Comparative Functional Outcomes After Corneal Crosslinking Using Standard, Accelerated, and Accelerated With Higher Total Fluence Protocols

Paul Z. Lang, BA, Nikki L. Hafezi, MASIP ETHZ,† Sumitra S. Khandelwal, MD,‡ Emilio A. Torres-Netto, MD,†§¶ Farhad Hafezi, MD, PhD,*†§||** and J. Bradley Randleman, MD****

Purpose: To compare the relative 12-month corneal crosslinking (CXL) functional outcomes using standard protocol and accelerated protocols in patients with progressive keratoconus.

Methods: CXL was performed using 3 epithelium-off protocols: standard [3 mW/cm^2 for 30 minutes, 5.4 J/cm^2 ($S_{3/30}$-CXL)], accelerated with equivalent total irradiance [9 mW/cm^2 for 10 minutes, 5.4 J/cm^2 ($A_{9/10}$-CXL)], and accelerated with increased total irradiance [30 mW/cm^2 for 4 minutes, 7.2 J/cm^2 ($A_{30/4}$-CXL)]. Efficacy measurements were evaluated 12 months after treatment with Scheimpflug imaging (Pentacam HR) and included change in maximum keratometry (K Max), corrected distance visual acuity (CDVA), other keratometric variables, pachymetry, keratoconus indices, astigmatism, asphericity, manifest refraction, and higher order aberrations.

Results: Ninety-three eyes (67 patients) were evaluated: 35 eyes (26 patients) with $S_{3/30}$-CXL, 29 eyes (19 patients) with $A_{9/10}$-CXL, and 29 eyes (22 patients) with $A_{30/4}$-CXL. Mean ΔK Max was −1.53 ± 2.1 diopter (D) for $S_{3/30}$-CXL, −0.71 ± 1.3 D for $A_{9/10}$-CXL, and −0.70 ± 2.3 D for $A_{30/4}$-CXL (P = 0.37). Mean ΔCDVA(logMAR) was −0.18 ± 0.2 for $S_{3/30}$-CXL, −0.13 ± 0.2 for $A_{9/10}$-CXL, and −0.18 ± 0.2 for $A_{30/4}$-CXL (P = 0.79). ΔK Mean (r = −0.29 to −0.46), anterior asphericity (r = −0.34 to −0.40), and central keratoconus index (r = −0.18 to −0.38) best correlated with ΔCDVA. $S_{3/30}$-CXL had greater changes in index of surface variance, index of vertical asymmetry, keratoconus index, and regularization index compared to $A_{9/10}$-CXL and $A_{30/4}$-CXL. There were no other differences between protocols.

Conclusions: All 3 protocols showed improvements in K Max, CDVA, and other variables, with similar functional outcomes for each despite greater change in keratoconus indices after $S_{3/30}$-CXL. Correlations between change in measured variables and CDVA were poor overall; however, K Mean, central keratoconus index, and anterior asphericity were better correlated with CDVA than K Max.

Key Words: corneal crosslinking, keratoconus, Scheimpflug, Pentacam, accelerated CXL

(*Cornea* 2019;38:433–441)

Received for publication July 17, 2018; revision received December 5, 2018; accepted December 8, 2018. Published online ahead of print January 23, 2019.

From the *Keck School of Medicine of USC, Los Angeles, CA; †ELZA Institute, Dietikon, Zurich, Switzerland; ‡Baylor College of Medicine, Cullen Eye Institute, Houston, TX; §Ocular Cell Biology Group, University of Zurich, Zurich, Switzerland; ¶Paulista School of Medicine, Federal University of Sao Paulo, Sao Paulo, Brazil; ||University of Wenzhou, Wenzhou, China; and **University of Southern California, Roski Eye Institute, Los Angeles, CA.

Supported in part by unrestricted departmental grants to the USC Roski Eye Institute and Baylor College of Medicine from Research to Prevent Blindness (New York, NY).

F. Hafezi—Shareholder/investor for EMAGine AG (Zug, Switzerland), consultant for GroupAdvance Consulting GmbH (Zug, Switzerland), exclusive patent owner for PCT patent/application (corneal apparatus used for CXL and chromophore for CXL application), recipient of travel funds from Light for Sight Foundation (Zurich, Switzerland), directed research funds from Light for Sight Foundation (Zurich, Switzerland), Schwind eye-tech-solutions (Kleinostheim, Germany), Velux Foundation (Søborg, Denmark), Gelbert Foundation (Geneva, Switzerland), and in-kind financial contribution for research materials from Sooft Italia (Montegiorgio, Italy). N. L. Hafezi—Shareholder/investor for EMAGine AG (Zug, Switzerland), consultant for GroupAdvance Consulting GmbH (Zug, Switzerland), recipient of travel funds from Light for Sight Foundation (Zurich, Switzerland), directed research funds from Light for Sight Foundation (Zurich, Switzerland). The remaining authors have no funding or conflicts of interest to disclose.

Correspondence: J. Bradley Randleman, MD, USC Roski Eye Institute, Keck School of Medicine of USC, 1450 San Pablo St., Los Angeles, CA, 90033 (e-mail: randlema@usc.edu).

Corneal crosslinking (CXL) has been proven effective in halting the ectatic process in the vast majority of patients with progressive keratoconus[1] and post-LASIK ectasia.[2,3] The treatment parameters used in most studies were identical to the original protocol described by Wollensak and colleagues,[4] now commonly referred to as the standard or Dresden protocol. More recently, great interest has developed in altering treatment parameters to reduce overall treatment time while maintaining ability to halt progression of keratoconus. Various protocols have been developed that accelerate treatment but maintain total irradiance or accelerate treatment while increasing total irradiation. Comparatively less is known about these accelerated protocols.[5–10]

Although CXL effects are straightforward to identify in the laboratory setting,[11,12] clinical outcomes after CXL have proven more challenging to evaluate. The primary variable evaluated has been change in maximum anterior corneal steepness (K Max) after treatment, but this is problematic for a variety of reasons. There has been a disconnect between Scheimpflug imaging metrics, such as change in K Max or corneal thickness, and

patient-related metrics, such as change in CVDA, refraction, and/or visual symptoms.[13]

The purpose of this study was to compare the relative 12-month efficacy of 3 CXL protocols (standard 5.4 J/cm^2, accelerated with equal total irradiation 5.4 J/cm^2, and accelerated with increased total irradiation of 7.2 J/cm^2) by evaluating a wide range of objective variables obtained using Scheimpflug imaging and to determine the variables that best correlate with change in visual acuity after CXL.

MATERIALS AND METHODS

Data were retrospectively collected from 3 separate study groups. For the standard protocol CXL ($S_{3/30}$-CXL), data were generated from patients who were enrolled in a multicenter, prospective, randomized controlled clinical trial at the Emory University, Atlanta, GA, to evaluate the efficacy of CXL in patients with progressive keratoconus (ClinicalTrials.gov identifier #NCT00567671), and treated by one surgeon (J.B.R.).[1,2] Inclusion criteria were based on those used in the clinical trial and included patients with a diagnosis of progressive keratoconus, defined as 2 or more of the following changes after 24-month period: an increase of 1.00 diopter (D) or more in the steepest topographic keratometry (K) measurements, increase of 1.00 D or more in manifest cylinder, or an increase of 0.50 D or more in manifest spherical equivalent (MRSE). For the accelerated protocol CXL with equivalent total irradiance ($A_{9/10}$-CXL), data were generated from patients who were treated at the ELZA Institute, Dietikon/Zurich, Switzerland using similar inclusion and exclusion criteria for treatment. For the accelerated protocol CXL with increased total irradiance ($A_{30/4}$-CXL), data were generated from patients who were enrolled at the Emory University as part of a prospective, multicenter, randomized, placebo-controlled study of the KXL System with VibeX (Riboflavin Ophthalmic Solution) for corneal crosslinking in eyes with keratoconus (ClinicalTrials.gov Identifier: NCT01643226, Avedro Inc, Waltham, MA) using similar inclusion criteria. Institutional Review Board approval for comparative analysis was granted by the University of Southern California. Full details for each treatment protocol are found in Table 1.

Clinical Examination

All patients discontinued contact lens wear for a minimum of 1 week (soft contact lenses) or 2 weeks (rigid contact lenses) before preoperative eye examination. A stable refraction was determined in which the MRSE and K measurements at the first visit did not differ by more than 0.75 D from the respective measurements at the second visit.

Measurements for this study were taken from evaluations at screening/baseline and 1, 3, 6, and 12 months after treatment. Measurement of uncorrected distance visual acuity, best spectacle-corrected distance visual acuity (CDVA), and manifest refraction were obtained at multiple time points after CXL treatments. Visual acuity measurements were obtained under controlled lighting conditions using a modified Lighthouse Early Treatment of Diabetic Retinopathy Study visual acuity test with Sloan letters. Patients were tested 4 m from the visual acuity chart. Visual acuity was recorded in Early Treatment of Diabetics Retinopathy Study notation and converted to Logarithm of the Minimum Angle of Resolution (logMAR) for analysis. Slit-lamp examination was performed at all visits.

CXL Treatment Protocols

CXL treatment protocol parameters are listed in Table 1. For each protocol, epithelium was mechanically removed to a 9.0 mm corneal diameter. After riboflavin application with the $S_{3/30}$-CXL and $A_{30/4}$-CXL protocols, corneal stroma and anterior chamber saturation were confirmed by slit-lamp examination. Ultrasound (US) pachymetry was performed, and if the corneal thickness was less than 400 μm, hypotonic riboflavin (0.1% in sterile water) was administered, 1 drop every 10 seconds for 2 minutes per treatment, and repeated until US pachymetry confirmed corneal thickness greater than 400 μm. The cornea was then aligned and exposed to UVA 365 nm light for the protocol duration.

For the $A_{9/10}$-CXL protocol, each cornea was soaked with 0.1% hypoosmolar riboflavin (Ricrolin Plus, SOOFT Italia, Italy) for 20 minutes following de-epithelialization. As the speculum was left in place during the entire procedure, hypoosmolar riboflavin was used in every patient to counterbalance the corneal thinning that occurs due to evaporation.[14] The corneas were then subsequently treated with UV-A light (CXL-365, Schwind eye-tech-solutions, Kleinostheim, Germany) irradiation at 9 mW/cm^2 for 10 minutes.

Postoperatively, antibiotic and corticosteroid drops were administered, a soft contact lens placed, and the eye re-examined at the slit lamp. Patients were seen at one day and within one week after treatment, with contact lens removal once the epithelial defect was resolved. Postoperative drops included fourth-generation fluoroquinolone antibiotics 4 times per day for 7 days, prednisolone acetate 1% 1 drop 4 times per day for 1 to 2 weeks, and preservative-free artificial tears as needed.

Scheimpflug Analysis

Scheimpflug imaging (Pentacam HR; Oculus, Wetzlar, Germany) was obtained at baseline and 1, 3, 6, and 12 months after treatment. Scheimpflug measurements analyzed included maximum keratometry values (K Max), mean keratometry at 4 mm (K Mean), elevation, astigmatism, asphericity, pachymetry, keratoconus indices [index of surface variance (ISV), index of vertical asymmetry (IVA), central keratoconus index (CKI), keratoconus index, and index of height asymmetry (IHA)], and inferior–superior value (IS value). Root mean square and coma higher order aberration values were analyzed between $A_{9/10}$-CXL and $A_{30/4}$-CXL only because the Scheimpflug imaging utilized at the time for the $S_{3/30}$-CXL group was not capable of Zernike wavefront analysis. For analysis, all data were exported as U12 files and uploaded into one software system at the University of Southern California Ocular Cell Biology and Biomechanics Laboratory to standardize all scales.

TABLE 1. CXL Protocol Treatment Parameters

	Standard ($S_{3/30}$-CXL)	Standard Accelerated ($A_{9/10}$-CXL)	Increased Accelerated ($A_{30/4}$-CXL)
Treatment target	Keratoconus	Keratoconus	Keratoconus
Fluence (total) (J/cm^2)	5.4	5.4	7.2
Riboflavin soak time and interval (minutes)	30 (q2)	20 (q2)	20 (q2)
Intensity (mW/cm^2)	3	9	30
Treatment time (min)	30	10	4
Epithelial status	Off—9 mm removal	Off—9 mm removal	Off—9 mm removal
Chromophore	0.12% riboflavin	0.1% riboflavin	0.1% riboflavin
Riboflavin carrier	Dextran	None	Dextran
Light source	UV-X (Peshke)	CXL-365 (Schwind)	KXL (Avedro)
Irradiation mode	Continuous	Continuous	Continuous
Protocol abbreviation in manuscript	$S_{3/30}$-CXL	$A_{9/10}$-CXL	$A_{30/4}$-CXL

Statistical Analysis

Statistical analysis was conducted using SPSS Statistics for Windows, Version 24.0 (IBM Corp, Armonk, NY). Tests of normality were conducted using the Shapiro–Wilk test. Within-group comparisons of baseline variables and 12-month post-operative variables were conducted using a paired *t* test for normally distributed variables and the Wilcoxon signed-rank test for non-normally distributed variables. A Levene test was used to test homogeneity of variance. For variables with homogeneity of variances and normal distribution, between-group comparisons for baseline variables and also change in variables at 12 months compared to baseline were made using a 1-way analysis of variance with Tukey HSD as post-hoc analysis for significant results. A Welch F test was performed for normally distributed variables without homogeneity of variances with a Games–Howell test as post-hoc analysis for significant results. A Kruskal–Wallis test with post-hoc Dunn test was conducted for non-normally distributed data. Between-group comparisons for higher order aberration variables were conducted with a Student *t* test. Correlation between measured variables with CDVA was calculated using Pearson r correlation.

RESULTS

Ninety-three eyes from 67 total patients were evaluated, including 35 eyes (26 patients) with $S_{3/30}$-CXL, 29 eyes (19 patients) with $A_{9/10}$-CXL, and 29 eyes (22 patients) with $A_{30/4}$-CXL. Baseline values for patient groups from each protocol are shown in Table 2. At baseline, all 3 protocols differed in CDVA, with the $S_{3/30}$-CXL group having worse CDVA before treatment. Patients in the $S_{3/30}$-CXL group were older and had higher ISV than the $A_{9/10}$-CXL group and lower IHA and IHD than the $A_{30/4}$-CXL group. The $A_{9/10}$-CXL group had less negative anterior asphericity, higher pachymetry, and lower K Mean values than both the $S_{3/30}$-CXL and $A_{30/4}$-CXL groups. The $A_{9/10}$-CXL group had lower CKI, K Max, and elevation values than the $A_{30/4}$-CXL group. No differences were observed for higher order aberration values.

Visual Acuity and Refractive Outcomes after CXL

Mean change in CDVA (logMAR) and MRSE are shown in Table 3. Significant differences from within-group analysis of baseline and postoperative variables were found for CDVA for $S_{3/30}$-CXL, CDVA and SE for $A_{9/10}$-CXL, and CDVA for $A_{30/4}$-CXL (Table 4). Between-group analysis found no significant differences between protocols in CDVA or refractive outcomes (Table 3). Figure 1 shows the distribution of outcomes in CDVA between the 3 protocols. Improvements to CDVA were seen in 85.7% (n = 30) of the $S_{3/30}$-CXL patients, 75% (n = 18) of $A_{9/10}$-CXL patients, and 82.8% (n = 24) of $A_{30/4}$-CXL patients.

Scheimpflug Parameters After CXL

Mean change in measured Scheimpflug parameters are shown in Table 3. Significant differences found from within-group analysis of baseline and postoperative variables are shown in Table 4. Figure 2 shows the distribution of outcomes for K Max for the 3 protocols. K Max was found to be stabilized in 97.1% (n = 34) of $S_{3/30}$-CXL patients, 93.10% (n = 27) of $A_{9/10}$-CXL patients, and 86.2% (n = 25) of $A_{30/4}$-CXL patients. Figure 3 shows the distribution of outcomes for K Mean between the 3 protocols. K Mean stabilized for 94.3% (n = 33) of $S_{3/30}$-CXL patients, 96.6% (n = 28) of $A_{9/10}$-CXL patients, and 93.1% (n = 27) of $A_{30/4}$-CXL patients.

Between-group analysis found no significant differences in change in average anterior and posterior elevation. The mean change in topometric indices was significantly greater in the $S_{3/30}$-CXL group compared to both the $A_{9/10}$-CXL and $A_{30/4}$-CXL groups for ISV, IVA, and keratoconus index (Fig. 4).

Visual Acuity Correlation Analysis

The strongest correlations between preoperative parameters and improved CDVA after CXL were found for IHA (r = −0.42), IVA (r = −0.42), and ISV (r = −0.41) for $S_{3/30}$-CXL, K Mean (r = −0.58), K Max (r = −0.51), and CKI (r = −0.47) for $A_{9/10}$-CXL, and posterior astigmatism (r = −0.45), anterior astigmatism (r = −0.36), and Z^3_1 horizontal coma (r = 0.32) for $A_{30/4}$-CXL.

The strongest correlations between change at 12 months and CDVA were found for anterior asphericity (r = 0.395), posterior astigmatism (r = 0.310), and K Mean (r = −0.307)

TABLE 2. Baseline Values for 3 CXL Protocols

	$S_{3/30}$-CXL (n = 35)	$A_{9/10}$-CXL (n = 29)	$A_{30/4}$-CXL (n = 29)	*P*
Age	39.6 ± 12*	31.9 ± 9.4	34.7 ± 9.3	0.02
CDVA (logMAR)	1.29 ± 0.27*†	0.331 ± 0.32†	0.743 ± 0.30	<0.001
MRSE	−5.18 ± 4.8	−3.10 ± 4.2	−5.89 ± 5.6	0.08
Asph. Q F	−0.960 ± 0.51*	−0.450 ± 0.60†	−1.00 ± 0.52	0.002
Pachy min	452 ± 40*	492 ± 59†	450 ± 38	0.002
ISV	106 ± 36*	81.2 ± 44	103 ± 36	0.03
IVA	1.19 ± 0.45	0.911 ± 0.60	1.03 ± 0.41	0.08
KI	1.29 ± 0.13	1.21 ± 0.18	1.25 ± 0.12	0.08
CKI	1.06 ± 0.039	1.03 ± 0.049†	1.09 ± 0.068	0.005
IHA	23.5 ± 17†	32.3 ± 28	44.9 ± 31	0.021
IHD	0.968 ± 0.044†	0.116 ± 0.081	0.156 ± 0.074	0.004
K Max sagittal	56.3 ± 6.1	53.1 ± 6.8†	59.6 ± 7.5	0.007
K Mean (4 mm)	49.4 ± 4.2*	45.8 ± 4.3†	51.1 ± 5.8	0.001
F Ele (3 mm)	5.97 ± 5.1	3.13 ± 5.8†	9.12 ± 5.7	<0.001
F Ele (5 mm)	−1.09 ± 1.9	−0.61 ± 1.4†	−2.00 ± 2.3	0.02
B Ele (3 mm)	15.4 ± 11	8.43 ± 11†	19.7 ± 13.4	0.02
B Ele (5 mm)	−1.91 ± 4.0	−1.10 ± 3.4†	−3.72 ± 4.7	0.045
IS value	8.12 ± 4.4	6.33 ± 5.2	6.61 ± 3.3	0.21
RMS HOA	N/A	2.92 ± 3.2	3.18 ± 1.3	0.69
Z^3_1	N/A	−0.0988 ± 0.60	−0.183 ± 1.6	0.79
Z^3_{-1}	N/A	−2.24 ± 2.2	−2.22 ± 1.2	0.98

*Significantly different than 9 mW/cm^2 for 10 minutes group ($P < 0.05$).
†Significantly different than 30 mW/cm^2 for 4 minutes group ($P < 0.05$).
Asph F, anterior asphericity; B Ele, average posterior elevation in a zone; F Ele, average anterior elevation in a zone; IHD, index of height decentration; IS-Value, inferior–superior value; K Max, maximum keratometry; K Mean, average keratometry; KI, keratoconus index; MR(Cyl), manual refraction cylinder; MR(Sph), manual refraction sphere; Pachy min, minimum pachymetry; RMS HOA, root mean square of higher order aberrations; SE, spherical equivalent; Z^3_1, horizontal coma; Z^3_{-1}, vertical coma.

for $S_{3/30}$-CXL; $Pachy_{pupil}$ (r = −0.710), $Pachy_{min}$ (r = −0.670), and $Pachy_{apex}$ (r = −0.662) for $A_{9/10}$-CXL; and K Mean (r = −0.458), anterior asphericity (r = 0.395), CKI (r = −0.383), and posterior asphericity (r = 0.383) for $A_{30/4}$-CXL.

Although some of the changes in the preoperative and postoperative CXL parameters were moderately correlated, particularly for the $A_{9/10}$-CXL protocol, no single metric correlated well with all 3 protocols. Additionally, a variety of metrics were more strongly correlated with change in CDVA than both preoperative and change in K Max.

DISCUSSION

The results of this study demonstrate that all 3 protocols evaluated were effective at halting the progression of keratoconus for most patients. The standard protocol ($S_{3/30}$-CXL) had more parameters that showed significant improvements at 12 months compared to either accelerated protocol and also showed significantly better improvements in some of the keratoconus indices. However, visual acuity, refractive outcomes, and proportion of total patients who experienced stabilization of disease were similar between all protocols.

The standard CXL protocol has been shown to be a successful treatment for patients with ectasia, which is capable of stabilizing and flattening the cornea.[1,3] Current protocol development efforts involve reducing overall treatment time with various accelerated protocols while maintaining ability to halt the progression of keratoconus. The Bunsen–Roscoe law states a biological effect is directly proportional to the total energy dose, and thus decreasing total crosslinking treatment time would be compensated by increasing UV light intensity. Multiple studies, however, have found that this law is not perfectly applicable to the human cornea, as increasing UV intensity results in decreased stiffness and a more shallow demarcation line, a possible marker of treatment depth, despite equivalent irradiance of 5.4 J/cm^2.[7,10,15,16]

Despite their apparent limitations in biomechanical analysis in the laboratory setting, accelerated protocols have been found to be safe and effective at halting progression of ectasia, at least in the short term.[17,18] Hashemi et al[19] compared an 18 mW/5-minute protocol with standard protocol 15 months after treatment and found that the standard protocol led to more measurable corneal flattening but otherwise had similar safety and efficacy compared to the accelerated protocol. Chow et al[20] compared the 18 mW/cm^2 for 5 minutes protocol with standard CXL at 12 months post-treatment and also found that both protocols produce similar clinical results and were capable of stabilizing keratoconus. Hashemian et al[9] compared results from a 30 mW/cm^2 for 3 minute (5.4 J/cm^2) accelerated protocol with standard protocol 15 months after treatment and concluded that outcomes were similar between the 2 protocols by 12 months post-treatment. Tomita et al[21] reported the same results comparing 30 mW/cm^2 for 3 minutes protocol with standard protocol at 12 months post-treatment. Studies that have looked at a 9 mW/cm^2 for 10 minutes protocol have similarly

TABLE 3. Change in Clinical and Scheimpflug Imaging Parameters at 12 Months After CXL

	$S_{3/30}$-CXL (n = 31)	$A_{9/10}$-CXL (n = 23)	$A_{30/4}$-CXL (n = 25)	*P*
CDVA (logMAR)	−0.183 ± 0.19	−0.129 ± 0.24	−0.183 ± 0.21	0.79
MRSE	0.911 ± 2.8	0.859 ± 2.3	1.52 ± 4.7	0.38
Asph. Q F	0.0851 ± 0.26	0.0300 ± 0.13	0.0293 ± 0.11	0.48
Pachy min	−12.1 ± 25	−8.83 ± 19	−10.6 ± 16	0.99
ISV	−11.7 ± 15*†	−0.760 ± 11	−2.00 ± 8.6	0.002
IVA	−0.144 ± 0.25*†	−0.0162 ± 0.16	−0.0231 ± 0.17	0.004
KI	−0.0397 ± 0.065*†	−0.00621 ± 0.036	−0.00621 ± 0.035	0.006
CKI	−0.00943 ± 0.020	−0.00931 ± 0.015	−0.00703 ± 0.025	0.99
IHA	2.57 ± 15	−6.29 ± 22	−2.43 ± 21	0.19
IHD	−0.0115 ± 0.023	−0.00400 ± 0.021	−0.00572 ± 0.021	0.35
K Max sagittal	−1.53 ± 2.1	−0.707 ± 1.3	−0.697 ± 2.3	0.37
K Mean (4 mm)	−0.859 ± 1.6	−0.549 ± 0.85	−0.524 ± 1.7	0.86
F Ele (3 mm)	−0.503 ± 2.5	0.148 ± 1.6	0.452 ± 4.4	0.45
F Ele (5 mm)	0.260 ± 1.3	0.586 ± 1.0	0.772 ± 1.9	0.36
B Ele (3 mm)	−0.114 ± 4.0	1.02 ± 3.1	2.68 ± 7.8	0.12
B Ele (5 mm)	−0.309 ± 2.3	0.521 ± 2.7	0.779 ± 3.9	0.32
IS value	−1.25 ± 3.6†	−0.451 ± 2.4	0.0310 ± 0.63	0.001
Regularization index	4.85 ± 4.7*†	2.04 ± 2.7	2.53 ± 2.9	<0.001
RMS HOA	N/A	−0.592 ± 2.5	−0.0543 ± 0.43	0.27
Z^3_1	N/A	−0.025 ± 0.30	0.347 ± 1.2	0.12
Z^3_{-1}	N/A	0.414 ± 1.1	−0.0253 ± 0.35	0.06

*Significantly different than the 9 mW/cm^2 for 10 minutes group ($P < 0.05$).
†Significantly different than the 30 mW/cm^2 for 4 minutes group ($P < 0.05$).
Asph F, anterior asphericity; B Ele, average posterior elevation in a zone; F Ele, average anterior elevation in a zone; IHD, index of height decentration; IS value, inferior–superior value; K Max, maximum keratometry; K Mean, average keratometry; KI, keratoconus index; MR(Cyl), manual refraction cylinder; MR(Sph), manual refraction sphere; Pachy min, minimum pachymetry; RMS HOA, root mean square of higher order aberrations; SE, spherical equivalent; Z^3_1, horizontal coma; Z^3_{-1}, vertical coma.

found comparable clinical outcomes between the accelerated and standard protocols.[22,23]

Relatively fewer studies have compared increased irradiance accelerated protocols with standard protocol. Sherif[24] compared 12-month outcomes from a 30 mW/cm^2 for 4 minutes 20 seconds (7.2 J/cm^2) accelerated protocol with standard CXL and found comparable results between the 2 protocols. Choi et al[25] looked at a 30 mW/cm^2 for 3 minutes 40 seconds (6.6 J/cm^2) accelerated protocol and standard protocol outcomes at 12 months post-treatment and observed significant clinical improvements with standard protocol CXL only. Kymionis et al[26] compared the 30 mW/cm^2 for 3 minutes protocol with an increased irradiance 18 mW/cm^2 for 7 minutes protocol (7.5 J/cm^2) and found comparable outcomes in demarcation line depth and change in mean endothelial cell density.

To our knowledge, only 2 other studies have compared outcomes from multiple accelerated protocols and standard

TABLE 4. Summary of Significant Changes After CXL by Protocol

$S_{3/30}$-CXL (n = 31)	*P*	$A_{9/10}$-CXL (n = 23)	*P*	$A_{30/4}$-CXL (n = 25)	*P*
CDVA (logMAR)	<0.001	CDVA (logMAR)	0.014	CDVA (logMAR)	<0.001
K Max sagittal	<0.001	K Max sagittal	0.007	K Max sagittal	0.028
K Mean (4 mm)	0.003	K mean (4 mm)	0.002	K mean (4 mm)	0.004
CKI	0.01	F Ele (5 mm)	0.005	F Ele (5 mm)	0.037
Pachy min	0.008	CKI	0.003	CKI	0.024
Astig F(D)	0.01	Pachy min	0.02	Pachy min	0.005
ISV	<0.001	SE	0.03		
IVA	0.001				
KI	<0.001				
IHD	0.006				

Astig F, anterior astigmatism; F Ele, average anterior elevation in a zone; IHD, index of height decentration; K Max, maximum keratometry; K Mean, mean keratometry; KI, keratoconus index; Pachy Min, minimum pachymetry; SE, spherical equivalent.

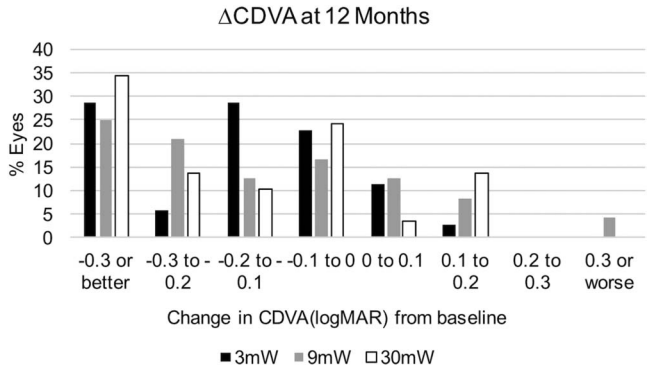

FIGURE 1. Distribution of CDVA outcomes 12 months following CXL.

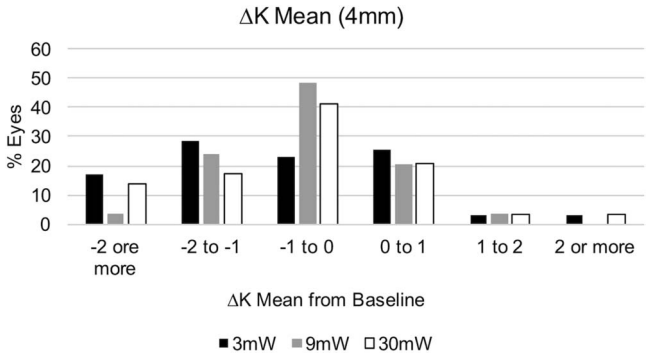

FIGURE 3. Distribution of K Mean outcomes 12 months following CXL.

CXL. Shetty et al[8] compared standard CXL with 3 accelerated protocols with equivalent total irradiance (9 mW/cm^2 for 10 minutes, 18 mW/cm^2 for 5 minutes, and 30 mW/cm^2 for 3 minutes). They concluded that all 4 protocols were safe and that standard 9 and 18 mW/cm^2 protocols showed clinical improvements by 12 months post-treatment, though minimal stabilization of disease was found in the 30 mW/cm^2 group. Toker et al[27] compared standard CXL with an accelerated protocol (9 mW/cm^2 for 10 minutes), an increased irradiance accelerated protocol (30 mW/cm^2 for 4 minutes, 7.4 J/cm^2), and a pulsed-light, increased irradiance accelerated protocol (30 mW/cm^2 for 8 minutes with 1 second on/1 second off). They also concluded that the 30 mW/cm^2 protocols appeared less effective compared to the standard and 9 mW/cm^2 protocols, but that all protocols were equally effective at halting disease progression.

The results of our study and previous studies seem to suggest a comparable clinical efficacy between standard and various accelerated protocols, at least with UV intensities up to 18 mW/cm^2. Although in our study $A_{30/4}$-CXL performed similarly to $A_{9/10}$-CXL, conflicting results exist in the literature regarding efficacy of 30 mW/cm^2 protocols of both equivalent and increased irradiance. It is unclear why there is such a variance in reported results for 30 mW/cm^2 protocols, though the shorter time of treatment likely contributes in some way. Overall, most studies, including our own, have found comparable improvements in visual acuity between all

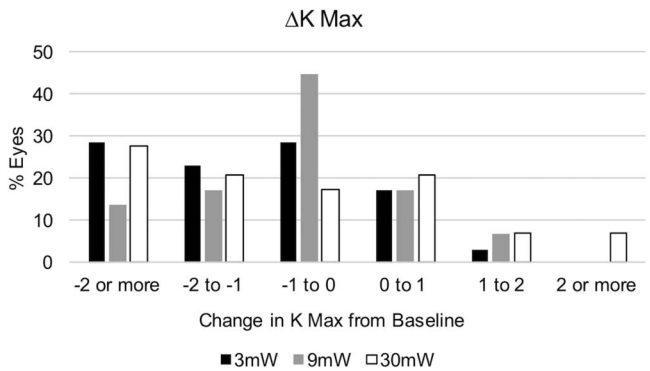

FIGURE 2. Distribution of K Max outcomes 12 months following CXL.

accelerated and standard protocols. This, in contrast with the smaller topographic improvements and more shallow demarcation line compared to standard protocol, raises the importance of determining not only how much treatment is adequate but also which metrics are the best to use for evaluating treatment success.[6]

One of the main concerns with using accelerated crosslinking protocols, especially with increased irradiance protocols, revolves around safety and crossing the endothelium safety threshold with the greater amount of energy delivered to the cornea. Endothelial damage has been shown to occur when a threshold of 0.65 J/cm^2 of energy reaches the endothelium.[28] In a cornea with a thickness of 400 μm, this translates to a total irradiance of 9.75 J/cm^2. Increasing the total irradiance in the $A_{30/4}$-CXL protocol from 5.4 J/cm^2 to 7.2 J/cm^2 is still well within this safety threshold, especially given that the mean minimum pachymetry in the $A_{30/4}$-CXL group was 450 μm. Although our study did not investigate change in the endothelial cell density, previous groups have found accelerated protocols of both equal and increased irradiance to be safe.[22,29] Ozgurhan et al[30] found that there was no significant change in the endothelial cell density when using an accelerated 30 mW/cm^2 protocol to treat keratoconus patients with a minimum corneal thickness of less than 400 μm.

Another concern regarding accelerated crosslinking protocols involves the shallower demarcation line after treatment compared to the standard protocol.[31] The demarcation line is a visible line in the corneal stroma that forms following crosslinking and is thought to represent keratocyte apoptosis and likely changes in refractive properties of the treated areas of the cornea.[32,33] Although some believe that the demarcation line is an indicator of treatment efficacy,[33] the clinical significance of the demarcation line is likely not a simple direct correlation with treatment depth. The depth of the demarcation line has been found to correlate poorly with other outcome metrics of crosslinking, and accelerated crosslinking protocols have been found to have comparable clinical outcomes despite more shallow demarcation lines.[34,35] Although this study did not have the available data, it may be helpful for future studies to investigate the significance of the demarcation line.

It is thought that oxygen is the rate limiting factor in the crosslinking reaction and that reactive oxidative species

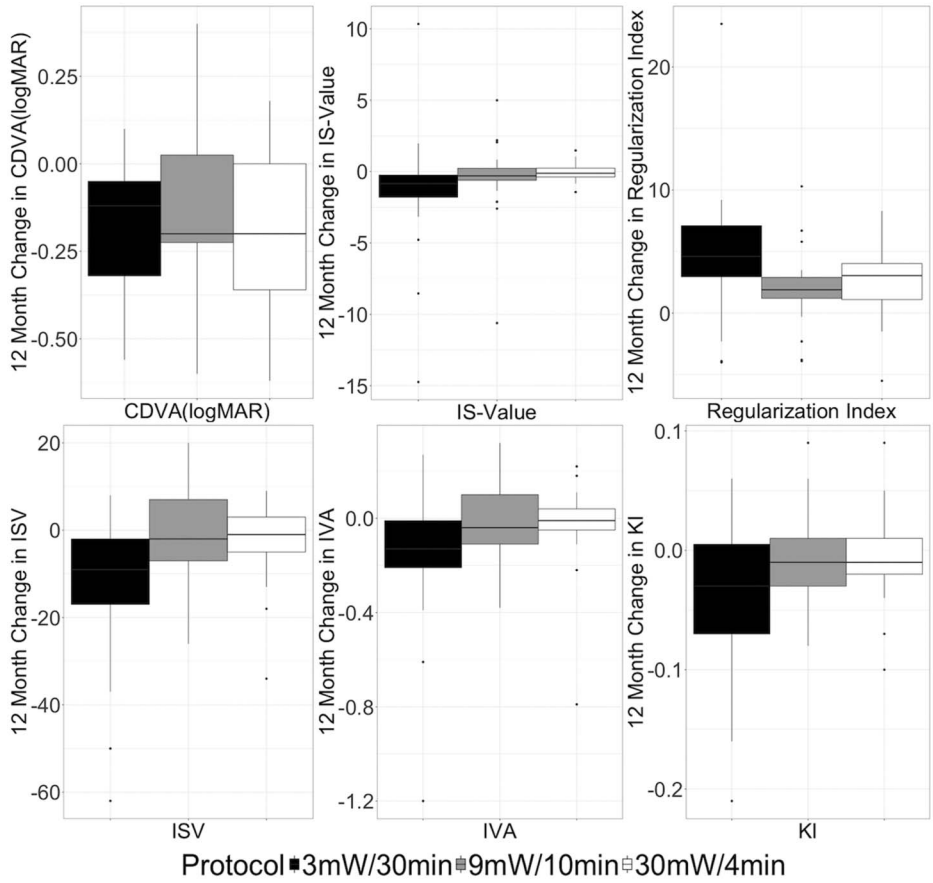

FIGURE 4. Boxplots for 12-month change in significant variables.

formed by the UV light reacting with the riboflavin are the catalyst for crosslink bond formation.[36] Time is a crucial factor allowing for oxygen to replenish in the reaction and may explain the shallower demarcation line and varied reported outcomes in accelerated crosslinking protocols. Indeed, lack of time for oxygen to replenish may be an explanation for our observed differences in patients receiving accelerated protocols whose keratoconus continued to progress compared to those receiving standard protocols. Although multiple solutions regarding this in accelerated protocols appear promising, such as pulsed UV light and increasing overall irradiance, no consensus on an effective solution has yet been made.[26,37–39] Although pulsed UV light has been shown to lead to a deeper depth of demarcation line compared to continuous light crosslinking, there is still a need for further comparison of clinical outcomes between the 2 protocols. Thus, the relative role of oxygen consumption in the crosslinking process is fundamental but should not be overestimated because this is not the only factor driving photochemical reactions during crosslinking.

K Max is generally considered to be an easy to measure but less than ideal variable to use in following crosslinking treatment.[40] In our study, we attempted to determine whether changes in any variable correlated well with changes in visual acuity outcomes. Few variables were satisfactorily correlated with changes in CDVA (logMAR), and even fewer still were consistently correlated among all 3 protocols. The variables that showed the best correlation overall included anterior asphericity, K Mean, and CKI; however, none of these were well correlated for any protocol. Of note, both K Max and regularization index, a metric introduced by Seiler et al to more robustly evaluate change in anterior curvature after CXL, had poor correlation with change in CDVA.[40,41] A possible explanation for the lack of correlation between measured variables and change in CDVA could be that the currently available measurements are limited in that they measure changes in the anterior stroma that are indirect consequences of the actual biomechanical disease process. Quantifying treatment effect may be better served by direct biomechanical measurements, using technology such as the Ocular Response Analyzer,[42] CorvisST,[43] or Brillouin microscopy.[7,11]

There are limitations to this study, including its retrospective design, smaller sample sizes, relatively short follow-up, and variation in some preoperative parameters between protocol cohorts. Additionally, we were not able to compare demarcation line depth and endothelial cell density between the protocols. Ideally, a randomized trial comparing multiple protocols would be conducted in one setting over a longer follow-up time and with well-matched preoperative patient characteristics. This, however, may prove challenging in clinical settings.

In conclusion, our study found that clinical outcomes after CXL using a standard protocol or accelerated

protocols with equivalent or increased total irradiance were all effective at halting keratoconus progression. Overall, change in visual acuity and most Scheimpflug parameters were relatively comparable to the standard CXL protocol with the notable exception of improvements in multiple surface asymmetry indices, which all showed greater improvement after standard protocol CXL. The results of our study are largely in line with the current literature and emphasize the need for not only a better understanding of the effects of accelerated crosslinking protocols but also an optimal metric to evaluate such effects consistently in vivo. Until such a goal is achieved, it is important to consider many different variables other than just K Max when studying different CXL protocols.

REFERENCES

1. Hersh PS, Stulting RD, Muller D, et al. United States multicenter clinical trial of corneal collagen crosslinking for keratoconus treatment. *Ophthalmology.* 2017;124:1259–1270.
2. Hersh PS, Stulting RD, Muller D, et al. U.S. Multicenter Clinical Trial of corneal collagen crosslinking for treatment of corneal ectasia after refractive surgery. *Ophthalmology.* 2017;124:1475–1484.
3. Hafezi F, Kanellopoulos J, Wiltfang R, et al. Corneal collagen crosslinking with riboflavin and ultraviolet A to treat induced keratectasia after laser in situ keratomileusis. *J Cataract Refract Surg.* 2007;33:2035–2040.
4. Wollensak G, Spoerl E, Seiler T. Riboflavin/ultraviolet-a-induced collagen crosslinking for the treatment of keratoconus. *Am J Ophthalmol.* 2003;135:620–627.
5. Randleman JB, Khandelwal SS, Hafezi F. Corneal cross-linking. *Surv Ophthalmol.* 2015;60:509–523.
6. Santhiago MR. Accelerated corneal cross-linking: we must acquire knowledge as fast. *J Refract Surg.* 2016;32:362–363.
7. Webb JN, Su JP, Scarcelli G. Mechanical outcome of accelerated corneal crosslinking evaluated by Brillouin microscopy. *J Cataract Refract Surg.* 2017;43:1458–1463.
8. Shetty R, Pahuja NK, Nuijts RM, et al. Current protocols of corneal collagen cross-linking: visual, refractive, and tomographic outcomes. *Am J Ophthalmol.* 2015;160:243–249.
9. Hashemian H, Jabbarvand M, Khodaparast M, et al. Evaluation of corneal changes after conventional versus accelerated corneal cross-linking: a randomized controlled trial. *J Refract Surg.* 2014;30:837–842.
10. Bao F, Zheng Y, Liu C, et al. Changes in corneal biomechanical properties with different corneal cross-linking irradiances. *J Refract Surg.* 2018;34:51–58.
11. Scarcelli G, Kling S, Quijano E, et al. Brillouin microscopy of collagen crosslinking: noncontact depth-dependent analysis of corneal elastic modulus. *Invest Ophthalmol Vis Sci.* 2013;54:1418–1425.
12. Wollensak G, Spoerl E, Seiler T. Stress-strain measurements of human and porcine corneas after riboflavin-ultraviolet-A-induced cross-linking. *J Cataract Refract Surg.* 2003;29:1780–1785.
13. Greenstein SA, Fry KL, Hersh PS. Corneal topography indices after corneal collagen crosslinking for keratoconus and corneal ectasia: one-year results. *J Cataract Refract Surg.* 2011;37:1282–1290.
14. Soeters N, van Bussel E, van der Valk R, et al. Effect of the eyelid speculum on pachymetry during corneal collagen crosslinking in keratoconus patients. *J Cataract Refract Surg.* 2014;40:575–581.
15. Wernli J, Schumacher S, Spoerl E, et al. The efficacy of corneal cross-linking shows a sudden decrease with very high intensity UV light and short treatment time. *Invest Ophthalmol Vis Sci.* 2013;54:1176–1180.
16. Hammer A, Richoz O, Arba Mosquera S, et al. Corneal biomechanical properties at different corneal cross-linking (CXL) irradiances. *Invest Ophthalmol Vis Sci.* 2014;55:2881–2884.
17. Badawi AE. Corneal endothelial changes after accelerated corneal collagen cross-linking in keratoconus and postLASIK ectasia. *Clin Ophthalmol.* 2016;10:1891–1898.
18. Marino GK, Torricelli AA, Giacomin N, et al. Accelerated corneal collagen cross-linking for postoperative LASIK ectasia: two-year outcomes. *J Refract Surg.* 2015;31:380–384.
19. Hashemi H, Miraftab M, Seyedian MA, et al. Long-term results of an accelerated corneal cross-linking protocol (18 mW/cm^2) for the treatment of progressive keratoconus. *Am J Ophthalmol.* 2015;160:1164–1170.e1161.
20. Chow VW, Chan TC, Yu M, et al. One-year outcomes of conventional and accelerated collagen crosslinking in progressive keratoconus. *Sci Rep.* 2015;5:14425.
21. Tomita M, Mita M, Huseynova T. Accelerated versus conventional corneal collagen crosslinking. *J Cataract Refract Surg.* 2014;40:1013–1020.
22. Cınar Y, Cingü AK, Türkcü FM, et al. Comparison of accelerated and conventional corneal collagen cross-linking for progressive keratoconus. *Cutan Ocul Toxicol.* 2014;33:218–222.
23. Males JJ, Viswanathan D. Comparative study of long-term outcomes of accelerated and conventional collagen crosslinking for progressive keratoconus. *Eye (Lond).* 2018;32:32–38.
24. Sherif AM. Accelerated versus conventional corneal collagen cross-linking in the treatment of mild keratoconus: a comparative study. *Clin Ophthalmol.* 2014;8:1435–1440.
25. Choi M, Kim J, Kim EK, et al. Comparison of the conventional dresden protocol and accelerated protocol with higher ultraviolet intensity in corneal collagen cross-linking for keratoconus. *Cornea.* 2017;36:523–529.
26. Kymionis GD, Tsoulnaras KI, Liakopoulos DA, et al. Corneal stromal demarcation line depth following standard and a modified high intensity corneal cross-linking protocol. *J Refract Surg.* 2016;32:218–222.
27. Toker E, Çerman E, Özcan DÖ, et al. Efficacy of different accelerated corneal crosslinking protocols for progressive keratoconus. *J Cataract Refract Surg.* 2017;43:1089–1099.
28. Wollensak G, Spoerl E, Wilsch M, et al. Endothelial cell damage after riboflavin-ultraviolet-A treatment in the rabbit. *J Cataract Refract Surg.* 2003;29:1786–1790.
29. Ozgurhan EB, Kara N, Cankaya KI, et al. Accelerated corneal cross-linking in pediatric patients with keratoconus: 24-month outcomes. *J Refract Surg.* 2014;30:843–849.
30. Ozgurhan EB, Akcay BI, Kurt T, et al. Accelerated corneal collagen cross-linking in thin keratoconic corneas. *J Refract Surg.* 2015;31:386–390.
31. Kymionis GD, Tsoulnaras KI, Grentzelos MA, et al. Corneal stroma demarcation line after standard and high-intensity collagen crosslinking determined with anterior segment optical coherence tomography. *J Cataract Refract Surg.* 2014;40:736–740.
32. Seiler T, Hafezi F. Corneal cross-linking-induced stromal demarcation line. *Cornea.* 2006;25:1057–1059.
33. Mazzotta C, Traversi C, Caragiuli S, et al. Pulsed vs continuous light accelerated corneal collagen crosslinking: in vivo qualitative investigation by confocal microscopy and corneal OCT. *Eye (Lond).* 2014;28:1179–1183.
34. Pircher N, Lammer J, Holzer S, et al. Correlation between central stromal demarcation line depth and changes in K values after corneal cross-linking (CXL). *Graefes Arch Clin Exp Ophthalmol.* 2018;256:759–764.
35. Gatzioufas Z, Balidis M, Kozeis N. Is the corneal stromal demarcation line depth a true indicator of corneal collagen crosslinking efficacy? *J Cataract Refract Surg.* 2016;42:804.
36. McCall AS, Kraft S, Edelhauser HF, et al. Mechanisms of corneal tissue cross-linking in response to treatment with topical riboflavin and long-wavelength ultraviolet radiation (UVA). *Invest Ophthalmol Vis Sci.* 2010;51:129–138.
37. Peyman A, Nouralishahi A, Hafezi F, et al. Stromal demarcation line in pulsed versus continuous light accelerated corneal cross-linking for keratoconus. *J Refract Surg.* 2016;32:206–208.
38. Mazzotta C, Baiocchi S, Bagaglia SA, et al. Accelerated 15 mW pulsed-light crosslinking to treat progressive keratoconus: two-year clinical results. *J Cataract Refract Surg.* 2017;43:1081–1088.
39. Krueger RR, Herekar S, Spoerl E. First proposed efficacy study of high versus standard irradiance and fractionated riboflavin/ultraviolet a cross-

linking with equivalent energy exposure. *Eye Contact Lens.* 2014;40: 353–357.
40. Seiler TG, Fischinger I, Koller T, et al. Customized corneal cross-linking: one-year results. *Am J Ophthalmol.* 2016;166:14–21.
41. Lang PZ, Thulasi P, Khandelwal SS, et al. Comparing change in anterior curvature after corneal cross-linking using scanning-slit and Scheimpflug technology. *Am J Ophthalmol.* 2018;191:129–134.
42. Hallahan KM, Rocha K, Roy AS, et al. Effects of corneal cross-linking on ocular response analyzer waveform-derived variables in keratoconus and postrefractive surgery ectasia. *Eye Contact Lens.* 2014;40:339–344.
43. Vinciguerra R, Romano V, Arbabi EM, et al. In vivo early corneal biomechanical changes after corneal cross-linking in patients with progressive keratoconus. *J Refract Surg.* 2017;33:840–846.