Iontophoresis Corneal Cross-linking With Enhanced Fluence and Pulsed UV-A Light: 3-Year Clinical Results

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ABSTRACT

PURPOSE: To assess 3-year safety and efficacy of enhanced-fluence pulsed-light iontophoresis cross-linking (EF I-CXL) in patients with progressive keratoconus.

METHODS: This prospective interventional pilot study included 24 eyes of 20 patients, with a mean age of 23.9 years (range: 15 to 36 years). Iontophoresis with riboflavin solution was used for stromal imbibition. The treatment energy was optimized at 30% (7 J/cm^2) and ultraviolet-A power set at 18 mW/cm^2 × 6.28 minutes of pulsed-light on-off exposure, with a total irradiation time of 12.56 minutes. Uncorrected distance visual acuity (UDVA), corrected distance visual acuity (CDVA), corneal tomography, and corneal optical coherence tomography (OCT) at baseline and 1, 3, 6, 12, 24, and 3 years postoperatively were evaluated.

RESULTS: At 3 years, average UDVA decreased from 0.50 ± 0.10 to 0.36 ± 0.08 logMAR (P < .05), average maximum keratometry decreased from 52.94 ± 1.34 to 51.4 ± 1.49 diopters (D) (Delta: -1.40 ± 0.80 D; P < .05), average coma improved from 0.24 ± 0.05 to 0.12 ± 0.02 µm (P = .001), and symmetry index decreased from 4.22 ± 1.01 to 3.53 ± 0.90 D. Corneal OCT showed demarcation line detection at 285.8 ± 20.2 µm average depth in more than 80% at 1 month postoperatively.

CONCLUSIONS: The 3-year results of EF I-CXL showed satisfactory I-CXL functional outcomes, increasing the visibility and the depth of demarcation line closer to epithelium-off standard CXL.

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Epithelium-off standard corneal cross-linking (S-CXL) is currently considered the gold standard treatment for early progressive keratoconus with the ability to halt or delay its progression in the long-term follow-up in both adults and pediatric patients.[1-4] Induced CXL of corneal stroma depends on riboflavin stromal concentration and ultraviolet-A (UV-A) light distribution within the stroma and its absorption capable of generating free radicals initiating the collagen chain's photopolymerization process.[5]

Photodynamic CXL of the corneal stroma is an oxygen-dependent reaction[6] and the amount of singlet oxygen released in the stroma basically depends on the UV-A energy (fluence) transfer from the activated riboflavin molecules and the stromal oxygen concentration available for this transfer. Corneal epithelium and the Bowman's lamina represent a physical barrier against UV light[7] and riboflavin molecules interacting with main CXL parameters, thus influencing its corneal biological impact and therapeutic efficacy.[8] Overall

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CXL efficiency depends on basic physiochemical parameters such as homogeneous stromal concentration of riboflavin, adequate transmission of UV-A energy, intrastromal oxygen concentration, and intraoperative diffusion, leading to indispensable stromal penetration and volume of treatment, ensuring a sufficient cross-link density and saturation, thus achieving a long-term biomechanical corneal reinforcement necessary for the long-term ectasia stabilization.[9-11]

The original Dresden protocol of S-CXL[1] and Siena modifications[2] requires the removal of corneal epithelium to allow the passive diffusion and homogeneous concentration of riboflavin (a large hydrophilic molecule that cannot penetrate an intact epithelium) before continuous light UV-A irradiation of the cornea with UV-A power of 3 mW/cm^2 and total energy dose (fluence) of 5.4 J/cm^2.[12]

The potential benefit of a transepithelial CXL therapy (epithelium-on CXL) includes a reduced risk of infectious keratitis and postoperative stromal wound-related complications (haze), and faster recovery and visual rehabilitation.[13-16] However, the original epithelium-on CXL protocols with chemically enhanced riboflavin solutions failed in reaching a mid to long-term ectasia stabilization, with a reported failure rate between 50% and 100% of treated patients in a 1- to 2-year follow-up, thus requiring an epithelium-off re-treatment.[17-21]

The scientific demonstration of the corneal epithelium photoattenuation against UV light was reported in the photobiology laboratory studies by Kolosvári et al[22] on the UV absorbance of the human cornea in the 240- to 400-nm range. The study proved that the UV-A energy photoattenuation provided by the antioxidant systems of the human corneal epithelium and Bowman's lamina,[7] in the waveband of 365 nm set in the S-CXL therapy principles,[9] was approximately 30% of UV-A energy radiation.

To overcome the limitations of the original epithelium-on CXL techniques[23-26] based on chemical disruption and uneven alteration of the epithelial barrier, an electric-assisted methodology of riboflavin transport with epithelium in situ called iontophoresis-assisted CXL (I-CXL) was used with interesting results compared to S-CXL.[27-30] Vinciguerra et al[31] reported a comparative 2-year follow-up study of three groups of patients with keratoconus treated with I-CXL, iontophoresis with epithelial removal, and S-CXL for progressive keratoconus. The comparative prospective clinical studies demonstrated the 2-year efficacy and safety of the original I-CXL technique to treat progressive keratoconus[31] with even less corneal flattening compared to epithelium-off S-CXL.[32]

Aldahlawi et al[33] demonstrated that a 25% increase in UV-A radiance (from 5.4 J to 6.5 J/cm^2) significantly increased the corneal resistance against enzymatic degradation. The study concluded that, although epithelium-on CXL appeared to be inferior to epithelium-off S-CXL in terms of enzymatic resistance to pepsin digestion, the outcome of epithelium-on CXL may be significantly improved by increasing the UV-A radiance and exposure time of 25%.

The first modification Mazzotta et al[34] did to the original I-CXL protocol was increasing the UV-A energy dose from 5.4 J to 7 J/cm^2. The second modification was the use of the pulsed-light UV-A irradiation to partially compensate for the intraoperative oxygen diffusion and treatment penetration. The new technique, named enhanced fluence pulsed light iontophoresis (EF I-CXL), was developed by Mazzotta et al[34] to improve the outcomes of whole epithelium-on CXL procedures, considering that oxygen diffusion was hypothesized to be a limiting factor of the biomechanical effectiveness of the original I-CXL protocol.[35]

The purpose of this study was to assess 3-year safety and efficacy of EF I-CXL in patients with progressive keratoconus.

PATIENTS AND METHODS

This 3-year follow-up pilot interventional study included 24 eyes of 20 patients (22 unilateral, 2 bilateral) affected by progressive stage II keratoconus according to Krumeich's classification. All patients underwent the EF I-CXL protocol at the Siena Crosslinking Center. All patients included in the study completed the entire 3-year follow-up period. The EF I-CXL method is summarized in **Table 1** and baseline demographic data are summarized in **Table 2**.

Approval for this study was obtained from the local ethical committee of the Siena Crosslinking Center and the study was conducted according to the tenets of the Declaration of Helsinki. A written dedicated informed consent was obtained from all participants before enrollment in the study. Participants had no systemic disease and had a confirmed diagnosis of progressive keratoconus; keratoconus was classified as progressive if there was an increase of at least 1.00 diopter (D) in the maximum keratometry derived by a dual-system corneal tomographer based on Scheimpflug analysis and computerized Placido-disk corneal tomography (Sirius; Costruzione Strumenti Oftalmici) during the 12 months preceding the operation. Exclusion criteria included a minimum corneal thickness of less than 400 µm, a maximum keratometry value steeper than 58.00 D, any corneal scarring, previous refractive or other corneal or ocular surgery, and other ocular disorders such as previous

TABLE 1
EF I-CXL Method

Parameter	Variable
Treatment target	Keratoconus stabilization
Fluence (total) (J/cm^2)	7
Soak time and interval (minutes)	Iontophoresis (5 minutes)
Intensity (mW)	18
Epithelium status	On
Chromophore	Riboflavin
Chromophore carriers	Trometamol, Na-EDTA, no dextran
Chromophore osmolarity	Hypotonic
Chromophore concentration	0.1%
Epithelial rinsing	30 seconds with BSS
Light source	New KXL I (Avedro)
Irradiation mode (interval)	Pulsed (1 sec on – 1 sec off)
Protocol modifications	I-CXL
Protocol abbreviation	EF I-CXL

EF I-CXL = enhanced-fluence pulsed-light iontophoresis corneal cross-linking; BSS = balanced salt solution

TABLE 2
Demographic Baseline Data

Parameter	Value
Patients	20 (18 males/2 females)
Eyes	24 (2 simultaneous bilateral treatments)
Mean age (y)	23.9 (range: 15 to 36)
Male/female ratio	18/2 (90% male/10% female)
UDVA (logMAR)	0.51 ± 0.10
CDVA (logMAR)	0.24 ± 0.14 logMAR
Kmax (D)	52.94 ± 1.32
SAI (D)	2.20 ± 1.00
SI (D)	4.22 ± 1.01
Coma (µm)	0.25 ± 0.05
MCT (µm)	472.5 ± 37.1
Follow-up (months)	36

UDVA = uncorrected distance visual acuity; CDVA = corrected distance visual acuity; Kmax = maximum keratometry; D = diopters; SAI = surface asymmetry index; SI = symmetry index; MCT = minimum corneal thickness

herpetic keratitis and severe dry eye. Patients who were pregnant or breastfeeding at the time of enrollment were also excluded. Treatment failure was defined as an increase in maximum keratometry value of more than 1.00 D from preoperatively at 1 year postoperatively or later.

EF I-CXL Technique

Iontophoresis was used for stromal imbibition and performed with the Ricrolin+ riboflavin solution (Sooft) delivered by the electric generator I-ON CXL (Sooft) at 1 mAmpere × 5 minutes, 0.8 mm of corneal surface via suction ring and inox electrode placement, and 0.35 mL of riboflavin 0.1% volume. The fluence was optimized at 30% (7 J/cm^2) and UV-A power set at 18 mW/cm^2 × 6.28 minutes of exposure time, pulsing the light 1 second on/1 second off with a total prolonged UV-A irradiation time of 12.56 minutes (30%).

After a full patient informed consent was obtained concerning the treatment goals (stabilization of corneal ectasia, reduction of infectious risk, prevention of the complications related to wound healing, and specific informed consent subscription), the treatments were conducted at the Siena Crosslinking Center by the same operator (CM).

After topical anesthesia (oxybuprocaine hydrochloride drops) instilled 10 minutes before treatment and a Barraquer's closed valve eyelid speculum application, the ocular surface was dried with a micro-sponge, an iontophoresis suction ring was placed and centered on the corneal surface, and Ricrolin+ solution was administered and transferred by the I-ON CXL electric delivering system. After 5 minutes of imbibition, the excess solution was aspirated by a syringe, suction was interrupted, and the ocular surface was rinsed abundantly with sterile saline sodium chloride solution, eliminating all of the residual riboflavin on the corneal surface, and then pulsed light UV-A exposure was started. The UV-A source used was the KXL I system (Avedro), setting a fluence of 7 J/cm^2 delivered by a UV-A power of 18 mW/cm^2 with pulsed light emission (1 second on/1 second off) in a total UV-A exposure time of 12.56 minutes to maintain the overall treatment time of less than 20 minutes.

Before starting UV-A irradiation, the corneal surface was rinsed for 30 seconds to completely remove the precorneal riboflavin solution, thus avoiding further UV-A energy photoattenuation. During the pulsed UV-A light emission, the epithelial surface was washed with the sodium chloride solution every 2 minutes. At the end of the UV-A irradiation, the corneal surface was treated with antibiotics (ofloxacin drops) and hyaluronic acid + amino acids (Trium Free; Sooft), and dressed with a therapeutic soft contact lens (Schalcon) for 48 hours.

Patients underwent preoperative full ophthalmological examination, including uncorrected distance visual acuity, corrected distance visual acuity, Scheimpflug corneal tomography (Sirius), corneal optical coherence tomography examination (OptoVue), and endothelial cell count (I-Conan, Noncon Robo, NSP-9900; Konan Medical). According to the study purpose, the follow-up examinations were performed at 3 days and 1, 3, 6, 12, 24, and 36 months. All patients completed the

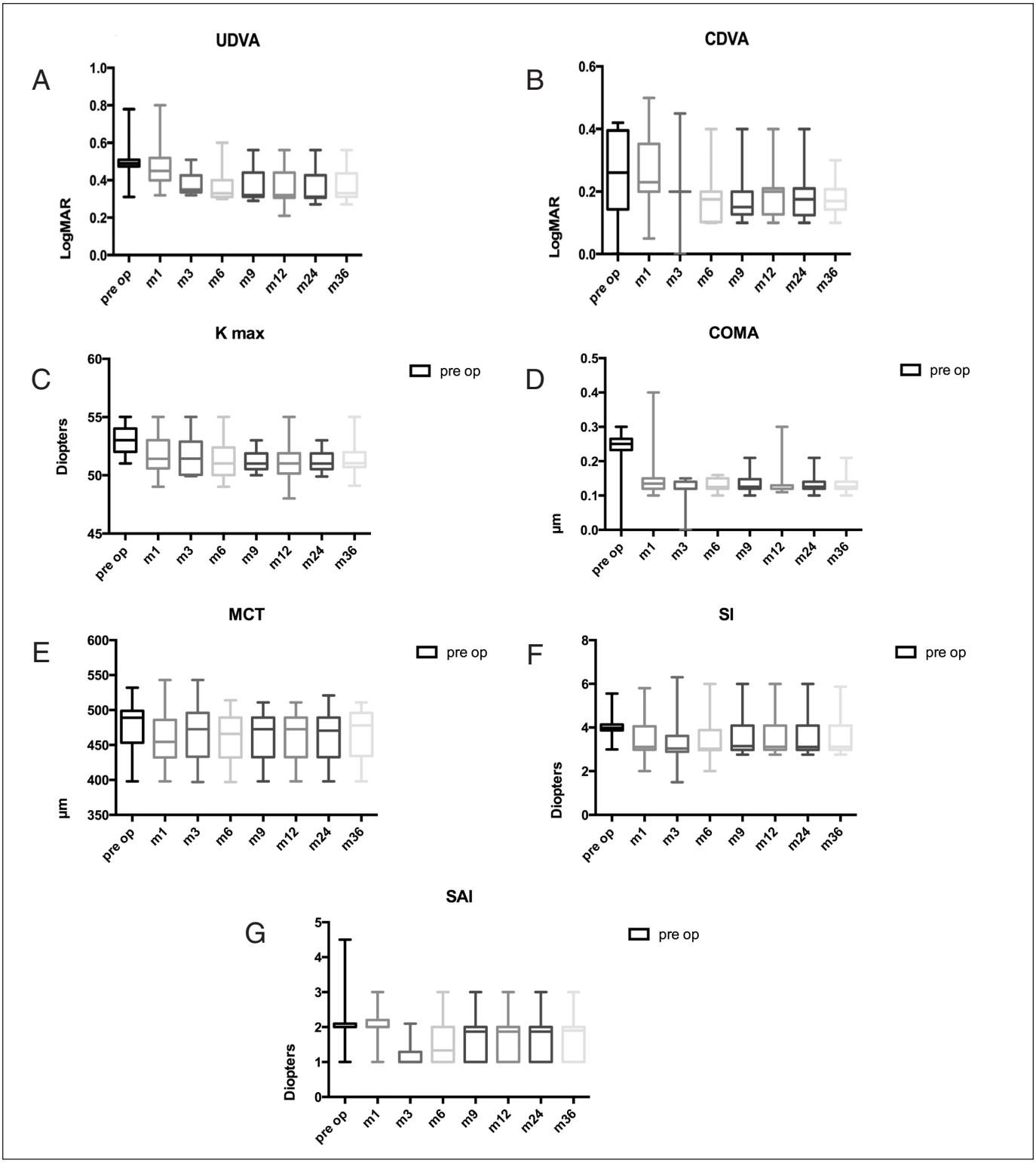

Figure 1. Box and whiskers plot for (A) uncorrected distance visual acuity (UDVA), (B) corrected distance visual acuity (CDVA) (logMAR), (C) maximum keratometry (Kmax) (diopters) (D), (D) coma value (µm), (E) minimum corneal thickness (MCT) (µm), (F) topographic symmetry index (SI), and (G) topographic surface asymmetry index (SAI) after enhanced-fluence pulsed-light iontophoresis corneal cross-linking (EF I-CXL).

3-year follow-up. Paired Student *t* test statistical analysis was performed using GraphPad Prism version 6.0 (GraphPad Software). Differences with a *P* value of less than .05 were considered statistically significant.

TABLE 3
3-Year Average Functional Outcomes After EF I-CXL

Parameter	Preoperative	Month 1	Month 3	Month 6	Month 9	Month 12	Month 24	Month 36
UDVA (logMAR)	0.51 ± 0.10	0.47 ± 0.11[a]	0.37 ± 0.06[a]	0.36 ± 0.08[a]	0.36 ± 0.084[a]	0.36 ± 0.09[a]	0.36 ± 0.08[a]	0.36 ± 0.08[a]
CDVA (logMAR)	0.24 ± 0.14	0.26 ± 0.11	0.22 ± 0.09	0.18 ± 0.09	0.17 ± 0.07	0.18 ± 0.07	0.18 ± 0.07	0.18 ± 0.07
Kmax (D)	52.94 ± 1.34	51.7 ± 1.54[a]	51.40 ± 1.40[a]	51.26 ± 1.56[a]	51.14 ± 1.56[a]	51.00 ± 1.20[a]	51.00 ± 1.43[a]	51.40 ± 1.49[a]
Coma (µm)	0.24 ± 0.05	0.14 ± 0.05[a]	0.11 ± 0.03[a]	0.12 ± 0.04[a]	0.12 ± 0.03[a]	0.12 ± 0.03[a]	0.12 ± 0.02[a]	0.12 ± 0.02[a]
MCT (µm)	472.5 ± 37.1	459.9 ± 34.6	467.3 ± 35.7	464.2 ± 32.5	465.3 ± 31.8	465.78 ± 32	466.52 ± 32	467.08 ± 32.9
SI (D)	4.22 ± 1.01	3.48 ± 0.97[a]	3.35 ± 1.04[a]	3.40 ± 0.85[a]	3.57 ± 0.91[a]	3.56 ± 0.90[a]	3.56 ± 0.90[a]	3.53 ± 0.90[a]
SAI (D)	2.39 ± 1.20	2.33 ± 1.00[a]	1.70 ± 0.82[a]	1.85 ± 0.98[a]	2.20 ± 1.13	2.13 ± 1.12	2.00 ± 1.10	2.07 ± 1.10

EF I-CXL = enhanced-fluence pulsed-light iontophoresis corneal cross-linking; UDVA = uncorrected distance visual acuity; CDVA = corrected distance visual acuity; Kmax = maximum keratometry; D = diopters; MCT = minimum corneal thickness; SI = topographic symmetry index; SAI = topographic surface asymmetry index
[a]Indicates statistically significant values (P < .05).

TABLE 4
Comparative Outcomes Summary of Iontophoresis Long-term Studies

Authors	Iontophoresis Fluence/ Irradiance (mW)	Eyes	Follow-up (mo)	Kmax Flattening (D)	CDVA (logMAR)	Coma (µm)	Demarcation Line: Visibility (%)/ Mean Depth (µm)
Boueheraoua et al,[30] 2015	5.4/10	15	24	-0.40 ± 0.28	0.06 ± 0.50	Not reported	35%/212 ± 36.5
Lombardo et al,[32] 2019	5.4/10	34	24	-1.05 ± 0.20	0.08 ± 0.15	0.1 ± 1	Not reported
Vinciguerra et al,[31] 2019	5.4/10	20	24	0.40 ± 0.50	0.10 ± 0.03	-1.74 ± 0.71[a]	Not reported
Current study, 2020	7/18 pulsed 1:1	24	36	-1.40 ± 0.80	0.06 ± 0.07	0.12 ± 0.03[a]	80%/285.8 ± 20.2

Kmax = maximum keratometry; D = diopters; CDVA = corrected distance visual acuity
**Indicates statistically significant values (P < .05).*

RESULTS

Average uncorrected distance visual acuity (**Figure 1A**) decreased from a baseline value of 0.50 ± 0.10 to 0.36 ± 0.08 logMAR at 3 years postoperatively, becoming statistically significant from the 3-month follow-up until the end of follow-up ($P = .001$). Average corrected distance visual acuity (**Figure 1B**) showed a not statistically significant reduction, decreasing from a baseline average value of 0.24 ± 0.14 to 0.18 ± 0.07 logMAR at 3 years postoperatively.

Average maximum keratometry value, measured with tangential algorithm, showed a reduction from 52.94 ± 1.34 D at baseline to 51.4 ±1.49 D at 3-year follow-up (Delta: -1.40 ± 0.80 D), becoming statistically significant at 1 month postoperatively ($P = .0091$) (**Figure 1C**). Average coma value showed a statistically significant improvement in the overall follow-up, starting at 1 month postoperatively and changing from an average baseline value of 0.24 ± 0.05 to 0.12 ± 0.02 µm ($P = .001$) at 3 years postoperatively (**Figure 1D**). Average minimum corneal thickness showed a not statistically significant reduction from a baseline value of 475.3 ± 37.1 to 467.08 ± 32.9 µm at 3 years postoperatively (**Figure 1E**). The average topographic symmetry index decreased from a baseline value of 4.22 ± 1.01 to 3.53 ± 0.90 D at 3 years postoperatively, becoming statistically significant at 1 month postoperatively ($P = .046$) (**Figure 1F**). Average topographic surface asymmetry index value showed a statistically significant reduction at 1, 3, and 6 months postoperatively, respectively ($P < .05$), decreasing from a baseline value of 2.39 ± 1.20 to 2.07 ± 1.10 D at 3 years postoperatively (**Figure 1G**).

According to the Visual Analogue Scale pain scale, the average value reported in our series after the EF I-CXL pulsed light protocol was 0 (range: 0 to 10: no pain).

The overall functional outcomes are summarized in **Table 3** and comparative I-CXL long-term results are reported in **Table 4**.

DISCUSSION

The EF I-CXL protocol showed its capability in stabilizing progressive keratoconus in the absence of adverse events over 3 years of follow-up. The new iontophoresis method reached clinical results closer to S-CXL and superior to the original epithelium-on I-CXL protocol with standard fluence of 5.4 J/cm^2 in terms of average maximum keratometry flattening, higher order aberration reduction, and functional outcomes. No additional intraoperative oxygen supplementation was used for this "compensated" epithelium-on method.

The 3-year clinical results were similar to those published by Lombardo et al[33] after epithelium-off

S-CXL. The EF-ICXL[34] increased the kinetic of CXL, optimizing the treatment's energy and intraoperative oxygen diffusion overcoming the main photochemical and physical limitations of the epithelium-on protocols in general, including the protocols where iontophoresis with electrically assisted riboflavin stromal soaking is used.

The problems were not correlated to iontophoresis itself (as imbibition technique) but to the UV-A photoabsorption[22] and higher oxygen consumption by the corneal epithelium when left in situ.[24,25] The EF I-CXL protocol[34] reduced postoperative pain, allowed well-tolerated simultaneous bilateral treatments eliminating the risk of postoperative infections, reduced stromal wound-related stimuli and complications, and increased the depth of demarcation line that was shallower and less visible in the original protocol.

The 30% of the UV-A photoattenuation provided by the corneal epithelium[25] and Bowman's lamina antioxidant systems[7] was compensated for by increasing the UV-A energy dose from 5.4 J to 7 J/cm^2.[34]

Pulsed-light irradiation was included in the new protocol because of its several advantages according to the literature: increasing intraoperative oxygen diffusion[36] and CXL treatment penetration[37] and inducing less microstructural damage.[38] These two variations from the original I-CXL protocol allowed a superior and repeatable visualization of the demarcation line that was considered a potential cause of less efficacy of epithelium-on I-CXL compared to epithelium-off S-CXL.[29,39]

The demarcation line was clearly visible in more than 80% of the examined patients at an average depth of 285.8 ± 20.2 µm in the first postoperative month, in the absence of wound-related complications (haze) and endothelial damage.[34] A previous study comparing I-CXL and S-CXL reported that the demarcation line after I-CXL was inconsistent and superficially visible only in 35% of treated corneas versus the 95% after S-CXL.[29] In another study, the same authors reported a visible demarcation line in 47.7% at a mean depth of 212 ± 36.5 µm, which was shallower compared with 97% with S-CXL at 345 ± 24.5 µm.[39]

The capability of this compensated epithelium-on CXL method to induce a consistent demarcation line visibility and higher depth, together with the 3-year functional results closer to epithelium-off S-CXL, showing a statistically significant improvement of UDVA and CDVA associated with a significant reduction of coma aberration leading to mid- to long-term ectasia stability, can be favorably considered. Moreover, the average topographic corneal symmetry index and surface asymmetry index were significantly improved due to keratoconus apex flattening and compensatory steepening of the superior flattest area, thus resembling the behavior of epithelium-off S-CXL.

According to the clinical results and absence of adverse events, the EF I-CXL protocol could definitively replace the original I-CXL technique, paving the way to a more efficacious epithelium-on approach for early progressive corneal ectasia and providing the opportunity to perform the CXL treatment safely and comfortably outside the operating room and at the slit lamp, without the necessity for intraoperative supplemental oxygen. Because photochemistry cannot be circumvented, when epithelium is left in situ, the accelerated CXL treatment necessarily requires compensations such as the optimization of the treatment's fluence and improvement of oxygen diffusion.

AUTHOR CONTRIBUTIONS

Study concept and design (CM, PV); data collection (CM, AS, AD, MF, AR, GMT); analysis and interpretation of data (CM, SAB, RV); writing the manuscript (CM, SAB, PV); critical revision of the manuscript (CM, AS, AD, MF, AR, RV, GMT); statistical expertise (SAB); administrative, technical, or material support (MF, AR, GMT); supervision (CM, SAB, PV)

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