D). Repeated IVCM failed to show pathological findings, and topical therapy was gradually tapered in the following weeks. Currently, 1 year postoperatively, Snellen corrected distance visual acuity with rigid gas-permeable contact lens was 20/25, and the slitlamp examination revealed a clear graft.

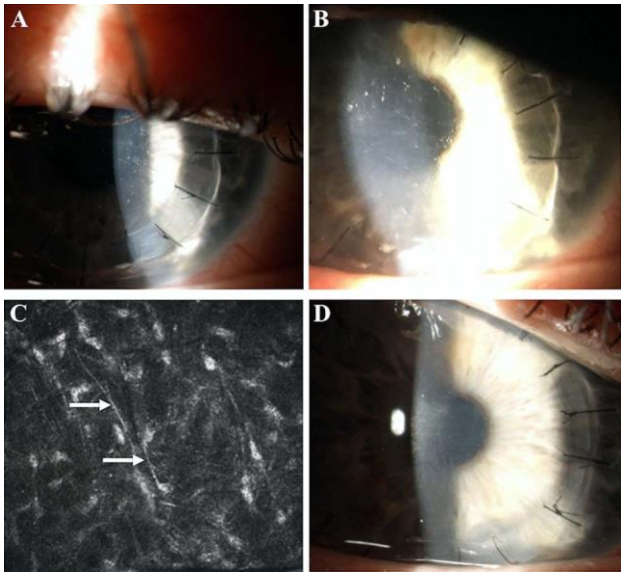


Figure 1. Slitlamp and in vivo confocal microscopy (IVCM) images of patient before and after ex vivo photoactivated chromophore for keratitis-corneal crosslinking (PACK-CXL). *A*: Slitlamp photograph taken 2 weeks after deep anterior lamellar keratoplasty (DALK) when grayish-white lesions located in the donor–host interface suspected for infection were noted. *B*: Slitlamp photograph taken 4 weeks after DALK showing a progressively worsening of the clinical picture with denser infiltrates and loss of graft transparency. *C*: IVCM (Heidelberg Retina Tomograph III- Rostock Cornea Module) images (300 mm × 300 mm) of the cornea showing high-contrast elongated particles resembling *Candida* pseudofilaments (*arrows*) in the posterior stroma at approximately 490 mm of depth. *D*: Slitlamp photograph taken 1 month after "ex vivo PACK-CXL on inverted graft" showing a marked improvement of graft transparency and the disappearance of the previous corneal infiltrates.

## DISCUSSION

In lamellar keratoplasty techniques, the presence of a corneal interface between the donor graft and the host cornea creates a closed environment for the development of interface infectious keratitis, which is particularly difficult to treat. Recently, several reports have described this complication in the setting of uneventful both anterior and posterior lamellar surgeries, and *Candida* species were isolated in most cases.[3–5,7–9] In a literature review, topical and systemic treatments were found to be not successful as alone on halting the progression, and irrigation of the interface with intrastromal injections in the deep stroma have been described as a salvage attempt that sometimes resulted effective.[1] In unresponsive cases, donor graft exchange or excisional penetrating keratoplasty were required to control the infection.[5] Relatively better results of visual outcomes and complications seem to be achievable in cases of interface infectious keratitis after DALK compared with those postendothelial keratoplasty because the host Descemet membrane is temporarily capable to withhold the infection and avoid its intraocular dissemination.[1]

In the case presented in this study, the detection in the early postoperative phase of small infiltrates located in the interface mimicking epithelial ingrowth alerted us about the possible risk of interface infectious keratitis. The progressive worsening of the clinical picture represented a further warning sign of infection that convinced us to proceed with a surgical approach. After peeling off the DALK graft, the infection seemed to be localized in the posterior side of the graft, whereas the recipient bed appeared clear and not infiltrated. Therefore, we decided to irrigate with antibiotics and antifungals the recipient bed and to perform PACK-CXL on the inverted (epithelium facing down) DALK graft that was then sutured again into the bed.

The antimicrobial effect of the adjuvant use of PACK-CXL has been demonstrated by both in vitro and in vivo studies, and its efficacy was confirmed by 2 recent meta-analyses.[10,11] Several mechanisms of action have been proposed for explaining its effects in the setting of infectious keratitis: (1) interferes with the enzymatic digestion caused by the pathogenic microorganisms that results in corneal melting; (2) induces keratocyte apoptosis followed by repopulation by proliferating cells; and (3) interferes with the microbial cell wall through free radical production.[10]

To the authors' knowledge, this report represents the first application of ex vivo PACK-CXL in a case of in- terface infectious keratitis. Our modified technique, ap- plied directly to the posterior side of the inverted DALK graft, allowed to obtain the maximum effect at the site of infection that otherwise is difficult to be reached with a conventional procedure performed through the anterior side of the cornea. Because corneal surgeons from many countries face with donor shortage, a further advantage of this approach is that it is tissue sparing.[12] This aspect might become even more crucial considering that the current COVID-19 pandemic could further exacerbate this shortage.

We cannot ascertain whether the final therapeutic effect depended exclusively on PACK-CXL procedure or washing the interface with antifungals provided a synergetic effect. However, it should be pointed out that irrigation with antimicrobial drugs is rarely efficacious as alone for the eradication of interface infectious keratitis, and the combination with graft removal or exchange is often required.[2] Another open issue is related to long-term effects of the direct application of PACK-CXL on the posterior stroma of the cornea because it is structurally different from the more superficial one that is conventionally treated in the setting of both keratoconus and infectious keratitis.[13] However, we did not detect alterations either in the curvature or in the transparency of the treated cornea throughout the entire period of examination. Further studies including post-DALK eyes affected by interface infectious keratitis of different severity and etiology are needed to confirm the safety and the efficacy of this new approach.

### WHAT WAS KNOWN

- Interface infectious keratitis is a rare but sight-threatening complication that can occur after any type of lamellar keratoplasty.
- Donor–host interface provides a hypoxic sequestered environment for microorganisms to thrive protected from the host immune response, thus making medical treatment likely unsuccessful.
- Early surgical intervention is recommended because of the potential for an adverse outcome, and in most severe cases, excisional penetrating keratoplasty can be required.

### WHAT THIS PAPER ADDS

- Ex vivo photoactivated chromophore for infectious keratitis-corneal crosslinking (PACK-CXL) on inverted graft is a new surgical approach for the management of interface infectious keratitis after deep anterior lamellar keratoplasty (DALK).
- According to this technique, PACK-CXL is applied directly to the posterior side of the inverted DALK graft (epithelium facing down), and then, the graft is sutured again in the recipient bed.
- This approach enabled the resolution of the infection while sparing the use of new donor tissue.

## REFERENCES


1. Fontana L, Moramarco A, Mandara` E, Russello G, Iovieno A. Interface infectious keratitis after anterior and posterior lamellar keratoplasty. Clinical features and treatment strategies. A review. Br J Ophthalmol 2019;103:307–314
2. Gao Y, Li C, Bu P, Zhang L, Bouchard CS. Infectious interface keratitis (IIK) following lamellar keratoplasty: a literature review. Ocul Surf 2019;17:635–643
3. Kitzmann AS, Wagoner MD, Syed NA, Goins KN. Donor-related *Candida* keratitis after Descemet stripping automated endothelial keratoplasty. Cornea 2009;28:825–828
4. Tu EY, Hou J. Intrastromal antifungal injection with secondary lamellar interface infusion for late-onset infectious keratitis after DSAEK. Cornea 2014;33:990–993
5. Busin M, Giannaccare G, Myerscough J, Bovone C, Scorcia V, Campos EC. Donor-to-host transmission of infection: contrasting outcomes of lamellar and penetrating keratoplasty. Transpl Int 2020;33:462–464
6. Hafezi F, Randleman JB. PACK-CXL: defining CXL for infectious keratitis. J Refract Surg 2014;30:438–439
7. Le Q, Wu D, Li Y, Ji J, Cai R, Xu J. Early-onset *Candida* glabrata interface keratitis after deep anterior lamellar keratoplasty. Optom Vis Sci 2015;92:e93–e96
8. Lyall DA, Srinivasan S, Roberts F. A case of interface keratitis following anterior lamellar keratoplasty. Surv Ophthalmol 2012;57:551–557
9. Nahum Y, Russo C, Madi S, Busin M. Interface infection after Descemet stripping automated endothelial keratoplasty: outcomes of therapeutic keratoplasty. Cornea 2014;33:893–898
10. Papaioannou L, Miligkos M, Papathanassiou M. Corneal collagen cross-linking for infectious keratitis: a systematic review and meta-analysis. Cornea 2016;35:62–71
11. Ting DSJ, Henein C, Said DG, Dua HS. Photoactivated chromophore for infectious keratitis—corneal cross linking (PACK-CXL): a systematic review and meta-analysis. Ocul Surf 2019;17:624–634
12. Romano V, Dinsdale M, Kaye S. Compensating for a shortage of corneal donors after Brexit. Lancet 2019;394:732
13. Meek KM, Knupp C. Corneal structure and transparency. Prog Retin Eye Res 2015;49:1–16



Disclosures: *None of the authors has a financial or proprietary interest in any material or method mentioned.*