Collagen Cross-Linking with Photoactivated Riboflavin (PACK-CXL) for the Treatment of Advanced Infectious Keratitis with Corneal Melting

Dalia G. Said, MD, FRCS,[1,*] *Mohamed S. Elalfy, MSc,*[1,*] *Zisis Gatzioufas, MD, PhD,*[2] *Ehab S. El-Zakzouk, PhD,*[1] *Mansour A. Hassan, MD,*[3] *Mohamed Y. Saif, MD,*[3] *Ahmed A. Zaki, MD,*[1] *Harminder S. Dua, MD, PhD,*[4] *Farhad Hafezi, MD, PhD*[2,5]

Purpose: To investigate the efficacy and safety of corneal collagen cross-linking (CXL) with photoactivated riboflavin (photoactivated chromophore for infectious keratitis [PACK]−CXL) in the management of infectious keratitis with corneal melting.

Design: Prospective clinical trial.

Participants: Forty eyes from 40 patients with advanced infectious keratitis and coexisting corneal melting.

Methods: Twenty-one patients (21 eyes) underwent PACK-CXL treatment in addition to antimicrobial therapy. The control group consisted of 19 patients (19 eyes) who received only antimicrobial therapy.

Main Outcome Measures: The slit-lamp characteristics of the corneal ulceration, corrected distance visual acuity, duration until healing, and complications were documented in each group. The Mann−Whitney *U* test was used for statistical analysis. *P* values less than 0.05 were considered statistically significant.

Results: The average time until healing was 39.76±18.22 days in the PACK-CXL group and 46.05±27.44 days in the control group ($P = 0.68$). After treatment and healing, corrected distance visual acuity was 1.64±0.62 in the PACK-CXL group and 1.67±0.48 in the control group ($P = 0.68$). The corneal ulceration's width and length was significantly bigger in the PACK-CXL group ($P = 0.004$ and $P = 0.007$). Three patients in the control group demonstrated corneal perforation; infection recurred in 1 of them. No serious complications occurred in the PACK-CXL group.

Conclusions: Corneal CXL with photoactivated riboflavin did not shorten the time to corneal healing; however, the complication rate was 21% in the control group, whereas there was no incidence of corneal perforation or recurrence of the infection in the PACK-CXL group. These results indicate that PACK-CXL may be an effective adjuvant therapy in the management of severe infectious keratitis associated with corneal melting. *Ophthalmology 2014;■:1−6 © 2014 by the American Academy of Ophthalmology.*

Infectious keratitis is a potentially sight-threatening condition of the cornea often presenting as an ophthalmic emergency. Delayed treatment of infectious keratitis can lead to significant visual loss in as many as 50% of cases.[1] A wide range of fungi, bacteria, protozoa, and viruses has been identified as infectious agents in microbial keratitis. Most community-acquired cases of microbial keratitis resolve with empiric treatment using broad-spectrum topical antimicrobials.[2] However, the emergence and spread of antimicrobial-resistant organisms remain a serious clinical and public health concern.[3−6] In infectious keratitis, antimicrobial-resistant strains are associated with a worse clinical presentation and marked visual impairment. A considerable amount of research is directed toward developing newer antibiotics or defining alternative methods of treatment. Corneal degradation and melting occurs when specific proteinases are upregulated after corneal damage. These matrix metalloproteinases are synthesized either in the keratocytes (matrix metalloproteinase 2) or by corneal epithelial cells (matrix metalloproteinase 9) and are also responsible for delayed epithelial wound healing.[7−10]

Corneal collagen cross-linking (CXL) is a novel technique that was developed and introduced in 1999 for the treatment of keratoconus and postoperative ectasia.[11−13] During the standard CXL procedure, the corneal epithelium is removed and the corneal tissue is irradiated with ultraviolet A light after instillation of the photosensitizer riboflavin. The induced photochemical reaction facilitates the strengthening of the collagen lamellae in corneal stroma, thereby stabilizing the cornea biomechanically.

It has been shown that CXL demonstrates excellent antimicrobial efficacy against a variety of common pathogens in vitro.[14] The evidence that CXL can treat clinical microbial keratitis effectively and can arrest the progression of corneal melting, however, is limited.[15−17] To distinguish better the use of CXL for the treatment of infectious keratitis from CXL for progressive keratoconus, the term photoactivated chromophore for infectious keratitis (PACK)-CXL was created at the ninth cross-linking

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congress in Dublin, Ireland, in 2013. Within this context, the aim of our study was to investigate the clinical efficiency and safety of PACK-CXL in the management of infectious keratitis associated with corneal melting.

Methods

This prospective clinical trial was conducted between January 2010 and April 2013 at the Cornea Clinic of the Research Institute of Ophthalmology, Cairo, Egypt, in collaboration with the Department of Ophthalmology, Beni-Suef University Hospitals, Beni-Suef, Egypt; the Rowad Correction Centre, Cairo, Egypt; the Department of Ophthalmology, University of Nottingham, Nottingham, United Kingdom; and the Department of Ophthalmology, University of Geneva, Geneva, Switzerland. The Research Institute of Ophthalmology Institutional Review Board approved the study protocol, which adhered to the tenets of the Declaration of Helsinki, and written informed consent was obtained from all participants before inclusion.

Participants

The study included adult patients (>18 years of age) seeking treatment at the Cornea Clinic of the Research Institute of Ophthalmology with infective corneal ulcer with a possible bacterial, fungal, *Acanthamoeba*, or mixed origin with evident corneal melting. Exclusion criteria were age younger than 18 years, corneal ulceration in proximity (1 mm) to the corneal limbus, underlying autoimmune disease, history of herpetic eye disease, corneal thickness less than 400 μm with epithelium, or pregnancy or nursing. Patients who agreed to be enrolled in the study and provided informed consent were randomized according to the order of presentation alternately into either of the 2 groups: a group treated with PACK-CXL and medical treatment and a control group that received medical treatment alone.

Ophthalmologic Examination and Medical Treatment

After admission, the initial examination included the patient's medical history, specifically the history of contact lens wear, the duration and type of treatment before the first visit, the presence of systemic diseases, measurement of corrected distance visual acuity, slit-lamp examination, slit-lamp photography, and ultrasound pachymetry of corneal thickness. The parameters evaluated during slit-lamp examination included the localization and extent (longest diameter and at right angles to it) of corneal ulcer, the site and extent (diameter) of infiltrate, the localization of corneal vascularization, and the presence of hypopyon.

At presentation, all pre-existing treatment was interrupted for 24 hours and corneal scrapes for direct smears and cultures were obtained. Initial antimicrobial therapy for both groups consisted of fortified vancomycin eye drops 50 mg/ml, fortified ceftazidime eye drops 50 mg/ml hourly, and the antifungal agent itraconazole 100 mg orally twice daily. This regimen was subject to change according to response or culture results.

Corneal Collagen Cross-Linking with Photoactivated Riboflavin Treatment

Patients allocated to the PACK-CXL group were assessed and treated with PACK-CXL within 48 hours. Topical anesthesia was achieved using 0.4% benoxinate hydrochloride drops. Epithelium was removed up to 9 mm in diameter. Corneal thickness of the area to be treated was measured (without epithelium), aiming for a starting thickness of no less than 350 μm and no more than 500 μm. Corneas thicker than 500 μm were deswelled using 70% glycerol drops (prepared at our local pharmacy) applied topically at intervals of 2 to 3 seconds for 5 minutes.

Iso-osmolar riboflavin drops (Medio-Cross 0.1% riboflavin/dextran solution; Peschke Meditrade GmbH, Huenenberg, Switzerland) were instilled topically on the cornea every 2 minutes for over 30 minutes, and the thickness was remeasured every 5 minutes to ensure that it remained less than 500 μm during the course of instillation. In 1 case, the cornea deswelled to 260 μm and hypo-osmolar riboflavin was instilled until the corneal thickness reached 350 μm.

The cornea was illuminated using a UVX lamp (Peschke Meditrade GmbH), 365-nm ultraviolet A with an irradiance of 3 mW/cm^2 for 30 minutes and a total dose of 5.4 J/cm^2, during which riboflavin was instilled every 2 minutes and corneal pachymetry performed every 5 minutes. Corneal CXL was performed in a 9-mm-diameter zone. After PACK-CXL treatment, antimicrobial treatment was continued as before and daily follow-up examination was performed until healing was complete. Complete healing was defined as re-epithelialization of the corneal epithelial defect with disappearance of hypopyon with no anterior chamber activity and clearing of stromal infiltrate. All complications, including perforation of the corneal stroma, were recorded.

Statistical Analysis

Data were analyzed with the Statistical Package for Social Sciences version 17.0 (IBM Corp, Armonk, NY). All data were expressed as the mean±standard deviation. Normal distribution of data was evaluated by the Shapiro-Wilk test. Groups were compared using the Mann−Whitney *U* test, and *P* values less than 0.05 were considered statistically significant.

Results

The PACK-CXL group included 21 patients (8 men and 13 women) with a mean age of 37.3 years. The control group included 19 patients (10 men and 9 women) with a mean age of 49.8 years. The baseline corrected distance visual acuity at presentation was 2.16±0.35 logarithm of the minimum angle of resolution (logMAR) units in the PACK-CXL group and 2.01±0.44 logMAR in the control group ($P = 0.11$). The isolated causative microorganisms for each group are shown in Table 1 for the PACK-CXL group and in Table 2 for the control group. *Staphylococcus* and *Aspergillus* were the most common bacterial and fungal genera isolated in culture, respectively. Slit-lamp findings of the corneal ulcer in both groups are depicted in Tables 1 and 2. The mean size of the ulcer was larger in the PACK-CXL group (5.62±1.88×6.22±1.98 mm; $P = 0.004$) than in the control group (3.97±2.5×4.22±2.18 mm; $P = 0.007$). The mean duration to complete healing was 39.76±18.22 days in the PACK-CXL group and 46.05±27.44 in the control group ($P = 0.68$). The average corrected distance visual acuity after complete healing was 1.64±0.62 logMAR in the PACK-CXL group and 1.67±0.48 logMAR in the control group ($P = 0.68$). Figures 1 and 2 show color fundus photographs and fluorescein staining images from patients 19 and 20, respectively, before and after PACK-CXL.

Three patients in the control group had corneal perforation, whereas patients treated with PACK-CXL did not experience this complication. Infection did recur in 1 patient from the control group, making the total complication rate 21%. In contrast, no severe complications occurred in the PACK-CXL group. Corneas that exceeded 500 μm in thickness were treated with glycerol, which caused conjunctival chemosis in some patients. In 1 eye, glycerol application caused excessive

Table 1. Corneal Collagen Cross-Linking Group

Patient No.	Visual Acuity (Logarithm of the Minimum Angle of Resolution)	Ulcer Size (mm)	Area of Infiltrate (mm)	Site	Length of Treatment before Presentation (Weeks)	Organism	Time to Healing (Days)	Final Visual Acuity (Logarithm of the Minimum Angle of Resolution)
1	2.28	6×5	9×9	Central	24	*Staphylococcus epidermidis*	42	1.78
2	2.28	8×7.5	8×8	Central	3	*S. epidermidis*	40	0.78
3	0.78	3×3	4×4	Paracentral	2	*Acanthamoeba* cysts	26	0.48
4	1.98	4.5×6	6×6	Central	6	*Staphylococcus aureus*	35	0.78
5	2.28	5×7	5×7	Central	8	Mucor	42	1.48
6	2.28	3×4.5	8×8	Central	3	*S. epidermidis*	26	1.98
7	2.28	4.5×4	6×7	Paracentral	8	*Pseudomonas*	31	1.98
8	2.28	6×7	7×8	Paracentral	4	*S. epidermidis*	19	2.28
9	2.28	5×5.5	7×7	Central	4	*S. epidermidis*, green fungus	35	1.3
10	1.98	4.5×4	7×8	Paracentral	16	*S. epidermidis*	14	1.02
11	1.98	1×3	2×4	Paracentral	1	No growth	47	1.00
12	2.28	5×6	6×6	Central	16	*S. epidermidis*, *Aspergillus niger*	40	1.98
13	2.28	7×8	8×8	Central	1	No growth	26	1.98
14	1.98	6×6	7×7	Central	2	No growth	19	1.08
15	2.28	8×9	9×9	Central	4	*Fusarium*	31	1.48
16	2.6	9×10	10×10	Central	12	*Pseudomonas*, *Aspergillus*	33	2.28
17	2.28	7×9	7×9	Central	24	*S. epidermidis*, *Aspergillus*	63	2.28
18	2.28	7×8	9×9	Central	16	*Moraxella*, *Aspergillus*	67	2.28
19	2.28	8×6	7×6.5	Central	4	*Aspergillus terreus*, *Pneumococci*, gram-negative bacilli	53	0.80
20	2.28	7×6	6×5	Central	4	*Candida*, *Pneumococci*	56	2.28
21	2.28	8×8	6×6	Central	4	*Fusarium*	90	2.28

deswelling that was treated with hypo-osmolar riboflavin before PACK-CXL. All patients treated with PACK-CXL exhibited limbitis after treatment, which resolved in 5 to 7 days in all but 1 case. In 1 patient (*Acanthamoeba* keratitis), limbitis persisted for 3 weeks after the PACK-CXL treatment, despite a reduction in the size of the ulcer.

Discussion

Infectious keratitis is a severe ocular infection and one of the leading causes of monocular blindness worldwide.[18] The incidence of microbial keratitis ranges from 6.3 to 710 cases per 100 000 population per year and is even more common in contact lens wearers.[19] Various microorganisms, including bacteria, viruses, fungi, and parasites, may cause infectious keratitis. This infection and inflammatory reaction may lead to ulceration, corneal melting, and perforation if not treated adequately. The increasing resistance to antimicrobial agents has contributed to a dramatic increase in keratitis-related complications with devastating consequences.[4–6] Therefore, current research focuses on innovative treatment options beyond antimicrobials for the management of infectious keratitis, particularly for the treatment of resistant forms.

There is a body of clinical evidence that supports the antimicrobial efficacy of PACK-CXL.[15–17] Iseli et al[15] demonstrated immediate regression of the corneal melting process and a significant decrease in infiltrate size after PACK-CXL in 5 patients with therapy-resistant bacterial or fungal ulcerative keratitis. Makdoumi et al[16] used PACK-CXL as primary therapy in patients (7 eyes, 6 patients) with bacterial keratitis and reported symptomatic relief and arrest of progression of melting in all cases. Panda et al[17] treated patients with antimicrobial-refractory, keratitis-associated corneal melting with PACK-CXL. The melting was halted, and emergency keratoplasty was avoided in all 7 eyes. Similarly, Kozobolis et al[20] presented excellent clinical outcomes after PACK-CXL in 2 patients with combined bullous keratopathy and ulcerative keratitis. Skaat et al[21] reported good results of PACK-CXL in the management of refractory infectious keratitis in 6 patients, whereas PACK-CXL also has been applied successfully in the treatment of fungal keratitis and post-LASIK keratitis associated with corneal melt.[22,23] Finally, PACK-CXL healed corneal ulceration and eliminated edema and painful symptoms in a patient with *Escherichia coli* keratitis.[24] Our results concur with published reports; however, to our knowledge, we are the first to report a series of more than 7 eyes that includes a control comparator. This control group afforded us the opportunity to estimate the rate of serious complications. Patients receiving only antimicrobial therapy demonstrated a significant complication rate compared with those who additionally underwent PACK-CXL (21% vs. 0%). No significant differences regarding the time to healing and the final visual outcome were observed between the 2 groups. Corneal CXL was associated with transient limbitis, a nonserious complication not seen in the control group.

Table 2. Medication Only Group (Controls)

Patient No.	Visual Acuity (Logarithm of the Minimum Angle of Resolution)	Ulcer Size (mm)	Area of Infiltrate (mm)	Site	Length of Treatment before Presentation (Weeks)	Organism	Time to Healing (Days)	Final Visual Acuity (Logarithm of the Minimum Angle of Resolution)
1	1.98	3×6	3×4	Central	8	*Staphylococcus epidermidis*	28	1.48
2	2.28	4×3	4×3	Central	24	No growth	63	1.98
3	1.18	3×1	2×1	Paracentral	4	*S. epidermidis*, *Neisseria*	20	1.02
4	2.28	5×4	4×4	Central	3	*Candida*, *Aspergillus*	55	1.98
5	1.98	3×4	3×2	Central	4	No growth	35	1.98
6	2.28	2×3	2×3	Central	1	*S. epidermidis*	21	1.98
7	2.28	7×8	7×8	Central	4	*S. epidermidis*	120 (60 after perforation)	2.28
8	2.28	6×5	5×5	Central	4	*S. epidermidis*	60	1.98
9	1.98	3×4	3×4	Central	4	Diphtheroids	35	1.98
10	1	1×2	1×2	Paracentral	8	*Acanthamoeba*	60	0.78
11	1.3	1×2	1×2	Paracentral	2	No growth	21	0.98
12	1.98	3×4	3×3	Central	4	No growth	60	1.98
13	2.6	8×8	6×5	Central	16	*S. epidermidis*, *Candida*	90	1.98
14	2.28	3×4	3×4	Central	2	*Acanthamoeba*	21	1.3
15	2.6	9×8	7×6	Central	4	*Staphylococcus*	60	1.98
16	1.98	0.5×2	1×2	Central	12	*Candida*	35	1.78
17	2.28	8×7	8×8	Central	4	*Pseudomonas*	56	2.28
18	1.98	3×3	3×3	Paracentral	4	No growth	21	1.02
19	1.78	3×4	3×4	Central	2	No growth	14	1.08

We also noted a transient increase in the size of hypopyon in the first 24 hours after PACK-CXL in some patients. Thereafter, the hypopyon decreased gradually until it disappeared (Fig 1A, B). This may be the result of a reaction to the massive and simultaneous death of micro-organism and release of endotoxins, similar to a Jarisch-Herxheimer reaction.[25]

Treatment with PACK-CXL halts corneal melting and improves infectious keratitis through at least 2 mechanisms, and these probably operate in synergy. First, it is well established that pathogens implicated in corneal melting may act by enzymatic digestion.[26,27] Because PACK-CXL increases tissue resistance to enzymatic digestion, the cross-linking procedure may help the corneal stroma resist proteolysis by enzymes from polymorphonuclear leukocytes participating in the inflammatory process.[28] A fortified stroma also may block the penetration or effect of toxins from the pathogenic organism. Second, the phenomenon of apoptosis induced by PACK-CXL likely not only kills keratocytes but also kills microbes, which decelerates the infectious process.[29] Indeed, in vitro studies support this latter antimicrobial mechanism. Treatment with PACK-CXL has documented bactericidal

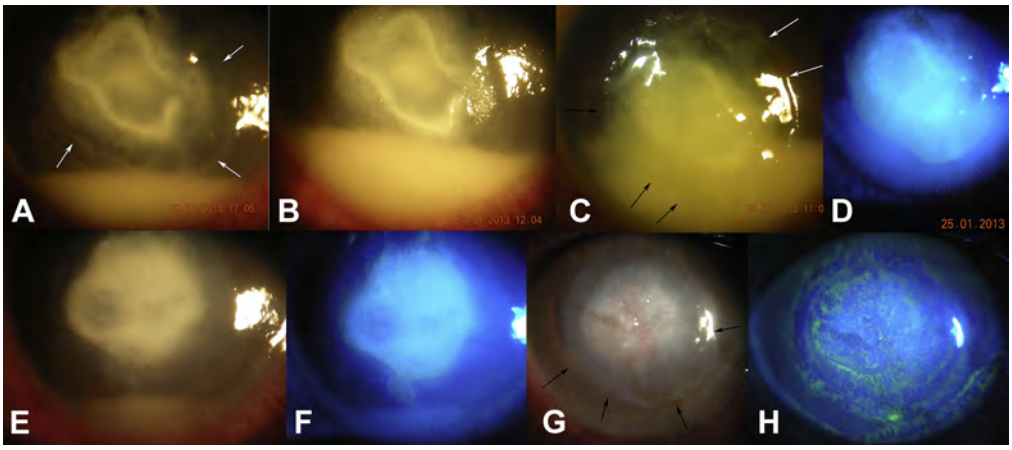

Figure 1. Images from patient 19 obtained at presentation. **A**, Note the central shield over the infiltrate. The epithelial defect was bigger than the infiltrate (arrows indicate the extent of epithelial defect). **B**, Two days later, the infiltrate and hypopyon had increased. **C**, **D**, Two days after corneal collagen cross-linking (CXL), the epithelial defect (arrows) corresponding to the area of treatment had increased. The hypopyon increased with increased inflammatory reaction after CXL. **E**, Nine days after CXL, the infiltrate changed its shape and the hypopyon was reduced. **F**, Fluorescein staining showed that the ulcer had reduced to be equal to the size of the infiltrate. **G**, Complete healing 53 days after CXL. Arrow indicates the scar. **H**, Fluorescein staining showing residual pooling effect with no active ulceration.

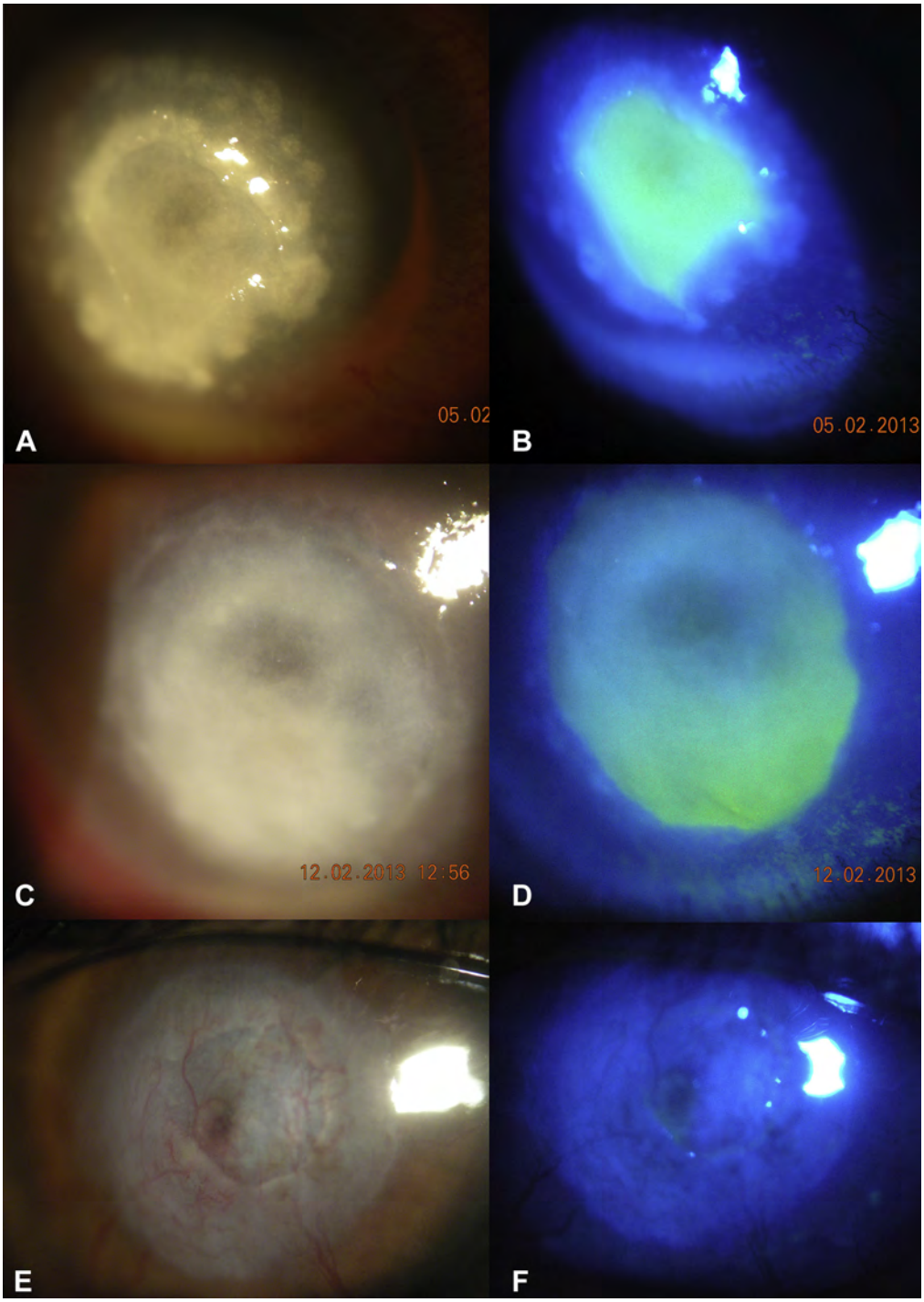

Figure 2. Images from patient 20 obtained at presentation. **A**, **B**, Well-demarcated deep central defect with multiple surrounding satellites. **C**, **D**, Four days after corneal collagen cross-linking (CXL), the defect was less demarcated, and satellites could not be distinguished from the central lesion anymore. **E**, **F**, Sixty days after CXL, the stroma has transformed into a vascularized scar in the absence of fluorescence staining.

activity against some common pathogens in vitro, such as *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, methicillin-resistant *S. aureus*, multidrug-resistant *P. aeruginosa*, and drug-resistant *Streptococcus pneumoniae*.[14] Also, PACK-CXL has exhibited antimicrobial effects against fungal pathogens in vitro, such as *Candida albicans*, *Fusarium* species, and *Aspergillus fumigatus*.[30]

Our results demonstrated the beneficial effect of PACK-CXL in cases of infectious keratitis with corneal melting. In the management of infectious keratitis with corneal melting, PACK-CXL could serve as valuable adjuvant therapy. This treatment may minimize or avoid severe complications, such as corneal perforation, recurrence of the infection, or both.

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[1] Research Institute of Ophthalmology, Cairo, Egypt.

[2] Division of Ophthalmology, Department of Clinical Neurosciences, Geneva University Hospitals, Geneva, Switzerland.

[3] Department of Ophthalmology, Beni-Suef University Hospitals, Cairo, Egypt.

[4] Department of Ophthalmology and Visual Sciences, Nottingham University Hospitals, Nottingham, United Kingdom.

[5] Doheny Eye Institute, Keck School of Medicine, University of Southern California, Los Angeles, California.

*Both Dr. Said and Dr. Elalfy contributed equally as first authors.

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Abbreviations and Acronyms:
CXL = corneal collagen cross-linking; **logMAR** = logarithm of the minimum angle of resolution; **PACK** = photoactivated chromophore for infectious keratitis.

Correspondence:
Farhad Hafezi MD, PhD, Professor and Chair of Ophthalmology, Geneva University Hospitals, Rue Alcide Jentzer 22, 1211 Geneva, Switzerland. E-mail: farhad@hafezi.ch.