Correlation of the Corneal Collagen Cross-Linking Demarcation Line Using Confocal Microscopy and Anterior Segment Optical Coherence Tomography in Keratoconic Patients

GEORGE D. KYMIONIS, MICHAEL A. GRENTZELOS, ARGYRO D. PLAKA, KONSTANTINOS I. TSOULNARAS, VASILIOS F. DIAKONIS, DIMITRIOS A. LIAKOPOULOS, VARDHAMAN P. KANKARIYA, AND ARISTOPHANIS I. PALLIKARIS

• PURPOSE: To evaluate and compare the depth of the corneal stromal demarcation line after corneal collagen cross-linking (CXL) using 2 different methods: confocal microscopy and anterior segment optical coherence tomography (AS OCT).

• DESIGN: Prospective, comparative, interventional case series.

• METHODS: Seventeen patients (18 eyes) with progressive keratoconus were enrolled. All patients underwent uneventful CXL treatment according to the Dresden protocol. One month after surgery, corneal stromal demarcation line depth was measured in all patients by 2 independent observers using confocal microscopy and AS OCT.

• RESULTS: Mean corneal stromal demarcation line depth measured using confocal microscopy by the first observer was 306.22 ± 51.54 μm (range, 245 to 417 μm) and that measured by the second observer was 303.5 ± 46.98 μm (range, 240 to 390 μm). The same measurements using AS OCT were 300.67 ± 41.56 μm (range, 240 to 385 μm) and 295.72 ± 41.01 μm (range, 228 to 380 μm) for the first and second observer, respectively. Pairwise comparisons did not reveal any statistically significant difference between confocal microscopy and AS OCT measurements for both observers ($P = .3219$ for the first observer and $P = .1731$ for the second observer).

• CONCLUSIONS: Both confocal microscopy and AS OCT have similar results in evaluating the depth of the corneal stromal demarcation line after CXL. (Am J Ophthalmol 2014;157:110–115. © 2014 by Elsevier Inc. All rights reserved.)

CORNEAL COLLAGEN CROSS-LINKING (CXL) IS A minimally invasive surgical treatment used to increase corneal strength (corneal stiffness and stiffening effect) and to stabilize the ectatic cornea leading to inhibition of keratoconus progression.[1,2] Several clinical and laboratory studies already have reported the stiffening effect of CXL treatment.[2–9]

A corneal stromal demarcation line is detectable on slit lamp as early as 2 weeks after CXL at a depth of approximately 300 μm.[10] The corneal stromal demarcation line after CXL also can be detected using confocal microscopy and anterior segment optical coherence tomography (AS OCT).[11–14] Confocal microscopy after CXL has demonstrated a series of corneal macrostructural alterations, which include an absence of corneal keratocytes (acellular zone) up to a depth of approximately 300 μm.[11,15] The depth of the acellular zone has been correlated with the effective depth of the CXL treatment.[11] The corneal stromal demarcation line after CXL also can be evaluated using AS OCT as a hyperreflective line within the corneal stroma; its depth also has been associated with the effectiveness of CXL treatment.[12–14]

Until now, the corneal stromal demarcation line has been evaluated independently using either confocal microscopy or AS OCT. Therefore, whether the corneal stromal demarcation line visualized using AS OCT represents the transition zone between acellular (treated) and cellular (untreated) corneal stroma using confocal microscopy after CXL remained to be determined. The aim of this study was to evaluate and compare the depth of the corneal stromal demarcation line after CXL using 2 different methods, AS OCT and confocal microscopy.

METHODS

INSTITUTIONAL REVIEW BOARD APPROVAL FROM UNIVERsity of Crete for this prospective study was obtained, and all patients were informed appropriately before their participation in the study and gave written informed consent in accordance with institutional guidelines, according to the tenets of the Declaration of Helsinki.

• PATIENT POPULATION: In this prospective, comparative, interventional case series, 17 patients (12 men and 5 women; 18 eyes) with progressive keratoconus who

Accepted for publication Sep 11, 2013.

From the Vardinoyiannion Eye Institute of Crete, Faculty of Medicine, University of Crete, Heraklion, Crete, Greece.

Inquiries to George D. Kymionis, Vardinoyiannion Eye Institute of Crete, Faculty of Medicine, University of Crete, 71003 Heraklion, Crete, Greece; e-mail: kymionis@med.uoc.gr

0002-9394/$36.00
http://dx.doi.org/10.1016/j.ajo.2013.09.010

underwent CXL were included. The clinical diagnosis of keratoconus was based on corneal topography data (Technomed C-Scan [Baesweiler, Germany], iTrace [Tracey Tech, Houston, Texas, USA] or both), stromal thinning, and conical protrusion. Inclusion criteria were progressive keratoconus, patient age older than 18 years, and corneal thickness more than 400μm. Exclusion criteria were history of intraocular or corneal surgery, herpetic keratitis, pregnancy, lactation, and other cornea or anterior segment pathologic signs. Keratoconus was described as progressive when there was an increase in the cone apex keratometry of 0.75 diopters (D) or alteration of 0.75 D in the spherical equivalent refraction in the previous 6 months.

Data obtained from the patient records included age, sex, AS OCT scan results (Visante OCT 3.0; Carl Zeiss Meditec, Inc, Jena, Germany) and confocal microscopy findings (HRT II; Heidelberg Engineering, Heidelberg, Germany) at 1 month after CXL.

• **SURGICAL TECHNIQUE:** All procedures were performed by the same surgeon (G.D.K.) under sterile conditions. After topical anesthesia with proxymetacaine hydrochloride 0.5% eyedrops (Alcaine; Alcon Laboratories, Inc, Fort Worth, Texas, USA) was applied, the corneal epithelium was removed mechanically using a rotating brush within an 8.0- to 9.0-mm diameter. After epithelial removal, riboflavin (0.1% solution of 10 mg riboflavin-5-phosfate in 10 ml dextran-T-500 20% solution; Medicross, Medio-Haus, Behrensbrook, Neudorf, Germany) was instilled on the center of the cornea every 3 minutes for approximately 30 minutes. Ultraviolet A (UVA) irradiation was performed using a commercially available UVA optical system (UV-X illumination system version 1000; IROC, Zurich, Switzerland). Before treatment, an intended irradiance of 3.0 mW/cm^2 was calibrated using the UVA light meter YK-34UV (Lutron Electronic, Enterprise Co, Ltd, Taipei, Taiwan), which is supplied with the UV-X device. Irradiance was performed for 30 minutes, corresponding to a total surface dose of 5.4 J/cm^2. During UVA irradiation, riboflavin solution was applied every 3 minutes to maintain corneal saturation with riboflavin. At the end of the procedure, a silicone-hydrogel bandage contact lens (14.0-mm diameter; 8.6 base curvature; Dk = 140 barriers; Lotrafilcon B, Air Optix, Ciba Vision, Duluth, GA, USA; 30-day recommended replacement) was applied until full re-epithelialization.

Postoperative medication included nepafenac suspension 0.1% (Nevanac, Alcon Laboratories, Inc, Athens, Greece) for 2 days and chloramphenicol/dexamethasone drops (Dispersadron C; Thea Laboratories, Inc, Athens, Greece) 4 times daily until the removal of the bandage contact lens. After the removal of the contact lens, patients received corticosteroid drops (FML 0.1%; Falcon Pharmaceuticals, Athens, Greece) tapering for the next 2 weeks. Patients were encouraged to use artificial tears at least 6 times daily for 3 months after surgery.

• **CONFOCAL MICROSCOPY:** Confocal microscopy was performed using a modified confocal scanning laser ophthalmoscope (HRT II) in all patients. With the addition of the Rostock Cornea Module, the HRT II was converted into a confocal corneal microscope that allowed the acquisition of 2-dimensional images of the various layers of the cornea by sequentially scanning a 670-nm laser beam over the cornea. After topical anesthesia with proxymetacaine hydrochloride 0.5% eyedrops (Alcaine; Alcon Laboratories, Inc) and instillation of eye high-viscosity gel (carbomer 3.0 mg/g; Thilogel; Alcon Laboratories, Inc), patients were asked to fixate using an external fixation target. The instrument objective then was brought into optical contact with the corneal tissue by a disposable sterile polymethyl methacrylate cup and a high-viscosity gel (carbomer 3.0 mg/g; Thilogel). Depth scans across the entire cornea were performed manually while an external electronic unit kept track of the focal plane. The external interface of the polymethyl methacrylate cup was taken as the reference point for thickness measurements. Images of the various layers of the cornea were acquired at the optical center of the cornea. The acquired images consisted of 384 × 384 pixels over a 400 × 400-μm field of view with a transversal resolution of approximately 2 μm and a longitudinal resolution of approximately 4 μm. The central corneal depth of the transition area between the acellular and cellular zones was assessed using the software provided by Heidelberg Engineering by the same 2 independent observers (M.G. and A.P.).

• **ANTERIOR SEGMENT OPTICAL COHERENCE TOMOGRAPHY:** All scans were performed under the same light conditions. All patients were asked to fixate at the optical target in the system. The image was captured when the corneal reflex, a vertical white line along the center of the cornea, was visible. The high-resolution corneal scan was used to produce an enhanced image of the cornea on the horizontal meridian (0–180). The corneal stromal demarcation line was identified in the enhanced corneal image and the demarcation line depth was measured using the flap tool as provided by the manufacturer. The demarcation line depth was measured by two independent observers (M.A.G. and A.D.P.). The visibility of the demarcation line was scored to obtain accuracy of the measurements (0 = line not visible; 1 = line visible, but measurement not very accurate; 2 = line clearly visible). Only measurements with scores of 2 were included in the study.

• **STATISTICAL ANALYSIS:** All data were collected in an Excel spreadsheet (Microsoft, Redmond, Washington, USA). Stata software version 12.0 (StataCorp LP, Lakeway Drive, Texas, USA) was used for statistical analysis of the results. Continuous variables are presented as mean ± standard deviation. A sample size analysis (G*Power 3.1.3; Franz Paul, Universität Kiel, Germany) was performed for a