Refractive, Topographic, Tomographic, and Aberrometric Analysis of Keratoconic Eyes Undergoing Corneal Cross-Linking

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Purpose: To report refractive, topographic, tomographic, and aberrometric outcomes 12 months after corneal cross-linking (CXL) in eyes with progressive advanced keratoconus.

Design: Prospective, nonrandomized, single-center clinical study.

Participants: Twenty-eight eyes undergoing CXL between April and June 2006.

Intervention: Riboflavin-ultraviolet A (UVA)-induced CXL included instillation of 0.1% riboflavin–20% dextrane solution 30 minutes before UVA irradiation and every 5 minutes for an additional 30 minutes during irradiation.

Main Outcome Measures: Uncorrected visual acuity (UCVA), best spectacle-corrected visual acuity (BSCVA), sphere and cylinder refraction, topography, tomography, aberrometry, and endothelial cell count were evaluated at baseline and at 1, 3, 6, and 12 months follow-up.

Results: Mean baseline UCVA and BSCVA were 0.17 ± 0.09 and 0.52 ± 0.17, respectively; 12-month mean UCVA and BSCVA were 0.27 ± 0.08 and 0.72 ± 0.16, a statistically significant difference ($P<0.05$). Mean spherical equivalent refraction showed a significant decrease of 0.41 diopters (D). Mean baseline simulated keratometry (SIM K) flattest and steepest meridians and SIM K average were 46.10, 50.37, and 48.08 D, respectively; at 12 months, 40.22, 44.21, and 42.01 D, respectively, were recorded, a difference that was significant for all 3 indices ($P<0.05$). Mean average pupillary power (APP) changed significantly from 47.50 to 41.04 D at 12 months ($P<0.05$) and apical keratometry (AK) from 58.94 to 55.18 D ($P<0.05$). The treated eyes showed no deterioration of the Klyce indices at 6 months postoperatively, whereas the untreated (contralateral) eyes did show deterioration. For a 3-mm pupil, there was a significant reduction ($P<0.05$) in whole eye (total), corneal, higher order, and astigmatic wavefront aberrations. A significant difference ($P<0.05$) in total coma and total spherical aberration after CXL was also observed. Mean baseline pupil center pachymetry and total corneal volume decreased significantly ($P<0.05$) to 470.09 ± 29.01 μm and 57.17 ± 3.21 mm^3 from baseline values of 490.68 ± 30.69 μm and 59.37 ± 4.36 mm^3, respectively. Endothelial cell counts did not changed significantly ($P = 0.13$).

Conclusions: Corneal cross-linking seems to be effective in improving UCVA and BSCVA in eyes with progressive keratoconus by significantly reducing corneal APP, AK, and corneal and total wavefront aberrations at 1 year postoperatively.

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Keratoconus is considered a slowly progressive, noninflammatory corneal dystrophy characterized by changes in corneal collagen structure and organization.[1–3] A reduced number of collagen cross-links and a pepsin digestion higher than normal induce an overall structural weakness of the corneal tissue, resulting in a stiffness that is only 60% that of the normal cornea.[4] Decreased mechanical corneal stability plays an important role in the progressive protrusion of the keratoconic cornea, resulting in mild to marked impairment of visual acuity owing to irregular astigmatism, progressive myopia, corneal thinning, and central corneal scarring.[5]

Common methods of vision correction for keratoconus range from spectacles to rigid gas-permeable contact lenses and more recently to wavefront-corrected spectacles and soft contact lenses.[6] Intracorneal ring segments implantation improves uncorrected visual acuity (UCVA) and best spectacle-corrected visual acuity (BSCVA) in patients with mild to moderate keratoconus and contact lens intolerance.[7,8] However, long-term follow-up shows that intracorneal ring segments fail to provide a permanent flattening effect. A significant progression of K-values in treated corneas has been observed,[9] demonstrating that this device can only temporarily correct the keratoconic eye's refractive errors; keratoconus is a progressive disease that requires corneal grafting in the most advanced cases. Recently, a new technique, corneal collagen cross-linking (CXL), has been introduced by Wollensak et al[10–12] to stabilize progressive keratoconus, prevent some of the underlying pathophysiologic mechanisms of the disease, and avoid the need for penetrating keratoplasty.

Collagen cross-linking increases the biomechanical strength of the human cornea by about 300% by the combined action of a photosensitizing substance (riboflavin) and ultraviolet (UV) light from a solid-state UVA source.[13] The treatment creates additional chemical bonds inside the anterior 200–300 microns of the corneal stroma by means of photopolymerization. There is minimal exposure to the surrounding structures of the eye.[14–16] Collagen cross-linking increases the resistance to pepsin digestion by enhancing corneal anticollagenase activity, and induces a thicker collagen fiber diameter.[17] Confocal microscopy studies have also shown apoptosis of keratocytes in the anterior and intermediate stroma followed by a gradual keratocytes repopulation.[16,18]

In this study, we examined the refractive, topographic, tomographic, and aberrometric outcomes at 12 months after CXL in eyes with progressive stage III keratoconus.

Materials and Methods

Population

Twenty-eight eyes of 28 consecutive patients (8 females, 20 males) in which keratoconus progression in 1 eye was detected in the preceding 6 months were enrolled at the Cornea Service of the Ophthalmology Department of Istituto Clinico Humanitas (Rozzano, Milano, Italy) from April to June 2006 in this prospective, nonrandomized, single-center study.

The contralateral keratoconic eyes were also observed during the 1-year study and the corneal parameters compared with those of the treated eyes.

Preoperative keratoconus progression was confirmed by serial differential corneal topographies and by differential optical pachymetry analysis in all eyes included in the study.[19] Keratoconus progression was defined as a change in either myopia and/or astigmatism of ≥3 diopters (D) in the previous 6 months, or a mean central K-reading change of ≥1.5 D observed in 3 consecutive topographies during the preceding 6 months, or a mean central corneal thickness decrease of ≥5% in 3 consecutive tomographies performed in the previous 6 months. The Amsler–Krumeich classification, based on patients' refraction, mean central K-reading, corneal signs, and corneal thickness, has been used for keratoconus grading.[20,21] The corneal higher order aberration scale[21–23] was not used because ophthalmologists referring patients to us for keratoconus progression provided only topography maps.

Inclusion criteria were a documented keratoconus progression in the previous 6 months, corneal thickness of ≥400 μm at the thinnest point, and age 18–60 years. The age of the patients included in the study ranged from 24–52 years. Of the treated eyes, 8 were right and 20 were left eyes. All treated eyes were graded stage III according to the Amsler–Krumeich classification. The untreated contralateral eyes were graded stage I–II.

Exclusion criteria included corneal thickness <400 microns at the thinnest point,[13,24–27] a history of herpetic keratitis, severe eye dry, concurrent corneal infections, concomitant autoimmune diseases, and any previous ocular surgery. Also excluded were pregnant or nursing women, patients with central or paracentral opacities, patients with poor compliance, and patients wearing rigid gas-permeable lenses for ≥4 weeks before baseline examination.

Criteria for aborting the study itself included a reduction in endothelial cell density of >50% in >2 eyes, the development of corneal haze grade 3–4 (Hanna scale) in >5% of the eyes, a loss of >2 lines of BSCVA in >5% of the eyes, and the detection of lens opacities in >1% of the treated eyes when compared with the untreated eyes (LOCS II classification). The study received an Institutional Review Board approval from the ethical committee of Istituto Clinico Humanitas and was conducted accordingly to the ethical standards set in the 1964 Declaration of Helsinki, as revised in 2000. All patients provided informed consent.

At baseline and each of the postoperative follow-up examinations (1, 3, 6, and 12 months) all patients underwent UCVA and BSCVA assessment, slit-lamp biomicroscopy, basal Schirmer test, Goldmann tonometry, dilated fundus examination, endothelial biomicroscopy (Konan Specular Microscope, Konan Medical Inc, Hyogo, Japan), corneal topography (Costruzione Strumenti Oftalmici [C.S.O.], Florence, Italy), corneal, internal, and total aberrometry with the Optical Path Difference Platform (OPD; Nidek, Gamagori, Japan), and Pentacam central pachymetry and optical tomography (Oculus Inc, Lynnwood, WA). Corneal higher order aberrations for a 3-, 5-, and 7-mm pupil were also measured with the C.S.O EyeTop Topographer corneal aberrometry program. In addition, the Nidek OPD scan was used to supply data on zonal refraction, topography, and aberrometry.

All 28 treated eyes underwent corneal topography with the C.S.O. device before epithelial scraping and immediately after epithelial debridement.

Visual Acuity Assessment

Visual acuity was assessed with the Early Treatment Diabetic Retinopathy Study logarithm of the minimum angle of resolution charts (Lighthouse International, New York, NY) based on the design suggested by Bailey and Lovie[28] and incorporating the recommendations of the US National Academy of Sciences—National Research Council.[29] The chart has been described in detail by Ferris et al.[30] Measurements were made with best correction after a noncycloplegic refraction at 4 m.

Corneal Topography

Corneal topography with the C.S.O. EyeTop Topographer analyzes 6144 points (24 rings each with 256 radial spots) over a 9.5-mm^2 corneal surface area. Repeatability is ±0.03 mm for axial and instantaneous maps and ±0.5 μm for elevation maps. The examination was performed under photopic conditions. The sensitivity of the technique for keratoconus detection is 98.5%.[31]

The Nidek OPD was also used to supply data on topography. Specifically, it was used to study the 21-Klyce indices provided by the Corneal Navigator Topo-Classifier Map. In keratoconus diagnosis, the navigator was found to be more specific and sensitive than the Rabinowitz–McDonnel test, and more specific and sensitive than central corneal power >47.2 D or inferior–superior asymmetry >1.4 D.[32–35]

Wavefront Analysis

Total (corneal and internal) wavefront analysis was performed with the Nidek OPD-Scan. The device was also used to objectively analyze mean refraction.

Mean refraction in the treated and contralateral eyes was studied using the OPD Zonal Refraction Map, which analyzes corneal refraction based on aberrometry. The zonal refraction map provides a mean refraction expressed in terms of sphere, cylinder, and axis of the overall cornea. It also supplies a refraction analysis of the central 3 mm, the midperipheral 5 mm, and the peripheral 7 mm of the cornea.

The OPD further provides an aberrometric analysis of the eye, decomposing whole eye (total) aberrations into corneal aberrations owing to the anterior corneal surface and internal aberrations owing to the posterior corneal surface, the anterior chamber, the lens, the vitreous body, and the retina.

Mean corneal total higher order aberrations, mean corneal spherical aberration, mean corneal astigmatic aberration, and mean corneal coma for a 3-, 5-, and 7-mm pupil were also measured with the C.S.O. EyeTop Topographer (C.S.O., Florence, Italy) corneal aberrometry program.

Anterior Chamber Analysis

An anterior chamber analysis was performed with the Oculus Pentacam HR, a reliable tool to image and measure the anterior segment of the eye using a rotating Scheimpflug camera (Oculus Inc, Lynnwood, WA).[36–40] The analyses performed with the Pentacam included pupil center pachymetry and the pachymetry of the thinnest point of the cornea. The *x*- and *y*-coordinates show the distance of these 2 points from the corneal apex.

Total and partial corneal volume is calculated in a ring around the apex, using diameters of 3, 5, 7, and 10 mm. Anterior chamber volume is calculated by measuring the distances between the back surface of the cornea and the iris–lens plane over a 12-mm diameter.

Anterior chamber depth (ACD) is measured from the endothelium of the corneal apex to the iris–lens plane. Anterior and posterior elevation maps use a toric reference body, with calculations based on the central radii and the eccentricity of the keratometry measurements. The advantage of the toric reference shape is its good approximation to astigmatic corneas.

Endothelial Cell Count

Endothelial biomicroscopy was performed manually according to the method described by Prinz et al.[41] Cell centers of ≥50 contiguous cells were identified by aligning a cursor on the cell apices. Endothelial cell density was then recorded.

Cross-Linking Procedure

All patients underwent the cross-linking procedure on a day surgery basis. Thirty minutes before the procedure, pain medication was administered and 2% pilocarpine drops were instilled in the eye to be treated. Because the amount of light rays reaching the retina is proportional to the square of the pupil diameter, the use of pilocarpine reduces the thermal and photochemical UVA light irradiation, which is potentially harmful to the lens and retina.

The procedure was conducted under sterile conditions in the operating suite. After topical anesthesia with 2 applications of 4% lidocaine drops and oxybuprocaine hydrochloride 0.2%, the patient was draped, the ocular surface was rinsed with sterile physiologic balanced salt solution and a lid speculum applied. The corneal epithelium was abraded in a central, 9-mm diameter area with the aid of an Amoils brush (Amoils Brush Ephthential Scrubber; Vision Technology Co, Seoul, Korea).

Before beginning UVA irradiation, photosensitizing riboflavin 0.1% solution (10 mg riboflavin-5-phosphate in 20% dextran-T-500 10 mL solution) was applied onto the cornea every minute for 30 minutes to achieve adequate penetration of the solution. Using a slit lamp with the blue filter, the surgeon confirmed the presence of riboflavin in the anterior chamber before UV irradiation was started. The cornea was exposed to a UV source emanating from a solid-state device (UV-X System, Peschke Meditrade GmbH, Huenenberg, Switzerland), which emits light at a wavelength of 370 ± 5 nm and an irradiance of 3 mW/cm^2 or 5.4 J/ cm^2. Exposure lasted for 30 minutes, during which time riboflavin solution was again applied, this time once every 5 minutes. The cropped light beam has a 7.5 mm diameter. A calibrated UVA meter (LaserMate-Q; Laser 2000, Wessling, Germany) was used before treatment to check the irradiance at a 1.0 cm distance. The Peschke laser emission probe has 1 central and 6 peripheral light-emitting diodes. Fixation during irradiation was achieved by instructing the patient to focus on the central light-emitting diode of the probe. During the procedure the surgeon also controlled for centration of treatment. Both topical anesthetics were added as needed during irradiation.

Postoperatively, patients received cyclopentolate (Ciclolux, Allergan, Rome, Italy) and levofloxacin drops (Oftaquix, Tubilux Pharma, Pomezia, Italy). A soft bandage contact lens was applied until reepithelialization was complete. Topical levofloxacin was given 4 times daily for 7 days, dexamethasone 21-phosphate 0.15% drops (Etacortilen, Sifi, Lavinaio, Italy) 3 times daily for 20 days, and 0.15 % sodium hyaluronate drops (BluYal, Sooft, Montegiorgio, Italy) 6 times daily for 45 days. In addition, all patients received oral amino acid supplements (Trium, Sooft, Montegiorgio, Italy) for 7 days.[42]

Mean reepithelialization time was 46 ± 11 hours. Patients were examined every day until reepithelialization and then at 1, 3, 6, and 12 months.

Data Analysis

Statistical analyses were performed with the Statistica (StatSoft Inc, Tulsa, OK) computer package. All data are reported as mean values ± standard deviation. Normality of the data was tested using the Kolmogorov and Smirnov tests and the normal probability plot. The level of statistical significance was set at $P<0.05$.
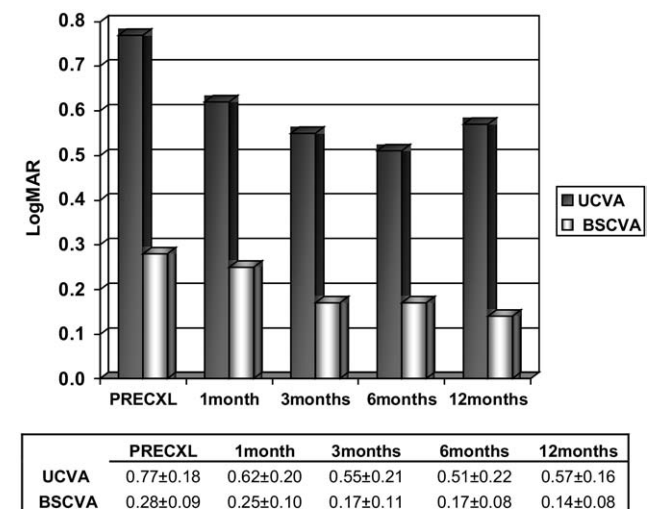
Results

Follow-up time was 12 months for all patients included in the study.

Visual Acuity Results

Figure 1 summarizes the UCVA and BSCVA data, expressed in logarithm of the minimum angle of resolution and covering the entire follow-up period.

Mean baseline UCVA was 0.77 ± 0.18. At 1 month post CXL, mean UCVA was 0.62 ± 0.21; at 3 months, 0.55 ± 0.19; at 6 months,

	PRECXL	1month	3months	6months	12months
UCVA	0.77±0.18	0.62±0.20	0.55±0.21	0.51±0.22	0.57±0.16
BSCVA	0.28±0.09	0.25±0.10	0.17±0.11	0.17±0.08	0.14±0.08

LogMAR = logarithm of the minimum angle of resolution; PRECXL = before cross-linking.

Figure 1. Uncorrected visual acuity (UCVA) and best spectacle-corrected visual acuity (BSCVA) in the 28 treated eyes before and after cross-linking.

Table 1. Zonal Refraction Analysis of Mean Spherical Equivalent (SE) of the Overall, Central, Mid, and Peripheral Cornea

	CXL Treated Eye				CXL Control Eye			
	PRECXL	SD	12 Months	SD	PRECXL	SD	12 Months	SD
Overall SE	−6.73	1.03	−6.3*	0.78	−3.52	0.82	−4.21[†]	0.56
Central SE	−6.47	0.76	−6.1*	0.25	−3.47	0.29	−4.24[†]	0.92
Mid SE	−5.33	0.89	−5.07*	1.01	−3.19	0.78	−3.53[†]	0.65
Peripheral SE	−4.63	0.95	−4.99[†]	0.36	−2.46	0.67	−3.45[†]	0.62

CXL = cross-linking; PRECXL = before cross-linking; SD = standard deviation.
*Decreased values at 12-month follow-up.
[†]Increased values at 12-month follow-up.

0.51±0.20; and at 12 months, 0.57±0.16. Mean baseline BSCVA was 0.28±0.09. At 1 month after the procedure, mean BSCVA was 0.25±0.12; at 3 months, 0.18±0.10; at 6 months, 0.17±0.11; and at 12 months, 0.14±0.08.

The improvements in UCVA and BSCVA were statistically significant ($P<0.05$ and $P<0.0001$, respectively) throughout the entire postoperative period when compared with preoperative levels. Both UCVA and BSCVA slowly improved during the first 6 months after CXL and remained unchanged between 6 and 12 months postoperatively.

Refractive Results

The mean preoperative spherical equivalent (SE) was −3.37±2.64 D, with a mean sphere of −1.86±2.58 D and a mean cylinder of −3.02±1.74 D. One year after CXL mean SE was −2.96±2.68 D, mean sphere, −1.58±2.64 D, and mean cylinder −2.76±1.11 D. The difference in mean cylinder was significant ($P<0.05$). Vector analysis showed a significant axis shift from 93.15°±43.26° to 102°±33.59° after CXL ($P<0.05$).

Mean refraction in the treated and contralateral eyes was also studied using the OPD Zonal Refraction Map. Table 1 shows mean SE data for each zone in treated and untreated eyes at baseline and 1 year after CXL. Central and midperipheral mean SEs were significantly reduced ($P<0.05$) in all treated eyes at 12 months postoperatively. In contrast, mean SE in the untreated eyes increased significantly in all 3 zones during the same period ($P<0.05$).

Topographic Results

Topographic astigmatism measured with the C.S.O. Topographer during follow-up is shown in Table 2. Mean baseline flattest

meridian keratometry, steepest meridian keratometry and average keratometry were 46.10, 50.37, and 48.08 D, respectively. At 12 months, these readings were 40. D, 44.21, and 42.01 D, respectively, a difference that was statistically significant for all 3 parameters ($P<0.05$).

Table 3 lists keratoconus indices obtained with the C.S.O. Topographer during follow-up. Mean baseline average pupillary power, apical keratometry, apical gradient curvature, inferior–superior index, and cone area were 47.50 D, 58.94 D, 8.41 D, and 11.66 and 9.53 mm^2, respectively. At 12 months these indices were 41.04 D, 55.18 D, 7.2 D, and 10.84 and 7.52 mm^2, respectively. All 5 indices were significantly lower ($P<0.05$) at 12 months postoperatively, showing a flattening effect over the keratoconic cornea.

The Klyce indices[35] obtained with the Nidek OPD platform were analyzed in treated and untreated eyes at baseline and at 12 months. Preoperative differences between the 2 groups with respect to the indices were statistically significant (Table 4). At 12 months postoperatively, the Klyce indices of the treated group had significantly decreased ($P<0.05$). In contrast, the Klyce indices of the untreated group were significantly higher ($P<0.05$) at the 1-year follow-up.

Aberrometric Results

Corneal higher order aberrations for a 3-, 5-, and 7-mm pupil were measured preoperatively and at 12 months, using the C.S.O. EyeTop corneal aberrometry program. The results are shown in Table 5.

At 12 months postoperatively, mean corneal spherical aberrations had significantly decreased from 0.03 μm at 3 mm, 0.13 μm at 5 mm, and 0.38 μm at 7 mm to −0.06 μm ($P = 0.0003$), −0.28 μm ($P = 0.0006$), and −0.44 μm ($P = 0.0048$), respectively. Mean corneal astigmatism and mean corneal coma had also de-

Table 2. Topographic Astigmatism as Measured with C.S.O. Topographer at Different Time Points

	PRECXL	1 Month	3 Months	6 Months	12 Months	P PRECXL-12 Months*
SIMK_Kf	46.10	46.57	46.31	45.45	40.22	0.0003[†]
SIMK_Ks	50.37	51.62	50.87	49.89	44.21	0.0011[†]
SIMK_AVG	48.08	48.87	48.37	47.50	42.01	0.0004[†]
SIMK_CYL	−4.27	−5.06	−4.56	−4.44	−3.99	0.5812
KR_CYL3	−4.73	−4.96	−4.94	−4.69	−4.10	0.2884
KR_CYL5	−3.64	−3.92	−3.68	−3.60	−3.27	0.4560

C.S.O. = Costruzione Strumenti Oftalmici; CXL = cross-linking; KR CYL3 = keratometry readings at 3 mm; KR CYL5 = keratometry readings at 5 mm; PRECXL = before cross-linking; SIMK Kf = simulated keratometry flattest meridian; SIMK Ks = simulated keratometry steepest meridian; SIMK AVG = average simulated keratometry; SIMK CYL = simulated keratometry cylinder.
*Differences between preoperative and 12 months postoperative values.
[†]$P<0.05$.

Table 3. Changes in Keratoconus Indices Changes Documented with the C.S.O. Topographer at Different Time Points

	PRECXL	1 Month	3 Months	6 Months	12 Months	P PRECXL-12 Months*
KR_AVG PUP PWR	47.50	48.34	47.99	47.09	41.04	0.0002[†]
KC_AK	58.94	60.08	59.40	58.83	55.18	0.0194[†]
KC_AGC	8.41	9.22	8.85	8.74	7.20	0.0465[†]
KC_SI	11.66	12.10	11.17	10.86	10.84	0.0391[†]
KC_RND	1.27	1.16	1.20	1.26	4.07	0.1926
KC_AREA	9.53	9.30	9.50	8.86	7.52	0.0475[†]
KC_PERIM	12.16	11.56	11.83	11.68	10.49	0.1161

C.S.O. = Costruzione Strumenti Oftalmici; CXL = cross-linking; KC AREA = keratoconus area surface; KC AK = keratoconus apical keratometry; KC AGC = keratoconus apical curvature gradient; KC PERIM = keratoconus perimeter; KC RND = keratoconus circular factor; KC SI = keratoconus symmetry index; KR AVG PUP PWR = keratometry average pupillary power; PRECXL = before cross-linking.
*Differences between preoperative and 12 months postoperative values.
[†]$P < 0.05$.

creased by the 1-year follow-up, but the difference was not statistically significant when compared with the preoperative data.

Wavefront analysis performed with the Nidek OPD-Scan showed that in the treated eyes mean baseline total (corneal and internal) aberrations at 3 mm were 2.48 ± 0.42 μm. They decreased to 2.15 ± 0.48 μm at 12 months. Mean baseline total higher order aberrations were 0.46 ± 0.12 μm, decreasing to 0.38 ± 0.07 μm at 12 months. Mean baseline total astigmatism decreased from 0.08 ± 0.02 μm to 0.07 ± 0.01 μm. Mean baseline total coma was down from 0.68 ± 0.13 μm to 0.65 ± 0.08 μm and mean baseline total spherical aberrations decreased from 0.16 ± 0.02 μm to 0.14 ± 0.01 μm. All these differences were significant ($P < 0.05$).

In the untreated group, we observed a significant ($P < 0.05$) increase in total astigmatism and total coma from 0.05 ± 0.02 μm to 0.07 ± 0.02 μm and from 0.37 ± 0.12 μm to 0.41 ± 0.10 μm, respectively. No statistically significant changes were observed in the untreated eyes with respect to total, higher order, or spherical aberrations.

Mean baseline corneal aberrations at 3 mm decreased from 2.38 ± 0.71 μm to 2.19 ± 0.54 μm, mean baseline corneal higher order aberrations decreased from 0.87 ± 0.13 μm to 0.73 ± 0.15 μm, and mean baseline corneal astigmatism decreased from 1.14 ± 0.32 μm to 1.09 ± 0.29 μm. All differences were significant ($P < 0.05$) at 12 months. No significant change in corneal coma or corneal spherical aberration were observed. In the untreated eyes, a significant ($P < 0.05$) increase in corneal higher order aberrations and in corneal astigmatism were noted, with readings changing from 8.32 ± 1.72 μm to 13.04 ± 3.56 μm and from 4.41 ± 0.45 μm to 5.06 ± 0.62 μm, respectively.

Tomographic Results

Mean pupil center pachymetry and total corneal volume measured by means of Pentacam optical pachymetry at baseline, were 490.68 ± 30.69 μm and 59.37 ± 4.36 mm^3, respectively. At 12 months they had decreased to 470.09 ± 29.01 μm and 57.17 ± 3.21 mm^3, respectively, a difference that was significant ($P < 0.05$). Partial corneal volume at 3, 5, and 7 mm was also significantly reduced from 3.53 ± 0.20 mm^3, 10.7 ± 0.57 mm^3, and 23.73 ± 1.41 mm^3 to 3.40 ± 0.17 mm^3, 10.35 ± 0.44 mm^3, and 22.86 ± 1.01 mm^3, respectively ($P < 0.05$).

Corneal pachymetry at the thinnest point changed from 451.14 ± 25.97 to 436.23 ± 29.38 μm, anterior chamber volume decreased from 202.39 ± 37.19 to 192.59 ± 31.97 mm^3, ACD decreased from 3.42 ± 0.15 to 3.28 ± 0.08 mm, and anterior and posterior elevation changed from 7.20 ± 0.47 mm and 6.08 ± 0.74 mm to 7.27 ± 0.56 mm and 5.99 ± 0.73 mm, respectively. Despite the changes in these parameters, the differences were not significant ($P > 0.05$), except for the ACD where the pre- to postoperative change was significant ($P < 0.05$). In the differential ACD map, the highest postoperative ACD reduction matched the exact position of the cone apex, the thinnest point, and the greatest corneal flattening.

Table 4. Changes in Klyce Indices Measured with the Nidek OPD Scan

	CXL Treated Eye		CXL Control Eye	
	PRECXL	*12 Months*	*PRECXL*	*12 Months*
SimK1 D	50.53	50.13*	45.81	46.21[‡]
SimK2 D	45.89	45.76*	43.29	43.57[‡]
MinK D	41.18	41.32[‡]	40.83	40.92[‡]
ACP	49.61	49.23*	44.8	45.33[‡]
CYL	4.62	4.27*	2.49	2.64[‡]
CVP	104.78	102.9*6	59.99	64.15[‡]
SDP	5.07	4.96*	2.77	2.94[‡]
CEI	1.13	1.09*	0.36	0.54[‡]
LogMAR	0.26	0.25*	0.1	0.11[†]
DSI	13.18	12.88*	7.9	8.3[‡]
SRI	1.64	1.61*	1.13	1.17[‡]
SRC	1.47	1.45*	1.05	1.06[†]
SAI	3.02	2.83*	1.69	1.78[‡]
IAI	0.62	0.62[†]	0.53	0.53[†]
OSI	10.61	10.46*	6.2	6.57[‡]
CSI	3.08	2.92*	0.73	1.02[‡]
KCI	0.9	0.88*	0.44	0.45[‡]
KPI	0.44	0.43*	0.3	0.31[‡]
EDP	4.23	3.84*	2.31	2.36[‡]
EDD	16.4	16.24*	9.09	9.43[‡]

AA = analyzed area; ACP = average corneal power; CEI = corneal eccentricity index; CSI = center surround index; CVP = coefficient of variation of corneal power; CXL = cross-linking; CYL = simulated keratometry cylinder; DSI = differential sector index; EDP = elevation/depression power; EDD = elevation/depression diameter; IAI = irregular astigmatism index; KPI = keratoconus prediction index; LogMar = logarithm of the minimum angle of resolution; MINK = minimum keratometry value; OPD = Optical Path Difference Platform; OSI = opposite sector index; PRECXL = before cross-linking; SAI = surface asymmetry index; SDP = standard deviation of corneal power; SIMK1 = simulated keratometry 1; SIMK2 = simulated keratometry 2; SRC = area compensated surface regularity index; SRI = surface regularity index.
*Decreased values at 12 months postoperatively.
[†]Equivalent values at 12 months postoperatively.
[‡]Increased values at 12 months postoperatively.

Table 5. Corneal Higher Order Aberrations for a 3-, 5-, and 7-mm Pupil Were Measured Preoperatively and at 12 months Using the C.S.O. EyeTop Corneal Aberrometry Program

	Astigmatism			Coma			Spherical Aberration		
	3.00 mm	5.00 mm	7.00 mm	3.00 mm	5.00 mm	7.00 mm	3.00 mm	5.00 mm	7.00 mm
PRECXL	1.38	2.42	3.22	0.91	2.81	5.15	0.03	0.13	0.38
1 Month	1.38	2.55	3.12	0.94	3.05	5.65	0.07	0.27	0.78
3 Months	1.33	2.43	3.06	0.84	2.76	5.15	0.05	0.20	0.60
6 Months	1.24	2.25	2.86	0.79	2.59	4.83	0.04	0.13	0.44
12 Months	1.13	2.02	2.59	0.59	2.15	4.20	−0.06	−0.28	−0.44

C.S.O. = Costruzione Strumenti Oftalmici; PRECXL = before cross-linking.

Mean baseline corneal thickness at 2, 4, and 6 mm was 478 ± 24.91, 542.96 ± 28.41, and 619.04 ± 36.41 μm, respectively. At 12 months, mean corneal thickness was 462.5 ± 24.07, 524.27 ± 20.90, and 594.95 ± 34.78 μm, respectively, a difference that was significant ($P < 0.05$). In contrast, there was no statistically significant difference in corneal thickness at 0 or 8 mm.

Endothelial Results

Mean baseline endothelial cell count was 2651 ± 321.36 cell/mm^2. One month after the procedure, it was 2485 ± 599.87 cell/mm^2, at 3 months 2390 ± 624.83 cell/mm^2, at 6 months 2512 ± 587.36 cell/mm^2, and at 12 months endothelial cell count was $2598 \pm$
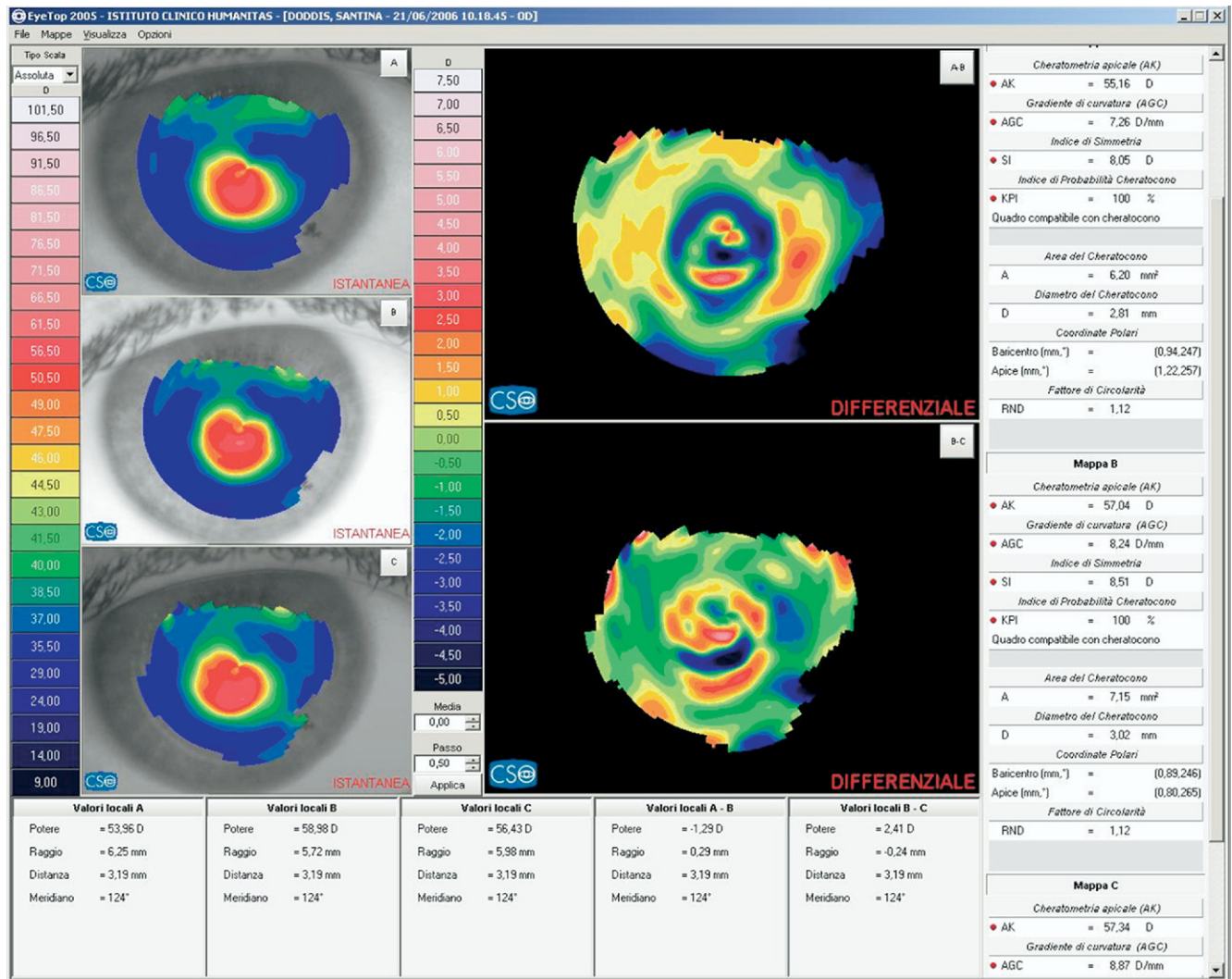

Figure 2. A, C.S.O. topographic instantaneous map before cross linking (PRECXL). **B**, C.S.O. topographic instantaneous map 1 month after CXL (1 month). **C**, C.S.O. topographic instantaneous map 12 months after CXL (12 months). **B-A**, Differential instantaneous map. **C-B**, Differential instantaneous map. C.S.O. = Costruzione Strumenti Oftalmici.

563.84 cell/mm^2. The difference between baseline and 12 months was not significant ($P>0.05$), indicating that CXL did not induce endothelial damage in the 1-year follow-up period.

No ocular or systemic adverse events were observed, and no significant intraocular pressure changes were seen. Of the treated eyes, 43.5% developed CXL-specific golden striae[10] and 12.7% of the treated eyes had 1+ haze (Hanna scale). The haze regressed after 1 month with a topical steroids regimen. We also observed a reduction of Vogt striae after cross-linking.

Patients complained of night glare and haloes, but only in the first 3 months. Subjectively, patients perceived improvement of UCVA during the first 6 postoperative months. Between 6 and 12 months, they reported a continuing improvement in BSCVA.

Discussion

To the best of our knowledge, this is the first study in which preoperative and postoperative refractive, topographic, tomographic, and aberrometric outcomes have been analyzed in eyes with progressive stage III keratoconus.

Long-term follow-up showed that, after an initial worsening of all keratoconus indices (probably because of epithelial debridement),[43–49] there was a slow but continuous improvement of the indices up to 12 months postoperatively. As Figure 2 shows, despite a dramatic change observed in corneal power with an increase in steepest meridian keratometry, simulated cylinder and AK values at 1 month after CXL (Fig 2, *C, B, B-C*), from the 3rd to the 12th postoperative month instantaneous topography maps showed that the procedure had a significant effect in flattening and regularizing corneal curvature (Fig 2, *A, A-B*).

The procedure lead to corneal flattening with a slow but significant UCVA and BSCVA improvement during the first 6 months.

Mean refraction showed a statistically significant decrease in cylinder ($P<0.05$) and a significant shift in axis after CXL ($P<0.05$). K-Readings decreased, as did corneal asymmetry and spherical aberration.

The significant reduction of simulated keratometry, APP, AK, apical gradient curvature, inferior–superior index, cone area, total and corneal aberrations, and the Klyce indices
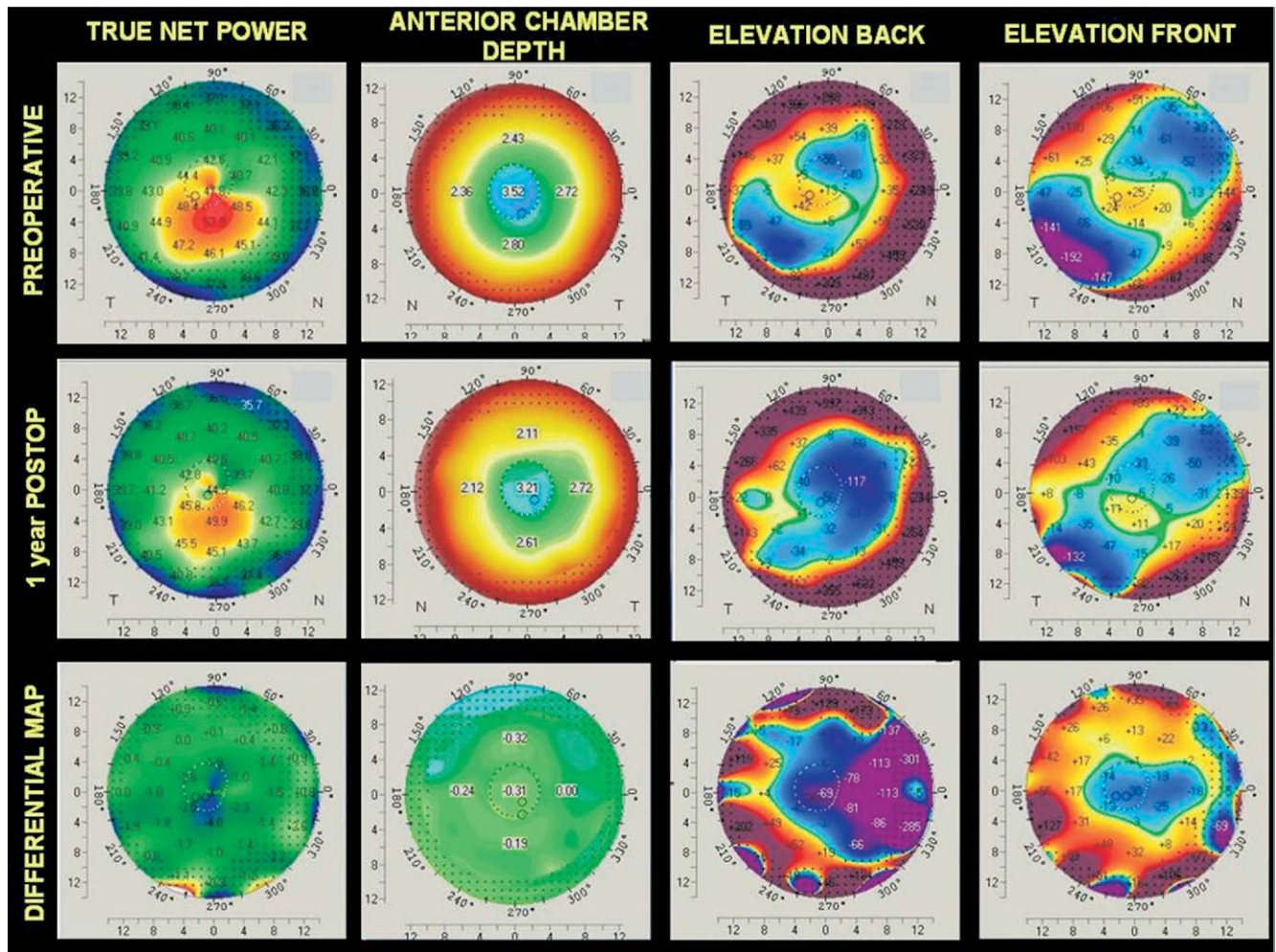

Figure 3. Pentacam true net power map, anterior chamber depth map, back elevation map and front elevation map before cross-linking and at 1 year after the procedure. Differential maps are shown in the bottom row.

explain the improvement in the postoperative visual acuity. Similar results were found by Caporossi et al,[50] and Wollensak et al.[10] Both groups showed a postoperative decrease in mean keratometry as well as and a reduction of manifest SE. We conclude that the refractive outcomes were achieved by both a flattening of the cone apex and a steepening of the part of the cornea symmetrically opposite the cone.

Analysis of total (whole eye) aberrations showed a significant reduction in astigmatism, coma, and spherical aberrations. However, corneal surface aberrometric analysis did not show an improvement in coma, indicating that there is a significant change in the posterior surface of the cornea, which was masked by the total aberration status. The Klyce topographic indices showed better sensitivity and specificity in detecting keratoconus regression after CXL than the suggested corneal aberrometry grading system.

Keratoconus does not involve only the anterior surface of the cornea and its thickness. Rather, it affects all anterior segment parameters.[51] This study demonstrates a significant decrease in total corneal volume and ACD 12 months after CXL (Fig 3). In view of the study by Emre et al,[51] these findings may indicate that the cornea becomes stiffer. Cross-linking induces the creation of new bonds between the collagen fibers.[10–14,52] The reduction of ACD at the cone apex matched the maximum reduction in elevation and the increased packing of collagen fibers, as demonstrated by Mencucci et al.[53]

We found no significant difference in corneal endothelial cell counts when comparing the preoperative with the 12-month measurements, a finding supported by Wollensak et al.[13,17,54,55] The lack of evidence for endothelial cell loss is an important safety consideration in assessing this new procedure.

The treated eyes showed no deterioration of the Klyce indices. In the untreated eyes, the same indices slowly deteriorated.

Follow-up in this patient cohort was not sufficiently long to assess the long-term effectiveness of CXL. However, the results are promising and suggest that it would be worthwhile to further investigate the application of this procedure, not only in keratoconus, but also in iatrogenic ectasia after corneal refractive surgery. If the cross-linking effect turns out to be stable over a longer period, the procedure could be combined with ring segments or surface customized refractive surgery to partially correct the refractive error of patients with keratoconus.[56]

In conclusion, cross-linking seems to be effective in improving UCVA and BSCVA in eyes with progressive keratoconus by significantly reducing corneal APP, AK, as well as total and corneal aberrations. No permanent side effects were noted after CXL. After an apparent initial keratoconus "progression," probably owing to epithelial debridement, there was a slow but continuous improvement of keratoconus indices, first because of reepithelialization and then because of the cross-linking effect on the cornea. No deterioration of the Klyce indices was seen in the treated eyes, whereas in the untreated eyes the same indices showed a slow deterioration. Although total and corneal aberrations decreased in the treated eyes, they increased in the untreated eyes during the same follow-up period.

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Footnotes and Financial Disclosures

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