Natural history of corneal haze after collagen crosslinking for keratoconus and corneal ectasia: Scheimpflug and biomicroscopic analysis

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PURPOSE: To determine the natural history of collagen crosslinking (CXL)–associated corneal haze measured by Scheimpflug imagery and slitlamp biomicroscopy in patients with keratoconus or ectasia after laser in situ keratomileusis.

SETTING: Cornea and refractive surgery subpecialty practice, United States.

DESIGN: Prospective randomized controlled clinical trial.

METHODS: The treatment group received ultraviolet-A/riboflavin CXL therapy. The control group received riboflavin alone without epithelial debridement. To objectively measure CXL-associated corneal haze, corneal densitometry using Scheimpflug imagery was measured and the changes in haze were analyzed over time. A similar analysis was performed using clinician-determined slitlamp haze. Correlation of CXL-associated corneal haze with postoperative outcomes was analyzed.

RESULTS: The mean preoperative corneal densitometry was 14.9 ± 1.93 (SD) (Pentacam Scheimpflug densitometry units). Densitometry peaked at 1 month (mean 23.4 ± 4.40; $P<.001$), with little change at 3 months (mean 22.4 ± 4.79; $P = .06$) and decreased between 3 months and 6 months (19.4 ± 4.48; $P<.001$) and between 6 months and 12 months. By 12 months, densitometry had not completely returned to baseline in the entire cohort (mean 17.0 ± 3.82; $P<.001$) and the keratoconus subgroup; however, it returned to baseline in the ectasia group (16.1 ± 2.41; $P = .15$). The postoperative course of slitlamp haze was similar to objective densitometry measurements. Increased haze, measured by densitometry, did not correlate with postoperative clinical outcomes.

CONCLUSIONS: The time course of corneal haze after CXL was objectively quantified; it was greatest at 1 month, plateaued at 3 months, and was significantly decreased between 3 months and 12 months. Changes in haze did not correlate with postoperative clinical outcomes.

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Corneal collagen crosslinking (CXL) is a treatment designed to decrease the progression of keratoconus[1] in particular as well as other corneal-thinning processes, such as post-laser in situ keratomileusis (LASIK) ectasia.[2] Studies suggest that CXL can also have beneficial visual and optical effects by decreasing corneal steepness, improving corrected distance visual acuity (CDVA) and uncorrected distance visual acuity (UDVA), and improving topography irregularity indices.[3–8]

In the CXL procedure, riboflavin (vitamin B2) is administered in conjunction with ultraviolet-A (UVA) (365 nm) irradiation. Riboflavin acts as a photosensitizer for the production of reactive oxygen species (singlet oxygen).[9] The free radicals produced by the interaction of riboflavin and UVA light cause the formation of chemical bonds within the corneal stroma and consequent mechanical stiffening of the cornea.[10,11]

Collagen crosslinking appears to have its predominant effect in the anterior 300 µm of the cornea.[12] Studies of the cornea after CXL report several changes. These include increased collagen fiber diameter,[13] keratocyte apoptosis and subsequent keratocyte changes,[14] resistance to thermal shrinkage,[15] change

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in corneal-swelling properties,[16] and increased resistance to collagenase degradation.[17]

A typical corneal haze has generally been noted on clinical examination after CXL. Studies show that the depth of the CXL can be observed by following the demarcation line seen in the corneal stroma[18] or by grading the corneal haze at the slitlamp.[19] Moreover, corneal haze after CXL has been confirmed[4] and its etiology, in part, has been defined using confocal microscopy.[20–23]

In the cohort in the present study, more than 90% of eyes had the clinical appearance of stromal haze on slitlamp examination after CXL. Subjective grading of corneal haze at the slitlamp, however, is subject to observer interpretation and is difficult to measure objectively. Therefore, to better quantitate and explore the natural history of this CXL-associated corneal haze, we used Scheimpflug image densitometry measurements in a prospective randomized controlled trial. We also sought to analyze the correlation between densitometry and visual acuity after CXL.

PATIENTS AND METHODS

Patients were enrolled as part of a multicenter prospective randomized controlled clinical trial conducted under guidelines of the U.S. Food and Drug Administration[A] (trials NCT00647699 and NCT00674661) and approved and monitored by an investigational review board. All patients provided informed consent, and all work performed for this study was compliant with the U.S. Health Insurance Portability and Accountability Act. Two patient cohorts were treated, 1 with progressive keratoconus and 1 with corneal ectasia after LASIK.

The inclusion criteria included age 14 years or older, axial topography consistent with keratoconus or corneal ectasia, inferior–superior ratio greater than 1.5 on topography mapping, CDVA worse than 20/20, removal of contact lenses for a specified period of time depending on the type of lens, and a diagnosis of progressive keratoconus or LASIK-induced ectasia. Progressive keratoconus was defined as 1 or more of the following changes over 24 months: an increase of 1.00 diopter (D) or more in the steepest keratometry (K), an increase of 1.00 D or more in manifest cylinder, or an increase of 0.50 D or more in the manifest refraction spherical equivalent. Exclusion criteria included a history of corneal surgery, corneal pachymetry less than 300 μm, a history of chemical injury or delayed epithelial healing, and pregnancy or lactation during the course of the study.

Surgical Technique

Patients were initially randomized to a treatment group (riboflavin–UVA) or a control group (riboflavin only). All control patients had CXL (riboflavin–UVA) after 3 months, at which time a new baseline was established for them. Corneal crosslinking was performed according to the methodology described by Wollensak et al.[1] In brief, a topical anesthetic agent was administered and the central 9.0 mm epithelium removed by mechanical debridement. Riboflavin was then administered topically every 2 minutes for 30 minutes. Complete riboflavin absorption throughout the stroma and into the anterior chamber was confirmed by slitlamp examination. Ultrasonic pachymetry was performed to confirm corneal thickness of 400 μm or more. If the cornea was thinner than 400 μm, hypotonic riboflavin was administered, 1 drop every 10 seconds for 2-minute sessions, until the stroma had swelled to more than 400 μm. The cornea was then exposed to UVA 365 nm light for 30 minutes at an irradiance of 3.0 mW/cm^2, with continued administration of riboflavin drops every 2 minutes. At the conclusion of the procedure, antibiotic and corticosteroid drops were administered and a bandage soft contact lens was placed. The contact lens was removed after epithelialization. Antibiotic drops and corticosteroid drops were continued 4 times daily for 1 week and 2 weeks, respectively. In the control group, the epithelium was not removed. Riboflavin drops were administered every 2 minutes for 30 minutes. For the next 30 minutes, the patient had a sham treatment with continued administration of riboflavin drops.

Postoperative Follow-up

Scheimpflug images of all eyes were taken with a Pentacam rotating Scheimpflug camera (Oculus, Inc.) before the procedure and at the 1-, 3-, 6-, and 12-month follow-up visits. The Scheimpflug device generates a 3-dimensional model of the cornea and anterior segment. As an objective measure of CXL-associated corneal haze, corneal densitometry was measured over the central 4.0 mm along 1 meridian using the Scheimpflug image. The meridian of the image used was determined as follows: At the initial visit, the coordinates of maximum steepness (maximum keratometry [K] value) were identified on the Scheimpflug device. The axis nearest to the maximum K value was determined, and the Scheimpflug image at this axis was used for analysis. A central 4.0 mm segment of the cornea was delineated manually using perimetry software included with the device (Figure 1). The tracing encompassed the entire thickness of the cornea, and the perimetry software automatically calculated the mean density of that area. The Scheimpflug device quantifies the density of the cornea on a scale from 0 to 100.

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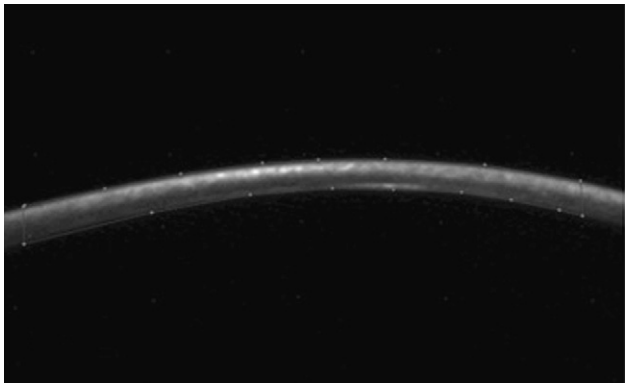

Figure 1. Technique used to manually trace the central 4.0 mm segment of the cornea using Scheimpflug imagery.

These measurements were obtained at 1, 3, 6, and 12 months using the Scheimpflug image taken at the same axis as at the baseline visit.

As a clinical correlate, corneal haze was observed at each visit by slitlamp biomicroscopy by the same investigator (P.S.H.) and graded on a scale from 1 to 4. The slitlamp examination grading was as follows: 0+ = clear cornea; 1+ = focal areas of minimal stromal clouding or reticulation; 2+ = diffuse mild stromal clouding or reticulation; 3+ = diffuse stromal clouding or reticulation somewhat obscuring view of iris details; 4+ = focal or diffuse areas of dense stromal haze obscuring iris detail (Figure 2). Similar to the analyses using densitometry measurements, the change in slitlamp haze was compared with baseline grading and analyzed over time.

To determine whether CXL-associated corneal haze affected clinical outcomes, an analysis was performed to determine whether densitometry-measured absolute corneal haze or change in haze had an association with any of the following parameters: CDVA, mean K value, maximum K value, and thinnest pachymetry. The latter 3 parameters were measured with the Scheimpflug device.

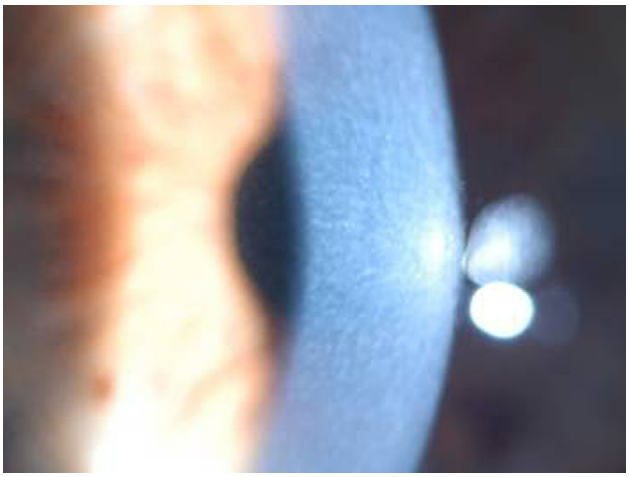

Figure 2. Representative slitlamp biomicroscopy image of clinical haze after CXL (original magnification ×16).

Statistical Analysis

The data are presented as the mean density ± SD or the mean slitlamp haze grade ± SD. Analysis was performed using PASW software (version 18, SPSS, Inc.). Three groups were analyzed: the entire cohort, the keratoconus subgroup, and the ectasia subgroup. A paired 2-tailed Student *t* test was used to analyze the postoperative change in haze from baseline. An independent *t* test was used to compare postoperative haze in the keratoconus subgroup and the ectasia subgroup and in patients who received hypotonic riboflavin intraoperatively and those who did not. Analysis of variance was used to compare the entire cohort and the keratoconus and ectasia subgroups with the corresponding control groups at baseline, 1 month, and 3 months. Pearson correlation coefficients were used to analyze the possible correlation between haze severity and haze change and the clinical outcomes. A *P* value less than 0.05 was used to determine statistical significance.

RESULTS

Fifty eyes of 44 patients had CXL and were followed for 1 year. These eyes were divided into 2 subgroups: keratoconus (n = 31) and post-LASIK ectasia (n = 19). These groups were analyzed together and individually.

The control group comprised 41 eyes (28 keratoconus and 13 ectasia). These eyes were followed for 3 months and analyzed together and within the individual groups. They were also compared at baseline and at the 1- and 3-month follow-up with the patients who received riboflavin–UVA therapy. Table 1 shows the demographics in the treatment group and control group.

Scheimpflug Densitometry

Control In the control group, the mean densitometry 1 month and 3 months after CXL was unchanged from baseline in all eyes, in the keratoconus subgroup, and in the ectasia subgroup. The preoperative mean densitometry was 14.7 ± 2.04 (Figure 3, upper left). At 1 month, mean densitometry was 14.9 ± 2.44 ($P = .62$), 14.7 ± 2.28 ($P = .75$), and 15.3 ± 2.82 ($P = .059$) in all eyes, in the keratoconus subgroup, and in the ectasia subgroup, respectively. At 3 months, the mean densitometry was 14.4 ± 1.84 ($P = .27$), 14.3 ± 1.77 ($P = .49$), and 14.6 ± 2.04 ($P = .35$), respectively.

Combined Keratoconus and Ectasia Table 2 shows the Scheimpflug densitometry measurements in the combined keratoconus and ectasia cohort. There was a significant increase in mean densitometry between baseline and 1 month ($P < .001$) (Figure 3, upper right). There was no significant change between 1 month and 3 months (change −1.01 ± 4.57; $P = .15$). Between 3 months and 6 months (change −3.0 ± 4.69; $P < .001$) and between 6 months and 12 months

Table 1. Baseline characteristics.

Parameter	Treated Group			Control Group		
	Entire Cohort	Ectasia	Keratoconus	Entire Cohort	Ectasia	Keratoconus
Eyes/patients (n)	50/44	19/15	31/29	41	13	28
Age (y)						
Mean ± SD	36.8 ± 11.1	44.2 ± 7.5	32.3 ± 10.3	34.8 ± 10.3	44.2 ± 7.5	32.4 ± 9.6
Range	15 to 53	27 to 54	15 to 52	15 to 55	27 to 54	15 to 51
Male, eyes/patients (n)	30/28	11/10	19/18	30	9	21
Female, eyes/patients (n)	20/16	8/5	12/11	11	4	7
Mean UDVA ± SD	20/151	20/123	20/172	20/179	20/162	20/187
Mean CDVA ± SD	20/44	20/37	20/48	20/52	20/41	20/58
Steep K (D)						
Mean ± SD	50.6 ± 6.1	47.2 ± 4.2	52.6 ± 6.2	50.3 ± 6.9	45.3 ± 4.4	52.6 ± 6.6
Range	39.5 to 64.0	39.5 to 57.3	45.0 to 64.0	38.8 to 66.0	38.8 to 54.0	45.0 to 66.0
Flat K (D)						
Mean ± SD	45.3 ± 5.91	40.9 ± 3.2	48.0 ± 5.5	46.1 ± 6.5	41.2 ± 3.5	48.5 ± 6.3
Range	34.0 to 58.0	34.0 to 47.5	38.0 to 58.0	37.0 to 64.0	37.0 to 50.0	41.5 to 64.0
Cylinder (D)						
Mean ± SD	5.31 ± 4.00	6.31 ± 5.21	4.60 ± 2.87	4.14 ± 2.35	4.13 ± 2.56	4.14 ± 2.29
Range	−0.50 to 22.50	0.50 to 22.5	0.90 to 13.00	0.25 to 9.50	0.25 to 8.25	1.00 to 9.50
Thinnest pachymetry (μm) (Scheimpflug)						
Mean ± SD	441.10 ± 54.31	433.60 ± 53.47	446.90 ± 54.63	434.50 ± 48.79	417.90 ± 51.28	442.20 ± 46.50
Range	320.0 to 571.0	320.0 to 536.0	333.0 to 571.0	306.0 to 535.0	324.0 to 484.0	306.0 to 535.0

CDVA = corrected distance visual acuity; K = keratometry; UDVA = uncorrected distance visual acuity

(change −2.43 ± 3.21; $P<.001$), there was a statistically significant decrease in mean densitometry. Although the mean densitometry decreased at 6 months and 12 months, it remained elevated compared with baseline values ($P<.001$).

Keratoconus In the keratoconus subgroup, there was a statistically significant increase in mean densitometry between baseline and 1 month ($P<.001$) (Table 2 and Figure 3, lower left). There was no significant change between 1 month and 3 months (change −1.49 ± 4.65; $P = .08$) or between 3 months and 6 months (change −1.67 ±5.02; $P = .07$). There was a statistically significant decrease in mean densitometry between 6 months and 12 months (−2.24 ± 3.49; $P = .001$). Although the mean densitometry decreased at 6 months and 12 months, it remained elevated compared with baseline values ($P<.001$).

Ectasia In the ectasia subgroup, there was a statistically significant increase in mean densitometry between baseline and 1 month ($P<.001$) (Table 2 and Figure 3, lower right). There was no significant change between 1 month and 3 months (change −0.22 ± 5.17; $P = .86$). Between 3 months and 6 months (change −5.16 ± 3.17; $P<.001$) and between 6 months and 12 months (change −2.73 ± 2.77; $P<.001$), there was a statistically significant decrease in mean densitometry. In contrast to the combined group and keratoconus subgroup, there was no significant difference in mean densitometry between 12 months and baseline ($P = .15$).

Slitlamp Biomicroscopy

The findings of 1-year slitlamp haze analysis corroborated the results of the Scheimpflug densitometry measurements. Natural history over time followed a similar course and, at 1 year, there was remaining CXL-associated corneal haze in the combined group and the keratoconus subgroup; however, CXL-associated corneal haze had returned to baseline levels in the ectasia group (Figure 4).

Combined Keratoconus and Ectasia In the combined group, the mean preoperative slitlamp-graded haze was 0.2 ± 0.64 (scale 0 to 4). At 1 month, the mean increased to 1.6 ± 0.75 ($P<.001$). Between 1 month and 3 months, there was no significant change (change −0.10 ± 0.87; $P = .3$). Between 3 months and 6 months (change −0.4 ± 0.86; $P = .001$) and between 6 months and 12 months (change −0.5 ± 0.81; $P<.001$), there was a significant decrease in mean slitlamp haze. At 1 year, slitlamp haze remained significantly elevated compared with baseline values (mean 0.6 ± 0.88; $P = .001$).

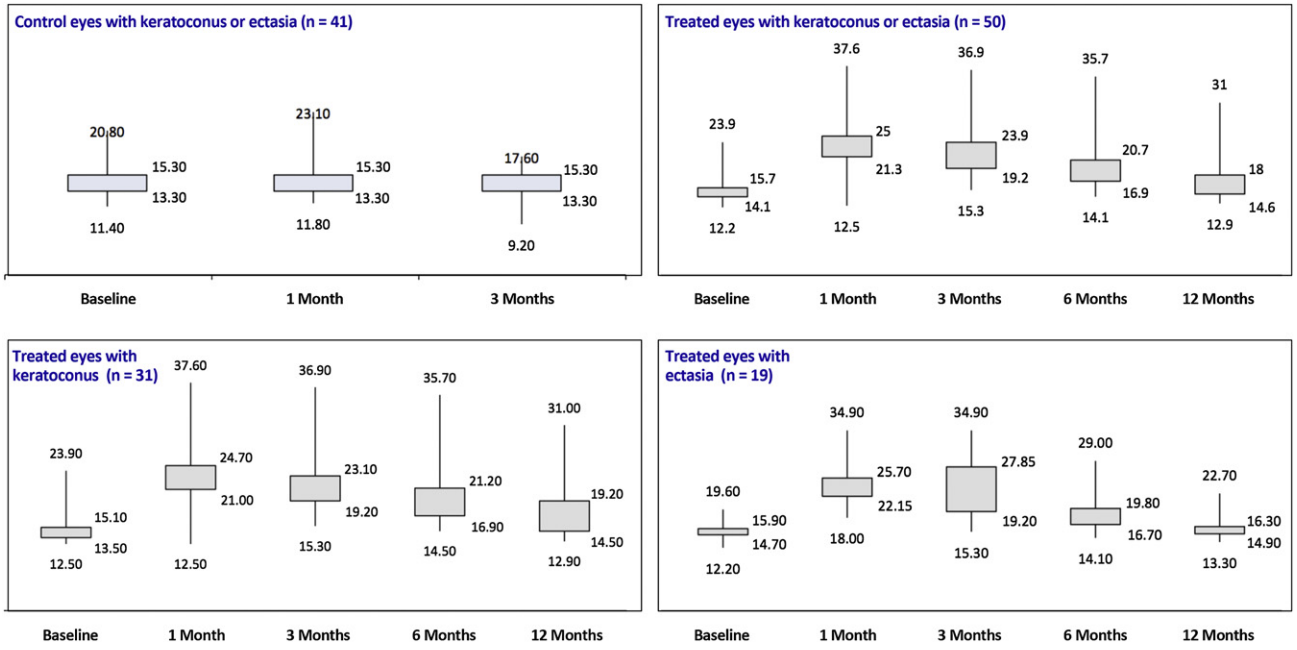

Figure 3. Time course of CXL-associated corneal haze using Scheimpflug densitometry measurements depicted by box-and-whisker plots. The upper bar represents the 4th quartile and the lower bar, the 1st quartile.

Keratoconus In the keratoconus subgroup, the mean preoperative slitlamp haze was 0.3 ± 0.73. At 1 month, the mean haze increased significantly to 1.6 ± 0.76 ($P < .001$) (Figure 4). There was no significant change in mean slitlamp haze between 1 month and 3 months (change 0.0 ± 0.78; $P = 1.0$). Between 3 months and 6 months (change −0.4 ± 0.80; $P = .01$) and between 6 months and 12 months (change −0.5 ± 0.81; $P = .001$), there was a significant decrease in slitlamp haze. At 1 year, slitlamp haze remained significantly elevated compared with baseline values (mean 0.7 ± 0.91; $P = .01$).

Ectasia In the ectasia subgroup, the preoperative mean slitlamp haze was 0.1 ± 0.46. At 1 month, the mean slitlamp haze increased to 1.6 ± 0.83 ($P < .001$) (Figure 4). There was no significant change between 1 month and 3 months (change −0.3 ± 1.00; $P = .2$). Between 3 months and 6 months (change −0.5 ± 0.96, $P = .04$), there was a significant decrease in slitlamp haze, followed by no significant change between 6 months and 12 months (change −0.4 ± 0.83; $P = .07$). At 1 year, slitlamp haze returned to baseline levels (mean 0.4 ± 0.90; $P = .06$).

Comparison Between Groups

Treatment Versus Control There were no significant differences between the treatment group and the control group at baseline in mean Scheimpflug densitometry measurements ($P = .99$). However, at 1 month and 3 months, there was a significant difference between the treatment group and the control group in all eyes, in the keratoconus subgroup, and in the ectasia subgroup (all $P < .001$).

Keratoconus Versus Ectasia Between 3 months and 6 months, there was a significant difference in the change in densitometry between the keratoconus subgroup and the ectasia subgroup. During this period, the mean CXL-associated corneal haze measured by densitometry decreased significantly more in the ectasia subgroup (mean change −5.16 ± 3.17) than in the keratoconus subgroup (change −1.67 ± 5.01) ($P = .01$). Changes in densitometry over other time periods were not significant between the 2 subgroups. At 12 months, there was a statistically significant difference in postoperative haze measured by densitometry compared with baseline measurements between the keratoconus subgroup and the ectasia subgroup ($P = .01$). The mean densitometry was 17.5 ± 4.41 and 16.1 ± 2.41, respectively.

Effect of Hypotonic Riboflavin on Haze

There was no significant difference between patients who received hypotonic riboflavin and those who did not in the change in densitometry from baseline to 1 month ($P = .3$), from 1 month to 3 months ($P = .7$), from 3 months to 6 months ($P = .4$), or from 6 months to 12 months ($P = .9$).

Table 2. Scheimpflug densitometry measurements over time.

	Densitometry Measurement							
	Preoperative			1 Mo Postoperative			3 Mo Postoperative	
		r Value			*r* Value			*r* Value
Treatment Group	Mean ± SD (95% CI)	With UDVA	With CDVA	Mean ± SD (95% CI)	With UDVA	With CDVA	Mean ± SD (95% CI)	With UDVA
All eyes* (n = 50)	14.9 ± 1.9 (14.4 to 15.5)	0.27‖	0.51‖	23.4 ± 4.4§,¶ (22.2 to 24.6)	0.37‖	0.42‖	22.4 ± 4.8§ (21.0 to 23.8)	0.28‖
Keratoconus (n = 21)	14.6 ± 2.1 (13.8 to 15.4)	0.32‖	0.56‖	22.9 ± 4.7§,¶ (21.2 to 24.7)	0.39‖	0.47‖	21.4 ± 3.9§ (20.0 to 22.9)	0.34‖
Ectasia (n = 19)	15.4 ± 1.6 (14.7 to 16.2)	0.22	0.43	24.2 ± 3.8§,¶ (22.3 to 26.0)	0.42	0.34	23.9 ± 5.7§ (21.2 to 26.7)	0.28

CDVA = corrected distance visual acuity (spectacles); CI = confidence interval; n = eyes; *r* Value = correlation coefficient; UDVA = uncorrected distance visual acuity
*Keratoconus and ectasia
†Keratoconus versus ectasia
‡Treatment versus sham
§Significant change compared with baseline measurement ($P < .05$)
¶Significant change compared with previous visit measurement ($P < .05$)
‖Significant correlation between densitometry measurement and visual acuity measurement ($P < .05$)

Clinical Outcomes Correlations

In the entire cohort, the absolute measurement of CXL-associated corneal haze measured by densitometry at 12 months was significantly correlated with CDVA ($r = -0.71$), the maximum K value ($r = 0.53$), the mean K value ($r = 0.70$), and the thinnest pachymetry ($r = -0.68$). However, the changes in densitometry both between baseline and 1 month and between baseline and 12 months were not correlated with the change in any clinical outcome from baseline to 12 months in any group. These correlation patterns were similar in the keratoconus subgroup and the ectasia subgroup. Table 2 shows the data for all clinical correlations.

DISCUSSION

Collagen crosslinking is a promising new treatment for stabilizing and strengthening the cornea in keratoconus and ectasia.[1,24] In the clinical setting, a typical corneal haze is noted after CXL in most cases.[20,21,23] Koller et al.[19] evaluated anterior stromal haze, which was graded on a scale used in cases after PRK[25]; the mean grade was 0.78, 0.18, and 0.06 at 1 month, 6 months, and 12 months, respectively. Previous confocal microscopy studies[4,21,26,27] report that a dense extracellular matrix (ECM) that is seemingly compatible with subclinical haze forms between 2 months and 3 months postoperatively. Raiskup et al.[22] found a greater tendency toward stromal haze in patients with more advanced keratoconus.

Although corneal haze has been described after CXL and its etiology explored in confocal microscopy studies,[4,21,26,27] the natural course of clinical corneal haze after CXL has not been fully elucidated or objectively quantified to date. Therefore, the purpose of this randomized controlled prospective study was to define the natural course of this haze to guide the clinician in his or her expectations when examining a patient at different time points after CXL. To do this, we used densitometry measurements obtained from Scheimpflug imagery as an objective measure of corneal haze. Moreover, to corroborate the clinical relevance of the Scheimpflug densitometry measurements, we performed a similar analysis using slitlamp biomicroscopy grading. Haze after CXL is different in clinical character from haze after other procedures, such as excimer laser photorefraction keratomy. The former is a dust-like change in the corneal stroma or a midstromal demarcation line, whereas the latter has a more reticulated subepithelial appearance.[28] Similarly, the mechanisms leading to haze formation may be different,[29] and further studies should help to clarify the molecular and cellular changes over time after CXL. To differentiate the unique corneal haze after CXL from haze and scarring after other corneal surgeries and diseases, we refer to it in this paper as CXL-associated corneal haze.

Regarding the occurrence and natural course after CXL, we found a significant postoperative increase in haze measured by both Scheimpflug densitometry and slitlamp assessment. The increase peaked at

Table 2. (Cont.)

Densitometry Measurement								
3 Mo Postoperative	6 Mo Postoperative			12 Mo Postoperative			*P* Value, Change from Baseline	
r Value		*r* Value			*r* Value			
With CDVA	Mean ± SD (95% CI)	With UDVA	With CDVA	Mean ± SD (95% CI)	With UDVA	With CDVA	To 12 Mo[†]	To 3 Mo[‡]
0.48[‖]	19.4 ± 4.8[§,¶] (18.1 to 20.7)	0.41[‖]	0.60[‖]	17.0 ± 3.8[§,¶] (15.9 to 18.1)	0.55[‖]	0.71[‖]	.01	.001
0.61[‖]	19.8 ± 4.8[§] (18.0 to 21.5)	0.46[‖]	0.63[‖]	17.5 ± 4.4[§,¶] (15.9 to 19.1)	0.58[‖]	0.73[‖]	–	.001
0.32	18.8 ± 4.1[§,¶] (16.8 to 20.7)	0.01	0.54[‖]	16.1 ± 2.4[¶] (14.9 to 17.2)	0.26	0.45	–	.001

1 month (Figure 5, *A* and *B*) and plateaued between 1 month and 3 months (Figure 5, *C*). Between 3 months and 6 months, the cornea began to clear and there was a significant decrease in CXL-associated corneal haze. From 6 months to 1 year postoperatively, there continued to be a decrease in haze measurements (Figure 5, *D* and *E*). Although CXL-associated corneal haze persisted above baseline levels at 1 year based on slitlamp grading and Scheimpflug densitometry measurements, a statistically significant finding, the actual change from preoperative measurements was small and its clinical significance requires further study.

Our findings indicated a possible difference in the natural history of CXL-associated corneal haze between the keratoconus subgroup and the ectasia subgroup. Although the maximum CXL-associated corneal haze measured by Scheimpflug densitometry and slitlamp biomicroscopy was similar in the 2 subgroups at 1 month, there was a difference in the rate of clearing of the haze. Between 3 months and 6 months, the decrease in corneal haze was more significant in the ectasia group than in the keratoconus group. A significant decrease in CXL-associated corneal haze was not observed in the keratoconus groups until 6 months, whereas haze decreased after 3 months in the ectasia group. At 12 months, there was a statistically significant difference in postoperative CXL-associated corneal haze compared with baseline measurements when comparing the keratoconus and ectasia groups; haze remained somewhat increased in the keratoconus group and returned to baseline in the ectasia group. These distinctions could suggest actual differences in the pathophysiology of the 2 diseases or simply statistical anomalies resulting from a smaller number of eyes in the ectasia group. Further follow-up is required to determine whether the values fully return to baseline in the keratoconus eyes as well.

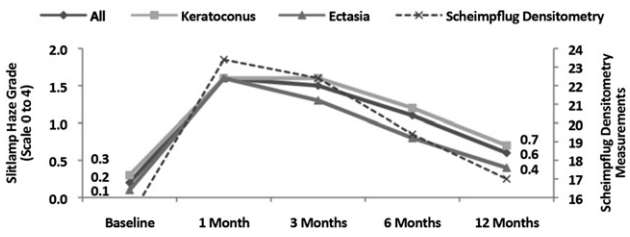

Figure 4. Time course of CXL-associated corneal haze measured by slitlamp biomicroscopy. For comparison, the CXL-associated corneal haze measured by Scheimpflug densitometry is shown.

To protect the corneal endothelium during CXL, it is suggested that the corneal thickness before UV exposure should be more than 400 µm.[1] Therefore, per the study protocol, if corneal thickness was less than 400 µm on ultrasound pachymetry after the initial 30-minute riboflavin loading, hypotonic riboflavin was used to swell the cornea to the 400 µm limit. We found no difference in CXL-associated corneal haze densitometry measurements between eyes requiring hypotonic riboflavin and those not requiring hypotonic riboflavin.

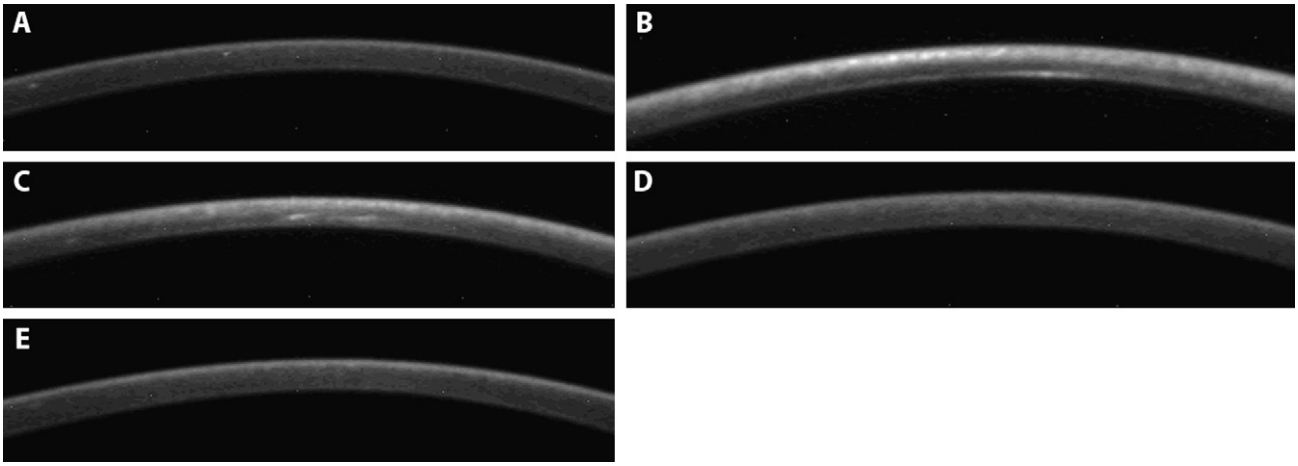

Figure 5. Example of CXL-associated corneal haze over time using Scheimpflug imagery. *A*: Preoperative visit. *B*: One month postoperatively. *C*: Three months postoperatively. *D*: Six months postoperatively. *E*: One year postoperatively.

We found clinical correlations between CXL-associated corneal haze and some outcome parameters. The absolute degree of haze was correlated with poorer UDVA and CDVA, thinner pachymetry, and higher maximum K and mean K values. However, in this analysis, we did not differentiate between patients who had increased densitometry at baseline and those who had increased CXL-associated corneal haze postoperatively. Higher baseline haze likely results from the keratoconus severity per se, and the latter likely will most affect the absolute measurements of clinical outcomes. Therefore, we performed a further analysis to evaluate the correlation between the change in densitometry between baseline and 1 month (the peak change in haze) and baseline and 12 months and the change in clinical outcome measurements at 12 months. Using this methodology, there was no correlation between the change in CXL-associated corneal haze and the change in visual acuity and topographic clinical outcomes in any group. It is noteworthy that the increased stromal haze after CXL observed in confocal microscopy studies did not appear to affect visual acuity outcomes as well.[26] Although increased CXL-associated corneal haze might be thought of as an indication of the efficacy of the CXL action (ie, more CXL-associated corneal haze = greater crosslinking response) or, conversely, as an adverse outcome (ie, more CXL-associated corneal haze causing decreased visual function), the data indicate that CXL-associated corneal haze is not a predictor of patient outcomes, belying both hypotheses. Further study, for instance assessing contrast sensitivity or low-contrast acuity, may help elucidate the clinical sequelae of corneal haze induced by CXL.

Regarding the mechanism of haze formation after CXL, it may be a result of back-scattered and reflected light, which decreases corneal transparency.[30] Transparency of the cornea is a result of the regular spacing and small uniform diameter of the collagen fibrils[31] and the cellular structure of stationary keratocytes.[32] After CXL, the cornea initially thins and then thickens toward baseline over 1 year,[B] a time course which parallels the haze density measurements found in this study. Thus, this supports a hypothesis that concomitant changes in the corneal lamellar array and spacing may lead to an increase in light scatter and a decrease in transparency. Furthermore, Wollsenak et al.[13] report a significant increase in collagen fibril diameter, with increased spacing between collagen fibrils, after CXL. This may also play a role in decreased corneal transparency.

In vitro and ex vivo studies[14,33] show that CXL leads to an almost immediate loss of keratocytes in the corneal stroma. In a confocal microscopy study, Mazzotta et al.[26] found that in eyes with keratoconus, activated keratocytes repopulated the corneal stroma starting at 2 months and that the repopulation was almost complete at 6 months. It is possible that these activated keratocytes contribute to the development of CXL-associated corneal haze. Moreover, stationary keratocytes have crystallins in their cytoplasm; the crystallins have a refractive index similar to that of the ECM. During wound healing, migratory keratocytes have changes in their crystalline proteins, leading to an increased scattering of light and a possible increase in haze.[32]

Other factors also may contribute to CXL-associated corneal haze. These include stromal swelling pressure changes,[16] proteoglycan–collagen interactions,[34] and glycosaminoglycan hydration.[35] Further study is needed to elucidate the pathophysiology of the development and time course of CXL-associated corneal haze.

Although our study shows an objective measurement of CXL-associated corneal haze over time, the haze measurements have some limitations. Densitometry was measured over a 4.0 mm central image of the cornea and along only 1 meridian. Furthermore, although Scheimpflug densitometry affords a quantitative measurement, its specific correlation to clinical corneal haze remains to be assessed. However, the close approximation of our densitometry findings to our results using slitlamp haze grading suggests that densitometry, indeed, does measure clinical corneal haze.

A further limitation of this study was the short follow-up in the control group. Because the study protocol allowed crossover of control eyes to full CXL treatment after the 3-month follow-up, the control group was followed for only 3 months, compared with the 12-month follow-up in the treated group. However, no significant changes were observed in any of the control groups at any time period.

The study protocol also did not allow deepithelialization of the control group corneas. Because epithelial removal alone could cause haze formation during the healing process, further study using a control group in which the epithelium is removed during the sham procedure may further elucidate the source of the haze response.

In conclusion, this study quantitatively evaluated the natural history of corneal haze after corneal collagen crosslinking. After CXL, the corneas in our study developed haze that peaked between 1 month and 3 months and diminished over time, approaching baseline at 1 year.

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