Long-term Results of Riboflavin Ultraviolet A Corneal Collagen Cross-linking for Keratoconus in Italy: The Siena Eye Cross Study

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• PURPOSE: To report the long-term results of 44 keratoconic eyes treated by combined riboflavin ultraviolet A collagen cross-linking in the first Italian open, nonrandomized phase II clinical trial, the Siena Eye Cross Study.

• DESIGN: Perspective, nonrandomized, open trial.

• METHODS: After Siena University Institutional Review Board approval, from September 2004 through September 2008, 363 eyes with progressive keratoconus were treated with riboflavin ultraviolet A collagen cross-linking. Forty-four eyes with a minimum follow-up of 48 months (mean, 52.4 months; range, 48 to 60 months) were evaluated before and after surgery. Examinations comprised uncorrected visual acuity, best spectacle-corrected visual acuity, spherical spectacle-corrected visual acuity, endothelial cells count (I Konan, Non Con Robo; Konan Medical, Inc., Hyogo, Japan), optical (Visante OCT; Zeiss, Jena, Germany) and ultrasound (DGH; Pachette, Exton, Pennsylvania, USA) pachymetry, corneal topography and surface aberrometry (CSO EyeTop, Florence, Italy), tomography (Orbscan IIz; Bausch & Lomb Inc., Rochester, New York, USA), posterior segment optical coherence tomography (Stratus OCT; Zeiss, Jena, Germany), and in vivo confocal microscopy (HRT II; Heidelberg Engineering, Rostock, Germany).

• RESULTS: Keratoconus stability was detected in 44 eyes after 48 months of minimum follow-up; fellow eyes showed a mean progression of 1.5 diopters in more than 65% after 24 months, then were treated. The mean *K* value was reduced by a mean of 2 diopters, and coma aberration reduction with corneal symmetry improvement was observed in more than 85%. The mean best spectacle-corrected visual acuity improved by 1.9 Snellen lines, and the uncorrected visual acuity improved by 2.7 Snellen lines.

• CONCLUSIONS: The results of the Siena Eye Cross Study showed a long-term stability of keratoconus after cross-linking without relevant side effects. The uncorrected visual acuity and best spectacle-corrected visual acuity improvements were supported by clinical, topographic, and wavefront modifications induced by the treatment. (Am J Ophthalmol 2010;149:585–593. © 2010 by Elsevier Inc. All rights reserved.)

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KERATOCONUS IS A DEGENERATIVE, NONINFLAMMATORY disease of the cornea.[1] Changes in corneal collagen structure and organization, alterations of the extracellular matrix,[2] as well as keratocyte apoptosis involving the anterior stroma and Bowman lamina partially explain the biomechanical corneal weakening typical of the disease.[3] Biochemical alterations with increased expression of proteolytic enzymes and decreased concentrations of protease inhibitors, decreased stromal thickness, and modified configuration of collagen lamellae also have been reported among the pathophysiologic mechanisms of keratoconus.[4] The technique of corneal collagen cross-linking,[5] conceived in Dresden,[6] consists of a photopolymerization of stromal collagen fibers induced by the combined action of a photosensitizing substance (riboflavin or vitamin B_2) and ultraviolet (UV) A light that induces corneal stiffening by increasing the number of intrafibrillar and interfibrillar covalent bonds and corneal collagen resistance against enzymatic degradation.[7,8] This therapy was introduced for the first time in Italy by us[9] in 2004 at the Department of Ophthalmology of Siena University to slow the progression of keratoconus and to reduce the demand for penetrating keratoplasty.

METHODS

TO DATE, 363 EYES WITH PROGRESSIVE KERATOCONUS have been treated in Siena with the riboflavin UV A corneal collagen cross-linking procedure. The Siena Eye Cross Study[9,10] (phase II nonrandomized open trial) included 44 patients with keratoconus between 10 and 40 years of age with disease progression documented clinically and instrumentally in the last 6 months, minimum corneal thickness of 400 μm in the thinnest point evaluated by Orbscan IIz (Bausch & Lomb, Inc., Rochester, New York, USA), topographic mean *K* value of less than 55 diopters (D), clear cornea by slit-lamp examination (absence of Vogt striae, subepithelial, and stromal scars), and absence

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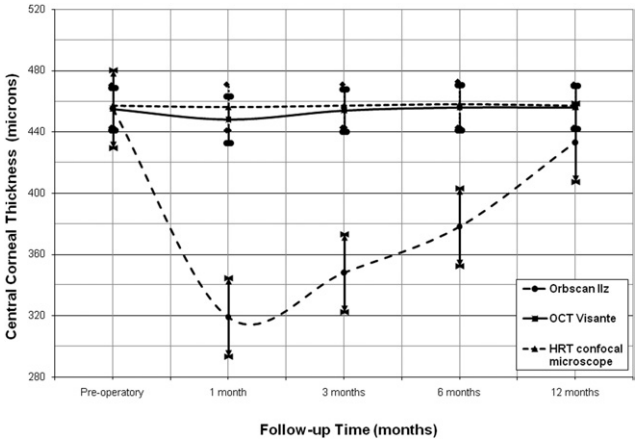

FIGURE 1. Graph showing comparative pachymetric findings recorded with Orbscan IIz, Visante OCT, and HRT II confocal microscope. Postoperative Orbscan measurement showed a significant underestimation of corneal thickness with a mean value of −120 μm in the first 6 months and −70 μm between 6 and 12 months (broken line), compared with confocal microscopic postoperative pachymetric examinations (dotted line). Postoperative confocal pachymetry data did not show significant differences with respect to preoperative values. Visante OCT differential pachymetry (unbroken line) confirmed the statistically nonsignificant difference between preoperative and postoperative corneal thickness after riboflavin UV A-induced corneal collagen cross-linking.

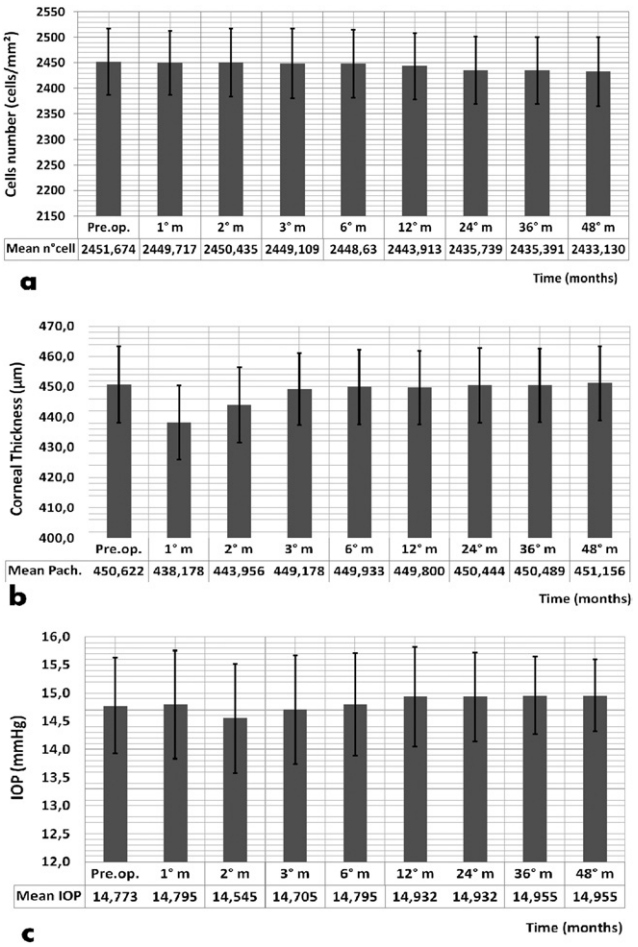

FIGURE 2. Graphs showing endothelial cell counts, central corneal pachymetry, and intraocular pressure (IOP) during follow-up. (Top) No statistically significant reduction in endothelial cells count was observed after treatment. (Middle) Postoperative ultrasound pachymetry did not show significant differences between preoperative (Pre.op.) and postoperative corneal central thickness. (Bottom) No statistically significant modifications were observed in IOP during the entire follow-up.

of eye infections, herpetic clinical history, autoimmune disease, and pregnancy. All patients reported in this series were enrolled in the first 6 months of the study and reached a minimum follow-up of 48 months (mean, 52.4 months; range, 48 to 60 months). Statistical analysis was conducted by the Mann–Whitney *U* test for nonparametric data (uncorrected visual acuity [UCVA] and best spectacle-corrected visual acuity [BSCVA]) and by the paired *t* test for parametric data (refraction, mean curvature power, central corneal thickness, and intraocular pressure [IOP]). Patients gave their specific informed consent before inclusion in the study. Riboflavin UV A corneal collagen cross-linking was performed according to the Siena protocol[10] using the CSO Vega CBM X linker (CSO, Florence, Italy), developed in Italy at the Department of Ophthalmology of Siena University by Mazzotta and associates.[10] The treatment was conducted under topical anesthesia (4% lidocaine drops). After applying the eyelid speculum, a 9-mm diameter marker was used to mark the corneal epithelium in a central circle, then epithelium was removed with a blunt metal spatula. After epithelial scraping, a disposable solution of riboflavin 0.1% and dextrane 20% (Ricrolin Sooft, Montegiorgio, Italy) was instilled for 15 minutes of corneal soaking before starting UV A irradiation. The riboflavin and dextrane solution was administered every 2 minutes for a total of 30 minutes of UV A exposure (3 mW/cm^2). Treated eyes were dressed with a soft contact lens bandage for 4 days and were medicated with antibiotics (ofloxacin drops 4 times/day), nonsteroidal anti-inflammatory drugs (diclofenac drops 4 times/day), and lacrimal substitutes (phospholipidic microemulsion drops tapered 4 times/day). In the first postoperative month, after therapeutic corneal lens removal, fluorometholone 0.2% drops (4 times/day) and lacrimal substitutes (phospholipidic microemulsion drops, 4 times/day) were administered for 4 to 6 weeks. Preoperative and follow-up examinations included: UCVA, BSCVA, spherical spectacle-corrected visual acuity measured by Snellen decimal equivalent, biomicroscopy (CSO, 990 SL, Florence, Italy), endothelial cells count (I Konan, Non Con Robo V; Konan Medical, Inc., Hyogo, Japan), IOP (Medtronic Tono-Pen; Medtronic Ophthalmics, Jacksonville, Florida, USA), optical pachymetry (Visante OCT; Zeiss, Jena, Germany), ultrasound

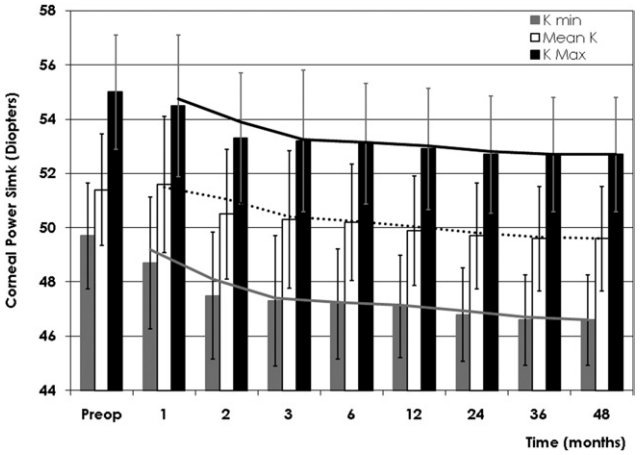

FIGURE 3. Graph showing computerized corneal topography results. Postoperative topographic analysis showed a mean *K* reading reduction of −1.96 diopters (D) after 1 year, −2.12 D after 2 years, −2.24 D after 3 years, and −2.26 D after 4 years of follow-up (white bars). In the first postoperative month, mean *K* showed a worsening that reasonably might have been the result of the initial epithelial thinning and relative topographic reading. After the third postoperative month, mean *K* improved progressively, reaching stability between 24 and 48 months of follow-up. A constant reduction in K_{max} (black bars) and K_{min} (gray bars) was recorded after the first 3 postoperative months.

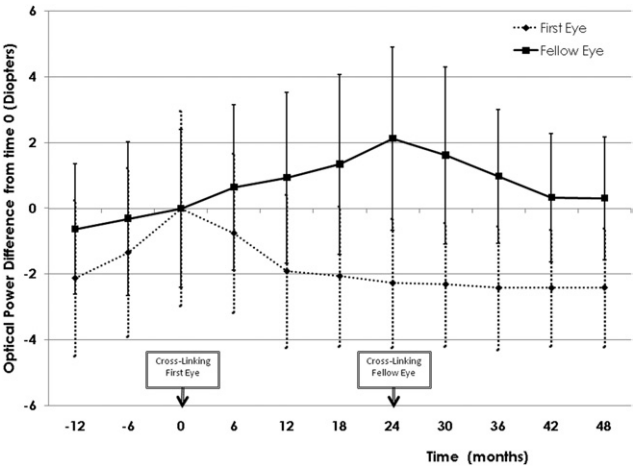

FIGURE 4. Graph showing comparative analysis of central dioptric power between cross-linked and fellow eyes (control group). The comparative topographic study of untreated fellow eyes (continuous black line) used as control group for 24 months showed a mean *K* increase of +1.2 diopters (D) and +2.2 D after 1 and 2 years of follow-up, respectively, versus a similar decrease in dioptric power in treated eyes (dotted black line). Fellow control eyes were cross-linked after 24 months of observation (see arrow) and showed a decrease in corneal power identical to that observed after treatment of first eyes in the first and second year of follow-up.

(US) pachymetry (DGH Pachette; DGH Technology, Inc., Exton, Pennsylvania, USA), corneal topography (CSO EyeTop, Florence, Italy) and tomography (Orbscan IIz; Bausch & Lomb, Inc.), surface aberrometry (CSO EyeTop), macular optical coherence tomography (Stratus OCT; Zeiss, Jena, Germany), and in vivo confocal microscopy (HRT II, Rostock Cornea Module; Heidelberg Engineering, Rostock, Germany).

RESULTS

THE MEAN PREOPERATIVE PACHYMETRIC VALUE MEASURED by central US pachymetry was 450 ± 14.54 μm (range, 422 to 512 μm) and that by optical Orbscan IIz system in the thinnest point was of 438 ± 13.87 μm (range, 408 to 503 μm). During follow-up, the Orbscan II z measurement showed significant underestimation of corneal thickness compared with US and confocal microscopic pachymetric examinations, with a mean of −120 μm in the first 6 months and −70 μm between 6 and 12 months. US and confocal postoperative pachymetric data did not show significant differences with respect to preoperative values. Because the Visante OCT system became available in our department 24 months ago, we checked the same data measured by US and confocal pachymetric examinations, confirming that preoperative and postoperative thickness data were superimposable (Figure 1).

Preoperative mean endothelial cell density was 2451 ± 130.444 cells/mm^2 (range, 2092 to 3016 cells/mm^2). A statistically insignificant reduction in endothelial cells count with respect to physiologic reduction was observed after treatment, namely a mean of 2% per year (Figure 2, Top). Preoperative mean US central corneal pachymetry was 450 ± 14.54 μm (range, 422 to 512 μm). Statistically nonsignificant corneal thinning was recorded in the first 2 postoperative months (438.177 ± 15.118 μm in the first month and 443.955 ± 15.349 μm in the second month). No statistically significant difference in central corneal thickness measured by US pachymetry was observed over the third 3-month of follow-up (Figure 2, Middle). Mean preoperative IOP measured by Tono-Pen II XL (Medtronic, Jacksonville, Florida, USA) was 14.773 ± 1.696 mm Hg (range, 11 to 18 mm Hg). No statistically significant modifications were observed in IOP values during the entire follow-up (Figure 2, Bottom).

No persistent early or late side effects were observed after the cross-linking procedure. Stromal edema, clinically detectable by slit-lamp examination in 70% of patients, occurred in the first 30 postoperative days. Temporary haze occurred in 9.8% of cases, 14 cases in the first 3 months and 2 cases after 6 months, disappearing progressively after topical preservative-free steroid therapy (fluorometholone preservative-free drops for 1 to 3 months). No delayed re-epithelialization or endothelial damage was detected during follow-up. No adverse events were recorded during the mean follow-up of 52.4 months (range, 48 to 60

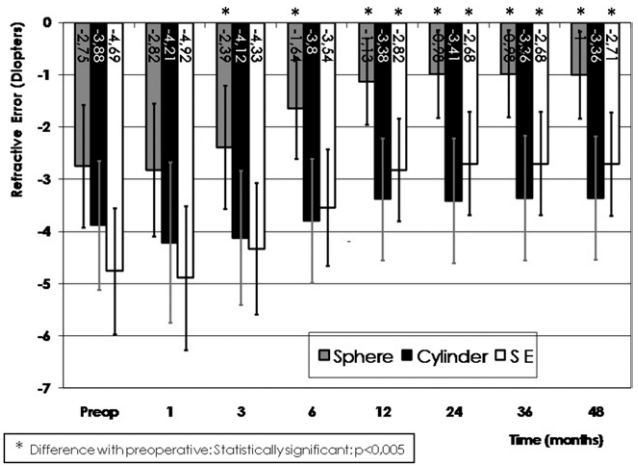

FIGURE 5. Graph showing refractive results after riboflavin ultraviolet A-induced corneal collagen cross-linking. Spherical refraction (gray bars) calculated in the spectacle plane showed a mean hyperopic shift of +1.62 diopters (D) at 1 year of follow-up (range, 0 to +3.75 D), increasing to +1.87 D (range, +0.25 to +3.75 D) after 2 years, and maintaining this value in the longer period (+1.86 after 3 years and +1.87 after 4 years). Cylinder refraction (black bars) showed an apparent postoperative increase in the first 3 months of follow-up, then a mean reduction of −0.52 D after 1 year, −0.53 D after 2 years, −0.53 D after 3 years, and −0.55 D after 4 years. Cylinder values were not statistically significant during the entire follow-up. Spherical equivalent (SE; white bars), increased in the first postoperative month, then showed hyperopic shift with the following values after 1, 2, 3, and 4 years, respectively: +1.87 D (range, +0.5 to +4.37 D), +2.12 D (range, +0.5 to +4.00 D), +2.13 D (range, +0.5 to +4.12 D), and +2.15 D (range, +0.87 to +4.12 D). The reduction in SE values became statistically significant after 12 months.

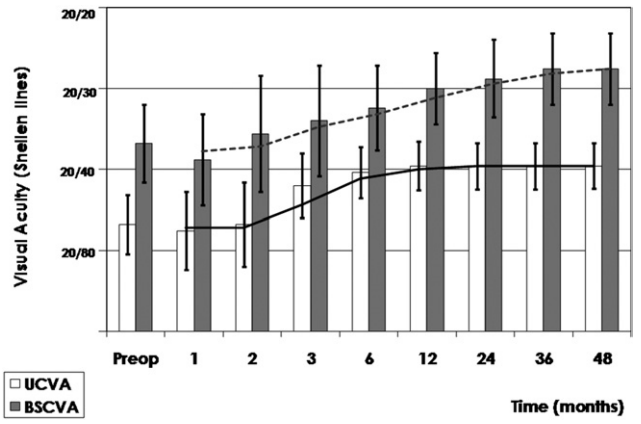

FIGURE 6. Graph showing visual performance during follow-up. After cross-linking, uncorrected visual acuity (UCVA; white bars) improved by a mean of +2.41 Snellen lines after 12 months, +2.75 lines after 24 months, +2.80 lines after 36 months, and +2.85 lines after 48 months. Postoperative best spectacle-corrected visual acuity (BSCVA; grey bars) improved by a mean of +1.34 Snellen lines after 12 months, +1.93 lines after 24 months, +1.91 lines after 36 months, and +2.03 lines after 48 months. Preop = preoperative.

months). Topographic analysis conducted 1 year after treatment by the CSO EyeTop system showed a mean reduction of −1.96 ± 0.63 D (range, −0.92 to −3.24 D) in mean *K* readings. The mean *K* reduction increased to −2.12 ± 0.65 D after 2 years, −2.24 ± 0.61 D after 3 years, and −2.26 ± 0.68 D after 4 years of follow-up (Figure 3). On the contrary, comparative topographic study of fellow eyes (control group) showed a mean *K* increase of +1.2 ± 0.96 D and 2.2 ± 1.24 D, respectively, after 1 and 2 years, against a similar decrease in mean *K* power in treated eyes. Fellow control eyes (initially untreated) were treated after 24 months of observation and showed a decrease in corneal power similar to that observed after treatment of the first (worst) eyes in the first and second year of follow-up (Figure 4). According to topographic data, the decrease in corneal curvature and spherical refraction, calculated in the spectacle plane, showed a mean hyperopic shift of +1.62 ± 1.03 D at 1 year of follow-up (range, 0 to 3.75 D), increasing at +1.87 ± 1.06 D (range, 0.25 to 3.75 D) after 2 years and maintaining this value in the longer period (+1.86 ± 0.97 D after 3 years and 1.87 ± 0.98 D after 4 years). Cylinder refraction showed a similar course, but with a reduction smaller than the spherical one: at 1 year, we observed a mean reduction of −0.52 ± 0.38 D (range, 0.75 to −2 D)

FIGURE 7. Graph showing topographic analysis of corneal symmetry using the superior–inferior index (SI). An improvement in corneal symmetry was documented starting from the third postoperative month through reduction in the mean difference (rainbow bars) between the superior and inferior hemimeridians index (SI) produced automatically by the CSO EyeTop corneal topographer. Each colored line indicates the course of single cases. The thick black-white dotted line indicates the group trend calculated in 2 consecutive periods. During follow-up, a statistically significant improvement of corneal symmetry calculated by the superior–inferior index (SI) was recorded starting from the third postoperative month. Preop = preoperative.

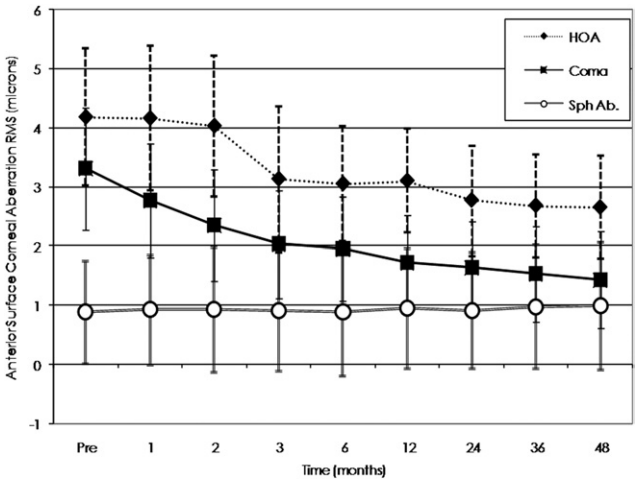

FIGURE 8. Graph showing anterior corneal surface aberrometry after corneal cross-linking. A statistically significant reduction in coma aberration (black solid line, square dot) between preoperative (pre) and postoperative values ($P = .032$, paired t test on 44 eyes) was recorded just 1 month after treatment and showed a constant statistically significant increase up to 48 months of observation. Starting from the third postoperative month, a reduction in total wavefront higher order aberrations (HOAs) was detected (black dotted line, rhombus dot), starting with a small, statistically significance difference that became highly significant after 1 year and increased 2 years after cross-linking. No more significant changes in HOA data were observed at 3 and 4 years of follow-up. No significant modifications of spherical aberration (SphAb.; double thin line, round white dot) were recorded during follow-up.

that maintained similar values after 2, 3, and 4 years: −0.53 ± 0.37 D, −0.53 ± 0.38 D, and −0.55 ± 0.38 D, respectively.

The spherical equivalent resulted in hyperopic shift with the following values at 1, 2, 3, and 4 years of follow-up, respectively: +1.87 ± 1.24 D (range, 0.5 to 4.37 D), +2.12 ± 1.27 D (range, 0.5 to 4.00 D), +2.13 ± 1.12 D (range, 0.5 to 4.12 D), and 2.15 ± 1.19 D (0.87 to 4.12 D; Figure 5). Statistical analysis with the paired t test showed a significant difference in sphere values between preoperative evaluation and 6, 12, 24, and 48 months of follow-up (P value, 3.7×10^{-6} at 6 months, 1.8×10^{-7} at 12 months, 1.4×10^{-9} at 24 months, 1.1×10^{-9} at 36 months, and 5.1×10^{-10} at 48 months; Figure 5).

UCVA improved by a mean of +2.41 ± 0.88 Snellen lines after 12 months, +2.75 ± 0.79 Snellen lines after 24 months, +2.80 ± 0.76 Snellen lines after 36 months, and +2.85 ± 0.81 Snellen lines after 48 months (Figure 6). BSCVA improved by a mean of +1.34 ± 1.13 Snellen lines after 12 months, +1.93 ± 1.04 Snellen lines after 24 months, +1.91 ± 1.03 Snellen lines after 36 months, and +2.03 ± 1.04 Snellen lines after 48 months (Figure 6).

Clinical and topographic improvements were recorded between the second and third month after the operation and continued thereafter, reaching reliable stability after 24 months. Refractive stability was confirmed in our series after 4 years without progression of keratoconus. A major finding recorded in all treated eyes during follow-up was reduction in the difference between superior and inferior hemimeridians (flattest vs steeper) expressed by preoperative and postoperative comparative values of the topographic symmetry index (Figure 7).

Statistical analysis comparing the preoperative and postoperative superior–inferior index provided by the CSO EyeTop system showed a nonsignificant reduction in this parameter in the first 3 months ($P = .136$ after 1 month; $P = .053$ after 3 months), whereas the reduction in superior–inferior topographic index became statistically significant after 6 to 48 months with a constant increase (from $P = 1.8 \times 10^{-4}$ comparing the preoperative and sixth postoperative month, to $P = 2.4 \times 10^{-8}$ comparing preoperative and forty-eighth postoperative month; Figure 7). Other important data provided by topographic analysis were the surface wavefront results (Figure 8). A statistically significant reduction in coma aberration between preoperative and postoperative values ($P = .032$, paired t test on 44 eyes) was recorded 1 month after treatment with a constant statistically significant increase up to 24 months of observation ($P = 1.4 \times 10^{-8}$, paired t test on 44 eyes). The statistical significance of these values was maintained at 36 and 48 months of follow-up. After the third postoperative month, a reduction in total wavefront higher order aberrations was recorded, starting with a low statistical significance ($P = .044$, paired t test on 44 eyes), which became high at 1 year ($P = 2.5 \times 10^{-5}$, paired t test on 44 eyes) and further increase at 2 years ($P = 1.3 \times 10^{-5}$). Nonsignificant changes in these data were observed at 3 years ($P = 3.6 \times 10^{-7}$) without appreciable modification at 4 years of follow-up ($P = 4.3 \times 10^{-7}$). We did not observe any significant changes in spherical aberration in our analysis (Figure 8).

DISCUSSION

RIBOFLAVIN UV A CORNEAL COLLAGEN CROSS-LINKING CURrently represents the only pathogenetic approach to progressive keratoconus to delay its progression[6,9,10] and to reduce the need for donor keratoplasty. The long-term results recorded in the Siena Eye Cross Study confirm another encouraging long-term report in the literature[11] and our pilot study[9] on riboflavin UV A-induced corneal collagen cross-linking, as far as safety and effectiveness are concerned.[12] Analyzing the results, an important finding was the pachymetric evaluation. Optical pachymetric data recorded by Orbscan analysis in the first 3 postoperative months was affected negatively by epithelial thinning, corneal subedema, and keratocytes loss in the anterior–mid stroma[13] determining a pseudoreduction in postoperative corneal thickness resulting from a measurement underestimation. Beyond the third postoperative month,

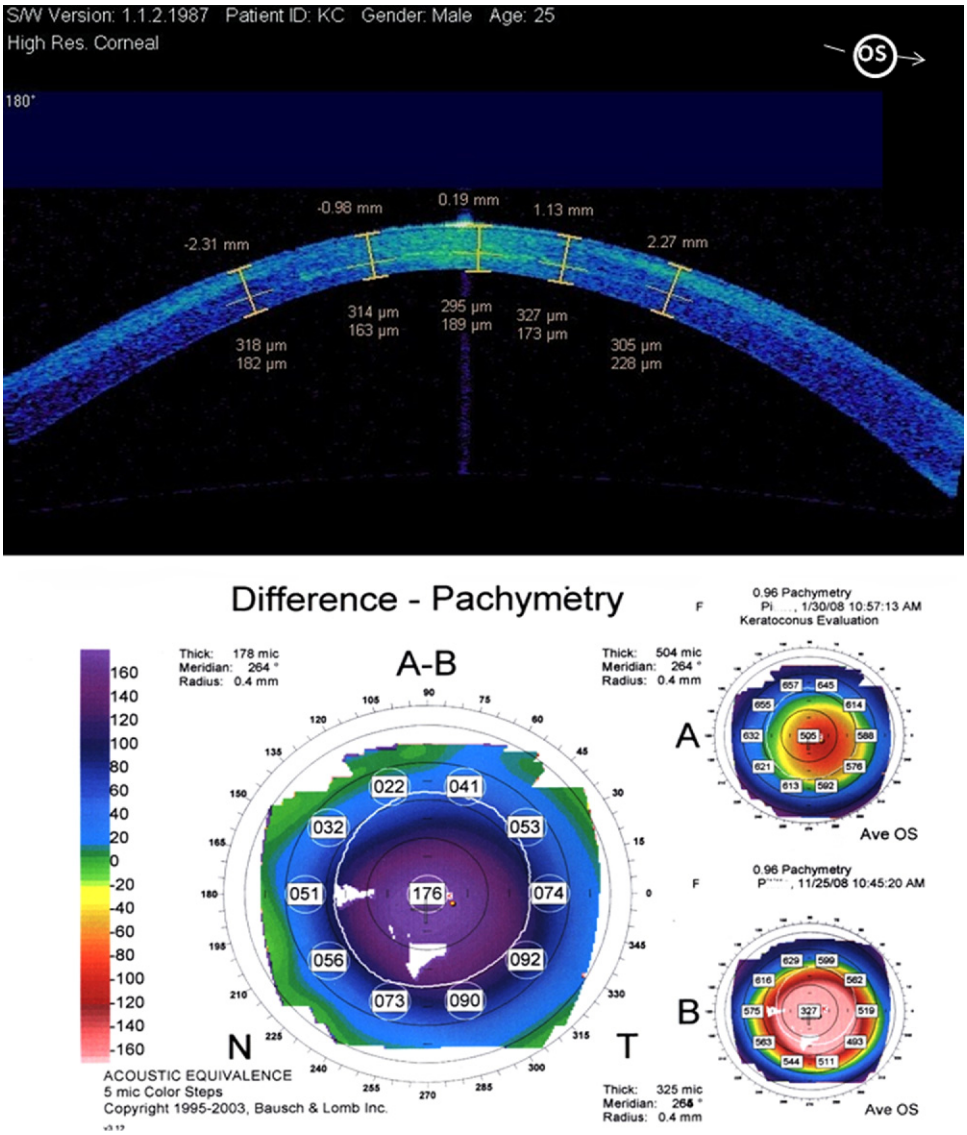

FIGURE 9. Comparative optical corneal thickness measurements after corneal collagen cross-linking. (Top) Visante OCT high-resolution image showing a detectable hyperreflective demarcation line between the anterior mid and deep stroma at a mean depth of approximately 310 µm with a corneal thinnest point of 484 µm. (Bottom) Pachymetric difference detected by the Orbscan IIz system in the same eye (3 months after the treatment) showing a significant underestimation of corneal thickness (−176 µm), presumably related to nonhomogeneous changes in stromal reflectivity. (Bottom right) Orbscan II B-map showing a postoperative central thickness of 327 µm, very near the depth value of the demarcation line indicated by the Visante OCT image.

reduction seemed also to be influenced by changes in stromal reflectivity documented, after cross-linking procedure, by in vivo confocal analysis at early and late follow-up.[10,13] Confocal data and US pachymetry seemed to be less influenced by corneal collagen cross-linking–induced stromal microstructural changes, providing more reliable postoperative pachymetric measurements.[10,13,14] Indeed, according to our results, there were no statistically significant differences between preoperative and postoperative corneal thickness after the riboflavin UV A corneal collagen cross-linking procedure. These data are confirmed by the Visante OCT differential pachymetric analysis that demonstrated insignificant differences between preoperative and post operative thickness measurements, also in the first 3 months of evaluation. These data reasonably may be influenced by the accurate measurement provided by high wavelength optical systems.[15] The Visante OCT (1350-nm wavelength and partially coherent light) is an easy noncontact system for postoperative pachymetric evaluation after cross-linking, relatively unaffected by limitations of other optical sources using white or blue light. White light seems to be influenced negatively by multiple scattering, and blue light seems to be influenced negatively by the short wavelength (420 to 440 nm).[15]

Light transmission in these cases is modified by the nonhomogeneous optical changes induced by corneal col-

lagen cross-linking, and this finding may explain the underestimation of corneal thickness recorded in the early postoperative period. Moreover, according to our unpublished observations, there is a high probability that the underestimation recorded by Orbscan optical analysis is related to the presence of a demarcation line between treated and untreated stroma, which was evident clearly in confocal analysis[10] and Visante OCT scans (Figure 9). The higher reflectivity (hyperdensity) of this line should be interpreted as a pseudoposterior corneal surface. In this context, the pachymetric value provided by Orbscan should express the penetration depth of the treatment. In our experience, there are significant correlations for these data with the depth of the demarcation line observed and measured by Visante OCT and in vivo confocal microscopy[10,13] (± 20 μm). The demarcation is especially visible in the first 6 months after treatment. Over sixth months, stromal reflectivity becomes more homogeneous, reducing the visibility of the line in some eyes much more than in others with increasing of the Orbscan II pachymetric reading.

Even if an in vitro study[16] on human corneas reported an overestimation of true IOP values measured by Goldmann applanation tonometer after cross-linking, no statistically significant modifications were observed in the IOP values measured in vivo by the Tono-Pen XL during the entire follow-up of this study. The procedure demonstrated few side effects for the cornea, both in early and late biomicroscopic controls. Biomicroscopically evident stromal edema occurred in the first 4 to 6 postoperative weeks in 70% of treated eyes. Subclinical corneal edema was evident in 100% of cases by confocal microscopy in the first postoperative 3 months without increasing postoperative corneal pachymetry values.[10] Temporary haze[17] with early onset in the first 3 postoperative months occurred in 9.8% of cases, with slight negative influences on visual performance (mean loss of 1.5 Snellen lines in UCVA and BSCVA; range, 1 to 3 lines) confined to the first 1 to 3 months of clinical evaluation and disappearing progressively after topical preservative-free steroid therapy (fluorometholone 0.2% preservative-free drops, from 1 to 3 months). Visual improvement generally started 3 months after treatment.[17] The temporary visual reduction observed in 65% of treated patients in the first 2 postoperative months (range, 1 to 3 months) is in line with the transient presence of corneal edema, clinically detectable by slit-lamp examination or with confocally detectable subclinical edema. After the third month, stromal hyperdensity or sometimes a reflective demarcation line was visible by biomicroscopy,[18] anterior segment OCT, and especially in vivo confocal scans,[10] as indirect clinical and instrumental signs of cross-linked tissue in the anterior midstroma. No delayed re-epithelialization was detected during follow-up. No adverse events were recorded in the study. No statistically significant numerical modifications of the endothelium[10,13] were found at 48 months of follow-up. Indeed, endothelial cell loss after cross-linking averaged 2% per year in our series, similar to the physiologic reduction reported in the literature.[19–21] Posterior segment OCT examination conducted before surgery and at 3, 6, 12, 24, and 48 months after treatment showed identical retinal macular thicknesses, without any detectable retinal damage.[9] These results confirm that, if epithelium is removed before cross-linking and a minimum preoperative thickness of 400 μm is observed for patient inclusion, according to the standardized parameters recommended in the literature for this technique,[6,10,12,22] the cross-linking procedure is well calibrated in energy density to produce apoptosis and hence necrosis of keratocytes confined to the anterior–mid stroma to a depth of 300 μm, besides being completely absorbed by riboflavin beyond the programmed dose and necessary thicknesses, avoiding damage to the endothelium, lens, and retina. Moreover, according to our recently published laboratory studies,[22] the transepithelial cross-linking procedure[23] fails to meet certain theoretical indicators of efficacy.[12]

Further preclinical and clinical evidence of the safety and effectiveness of the transepithelial method compared with the standardized epithelium-off procedure are needed, and ultimately, a prospective clinical trial of both techniques would be valuable for deciding the issue.[22] According to our clinical, topographical, and wavefront results, riboflavin UV A corneal cross-linking seems to be an effective and safe therapy to induce and maintain keratoconus stability after 4 years of evaluation, influencing collagen biomechanical resistance and its turnover. Moreover, average visual improvements of approximately +2.7 Snellen lines in UCVA and +1.9 Snellen lines in BSCVA were recorded in the Siena Eye Cross Study, in line with other long-term studies reported in the literature[11] and our preliminary experience.[9] The improved UCVA recorded during the follow-up is, in our opinion, partially explained by the sphere and spherical equivalent reduction. However, these data also may be related to progressive reduction of mean *K* power. Furthermore, the increased BSCVA may be linked to reduction in the difference between superior and inferior corneal hemimeridians (flattest vs steeper), well expressed by the improvement in corneal symmetry[9] indexes. Moreover, increased BSCVA may be sustained by the statistically significant early reduction in coma aberration[9] starting just 1 month after treatment and increasing constantly and significantly up to 24 months of observation. The statistical significance of these values was maintained after 36 and 48 months of follow-up. Variations in refractive effect seems to be related to certain morphologic changes occurring after treatment such as epithelial thickening, edema reduction, and symmetry indexes changes, with a variable improvement in corneal optical properties. The reduction in total wavefront higher order aberrations seemed more in keeping with the modifications in coma values induced by cross-linking. We did not observe any significant modification in spherical aberration in our analysis,

probably because of the data dispersion existing before surgery and during follow-up.

Biochemical and microstructural changes induced by the photopolymerization reaction and corneal collagen replacement recently reported in the literature[10,24] are in line with the clinical and topographic results recorded in this study and, in our opinion, may be an expression of cross-linking–induced flattening and improved corneal symmetry. Indeed, corneal reshaping[10] seems to be a more reliable expression of the cross-linking–induced clinical and topographic changes. Mean clinical and topographic improvements were recorded from the end of the third postoperative month and continued thereafter, reaching reliable stability in 24 months in all cases. Refractive stability was confirmed in 44 patients after 4 years of follow-up, without progression of keratoconus. Because riboflavin UV A-induced corneal collagen cross linking-was a treatment option for keratoconus,[6,9] on the basis of our patient selection, the disease should be distinguished into 2 clinical categories: progressive and stationary. Cross-linking is most indicated in young patients, especially those younger than 26 years with clinical and instrumental documented evidence of keratoconus progression, a minimum thickness (in the thinnest point) of 400 μm, and biomicroscopic evidence of clear cornea (absence of scars and accentuated Vogt striae). In younger patients, the progression of keratoconus generally is faster and the risk of requiring keratoplasty high.[25] Corneal collagen cross-linking also is indicated in patients older than 26 years if keratoconus progression is documented. In patients older than 36 years with generally stationary keratoconus[25] and rigid gas-permeable contact lens intolerance, the procedure should be performed to improve visual acuity, albeit slightly, although these patients should be fully informed that outcome may be unpredictable and even unsuccessful. Further clinical studies with longer follow-up are needed to determine the efficacy of the cross-linking in time, despite the fact that the results of this study are promising for a long-term efficacy.

THE AUTHORS INDICATE NO FINANCIAL SUPPORT OR FINANCIAL CONFLICT OF INTEREST. INVOLVED IN DESIGN AND conduct of study (A.C., C.M., S.B.); Analysis and interpretation of the data (A.C., C.M., S.B.); Data management and statistical analysis (S.B., T.C.); Collection of data (A.C., C.M., S.B., T.C.); Preparation and writing of the manuscript (A.C., C.M., S.B.); and Critical revision of the manuscript (A.C., C.M., S.B.). The Siena Eye Cross Study was approved for the first time in Italy in September 2004 by the Siena University Medical Hospital Institutional Review Board and by the Local Ethical Committee of the University of Siena. The study (head, Aldo Caporossi, MD) was conducted under the between September 15, 2004, and September 15, 2008, in accordance with the ethical standards set in the 1964 Declaration of Helsinki as renewed in 2000. All patients gave their written informed consent prior to their inclusion in the study.

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AJO History of Ophthalmology Series

Computed Tomography introduced to United States

Mayo Clinic neuro-radiologists were enthusiastic after seeing the first images from Geoffrey Hounsfield's prototype computed tomography (CT) scanner (made by the EMI organization in England) in the spring of 1972. One of them, Bud Baker, went to England in the summer of 1973 to learn more about the machine, with permission from the Mayo Clinic Board of Governors to buy a machine "on the spot" if he saw fit (a little unusual because CTs cost about $350,000 at the time, enough to outfit several labs). He promptly made an offer on an EMI scanner. The National Hospital at Queen's Square had already ordered the first non-prototype machine but the hospital was 150 years old and the floors and elevators couldn't support the machine's weight. It would take two years for them to refurbish the hospital to accomodate the scanner. Thus the Mayo Clinic obtained the first EMI CT scanner outside the United Kingdom. EMI asked Baker how many machines he thought would sell in the U.S. He guessed 3,000. "That kind of flabbergasted them. They didn't really know what they had," Baker said. This number was quickly surpassed as the CT scanner proved its revolutionary utility for central nervous system diagnosis.

Submitted by Jacqueline A. Leavitt from the Cogan Ophthalmic History Society.