High-irradiance CXL combined with myopic LASIK: flap and residual stroma biomechanical properties studied ex vivo.

Anastasios John Kanellopoulos,1,2 George Asimellis,1 Joseph B Ciolo,3 Borja Salvador-Culla,2 James Chodosh1

ABSTRACT
Background/aims: To evaluate ex vivo biomechanical and enzymatic digestion resistance differences between standard myopic laser in-situ keratomileusis (LASIK) compared with LASIK+CXL, in which high-irradiance cross-linking (CXL) is added.

Methods: Eight human donor corneas were subjected to femtosecond-assisted myopic LASIK. Group A (n=4) served as a control group (no CXL). The corneas in LASIK+CXL group B were subjected to concurrent prophylactic high-irradiance CXL (144). Saline-diluted (0.1%) riboflavin was instilled on the stroma, subsequently irradiated with UV-A through the repositioned flap. The corneal stroma and flap specimens were separately subjected to transverse strain resistance measurements; biomechanical differences were assessed via stress and Young’s modulus. Subsequently, the specimens were subjected to enzymatic degradation. Results: For the corneal stroma specimens, stress at 10% strain was 128±11 kPa for control group A versus 293±20 kPa for the LASIK+CXL group B (relative difference Δ±Δ+129%, p<0.05). The stress in group B was also increased at 20% strain by +48% (p<0.05). The strain modulus of group B was increased at 10% strain by +79%, and at 20% strain by +48% (both statistically significant, p<0.05). The enzymatic degradation time to dissolution was 157±51.5 min in group A versus 186.2±7.5 min in group B (Δ±Δ+45%, p<0.05). For the flaps, both biomechanical, as well as enzymatic degradation tests showed no significant difference.

Conclusions: LASIK+CXL appears to provide significant increase in underlying corneal stromal rigidity, up to +120%. Additionally, there is significant resistant enzymatic digestion resistance confirming to the above. LASIK flaps appear unaffected biomechanically by the LASIK+CXL procedure, suggesting effective CXL just under the flap.

INTRODUCTION

Corneal collagen cross-linking (CXL) has been clinically employed for stabilizing progressive keratoconus. This photochemical reactive process is induced by peak 365 nm ultraviolet (UV-A) radiation absorbed by riboflavin, a photosensitive vitamin B2 molecule. The procedure is broadly known as corneal cross-linking—despite the fact that there are some reports suggesting that the mechanism responsible for biomechanical strengthening within the stroma is related not to interfascicular cohesion increase, but to interstitial and intra-fascicular cohesion. In addition, increased collagen resistance against enzymatic degradation has been associated with CXL. We have introduced an alternative CXL application, adjacent to myopic laser in-situ keratomileusis (LASIK+CXL). The application aims to improve long-term keratometric stability and to reduce regression likelihood following moderate and high myopic LASIK by proactively restoring corneal biomechanical strength. Riboflavin solution is briefly applied on the exposed stromal bed at the completion of the excimer ablation; the flap is then repositioned, followed by superficial UV-A irradiation. To the best of our knowledge, the biomechanical and/or enzymatic degradation resistance modulations achieved via CXL application concurrent with LASIK have not been studied in human corneas. The purpose of this study is to evaluate ex vivo biomechanical and enzymatic degradation resistance differences in such application.

MATERIALS AND METHODS

Eight human donor corneas were involved, obtained by the Eye-Bank of Sight Restoration Inc (New York, USA), an accredited member of the Eye-Bank Association of America. The corneas were donated by eight different donors (four men, four women) of average age 62.0±9.5 (43–72) years, stored in 4°C Optisol solution (Bausch & Lomb, Rochester, New York, USA).

Surgical technique

All corneas were subjected to femtosecond-laser assisted myopic treatment. The corneas were mounted on an artificial anterior chamber (Barron, Katena Products Inc, Demville, New Jersey, USA). Flaps (120 μm thick, 8.5 mm diameter) were treated with the WaveLight FS200 femtosecond laser (Alcon Surgical, Ft Worth, Texas, USA), observing standard docking, applanation and vacuum-suction procedures (figure 1A). After flap lifting (figure 1B), the WaveLight EX500 excimer laser (Alcon) was employed to create a −8.00 D myopic ablation over a 6.3 mm diameter optical zone (figure 1C). During the procedure, interferometric pachymetry embedded in the EX500 provided corneal thickness data.

Isotonic saline 0.1% riboflavin solution (Vibex Rapid, Icedro Inc, Wallams, Massachusetts, USA) was instilled on the exposed stromal bed afforded by the lifted flap (figure 1C). Soaking time was 1 min (figure 1E); then excess riboflavin was wiped from the cornea surface. Special care was taken to minimise potential riboflavin soaking to the folded LASIK flap.
The corneas were then randomly formed into two groups, four in each. Control group A received no further treatment. Group B (LASIK+CXL) was subjected to cross-linking with the flap repositioned, the cornea was UV-A irradiated at 30 mW/cm² for 30 s (total fluence 2.4 J/cm²), employing the KXL device (Avedro) (figure 1B). The specimens were then amputated from all corneas; stroma and flap specimens were stored back to OptiSol (4°C) until testing.

Biomechanical strength testing

The stroma specimens were prepared by razor blade manual dissection to approximately 12×12 mm. The amputated flaps were tested with additional preparation. Transmission bilateral load cell resistance measurements were accomplished by tangential shear force employing the Biostress 5000 (CellBiac Biomaterials Testing, Waterloo, Canada). The device records the simultaneous x-axis and y-axis displacement, applied force, and time. An integrated camera captures still, 1280×960-pixel images, which provide precise x-displacement and y-displacement measurements, analysed by custom software (LabJoy V.21.0 (IBM Corporation, New York, USA). The exponential fitting for stress calculation was conducted at the 10% and 20% strain. Linear regression fitting was performed to calculate, by means of the slope function (gradient), the stress/strain ratio, an expression of the shear (Young’s) modulus. The shear modulus was calculated as the gradient at 10% and 20% strain. To ensure proper linear fit, we sought a minimum of 0.98 for the trend-line determination coefficient (r²). Statistical significance was assessed employing Student’s t test. Values less than 0.05 were indicative of statistically significant results in this study. Results are reported in the form: average ±SD (minimum to maximum).

RESULTS

In group A the donor age the time of cornea harvest was 61.3 ± 13.7 years (48–72) and in group B, 60.8 ± 4.3 years (43–69).

Biomechanical strength results

The distinct datasets during shear-strength measurements on stroma specimens (all stroma specimens) were on average 290 ± 37 (240–340), while average displacement was 1205±155 μm (1008–1868). Mean maximum applied force was 3996±517 mN (3469–5247). Average value of maximum strain was 10.6±3.4% (6.3–15%).

For the stroma specimens in control group A: stress at 10% strain was 128±11 kPa and at 20% strain 804±31 kPa; Young’s modulus at 10% strain was 3.7±2.5 MPa and at 20% strain 9.5±1.8 MPa. In the LASIK+CXL group B, stress at 10% strain was 182±21 kPa and at 20% strain 1534±134 kPa; Young’s modulus at 10% strain was 6.6±2.4 MPa and at 20% strain 14.0±5.2 MPa. The relative increase of biomechanical properties in LASIK+CXL group B in comparison to control group A stress ±129% and ±68% for 10% and 20% strain, and Young’s modulus ±79% and ±48%, respectively. All differences were statistically significant (table 1). For the flap specimens in control group A: stress at 10% strain was 228±17 kPa and at 20% strain 1566±46 kPa; Young’s modulus at 10% strain was 5.9±3.9 MPa and at 20% strain 13.6±6.2 MPa. In the LASIK+CXL group B, stress at 10% strain was 182±21 kPa and at 20% strain 1134±134 kPa; Young’s modulus at 10% strain was 6.5±3.2 MPa and at 20% strain 11.9±1.1 MPa. The relative changes of these biomechanical properties in LASIK+CXL group B in comparison to control group A were not statistically significant (table 2).

Enzymatic digestion results

Regarding the stroma specimens, the mean time to complete dissolution in control group A was 157±15 min, while in group B (LASIK+CXL) it was 186±7.5 min. The relative difference

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<tr>
<th>Table 1</th>
<th>Biomechanical comparative measurements between the two groups</th>
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<td>Stress (kPa)</td>
<td>Young’s shear modulus (MPa)</td>
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<td></td>
<td>10% strain</td>
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<td></td>
<td>10% strain</td>
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<tr>
<td>Group A (control)</td>
<td>128.0 ±11.3</td>
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<tr>
<td>Group B (CXL)</td>
<td>182.8 ±20.8</td>
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<tr>
<td>Δ</td>
<td>52.8%</td>
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<td>p</td>
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<th>Biomechanical comparative measurements between the two groups</th>
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<td>Stress (kPa)</td>
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<td>10% strain</td>
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<td>10% strain</td>
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<tr>
<td>Group A (control)</td>
<td>158.3 ±16.8</td>
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<tr>
<td>Group B (CXL)</td>
<td>162.8 ±20.8</td>
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<tr>
<td>Δ</td>
<td>11%</td>
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<tr>
<td>p</td>
<td>0.004</td>
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\(\Delta\), relative (%) difference between metrics; Δ, Student t test p value; t, Student t test t value. Results from corneal stroma specimens.
UV-A absorption in high-myopic LASIK. Cross-linking techniques in the in-vivo evaluation of the effect of corneal biomechanics and inhomogeneous riboflavin starch, and thus affects UV-A transmission to deeper layers, due to increased UVA absorption by the superficially concentrated riboflavin.

The second issue that affects epithelium-on CXL relates to the in-situ riboflavin application. The in-situ riboflavin application is to provide prophylactic corneal strengthening to counter weakening due to tissue removal, we view this very significant biomechanical effect as a very important finding. These findings are confirmatory to our previous clinical effects reported on myopic and hyperopic LASIK+CXL applications. CXL may therefore be used as a biomodulator offering refractive stability following CXL.

There was no indication that the flaps had any statistically significant biomechanical difference from the control group, but the findings are very important indicative that no actual cross-linking of the flap occurs during the CXL-CXL technique. There are two main reasons that we carefully record any cross-linking effect on the flap. First is that the flap does not contribute to the biomechanical properties of the cornea after cross-linking. Therefore, there is no benefit from a potential cross-linking of the flap. Second, and perhaps even more important, if such a thin (the CXL flap consists of ~50 μm of epithelium and ~40 μm of stroma) stromal content may lead to undesired effects on the corneal transparency and optical properties of the flap.

Additionally, the findings in the collagen-enzymatic digestion part of this work provide circumstantial evidence of the differential effects of cross-linking on the stroma and not on the flap. This, to the best of our knowledge, is the first manuscript presenting both techniques, biomechanical stress measurements and enzymatic digestion, of ex-vivo human assessment of cross-linking effects.

Additional studies with larger sample size, differentiation of UVA irradiance, as well as extending the testing period to longer times intervals followed in order to validated and further investigate the findings in this preliminary study.

Table 3 Enzymatic digestion time to complete dissolution

<table>
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<tr>
<th>Corneal Region</th>
<th>Flaps Tissue</th>
<th>Time (min)</th>
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<tr>
<td>A</td>
<td>157 ± 10</td>
<td>17.3 ± 1</td>
</tr>
<tr>
<td>Group B (CXL)</td>
<td>186 ± 7</td>
<td>17.3 ± 1</td>
</tr>
<tr>
<td>A</td>
<td>18%</td>
<td>18%</td>
</tr>
<tr>
<td>B</td>
<td>0.014</td>
<td>0.0094</td>
</tr>
<tr>
<td>I</td>
<td>0.045</td>
<td>0.02</td>
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A = anterior, I = inferior, α = standard deviation, β = standard error of beta, δ = Student's t-test.

A was 18% for group B, a statistically significant difference (p=0.014). Regarding the flaps, the mean time to complete dissolution in group A was 48.7 ± 17.3 min, while in group B it was 50.8 ± 28.4 min. The relative difference A was ±11% for group B, a difference which, however, was not statistically significant (table 3).

DISCUSSION

Epithelial-on, in-situ CXL is reported in the peer review literatures as providing a significantly weaker biomechanical effect in comparison to epi-off CXL. A study by et al. demonstrated that the effective CXL stromal crosslinking effects in the in-vivo evaluation of the effect of corneal biomechanics and inhomogeneous riboflavin starch, and thus affects UV-A transmission to deeper layers, due to increased UVA absorption by the superficially concentrated riboflavin.

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There was no indication that the flaps had any statistically significant biomechanical difference from the control group, but the findings are very important indicative that no actual cross-linking of the flap occurs during the CXL-CXL technique. There are two main reasons that we carefully record any cross-linking effect on the flap. First is that the flap does not contribute to the biomechanical properties of the cornea after cross-linking. Therefore, there is no benefit from a potential cross-linking of the flap. Second, and perhaps even more important, if such a thin (the CXL flap consists of ~50 μm of epithelium and ~40 μm of stroma) stromal content may lead to undesired effects on the corneal transparency and optical properties of the flap.

Additionally, the findings in the collagen-enzymatic digestion part of this work provide circumstantial evidence of the differential effects of cross-linking on the stroma and not on the flap. This, to the best of our knowledge, is the first manuscript presenting both techniques, biomechanical stress measurements and enzymatic digestion, of ex-vivo human assessment of cross-linking effects.

Additional studies with larger sample size, differentiation of UVA irradiance, as well as extending the testing period to longer times intervals followed in order to validated and further investigate the findings in this preliminary study.

CONCLUSIONS

Adherent intrastromal in-situ CXL combined with myopic LASIK appears in ex-vivo human study to be a significant biomechanical modulator.

References: 17, 20, 23, 26, 33, 34, 37, 85. Laboratory science.
Laboratory science
Purpose: To evaluate biomechanical changes induced by in situ corneal cross-linking (CXL) with stromal pocket delivered enhanced concentration riboflavin and high-fluence, high-energy UV-A irradiation.

Methods: Eight human donor corneas were subjected to intrastromal lamellar corneal tissue removal of anterior 140-μm deep, 80-μm thick × 5-mm diameter central stromal buttons, extracted through a 3.5-mm width tunnel, surfacing in the superior cornea parenhyma. Enhanced concentration riboflavin solution (0.25%) was instilled in the pocket. In study group A (CXL), superficial high-fluence UV-A irradiation was applied, whereas in control group B (no CXL), none. To computationally assess changes in corneal rigidity, corneal specimens were subjected to transverse biaxial resistance measurements by application of a unidirectional tangential shear force. Biomechanical differences were evaluated through stress and Young shear modulus.

Results: Stress at 10% strain was 301 ± 24 kPa in study group A versus 157 ± 11 kPa in control group B (relative difference Δ = 107%; P = 0.021). Stress at 20% strain was 1248 ± 34 kPa in study group A versus 874 ± 29 kPa in control group B (Δ = 47%; P = 0.043). Average shear modulus in study group A at 10% strain was 6.98 ± 1.12 MPa versus 4.04 ± 0.85 MPa in control group B (Δ = 73%; P = 0.036). Average shear modulus in study group A at 20% strain was 11.46 ± 0.75 MPa versus 8.80 ± 0.72 MPa in control group B (Δ = 30%; P = 0.047).

Conclusions: Adjunct CXL, in this ex vivo simulation refractive lens extraction procedure seems to provide significant increase in corneal rigidity, up to 107%. These findings also support our previous reported work on laser in situ keratomileusis combined with CXL.

Key Words: femtosecond laser, refractive lens extraction, biomechanical simulation, in situ CXL, epithelium on, high-fluence CXL, high-energy CXL, higher riboflavin concentration CXL, corneal biomechanics, Young shear modulus, corneal stress-strain (Cornea 2015;0:1–7)

Corneal Collagen Cross-linking Combined With Simulation of Femtosecond Laser–Assisted Refractive Lens Extraction: An Ex Vivo Biomechanical Effect Evaluation

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MATERIALS AND METHODS

This laboratory (ex vivo) study was approved by the Laservision.gr Clinical and Research Eye Institute Ethics Committee.

Eight human donor corneas were used in the study, which were obtained for research purposes from the Eye Bank for Sight Restoration Inc (New York, NY). The corneas were donated by 8 different organ donors (4 men, 4 women), whose mean age was 64.25 ± 11.96 years (range, 50–79 years).

Experimental Technique

All corneas were subjected to creation and removal of a central lamellar intrastromal button through a custom-designed application consisting of a double intrastromal bed cut. A WaveLight FS200 femtosecond laser (Alcon Surgical, Ft Worth, TX) was used for this purpose. The corneas were mounted on an artificial chamber (Baron, Katena Products, Inc, Denville, NJ). To achieve high precision intrastromal cuts, we implemented standard docking, application, and vacuum suction procedures appropriate for the use of the FS200 laser along with the clear cone-patient interface.23 Subsequently, the following 2 FS200 procedures were applied.

1. Procedure A, a circular posterior lamellar bed dissection of 9-mm diameter 220-μm depth from the anterior corneal surface (Fig. 1A). To achieve this, the “flip” mode with no side cut was used. A 0.4-mm wide and 3.0-mm long canal was designed to serve as the channel through which the “button” to be created would be extracted.

2. Procedure B, a circular anterior lamellar dissection of 5-mm diameter 140-μm depth (Fig. 1B). To achieve this, the “posterior lamellar” mode was used. In addition, the circumferential “side-wall” cut was extended to 180 μm to ensure that the vertical dissections of the cut would transcend the posterior lamellar level.

The combination of these 2 procedures resulted in the creation of an intrastromal “button.” 5-mm diameter, 80-μm thick, located 148 μm inside the cornea (Fig. 1C). In addition, a “channel” was created, enabling button extraction. The button was extracted using a Smiley hook and toothless refractive correction application aiming to restore corneal biomechanical strength has not yet been reported.

The purpose of this study was to evaluate ex vivo the biomechanical changes resulting from a simulated refractive lens extraction procedure combined with CXL.

FIGURE 1. A, Posterior lamellar cut: left, detail from the system software report, right, schematic drawing. B, Superior lamellar cut: left, detail from the system software report, right, schematic drawing. C, Intrastromal “button” creation: left, detail from the system software report, right, schematic drawing.

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Data Collection and Analysis
A central square block (approximately 12 × 12 mm) was obtained from each treated corneal specimen using a disposable razor blade. A Biotester 5000 device (CellScale Biomaterials Testing, Waterloo, Canada) was used to provide biaxial testing resistance testing.

The specimens were fixed (random orientation) on a 4 × 5 time rake arrangement (Fig. 2) at their center 3.5 × 3.5 mm section. The times (258-μm diameter) were spaced apart by 0.7 mm. The specimens on the fixation unit were submerged into an isotonic saline bath, temperature controlled at 37°C for 5 minutes before testing (to allow for temperature stabilization), as well as during testing (to eliminate any temperature-related variability). 1° Shear rate was fixed to 4.16 μm/s. The following data, time (t), x and y size (μm), x and y displacement (μm), x and y force (mN), and x and y strain (unitless), were recorded every second. An integrated CCD camera captured still images at 1280 × 960 pixel resolution, analyzed by custom software (Labphy v. 9.05, CellScale Biomaterials Testing).

Linear regression fitting using Microsoft Excel 2013 (version 15.0, Microsoft, Redmont, WA) was applied on the linear section of the loading curve to calculate, by means of the slope function (gradient), the stress-strain ratio, an expression of the Young modulus E. To ensure proper linear relationship fit, a minimum value coefficient of determination (R²) of the trend line of 0.98 was sought. Stress at 10% and 20% strain was recorded. Young shear modulus E was calculated as the gradient at the vicinity of 10% and 20% strain at the stress-strain graph. The following definitions have been adopted: stress, a pressure metric. As the applied force F divided by cross-section A of the corneal specimen test area (F/A, units = mN/mm² = kPa = 10⁻³ Pa; MPa = 10⁶ Pa, where Pa is Pascal, SI unit for pressure), and strain as the unitless relative elongation (Δ/Δl), expressed as percentage. Cross-section of the test area was defined by 3.5-mm x (or y, respectively) width multiplied by the corneal thickness.
fluence of 5.4 μm, aiming to address inherently biomechanically weaker thin corneas suffering from kerat...n studies indicate approximately one-third epithelial UV-A absorption, whereas other studies suggest that human corneal epithelium and the underlying basement membrane absorb strongly only at wavelengths less than 310 nm. The efficacy of multiple epithelial-on-CXL techniques may warrant standardization and extensive investi...Ex vivo evaluation of in situ (through a stromal pocket) CXL in porcine corneas (which do not have Bowman layer) indicates that the biomechanical strengthening is reduced by approximately 50%, in comparison with standard protocol CXL. There is thus inconclusive evidence in the peer-reviewed literature on the aspect of efficacy of some epithelial-on-CXL variations.

The in situ riboflavin application naturally overcomes the first of the two obstacles, related to riboflavin penetration through the intact epithelium and in acting as a “blocking” agent against UV-A propagation. This is because the epithelium and Bowman layer, that the UV-A light has to be transmitted, are not soaked in riboflavin. In this study, we conducted an ex vivo investigation to biomechanically address this issue, as in vivo biomechanical measurements in our experience, have shown less specificity and sensitivity. We investigated the effect on corneal biomechanical properties by using objective biaxial stress–strain measurements. This technique may be superior to corneal strip extension studies in earlier experiments, considering the nonuniform topographic distribution of the corneal strength profile due to collagen anisotropy. To address the fact that corneal stromal modulus naturally varies among different corneal meridians, the specimens were also randomly oriented during testing. It is known that the cornea is stronger at the anterior third. This transverse depth dependence suggests that tissue removal from the upper third may affect corneal rigidity the most. In our study, we demonstrated that the effective corneal rigidity increased for cross-linked corneas was of the order of 100% at the 10% strain point, in comparison with the non-CXL corneas that received the same treatment. This is an important finding, considering that the anterior stromal tissue was identified to provide corneal stiffening to compensate for stromal weakening due to anterior stromal tissue removal, and thus to offer long-term corneal stability. The data in this study provide substantial ex vivo evidence that significant stromal strengthening may occur even when UV-A is projected through the intact corneal epithelium, Bowman membrane, and superficial stroma, to reach the underlying riboflavin-soaked stroma. These findings support our previous clinical investigation conducted in vivo of refractive stability in high myopic LASIK cases treated with prophylactic CXL, as well as the compelling steepening refraction effect stabilization in clinical hyperopic LASIK cases treated with prophylactic CXL.

Recent advances in variable flaps, customized pattern CXL may add another element to potential applications of this proposed technique: “hypercyc” style pattern in situ CXL may be combined to enhance cornea aphakia, overcorrect, and even address future presbyopia in myopic refractive lens extraction.

**CONCLUSIONS**

Adjunct intrastromal cross-linking may be a valuable biomechanical modulator providing titratable increased rigidity in corneas undergoing intrastral tissue removal such as femto-LASIK, myopic LASEK, or radial keratotomy.

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Clinical Ophthalmology

Comparison of prophylactic higher fluence corneal cross-linking to control, in myopic LASIK, one year results

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Purpose: To compare 1-year results: safety, efficacy, refractive, keratometric stability, of femtosecond myopic laser-assisted in situ keratomileusis (LASIK) with and without concurrent prophylactic high-fluence cross-linking (CXL) (LASIK-CXL).

Methods: We studied a total of 155 consecutive eyes planned for LASIK myopic correction. Group A represented 73 eyes that were treated additionally with concurrent prophylactic high-fluence CXL, group B included 82 eyes subjected to the stand-alone LASIK procedure. The following parameters were evaluated preoperatively and up to 1-year postoperatively: manifest refractive spherical equivalent (MRSE), refractive astigmatism, visual acuity, corneal keratometry, and endothelial cell counts. We plotted keratometry measurements postoperatively and its changes in the early, interim and later post-operative time for the two groups, as a means of keratometric stability comparison.

Results: Group A (LASIK-CXL) had an average postoperative MRSE of −0.25, −0.19, and −0.19 D for the 3-, 6-, and 12-month period, respectively, compared to −5.81 ± 0.98 D preoperatively. Flat keratometry was 37.69, 37.66, and 37.67 D, compared to 43.94 D preoperatively, and steep keratometry was 38.35, 38.36, and 38.37 D, compared to 45.17 D preoperatively.

Conclusion: Application of prophylactic CXL concurrently with myopic LASIK surgery appears to contribute to improved refractive and keratometric stability compared to standard LASIK. The procedure appears safe and provides a new potential for LASIK correction.

Keywords: myopic LASIK regression, femtosecond myopic LASIK, LASIK-CXL, high myopia, accelerated high-fluence collinear cross-linking

Introduction

Laser-assisted in situ keratomileusis (LASIK) is the most common form of refractive surgery,1 offering predictable and stable refractive and visual outcomes.2 Specifically, in correcting moderate to high myopia (equal or more than −6.00 D in the least minus meridian),2,3,4 there have been reports in the past indicating significant long-term regression.5,6 The work by Alió et al7 reported that 20.8% of high myopia cases required...
retreatment because of over- or undercorrection, or regression. Other studies have shown that the risk of regression may be between 5% and 27%.15 Our team’s experience with high myopia LASIK correction suggests a slight (0.5 D) long-term postoperative corneal thinning trend.16 This was the motivation behind attempting to apply prophylactic in situ cross-linking (CXL) on the stromal bed, concurrently with the LASIK procedure, particularly in high-myopic eyes with thin residual stroma and in younger patients who may not yet have exhibited ectasia risk factors.17,18 The application aims to enhance corneal rigidity and thus reduce the possibility of long-term myopic shift.19

This study aimed to investigate potential differences in safety and efficacy, as well as in the refractive and keratometric results of myopic LASIK utilizing the WaveLight® FS200 Femtosecond Laser (Alcon Laboratories Inc, Fort Worth, TX, USA) and the WaveLight® EX500 Excimer Laser (Alcon Laboratories Inc) refractive surgery platforms. The study evaluated 1-year refractive and keratometric results from two groups, a “LASIK-CXL” and a stand-alone “LASIK” group, in which no concurrent CXL was applied.

Materials and methods

This prospective, observational, longitudinal study received approval by the Ethics Committee of LaserVision.gr Eye Institute and adhered to the tenets of the Declaration of Helsinki. Informed written consent was provided and documented from each subject at the time of the first study visit. The inclusion criteria comprised: no other previous ocular surgery, documented refractive stability for at least 3 months, no previous ocular disease, eyes with history of corneal dystrophy or herpetic eye disease, topographic evidence of ectatic corneal disorder, epithelial warpage from contact lens use, corneal scarring, glaucoma, severe dry eye, and collagen vascular disease. As per our clinical protocol for the last 6 years, in this study, the LASIK treatments for myopia were allocated to include LASIK-CXL if the patient had either of the following preoperative measurements: mean manifest refraction spherical equivalent (MRSE) over −5.00 D or keratometric astigmatism over 1.50 D on Scheimpflug-derived simulated keratometry, or predicted residual postoperative stromal thickness less than 330 μm. No similar restrictions were applied in the inclusion criteria for the standard LASIK group B, other than than applying it for myopia not exceeding −10.00 D.

Surgical technique

In both groups, the FS200 Femtosecond Laser was employed to provide a corneal flap of 110 μm thickness and 8.00 mm diameter. The average pulse energy for the flap bed cut was 0.8 μJ, the side cut angle was 70°, and the hinge position was superior. For the bed cut, the spot and line separations were 8 μm. For the side cut, the spot separation was 5 μm and line separation 3 μm. The myopia ablation (6 mm to 6.50 mm ablation zone diameter) treatment was accomplished with the EX500 Excimer Laser. Specifically for the LASIK-CXL, after the excimer laser ablation, and with the flap folded onto itself and protected with a dry Wexel sponge, one drop of Vibex Rapid™ (Avedro, Inc, Waltham, MA, USA), consisting of 0.1% sodium riboflavin (a very slightly hypotonic solution, mixed with hydroxypropyl methylcellulose [HPMC], a dextran substitute), was placed on the exposed stromal bed afforded by the open LASIK flap and carefully spread over the bed area with an irrigating cannula for 60 seconds. It is important to avoid riboflavin immersion of the flap and its hinge – for this purpose, the flap was protected, while remaining in a folded shape, as indicated in our earlier work.14 The reason for this is to inhibit flap collagen CXL. However, a small amount of riboflavin absorption, and thus CXL, will inevitably occur as a result of osmotic during the (however short) ultrafast A (UVa) exposure as the flap is in contact with the riboflavin-soaked stroma. One has to consider the following aspects: a riboflavin-presoaked flap will participate strongly in the UV A absorption (as it precedes the residual stroma along the illumination propagation path), however, it will not contribute any farther to the corneal biomechanical stability and may negatively affect the postoperative outcome, given that a 110 μm thick flap has perhaps only 40 μm stromal (collagen) content. The application of CXL to such a thin stromal layer may lead to undesirable flap shrinking. Regarding collateral benefits, a “CXL” flap–stromal interface might positively affect flap adherence.20

Following stromal soaking, the flap was properly repositioned into place and the residual riboflavin-irrigated; then a UVA fluence of 30 mW/cm² was applied for 80 seconds (total energy 2.4 J/cm²) provided by the KXL-CXL system (Avedro, Inc).

The selection of the UVR irradiation parameters (fluence and exposure time) was influenced by the following considerations: (a) provision of about half of the full “treatment” energy in comparison with the traditional CXL protocol, and (b) minimization of UVA exposure in order to constrain CXL within the overlying flap, and (c) minimization of flap dehiscence and possible shrinkage. The superficial application of UVA following the in situ installation of riboflavin was selected taking into account the following aspects:

- Application of CXL to the underlying stroma increases flap dehiscence and potential predisposition for fragility.
- CXL through the repositioned flap results in some riboflavin reflux in the dehydrated flap and CXL of the inner-flap collagen and the surface underlying the stroma. This may increase flap–underlying stroma adherence and additionally, potentially reduce or eliminate the inadvertent space created between them (which, in postmortem standard LASIK histopathology, has been shown to be filled with amorphous deposits).
- CXL has well-known disinfection, if not antimicrobial activity, thus, conducting the CXL through a repositioned flap reduces the chance of flap contamination by airborne microorganisms or fomites in the operating room environment, and/or acts as an adjunct disinfectant of the LASIK procedure.

Common to both groups, to avoid any risk of same-day accidental flap contact or rubbing, a bandage planar contact lens was then placed on the ocular surface, to be removed the following day. All patients were treated with moxifloxacin (Vigamox; Alcon Laboratories Inc) and 0.8% sof rotiblavin (a very slightly hypotonic solution, mixed with hydroxypropyl methylcellulose [HPMC], a dextran substitute) on each eye. In addition, we measured endothelial cell counts preoperatively and 1-month postoperatively, employing noncontact specular microscopy (CF-709; Konan Medical, Irvine, CA, USA) and postoperative examinations were conducted at 1 day, 1 week, 3 months, 6 months, and up to 1 year. This report presents the refractive and keratometric data analysis from the 1-year follow-up visits. Data were processed using web-based ophthalmic outcome analysis software (IBRA; Zubischo GmbH, Oberhaelris, Switzerland). Descriptive statistics and analysis were performed using Minitab®

Data collection

All eyes were measured for uncorrected distance visual acuity (UDVA), best (spectacle) corrected distance visual acuity (CDVA), and MRSE via manifest refraction and autorefractometry measurements (Speedy-4, K-Mold Auto Refractometer/Keratometer; Nidek, Gamagori, Japan), visual acuity (Functional Vision Analyzer®), Stereo Optical Co Inc, Chicago, IL, USA), corneal topography, for steep and flat keratometry within the 3 mm radius, employing Placido topography (WaveLight® Allegro Topolyzer™; Vario), Alcon Laboratories Inc) and Scheimpflug imaging (WaveLight® OcuLMZ® II Diagnostiv Device; Alcon Laboratories Inc), and corneal thickness, employing the Ocularer II and optical coherence tomography (OCT) (RTVue-100; Optovue, Inc, Fremont, CA, USA). In addition to pachymetry, OCT was employed to obtain meridional scans – the hyperreflectivity lines are considered an indirect indication of CXL efficiency,20 as demonstrated by our group’s study, which showed CXL dimunation lines were evident as late as 3 years postoperatively.20 An example of an OCT meridional image scan of a treated cornea is provided in Figure 1 and shows an increased reflectivity of the upper stromal bed. This is supportive of our argument that CXL mainly affects the underlying stroma and not the superflacent flap.

The postoperative evaluation additionally included slitlamp examination, clinical evaluation of dry eye, indications of epithelial ingrowth,21 and corneal haze. In addition, we measured endothelial cell counts preoperatively and 1-month postoperatively, employing noncontact specular microscopy (CF-709; Konan Medical, Irvine, CA, USA) and postoperative examinations were conducted at 1 day, 1 week, 3 months, 6 months, and up to 1 year. This report presents the refractive and keratometric data analysis from the 1-year follow-up visits. Data were processed using web-based ophthalmic outcome analysis software (IBRA; Zubischo GmbH, Oberhaelris, Switzerland). Descriptive statistics and analysis were performed using Minitab®

Figure 1 Anterior segment optical coherence tomography diagonal cross-sectional (60 μm) image of an eye treated with LASIK-CXL for −2.25 D of sphere and −0.125 D of astigmatism, obtained 1-year postoperatively. Blue arrow indicates the LASIK flap, while yellow arrows indicate the stromal suprachoroidal line, which correlates with the depth of the prophylactic cross-linking effect.

Abbreviations: CXL, cross-linking; LASIK, laser-assisted in situ keratomileusis.
that in the LASIK-CXL group-A, 90.4% of the eyes had postoperative UDVA 20/20 (1.0 decimal) or better, and 94.3% had 20/25 (0.8 decimal) or better. In the stand-alone LASIK group, 85.4% of the eyes had postoperative UDVA better than 20/20 (1.0 decimal) and 89.0% had better than 20/25 (0.8 decimal). The differences between the two groups at the 20/20 and the 20/25 levels were statistically significant (P=0.042 and P=0.037, respectively).

Efficacy of CDVA

The gain–loss data (preoperative CDVA versus postoperative UDVA) (Figure 3) indicate that in the LASIK-CXL group, 35.6% of the eyes were unchanged, 56.2% gained one Snellen line, and 8.2% (six eyes) gained two more Snellen lines. No eye lost any line. In the stand-alone LASIK group, 37.8% of the eyes were unchanged, 56.1% gained one Snellen line, and 4.9% (four eyes) gained two or more lines. Only 1.2% (one eye) lost one line.

Refractive predictability and accuracy

The refractive prediction results are presented in the form of linear regression scatterplots (Figure 4), in which the vertical axis corresponds to the achieved MRSE, and the horizontal axis corresponds to the attempted MRSE. The data for the LASIK-CXL group had a coefficient of determination ($r^2$) of 0.979, while for the stand-alone group-B, this was 0.970. The postoperative MRSE refractive results are presented, within 0.50 D intervals, in Figure 5. In the LASIK-CXL group, MRSE refraction between $-0.50$ and $0.00$ D was achieved in 82.2% of the eyes, and in the stand-alone group, this was achieved in 81.7% (no statistically significant difference [P=0.079]).

Postoperative refractive astigmatism results, within intervals of 0.50 D representing the accuracy of the cylinder correction, are illustrated in Figure 6. The LASIK-CXL group had a mean preoperative cylinder of $-0.93±0.04$ D, while the stand-alone LASIK group had a mean preoperative cylinder of $-0.82±0.03$ D. The LASIK-CXL group had postoperatively, 90.4% of eyes with less than 0.25 D of refractive astigmatism, and mean cylinder of $-0.16±0.04$ D. The stand-alone LASIK group had 91.5% with less than 0.25 D of refractive astigmatism, and mean cylinder of $-0.15±0.04$ D.

Refractive and keratometric stability

Refractive stability was demonstrated by the MRSE correction, as followed during the 1-, 3-, 6-, and 12-month postoperative visits (Figure 7). The 1-year mean postoperative MRSE was $-0.19±0.17$ D in the LASIK-CXL group and $-0.27±0.23$ D in the stand-alone LASIK group. These findings indicate a reduced refractive shift in the LASIK-CXL group in comparison with the stand-alone group (P=0.063). The keratometric stability, demonstrated by the K-flat and K-cylinder average values up to the 1-year postoperative visit, is illustrated in Figure 8. The results indicate an increased keratometric stability in the LASIK-CXL group (1-year at $+0.03$ D in the flat and $+0.05$ D in the steep compared to the stand-alone LASIK group).

**Figure 2** Postoperative uncorrected distance visual acuity (line contacts) versus preoperative corrected distance visual acuity (Snellen columns) 1 year postoperatively, in (A) the LASIK-CXL group and (B) the stand-alone LASIK group.

**Abbreviations:** CDVA, corrected distance visual acuity; CIL, cross-linking; LASIK, laser-assisted in situ keratomileusis; UDVA, uncorrected distance visual acuity.

**Figure 3** Change in corrected visual acuity, as a percentage of eyes with gain/loss in Snellen lines of normalized distance visual acuity 1 year postoperatively.

**Abbreviations:** CDVA, corrected distance visual acuity; CIL, cross-linking; LASIK, laser-assisted in situ keratomileusis.

**Figure 4** Predictability of spherical equivalent correction, measured at 1-year postoperatively, showing achieved spherical equivalent (horizontal axis) versus attempted spherical equivalent (horizontal axis) in (A) the LASIK-CXL group and (B) the stand-alone LASIK group.

**Abbreviations:** CDVA, corrected distance visual acuity; Q, coefficient of determination; R², coefficient of determination; $R^2$ adjusted, coefficient of determination.
with the 1-month baseline) compared with the stand-alone LASIK group (p = 0.67 D and +0.55 D, respectively), which was statistically significant (p = 0.039).

**Discussion**

Improved diagnostics, ablation profiles, and laser-beam tracking refinements of the LASIK procedure,27 and improvements attributed to femtosecond laser-assisted flap creation28 all have contributed to an excellent track record in myopia correction. However, refractive regression in high myopic corrections remains a possibility. There are several possible mechanisms leading to post-LASIK regression. For example, a correlation between increased epithelial thickness and myopia correction up to 1-year postoperatively was noted, in a study by Spada et al29 in high-myopic myopes between –8.50 and –12.25 D patients. This epithelial thickening in high myopia has been corroborated recently in femtosecond LASIK correction of high myopia.6 Furthermore, a comparison of stand-alone LASIK to LASIK-CXL in high myopia verified that the observed post-LASIK epithelial thickening changes are significantly less prominent in LASIK-CXL cases. This difference may correlate with higher regression rates and/or may depict increased biomechanical instability in stand-alone LASIK.30 We believe that this finding is a manifestation of the same aspect, which is the distinct difference between the two groups – the application of prophylactic CXL in this group. As we have previously demonstrated,31 CXL affects epithelial thickness, leading to reduced overall thickness.

In the present study, we investigated up to 1-year postoperative refractive stability and results of 155 eyes subjected to femtosecond-laser LASIK for myopia between two groups – group A in which prophylactic high-fluence CXL was incorporated and group B that received stand-alone LASIK. The two groups in the study were by all other means matched: ablation zone, flap thickness, surgeon, lasers employed, and postoperative medication and treatment. Our research on this subject matter is ongoing, and we do hope to report long-term follow-up results in the future. The postoperative evaluation in the LASIK-CXL group did not indicate clinical or topographic evidence of complications in comparison with the stand-alone group. Visual rehabilitation between the two groups, as expressed by CDVA and contrast sensitivity evaluation, was at similar levels, without induction of side effects or compromise of visual safety. The refractive outcome, predictability, and stability were completely satisfactory, and in some cases superior to standard LASIK (for example, data shown in Figure 2, subgroup of achieved visual acuity of 20/16).

Comparison of the stability results between the two groups indicates that in the stand-alone group, there was a slight positive slope in the keratometric readings, both at the flat and the steep meridian, as illustrated in Figure 8, which is suggestive of a mild progressive corneal steepening. The recorded changes correspond to +0.67 D for the flat meridian and +0.54 D for the steep meridian. The data clearly show a trend toward mild corneal steepening in the long-term postoperative period. A similar refractive shift has been reported previously by our team in LASIK corrections of high myopia with no prophylactic CXL application.32

There was no such trend of keratometric shift in the LASIK-CXL group (+0.63 D and +0.65 D respectively). Other differences between the two groups were the slightly increased stability of the MRESE (Figure 7), as well as the improved predictability (Figure 4), despite the larger range of attempted correction and increased preoperative astigmatism (Figure 6). It is worth noting that the mean spherical error (S), as well as cylinder error (C) treated in group A (mean S = –6.62 D, maximum S = –11.50 D, mean C = –1.35 D, maximum C = –5.25 D) was significantly greater than in the LASIK stand-alone group (mean S = –5.65 D, maximum S = –9.50 D, mean C = –0.85 D, maximum C = –3.50 D). Despite the apparently more challenging cases included in group A (LASIK-CXL) compared to group B (Stand-alone LASIK), the refractive results in the LASIK-CXL group were equally good and, in some cases, slightly better.

One aspect that needs consideration is the possibility of refractive flattening as a result of the CXL applied. Our clinical experience, as well as the peer-review literature, suggests the continued progression of the CXL effect over time.33 We have indicated that the long-term keratometry flattening progression in the fully cross-linked corneas is of the order of +0.30 D. One has to acknowledge the following two parameters that differentiate this finding, when considering the LASIK-CXL:

- The keratoconus management cases were fundamentally unstable ectatic corneas, whereas in the present work only healthy corneas were included, and
- The keratoconus management cases received the “full energy” treatment (up to 6 J/cm²), whereas in the present work, the LASIK-CXL eyes received only a “partial energy” treatment (2.4 J/cm²), corresponding to less than half of the standard protocol energy.

It may be thus estimated that the possibility of long-term keratometric flattening may well be restricted. Additional long-term studies are required to investigate this aspect.

In view of the expressed skepticism by colleagues regarding possible regression of the refractive effect,34 sometimes, despite low preoperative risk (for example, classified as low...
Author contributions
AJK design and conduct of the study, and collection and interpretation of the data. AJK performed data management, and CK and GA analyzed the data. AJK, CK and GA prepared the manuscript and AJK, CK, and GA participated in manuscript review. All authors provided manuscript approval.

Disclosure
AJK has consulted or advisory positions with Alcon/WaveLight, Allergan, Avedro, and i-Optics. The other authors report no other conflicts of interest in this work.

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Article

Epithelial remodeling after partial topography-guided normalization and high-fluence short-duration crosslinking (Athens protocol): Results up to 1 year

Anastasios John Kanellopoulos, MD, George Asimellis, PhD

PURPOSE: To compare epithelial remodeling in keratoconic eyes that had photorefractive keratectomy and corneal collagen crosslinking (Athens protocol) with that in untreated keratoconic eyes and healthy eyes.

SETTING: Private clinical practice, Athens, Greece.

DESIGN: Comparative case series.

METHODS: Fourier-domain anterior segment optical coherence tomography (AS-OCT) was used to obtain in vivo 3-dimensional epithelial thickness maps and center, superior, inferior, maximum, minimum, mean, midperipheral, and variability data.

RESULTS: Group A comprised 175 keratoconic eyes (Athens protocol); Group B, 193 untreated keratoconic eyes; and Group C, 169 healthy eyes. The 1-year mean center epithelial thickness in Group A was 47.78 μm ± 7.36 μm (SD range 33 to 64 μm). At the first clinical visit, it was 52.09 ± 6.80 μm (range 36 to 72 μm) in Group B and 52.54 ± 3.23 μm (range 45 to 59 μm) in Group C. The mean thickness range in Group A at 1 year was −19.84 ± 7.21 μm (range −6 to −34 μm). It was −21.83 ± 12.07 μm (range −4 to −46 μm) in Group B and −6.68 ± 3.33 μm (range −3 to −29 μm) in Group C. The mean topographic thickness variability in Group A at 1 year was 4.64 ± 1.63 μm (range 1.6 to 8.1 μm) (P<0.05). It was 5.77 ± 3.39 μm (range 1.3 to 17.8 μm) in Group B and 1.59 ± 0.79 μm (range 0.6 to 5.6 μm) in Group C.

CONCLUSION: Anterior segment OCT indicated a thinner and more homogeneous remodeled epithelium in the keratoconic eyes treated using the Athens protocol.

Financial Disclosure: Dr. Kanellopoulos is a consultant to Alcon Surgical, Inc.; Wavelight Laser Technologie AG; Avedro, Inc.; and i-Optics Optikgerate GmbH. Dr. Asimellis has no financial or proprietary interest in any material or method mentioned.


We previously reported overall reduced corneal epithelial thickness in keratoconic eyes that were treated with (1) excimer laser debridement of the top 50 μm of the epithelium, (2) partial topography-guided excimer ablation, and (3) immediate high-fluence ultraviolet-A radiation (10 mW/cm²) and short-duration (10 minutes) corneal collagen crosslinking (CXL) with riboflavin in a procedure known as the Athens protocol.1-3 Our goal was to arrest the keratectasia progression4 and provide a less irregular anterior corneal surface. In this study,1 which was performed using high-frequency scanning ultrasound biomicroscopy (UBM), the epithelial thickness in a group of untreated keratoconic eyes was compared with that in a group of keratoconic eyes treated using the Athens protocol.

Epithelial thickness assessment has been facilitated by the development of anterior segment optical coherence tomography (AS-OCT).5 Although there are studies of AS-OCT epithelial measurement in the peer-reviewed literature, until recently and to our knowledge, the methodology and instrumentation mainly used an on-screen caliper tool; thus, only focal point-thickness measurements were reported. The recent availability of in vivo, 3-dimensional (3-D) corneal epithelial mapping by AS-OCT in clinical practice6-8 allows easy capture of optical images and high-speed measurements conferred by Fourier-domain signal processing.9

This study used this new clinical modality to evaluate the longitudinal postoperative changes in epithelial thickness distribution as well as the epithelial layer topographic variability in a large group of keratoconic cases treated using the Athens protocol. The results in these eyes were compared with those in untreated keratoconic eyes and in healthy control eyes.

PATIENTS AND METHODS

This observational comparative prospective study received approval by the Ethics Committee, LaserVision.gr Institute, and adhered to the tenets of the Declaration of Helsinki. All patients provided informed written consent at the time of the first clinical visit. Exclusion criteria were systemic disease, previous corneal surgery, history of chemical injury or delayed epithelial healing, and pregnancy or lactation.

Patient Enrollment and Surgical Technique

Group A In Group A, eyes were treated for keratoconus with the Athens protocol. All procedures were performed by the same surgeon (A.J.K.) using an EX500 excimer laser7 (Alcon Surgical, Inc.) with topography-guided custom partial ablation. Figure 1 shows an example of treatment planning, the distribution of the ablation depth, a preoperative axial curvature map, a postoperative axial curvature map, and the difference in axial curvature map between preoperatively and postoperatively. Immediately after surface normalization, accelerated CXL was applied using the KXL System (Avedro, Inc.). The patients were followed up to 1 year.

Group B Group B comprised eyes with keratoconus that had not received surgical treatment. Inclusion criteria were a clinical diagnosis of progressive keratoconus (confirmed by a complete ophthalmologic evaluation), minimum age 17 years, and corneal thickness of at least 300 μm. The keratoconus diagnosis was further confirmed using the WaveLight Ocuzyler II (Alcon Surgical, Inc.) and the Pentacam high-resolution Scheimpflug imaging camera6 (Oculus Optikgerate GmbH).

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Imaging Instrumentation

The RTVue-100 Fourier-domain AS-OCT system (Optovue, Inc.), running on analysis and report software version A6 (9.0.27), was used in the study. Data output included total corneal and epithelial thickness maps corresponding to a 6.0 mm diameter area. In all cases, to avoid potential artifacts (eg, due to eyelid instillation), OCT imaging preceded the ocular clinical examination and was performed by the same trained investigator. The settings were as follows: L-Cam lens and 8 radial meridional B-scans per acquisition consisting of 1024 A-scan each with a 5 μm axial resolution. These 8 radial meridional scans, all acquired in less than 0.5 second, were used by the system software to produce by interpolation 3-D thickness maps. Images with quality greater than 30, determined using the signal strength index parameter, were considered in the study. The signal strength index parameter measures the average signal strength across the scan. Two consecutive individual acquisitions were obtained in each eye (eye) to ensure data validity; the mean value of 2 was used in this study.

Data Collection and Statistical Analysis

In Group A, the postoperative measurements were performed at 1 and 6 months as well as at 1 year. Imaging in
Figure 2. Comparative AS-OCT epithelial thickness (µm): 3-D maps show an image from Group A taken 1 year postoperatively and an image from Group B (untreated keratoconic) comprised 193 eyes, 92 of women and 101 of men. The mean patient age at the time of surgery was 26.9 years ± 7.2 (SD) (range 18 to 48 years). There were 97 right eyes and 98 left eyes. The Athens protocol treatment was uneventful in all cases.

Group A (Athens protocol) comprised 193 eyes, 92 of women and 101 of men. The mean patient age at the time of examination was 31.1 ± 9.9 years (range 18.0 to 51.0 years). There were 95 right eyes and 102 left eyes.

Group C (control) comprised 160 eyes, 67 of women and 93 of men. The mean patient age at the time of examination was 35.45 ± 9.55 years (range 18.0 to 52.0 years). There were 74 right eyes and 86 left eyes.

Epithelial Thickness

In Group A, the difference in the center epithelial thickness between each postoperative timepoint was statistically significant (all P < 0.05). The difference in the mean center epithelial thickness (–4.31 µm; 95% confidence interval [CI], –6.31 to –2.30) between Group A 1 year after treatment and Group B at the time of examination was statistically significant (P < 0.05, 2-sample t test). The difference in the mean center epithelial thickness (–4.75 µm, 95% CI, –6.59 to –2.92) between Group A 1 year after treatment and Group C at the time of examination was also statistically significant (P < 0.05) (Figure 3).

In Group A, the difference in topographic thickness variability between each postoperative timepoint was statistically significant (all P < 0.05). Figure 4 shows the epithelial thickness variability and range by group.

DISCUSSIONS

Until recently, high-frequency UBM had been the gold standard for in vivo corneal epithelial 3-D imaging. 7 The recent, rapid development and current high-speed imaging capabilities of AS-OCT 8–10 have made acquisition of in vivo 3-D pachymetry corneal maps reliable and fast. 11,12 Software refinement also enables clinical assessment of corneal asymmetry and focal thinning parameters for keratoconus classification. 13 In addition, the higher axial resolution increased accuracy, and finer image-processing capabilities of the current AS-OCT imaging systems have enabled, among other things, 3-D imaging of epithelial thickness. 7

Epithelial thickness and irregularity indices (eg, center and mean epithelial thickness, epithelial thickness topographic irregularity, and epithelial thickness range measured quantitatively with AS-OCT can serve as possible indicators of cornea instability, including ex- tasia and keratoconus. 14 In this study, we evaluated these parameters with a Fourier-domain AS-OCT system in a large group of keratoconic patients who had combined treatment of excimer laser anterior surface normalization and simultaneous high-fluence accelerated CXL. This study adds new information based on the large group of treated keratoconic eyes and its comparison with untreated keratoconic eyes and healthy eyes. In addition, our study was performed with a commercially available AS-OCT system whose use may become more widespread in clinical settings. Our findings confirm compensatory epithelial thickness changes previously described after various refractive corneal ablation procedures. 15,21,22

The epithelial thickness and irregularity assessment in the Athens protocol-treated Group A suggests short-term variability in corneal thickness distribution between the third month and the sixth month. Specifically, in our study, the corneal and epithelial thickness distributions were characterized by large deviations that gradually became less irregular. The mean SD at the center epithelial thickness of 7.36 µm at 1 month gradually decreased to 6.80 at 3 months and to 4.57 µm at 1 year. The SD in Group B (untreated keratoconic) and in Group C (control) was 6.79 µm and 3.25 µm, respectively. In addition to fluctuating less between different eyes, the epithelial thickness distribution in Group A progressed toward a reduced mean topographic variability and mean thickness range (from 5.43 µm and 24.69 µm to 4.64 µm and 19.94 µm, respectively). These results indicate that the epithelial thickness distribution was more uniform in the Athens protocol-treated group than in the untreated group of keratoconic eyes and had less overall thickness range, as suggested by the reduced mean and center thickness values.
The findings in the current study agree with those in our previous study that, although an overall thicker epithelium with larger variations can be observed clinically and topographically in eyes with keratoconus, in eyes treated with CXL, the variability in epithelial thickness and topographic thickness decreased by a statistically significant margin and was more uniform.

In conclusion, we present the results in a comprehensive study of the postoperative development of corneal epithelial thickness distribution after keratoconus management using combined anterior corneal normalization by topographic-guided excimer ablation and accelerated CXL. The epithelial healing processes can be monitored by AS-OCT with ease in a clinical setting, expanding the clinical application of this technology. Our findings suggest less topographic variability and overall reduced epithelial thickness distribution in keratoconus eyes treated with CXL using the Athens protocol.

REFERENCES


OTHER CITED MATERIAL

Keratoconus Management: Long-Term Stability of Topography-Guided Normalization Combined With High-Fluence CXL Stabilization (The Athens Protocol)

Anastasios John Kanellopoulos, MD; George Asimellis, PhD

**ABSTRACT**

**PURPOSE:** To investigate refractive, topometric, pachymetric, and visual rehabilitation changes induced by anterior surface normalization for keratoconus by partial topography-guided excimer laser ablation in conjunction with accelerated, high-fluence cross-linking.

**METHODS:** Two hundred thirty-one keratoconic cases subjected to the Athens Protocol procedure were studied for visual acuity, keratometry, pachymetry, and anterior surface irregularity indices up to 3 years postoperatively by Scheimpflug imaging (Oculus Optikgeräte GmbH, Wetzlar, Germany).

**RESULTS:** Mean visual acuity changes at 3 years postoperatively were −0.38 ± 0.31 (range: −0.34 to +1.10) for uncorrected distance visual acuity and −0.20 ± 0.21 (range: −0.32 to +0.90) for corrected distance visual acuity. Mean K1 (flat meridian) keratometric values were 46.56 ± 3.83 diopters (D) (range: 39.75 to 58.30 D) preoperatively, 44.44 ± 3.97 D (range: 36.10 to 55.01 D) 1 month postoperatively, and 43.22 ± 3.80 D (range: 36.00 to 53.70 D) up to 3 years postoperatively. The average Index of Surface Variance (range: −0.27 to +0.90) was 0.18 ± 0.42 preoperatively, and 0.29 ± 0.55 (range: 0.19 to 0.49) 1 month postoperatively. The average Index of Surface Decentration and Index of Surface Decentration, calculated with Fourier analysis of topographic maps, were −0.07 ± 0.14 (range: −0.18 to +0.19) and −0.07 ± 0.14 (range: −0.18 to +0.19) 1 month postoperatively, respectively. These graphs (the 95% median confidence range plots, visual acuity gained or lost 3 years postoperatively) are indicative of the long-term stability of the procedure.

**CONCLUSIONS:** The Athens Protocol to arrest keratoconic progression and improve corneal regularity demonstrates safe and effective results as a keratoconus management option. Progressive potential for long-term flattening validates using caution in the surface normalization to avoid overcorrection.

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Dr. Kanellopoulos is a consultant for Alcon/WaveLight. The remaining author has no financial or proprietary interest in the materials presented herein.

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**Table 1**

**Visual Acuity Data (N = 231 Eyes)**

<table>
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<tr>
<th>Value</th>
<th>Preop 1 Month</th>
<th>Preop 3 Months</th>
<th>Preop 6 Months</th>
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<tr>
<td>5D (+)</td>
<td>±0.20</td>
<td>±0.27</td>
<td>±0.29</td>
<td>±0.29</td>
<td>±0.28</td>
<td>±0.28</td>
</tr>
<tr>
<td>Gain/loss</td>
<td>n/a</td>
<td>+0.23</td>
<td>+0.30</td>
<td>+0.36</td>
<td>+0.36</td>
<td>+0.39</td>
</tr>
<tr>
<td>CDVA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td>0.62</td>
<td>0.69</td>
<td>0.76</td>
<td>0.80</td>
<td>0.81</td>
<td>0.82</td>
</tr>
<tr>
<td>5D (+)</td>
<td>±0.23</td>
<td>±0.22</td>
<td>±0.20</td>
<td>±0.20</td>
<td>±0.19</td>
<td>±0.19</td>
</tr>
<tr>
<td>Gain/loss</td>
<td>n/a</td>
<td>−0.07</td>
<td>+0.14</td>
<td>−0.18</td>
<td>+0.19</td>
<td>−0.20</td>
</tr>
</tbody>
</table>

**Gain/loss** = preoperative − uncorrected distance visual acuity; 5D = standard deviation; n/a = not applicable; CDVA = corrected distance visual acuity.

Expressed as the difference of postoperative minus preoperative values (gain/loss). ĉ, N = number of eyes.
was ±0.20 preoperatively and ±0.28 postoperatively. Likewise, the standard deviation of CDVA was ±0.23 preoperatively and ±0.28 postoperatively.

We theorize that the reason for visual acuity in keratoconic cases having such large fluctuations (and often being unexpectedly good) can be attributed to a ‘multifocal’ and ‘soft’ (ie, adaptable) cornea, in addition to advanced neural processing in the individual visual system. However, these ‘advantages’ are essentially negated with CXL treatment, which stiffens the cornea. Over time, possibly due to further topography improvement and adaptation to the partially normalized cornea, a noteworthy improvement in visual acuity is observed.

The two anterior surface indices, Index of Surface Variance and Index of Height Decentration, less irregular surface, lower Index of Surface Variance, less steep and more cone less steep and more apex (front and back). These changes are therefore suggestive of keratometric improvement, in agreement with other smaller sample studies.27,31 Such changes in Index of Surface Variance and Index of Height Decentration have been reported only recently.28

The initial more ‘drastic’ change of the Index of Height Decentration can be justified by the chief objective of surface normalization, cone centering, which is noted even by the first month. The subsequent surface normalization, with gradual and continuous improvement toward the 3-year visit. These visual rehabilitation improvements appear to be superior to those reported in cases of simple CXL treatment.19,20 However, it is noted that the visual acuity presented in this table may be warranted. In addition, corneal biomechanical analysis and corneal volume studies may be necessary to further validate such findings.

We note, however, that CXL alone results not just in corneal reshaping, but also in stromal density and refractive index of Height Decentration (µm) as measured by the Scheimpflug imaging device (OCular II, WaveLight AG, Erlangen, Germany) (preoperatively) up to 3 years postoperatively. All units in keratometric diopters (D).

**Keratometric and Anterior Surface Indices Progression**

After the 1-month visit, keratometric values are reduced. This progressive potential for long-term flattening has been clinically observed in many cases over at least 10 years. Peer-reviewed reports on this matter have been rare and only recently.27-29

The two anterior surface indices, Index of Surface Variance and Index of Height Decentration, less irregular surface, lower Index of Surface Variance, less steep and more centra. These changes are therefore suggestive of corneal topography improvement, in agreement with other smaller sample studies.27,31 Such changes in Index of Surface Variance and Index of Height Decentration have been reported only recently.28

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Our study indicates a significant improvement in all parameters studied. The changes induced by the procedure indicate a consistent trend toward improved visual rehabilitation, corneal flattening (validating ectasia arrest), and anterior surface improvement. The Athens Protocol procedure demonstrates impressive refractive, keratometric, and topometric results. Progressive potential for long-term flattening documented.
in this study suggests employment of caution in the surface normalization process to avoid overcorrection.

REFERENCES

Purpose: The aim of this study was to evaluate the safety and efficacy of high-fluence collagen crosslinking (CXL) as a means of achieving increased corneal rigidity and reduced enzymatic digestion in the vehicle cornea of Boston keratoprosthesis (KPro) type 1.

Methods: Eleven consecutive patients fitted with a KPro (5 with a previous repeat cornea graft failure, 4 with ocular cicatricial pemphigoid, and 2 with chemical burn) underwent donor vehicle corneal pretreatment with high-fluence prophylactic CXL in a 2-step procedure. First, the donor cornea was crosslinked with an intrastromal riboflavin instillation through a femtosecond laser-created pocket. This was followed up with a superficial CXL treatment. On the completion of the CXL pretreatment, the donor cornea was trephined with the femtosecond laser, and the KPro was fitted onto the crosslinked donor cornea. Visual acuity, corneal surface, and donor vehicle cornea stability were evaluated. Follow-up evaluations were conducted over the next 9 years with a mean of 7.5 years.

Results: Mean uncorrected visual acuity improved from light perception to 20/60. One patient required a follow-up surgery, which the KPro was refitted. Pretreatment with intrastromal and superficial high-fluence CXL in the donor vehicle cornea that would be used in the KPro procedure was presented to them by the operating surgeon with a comprehensive explanation of the benefits and risks of this procedure and the potential endophthalmitis, which will signiﬁcantly increase the risk of potential endophthalmitis and may predispose prosthesis exposure and/or infection. We hypothesized that the application of a prophylactic crosslinking treatment on the donor vehicle cornea might help reduce the susceptibility of these corneas to enzymatic digestion and corneal infection. This work presents an evaluation of a longitudinal case series to study the advantages of using CXL as a prophylactic intervention to Boston KPro surgery.

Conclusions: Pretreatment with intrastromal and superficial high-fluence CXL in conjunction with Boston type 1 KPro seems to be a safe and effective adjunctive treatment for achieving increased vehicle donor cornea rigidity. Additionally, there is an increased resistance to enzymatic degradation. This application may serve to enhance the biomechanical stability and external disease resistance of the donor vehicle cornea in patients with advanced external disease.

Key Words: prophylactic pretreatment with collagen crosslinking, Boston keratoprosthesis type 1, Dohlman keratoprosthesis, severe external disease, ocular cicatricial pemphigoid, chemical burn, repeat corneal transplantation failure (Cornea 2014;33:914-918)

E xtreme external disease has been successfully treated over the last few decades with all-corrug cornea transplantation, relatively histocompatible limbal stem-cell transplantation, or keratoprosthesis (KPro). There are several KPro variations. Currently, the most prevalent in clinical practice are the Boston KPro, the AlphaK, 1 the odonto-KPro,2 the Fyodorov KPro,3 and the Keraclear implant KPro.4 Our team has introduced the concept of accelerated, high-fluence collagen crosslinking (CXL) in post-laser in situ keratomeunis (LASIK), 6 and its use of prophylactic CXL in routine LASIK, 7 in treatment of cornea ectasia,8 and in attemptng corneal deturgescence9 in bulbar keratopathy.10

In our 20 years of experience in using the Boston KPro, we encountered 2 significant complications (1) melts and (2) erosion of the donor and host cornea interface.11 When the latter occurs, there is an increase in the risk of developing infectious keratitis, which will significantly increase the risk of potential endophthalmitis11 and may predispose prosthesis exposure and/or infection. We hypothesized that the application of a prophylactic crosslinking treatment on the donor vehicle cornea might help reduce the susceptibility of these corneas to enzymatic digestion and corneal infection. This study describes the outcomes from 11 different cases belonging to 11 different patients with external disease and almost total visual disability who opted to undergo Boston KPro type 1 in conjunction with prophylactic high-fluence CXL in the donor vehicle cornea that would be used for their KPro surgery. All the patients had debilitating visual compromise. The most common preoperative diagnosis in this group was repeat cornea graft failure (5 patients) with at least 3 failed grafts in the past for each. The remaining patients were diagnosed with ocular cicatricial pemphigoid (4 patients) and chemical corneal trauma (2 patients).

Preoperatively and postoperatively, we evaluated uncorrected distance visual acuity (CDVA), best spectacle-corrected distance visual acuity (CDVA), subjective refraction, keratometry, and used anterior-segment optical coherence tomography (OCT).12 These examinations were performed to evaluate the integrity of the donor vehicle cornea on which the KPro was mounted, the host cornea, and the donor-host interface. Macula and optic nerve OCT screening was also performed to evaluate potential glaucoma damage and/or macular edema.

Surgical Technique

The donor cornea was mounted on an artificial chamber (Baron, Katena Products, Inc, Denville, N.J.). A deep (350–400 μm) lamellar central corneal pocket of an 8-mm diameter was created using a femtosecond laser (IntraLase FS60; AMO, Abbott Park, IL, in the first 4 cases, and the WaveLight FS200; AMO, Ft Worth, TX, in the subsequent 7 cases). This pocket was accessed from the anterior corneal surface through a 3-mm channel. After a blunt olive-tip cannula was placed into the pocket to facilitate injection, 0.1 mL of 0.1% rifabutin sodium phosphate solution was injected into the intrastromal pocket. Immediately after this, 4 minutes of 30 mW/cm² ultraviolet-A radiation for a total energy of 7.2 J/cm² was directed onto the donor vehicle cornea.

After completing this step, the corneal epithelium was scraped with a blunt crescent blade, and drops of 0.1% rifabutin sodium phosphate solution were administered every minute for a total of 10 minutes until the anterior donor cornea was briskly color yellow. The central CXL session was also performed with a 30-mW/cm² fluence for 4 minutes until a total of 7.2 mJ/cm² of energy was reached. Ultraviolet-A in all cases was administered by using the KXL crosslinking device (Avedro Inc, Waltham, MA).

The 3.3-mm trephination of the donor cornea button was performed with a femtosecond laser. Before mounting the KPro, the donor cornea was trephined on a disposable Barron donor block (9.00 mm). Then, the KPro was sutured in place with 16 interrupted 1.0 nylon sutures, and the wound was deemed watertight. After the surgery, all cases were fitted with an 18-mm-diameter bandage contact lens to reduce the friction between the eyelids and the operated ocular surface. All patients were treated postoperatively with a drop of ofloxacin and 50 mg/mL of a solution of vancomycin once a day. In the later 6 years, all patients were treated with amoxycillin 4 times a day and with vancomycin once as well. In addition, it should be noted that antifungal prophylaxis, 1 drop of natamycin (Natracyn, Alcon, Ireland), was administered weekly to all the patients.

At all visits, we performed the first postoperative day, every month for 6 months, and every 3 months thereafter to assess visual acuity and the condition of the external surface. Because the capability of assessing intracocular pressure was limited to finger tip application over the eyelids, meticulous optic nerve visualization every 3 months was used as a means to evaluate possible glaucomatous optic nerve damage.
RESULTS

The mean age of the patients was 67 ± 14 years. Six patients were female and 5 were male. The visual acuity assessed from preoperative light perception and/or hand motion showed a 6-month postoperative improvement. The average UDVA was 20/80 (range: 20/100–20/40), and the CDVA was 20/70 (range: 20/80–20/32). These patients are still being followed up. At least annually. During the long follow-up time that these patients have been continuously monitored (minimum 1 year, maximum 9 years), 2 of the patients required subsequent injection of intracameral and triamcinolone and bevacizumab injection (Avastin, Genentech/Roche, San Francisco, CA) because of cystoid macular edema. Additionally, 1 patient needed intravitreal aluminum doped laser intervention for a retinopathy inflammatory membrane that was quite dense and had resulted in a CDVA reduction from 20/100 to 20/400. After this procedure, the patient’s vision returned to 20/30.

![FIGURE 2. CXL of the cornea, the anterior part of the donor cornea after epithelial debridement, and installation of riboflavin solution with very high-fluence CXL. A, The first cross-linking session of the donor cornea through intact epithelium and riboflavin solution injected in the lamellar pocket with 30 mW/cm² for 4 minutes. B, Scraping the donor corneal epithelium with a crescent blade before soaking the stromal surface, in preparation for the second cross-linking session. C, Soaking the deepithelialized donor cornea with riboflavin solution as a preparation for the second cross-linking session.](image1)

![FIGURE 3. Donor Cornea after 2.8-mm central trephination and just before the peripheral 9.5-mm trephination.](image2)

![FIGURE 4. Four years postoperative anterior-segment OCT imaging of the patient who had received prophylactic CXL. Top panel, meridional scan at 9:00; bottom panel top view of the cornea showing the scan orientation.](image3)

![FIGURE 1 demonstrates the procedure. Figure 1A shows the FS200 femtosecond laser programming interface. Figure 1B is a screen capture of the 8-mm diameter, 400-nm-deep femtosecond laser-assisted pocket creation. Figure 1C, is a screen capture of the intrastromal infusion of 0.1% riboflavin solution with the olive-tip cannula. Figure 2 illustrates the CXL of the cornea, the anterior part of the donor cornea after epithelial debridement, and installation of riboflavin solution. Figure 3 shows the trephination of the crosslinked vehicle donor cornea in its center to fit the KPro. Figure 4 shows results from a case 4 years postoperatively. This patient had an explosion-related injury. The right eye had to be enucleated as a result. The left eye sustained a chemical injury, which resulted in the visual acuity being light perception and severe cicatral changes occurring in both the conjunctiva and cornea. KPro was deemed to be the optimal treatment option for this left eye. The patient’s vision improved from light perception to 20/40 UDVA in the first week postoperatively.](image4)

DISCUSSION

In our 20 years of experience in external disease and the use of the Boston KPro to address it, the main obstacles of prophylaxis and visual rehabilitation stability that we have encountered and reported (Peralta RJ, Kanellopoulos AJ. Boston keratoprosthesis: A long-term prospective clinical study. A Long-Term Prospective Clinical Study. Poster Presentation, ARVO Meeting, May 6–10, 2007, Fort Lauderdale, FL) have been intraocular pressure control, infection (which was attributed partly on cornea melt around the prosthesis), and intraocular inflammation. One of the 2 major difficulties in managing these patients has been antibiotic prophylaxis, because these eyes are especially susceptible to microbial infections.17,18 which, after the KPro surgery, almost invariably leads to endophthalmitis in a uveitic eye with very poor prognosis.19

The second significant postoperative management problem is donor vehicle cornea and/or host cornea melts.20,21 especially near the graft-host interface. Using antiprotozoal, such as oral tetraacycline-type medications and/or topical prostaglandin, may be an effective alternative.22

The decision to incorporate this adjunct prophylactic treatment in these very challenging cases was based on significant experience with CXL techniques. The long-term safety and efficacy results of this technique as noted above suggest that there may exist a very significant advantage for the long-term prognosis of the Boston KPro.

This work presents 11 cases treated with adjunct crosslinking. The relative rarity of the parameters that led to the decision for inclusion in this study leads us to believe that our cases represent 1 of the largest groups in the literature. Among the reasons for crosslinking was our observation that up to 50% of these patients suffer from cornea melting, which may increase their likelihood of developing infections. The lack of a control group is a significant flaw of this study; in our defense, the seriousness and urgency for treatment and our strong belief in the benefit of crosslinking were reasons why we did not include a control group.

Questions on whether crosslinked corneas allow effective topical antibiotic penetration and or the apoptosis of cornea keratocytes,23 which is a side effect of CXL, and their effect on long-term microbial host defenses, are not answered in this study. However, there remain important considerations.

There are recent reports indicating that crosslinked corneas do allow for effective antibiotic penetration within the corneal stroma,25 and it is possible that the donor vehicle cornea may have reduced antigenicity to the host’s immune mechanisms after crosslinking, because most of the keratocytes will be killed in the crosslinking process. This has been shown in basic science studies.26,27 Because we have incorporated this novel pretreatment technique in our KPro routine, larger-scale future studies may further validate these findings. In conclusion, the very high-fluence CXL of the donor vehicle cornea as a prophylaxis against corneal melt and extreme external disease may be an efficacious adjunct that helps to reduce the susceptibility of these corneas to enzymatic digestion and cornea infection.

REFERENCES

Novel myopic refractive correction with transepithelial very high-fluence collagen cross-linking applied in a customized pattern: early clinical results of a feasibility study

Anastasios John Kanellopoulos
LaserVision gr, Institute Athens, Greece, and New York Medical School, New York, NY, USA

Background: The purpose of this study is to report the safety and efficacy of a new application of collagen cross-linking using a novel device to achieve predictable refractive myopic changes in virgin corneas.

Methods: Four cases were treated with a novel device employing very high-fluence collagen cross-linking applied in a myopic pattern. Prior to treatment, riboflavin solution was applied to the intact epithelium. The collagen cross-linking device was then engaged for a total of 12 J/cm² cross-linking applied in a myopic pattern. Prior to treatment, riboflavin solution was applied to the anterior segment following Boston keratoprosthesis. A mean keratometric change was from 44.90 diopters to 43.46 diopters. There was no significant change in endothelial cell counts or corneal clarity. There was some mild change in epithelial thickness distribution, with the treated area showing a slight but homogeneous reduction in mean thickness from 52 μm to 44 μm.

Conclusion: This report describes the novel application of very high-fluence collagen cross-linking with a predictable well defined myopic refractive (flattening) corneal effect. This technique has the advantages of essentially no postoperative morbidity, immediate visual rehabilitation, and the potential for tapering until the desired result is achieved.

Keywords: myopia, refractive correction, high-fluence collagen cross-linking, clinical results

Introduction
Collagen cross-linking has been used for many years as a means of stabilizing cornea ectasia. Although a multitude of treatments and techniques are available, it has been well documented that the procedure almost invariably results in some central anterior keratometry changes. Several aspects of this theory need further investigation. Is it possible to achieve predictable refractive changes? Can this be achieved through an intact epithelium? Can the human cornea tolerate higher fluence of ultraviolet light? This paper describes the use of a novel...
device employing very high-fluence collagen cross-linking, applied with a customizable pattern, in order to achieve myopic refractive changes.

**Materials and methods**

The study included four partially sighted volunteers with vision less than 20/400 (count fingers and worse), due to end-stage wet age-related macular degeneration, myopic refraction from –3 to –6 diopters, and astigmatism from –0.25 to –2.5 diopters. None had previously undergone cornea surgery. All patients signed informed consent forms, and the study received approval from the ethics committee. All eyes were imaged using Placido disc corneal topography (Vario Topolyzer, OcuLab, Wetzlar, Germany), Scheimpflug topography (Pentacam®, Oculus), autorefracration and keratometry (Speedy-K®, Nikon, Tokyo, Japan), and anterior segment imaging and mapping by spectral domain anterior segment optical coherence tomography (RTVue-100; Optovue, Irvine, CA, USA). A multitude of reports have established the significant refactive changes that accompany classic collagen cross-linking14 utilizing the classic Dresden protocol (3 mW/cm² for 30 minutes), as well as collagen cross-linking utilizing higher fluence,3 and even cross-linking delivered in eyes that have had riboflavin placed within a femtosecond laser-created pocket or intrastromal corneal ring segments.15,16 Over the years, most clinicians have referred to this process as “flattening,” which has often been interpreted as “disease regression.” We have long

![Image](https://example.com/image1.png)

**Figure 1** Placido disc topography for patient 1 preoperatively (left) and 6 months postoperatively (right) depicting the significant and regular central corneal flattening effect.

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**Table 1** Preoperative and postoperative keratometry, flattening at 1 week, and keratometric flattening results at 6 months along with corneal endothelial cell counts and age of each subject.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Pre-K1 (D)</th>
<th>Pre-K2 (D)</th>
<th>Post-K1 (D)</th>
<th>Post-K2 (D)</th>
<th>1 w flat (D)</th>
<th>6 m flat (D)</th>
<th>Pre-ECC (counts/mm²)</th>
<th>Post-ECC (counts/mm²)</th>
<th>Age (years)</th>
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<tbody>
<tr>
<td>Patient 1</td>
<td>44.50</td>
<td>45.50</td>
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<td>43.30</td>
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<td>1.45</td>
<td>1.800</td>
<td>1.850</td>
<td>72</td>
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<td>44.25</td>
<td>45.50</td>
<td>43.75</td>
<td>44.50</td>
<td>1.75</td>
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<td>44.80</td>
<td>45.50</td>
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<td>1.650</td>
<td>1.650</td>
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<td>43.76</td>
<td>43.76</td>
<td>2.30</td>
<td>1.44</td>
<td>1.600</td>
<td>1.625</td>
<td>71.25</td>
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</table>

**Abbreviations:** OD, right eye; OD, right eye; Pre-K1, preoperative keratometry value at 1 week; Pre-K2, preoperative keratometry value at 6 months; Post-K1, postoperative keratometry value at 1 week; Post-K2, postoperative keratometry value at 6 months; Pre-ECC, preoperative endothelial cell count (cells/μm²); Post-ECC, postoperative endothelial cell count (cells/μm²); age, age of each patient studied at the start of the trial.
Although longer follow-up will be necessary to determine the long-term stability of the present data, these findings are compelling with regard to the correction of small myopic refractive errors of the cornea without incision or tissue removal in an incision-like fashion or other previously described thermal techniques combined with collagen cross-linking.15

This novel procedure appears to be extremely simple for both patient and surgeon, and is essentially performed through intact epithelium. The interim epithelial remodeling in the first 6 months, when compared with normal eyes,16 appears to flatten slightly in a homogeneous way (average 8 microns). It appears to require extremely minor postoperative lifestyle adjustment on the part of the patient, and essentially no pain or discomfort occurs, even within the first few hours following the procedure. The topographic changes seen on Placido and Scheimpflug imaging as well as on anterior segment optical coherence tomography are compelling evidence of the specificity and topography-guided precision of the KXL II device. This application may have greater efficacy in younger corneas, given that the corneas studied here were from older patients and possibly less sensitive to collagen cross-linking. In patients with early keratoconus where stabilization and correction of myopic refractive error is desired, the potential advantages of this procedure would be another possible future application. These cases were specifically chosen for an initial feasibility study that would establish the safety and possible efficacy of this procedure. We introduce herein the clinical use of ultra high fluence transpupillary CXL for refractive corneal change. The dose-effect relationship of this treatment is currently under investigation. Minimal patient discomfort compared to laser refractive surgery makes this procedure a possible future alternative for lower refractive errors. Hyperopic and toric corrections may be other novel applications currently being investigated. The ease of this procedure and the essentially zero associated morbidity it offers may also allow the possibility of titrating the effect over two or more treatments. Hyperopic and toric corrections may be other novel applications that could be attempted using this technology.

Conclusion

This paper introduces a novel technique based on refractive collagen cross-linking on virgin corneas as an alternative refractive correction technique for mild myopia. In the follow-up time evaluated, these patients showed an impressive and stable reduction in their myopia. This pilot work may represent a landmark study of a potentially revolutionary new refractive procedure.

Acknowledgment

The novel concept of refractive CXL named PXL was designed and implemented as concept and technology design by David Muller, PhD.

Disclosure

This work was presented in part at the International Society of Refractive Surgery subspecialty day, New Orleans, LA, USA, November 15–17, 2013. The author has no other conflicts of interest in this work.

References

Evaluation of Visual Acuity, Pacymetry and Anterior-Surface Irregularity in Keratoconus and Crosslinking Intervention Follow-up in 737 Cases

Anastasios John Kanellopoulos, Vasilliki Moustou, George Asimellis

ABSTRACT
Purpose: To investigate visual acuity, corneal pachymetry, and anterior-surface irregularity indices correlation with keratoconus severity in a very large pool of clinically-diagnosed untreated keratoconic eyes, and in keratoconic eyes subjected to cross-linking intervention.

Materials and methods: Total of 737 keratoconus (KCN) cases were evaluated. Group A was formed from 362 untreated keratoconic eyes, and group B from 375 keratoconic eyes subjected to partial normalization via topography-guided excimer laser ablation and high-fluence collagen crosslinking. A control group C of 145 healthy eyes was employed for comparison. We investigated distance visual acuity, uncorrected (UDVA), best-spectacle corrected (CDVA), and Scheimpflug-derived keratometry, pachymetry (central corneal thickness, CCT and thinnest, TCT), and two anterior-surface irregularity indices, the index of surface variance (ISV) and the index of height decentration (IHD). The correlations between these parameters vs topographic keratoconus classification (TKC) were investigated.

Results: Keratometry for group A was K1 (flat) 46.67 ± 3.80 D and K2 (steep) 50.76 ± 5.02 D; for group B K1 44.03 ± 3.64 D and K2 46.87 ± 4.61 D; for group C, K1 42.89 ± 1.45 D and K2 44.18 ± 1.88 D. Visual acuity for group A was UDVA 0.12 ± 0.18 DVA and CDVA 0.59 ± 0.25 (decimal), for group B, 0.51 ± 0.28 and 0.77 ± 0.22, and for group C, 0.81 ± 0.31 and 0.87 ± 0.12. Correlation between ISV and TCK (r²) was for group A 0.853, and for group B 0.886. Correlation between ISV and TKC was for group A r² = 0.731, and for group B r² = 0.777. The ROC analysis ‘area under the curve’ was for CDVA 0.550, TCT 0.596, ISV 0.876 and IHD 0.887.

Conclusion: Our study indicates that the traditionally employed metrics of visual acuity and corneal thickness may not be robust indicators nor provide accurate assessment on either keratoconus severity or postoperative evaluation. Two anterior surface irregularity indices, derived by Scheimpflug-imaging, ISV and IHD, may be more sensitive and specific tools.

Précis: Visual acuity, Scheimpflug-derived pachymetry and anterior-surface irregularity correlation to keratoconus severity in untreated cases (A), treated with crosslinking (B), and in a control group (C) reveals that visual acuity and pachymetry do not correlate well with keratoconus severity.

Keywords: Keratoconus, Scheimpflug-derived pachymetry, anterior-surface irregularity, keratoconus severity, keratoconus classification, Pentacam, Keratoconic Scheimpflug topometric indices, visual acuity, keratoconus, Grading anterior surface Pentacam indices, keratoconus, Astigmatism and Kruisimgadching, Corneal pachymetry, Receiver operating characteristic ROC analysis.


Source of support: Nil
Conflict of Interest: None declared

INTRODUCTION
Keratoconus (KCN), derived from the Greek words κερατός κονιός: cornea; κύμος: cone, meaning cone-shaped protrusion, is a corneal disorder, defined as a noninflammatory degenerative axial thinning of an ectatic cornea.1 Vision is affected by increased myopia due to the cone protrusion, and irregular astigmatism due to substantial corneal astigmatism.2,3

Our long clinical experience with keratoconic screening and rehabilitation34 indicates that neither corneal pachymetry nor visual acuity (uncorrected distance visual acuity, UDVA, and best-spectacle corrected distance visual acuity, CDVA) can be reliable indicators of ectasia and/or keratoconus progression assessment.5 One may expect that the presence of large amounts of corneal irregularities might hamper sufficient spectacle-correction of visual acuity. However, at least in our experience, often enough keratoconic patients present with surprisingly high CDVA, even near 20/20, despite severe topographic irregularity and/or pachymetric thinning present. This makes keratoconus diagnosis a difficult and potentially dangerous process, as most early, many advanced and even some severe cases can be missed with traditional screening methods.

We have also encountered cases with progressive keratoconus with no clinically significant reduction in visual acuity.

To the best of our knowledge, the subject of quantitative correlation of visual acuity with keratoconus grading67 has been reported only in very few post-review publications and/or pachymetric thinning present. This makes keratoconus diagnosis a difficult and potentially dangerous process, as most early, many advanced and even some severe cases can be missed with traditional screening methods.

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etasia severity classification, and clinical keratoconus management follow-up.

MATERIALS AND METHODS
This study received approval by the Ethics Committee of our Institution, according to the tenets of the Declaration of Helsinki. Informed consent was obtained from each subject at the time of the first clinical visit.

Patient Inclusion Criteria
A total of seven hundred thirty-seven (737) keratoconic eyes were evaluated, enrolled in the study over the course of the past 7 years. Each patient enrolled in the study was subjected to a complete ocular examination, including slit-lamp biomicroscopy for clinical signs of keratoconus.

Group A consisted of unoperated eyes clinically diagnosed with keratoconus. Mean age or patients in this group at the time of the examination was 30.3 ± 6.9 (19 to 55) years of age. In this ‘unoperated KCN’ group A, 362 different eyes were enrolled, of which 196 were right (OD) and 166 left (OS). Gender specifics were 124 eyes belonged to female patients, and 238 to male patients.

Exclusion criteria were a minimum age of 18 years and clinical diagnosis of keratoconus. Exclusion criteria were systemic disease, any previous corneal surgery, history of chemical injury or delayed epithelial healing, and pregnancy or lactation during the study (for the female patients).

Group B (AP-treated) was formed from keratoconic patients whose eyes received anterior surface normalization by partial topography-guided excimer ablation combined with an additional procedure we introduced and reported as the Athens Protocol.12,13 The same surgeon (AUK) performed the operations. Mean age or patients in this group, at the 6 months postoperative examination, was 31.2 ± 7.3 (20 to 57) years. In this ‘AP-treated KCN’ group, 375 different eyes were enrolled, of which 199 were right (OD) and 176 left (OS). 142 eyes belonged to female patients and 233 to male patients.

The noted preponderance in both groups toward male population is consistent with our clinical experience in male-female incidence in keratoconic patients, and keratoconus incidence large studies.14 Inclusion criteria for group B were uneventful Athens-protocol rehabilitation, and no other ocular complications.

The control group C (n = 145 different eyes, 75 right and 70 left, 83 belonging to male and 62 to female patients) consisted of unoperated, normal eyes with no current or past ocular pathology other than refractive error, no previous surgery and no present irritation or dry eye disorder, all confirmed by a complete ophthalmologic evaluation. Contact lens wearers were excluded from this group.

Imaging, Measurement and Analysis
In each case, clinical examination included monocular UDVA and subjective refraction and CDVA with the best spectacle refraction. Both UDVA and CDVA were measured in mesopic conditions.

Scheimpflug imaging was performed with the WaveLight OcuLyzer (WaveLight, Erlangen, Germany), a Pentacam (Oculus Optikgeräte GmbH, Wetzlar, Germany) Scheimpflug rotating camera.15,16 The device was calibrated according to manufacturer recommendations prior to undertaking the measurements. The measurements were obtained and processed via the Examination Software (Version 1.17v4). The default settings of twenty-five images per single acquisition was used. Scheimpflug imaging was conducted in order to provide anterior surface keratometry (K1 flat and K2 steep, measured, in keratometric diopters (D)), corneal pachymetry, (TCT, thinnest corneal thickness, measured in µm), and keratometric Amster & Krameich classification. The topographic keratoconus classification (TKC) scale with increasing severity, was: (-), K1, K1-1, K2, K3-3, K3-3, K3-4, and K4. Corneal surface irregularity was evaluated by two anterior-surface topometric indices, measured in the central 8 mm corneal zone. These indices were: the (an)index of surface variance (ISV), an expression of corneal surface curvature irregularity, expressing the standard deviation of the sagittal radius values from the mean; and the index of height decentration (HDC), calculated with Fourier analysis of corneal height data to quantify the degree of vertical cone decentration.17 The decentration is calculated on a ring of 3 mm radius.

For groups A and C, measurements from the most recent clinical visit has been included in the study. For group B, measurements from the closest to the one-year postoperative visit was considered. Linear regression analysis was performed to seek possible correlations. Descriptive and comparative statistics, analysis of variance between keratoconus TKC severity and regression analysis, and receiver operating characteristics (ROC) curve analysis were performed with statistic tools provided by MiniTab version 16.2.3 (MiniTab Ltd, Coventry, UK) and IBM SPSS Statistics version 21.0 (IBM Corporation, New York, NY).

RESULTS
Keratometric, Topometric, Pachymetric and Visual Acuity Results
As shown in Table 1, average keratometry for group A (unoperated KCN), K1 (flat) was 46.67 ± 3.80 D, and K2 (steep) 50.76 ± 5.02 D. For group B (AP treated) K1 was 44.03 ± 3.64 D and K2 46.87 ± 4.61 D, and for group C K1 was 42.89 ± 1.45 D and K2 was 44.18 ± 1.88 D.

Our analysis indicated that more than 95% of the sample population in group A (unoperated KCN eyes) had a steep meridian keratometry >46.025 D, consistent with the CLEK group standards.18

Corneal surface irregularity, as expressed by the indices ISV and ISH, was: for group A ISV 99.60 ± 43.28 and ISH 0.095 ± 0.052; for group B ISV 79.21 ± 36.58, and ISH 0.059 ± 0.037; and for group C ISV 31.83 ± 23.81 and ISH 0.031 ± 0.19.

Average thinnest corneal pachymetry for group A was 444.64 ± 37.14 µm, for group B 364.91 ± 61.51 µm, and for group C 525.15 ± 270.03 µm.

Visual acuity, as reported by the decimal expressions of UDVA and CDVA was, for group A, 0.12 ± 0.18 and 0.59 ± 0.25, for group B 0.31 ± 0.28 and 0.77 ± 0.22 and for group C 0.81 ± 0.31 and 0.87 ± 0.12.

Keratoconus Severity Grading
The histograms based on the Scheimpflug severity grading of each eye in seven alphanumerical TKC grades for groups A and B are presented in Figure 1. To facilitate statistical analysis we introduced a numeric conversion, that is grade (-) was set to 0, K1 to 1, K1-1 to 2, K2 to 3, K2-3 to 4, K3 to 5, K3-4 to 6 and K4 to 7. Based on this conversion, for group A average TKC grade was 3.81 ± 1.95 (the average was between K2 and K2-3, closer to the K2 grade), and for group B, average TKC grade was 3.39 ± 1.89, closer to the K2 grade. Group C, comprised of healthy, nonkeratoconic eyes, had average TKC (-).

Linear fit between Visual Acuity, Thinnest Pachymetry, Topometric TKC Grading
The linear fit between the various parameters studied (UDVA, CDVA, TCT, ISV and ISH) and the TKC severity grading was presented in the form of marginal plots (Figs 2 to 6) and the coefficients of determination (r²) are reported in Table 2. Figure 2 illustrates UDVA vs TKC grading for both groups, and Figure 3, CDVA vs TKC grading for both groups. Based on these graphs, and as reported in Table 2, the coefficient of determination (r²) was, for the group A, between UDVA and TCK and 0.617 and between CDVA and TCK, 0.292. Likewise, for the group B, between UDVA and TCK r² was 0.292 and between CDVA and TCK, 0.175.

The linear fit between thinnest cornea (TCT) and TKC grading is presented in Figure 4 for both groups. Based on these graphs, the coefficient of determination (r²) between TCT and TKC, was, for group A, 0.236 and for group B, 0.180.

The linear fit between the anterior-surface indices ISH and TKC grading is presented in Figures 5 and 6. Based on these graphs, the coefficient of determination (r²) between TCT and IKC was for group A, 0.853, and for group B, 0.886. Likewise, the coefficient of determination (r²) between IKC and TKC was for group A, 0.731 and for group B, 0.701 respectively.

Receiver Operating Characteristic Curve Analysis
Receiver operating characteristics (ROC) curve analysis, area under curve (area), standard error (Std. error),
Fig. 1: Histograms of keratoconus classification for the two groups under study. Left — group A, unoperated KCN eyes and, right — group B, Athens-protocol (AP) treated KCN eyes.

Table 2: Coefficient of determination ($r^2$) and Pearson correlation coefficient for the two groups in the study between UDVA and TKC, CDVA and TKC, TCT and TKC, ISV TKC, IHD and TKC

<table>
<thead>
<tr>
<th>Coefficient of determination ($r^2$)</th>
<th>Pearson correlation coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A, unoperated KCN eyes</td>
<td>0.071</td>
</tr>
<tr>
<td>Group B, AP-treated KCN eyes</td>
<td>0.263</td>
</tr>
<tr>
<td>Group A, unoperated KCN eyes</td>
<td>0.292</td>
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<tr>
<td>Group B, AP-treated KCN eyes</td>
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<td>Group A, unoperated KCN eyes</td>
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<tr>
<td>ISV vs TKC</td>
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<tr>
<td>Group A, unoperated KCN eyes</td>
<td>0.856</td>
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<tr>
<td>IHD vs TKC</td>
<td>0.731</td>
</tr>
<tr>
<td>Group A, unoperated KCN eyes</td>
<td>0.701</td>
</tr>
</tbody>
</table>

KCN: keratoconus; UDVA: uncorrected distance visual acuity (decimal); TKC: topographic keratoconus classification; CDVA: best-corrected distance visual acuity (units: decimal); TCT: thinnest corneal thickness (units: μm); ISV: index of surface variance; IHD: index of height decentration; AP: Athens-protocol

Fig. 2: Marginal plot of UDVA (expressed decimally) and TKC grading with overlying box plots showing mean levels and outliers. Left — group A, unoperated KCN eyes and, right — group B, Athens-protocol (AP) treated KCN eyes.

Fig. 3: Marginal plot of CDVA (expressed decimally) and TKC grading with overlying box plots showing mean levels and outliers. Left — group A, unoperated KCN eyes and, right — group B Athens-protocol (AP) treated KCN eyes.

Fig. 4: Marginal plot of TCT, thinnest corneal thickness (expressed in μm), and TKC grading with overlying box plots showing mean levels and outliers. Left — group A, unoperated KCN eyes and, right — group B, Athens-protocol (AP) treated KCN eyes.

Fig. 5: Marginal plot of ISV, index of surface variance, and TKC grading with overlying box plots showing mean levels and outliers. Left — group A, unoperated KCN eyes and, right — group B, Athens-protocol (AP) treated KCN eyes.

Evaluation of Visual Acuity, Pachymetry and Anterior-Surface Irregularity in Keratoconus and Crosslinking Intervention Follow-up
CDVA: best-spectacle corrected distance visual acuity; TCT: thinnest corneal thickness; ISV: index of surface variance; IHD: index of height decentration; Notes: (a) Under the nonparametric assumption (b) Null hypothesis: true area = 0.5

**DISCUSSION**

There have been several reports in the peer-review literature lately, regarding the keratocoma and keratoconus assessment[^1] and progression monitoring[^2],[^3] as well as postoperative follow-up due to various CXL interventions[^4]. The current options of the clinical investigator include quantitative evaluation of corneal morphologic parameters[^5] derived from topography or Scheimpflug tomography. The latter modality provides specific anterior-surface corneal irregularity indices developed for the grading and classification of keratoconus stages [...][^6]. The association of visual performance from optical quality metrics has been investigated in length for normal eyes and in highly aberrated eyes with keratoconus[^7].[^8]. Visual acuity, which is commonly measured in mesopic conditions, provides a high-contrast forced-choice test for establishing threshold values of visual performance, and it is highly sensitive to disturbances in the visual pathway, presenting challenges in the quantification.

To the best of our knowledge, we identified only two reports in this matter of correlation of the above Scheimpflug-derived indices with either best spectacle corrected distance visual acuity (CDVA[^9]) or with the severity of keratoconus classification[^10].

The assessment of keratoconus severity with visual function has yielded poor results in a number of front surface-derived parameters in keratoconic eyes. As indicated in results presented in[^11], for example, the average correlation coefficients (r) among CDVA and keratometric and anterior surface irregularity parameters were between 0.421 and 0.643, which, in turn, translate to coefficients of determination (R^2) 0.177 and 0.413. As noted in our results, the spread of CDVA measurements within the same ‘severity stage’, e.g. KC3, KC4-3 was found to be too large. The lower tier, as well as the upper end of either UDVA or CDVA values were fluctuating in several stages of TKC, from moderate (e.g. KC1 or lower) to severe (e.g. KC3 or higher), therefore lacking the continuum of measurements needed to provide a smooth gradation of the condition from low to severe stage. The correlation between CDVA and TCK (Table 2), had coefficients of determination 0.292 for the uncorrected CDVA eyes and 0.175 for the AP-treated CDVA eyes. The correlation between TCT and TCK was also poor (r^2 = 0.236 for the untreated TCK group A and 0.176 for the AP-treated TCK group B). These low coefficient of determination values indicate that visual acuity and/or corneal pachymetry may not be a dependable indicator of keratoconus severity and/or progression.

There are many possible reasons that may explain why visual performance is not well correlated to keratoconus. The large noted fluctuation of visual performance is partly determined by factors unrelated to corneal shape, such as tear film breakup, lenticular shape and opacities, and neurological factors (possible advanced neural processing development in the individual). The effects of optical aberrations on image formation are also very complex. A soft, keratoconic cornea may display ‘multifocality’, i.e. the cornea may be adaptable, which may further add variability in the measured visual acuity. Additionally, simple clinical reasons may exist as well, such as the fact that in clinical evaluation we refracet these young patients monocularly and thus allow them to tilt their head in many directions in order to benefit from the cornea multifocality, use significant accommodation and pinholing and well as squinting.

Likewise, corneal thickness has been suggested in our work as a poor indicator of keratoconus severity. Although it is true that keratoconus is a thinning disease, any individual thickness has large variance and poor sensitivity to distinguish keratoconus from normal corneas.

The data provided herein suggest that clinical assessment of keratoconus severity and/or progression based on visual acuity and/or thinnest pachymetry alone may be misleading. Moreover, the poor correlation found in the AP-treated group B indicates that visual acuity and corneal thickness also cannot be employed as specific disease staging markers in the postoperative assessment of interventions aiming to arrest the keratoconus progression such as cross-linking with riboflavin (CXL)[^12]. The possible advantages of a cornea ‘multifocality’ and ‘adaptation’ in an untreated keratoconic eye, are to a large degree compromised with a CXL procedure, since the cornea becomes stiff.

In this extremely large sample of patients evaluated, the compelling disease staging markers appear to be two anterior surface irregularity indices, namely the ISV and the IHD. This work establishes that a better approach may be the examination of quantitative indicators that reflect the anterior-surface variance across the cornea. These anterior shape-based indices provide positive results, and provide a quantitative tool for keratoconus classification and progression assessment. Specifically, the average coefficient of determination (r^2), as reported in Table 2, between ISV and the determined TKC keratoconus severity grade had an average of 0.793 for both keratoconic groups, and between IHD and TKC 0.716, respectively. In other words, our study indicates that there is a significant correlation (Table 2, Figs 5 and 6) between the two anterior-surface irregularity indices and keratoconus classification, which is within the same margins either the untreated keratoconic group A and the AP-treated group B.

These findings are also quantitatively supported by the receiver operating characteristics (ROC) analysis. Specifically, the area under the curve, indicative of the sensitivity of the index under study, as reported in Table 3, was found to be 0.55 for the CDVA, 0.596 for the TCT, and substantially larger for the ISV and IHD indices, whose respective values were 0.876 and 0.857, indicating that ISV and IHD are more sensitive indicators for keratoconus severity classification. In countries where keratoconus appears to be rampant -we estimate that in every 50 young adults has topographic signs of the disease- topography screening may be the most important public health diagnostic medical tool. With the time-proven disease course alteration by CXL and other technique introduced since, like the Athens Protocol, screening teenagers for KCN may prove a life changing medical assessment in regard to their visual function and adult life work and habitual opportunities.

**CONCLUSION**

Our study indicates that visual acuity and corneal thickness may be poor indicators for keratoconus severity grading and accurate assessment of postoperative assessment. The compelling disease staging markers appear to be anterior-surface irregularity indices derived by Scheimpflug imaging, namely the index of surface variance and the index of height
decentration, which appear to be more sensitive and specific tools to visual acuity or pachymetry in early diagnosis and possible progression in keratoconus and corneal ectasia. These indices may become a novel benchmark for future studies, and may aid in the development of new keratoconus diagnostic and follow-up criteria.

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