ESCRS, Copenhagen 2016

IC 54: Collagen Cross-Linking. Indications, Applications, results, complications and evolving technology

Senior Instructor: A. John Kanellopoulos, MD

Co-authors: B. Armstrong, MD  
R. Pineda, MD  
S. Jacob, MD  
D. Stulting, MD  
G. Pamel, MD

Course Description:

Synopsis: Didactic approach to the management of progressive cornea ectasia associated with keratoconus and refractive surgery. Several surgical treatment modalities utilized internationally will be presented, including: collagen cross-linking with ultraviolet radiation A in order to halt ectasia, combined in some cases with a customised excimer laser ablation to facilitate visual rehabilitation (as presented in previous ESCR S meetings by the author), these alternatives to Intracornea ring segment implantation, lamellar grafts as well as penetrating graft techniques will be analyzed. Surgical and medical treatment technique, indications, potential complications and their management as well as clinical experience pearls will be presented.

Objective:
The participants will share our vast experience in managing progressive keratoconus and post-LASIK ectasia in order to visually rehabilitate these patients. Pearls on indications, patient selection, surgical technique and complication management for safe and effective results will be presented and discussed with the participants.

Outline:

1-Keratoconus surgical management, Literature review:  
- Thermokeratoplasty  
- Lamellar grafts  
- INTACS  
- Penetrating keratoplasty  
- Cornea Collagen Cross-linking

2-Post-LASIK ectasia-surgical management, literature review:
- How to avoid risk factors
- Thermokeratoplasty
- Lamellar grafts
- INTACS
- Penetrating keratoplasty
- Cornea Collagen Cross-linking

3. Patient selection
   a) Indications
   b) Medical contraindications
   c) Pre-operative evaluation and refractive error

4. Stabilization of ectasia:
   - INTACS
   - Collagen cross-linking
   - Lamellar tissue support

5. Collagen cross-linking:
   - Energy source, luminance and duration
   - Protective riboflavin A
   - Corneal pachymetry issues
   - Topographic and elevation changes
   - Stabilization of ectasia
   - When is it best to intervene?
   - FDA issues

6. Customised enhancement techniques
   a) Wavefront-guided
   b) Topography-guided
   c) Asphericity adjustment

7. Microkeratome-assisted lamellar keratoplasty technique
   a) Basic principles
   b) Pre-operative evaluation parameters
   c) Surgical technique
   d) Possible complications and their management
   e) Clinical data and review of the literature

8. Penetrating keratoplasty considerations:
   a) Basic principles
   b) Pre-operative evaluation parameters
   c) Surgical technique
   d) Possible complications and their management
   e) Clinical data and review of the literature

9. Refractive surgery enhancements following these procedures

10. Surgery in action
    Step-by-step action on several procedures on tape, question-answer session and coverage of basic problem-shooting with the panelists
ABSTRACT

PURPOSE: To investigate preoperative and postoperative anterior and posterior keratometry and simulated corneal astigmatism in keratoconic eyes treated with collagen cross-linking combined with anterior surface refractive surgery, following treatment, they were assessed 1 year postoperatively.

RESULTS: Before treatment, average anterior keratometric value was 47.06 ± 6.02 diopters (D) for flat and 51.24 ± 6.75 D for steep. The posterior keratometric values were -6.70 ± 0.99 D (flat) and -7.67 ± 1.15 D (steep). Anterior astigmatism was on average with-the-rule (1.97 ± 6.21 D), whereas posterior astigmatism was against-the-rule (+1.53 ± 1.02 D). The posterior and anterior astigmatism were highly correlated (r = 0.830). After treatment, anterior keratometric values were 43.97 ± 5.81 D (flat) and 46.55 ± 6.82 D (steep). Posterior keratometric values were 6.58 ± 1.06 D (flat) and 7.19 ± 1.22 D (steep). Anterior astigmatism was on average with-the-rule (-1.56 ± 3.80 D), whereas posterior astigmatism was against-the-rule (+1.45 ± 1.29 D). The statistically significant P < .05 keratometric changes indicated anterior surface flattening (-3.09 ± 2.67 D flat and -4.19 ± 2.96 D steep). The posterior keratometric changes were not statistically significant (P > .05).

CONCLUSIONS: Before treatment, there was a strong correlation between posterior and anterior corneal astigmatism. After treatment, statistically significant anterior keratometric values flattened. The posterior surface keratometric values did not change statistically, and significant postoperative change was minimal. The anterior surface normalization significantly improved keratometric and astigmatic values.

PATIENTS AND METHODS

This study was performed in patients visiting our clinical practice and which received approval by the ethics committee of our institution and adhered to the tenets of the Declaration of Helsinki. Written informed consent was obtained from all patients at the time of the first clinical visit.

INCLUSION CRITERIA AND SURGICAL TECHNIQUE

The study group consisted of 267 eyes. Patients’ ages at the time of the screening ranged from 19 to 57 years. Each patient received a complete ophthalmic examination, including subjective refraction, visual acuity, and slit-lamp biomicroscopy for clinical signs of keratoconus. The Collaborative Longitudinal Evaluation of Keratoconus group.3 Exclusion criteria included systemic diseases, previous corneal surgery, history of chemical injury or delayed epithelial healing, and pregnancy or lactation (female patients) during the study.

The same cases received treatment with the Athens Protocol,2 which included excimer laser epithelial debridement (50 µm), partial (maximum ablation 90 µm) topography-guided excimer laser stromal ablation, and high-fluence ultraviolet-A irradiation (10 mW/cm²), accelerated (10%) CXL performed with the Avedro KXL System (Avedro, Inc., Waltham, MA). The Athens Protocol treatments employed the Ocularyzer II (WaveLight AG, Ehingen, Germany) for corneal topography imaging. The Ocularyzer II is a high-resolution Pentacam Scheimpflug imaging rotating camera (Oculus Optikgerate GmbH, Wetzlar, Germany) incorporated by a proprietary network in the WaveLight Refractive Suite (WaveLight AG) to provide corneal elevation data to the excimer laser, namely the Ex500 (Alcon Laboratories, Fort Worth, TX).

For a patient to be considered for the Athens Protocol procedure, the criteria included clinical diagnosis of progressive keratoconus, minimum age of 18 years, and 300-µm minimum corneal thickness. Further details and the protocol have been published elsewhere (1,2,13). In total, 267 patients were observed at 1 week and 1, 3, 6, and 12 months postoperatively, and on an annual basis thereafter. The casuall sample data reported in this study after treatment comprised measurements obtained at the 12-month postoperative visit.

IMAGING, MEASUREMENT, AND ANALYSIS

The Scheimpflug camera (Ocularyzer II) was regularly calibrated according to manufacturer recommendations. The measurements were obtained and processed via the Examination Software (Version 1.1747; WaveLight AG). The default settings and 25 images per single acquisition were implemented. Keratometric and simulated astigmatism data were obtained with the best fit toric ellipsoid reference surface.

Linear regression analysis was performed to seek possible correlations. Descriptive and comparative statistics and analysis of variance were performed with statistics tools provided by Minitab version 16.2.1 (Minitab Ltd., Coventry, UK) and Origin Lab version 9 (OriginLab Corp, Northampton, MA). A P value less than .05 was considered statistically significant.

RESULTS

The sample consisted of 267 eyes (82 female and 185 male). There was preponderance toward male gender, consistent with our clinical experience in male-to-female incidence in keratoconic patients19 and keratoconus incidence studies.19 Of the 267 eyes, 140 were right eyes and 127 were left eyes. The average age for all patients at the time of the procedure was 30.80 ± 7.25 years (range: 19 to 57 years). The average preoperative corrected distance visual acuity was 20/32, ranging from 20/200 to 20/20 (0.628 ± 0.241). One year postoperatively, average corrected distance visual acuity was 20/25, ranging from 20/100 to 20/16 (0.762 ± 0.225).

ANTERIOR AND POSTERIOR KERATOMETRY AND CORNEAL ASTIGMATISM BEFORE TREATMENT

Averaged standard deviation, and maximum and minimum anterior and posterior central corneal surface keratometric values before and after treatment with the Athens Protocol are reported in Table 1. The astigmatism sign is dependent on the flat meridian horizontal or vertical orientation: the sign is considered positive if the flat meridian orientation is between +45° and -135°, which is called “against-the-rule,” whereas it is considered negative if between -45° and +45°, which is called “with-the-rule.”

After treatment, the resulting anterior astigmatism was on average with-the-rule, whereas the posterior astigmatism was against-the-rule (Figure 1). Paired analysis of the differences regarding astigmatism change indicated a P value of 0.067 for the anterior surface and .398 for the posterior surface.

KERATOMETRIC CHANGES AND ANALYSIS

Figure 1 illustrates the correlation between posterior versus anterior astigmatism in the form of scatter and fitted line plots with 95% confidence intervals and 95% prediction intervals before and after treatment. Before treatment, the coefficient of determination (r²) was 0.839 with a P value less than .001. After
In diopters (D) versus anterior astigmatism (also expressed in D) with 95% differences regarding the flat anterior keratometric value less than .001 and the steep posterior keratometric values showed a postoperative flattening by -4.19 ± 2.96 D, or -8.19%, again statistically significant (P < .05). Our results indicate that this was due to the induced changes in the anterior surface. Specifically, there was a statistically significant reduction in the anterior keratometric values after the Athens Protocol procedure demonstrated by a flattening of -3.09 D for the flat and -4.19 D for the steep meridians, in agreement with other studies of keratoconic cases receiving CXL treatment. The observed changes in the anterior keratometric values were not statistically significant, and thus no measurable change in the posterior corneal surface could be established in our study.

The issue of posterior surface change following CXL, surface ablation, or a combination of both procedures has to be viewed with skepticism. We present these data with the reservation that measurements of the posterior surface might be influenced by the algorithms involving corneal thickness measurements, which, in the case of the densitometry principle-based imaging, might be influenced in the CXL-treated corneas. Scheimpflug imaging in its current form can only provide data for the posterior surface on clear, normal corneas. Any significant corneal irregularity or light conduction interference may bias the findings. Also possible is that the denser CXL effect observed with application of the Athens Protocol may mask posterior surface changes. Therefore, although we observed some posterior surface changes, as expected, due to the dramatic alteration of the anterior surface and corneal stromal changes, these appear to be non-significant in comparison to the anterior surface changes.

There is an obvious clinical dilemma in deciding to ablate thin corneas with ectasia. Traditional clinical experience in the pro-CXL era may consider this dangerous for the ectasia progression. We investigated the principle of posterior curvature stability in these eyes because of the critical factor of establishing the safety of ablating these corneas. As expected, the posterior surface keratometric values did not show statistically significant change after CXL. These data suggest that there is minimal change in the biomechanical behavior of the ectatic cornea after the Athens Protocol, despite the dramatic change in the anterior surface.

Before treatment the elevated anterior and posterior keratometric values were highly correlated. After treatment showed statistically significant anterior surface flattening, consistent with our previous studies. The posterior surface did not show a statistically significant change, validating the accomplished cornea stability despite the interventional thinning.

**TABLE 2**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>K1 Anterior</th>
<th>K2 Anterior</th>
<th>K1 Posterior</th>
<th>K2 Posterior</th>
</tr>
</thead>
<tbody>
<tr>
<td>Difference (%)</td>
<td>-0.05</td>
<td>-0.13</td>
<td>-1.75</td>
<td>-0.04</td>
</tr>
<tr>
<td>Average change</td>
<td>-3.09</td>
<td>-4.19</td>
<td>0.12</td>
<td>-0.02</td>
</tr>
<tr>
<td>SD</td>
<td>2.67</td>
<td>2.96</td>
<td>0.61</td>
<td>0.55</td>
</tr>
<tr>
<td>Min</td>
<td>3.01</td>
<td>1.70</td>
<td>2.60</td>
<td>2.50</td>
</tr>
<tr>
<td>Max</td>
<td>-17.30</td>
<td>-17.30</td>
<td>-1.50</td>
<td>-2.90</td>
</tr>
</tbody>
</table>

P<.01

**REFERENCES**


**DISCUSSION**

In this study, a rotating Scheimpflug camera was employed to measure both the anterior and posterior corneal curvature in a large number (267) of keratoconic cases, before and after (1 year postoperatively) a combined CXL and anterior surface excimer laser normalization. In the case of keratoconus, highly irregular keratometric values were present. For example, the simultaneous investigation of anterior and posterior corneal keratometric values has indicated statistically significant differences between normal and keratoconus-suspect eyes. In a study evaluating keratometric values in keratoconic compared to normal eyes, the mean value was 43.28 ± 1.17 D (range: 41.53 to 45.40 D) for normal eyes and 49.29 ± 4.37 D (range: 42.97 to 60.33 D) for keratoconic eyes. In our study, the average keratometric values before treatment were 47.06 ± 6.02 D (range: 33.7 to 78.5 D) for flat and 51.24 ± 6.75 D (range: 39.5 to 80.7 D) for steep. As shown in the corresponding box plots in Figures A-C (available in the online version of this article), 95% of the sample population had a steep keratometric value greater than 46.025 D, consistent with the Collaborative Longitudinal Evaluation of Keratoconus group standards.

In the current study, before treatment the results were characterized by a pattern of linear correlation between anterior and posterior astigmatism, as shown in the fitted line plot of Figure 1. The anterior astigmatism before treatment is normally with-the-rule and the posterior astigmatism is against-the-rule, with a linear fit coefficient of -0.2067 and a robust coefficient of determination (R2) of 0.839. Thus, posterior corneal keratometric values appeared in this large keratoconic population to be associated with the anterior keratometric values.

However, this pattern does not seem to be consistent after treatment (Figure 1). Although the ratio was similar (0.26), the coefficient of determination was considered poor (R2 = 0.405). Our results indicate that this was due to the induced changes in the anterior surface. Specifically, there was a statistically significant reduction in the anterior keratometric values after the Athens Protocol procedure demonstrated by a flattening of -3.09 D for the flat and -4.19 D for the steep meridians, in agreement with other studies of keratoconic cases receiving CXL treatment. The observed changes in the posterior keratometric values were not statistically significant, and thus no measurable change in the posterior corneal surface could be established in our study.

The issue of posterior surface change following CXL, surface ablation, or a combination of both procedures has to be viewed with skepticism. We present these data with the reservation that measurements of the posterior surface might be influenced by the algorithms involving corneal thickness measurements, which, in the case of the densitometry principle-based imaging, might be influenced in the CXL-treated corneas. Scheimpflug imaging in its current form can only provide data for the posterior surface on clear, normal corneas. Any significant corneal irregularity or light conduction interference may bias the findings. Also possible is that the denser CXL effect observed with application of the Athens Protocol may mask posterior surface changes. Therefore, although we observed some posterior surface changes, as expected, due to the dramatic alteration of the anterior surface and corneal stromal changes, these appear to be non-significant in comparison to the anterior surface changes.

There is an obvious clinical dilemma in deciding to ablate thin corneas with ectasia. Traditional clinical experience in the pro-CXL era may consider this dangerous for the ectasia progression. We investigated the principle of posterior curvature stability in these eyes because of a critical factor of establishing the safety of ablating these corneas. As expected, the posterior surface keratometric values did not show statistically significant change after CXL. These data suggest that there is minimal change in the biomechanical behavior of the ectatic cornea after the Athens Protocol, despite the dramatic change in the anterior surface.

Before treatment the elevated anterior and posterior keratometric values were highly correlated. After treatment showed statistically significant anterior surface flattening, consistent with our previous studies. The posterior surface did not show a statistically significant change, validating the accomplished cornea stability despite the interventional thinning.

**AUTHOR CONTRIBUTIONS**

Conception and design (AK); data collection (ABK); analysis and interpretation of data (AK, LK); writing the manuscript (GA); critical revision of the manuscript (ABK, GA).

**REFERENCES**


Figure A. Anterior and posterior surface keratometric values (K1 [ Flat], K2 [ Steep], and Km [ mean]) before treatment. (A, B) Box plots showing median level (indicated by ●, average symbol ○), 95% median confidence range box (black line boxes), and interquartile range box (red line boxes). (C, D) The corresponding histogram plots (for K1 and K2). All units in keratometric dipters (D).
Evaluation of Visual Acuity, Pachymetry and Anterior-Surface Irregularity in Keratoconus and Crosslinking Intervention Follow-up in 737 Cases

Anastasios John Kanellopoulos, Vassiliki 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 keratoconic (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 and CDVA 0.59 ± 0.25 (decimal), for group B, 0.51 ± 0.28 and 0.77 ± 0.23, and for group C, 0.81 ± 0.31 and 0.87 ± 0.12.

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.

INTRODUCTION

Keratoconus (KCN), derived from the Greek words κέραστος: cornea; κονιος: 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 asymmetry.2,3

Our long clinical experience with keratoconic screening and rehabilitation5 indicate 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.6 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 grading5,11 has been reported only in very few post-review publications.

This study aims to investigate the possible correlations of visual acuity (UDVA and CDVA), corneal pachymetry, and specific Scheimpflug-imaging derived anterior-surface topographic irregularity indices with keratoconus severity, in a large pool of clinically-diagnosed keratoconic eyes, and in a group of keratoconic eyes subjected to cross-linking and anterior-surface normalization intervention, and examine the applicability of these indicators in keratoconus screening,
etasia severity classification, and clinical keratoconus management follow-up.

MATERIALS AND METHODS

This study received approval by the Ethics Committee of our Institution, adherent 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) keratometric eyes were evaluated, enrolled in the study over the course of the last 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.

Inclusion 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 topographic-guided excimer ablation combined with a procedure that was previously 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,6 and keratoconus incidence large studies.14 Inclusion criteria for group B were all keratoconus patients, 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 C.

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.13,15 The device was calibrated according to manufacturer recommendations prior to undertaking the measurements. The measurements were obtained and processed via the Examination Software (Version 1.1747). 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, reported in keratometric diopters (D)), corneal pachymetry, (TCT, thinnest corneal thickness, measured in μm), and keratometric Astmèr & Kramlich classification. The topographic keratometric classification (TKC) scale with increasing severity, was: (-), K1C1-K2, K2C3-2, KC3, KC3, 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 (radiless) 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 deviation (HID), 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 keratometric TKC severity and regression analysis, and receiver operating characteristics (ROC) curve analysis were performed with stata statistical tools provided by Minibab 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.6

Corneal surface irregularity, as expressed by the indexes ISV and HID, was: for group A ISV 99.60 ± 43.28 and HID 0.093 ± 0.052, for group B ISV 79.21 ± 36.58, and HID 0.059 ± 0.037, and for group C ISV 31.83 ± 23.81 and HID 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 ± 27.93 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 0.081 ± 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, K1C1 to 1, K1C1-2, to 2, K2C3, C2C3-2, to 4, K2C3 to 5, K3C3-4 and K4 to 7. Based on this conversion, for group A average TKC grade was 3.81 ± 1.95 (the average was between K2C2 and K2C2-3, closer to the KC2 grade), and for group B, average TKC grade was 3.39 ± 1.89, closer to the KC2 grade. Group C, comprised of healthy, nonkeratoconic eyes, had average TKC (--)
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>UDVA vs TKC</td>
<td></td>
</tr>
<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>CDVA vs TKC</td>
<td></td>
</tr>
<tr>
<td>Group A, unoperated KCN eyes</td>
<td>0.202</td>
</tr>
<tr>
<td>Group B, AP-treated KCN eyes</td>
<td>0.175</td>
</tr>
<tr>
<td>TCT vs TKC</td>
<td></td>
</tr>
<tr>
<td>Group A, unoperated KCN eyes</td>
<td>0.236</td>
</tr>
<tr>
<td>Group B, AP-treated KCN eyes</td>
<td>0.175</td>
</tr>
<tr>
<td>ISV vs TKC</td>
<td></td>
</tr>
<tr>
<td>Group A, unoperated KCN eyes</td>
<td>0.853</td>
</tr>
<tr>
<td>Group B, AP-treated KCN eyes</td>
<td>0.856</td>
</tr>
<tr>
<td>IHD vs TKC</td>
<td></td>
</tr>
<tr>
<td>Group A, unoperated KCN eyes</td>
<td>0.731</td>
</tr>
<tr>
<td>Group B, AP-treated KCN eyes</td>
<td>0.701</td>
</tr>
</tbody>
</table>

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

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.

Fig. 2: Marginal plot of UDVA (expressed as decimal) 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 as decimal) 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 pm), 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.
CDVA; best-spectacle corrected distance visual acuity; TCT; thinnest corneal thickness; ISV; index of surface variance; IID; index of height deviation; 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 keratocasia and keratoconus assessment \(^{11-13}\), as well as postoperative follow-up due to various CXL interventions.\(^{31}\) The current options of the clinical investigator include quantitative evaluation of corneal morphologic parameters derived from topography or Scheimpflug topometry.\(^{24,25}\)

The latter modality provides specific anterior-surface corneal irregularity indices developed for the grading and classification of keratocasias.\(^{30-32}\)

The association of visual performance from optical quality metrics has been investigated in length for normal eyes and in highly aberrated eyes with keratoconus.\(^{29,30}\)

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)\(^{31}\) or with the severity of keratoconus classification.\(^{31}\)

The assessment of keratocasia 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,\(^{31}\) 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, KC3-4 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 KC 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 keratocasia 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 alterations 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 refract 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 keratocasia severity. Although it is true that keratoconus is a thinning disease, any individual thickness has large variance and poor sensitivity to distinguish keratoconics from normal corneas.

The data provided herein suggest that clinical assessment of keratocasia 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 keratocasus progression such as cross-linking with riboflavin (CXL).\(^{31}\) 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 stiffer.

In this extremely large sample of patients evaluated, the compelling disease staging markers appear to be the two anterior surface irregularity indices, namely the ISV and the IID. 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 TCK keratoconus severity grade had an average of 0.793 for both keratoconic groups, and between IID and TCK, 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 IID indices, whose respective values were 0.876 and 0.887, indicating that ISV and IID are more sensitive indicators for keratoconus severity classification. In countries where keratoconus appears to be rampant -we estimate that in 1 in every 50 young adults has topographic signs of the disease- topography screening \(^{31}\)

may be the most important public health diagnostic medical tool. With the time-proven disease course alteration by CXL and other techniques 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 grade and accurate assessment of postoperative measurement. The compelling disease staging markers appear to be two anterior-surface irregularity indices derived by Scheimpflug imaging, namely the index of surface variance and the index of height

---

**Table 3:** Receiver operating characteristics (ROC) curve analysis, area under curve, standard error, asymptotic signature and 95% confidence interval results

<table>
<thead>
<tr>
<th>Test result variable(s)</th>
<th>Area under curve</th>
<th>Std. error</th>
<th>Asymptotic signature</th>
<th>Asymptotic 95% confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CDVA</strong></td>
<td>0.550</td>
<td>0.039</td>
<td>0.000</td>
<td>0.524</td>
</tr>
<tr>
<td><strong>TCT</strong></td>
<td>0.596</td>
<td>0.070</td>
<td>0.009</td>
<td>0.535</td>
</tr>
<tr>
<td><strong>ISV</strong></td>
<td>0.876</td>
<td>0.035</td>
<td>0.000</td>
<td>0.808</td>
</tr>
<tr>
<td><strong>IID</strong></td>
<td>0.887</td>
<td>0.036</td>
<td>0.000</td>
<td>0.817</td>
</tr>
</tbody>
</table>

**Fig. 6:** Marginal plot of IID, index of height deceleration, and TCK grading with varying box plots showing mean levels and outliers. Left — group A, uncorrected KCN eyes and, right — group B, Athens-protocol (AP) treated KCN eyes

**Fig. 7:** Receiver operating characteristics plot for the four variables, CDVA, TCT, ISV and IID. (CDVA; best-spectacle corrected distance visual acuity; TCT; thinnest corneal thickness; ISV; index of surface variance; IID; index of height deceleration)
decentration, which appear to be more sensitive and specific tools to visualize 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.

REFERENCES
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 eximer 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 55.50 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, an expression of anterior keratometry, preoperatively and postoperatively, was 45.11 ± 8.22 µm (range: 21.8 to 500 µm) up to 3 years postoperatively.

**CONCLUSIONS:** The Athens Protocol to arrest keratoconus progression and improve corneal regularity dominates 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.

---

Keratoconus is a degenerative bilateral, noninflammatory disorder characterized by ectasia, thinning, and irregular corneal topography. The disorder usually has onset at puberty and often progresses until the third decade of life, may manifest asymptomatically in the two eyes of the same patient, and can present with unpredictable visual acuity, particularly in relation to corneal irregularities. One of the acceptable options for progressive keratoconus management is corneal collagen cross-linking (CXL) with riboflavin and ultraviolet-A.

To further improve the topographic and refractive outcomes, CXL can be combined with customized anterior surface normalization. Our team has developed a procedure involving sequentially excimer epithelial debridement (50 µm), partial topography-guided excimer laser stromal ablation, and high-fluence ultraviolet-A irradiation (10 mW/cm²), accelerated (10’, or minutes) CXL. Early results and anterior segment optical coherence tomography quantitative findings are indicative of the long-term stability of the procedure.

Detailed studies on postoperative visual rehabilitation and anterior surface topographic changes by such combined CXL procedures are rare, particularly those reporting results longer than 1 year. This study aims to investigate safety and efficacy of the Athens Protocol procedure by analysis of long-term (3-year) refractive, topographic, pachymetric, and visual rehabilitation changes on clinical keratoconus management with the Athens Protocol in a large number of cases.

**PATIENTS AND METHODS**

This clinical study was approved by the Ethics Committee of our Institution and adhered to the tenets of the Declaration of Helsinki. Written informed consent was obtained from each participant at the time of the intervention or the first clinical visit.

**RESULTS**

Two hundred thirty-one consecutive keratoconic cases subjected to the Athens Protocol procedure between 2008 and 2010 were investigated. All procedures were performed by the same surgeon (AJK) using the Alcon/Wavelight 400 Hz Eye-Q® or the X550 excimer lasers. Inclusion criteria were clinical diagnosis of progressive keratoconus, minimum age of 17 years, and corneal thickness of at least 300 µm. All participants completed an uneventful Athens Protocol procedure and 231 eyes were observed for up to 3 years. Exclusion criteria were systemic disease, previous eye surgery, chemical injury or delayed epithelial healing, and pregnancy or lactation (female patients).

**MEASUREMENTS AND ANALYSIS**

Postoperative evaluation included uncorrected distance visual acuity (UDVA), manifest refraction, corrected distance visual acuity (CDVA) with this refraction, and slit-lamp biomicroscopy for clinical signs of CXL. For the quantitative assessment of the induced corneal changes, postoperative evaluation was performed by a Pentacam Scheimpflug imaging device (Oculus II, Wavelight AG, Erlangen, Germany) and processed via Examination Software (Version 1.17r47). Specific anterior surface irregularity indices provided by the Scheimpflug imaging analysis were evaluated in addition to keratometric and pachymetric values. These indices are employed in grading and classification based on the Amsler and Krumeich criteria.
The Athens Protocol/Kanellopoulos & Asimellis

was ±0.20 preoperatively and ±0.28 postoperatively. Likewise, the standard deviation of CDVA was ±0.23 preoperatively and ±0.26 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.

This study aimed to address some of the above issues. The large sample and follow-up time permit sensitive analysis with confident conclusion of postoperative efficacy. We monitored visual acuity changes and for the quantitative assessment we chose to standardize on one Scheimpflug screening device and to focus on key parameters of visual acuity, keratometry, pachymetry, and anterior surface indices. All of these parameters reflect changes induced by the procedure and describe postoperative progression. However, variations in the two anterior surface indices (Index of Surface Variance and Index of Height Decentration) may provide a more valid analysis than keratometry and visual function.

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.25-27

The two anterior surface indices, Index of Surface Variance and Index of Height Decentration, also demonstrated postoperative improvement. A smaller value is indication of corneal normalization (lower Index of Surface Variance, less irregular surface, lower Index of Height Decentration, cone less steep and more central). These changes are therefore suggestive of postoperative topography improvement, in agreement with other smaller sample studies.28 Such changes in Index of Surface Variance and Index of Height Decentration have been reported only recently.29

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 flattening, which, 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.14,15

However, it is noted that the visual acuity presented with large variations. The standard deviation of UDVA

Figure 1. Change (gains/losses in visual acuity, expressed as the difference of postoperative minus preoperative values) expressed decimally, showing median level (indicated by ‘+’), average symbol (×), 95% median confidence range box (red borderlines box), and interquartile intervals range box (black borderline boxes).

Figure 2. Anterior keratometry (K1 flat and K2 steep) as measured by the Scheimpflug device (Oculyzer II, WaveLight AG, Erlangen, Germany) preoperatively up to 3 years postoperatively. All units in keratometric diopters (D).

Figure 3. Anterior surface topometric indices Index of Surface Variance (ISV) and Index of Height Decentration (IHD) as measured by the Scheimpflug imaging device (Oculyzer II, WaveLight AG, Erlangen, Germany) preoperatively, 1 month postoperatively, and up to 36 months postoperatively.

Pachymetric Progression

The thinnest corneal decreased as a result of excimer laser ablation but then stabilized over time without additional thinning (Table 2).

Discussion

Many reports describe the effects of CXL with or without same-session excimer laser ablation corneal normalization. There is general consensus that the intervention strengthens the cornea, helps arrest the ectasia progression, and improves corneal keratometric values, refraction, and visual acuity.

The key question is the long-term stability of these induced changes. For example, is the cornea ‘inactive’ after the intervention and, if not, is there steepening or flattening and/or thickening or thinning? These issues are even more applicable in the case of the Athens Protocol, due to the partial anterior surface ablation. Ablating a thin, ectatic cornea may sound unorthodox. However, the goal of the topography-guided ablation is to normalize the anterior cornea and thus help improve visual rehabilitation to a step beyond that a simple CXL would provide.

This study aims to address some of the above issues. The large sample and follow-up time permit sensitive analysis with confident conclusion of postoperative efficacy. We monitored visual acuity changes and for the quantitative assessment we chose to standardize on one Scheimpflug screening device and to focus on key parameters of visual acuity, keratometry, pachymetry, and anterior surface indices. All of these parameters reflect changes induced by the procedure and describe postoperative progression. However, variations in the two anterior surface indices (Index of Surface Variance and Index of Height Decentration) may provide a more valid analysis than keratometry and visual function.25 Our results indicate that the apparent disadvantage of thinning the cornea may sound unorthodox. However, the goal of the topography-guided ablation is to normalize the anterior cornea and thus help improve visual rehabilitation to a step beyond that a simple CXL would provide.

This study aims to address some of the above issues. The large sample and follow-up time permit sensitive analysis with confident conclusion of postoperative efficacy. We monitored visual acuity changes and for the quantitative assessment we chose to standardize on one Scheimpflug screening device and to focus on key parameters of visual acuity, keratometry, pachymetry, and anterior surface indices. All of these parameters reflect changes induced by the procedure and describe postoperative progression. However, variations in the two anterior surface indices (Index of Surface Variance and Index of Height Decentration) may provide a more valid analysis than keratometry and visual function.25 Our results indicate that the apparent disadvantage of thinning the cornea may sound unorthodox. However, the goal of the topography-guided ablation is to normalize the anterior cornea and thus help improve visual rehabilitation to a step beyond that a simple CXL would provide.
in this study suggests employment of caution in the surface normalization process to avoid overcorrection.

Table A

<table>
<thead>
<tr>
<th>Value</th>
<th>Preop</th>
<th>1 Month</th>
<th>3 Months</th>
<th>6 Months</th>
<th>12 Months</th>
<th>24 Months</th>
<th>36 Months</th>
</tr>
</thead>
<tbody>
<tr>
<td>R1 (flat)</td>
<td>48.56</td>
<td>44.44</td>
<td>43.99</td>
<td>43.73</td>
<td>43.54</td>
<td>43.35</td>
<td>43.22</td>
</tr>
<tr>
<td>SD</td>
<td>±3.83</td>
<td>±3.97</td>
<td>±3.86</td>
<td>±3.78</td>
<td>±3.73</td>
<td>±3.74</td>
<td>±3.80</td>
</tr>
<tr>
<td>Maximum</td>
<td>58.30</td>
<td>55.60</td>
<td>53.75</td>
<td>53.70</td>
<td>53.70</td>
<td>53.70</td>
<td>53.70</td>
</tr>
<tr>
<td>Minimum</td>
<td>39.75</td>
<td>36.10</td>
<td>36.10</td>
<td>36.10</td>
<td>36.10</td>
<td>36.10</td>
<td>36.00</td>
</tr>
<tr>
<td>R2 (slope)</td>
<td>50.71</td>
<td>47.61</td>
<td>47.03</td>
<td>46.84</td>
<td>46.63</td>
<td>46.44</td>
<td>46.30</td>
</tr>
<tr>
<td>SD</td>
<td>±5.14</td>
<td>±5.15</td>
<td>±5.09</td>
<td>±5.00</td>
<td>±4.92</td>
<td>±4.91</td>
<td>±4.88</td>
</tr>
<tr>
<td>Maximum</td>
<td>66.62</td>
<td>62.75</td>
<td>62.25</td>
<td>60.25</td>
<td>60.00</td>
<td>60.00</td>
<td>60.00</td>
</tr>
<tr>
<td>Minimum</td>
<td>42.80</td>
<td>38.00</td>
<td>37.90</td>
<td>37.00</td>
<td>37.90</td>
<td>37.90</td>
<td>37.20</td>
</tr>
</tbody>
</table>

Preop = preoperative; SD = standard deviation.

Table B

<table>
<thead>
<tr>
<th>Value</th>
<th>Preoperative</th>
<th>12 Months</th>
<th>24 Months</th>
<th>Postoperative</th>
<th>36 Months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Index of Surface Variance</td>
<td>98.48</td>
<td>83.08</td>
<td>81.08</td>
<td>79.51</td>
<td>78.19</td>
</tr>
<tr>
<td>SD</td>
<td>±4.47</td>
<td>±3.73</td>
<td>±3.13</td>
<td>±2.79</td>
<td>±2.64</td>
</tr>
<tr>
<td>Minimum</td>
<td>208.00</td>
<td>190.00</td>
<td>190.00</td>
<td>190.00</td>
<td>190.00</td>
</tr>
<tr>
<td>Maximum</td>
<td>17.00</td>
<td>18.00</td>
<td>17.00</td>
<td>16.15</td>
<td>14.15</td>
</tr>
</tbody>
</table>

Preop = preoperative; SD = standard deviation.

In this study, the Athens Protocol/Kanellopoulos & Asimellis
Revisiting keratoconus diagnosis and progression classification based on evaluation of corneal asymmetry indices, derived from Scheimpflug imaging in keratoconic and suspect cases

Anastasios John Kanellopoulos1,2
George Asimellis1

1Laservision Eye Institute, Athens, Greece, New York University School of Medicine, New York, NY, USA

Purpose: To survey the standard keratoconus grading scale (Pentacam®-derived Ameloz-Kramich stages) compared to corneal irregularity indices and best spectacles-corrected distance visual acuity (CDVA).

Patients and methods: Two-hundred and twelve keratoconus cases were evaluated for keratoconus grading, anterior surface irregularity indices (measured by Pentacam imaging), and subjective refraction (measured by CDVA). The correlations between CDVA, keratometry, and the Scheimpflug keratometry grading and the seven anterior surface Pentacam-derived topometric indices – index of surface variance, index of vertical asymmetry, keratoconus index, central keratoconus index, index of height asymmetry, index of height decentration, and index of minimum radius of curvature – were analyzed using paired two-tailed t-test, coefficient of determination (r²), and trendline linearity.

Results: The average ± standard deviation CDVA (expressed decimally) was 0.62 ± 0.244 for all eyes (range 0.10–1.00). The average flat meridian keratometry was (K1) 46.7 ± 5.93 D, the average steepest keratometry (K2) was 51.05 ± 6.59 D. The index of surface variance and the index of height decentration had the strongest correlation with topographic keratoconus grading (r² = 0.001). CDVA and keratometry correlated poorly with keratoconus severity.

Conclusions: It is reported here for the first time that the indices of surface variance and the index of height decentration may be the most sensitive and specific criteria in the diagnosis, progression, and surgical follow-up of keratoconus. The classification proposed herein may present a novel benchmark in clinical work and future studies.

Keywords: diagnosis and classification, Pentacam topometric indices, Ameloz-Kramich keratoconus grading, surface variance, vertical asymmetry, keratoconus index, central keratoconus index, index of height asymmetry, height decentration, minimum radius of curvature

Introduction

Keratoconus is described as a degenerative bilateral, progressive, noninflammatory corneal disorder characterized by ectasia, thinning, and increased curvature. It is associated with loss of visual acuity particularly in relation to progressive corneal irregularity, and usually is manifested asymmetrically between the two eyes of the same patient. Occasionally, the patient may present with symptoms of photophobia, glare, and monocular diplopia.

The problem of specificity and sensitivity of keratoconus assessment, particularly the diagnosis of early signs of ectasia and subclinical keratoconus, and for monitoring the progression of the disease, has been extensively studied. The commonly used options for the clinician include optically-based anterior segment imaging modalities, eg, Placido corneal topography and slit or Scheimpflug imaging, that provide corneal surface qualitative and quantitative data.

Anterior segment topometric indices

Rotating camera Scheimpflug imaging (Pentacam, Oculus Optikgeräte GmbH, Wetzlar, Germany), provides a multitude of corneal refractive (keratometry), topometric, tomographic, and pachymetric data. In addition, specific anterior-surface irregularity indices have been developed for the grading and classification of keratoconus development, as well as the post-operative assessment.

The aim of this study was to investigate the values of these indices, the repeatability of their measurement, and their correlation with best spectacle-corrected distance visual acuity (CDVA), keratometry, and commonly used keratoconus classification in a large pool of clinically diagnosed keratoconic eyes.

Patients and methods

This study received approval from the Ethics Committee of the authors’ institution, adherent to the tenets of the Declaration of Helsinki. Informed consent was obtained from each subject at the time of the first clinical visit. These cases were studied over the span of at least 2 years.

Patient inclusion criteria

The study group consisted of 212 cases that presented to the authors’ institution. Subjects’ ages ranged from 19–57 years (average 31.9 ± 7.5 years). Each case was subjected to a complete oculomotor examination, including subjective refraction, CDVA measurement with this refraction, and slit-lamp biomicroscopy for clinical signs of keratoconus.

Inclusion criteria included a minimum age of 18 years and definite findings consistent with keratoconus, such as those described by the CLECK (Collaborative Longitudinal Evaluation of Keratoconus) group. Exclusion criteria included systemic disease, previous corneal surgery, history of chemical injury or delayed epithelial healing, and pregnancy or lactation during the study (for the female patients).

Imaging, measurement, and analysis

Anterior segment evaluation, including anterior segment imaging measurements with the Pentacam Scheimpflug rotating camera, was performed on each case. The device was calibrated according to manufacturer’s recommendations prior to undertaking the measurements. The Pentacam measurements were obtained and processed via the examination software (version 1.1747).

For each eye, four consecutive measurements were obtained and processed to test for data acquisition repeatability. The default settings, and 25 images per single acquisition were employed.

Linear regression analysis was performed to seek possible correlations: Descriptive and comparative statistics, analysis of variance between keratoconus Ameloz-Kramich stage subgroups, and linear regression were performed with statistics tools provided by Minitab® version 1.6 (Minitab Inc, Coventry, UK) and Origin version 9 (OriginLab Corporation, Northampton, MA, USA). P-values less than 0.05 in the paired analyses were considered statistically significant.

Results

Keratoconetic and anterior surface topographic indices statistics

The sample population consisted of 212 cases, which consisted of 65 female and 147 male patients. The preponderance towards males in the population is consistent with the authors’ clinical experience in male/female incidence in keratoconic patients3,4 and keratoconus incidence studies5,6. Of the 212 eyes, 113 were the right eye and 99 were the left eye. The average age of all patients was 31.77 ± 7.23 (range 19–57) years.

Table 1: Collective average, standard deviation, maximum, and minimum anterior keratometric and topometric indices, as measured in the 8 mm zone

<table>
<thead>
<tr>
<th>Anterior keratoconus</th>
<th>Average</th>
<th>SD</th>
<th>Max</th>
<th>Min</th>
</tr>
</thead>
<tbody>
<tr>
<td>K1 – flat (D)</td>
<td>46.78 ± 0.08</td>
<td>58.90</td>
<td>80.70</td>
<td>42.10</td>
</tr>
<tr>
<td>K2 – steepest (D)</td>
<td>51.05 ± 0.09</td>
<td>62.50</td>
<td>78.00</td>
<td>45.60</td>
</tr>
<tr>
<td>K1 – steepest (D)</td>
<td>46.78 ± 0.08</td>
<td>58.90</td>
<td>80.70</td>
<td>42.10</td>
</tr>
<tr>
<td>K1 – mean (D)</td>
<td>48.80 ± 0.08</td>
<td>60.80</td>
<td>78.00</td>
<td>46.40</td>
</tr>
<tr>
<td>Asymmetry (D)</td>
<td>0.10 ± 0.01</td>
<td>0.13</td>
<td>0.20</td>
<td>0.03</td>
</tr>
<tr>
<td>Anterior surface topographic indices</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SIV</td>
<td>90.99 ± 47.43</td>
<td>262</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>IVA (mm)</td>
<td>1.05 ± 0.05</td>
<td>2.52</td>
<td>0.90</td>
<td></td>
</tr>
<tr>
<td>VX</td>
<td>1.30 ± 0.15</td>
<td>0.90</td>
<td>0.90</td>
<td></td>
</tr>
<tr>
<td>K1</td>
<td>1.06 ± 0.07</td>
<td>1.30</td>
<td>0.90</td>
<td></td>
</tr>
<tr>
<td>CRK (µm)</td>
<td>20.60 ± 20.21</td>
<td>100.00</td>
<td>0.20</td>
<td></td>
</tr>
<tr>
<td>HDA (µm)</td>
<td>0.911 ± 0.054</td>
<td>0.256</td>
<td>0.005</td>
<td></td>
</tr>
<tr>
<td>RDK (µm)</td>
<td>6.07 ± 0.22</td>
<td>3.73</td>
<td>3.20</td>
<td></td>
</tr>
<tr>
<td>CDVA</td>
<td>0.63 ± 0.25</td>
<td>1.00</td>
<td>0.10</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: CDVA, best spectacle-corrected distance visual acuity; CKI, central keratoconus index; HDA, index of height asymmetry; HDA, index of height decentration; SIV, surface irregularity; VX, index of vertex asymmetry; K1, keratometry index; Max, maximum; Min, minimum; RDK, radius of curvature; SD, standard deviation.
The average ± standard deviation CDVA (expressed decimally) for all eyes was 0.626 ± 0.244 (range 0.10–1.00). Average, standard deviation, maximum, and minimum corneal surface keratometry, and topometric indices for all eyes in the study are reported in Table 1. Intra-individual repeatability was assessed via the standard deviation of the four consecutive measurements undertaken for each eye. The average ± standard deviation of repeatability for the seven topometric indices in all 212 eyes measured is reported in Table 2.

The sample was presented with an average keratometry on the anterior surface flat axis of 46.7 ± 5.9 mm and 51.0 ± 6.6 mm on the steep axis. The statistical analysis showed that 95% of the sample population had a steep axis keratometry > 46.025 D, consistent with the CLEK group standards.

Table 3 Coefficient of determination (r²) and Pearson correlation data between best spectacle-corrected distance visual acuity and the seven anterior surface topometric indices within all eyes in the study group (n = 212)†

<table>
<thead>
<tr>
<th>CDVA (µm)</th>
<th>ISV (µm)</th>
<th>IVA (µm)</th>
<th>KI</th>
<th>CKI</th>
<th>IHA (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IHG</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pearson correlation</td>
<td>0.627</td>
<td>0.593</td>
<td>0.746</td>
<td>0.355</td>
<td></td>
</tr>
<tr>
<td>r²</td>
<td>0.391</td>
<td>0.878</td>
<td>0.91</td>
<td>0.911</td>
<td>0.485</td>
</tr>
<tr>
<td>ISV</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pearson correlation</td>
<td>0.584</td>
<td>0.340</td>
<td>0.680</td>
<td>0.403</td>
<td></td>
</tr>
<tr>
<td>r²</td>
<td>0.878</td>
<td>0.878</td>
<td>0.845</td>
<td>0.845</td>
<td>0.485</td>
</tr>
<tr>
<td>IVA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pearson correlation</td>
<td>0.642</td>
<td>0.542</td>
<td>0.596</td>
<td>0.507</td>
<td></td>
</tr>
<tr>
<td>r²</td>
<td>0.721</td>
<td>0.721</td>
<td>0.73</td>
<td>0.73</td>
<td>0.73</td>
</tr>
<tr>
<td>KI</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pearson correlation</td>
<td>0.514</td>
<td>0.514</td>
<td>0.514</td>
<td>0.514</td>
<td></td>
</tr>
<tr>
<td>r²</td>
<td>0.514</td>
<td>0.514</td>
<td>0.514</td>
<td>0.514</td>
<td>0.514</td>
</tr>
<tr>
<td>CKI</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pearson correlation</td>
<td>0.710</td>
<td>0.666</td>
<td>0.686</td>
<td>0.790</td>
<td></td>
</tr>
<tr>
<td>r²</td>
<td>0.777</td>
<td>0.777</td>
<td>0.777</td>
<td>0.777</td>
<td>0.777</td>
</tr>
</tbody>
</table>

*IHG = 0.588 in all cases.

The correlations between the seven keratometric anterior surface topographic indices with CDVA are illustrated in Figure 1A-G. Specifically, the scatter and fitted line plots of ISV (Figure 1A), IVA (Figure 1B), KI (Figure 1C), CKI (Figure 1D), IHA (Figure 1E), BID (Figure 1F), and Rmin (Figure 1G) versus CDVA are plotted, in addition to the 95% confidence (C3) and 95% prediction interval (PI) lines. The paired data present with coefficient of determination (r²) of linear correlation with CDVA of 0.557 for ISV, 0.34 for IVA, 0.424 for KI, 0.396 for CKI, 0.167 for IHA, 0.393 for IHD, and 0.516 for Rmin. The Pearson correlation values versus CDVA were -0.746 for ISV, -0.584 for IVA, -0.680 for KI, -0.642 for CKI, -0.344 for IHA, -0.627 for IHD, and 0.718 for Rmin. In all cases, the P-value was <0.0001 (Table 3).
Topographic indices correlation with keratoconus stages grading

The 212 eyes were subjected to keratoconus Amsler–Krumbein stages grading\(^a\) by the Pentacam software. The resulting grading designated n = ten eyes as Stage I, n12 = eleven eyes as Stage I–II, n22 = twelve eyes as Stage II, n23 = twenty-two eyes as Stage II–III, n33 = thirty-three eyes as Stage III, n34 = thirty-four eyes as Stage III–IV, and n4 = four eyes as Stage IV.

The correlations between CDVA and the seven Schimpff anterior surface keratometric and topographic indices with the above keratoconus grading are illustrated in Figure 2A–H.

Keratoconus classification


deviation by a factor

Figure 2

(A) CDVA versus keratoconus grading; (B) CDVA versus keratoconus grading; (C) CDVA versus keratoconus grading; (D) CDVA versus keratoconus grading; (E) CDVA versus keratoconus grading; (F) CDVA versus keratoconus grading; (G) CDVA versus keratoconus grading; (H) CDVA versus keratoconus grading.


deviation by a factor

For example, measured values which exceed the standard deviation by a factor of more than three, are highlighted in red. Namely, these indices are the following:

1. ISV: the unitless standard deviation of individual corneal sagittal radii from the mean curvature. ISV is thus an expression of the corneal surface irregularity. It is elevated in all types of corneal surface irregularity (eg, scars, astigmatism, deformities caused by contact lenses, pachymetry).

2. IVA: measured (expressed in mm) as the mean difference between superior and inferior corneal curvature (similar to the commonly used inferior/superior ratio).\(^b\) IVA is thus the value of curvature symmetry, with respect to the horizontal meridian as the axis of reflection. An IVA greater than 0.28 is considered abnormal and/or pathological.

3. KI: a unitless index is expressing the ratio between mean radius values in a peripheral meridian as the axis of reflection. An IVA larger than 0.28 is considered abnormal and/or pathological.

4. CKI: the ratio between mean radius values in a peripheral ring divided by a central ring: r sag (mean peripheral) to r sag mean center (no units). CKI is elevated especially in central pachymetric, and increases with the severity of central keratoconus. A CKI value larger than 1.03 is considered abnormal and/or pathological.

5. IHA: the mean difference between height values superior minus height values inferior with horizontal meridian as mirror axis (expressed in µm). IHA is calculated by the height data symmetry comparison of the superior and inferior area, and provides the degree of symmetry of height data with respect to the horizontal meridian as the axis of reflection. IHA is similar to the IVA, but based on corneal elevation, and is thus more sensitive. An IHA value larger than 19 is considered abnormal, and larger than 21 is pathological.

6. BID: the value of the deceleration of elevation data in the vertical direction (expressed in µm) and is calculated from a Fourier analysis. This index provides the degree of deceleration in the vertical direction, calculated on a ring with radius 3 mm. An BID value larger than 0.014 is considered abnormal, and larger than 0.016 is pathological.

7. Rmin: expressed in mm. It is a measurement of the smallest radius of sagittal corneal curvature (ie, the maximum steepness of the cone). Values of Rmin less than 6.71 are considered abnormal, and larger than 41 is pathological.

Association with these indices with clinical keratoconus observations is provided by the manufacturer and is listed in Table 6.

Discussion

The Pentacam software compares the measured values with the means and standard deviations of a normal population, and helps provide color-coded “flags.” For example, measured values which exceed the standard deviation by a factor of more than three, are highlighted in red. Namely, these indices are the following:

1. ISV: the unitless standard deviation of individual corneal sagittal radii from the mean curvature. ISV is thus an expression of the corneal surface irregularity. It is elevated in all types of corneal surface irregularity (eg, scars, astigmatism, deformities caused by contact lenses, pachymetry).

2. IVA: measured (expressed in mm) as the mean difference between superior and inferior corneal curvature (similar to the commonly used inferior/superior ratio).\(^b\) IVA is thus the value of curvature symmetry, with respect to the horizontal meridian as the axis of reflection. An IVA greater than 0.28 is considered abnormal and/or pathological.

3. KI: a unitless index is expressing the ratio between mean radius values in a peripheral meridian as the axis of reflection. An IVA greater than 0.28 is considered abnormal and/or pathological.

4. CKI: the ratio between mean radius values in a peripheral ring divided by a central ring: r sag (mean peripheral) to r sag mean center (no units). CKI is elevated especially in central pachymetric, and increases with the severity of central keratoconus. A CKI value larger than 1.03 is considered abnormal and/or pathological.

5. IHA: the mean difference between height values superior minus height values inferior with horizontal meridian as mirror axis (expressed in µm). IHA is calculated by the height data symmetry comparison of the superior and inferior area, and provides the degree of symmetry of height data with respect to the horizontal meridian as the axis of reflection. IHA is similar to the IVA, but based on corneal elevation, and is thus more sensitive. An IHA value larger than 19 is considered abnormal, and larger than 21 is pathological.

6. BID: the value of the deceleration of elevation data in the vertical direction (expressed in µm) and is calculated from a Fourier analysis. This index provides the degree of deceleration in the vertical direction, calculated on a ring with radius 3 mm. An BID value larger than 0.014 is considered abnormal, and larger than 0.016 is pathological.

7. Rmin: expressed in mm. It is a measurement of the smallest radius of sagittal corneal curvature (ie, the maximum steepness of the cone). Values of Rmin less than 6.71 are considered abnormal, and larger than 41 is pathological.

Association with these indices with clinical keratoconus observations is provided by the manufacturer and is listed in Table 6.

The clinical suspicion of early-stage keratoconus may be based on refraction criteria such as a change in refractive power and the axis of astigmatism, fluctuating refraction, and several clinical findings (eg, conspicuous retinoscopy signs). Optical imaging, such as topometry and topography, provides valuable supplementary information, and it has long been supported that the contribution of proper evaluation and analysis of anterior surface irregularity derived from topography,\(^c\) or more recently from Pentacam topometry (eg, the seven topographic indices studied in this manuscript), may provide an invaluable aid in the diagnosis and progression evaluation of the disease.\(^d\) Only two reports have been identified that address this matter of correlation of the above Pentacam-derived indices with CDVA\(^x\) and the severity of keratoconus classification.\(^x\)

The correlation between the seven anterior surface topographic indices and CDVA appears not very strong in our study. The Rmin (r = 0.516, P < 0.001) and ISV (r = 0.557, P < 0.001) were found to be the strongest correlated indices with CDVA, in comparison to the other indices. The least

The correlation between the seven anterior surface topographic indices and CDVA appears not very strong in our study. The Rmin (r = 0.516, P < 0.001) and ISV (r = 0.557, P < 0.001) were found to be the strongest correlated indices with CDVA, in comparison to the other indices. The least

The correlation between the seven anterior surface topographic indices and CDVA appears not very strong in our study. The Rmin (r = 0.516, P < 0.001) and ISV (r = 0.557, P < 0.001) were found to be the strongest correlated indices with CDVA, in comparison to the other indices. The least
The above results are in agreement with the authors’ past clinical experience with a significant number of keratoconic patients followed for over 15 years. The observation has been that visual acuity can present with large variations, and can sometimes be unexpectedly good for the corresponding clinical experience with a significant number of keratoconic patients followed for over 15 years. The observation has been that visual acuity can present with large variations, and can sometimes be unexpectedly good for the corresponding
Keratoconus classification

<table>
<thead>
<tr>
<th>CDVA</th>
<th>ISV</th>
<th>KI</th>
<th>CDVA</th>
<th>ISV</th>
<th>KI</th>
</tr>
</thead>
<tbody>
<tr>
<td>No clear light or shadow movement.</td>
<td>Clear corneal, umbilication.</td>
<td>Horizontal, notch, round, oval, or round shading nasal or temporal</td>
<td>No clear light or shadow movement.</td>
<td>Clear corneal, umbilication.</td>
<td>Horizontal, notch, round, oval, or round shading nasal or temporal</td>
</tr>
<tr>
<td>Distorted retinal reflex.</td>
<td>Scleral suction.</td>
<td>Clear corneas, Fleischer’s ring at the apex.</td>
<td>Clear corneas, Fleischer’s ring at the apex.</td>
<td>Decrease in apex thickness not visible, but measurable.</td>
<td>Clear corneas, Fleischer’s ring at the apex.</td>
</tr>
</tbody>
</table>

Notes: Best spectacle-corrected visual acuity abnormalities with topography-based gradation indices (index of surface variance; keratometry index) compared with keratoconus and normal corneas. Abbreviations: CDVA, best spectacle-corrected visual acuity; ISV, index of surface variance; KI, keratometry index; Rms, minimum value of curvature.

Conclusion

The ISV and IHA both derived from Scheimpflug corneal imaging may be more sensitive and specific tools than CDVA in early diagnosis and possible progression in keratoconus patients and corneal ectasia. They may become a novel benchmark for future studies, and may aid in the development of new keratoconus diagnostic and follow-up criteria.

Disclosure

Both AK and GA occasionally consult for Aclon/WaveLight. The authors report no other conflicts of interest in this work.

References


Clinical Ophthalmology 2013:7

Kondapalli and Aminwalla

Keratoconus classification

<table>
<thead>
<tr>
<th>Level</th>
<th>CDVA</th>
<th>ISV</th>
<th>KI</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>20/20-40</td>
<td>1.15-1.45</td>
<td>7.8-6.7</td>
</tr>
<tr>
<td>2</td>
<td>20/15-20/20</td>
<td>1.10-1.25</td>
<td>6.9-5.3</td>
</tr>
<tr>
<td>3</td>
<td>20/15-20/20</td>
<td>1.10-1.25</td>
<td>6.9-5.3</td>
</tr>
<tr>
<td>4</td>
<td>20/200</td>
<td>&lt;1/50</td>
<td>&lt;1.00</td>
</tr>
</tbody>
</table>

Notes: Best spectacle-corrected visual acuity abnormalities with topography-based gradation indices (index of surface variance; keratometry index) compared with keratoconus and normal corneas. Abbreviations: CDVA, best spectacle-corrected visual acuity; ISV, index of surface variance; KI, keratometry index; Rms, minimum value of curvature.
Introduction of quantitative and qualitative cornea optical coherence tomography findings induced by collagen cross-linking for keratoconus: a novel effect measurement benchmark

A John Kanellopoulos1,2
George Asimellis1

1Laservision Institute, Athens, Greece; New York University Medical School, New York, NY, USA

Video abstract

Correspondence: A John Kanellopoulos
Laservision Eye Institute, 17 Tsiapa Street, Athens 11243, Greece
Tel +30 210 976 2377
Fax +30 210 274 5795
Email ajk@brilliantvision.com

Clinical Ophthalmology 2013:7 329–335

© 2013 Kanellopoulos and Asimellis, publisher and licensee Dove Medical Press Ltd. This is an Open Access article which permits unrestricted noncommercial use, provided the original work is properly cited.

Introduction

Keratoconus (KC) is a degenerative bilateral, progressive, noninflammatory disorder characterized by ectasia, thinning, and increased curvature of the cornea, and is associated with loss of visual acuity, particularly in relation to high-order aberrations.1–4

Corneal collagen cross-linking (CXL) with riboflavin and ultraviolet-A irradiation is a common technique for tissue stabilization.5–9 Several studies have shown that CXL is an effective intervention to halt the progression of keratoconus and corneal ectasia.10

Anterior-segment optical coherence tomography (AS-OCT) is a promising imaging mode providing high-resolution cross-sectional images across a meridian of choices that can be employed in KCN diagnosis.8,9 The most advanced AS-OCT systems invariably employ Fourier spectral-domain signal processing. As of today, there are a number of different spectral domain OCT systems commercially available.10,11

The ability to provide real-time cross-sectional mapping, in conjunction with the very principle of operation, namely photon back scattering, provides the understudied application of quantitative assessment of the extent of stromal changes due to CXL.

Purpose

To introduce a novel, noninvasive technique to determine the depth and extent of anterior corneal stroma changes induced by collagen cross-linking (CXL) using quantitative analysis of high-resolution anterior-segment optical coherence tomography (OCT) post-operative images.

Methods

To introduce a novel, noninvasive technique to determine the depth and extent of anterior corneal stroma changes induced by collagen cross-linking (CXL) using quantitative analysis of high-resolution anterior-segment optical coherence tomography (OCT) post-operative images.

Patients and methods

Two groups of corneal cross-sectional images obtained with the OptoVue RTVue anterior-segment OCT system were studied: group A (control) consisted of unoperated, healthy corneas, with the exception of possible refractive errors. The second group consisted of keratoconic corneas with CXL that were previously operated on. The two groups were investigated for possible quantitative evidence of changes induced by the CXL, and specifically, the depth, horizontal extent, as well as the cross-sectional area of intrastromal hyper-reflective areas (defined in our study as the area consisting of pixels with luminosity greater than the mean +2 × standard deviation of the entire stromal cross section) within the corneal stroma.

Results

In all images of the second group (keratoconus patients treated with CXL) there was evidence of intrastromal hyper-reflective areas. The hyper-reflective areas ranged from 0.2% to 8.8% of the cross-sectional area (mean ± standard deviation: 3.46% ± 1.92%). The extent of the horizontal hyper-reflective area ranged from 4.42% to 99.2% (56.2% ± 23.35%) of the corneal image, while the axial extent (the vertical extent in the image) ranged from 40.00% to 86.67% (70.98% ± 7.85%). There was significant statistical difference (P < 0.02) in these values compared to the control group, on which, by application of the same criteria, the same hyper-reflective area (owing to signal noise) ranged from 0.00% to 2.51% (0.74% ± 0.63%).

Conclusion

Herein, we introduce a novel, noninvasive, quantitative technique utilizing anterior-segment OCT images to quantitatively assess the depth and cross-sectional area of CXL in the corneal stroma based on digital image analysis. Mean cross-sectional area showing evidence of CXL was 3.46% ± 1.92% of 4 mm long segment.

Keywords: Collagen cross-linking, keratoconus, optical coherence tomography, higher fluence cross-linking, corneal ectasia, Athens Protocol

OCT and CXL demarcation line observations

To date, the efficacy of CXL treatment can be monitored only indirectly by postoperative follow-up observations, such as with a Scheimpflug camera,12 or with corneal confocal microscopy.13

In addition, a corneal stromal demarcation line indicating the transition zone between cross-linked anterior corneal stroma and untreated posterior corneal stroma can be detected in slit-lamp examination as early as 2 weeks after treatment.14

In our clinical assessment, the presence of this finding over the anterior two-thirds of the stroma confirms that sufficient CXL treatment has occurred.

Following our presentation and the introduction in the peer-reviewed literature of the use of OCT imaging in order to evaluate the CXL-induced demarcation line, OCT has seen some recent interest as a tool for investigating CXL effects, such as corneal thickness before and after CXL for KCN, and demarcation line depth following CXL.15–21

The principle lies in the fact that although these lines do not appear to affect vision, as they correspond to changes in stromal density, they appear as brighter (hyper-reflective) areas on cross-sectional corneal OCT scans. However, the depth and extent of stromal changes induced by CXL has been difficult to evaluate quantitatively in the clinic.

The motivation for our study was to advance this aforementioned theory by examining not only the demarcation line depth between the suspected CXL and the deeper cornea with corneal OCT, but also to attempt to quantitatively assess the extent of this area on a large number of patients over a large postoperative interval. Our novel technique is based on digital signal processing on cross-sectional OCT images of corneas, and evaluates quantitatively and, in our opinion, free of examiner bias, the extent of CXL changes in the corneal stroma.

Patient inclusion criteria

The control group (50 patients, 100 eyes) consisted of patients with eyes with unoperated corneas (ie, normal eyes with no ocular pathology other than refractive error). Mean patient age was 35.2 ± 9.1 years (range 19–48), equally divided between males and females. Before OCT corneal mapping, a complete ocular examination and tomographic topography was performed to screen for corneal abnormalities.

The second group (47 patients, 94 eyes) consisted of KCN patients previously operated with CXL by employing the Athens Protocol, which combined same-day phototherapeutic keratectomy epithelial removal and partial topographically-guided phototherapeutic keratectomy normalization of the cornea ectasia, followed by high-fluence, short-duration riboflavin induced CXL.12

The mean patient age in this group was 28.1 ± 7.1 years (range 16–45 years). There is a bias towards males in this group (33 males, 14 females), which is consistent with our clinical experience of the male–female incidence of keratoconus

Figure 1 (A) Typical cornea cross-sectional meridian image of a patient with KCN. (B) The selected hyper-reflective intrastromal area is indicated in red.
OCT-assisted quantitative analysis of CXL in KCN patients

### Materials

The OptoVue RTVue (OptoVue Inc, Fremont, CA, USA) AS-OCT system was employed in the study. Using the Hi-Res Cross Line Scan, a 6 mm long Hi-Res Cross Line Scan, centered at the pupil center along the vertical meridian, was recorded. The meridional cross-sectional images were processed with the RTVue software (version 5.1.0, processing A5, 1, 0, 90). The software averages up to 32 successive acquisitions. In our study, we included images consisting of at least five averages.

### Results

#### Areal extent (depth and diameter) of demarcation

Cross-sectional meridian area measurements had an average of 59,183 pixels (±5778), ranging from 80,729 to 39,951 (maximum to minimum). This corresponds to an area of 2.88 mm². Mean luminosity values were, on a grayscale of 0 to 255, 63 ± 13, ranging from 89 to 25 (maximum to minimum).

As shown in Table 1, the intrastromal hyper-effective area found with this technique for group A (control) had a mean area of 427.25 ± 137.81 pixels (range, maximum to minimum, 1518.40), corresponding to a mean of 0.74% ± 0.63% (range, 2.50%–0.00%), corresponding to 0.02 mm². This contrasts with group B (KCN), in which the mean hyper-effective area had a mean of 2018.21 ± 1104.70 range, 4927–121, corresponding to a mean of 3.46% ± 1.92% (range, 8.80%–0.18%), or 0.09 mm² of the corneal cross-sectional area. Of the 94 cases examined, 72 had more than 2.50% hyper-effective areas, whereas six were close to and 16 were below this mark. The two groups were found to be statistically different (comparison p < 0.02). The over time development of the extent of the area of demarcation; that is, the CXL area over post-operative time, is plotted in Figure 2.

### Horizontal extent of demarcation

The horizontal extent of demarcation was assessed for the CXL group and was compared to the standard 860 pixel

<table>
<thead>
<tr>
<th>Group A (control)</th>
<th>Group B (KCN)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hyper-reflective area (pixels)</strong></td>
<td><strong>Hyper-reflective area (pixels)</strong></td>
</tr>
<tr>
<td>Mean</td>
<td>427.25</td>
</tr>
<tr>
<td>Min</td>
<td>0</td>
</tr>
<tr>
<td>Max</td>
<td>1518.40</td>
</tr>
<tr>
<td>SD</td>
<td>137.81</td>
</tr>
</tbody>
</table>

**Abbreviations:** KCN, keratoconus; Max, maximum; Min, minimum; SD, standard deviation.

#### Table 1

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Horizontal and axial extent of hyper-reflective area in group B (KCN)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CXL width (pixels)</strong></td>
<td><strong>Horizontal extent</strong></td>
</tr>
<tr>
<td><strong>Overall width</strong></td>
<td><strong>Vertical extent</strong></td>
</tr>
<tr>
<td>Mean</td>
<td>400.35</td>
</tr>
<tr>
<td>Min</td>
<td>150.00</td>
</tr>
<tr>
<td>Max</td>
<td>1104.70</td>
</tr>
<tr>
<td>SD</td>
<td>233.35</td>
</tr>
</tbody>
</table>

**Abbreviations:** KCN, keratoconus; CXL, cross-linking; V, vertical; Max, maximum; Min, minimum; SD, standard deviation.

#### Table 2

<table>
<thead>
<tr>
<th>Figure 2</th>
<th>Demarcation line area as a function of time elapsed since the CXL operation.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Note:</strong> The solid line shows the area of the area demarcation over time. The dotted line shows the mean and standard deviation of the area demarcation over time.</td>
<td></td>
</tr>
<tr>
<td><strong>Abbreviation:</strong> CXL, cross-linking.</td>
<td></td>
</tr>
</tbody>
</table>
quantitatively measured by the extent of the area of the group regarding the presence of a demarcation line, as difference between the control group and the KCN encountered statistically different findings between the study groups. By examining high-resolution corneal OCT images, we found to be 43.81 mm across the image corresponded to 860 pixels, in the 61,770 pixels (cornea cross-section), only 443 correspond to a hyper-reflective area. Example of corneal cross-sectional images examined in the study showing various degrees of demarcation line extent are presented in Figure 5.

Axial extent (depth) of demarcation
The axial extent of demarcation corresponds to what we describe as the depth of the CXL effect. The quantitative assessment is subject to the corneal thickness, which varies significantly among images. In each individual study, the corneal thickness was measured in pixels (vertical line total in Table 2), and was found to correspond to an average of 61.93 ± 8.18 pixels (max to min, 80–38). Considering that 6 mm across the image corresponded to 860 pixels, the 61.93 pixels corneal thickness translates to 452 μm of thickness. Having measured the corneal thickness of each individual section, the distance in pixels (vertical line CXL) from the anterior surface was measured. On average, it was found to be 43.81 ± 6.96 pixels (range, 35–303), corresponding to a 305.6 mm or 70.98% of the total corneal thickness. The over time (postoperative) development of the depth of the area of demarcation, that is to say the cornea area over time, is presented in Figure 3.

Discussion
By examining high-resolution corneal OCT images, we encountered statistically different findings between the treated group (KCN; group B) and the control group (A). It appears that there is a statistically significant difference between the control group and the KCN group regarding the presence of a demarcation line, as quantitatively measured by the extent of the area of the hyper-reflective effect, indicating a localized change in stromal (treated) density over the underlying (untreated) stroma. In 72 of 94 cases, the demarcation line area corresponded to more than 2.50% of the total corneal cross-sectional area, with a mean ± standard deviation of 3.40% ± 1.92%. In the entire control group A, by applying the same luminosity criteria, the similar area had a mean of 0.74% ± 0.63%.

We believe that these pixel counts represent merely signal noise rather than reflect actual changes in stromal density. Thus, we can ascertain that the demarcation line was viewed by OCT can be a good indication of the extent of collagen density changes induced by CXL.

To better understand this phenomenon, we measured the axial extent of demarcation line over time (postoperative) development. The depth of the demarcation line area, found to be on average of 105 μm, is consistent with the accepted notion that effective treatment occurs only in the first 300 μm of the corneal stroma. The depth of the demarcation line depth (relative to the corneal depth) is associated with thinner corneal thickness, as measured postoperatively. In the selected 12 thinner corneas, the depth of the demarcation area was found to be 83% of the total corneal thickness.

One clinical example of ineffective CXL action is presented in Figure 4, in which a case of a cornea treated in another institution with epithelium-on-CXL technique demonstrated minimal signs of hyper-reflective areas. This case, which was not part of the case study, was presented to our practice with progressive ectasia following operation, at approximately 70% of the corneal depth. However, a deeper demarcation line depth (relative to the corneal depth) is associated with thinner corneal thickness, as measured postoperatively. In the selected 12 thinner corneas, the depth of the demarcation area was found to be 83% of the total corneal thickness.

Conclusion
AS-OCT appears to demonstrate reproducible early (1 month) and long-term (up to 3 years) CXL cornea findings. The hyper-reflective lines may represent induced cornea density changes or sub-basal intrastromal cornea scarring. This novel quantitative and qualitative technique may constitute a possible benchmark for a noninvasive measurement to evaluate and treat the amount, extent, and depth of intrastral effects of the CXL treatment in KCN and possibly ectasia eyes. Disclosure
The authors report no conflicts of interest in this work.

References