Accelerated Corneal Cross-linking as an Adjunct Therapy in the Management of Presumed Bacterial Keratitis: A Cohort Study

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ABSTRACT

PURPOSE: To compare the outcomes of accelerated photoactivated chromophore for keratitis corneal cross-linking (PACK-CXL) as an adjunct treatment for bacterial keratitis (PACK-CXL plus standard antibiotic therapy) for patients receiving only standard antibiotic therapy.

METHODS: Retrospective cohort study of outcomes of patients with moderate infectious presumed bacterial keratitis (ulcer diameter 2 to 7 mm and stromal depth < 300 µm) were compared before and after initiation of a new treatment protocol of PACK-CXL in addition to standard antibiotic treatment.

RESULTS: A total of 70 eyes of 70 patients were included: 39 eyes in the PACK-CXL plus antibiotic (PACK-ABX) group and 31 eyes in the antibiotic only (ABX) control group. The PACK-ABX group showed shorter times to complete reepithelialization (9.3 ± 6.0 vs 16.0 ± 12.7 days, $P = .01$) and did not require tectonic emergency keratoplasty (0% versus 19.4%, $P = .006$). The PACK-ABX group also showed a higher percentage of eyes with complete reepithelialization in 6 days or less (46.2% vs 6.5%, $P < .001$) and a trend for shorter hospitalizations (6.3 ± 5.0 vs 8.5 ± 4.5 days, $P = .06$). A multivariate analysis controlling for age showed that PACK-ABX treatment remained significantly associated with early ulcer reepithelialization (odds ratio = 0.09, 95% confidence interval = 0.02 to 0.48, $P = .005$).

CONCLUSIONS: This study validates previous findings regarding the use of accelerated PACK-CXL in the treatment of bacterial keratitis. Adding PACK-CXL improved clinical outcomes (reducing healing time) when compared to antibiotics alone.

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Infectious bacterial keratitis is a major corneal disease that can lead to severe visual impairment and ocular morbidity.[1] The disease is one of the most common causes of monocular blindness worldwide and may account for 5.1% to 32.3% of all indications for penetrating keratoplasty.[2]

According to the guidelines of the American Academy of Ophthalmology (AAO), standard treatment includes broad-spectrum topical antibiotics and, after the identification of a pathogen, antibiotic treatment according to sensitivity. However, antimicrobial resistance is rising at an alarming rate, and the World Health Organization has published an urgent call to identify alternatives to antibiotic treatment in its global report on antimicrobial resistance.[3]

Recently, photoactivated chromophore for keratitis corneal cross-linking (PACK-CXL) had been proposed as an additional tool in the physicians' spectrum for

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treating infectious diseases.[4,5] First validated in a series of animal studies,[6-8] the clinical (human) studies published between 2008 and 2017 cover a total of 184 eyes treated with PACK-CXL as an adjunct to antimicrobial treatment, 182 of whom were treated with the Dresden protocol settings (CXL irradiation settings of 3 mW/cm² for 30 minutes).[9,10] However, only two cases were treated with the accelerated protocol, 9 mW/cm² for 10 minutes.[11-15] A recent meta-analysis[12] of PACK-CXL's efficacy in bacterial keratitis, which included almost entirely case reports and case series (23 of 25 included studies), reported a success rate of approximately 85%. The meta-analysis' authors concluded that further research is necessary to assess the efficacy of PACK-CXL.

In 2014, we showed in vitro that the antibacterial efficacy of PACK-CXL is preserved when the treatment is accelerated by a factor of 10 (ie, 30 mW/cm² for 3 minutes). Recently, we have used these accelerated PACK-CXL settings successfully in 20 eyes with moderate-sized, therapy-resistant bacterial keratitis.[14]

In this study, we further investigated accelerated PACK-CXL as an adjunct treatment in naïve patients with moderate presumed bacterial keratitis by expanding our treatment group and comparing the outcomes of patients treated before 2014 (standard antibiotic therapy) to those treated after the incorporation of PACK-CXL in our clinical routine.

PATIENTS AND METHODS

We retrospectively analyzed and compared the outcomes of all consecutive cases with infectious presumed bacterial keratitis treated with topical antibiotics prior to (2012–2014) and after (2015–2017) the initiation of an adjunct treatment protocol using PACK-CXL. Of the 116 cases treated between 2012 and 2017 that were reviewed, 70 eyes of 70 patients with moderate presumed bacterial keratitis were included: 39 eyes in the PACK-CXL with standard antibiotic therapy (PACK-ABX) treatment group and 31 eyes in the antibiotic only (ABX) treatment group. All 70 patients included in the study were treated in Soroka University Medical Center, Beer-Sheva, Israel.

Inclusion criteria comprised patients presenting with clinical signs of bacterial corneal ulcers with a diameter between 2 and 7 mm and a stromal depth of up to 300 µm, assessed by pachymetry (PachyPen; Accutome, Inc., Malvern, PA) and slit-lamp examination, to ensure that the ulcer did not extend beyond two-thirds of the cornea. The exclusion criteria were: keratitis of suspected viral, fungal, *Acanthamoeba*, or non-infectious origin, sterile infiltrates, descemetocele, corneal perforation, pregnancy or breastfeeding, systemic treatment involving steroids, immunosuppressed/immune-compromised patients, and diagnosed eczema (or atopic dermatitis). Patient enrollment in this study is presented in **Figure A** (available in the online version of this article).

The study protocol was approved by the institutional review board of the Ben-Gurion University of the Negev, Israel, and adhered to the tenets of the Declaration of Helsinki.

Antimicrobial Therapy

Antibiotic therapy was administered following AAO guidelines and consisted of fortified vancomycin eye drops (50 mg/mL) and fortified ceftazidime eye drops (50 mg/mL), after corneal scraping for bacterial and fungal analysis was obtained.[15] Both groups continued with the same initial treatment protocol (first day: hourly, second day: hourly excluding night time, third day: every 2 hours). Results of the culture sensitivity report determined whether we continued antibiotic therapy with either fortified vancomycin or fortified ceftazidime. Every patient in both groups was fitted with a therapeutic 14-mm diameter soft contact lens (PureVision, Balafilcon A; Bausch & Lomb, Rochester, NY) once 50% reepithelialization was achieved. In all cases, the decision to treat was made and the clinical management was performed by a single corneal specialist (BK).

All patients in the PACK-ABX group initially received antibiotic therapy for 72 hours and only received CXL treatment if clinical improvement was absent (defined as no reepithelialization, no resolving hypopyon or reduction in anterior chamber cells, visual loss, and/or increase in infiltrate size). In addition, 6 patients in the ABX group clinically deteriorated during their first days of hospitalization and underwent urgent penetrating keratoplasty. Their antibiotic regimen did not change and topical antimicrobial therapy was continued as per the standard department protocol detailed above.

CXL

Table 1 describes the CXL methods. The PACK-CXL procedure was performed using an accelerated protocol as described previously.[14] In brief, following the application of topical anesthesia, a 1-mm circumferential epithelium removal was performed around the borders of the infected corneal ulcer. Then, a hypo-osmolar 0.1% riboflavin solution (Medio-Cross 0.1%; Peschke Meditrade GmbH, Huenenberg, Switzerland) was instilled topically on the entire cornea every 2 minutes for 25 minutes. Because infection can result in tissue necrosis with consequent reduced corneal thickness, we used hypo-osmolaric 0.1% riboflavin solution to induce stromal swelling and reduce potential harm to the endothelium. The cornea was then ir-

TABLE 1
CXL Methods

Parameter	Variable
Treatment target	Corneal bacterial infiltrate/ulcer
Fluence (total) (J/cm^2)	5.4
Intensity (mW)	30
Treatment time (minutes)	3
Epithelium status	Off
Chromophore	Riboflavin (Medio-Cross 0.1%, Peschke Meditrade GmbH, Huenenberg, Switzerland)
Chromophore carrier	None
Chromophore osmolarity	Hypo-osmolar riboflavin solution
Chromophore concentration	0.1%
Light source	LightLink-CXL (LightMed, San Clemente, CA)
Irritation mode	Continuous
Protocol modification	Abrasion 1 mm around the border corneal infiltrate/ulcer; treatment 2 mm around the border corneal infiltrate/ulcer
Protocol abbreviation in manuscript	PACK-CXL

CXL = corneal cross-linking; PACK-CXL = photoactivated chromophore for keratitis corneal cross-linking

radiated at 365 nm with an intensity of 30 mW/cm^2 for 3 minutes (LightLink-CXL; LightMed, San Clemente, CA). PACK-CXL treatment was conducted once written informed consent had been obtained.

Cost Calculation

Treatment costs in the two groups were compared by calculating the cost of antimicrobial therapy, the PACK-CXL treatment, and each follow-up examination, as determined by the Israeli Ministry of Health's price list for ambulatory and hospitalization services. Prices were converted from New Israeli Shekel (NIS) to United States Dollar (USD) at the rate of 1 NIS = 0.28 USD.

Statistical Analysis

Patient characteristics are presented as mean ± standard deviation for continuous variables and as percentages for categorical variables. Categorical variables were compared using the chi-square or Fisher's exact test, as appropriate. Continuous variables were compared using either the Student's *t* test (normal distribution) or the Mann–Whitney *U* test (non-normal distribution). We used a paired *t* test to compare changes in visual acuity following treatment. Because the univariate results showed a significant age difference between the PACK-ABX and ABX groups, we subsequently used a logistic regression multivariate model including age adjustment and other relevant variables in a forward subset model to assess the outcome of reepithelialization by treatment. Statistical analyses were performed using SPSS software (version 24; IBM Corporation, Armonk, NY). *P* values less than .05 were considered statistically significant.

RESULTS

Baseline demographic and clinical characteristics are shown in **Table 2** and were similar between the ABX and PACK-ABX groups.

Clinical outcomes of the ABX and PACK-ABX groups are presented in **Table 3**. Both groups significantly improved in terms of CDVA following treatment. The difference in final visual acuities between each treatment group was not significant (*P* = .51). Complete reepithelialization of the cornea was achieved significantly more quickly in the PACK-ABX group than in the ABX group, and a greater proportion of patients in the PACK-ABX group achieved complete reepithelialization in 6 days or less than in the ABX group. **Figure B** (available in the online version of the article) shows three different cases prior to and at 7 and 21 days after PACK-CXL. In the PACK-ABX group, no patients required tectonic urgent keratoplasty, whereas 19.4% of patients underwent this urgent keratoplasty in the ABX group (*P* = .006). Finally, in the PACK-ABX group, the number of visits during the entire follow-up period was significantly lower and the overall follow-up duration was shorter when compared to the ABX group. No significant difference was observed regarding cost between the PACK-ABX and ABX groups (1,321.5 ± 339.2 vs 1,186.0 ± 1,081.7 USD per patient, *P* = .40).

The multivariate analysis showed that treatment with PACK-ABX remained significantly associated with early ulcer reepithelialization when controlling for age and other possible confounders such as diabetes mellitus status and positive bacterial analysis (odds ratio = 0.09, 95% confidence interval = 0.02 to 0.48, *P* = .005). Analysis of patients with positive cultures (n = 35) also showed that the PACK-ABX group (n = 22) fared better than the ABX group (n = 13) with regard to faster epithelialization (8.7 ± 6.4 vs 18.7 ± 15.5 days, *P* = .043) and hospitalization time (5.8 ± 5.5 vs 10.0 ± 5.7 days, *P* = .040), better visual acuity at the end of follow-up (CDVA = 0.8 ± 0.7 vs 1.3 ± 0.6 logMAR, *P* = .021; uncorrected distance visual acuity = 0.9 ± 0.7 vs 1.4 ± 5.7 logMAR, *P* = .018), and lower rates of urgent keratoplasty (0% vs 23%, *P* = .044).

TABLE 2

Basic Characteristics of Patients in the ABX and PACK-ABX Groups

Characteristic	ABX (n = 31)	PACK-ABX (n = 39)	*P*
Age (y), mean ± SD	71.23 ± 19.72	48.62 ± 26.75	< .001
Female gender, no. (%)	13 (41.9)	15 (38.5)	.76
Diabetes mellitus, no. (%)	10 (32)	9 (23)	.357
Cause = wearing of contact lenses, no. (%)	3 (9.7)	10 (25.6)	.092
Cause = infected suture, no. (%)	5 (16.1)	7 (17.9)	.8436
Cause = corneal erosions, no. (%)	11 (35.5)	10 (25.6)	.3727
Cause = bullous keratopathy, no. (%)	12 (38.7)	12 (30.8)	.4923
Overall difference in cause			.35
Size of infiltrate (mm), mean ± SD	3.08 ± 1.14	3.16 ± 1.32	.78
Anterior chamber reactions, no. (%)	11 (35.5)	15 (38.5)	.79
Hypopyon, no. (%)	5 (16.1)	8 (20.5)	.64
Positive culture, no. (%)	13 (41.9)	22 (56.4)	.23
Bacteria Gram (+), no. (%)	8 (25.8)	6 (15.4)	.07
Bacteria Gram (–), no. (%)	5 (16.1)	16 (41.0)	.035
Preoperative UDVA (logMAR), mean ± SD	1.43 ± 0.5	1.43 ± 0.5	.987
Preoperative CDVA (logMAR), mean ± SD	1.41 ± 0.6	1.34 ± 0.6	.677

ABX = antibiotic only; PACK-ABX = photoactivated chromophore for keratitis corneal cross-linking plus antibiotic; SD = standard deviation; UDVA = uncorrected distance visual acuity; CDVA = corrected distance visual acuity

TABLE 3

Clinical Outcomes of Patients in the ABX and PACK-ABX Groups

Characteristic	ABX (n = 31)	PACK-ABX (n = 39)	*P*
Final CDVA (logMAR), mean ± SD	0.97 ± 0.7	0.97 ± 0.7	.51
Delta CDVA (logMAR), mean ± SD	0.32 ± 0.5	0.37 ± 0.6	.709
Hospitalization (days), mean ± SD	8.52 ± 4.54	6.33 ± 4.95	.06
Urgent keratoplasty,[a] no. (%)	6 (19.4)	0 (0)	.006
Follow-up (months), mean ± SD	4.48 ± 2.65	2.51 ± 1.76	.001
Days of reepithelialization, mean ± SD	16 ± 12.7	9.3 ± 6.0	.01
Reepithelialization ≤ 6 days, no. (%)	2 (6.5)	18 (46.2)	< .001
No. of visits, median (IQR)	11 (5 to 20)	5 (2 to 7)	< .001

ABX = antibiotic; PACK-ABX = photoactivated chromophore for keratitis corneal cross-linking plus antibiotic; CDVA = corrected distance visual acuity; SD = standard deviation; IQR = interquartile range

[a]The decision to perform urgent keratoplasty was primarily based on severe descemetocele formation or when perforation was imminent or already occurred.

DISCUSSION

In this non-randomized cohort study, we evaluated the effect of PACK-CXL as an adjunct to the standard antimicrobial treatment of presumed bacterial keratitis by comparing cases prior to and following the implementation of a PACK-CXL protocol in our clinic. Our results show that adjunct treatment with accelerated PACK-CXL resulted in a significantly shorter time to reepithelialization, fewer follow-up visits, and a shorter total follow-up when compared to standard antimicrobial therapy alone. Moreover, accelerated PACK-CXL significantly reduced the need for tectonic urgent keratoplasty.

In 2008, Iseli et al.[4] successfully treated 5 eyes with infectious keratitis using the standard CXL settings of 3 mW/cm² for 30 minutes (Dresden protocol). In the same year, Martins et al.[16] used the same technical settings in vitro to demonstrate the effect of ultraviolet-A (UV-A) and riboflavin against a variety of pathogens involved in infectious keratitis. In 2014, our group published an in vitro study[3] in which CXL, using accelerated protocols of 18 mW/cm² for 5 minutes and

36 mW/cm² for 2.5 minutes, had similar killing rates (approximately 93%) for *Staphylococcus aureus* and *Pseudomonas aeruginosa* when compared to the initial 3 mW/cm² for 30 minutes protocol. Our previous work has also shown that adding accelerated PACK-CXL (30 mW/cm² for 3 minutes) to the standard antibiotic treatment regimen is safe.[17]

PACK-CXL is based on the use of riboflavin as a photosensitizer when activated by UV-A light at 365 nm to negatively influence pathogen survival and to locally strengthen the stroma. The elimination of pathogens may be a direct effect of UV-A light and the generation of reactive oxygen species during the photoactivation process, which affects the cell wall and nucleus. The photoactivated chromophore, riboflavin, can also intercalate with the pathogen's DNA and RNA, leading to nucleic acid degradation and inhibition of replication.[18,19] In addition, changes in the stromal collagen fibers and proteoglycans caused by CXL renders stroma more resistant to enzymatic degradation and melting.[20] PACK-CXL appears to maintain its efficacy when accelerated to 3 minutes, both under laboratory conditions[3] and in vivo, as demonstrated previously.[14]

It is worth noting that patients in the PACK-ABX group had higher rates of infection by Gram-negative bacteria (41.0% vs 16.1%, $P = .035$). Alió et al.[21] reviewed 104 cases of infectious keratitis and reported that CXL had the same effectiveness against Gram-negative (92%; 13 of 14 cases) and Gram-positive (84%; 37 of 44 cases) bacteria (the *P* value was not reported in the Alió et al. study, but we calculated it as .664 using Fisher's exact test). Additionally, it has previously been shown in vitro that the antibacterial effect of accelerated CXL (2.5 minutes at 36 mW/cm²) was similar for Gram-positive (killing rates for *S. aureus*: 94.4% ± 2.9%) and Gram-negative (killing rates for *P. aeruginosa*: 92.9% ± 5.0%) bacteria.[3]

Accelerated PACK-CXL may have several advantages over standard antimicrobial therapy. Our results show that PACK-CXL was associated with shorter hospitalizations, faster time to reepithelialization, and a reduced number of visits to the clinic, suggesting that PACK-CXL might be valuable economically, especially in rural areas or those with limited access to health care. Furthermore, the fact that this is a single, short therapy might prove advantageous in people who have low compliance with treatment regimens, because the continuous use of antibiotics requires a high degree of compliance and frequent follow-up visits.[22] In addition, in light of ever-increasing antimicrobial resistance, new treatment modalities are required as an adjunct and stand-alone alternatives to antibiotics.[17,23] For these reasons, it is also worth considering the possible advantages this treatment modality might have in developing countries. Maintaining frequent clinic visits and adherence to treatment regimens is impractical in many underdeveloped regions and access to health care is also limited by economic constraints. Coupled with the high prevalence of bacterial keratitis, this modality could have important implications for health care in the underdeveloped world and poses an interesting avenue for future research.

Another intriguing reason to use PACK-CXL in addition to antibiotic treatment is the fact that PACK-CXL treatment has two properties that set it apart from antibiotics. First, not only does PACK-CXL kill pathogens, but it also increases corneal enzymatic resistance to collagen digesting enzymes (pepsin, trypsin, and collagenase), which supports its role in the treatment of infected ulcers[20,24] (although demonstrating this effect in a clinical study would require a much higher number of eyes to be treated than were present in this study). Second, PACK-CXL kills not only bacterial pathogens but also, to a certain extent, fungal pathogens. This makes PACK-CXL particularly interesting in those cases where the clinician suspects a mixed (bacterial/fungal) infection.

Our study has several limitations. First, not all confounders could be accounted for due to the retrospective design of the study. Second, patient allocation was determined using a before-and-after method, which could introduce bias in the form of differing patient populations and treatment variations with time and different age, because age is also a factor for greater natural CXL, which occurs with aging.

In fact, one major difference in baseline characteristics between groups was age (mean difference of 21 years, range = 9.6 to 32.4 years, $P = .002$), which is a known factor for more severe keratitis with poorer visual outcomes.[25] We accounted for this by incorporating the patient's age into a multivariate analysis, which showed consistent results. Nevertheless, both groups were similar with regard to ulcer size and the occurrence of diabetes mellitus, which can affect the severity of keratitis and epithelialization time.[26] Also, patients treated after 2014 who improved with antibiotics alone and did not receive PACK-CXL were not available as a negative control group. Finally, the bacterial nature of the keratitis could not be confirmed by smear or culture in all cases. Negative smears and cultures are common in bacterial keratitis and represent one of the many challenges of keratitis treatment.

Finally, this study did not show any visual acuity benefit of adding antibiotic therapy to PACK-CXL; there was no difference in final visual outcomes in the PACK-ABX and ABX groups. Visual rehabilitation

as an outcome measure is more complex than reporting the arrest of melting, tectonic transplantation, or epithelialization time.[21,27] In this study, performed in patients with a moderately sized ulcer (between 2 and 7 mm in diameter), the resolution of the keratitis would end with a stromal scar, irregular astigmatism, and the necessity for future corneal surgeries.[28] In addition, the follow-up was shorter in the PACK-ABX group (2.51 ± 1.76 months compared with 4.48 ± 2.65 months in the ABX group, $P = .001$). Because visual acuity in patients with bacterial keratitis may continue to improve over a year of follow-up,[29] it might be that a longer follow-up period would be necessary to reveal any effect of adjunct CXL on visual acuity outcome.

A strength of this study is the fact that it was conducted in a single center and by a single corneal specialist, reducing the likelihood of variations in the clinical management of the individual cases.

Our results support the statement that the adjunctive use of an accelerated PACK-CXL protocol is associated with shorter time to reepithelialization, lower rates of keratoplasty, and a trend for shorter hospitalizations when compared to patients treated with antibiotics alone. These results may help in the clinical management of bacterial keratitis and support a role for PACK-CXL as an additional treatment to improve clinical outcomes.

AUTHOR CONTRIBUTIONS

Study concept and design (BK, YK); data collection (BK, YK); analysis and interpretation of data (BK, YK, MAT, AA, IH, TL, EAT-N, NLH, FH); writing the manuscript (BK, YK, AA, IH, EAT-N, NLH, FH); critical revision of the manuscript (BK, MAT, AA, IH, TL, EAT-N, NLH, FH); statistical expertise (MAT, AA, IH); supervision (BK, TL, EAT-N, NLH, FH)

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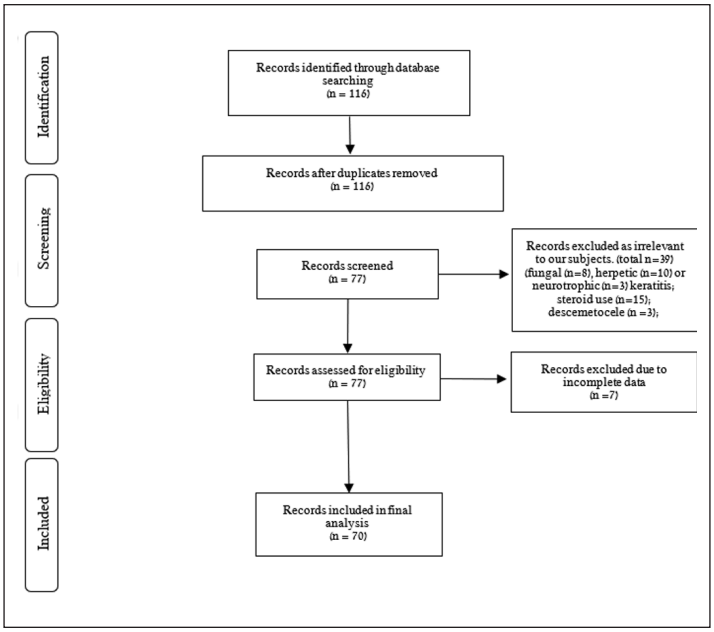

Figure A. Flow diagram of the inclusion process.

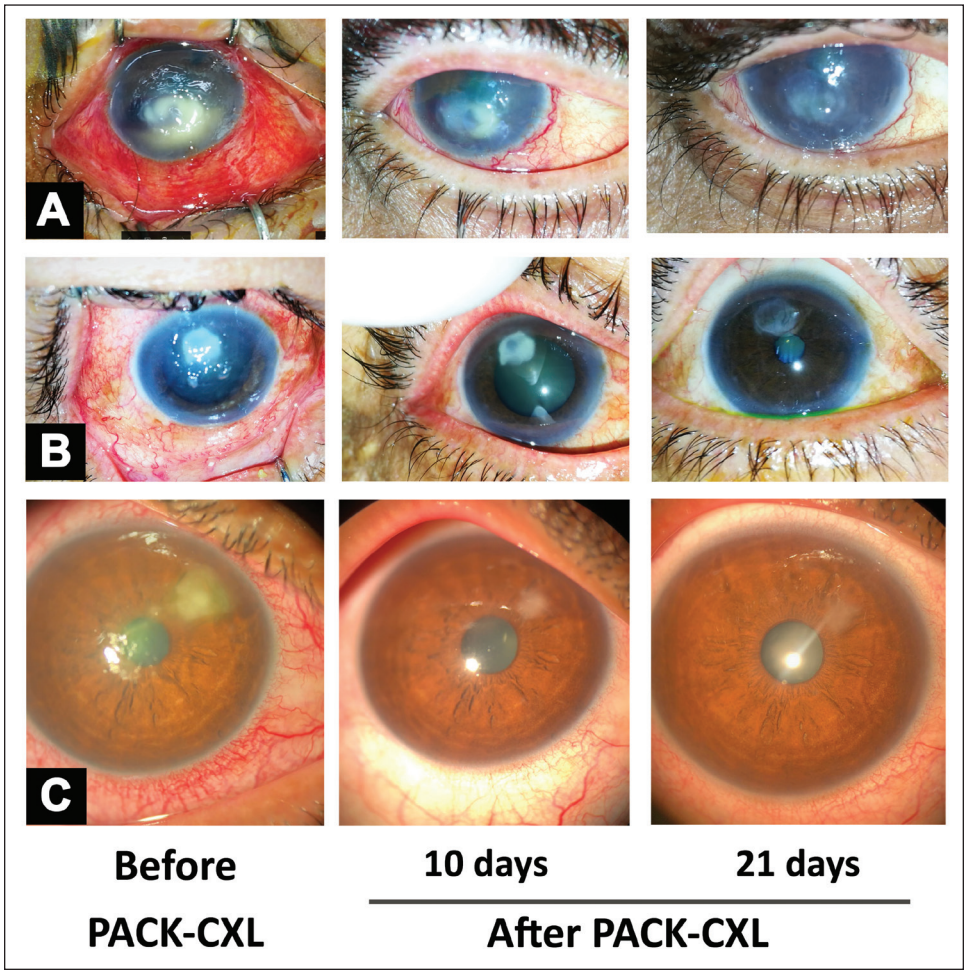

Figure B. Three cases of infectious keratitis (left) prior to and at (middle) 7 and (right) 21 days after photoactivated chromophore for keratitis corneal cross-linking (PACK-CXL). All cases showed complete reepithelialization and beginning scar formation at 21 days postoperatively.