Original Investigation

Increased Corneal Hysteresis After Corneal Collagen Crosslinking

A Study Based on Applanation Resonance Technology

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IMPORTANCE A reliable tool for quantification of the biomechanical status of the cornea in conjunction with corneal collagen crosslinking (CXL) treatment is needed.

OBJECTIVE To quantify the biomechanical effects of CXL in vivo.

DESIGN, SETTING, AND PARTICIPANTS A prospective, open, case-control study was conducted at the Department of Ophthalmology, Umeå University, Umeå, Sweden. Participants included 28 patients (29 eyes) aged 18 to 28 years with progressive keratoconus and corresponding age- and sex-matched healthy individuals serving as controls. All participants were monitored during a 6-month period between October 13, 2009, and November 5, 2012.

MAIN OUTCOMES AND MEASURES Corneal hysteresis after CXL for keratoconus.

RESULTS A difference in corneal hysteresis between the control group and the patients with keratoconus was found at baseline, both with an applanation resonance tonometer (ART) and an ocular response analyzer (ORA), at mean (SD) values of −1.09 (1.92) mm Hg (99% CI, −2.26 to 0.07; $P = .01$) and −2.67 (2.55) mm Hg (99% CI, −4.05 to −1.32; $P < .001$), respectively. Increased corneal hysteresis was demonstrated with an ART 1 and 6 months after CXL, at 1.2 (2.4) mm Hg (99% CI, −0.1 to 2.5; $P = .02$) and 1.1 (2.7) mm Hg (99% CI, −0.3 to 2.6; $P = .04$), respectively, but not with ORA. A decrease in corneal thickness was seen 1 and 6 months after treatment (−24 [26] µm, $P < .001$; and −11 [21] µm, $P = .01$, respectively), and a corneal flattening of −0.6 (0.7) diopters was seen at 6 months ($P < .001$). No significant change in intraocular pressure was identified in patients with keratoconus with any method, except for an increase at 1 month with Goldmann applanation tonometry ($P = .005$).

CONCLUSIONS AND RELEVANCE To our knowledge ART is the first in vivo method able to assess the increased corneal hysteresis after CXL treatment. Given the large-scale use of CXL in modern keratoconus treatment, a tool with this capacity has a great potential value. Refinement of the ART method of measuring and quantifying corneal biomechanical properties will be a subject of further studies.

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Keratoconus is a progressive corneal thinning disease with a decreased rigidity in the corneal structure, which leads to an abnormal corneal protrusion and causes myopia and irregular astigmatism, leading to decreased vision.[1] Refractive errors are corrected with glasses or rigid contact lenses and, in late stages, keratoplasty, but none of these interventions has any effect on the underlying causes of the disease.[2] Corneal collagen crosslinking (CXL) is an established treatment for progressive keratoconus, particularly in the early stages. Corneal collagen crosslinking combines riboflavin and UV-A photoactivation to halt the progression of the disease.[3] In this treatment, the postulated mechanism is that formation of crosslinks stabilizes the corneal stroma,[4] and increased corneal biomechanical stability has been demonstrated in vitro after CXL treatment in several studies,[4-7] although it has been more difficult to demonstrate in vivo. However, it is still not known where crosslinks occur in the extracellular matrix[8] and there has been no report demonstrating crosslinks directly in treated corneas, although several studies[4-7] have reported indirect evidence for the presence of crosslinks.

The ocular response analyzer (ORA) is an instrument designed for tonometry as well as for measurement of corneal biomechanical properties. Several studies have proven the usefulness of the ORA in detecting biomechanical changes related to aging,[9,10] smoking,[11] and corneal refractive procedures,[12,13] as well as to different pathologic conditions including keratoconus,[14,15] Fuchs dystrophy,[16] and diabetes mellitus.[17] However, repeated attempts with the device to assess the biomechanical effects of CXL have been less successful.[15,18,19]

Several studies[20,21] have shown that corneal thickness affects the measurement of intraocular pressure (IOP). Accordingly, keratoconus is a cause of error in applanation tonometry,[22] but the effect of CXL in this respect remains to be elucidated.[23]

Given these circumstances and the large-scale use of CXL in treatment of keratoconus today, there is a need for a reliable tool for in vivo quantification of the biomechanical status of the cornea in conjunction with CXL treatment. The aims of the present study were to quantify the biomechanical effects of CXL using ORA and applanation resonance tonometry (ART) technology. In addition, we wanted to assess the effect of CXL treatment on IOP measurement values using 3 different tonometry techniques.

Methods

This was a prospective, open, case-control study conducted at the Department of Ophthalmology, Umeå University, Umeå, Sweden, between October 13, 2009, and November 5, 2012. The study was approved by the regional ethics review board of Umeå University and was performed in accordance with the Declaration of Helsinki. All participants provided both written and oral informed consent. The participants in the control group received financial compensation.

The study comprised 30 eyes of 29 patients with keratoconus who were scheduled for routine CXL. Age- and sex-matched individuals serving as controls were paired with corresponding patients to rule out natural time-dependent changes in the various variables assessed. This study was part of a larger investigation[24] comparing standard CXL with a modified treatment protocol. In the present study, only the standard CXL treatment group was included.

The inclusion criteria were progressive keratoconus, documented with repeated astigmatic refraction and/or Scheimpflug tomography, planned CXL with a corneal thickness of 400 µm or more at the thinnest point after epithelial removal, age between 18 and 28 years, no previous ocular surgery, no corneal abnormalities except keratoconus, and no cognitive insufficiency interfering with informed consent. The Amsler-Krumeich classification[25] and the total deviation keratoconus quantification value obtained from the Pentacam high-resolution (HR) (Oculus Inc) measurements (described below) was used for grading keratoconus. All cases of keratoconus had a history of progression with an increase in the maximum local keratometric value of 1 diopter (D) or more in 1 year, documented by Pentacam HR measurements (43 eyes) or by repeated keratometry and refraction (17 eyes). In the latter cases, rapid keratoconus progression with increasing K values and declining best spectacle-corrected visual acuity was documented by the referring clinic, and we chose not to await further progression before treating. The healthy participants of the control group were matched with respective keratoconus patients on age and sex.

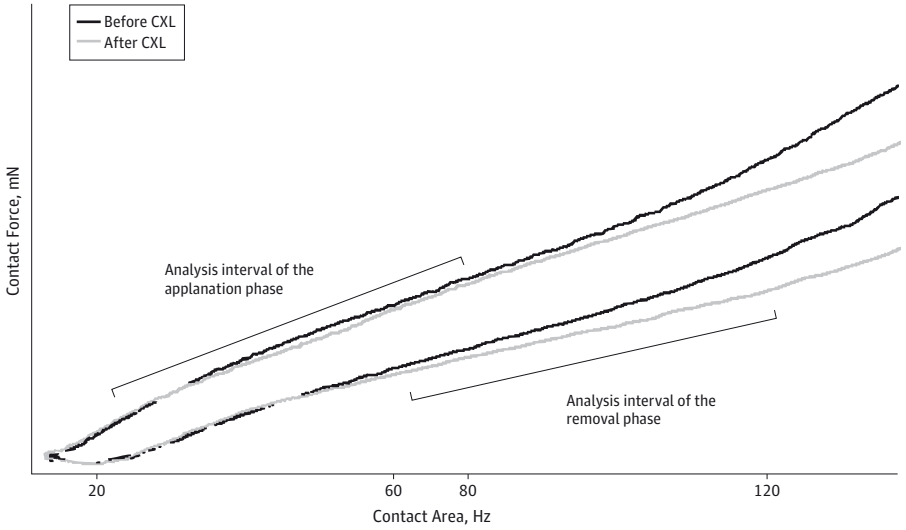
The CXL treatment for the patients with keratoconus at the baseline visit involved topical anesthesia with tetracaine hydrochloride, mechanical removal of the central 9.0-mm corneal epithelium, topical application of riboflavin, 0.1% (Ricrolin; Sooft), every 3 minutes during 30 minutes, and then UV-A irradiation for 30 minutes using a solid-state UV-A illuminator (Caporossi-Baiocchi-Mazzotta X-linker; Costruzione Strumenti Oftalmici). During the UV-A irradiation, riboflavin, 0.1%, was applied every 5 minutes. The diameter of the irradiation area was 8 mm, and the delivered energy was 3 mW/cm.[2,26]

Examinations were performed immediately before treatment and 1 month and 6 months after treatment. Each visit included slitlamp examination, corneal topography, and corneal thickness measurements at the thinnest point with Scheimpflug tomography (Pentacam HR) and IOP by Goldmann applanation tonometry (GAT), ORA software, version 1.02 (Reichert Inc), and the ART (described below). Corneal biomechanical assessments were made with the ORA and ART (described below). We compared preoperative baseline measurements with postoperative measurements at each visit, as well as with measurements in the control participants.

The IOP measurement obtained with GAT (IOP_{GAT}) was obtained with the tonometer tip prism set horizontally (horizontally split semicircles) and vertically. Each measurement was made in duplicate and the IOP_{GAT} was presented as the mean value of all 4 measurements.

The ORA is a tonometer using an electro-optical system to measure the corneal response to indentation by a rapid air

Figure 1. Measurement of Force and Contact Area with Applanation Resonance Tonometer (ART) Before and After Corneal Collagen Crosslinking (CXL) Treatment



Example of ART measurements of a keratoconus patient before and 1 month after CXL treatment. Intraocular pressure (IOP) during the applanation was calculated from data derived from the analysis interval between 20 and 80 Hz. The IOP during the removal was similarly calculated from data between 120 and 60 Hz. The slope between force and frequency in the same intervals was proportional to IOP. The scale on the x-axis highlights the analysis intervals for the applanation and removal phase. Corneal hysteresis was calculated as the difference in IOP during the applanation and removal phases. IOP before and after CXL was similar at applanation, whereas there was a difference in IOP (slope) during the removal. mN indicates milli-Newton.

pulse when measuring the IOP. It simultaneously measures the biomechanical properties of the cornea[27]: the corneal rigidity as corneal resistance factor (CRF) and the corneal viscoelasticity as corneal hysteresis (CH). The ORA provides 2 values for IOP: Goldmann-correlated IOP and corneal-compensated IOP. Corneal-compensated IOP is an IOP that is less dependent on corneal curvature and central corneal thickness.[28] For each eye we obtained 4 measurements and recorded the mean value for corneal-compensated IOP, CH, and CRF (in this study termed IOP_{ORA}, CH_{ORA}, and CRF_{ORA}).

The ART is a recently introduced tonometer[29,30] based on resonance technology.[31] A resonance sensor measures the contact area and a force transducer measures the contact force. Data for the contact area and contact force are sampled continuously (1000 Hz) during the corneal indentation, from the point the sensor contacts the cornea until the sensor is removed.[31,32] A prototype of ART Servo with a servo-controlled motion of the sensor providing a movement toward the cornea was used in this study. A new algorithm for control of the sensor movement was implemented: when the full contact area is reached, the sensor immediately reverses and moves away from the cornea. Applanation velocity was set to 4 mm/s and removal velocity was set to 7 mm/s. The slope of the curve between force and frequency within certain frequency ranges (ie, contact area intervals) is related to the IOP (**Figure 1**). This is valid for the applanation phase as well as for the removal phase. The analysis interval for the applanation was between 20 and 80 Hz in accordance with the standard instrument. A calibration constant transforms the slope of the curve between force and frequency into millimeters of mercury. For choosing an analysis interval during the removal phase an optimization algorithm was used[31] on data from baseline measurements in patients and controls. The algorithm stepped through all combinations of start points and interval lengths in steps of 10 Hz. In that frequency interval, a linear regression was used to calculate the slope between force and frequency. The slope was interpreted as proportional to the IOP and the same calibration constant as for the applanation was also used during the removal phase. The optimal frequency interval was determined as the frequency interval that produced the lowest SD between the slope and the reference IOP measured with GAT. The CH measured with the ART (CH_{ART}) was defined as the difference in IOP between the applanation phase and the removal phase.[32] Each eye was measured 4 times and the mean value was used as IOP assessed with the ART (IOP_{ART}) and CH_{ART}, respectively.

Data are presented as mean (SD). The differences between pretreatment and posttreatment values or between CXL patients and control participants were analyzed using a paired, 2-tailed *t* test, and $P < .05$ was considered statistically significant. All calculations were done using SPSS, version 18 (SPSS Inc).

Results

Of the original 29 patients, 1 was lost to follow-up, leaving 29 eyes of 28 patients (1 woman) to be evaluated. One of the patients developed transient keratitis after the treatment, but was able to continue the study. The mean (SD) ages of the patients and the controls were 23.9 (3.0) years (range, 18.1-28.0 years) and 23.8 (3.1) years (range, 18.2-27.8 years), respectively. The median baseline Amsler-Krumeich keratoconus grading was 2 (interquartile range, 2-3). The total deviation keratoconus quantification value based on Scheimpflug corneal tomography data was 8.5 (6.7) (reference range, −2.0 to 2.0). None of the healthy participants showed any signs of keratoconus according to these gradings.

Table. IOP and Corneal Biomechanical Results in Patients With CXL and Age- and Sex-Matched Controls

Characteristic[a]	ORA, mm Hg			ART, mm Hg		GAT, mm Hg	CT_{min}, µm[b]	Corneal Curvature, Diopters[c]
	IOP	CH	CRF	IOP	CH	IOP		
Patients								
Baseline, mean (SD)	14.0 (2.7)	8.5 (1.4)	7.4 (1.6)	10.6 (3.1)	3.8 (1.6)	11.0 (2.8)	481 (41)	45.9 (3.3)
No. of eyes	28	28	28	28	28	29	29	29
1 mo, mean (SD)	14.4 (2.5)	7.9 (1.5)	6.8 (1.5)	11.1 (3.1)	4.9 (2.1)	11.8 (2.6)	457 (44)	45.8 (3.9)
No. of eyes	29	29	29	28	27	28	29	29
P value (No.)	.30 (28)	.08 (28)	.10 (28)	.53 (27)	.02 (26)	.005 (28)	<.001 (29)	.85 (29)
6 mo, mean (SD)	13.9 (2.6)	8.5 (1.3)	7.3 (1.6)	10.2 (2.7)	4.8 (2.0)	11.1 (2.9)	470 (42)	45.3 (3.5)
No. of eyes	29	29	29	29	29	29	29	29
P value (No.)	.67 (28)	.86 (28)	.65 (28)	.13 (28)	.04 (28)	.78 (29)	.01 (29)	<.001 (29)
Control subjects								
Baseline, mean (SD)	14.1 (2.8)	11.1 (1.5)	10.6 (1.8)	10.8 (2.6)	4.3 (1.7)	12.0 (2.2)	553 (33)	43.6 (1.4)
No. of eyes	28	28	28	27	23	29	29	29
P value (No.)	.86 (27)	<.001 (27)	<.001 (27)	.56 (26)	.01 (22)	.13 (29)	<.001 (29)	.006 (29)
1 mo, mean (SD)	13.4 (2.8)	11.1 (1.4)	10.4 (1.8)	10.0 (2.9)	4.1 (2.0)	11.7 (2.3)	553 (32)	43.6 (1.4)
No. of eyes	29	29	29	28	26	29	29	29
P value (No.)	.08 (29)	<.001 (29)	<.001 (29)	.25 (27)	.16 (24)	.95 (28)	<.001 (29)	.01 (29)
6 mo, mean (SD)	13.6 (3.1)	10.9 (1.5)	10.2 (1.8)	10.6 (2.9)	4.3 (1.9)	11.6 (2.6)	554 (32)	43.6 (1.4)
No. of eyes	29	29	29	29	29	29	29	29
P value (No.)	.71 (29)	<.001 (29)	<.001 (29)	.59 (29)	.20 (29)	.54 (29)	<.001 (29)	.04 (29)

Abbreviations: ART, applanation resonance tonometer; CH, corneal hysteresis; CRF, corneal resistance factor; CT_{min}, corneal thickness at the thinnest point; CXL, corneal collagen crosslinking; GAT, Goldmann applanation tonometry; IOP, intraocular pressure; ORA, ocular response analyzer.

[a] The *P* values (paired *t* test) for the patients with keratoconus denote the difference between baseline and 1 or 6 months, respectively. The *P* values (paired *t* test) for the control participants denote the difference between patients who received CXL vs corresponding control participants at baseline and 1 or 6 months, respectively. The corresponding numbers are in parentheses; these indicate the number of participants available for comparison for that variable.

[b] Measurement was obtained with Pentacam high resolution (HR).

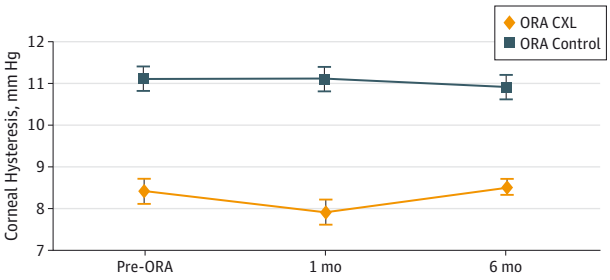
[c] Curvature was calculated as (K1 + K2)/2, obtained with Pentacam HR.

The optimization of ART-frequency interval for the removal phase showed the best result (lowest SD) between 120 and 60 Hz, which was used in all subsequent analyses. The IOP and corneal biomechanical results for patients are reported in the **Table**.

An increased CH was demonstrated in patients with keratoconus after CXL with the ART but not with the ORA. Between baseline and 1 month (immediate effect), the ART measured an increased CH at 1.2 (2.4) mm Hg (*P* = .02; 99% CI, -0.1 to 2.5). Between baseline and 6 months (persisting effect), the ART measured an increased CH, at 1.1 (2.7) mm Hg (*P* = .04; 99% CI, -0.3 to 2.6). No significant difference in IOP was identified after CXL with any method, except for a small increase at 1 month with GAT (1.0 mm Hg; *P* = .005) (Table). The corneal thickness decreased by −24 (26) µm and −11 (21) µm, respectively, 1 and 6 months after treatment compared with baseline (*P* < .001 and *P* = .01, respectively). A significant difference in corneal flattening of −0.6 (0.7) D was seen at 6 months (*P* < .001).

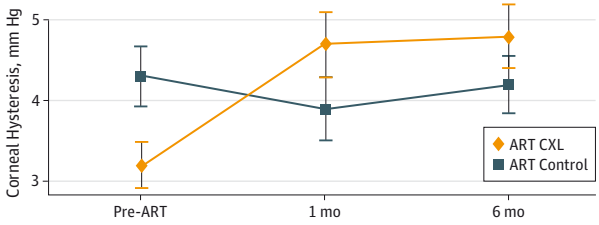
The Table reports IOP and biomechanical variables for the matched control participants and *P* values from paired *t* test comparisons between the control participants and the patients with keratoconus. A significant difference in CH between the controls and the keratoconus patients was found at baseline, both with the ART (−1.09 [1.92] mm Hg; *P* = .01; 99% CI, -2.26 to 0.07) and the ORA (−2.67 [2.55] mm Hg; *P* < .001; 99% CI, -4.05 to -1.32). The increase in CH after treatment assessed with the ART abolished the difference in CH between keratoconus patients and healthy participants at 1 and 6 months (*P* = .16 and *P* = .20, respectively), whereas the ORA difference persisted (both *P* < .001) because CH_{ORA} values were unaltered by CXL (**Figure 2** and **Figure 3**). Likewise, the CRF_{ORA} values were unaltered throughout the study.

Figure 2. Corneal Hysteresis Measured With the Ocular Response Analyzer (ORA) in Patients and Controls



Corneal hysteresis measured with ORA (mean [SE]) on corneal collagen crosslinking (CXL)–treated keratoconus in patients and corresponding age- and sex-matched individuals serving as controls. There was a significant difference between the 2 groups.

Figure 3. Corneal Hysteresis Measured With the Applanation Resonance Tonometer (ART) in Patients and Controls



Corneal hysteresis measured with ART (mean [SE]) on corneal collagen crosslinking (CXL)–treated keratoconus in patients and corresponding age- and sex-matched individuals serving as controls. There was a significant difference between the groups before treatment and a significant increase in corneal hysteresis measured with the ART after treatment.

Discussion

To our knowledge this is the first study demonstrating an increase in CH in vivo after CXL treatment for keratoconus. Today there is convincing evidence[3,19,33,34] that CXL is a successful treatment for keratoconus in that it slows, halts, or even reverses the progressive ectasia. It is generally accepted that the effect is caused by increased corneal biomechanical strength,[35] which has been confirmed in numerous in vitro studies.[4-7,36,37] However, repeated attempts to demonstrate an increased CH_{ORA} after CXL in clinical settings have failed.[15,18,19]

The novel device that we used in the present study for CH assessment was the ART, an instrument originally created for IOP measurements. In this study it was further developed and modified to measure CH. Both the ART and the ORA detected a significant difference in CH between keratoconus patients and healthy control participants at baseline (Figures 2 and 3), but the CH_{ART} increased significantly after CXL, while the CH_{ORA} was unaltered after CXL, in accordance with previous reports.[15,18,19]

The ART measures the corneal contact area using resonance technology, which is based on the assumption of constant acoustic impedance in the tissue. Theoretically, the CXL treatment might change the corneal acoustic impedance, but if this were the case, the measured IOP_{ART} would change accordingly, which did not occur in the present study. In addition, CH_{ART} was measured as the difference between applanation IOP and removal IOP. A change in acoustic impedance would not change this difference, and therefore it would not affect the CH_{ART}.

The ART assesses CH at a slower velocity than the ORA and the CH values in millimeters of mercury are therefore lower (Figures 2 and 3) because viscoelastic dampening is velocity dependent. In viscoelastic materials the counterforce diminishes at slow velocity (ie, the difference in measured pressure between indentation and removal decreases and CH at low velocity will be smaller). Consequently, CH_{ART} shows numerically lower values compared with CH_{ORA} (Table). One could speculate that measuring CH with a controlled indentation at a slower velocity better reveals the increased stability induced by CXL. The velocity of the motor-controlled indentation of the cornea induced by the ART is possible to alter over a wide range of velocities, and optimizing the indentation speed may reveal the stiffening from CXL even better. From the present data, our impression is that an even slower measurement rate could be an approach to make the difference between an untreated and a treated keratoconus cornea clearer. In addition to modifying the velocity, refined mathematical analyses of the ART raw data might enable a better distinction between untreated and treated corneas. Finally, altering the radius of the sensor tip could also affect the sensitivity of the ART measurement. Our sensor tip has a radius of curvature of 8 mm and was originally developed for IOP measurements. A tip optimized for measuring corneal biomechanical properties would probably benefit from a steeper curvature, since the tissue of interest is the central cornea only, rather than the entire globe. Murayama et al[38] have shown that resonance technology is useful to detect elastic properties of small-cell membranes using a sensor tip with a radius of curvature of only 10 µm. Similar reasoning is also used in other fields of science, for example, when the stiffness of rubber is determined using international rubber hardness degrees (International Organization for Standardization, ISO48:2010). Thus, reducing the radius of curvature of the ART probe may be a way to further increase its sensitivity to CXL-induced corneal changes. The ultimate goal is to optimize the ART device for detection and quantification of biomechanical changes induced by CXL, which would possibly also help us to further understand what happens in the cornea during and after a CXL treatment. Such optimization of the ART device will be the subject of future studies.

With the ORA the standardized air puff causes a rather large area of central corneal flattening, which means part of the corneal bending may occur in the peripheral cornea, even outside the CXL-treated area. Therefore, the stiffening effect from the treatment may be harder to detect. On the other hand, a significant difference in CH is seen between keratoconus and healthy corneas, which the present study also confirms, and the keratoconus disease mainly affects the central cornea, while the thickness of the peripheral cornea is preserved.[14]

The corneal thinning and flattening seen after CXL in the present study, as well as in many other studies,[19,33,34] cannot have contributed to the increase in CH demonstrated in the present study by the ART. On the contrary, a thinner, flatter cornea would lower the CH if all other factors were unaltered.[10,39]

The IOP_{ORA} and IOP_{ART} are 2 tonometry methods developed with the aim to be less sensitive to different biomechanical properties.[27,32] With these methods, the measured IOP was unaltered after CXL. It is generally accepted that thick corneas overestimate and thin corneas underestimate the applanation IOP.[21] Still, 1 month after CXL we found that the IOP_{GAT} was higher compared with the baseline level, despite the corneal thinning seen at the same time point. Applanation tonometry is also known to be affected by corneal rigidity.[21] Our interpretation is that this increase in IOP_{GAT} might be another sign of increased corneal stability induced by the CXL treatment.

Conclusions

Our present data indicate that what we measure with the ART is an increase in CH after CXL treatment in keratoconus. Given the widespread use of CXL in today's keratoconus treatment arsenal, a tool capable of quantifying the increased corneal stability in vivo after CXL has a great potential value. Further fine-tuning of the ART method of assessing corneal biomechanical properties, a subject of future studies, can make the ART a useful tool in future assessment of CXL treatment effects.

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Study concept and design: Beckman Rehnman, Behndig, Lindén.
Acquisition, analysis, or interpretation of data: All authors.
Drafting of the manuscript: All authors.
Critical revision of the manuscript for important intellectual content: All authors.
Statistical analysis: Beckman Rehnman, Behndig, Hallberg.
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Study supervision: Behndig, Hallberg, Lindén.

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REFERENCES

1. Krachmer JH, Feder RS, Belin MW. Keratoconus and related noninflammatory corneal thinning disorders. *Surv Ophthalmol*. 1984;28(4):293-322.

2. Tan DT, Por YM. Current treatment options for corneal ectasia. *Curr Opin Ophthalmol*. 2007;18(4):284-289.

3. Wollensak G, Spoerl E, Seiler T. Riboflavin/ultraviolet-A–induced collagen crosslinking for the treatment of keratoconus. *Am J Ophthalmol*. 2003;135(5):620-627.

4. 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(9):1780-1785.

5. Wollensak G, Spoerl E, Reber F, Seiler T. Keratocyte cytotoxicity of riboflavin/UVA-treatment in vitro. *Eye (Lond)*. 2004;18(7):718-722.

6. Spoerl E, Wollensak G, Dittert DD, Seiler T. Thermomechanical behavior of collagen-cross-linked porcine cornea. *Ophthalmologica*. 2004;218(2):136-140.

7. Wollensak G, Redl B. Gel electrophoretic analysis of corneal collagen after photodynamic cross-linking treatment. *Cornea*. 2008;27(3):353-356.

8. Meek KM, Hayes S. Corneal cross-linking—a review. *Ophthalmic Physiol Opt*. 2013;33(2):78-93.

9. Kamiya K, Shimizu K, Ohmoto F. Effect of aging on corneal biomechanical parameters using the ocular response analyzer. *J Refract Surg*. 2009;25(10):888-893.

10. Narayanaswamy A, Chung RS, Wu RY, et al. Determinants of corneal biomechanical properties in an adult Chinese population. *Ophthalmology*. 2011;118(7):1253-1259.

11. Hafezi F. Smoking and corneal biomechanics. *Ophthalmology*. 2009;116(11):2259.e1. doi:10.1016/j.ophtha.2009.07.039.

12. Chen S, Chen D, Wang J, Lu F, Wang Q, Qu J. Changes in ocular response analyzer parameters after LASIK. *J Refract Surg*. 2010;26(4):279-288.

13. Ryan DS, Coe CD, Howard RS, Edwards JD, Bower KS. Corneal biomechanics following epi-LASIK. *J Refract Surg*. 2011;27(6):458-464.

14. Fontes BM, Ambrósio R Jr, Velarde GC, Nosé W. Ocular response analyzer measurements in keratoconus with normal central corneal thickness compared with matched normal control eyes. *J Refract Surg*. 2011;27(3):209-215.

15. Gkika M, Labiris G, Giarmoukakis A, Koutsogianni A, Kozobolis V. Evaluation of corneal hysteresis and corneal resistance factor after corneal cross-linking for keratoconus. *Graefes Arch Clin Exp Ophthalmol*. 2012;250(4):565-573.

16. del Buey MA, Cristóbal JA, Ascaso FJ, Lavilla L, Lanchares E. Biomechanical properties of the cornea in Fuchs' corneal dystrophy. *Invest Ophthalmol Vis Sci*. 2009;50(7):3199-3202.

17. Goldich Y, Barkana Y, Gerber Y, et al. Effect of diabetes mellitus on biomechanical parameters of the cornea. *J Cataract Refract Surg*. 2009;35(4):715-719.

18. Spoerl E, Terai N, Scholz F, Raiskup F, Pillunat LE. Detection of biomechanical changes after corneal cross-linking using Ocular Response Analyzer software. *J Refract Surg*. 2011;27(6):452-457.

19. Goldich Y, Marcovich AL, Barkana Y, et al. Clinical and corneal biomechanical changes after collagen cross-linking with riboflavin and UV irradiation in patients with progressive keratoconus: results after 2 years of follow-up. *Cornea*. 2012;31(6):609-614.

20. Ehlers N, Bramsen T, Sperling S. Applanation tonometry and central corneal thickness. *Acta Ophthalmol (Copenh)*. 1975;53(1):34-43.

21. Whitacre MM, Stein R. Sources of error with use of Goldmann-type tonometers. *Surv Ophthalmol*. 1993;38(1):1-30.

22. Rask G, Behndig A. Effects of corneal thickness, curvature, astigmatism and direction of gaze on Goldmann applanation tonometry readings. *Ophthalmic Res*. 2006;38(1):49-55.

23. Gkika MG, Labiris G, Kozobolis VP. Tonometry in keratoconic eyes before and after riboflavin/UVA corneal collagen crosslinking using three different tonometers. *Eur J Ophthalmol*. 2012;22(2):142-152.

24. Beckman Rehnman J, Behndig A, Hallberg P, Lindén C. Initial results from mechanical compression of the cornea during crosslinking for keratoconus [published online March 15, 2014]. *Acta Ophthalmol*. doi:10.1111/aos.12380.

25. Krumeich JH, Daniel J, Knülle A. Live-epikeratophakia for keratoconus. *J Cataract Refract Surg*. 1998;24(4):456-463.

26. Mazzotta C, Traversi C, Baiocchi S, et al. Corneal healing after riboflavin ultraviolet-A collagen cross-linking determined by confocal laser scanning microscopy in vivo: early and late modifications. *Am J Ophthalmol*. 2008;146(4):527-533.

27. Luce DA. Determining in vivo biomechanical properties of the cornea with an ocular response analyzer. *J Cataract Refract Surg*. 2005;31(1):156-162.

28. Luce D. Methodology for corneal compensated IOP and corneal resistance factor for the Reichert ocular response analyzer: American Research in Vision and Ophthalmology (ARVO). Fort Lauderdale, FL, USA [abstract]. *Invest Ophthalmol Vis Sci*. 2006;47. http://abstracts.iovs.org/cgi/content/abstract/47/5/2266. Accessed July 16, 2014.

29. Hallberg P, Eklund A, Bäcklund T, Lindén C. Clinical evaluation of applanation resonance tonometry: a comparison with Goldmann applanation tonometry. *J Glaucoma*. 2007;16(1):88-93.

30. Jóhannesson G, Hallberg P, Eklund A, Lindén C. Introduction and clinical evaluation of servo-controlled applanation resonance tonometry. *Acta Ophthalmol*. 2012;90(7):677-682.

31. Eklund A, Hallberg P, Lindén C, Lindahl OA. An applanation resonator sensor for measuring intraocular pressure using combined continuous force and area measurement. *Invest Ophthalmol Vis Sci*. 2003;44(7):3017-3024.

32. Hallberg P, Lindén C, Lindahl OA, Bäcklund T, Eklund A. Applanation resonance tonometry for intraocular pressure in humans. *Physiol Meas*. 2004;25(4):1053-1065.

33. Raiskup-Wolf F, Hoyer A, Spoerl E, Pillunat LE. Collagen crosslinking with riboflavin and ultraviolet-A light in keratoconus: long-term results. *J Cataract Refract Surg*. 2008;34(5):796-801.

34. Vinciguerra P, Albè E, Trazza S, Seiler T, Epstein D. Intraoperative and postoperative effects of corneal collagen cross-linking on progressive keratoconus. *Arch Ophthalmol*. 2009;127(10):1258-1265.

35. Beshtawi IM, O'Donnell C, Radhakrishnan H. Biomechanical properties of corneal tissue after ultraviolet-A–riboflavin crosslinking. *J Cataract Refract Surg*. 2013;39(3):451-462.

36. Knox Cartwright NE, Tyrer JR, Marshall J. In vitro quantification of the stiffening effect of corneal cross-linking in the human cornea using radial shearing speckle pattern interferometry. *J Refract Surg*. 2012;28(7):503-508.

37. Dias J, Diakonis VF, Kankariya VP, Yoo SH, Ziebarth NM. Anterior and posterior corneal stroma elasticity after corneal collagen crosslinking treatment. *Exp Eye Res*. 2013;116:58-62.

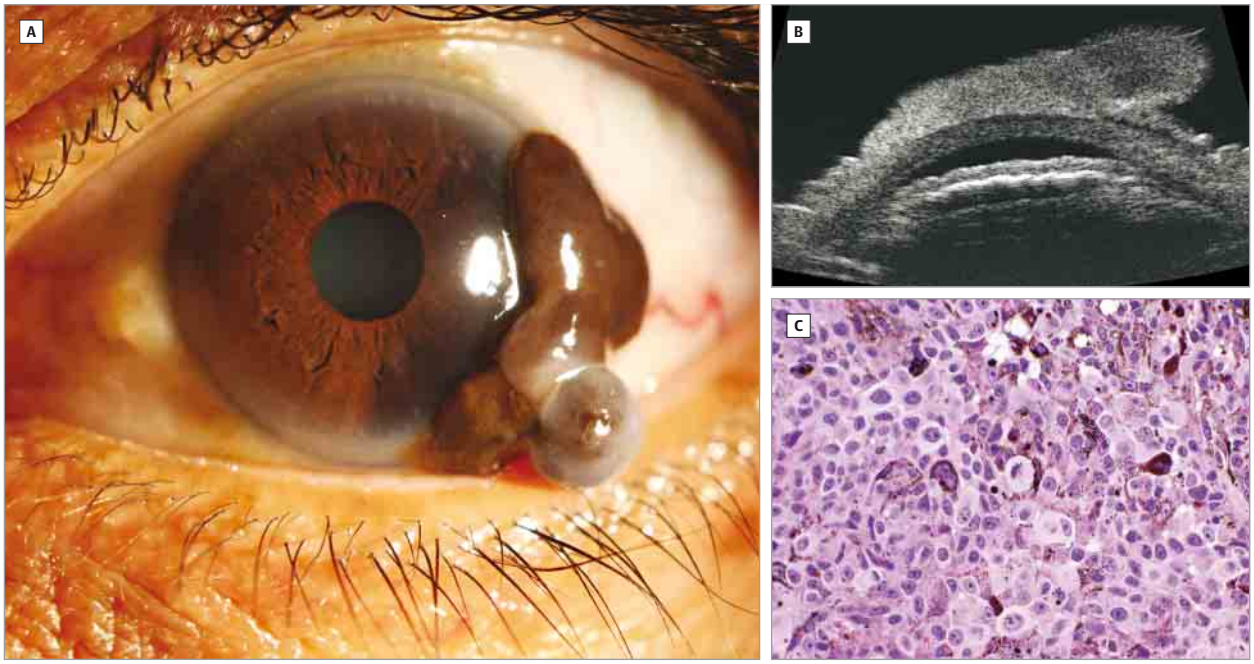
38. Murayama Y, Constantinou CE, Omata S. Micro-mechanical sensing platform for the characterization of the elastic properties of the ovum via uniaxial measurement. *J Biomech*. 2004;37(1):67-72.

39. Hwang HS, Park SK, Kim MS. The biomechanical properties of the cornea and anterior segment parameters. *BMC Ophthalmol*. 2013;13:49. doi:10.1186/1471-2415-13-49.

OPHTHALMIC IMAGES

Conjunctival Melanoma

Glenn Yiu, MD, PhD; Thomas J. Cummings, MD; Prithvi Mruthyunjaya, MD



A and B, A 64-year-old man presented with an elevated, darkly pigmented left conjunctival lesion with leukoplakia and a sentinel vessel (A) with no intraocular involvement on ultrasound biomicroscopy (B). C, Excisional biopsy with double freeze-thaw cryotherapy (**Video**) showed pigmented epithelioid cells and prominent nucleoli consistent with a primary conjunctival melanoma.