Corneal Crosslinking with Riboflavin and Ultraviolet A. Part II. Clinical Indications and Results

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ABSTRACT Changes in the biomechanical properties of the human cornea play an important role in the pathogenesis of corneal ectatic diseases. A variety of conditions in primary acquired (keratoconus and pellucid marginal degeneration) or secondary induced (iatrogenic keratectasia after excimer refractive laser surgery) corneal ectatic disorders lead to reduced biomechanical resistance. Corneal collagen crosslinking (CXL) has emerged as a promising technique to slow or even to stop the progression of these corneal ectatic pathologies. In this procedure, riboflavin (vitamin B_2) is administered in conjunction with ultraviolet A light (UVA, 365 nm). This interaction causes the formation of reactive oxygen species, leading to the formation of additional covalent bonds between collagen molecules, with consequent biomechanical stiffening of the cornea. Although this method is not yet accepted as an evidence-based medicine modality for the treatment of corneal primary or secondary ectasias, the results of prospective, randomized studies of CXL used in the treatment of these pathologic entities show significant changes in the properties of corneal tissue. This procedure is currently the only etiopathogenetic approach in ectatic eyes that can delay or stop the process of cornea destabilization, reducing the necessity for keratoplasty. Despite promising results, CXL is associated with issues that include long-term safety and duration of the stabilizing effect. Combination of CXL with vision-improving procedures, such as topography-guided custom ablation and implantation of intracorneal ring segments of phakic intraocular lenses, may expand the indications for this procedure.

KEY WORDS cornea, crosslinking, keratectasia, keratoconus, riboflavin, ultraviolet A

I. INTRODUCTION

Keratoconus is a degeneration of the cornea; 84% of all cases begin between the ages of 20 and 49 years,[1] usually (80-85%) with bilateral cone-shaped corneal bulging and stromal thinning. Disease manifestation is highly variable. It can vary from slightly irregular astigmatism to severe visual impairment because of increased corneal protrusion and subepithelial scarring. Because of the young age of the patients, this disease often has a dramatic effect on quality of life and life planning.[2,3] The treatment options available to date have not been encouraging, as they have not been able to halt the progression of the keratoconus. Thus, keratoconus, because it is a progressive disease, has been one of the most common indications for keratoplasty.[3]

The exact cause of the disease that leads to the biomechanical changes remains unknown. The biomechanical properties of the cornea are created by the collagen structure and the composition of the proteoglycans and their links with the collagen fibrils. The three-dimensional arrangement of the collagen lamellae is one of the important factors that determines the resistance of the cornea.[4] Biochemical and immunohistochemical studies of the proteoglycans of the matrix reveal differences between the normal cornea and the keratoconic cornea.[5-7] Enzyme changes, including increased expression of lysosomal and proteolytic enzymes and reduced levels of protease inhibitors,[5,8-10] and reduced number and thickness,[5] along with altered arrangement of the collagen lamellae of the stroma, have also been shown.[11,12]

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OUTLINE

To counter the process of progressive thinning of the cornea and thus inhibit the progression of the disease, photo-oxidative collagen crosslinking (**CXL**) with riboflavin/ultraviolet A (**UVA**) light was developed. In the course of this CXL process, additional covalent bonds between the collagen molecules, which stabilize the collagen structure, are formed.

II. CLINICAL INDICATIONS FOR CXL

A. Keratoconus

Keratoconus is a corneal noninflammatory degeneration characterized by bilateral conical protrusion and thinning. The average age of appearance of keratoconus is the second decade of life. The course of the disease varies from slight irregular astigmatism to severe visual impairment because of increasing protrusion and subepithelial scarring.

The biochemical characteristics of the cornea result from the collagen scaffold and collagen compound and their bonding with the collagen fibrils. Biochemical and immunohistochemical studies of the proteoglycans in the matrix reveal differences between normal and keratoconic corneas. Enzymatic alterations, including increased expression of lysosomal and proteolytic enzymes and decreased concentration of protease inhibitors, along with decreased thickness and modified configuration of the stromal collagen lamellae, have been observed in keratoconus. With CXL, additional covalent binding between collagen molecules can be achieved, which stabilizes the collagen scaffold and changes several tissue properties.[13] In 1998, the first keratoconus patients underwent CXL procedures at the Department of Ophthalmology, C.G.Carus University Hospital in Dresden, Germany. Because of its progression, keratoconus, the most common ectatic corneal pathology, is also the most frequent pathology treated by the CXL procedure.[14]

1. Clinical Studies

In recent years, several studies have been conducted in cornea centers in Europe and the USA that provide clinical data to support the efficacy and safety of the CXL procedure for keratoconus. In 2008, our group described the largest published series to date, consisting of 241 eyes from 130 patients followed in Dresden for up to 6 years after CXL. This retrospective study confirmed earlier findings with statistically significant improvements in astigmatism, best-corrected visual acuity (**BCVA**), and maximum simulated keratometry values (K_{max}).[13]

Later studies from several other centers had similar results. In 2008, Wittig-Silva from Australia showed similar results from her prospective, randomized, controlled trial of 66 eyes from 49 patients.[15] In 2009, Vinciguerra conducted a prospective, nonrandomized, single-center clinical study in Milan and reported improved uncorrected visual acuity (**UCVA**) and BCVA and reduced corneal and total wavefront aberrations at the 1-year postoperative timepoint.[16]

The Siena Eye Cross Study was a prospective, nonrandomized, open trial that analyzed results from 44 eyes and confirmed stability of keratoconus after CXL without relevant side effects up to 60 months postoperatively.[17] A multicenter prospective randomized controlled clinical trial performed according to the guidelines of the U.S. Food and Drug Administration is currently underway; its one-year results reported improving UCVA, BCVA, K_{max} and average K value.[18] Clinical results of all above mentioned studies are summarized in Table 1.[13-20]

2. Evidence of Progression

Not every cornea with keratoconus needs to be cross-linked. In each of the above-mentioned studies, the indication for the CXL procedure was progression of keratoconus at a particular, precisely defined time. The parameters indicating the progression and the period of time in which these parameters were observed varied. In Dresden, progression indicating the necessity for treatment was based on an increase in K_{max} at the apex of keratoconus of 1 diopter (**D**) in 1 year, deterioration of visual acuity, or the need for new contact lens fittings more than once in 2 years.[13] Vinciguerra defined keratoconus progression as a change in either myopia

Table 1. Reports of CXL in progressing keratoconus

Author	Country	Study design	No. of eyes	Follow-up (months)	Topography	Visual acuity
Wollensak et al. 2003[14]	Germany	Prospective, nonrandomized	23	3-47	K_{max} reduced by 2.01D in 70%, stable in 22%	BCVA improved by 1.26 lines in 65%
Raiskup-Wolf et al. 2008[13]	Germany	Retrospective, case series	241	6-72	K_{max} decreased by 2.68D in 62%, stable in 17%	BCVA improved by at least 1 line in 53%, stable in 20%
Wittig-Silva et al. 2008[15]	Australia	Prospective, randomized, controlled trial	66	3-12	K_{max} decreased by 1.45D	BCVA improved by 0.12 logMAR
Vinciguerra et al. 2009[16]	Italy	Prospective, nonrandomized	28	12	K_{max} decreased by 1.35D	BCVA improved by 0.15 logMAR
Caporossi et al. 2010[17]	Italy	Prospective, nonrandomized	44	48-60	K_{max} reduced by 2.0D	BCVA improved by 1.9 lines
Hersh et al. 2011[18]	USA	Prospective, randomized, controlled trial	49	12	K_{max} reduced by 2.0D	CDVA improved by 2 or more lines in 21.1%
Asri et al. 2011[19]	France	Retrospective, case series	142	12	K_{max} decreased by $>$ 2.0D in 21.3%, stable in 68.8%	CDVA improved in 40%, stable in 47.6%
Poli et al. 2012[20]	France	Prospective, comparative	45	12-36	K_{max} nonsignificant trend toward reduction	BCVA significant improvement

and/or astigmatism of $\geq$3 D in the previous 6 months, a mean central K-reading change of $\geq$1.5 D observed in 3 consecutive topographies during the preceding 6 months, or a mean central corneal thickness decrease of $\geq$5% in 3 consecutive tomographies performed in the previous 6 months.[16] The U.S. group found indication for the CXL procedure when 1 or more of the following changes over a period of 24 months were noted: an increase of 1 D or more in the steepest K measurement, an increase of 1 D or more in manifest cylinder, or an increase of 0.5 D or more in manifest refraction spherical equivalent.[18] Patients with a history of corneal surgery, corneal pachymetry less than 300 μm, a history of ocular surface pathologies, and pregnancy or lactation during the course of the study were excluded.[13]

3. Medical History

Medical history of keratoconus patients is important for deciding whether they should be classified as "high risk" or "low risk" for progression to corneal ectasia. If they are in a "high-risk" group, we should watch for progression over a short period of time and thus follow them very closely. Alternatively, if the patient is classified as "low risk" because we do not expect rapid progression, it is sufficient to conduct regular but less frequent check-ups, eg, yearly.

The information that should be considered in the medical history during the patient's first examination to classify him/her as a high risk or low risk includes the following: age, gender, regular medication use, allergy, pregnancy, sports and hobbies, and smoking habits.

From our personal experience (and that of an Italian group that has confirmed this observation), in pediatric patients up to the age of 18 years, there is a male/female ratio of 6:1, and keratoconus is more aggressive and with a possibility of progression higher than in older age groups.[21] Patients with neurodermitis also tend to have a rapid progression of ectasia that could be "additively" supported by a prolonged intake of steroids, which changes the biomechanics of the cornea and has a "stiffness-reducing" effect.[22] Other regular steroid medication use in patients with chronic systemic inflammatory diseases or the regular hormonal intake of estrogen (contraceptives) or anabolic steroids (top-performance sports, body building) can trigger progression of ectasia in a predisposed cornea.[23] Hormonal changes during pregnancy can affect corneal biomechanics negatively; thus, pregnant women who have a diagnosed keratoconus or have recently undergone corneal refractive surgery should be closely followed; after delivery, they should undergo CXL if progression occurs.[24] We avoid CXL during pregnancy because of possible postoperative complications (eg, infection, melting) that would require either systemic therapy or additional procedures requiring general anesthesia. Female patients should be informed that despite the CXL procedure, their ectasia could progress further during a subsequent pregnancy because of changes in estrogen levels.[25]

Specific sports and hobbies leading to a regular, long-standing increase in the intraocular pressure (body building with weight-lifting, yoga with upside-down posturing, playing wind instruments)[26] could also be risk factors for progression in pre-existing ectatic corneas.

In contrast, certain conditions or habits of patients cause natural CXL processes in the tissues of the human body, eg, diabetes mellitus and smoking. For these patients, clinicians do not expect rapid progression of keratoconus, or progression at all; thus, they do not need close followup. The protective effect that manifests in cases of diabetes may be explained by the induction of crosslinks in the stroma, preventing biomechanical weakening of the cornea.[27,28] Cigarette smoking is also negatively associated with keratoconus. A stiffening effect in the skin and blood vessels was observed among patients who smoked.[29,30] The toxic substances contained in the smoke cause chemical CXL in the cornea. However, despite the negative relationship between cigarette smoking and progression of keratoconus, the irreversible side effects and extensive organ damage caused by smoking completely contraindicate any suggestion that smoking should comprise any part of treatment.[31]

All the above-mentioned studies were performed according to the standard "Dresden" protocol.

4. Standard "Dresden" Protocol

CXL is performed as an outpatient service on a day-surgery basis. Thirty min before the procedure, pain medication and, when necessary, a tranquilizer are administered. Some surgeons instill 2% pilocarpine drops preoperatively to reduce the thermal and photochemical UVA light irradiation, which could be potentially harmful to the lens and retina. The procedure is conducted under sterile conditions in an operating room.

After topical anesthesia is administered, a lid speculum is applied, and the epithelial tissue is removed from a 9.0-mm diameter area. This is to ensure that the riboflavin can penetrate the stroma and that a high level of UVA absorption is achieved. Another modality of epithelial removal is transepithelial phototherapeutic keratectomy (**t-PTK**)using a 213-nm solid-state laser system followed by the standard CXL procedure described by Kymionis, which yields improvements in patient visual outcomes.[32]

As a photosensitizer, 0.1% riboflavin is applied to the cornea every 1-2 min for 30 min to achieve adequate penetration of the solution. During this saturation period, the lid speculum is removed. Using a slit lamp with a blue filter, the surgeon confirms the presence of riboflavin in the anterior chamber before UV irradiation begins. The corneal thickness is measured (ultrasound pachymetry) after removal of the epithelium (to decide which riboflavin solution should be used to saturate the cornea — iso-osmolar or hypo-osmolar) and before irradiation initiation to ensure that the thickness of the irradiated cornea is above 400 μm to protect corneal endothelium.

The 8.0-mm diameter of the central cornea is irradiated with UVA light with a wavelength of 370 nm and an irradiance of 3 mW/cm^2. A calibrated UVA meter is used before treatment to verify the irradiance. During the 30 min of irradiation, drops of riboflavin are applied to the cornea every five min. Fixation during irradiation is achieved by instructing the patient to focus on the central light-emitting diode of the probe. During the procedure, the surgeon also controls the centration of the treatment. Topical anesthetics are added as needed during the procedure.

Postoperatively, patients receive antibiotic drops and artificial tears. A soft bandage contact lens is applied until re-epithelization is complete. Analgesics are prescribed. After corneal re-epithelization, topical therapy with steroids is initiated and continued for 3-4 weeks. Patients are examined daily until re-epithelization, then at 1, 3, 6, and 12 months, and once a year thereafter. A new contact lens fitting can be performed after stabilization of the cornea approximately 6-8 months after CXL.

5. Thin Corneas

To protect the endothelium, CXL inclusion criteria require a minimum corneal thickness of 400 μm after removal of the epithelium. To assure adequate thickness, intraoperative pachymetry measurement during each procedure is necessary. However, in advanced keratoconus, progressive corneal thinning often leads to a remaining stromal thickness of less than 400 μm. In patients who still have good BCVA that allows them an adequate quality of life without any major restrictions, we can modify the current standard treatment protocol by preoperatively swelling thin corneas to a stromal thickness of at least 400 μm using hypo-osmolar riboflavin solution. This treatment modification safely broadens the spectrum of CXL indications to thin corneas that would otherwise not be eligible for treatment.[33] Yet, this modification has its limits, as described by Hafezi in one case report of the failure of CXL after preoperative stromal swelling with hypo-osmolar riboflavin solution in an extremely thin cornea (268 μm).[34] Although the swelling of the corneal stroma was effective and yielded a pre-irradiating thickness of more than 400 μm, the increase in biomechanical resistance was not sufficient to arrest the progression of the disease. This finding alerts us to the necessity of determining the lower limit for stromal thickness of the cornea after removal of epithelium that still allows us to treat with hypo-osmolar riboflavin solution and avoid complications or failure of the procedure. Based on the outcome in this case and the findings of the earlier study, it was suggested that CXL be performed when stromal thickness is 320 μm or greater prior to swelling with hypo-osmolar riboflavin solution.

Although CXL is technically an easy and relatively safe procedure, epithelial debridement using the standard protocol can cause severe pain and visual loss during the first few postoperative days until regrowth of the epithelium is complete. Additionally, infectious complications and melting processes with corneal perforations related to epithelial removal and bandage contact lens use have been reported.[35-38]

6. Transepithelial CXL

Because of the partially anecdotal (but still negative) above-mentioned reports, clinicians have attempted to find a way to treat without removing the epithelium — an "epithelium-on" CXL procedure. The transepithelial CXL

procedure has been described previously, and its principle rests on the use of enhancer substances that help riboflavin to penetrate the corneal stroma through an intact epithelium, avoiding the need for epithelial debridement. Experimental work of Wollensak showed that the biomechanical effect of epi-on CXL was only about 20% of the increase produced by standard CXL, probably because of restricted and inhomogeneous stromal distribution of riboflavin.[39] Animal experiments of our group with changed concentrations of BAC and NaCl induced sufficient epithelial permeability for the passage of riboflavin and resulted in increased corneal stiffening after CXL.[40,41]

Combinations of permeability enhancers have a multiplying effect, and the use of these combinations is advised to achieve better intraocular penetration of topical hydrophilic drugs.[42,43] Recent clinical studies conducted in the Netherlands, Italy, and the United Kingdom evaluated the clinical effects of transepithelial CXL on keratoconic eyes pretreated with substances enhancing epithelial permeability. CXL was performed by applying an enhanced riboflavin solution with trometamol, BAC, EDTA, and gentamicin. Statements concerning the results of clinical observations vary from "less effective than standard CXL" to "moderately effective" to "appearing to halt keratoconus progression, with a statistically significant improvement in visual and topographic parameters."[44-46] Alcohol 20% has also been applied to devitalize corneal epithelium in order to disturb tight epithelial junctions to increase riboflavin penetration into the stroma.[47] The question is whether this particular modality of epi-on CXL has real advantages over the epi-off procedure when the epithelium is not able to carry out its physiological barrier function anymore.

Optical coherence tomography (**OCT**) analysis of the corneas after the transepithelial CXL procedure revealed a demarcation line at a depth range of 90-140 μm from the epithelial surface. With the standard protocol procedure, the demarcation line in corneas is at a depth of approximately 250 μm. These findings indicate that the formation of crosslinks in the epithelium-on procedure appears to take place in the upper third of the corneal stroma, 20-30 μm beneath the Bowman membrane, compared with the "classic" procedure in which collagen crosslinks occur in much deeper layers of the stroma.

It is unknown whether transepithelial CXL treatment will produce satisfactory, long-term clinical effects, but in the reported studies, it appeared to halt keratoconus progression. Additionally, with a statistically significant improvement in visual and topographic parameters, no complications were reported. Although the effect of transepithelial CXL appears less pronounced than that described in the literature after CXL with de-epithelialization, its noninvasive nature makes it potentially useful in cases in which epithelial debridement is ideally avoided, such as in patients with additional ocular surface pathology, dry eyes, children, uncooperative patients, and patients with very thin corneas.[46,48]

7. CXL in Children

Pediatric age at the time of diagnosis represents a negative prognostic factor for keratoconus progression, with an increased probability of needing a corneal transplant. In particular, younger patients represent a population at high risk for more rapid progression of the disease.[49] The Siena CXL Pediatrics Study analyzed findings of 152 keratoconus patients from 10-18 years of age.[50] The parameters used to indicate that keratoconus was progressive were at least two of the following in the 3 months prior to the study: UCVA/BCVA deterioration $\geq$1 Snellen line, Sph/Cyl increase $>$0.5 D, K_{ave} increase $>$0.5 D, topographic surface asymmetry index (SAI)/symmetry index (SI) increase $>$0.5 D, reduction in corneal thickness (thinnest point) $\geq$10 μm, biomicroscopic and confocal microscopic evidence of clear cornea and clinical refractive instability that could not be corrected optically by spectacles or contact lenses. This study reported a 4:1 M:F ratio of patients, which differs from epidemiological findings in the literature of a 2:1 M:F ratio.[51,52] The results of this study demonstrated significant and rapid functional improvement in pediatric patients with progressive keratoconus who underwent CXL treatment. No adverse events were recorded.

The faster functional response in patients with lower corneal thickness ($<$450 μm) seems to be reasonably explained by early coma value improvements, likely because of the relatively higher percentage of crosslinked tissue. Patients with thicker ($>$450 μm) corneas improved more slowly, but at 36 months of followup, the functional gain was a mean of +1.5 Snellen lines in BCVA and +1.6 Snellen lines in UCVA, without statistically significant differences between the two groups. Final visual acuity was better in the group with thicker corneas because of the earlier stage of keratoconus at the time of enrollment. However, the poorer final visual acuity in the group with thinner corneas was well explained by their more advanced stage of keratoconus at the time of inclusion in the treatment protocol.

This study demonstrated the ability of CXL to slow the progression of keratoconus in pediatric patients, improving functional performance in 80% of patients 3 years after the procedure, irrespective of preoperative corneal thickness. In 4.6% of patients, worsening of functional and objective data was observed during the followup, which was reasonably explained by the greater aggressiveness and progression of keratoconus in pediatric patients. These outcomes suggest that CXL should be elective treatment for progressive keratoconus in pediatric patients. Analyzing the age-related long-term results after CXL, the Italian study showed the capacity of CXL to retard keratoconus progression in all age groups, with better functional response in patients under 26 years.[51] The treatment provided long-term stabilization in over 90% of treated cases. The lower functional response observed in patients over 27 years of age may be explained by a reduced collagen "plasticity" in adults, similar to what has been demonstrated in the literature.[21,53,54]

B. Pellucid Marginal Degeneration

1. Clinical Characteristics and Considerations

Pellucid marginal degeneration (**PMD**) is a rare ectatic disorder that typically affects the inferior peripheral cornea in a crescentic fashion. The condition occurs mostly in males and usually appears between the second and fifth decades of life, affecting all ethnicities. The prevalence and etiology of PMD remain unknown. Ocular signs and symptoms are often undetected, and in the later stages, PMD can be misdiagnosed as keratoconus. The initial treatment of the disease consists of optical correction.

Because of the large amounts of against-the-rule astigmatism, patients with PMD are much more difficult to fit with contact lenses than patients with keratoconus, although spherical or aspheric contact lenses with large overall diameter should initially be attempted in early to moderate cases. When the disease progresses to advanced stages, surgical procedures are necessary, such as wedge resection, penetrating keratoplasty, lamellar keratoplasty, and intracorneal segments. Surgery is considered for patients whose vision is not adequately corrected by contact lenses or in patients who are contact lens-intolerant.[55,56] Still, the intraoperative findings and postoperative results are unpredictable because of different forms and extensions of the thinning of the peripheral cornea. Thus, the CXL procedure faces challenges in halting progression to avoid surgery and to enable comfortable contact lens fitting. Unlike keratoconus, which can appear as early as the prepubescent age, PMD can be newly diagnosed in later decades. The clinician should carefully monitor progression in patients with later onset, scheduling half-yearly followup appointments to reveal early significant progression.

2. Case Reports

Three cases have been reported of treating PMD with the CXL procedure.[57-59] One report describes a case that was additionally treated with photorefractive keratectomy.[57] The impaired tissue was located in the periphery of the cornea inferior to the limbus. Thus, it is important during the CXL procedure not only to intentionally decenter the focus of the irradiation to treat the pathologic area but also to protect corneal epithelial limbal stem cells for use during the procedure as a mechanical protection ring. However, Wollensak demonstrated in recent experiments that direct irradiation of the limbal or conjunctival epithelium using UVA with or without the photosensitizer riboflavin with dextran and with the standard irradiance does not induce significant cellular conjunctival or corneal epithelial damage, as assessed by histological and immunohistological techniques in the early phase after irradiation. Therefore, he postulated that accidental or intentional irradiation of the limbal epithelium during CXL does not seem to pose a threat for the induction of degenerative or neoplastic changes.[58] All of the treated patients were in the third to fifth decade of age, and all reported improved keratometry parameters and BCVA, which allowed postponement of any radical tectonic surgical intervention.[57-59] No intraoperative or early postoperative complications occurred. The followup time for the reported cases was up to 12 months, but despite the encouraging results, a longer followup period is necessary to confirm the stability of the results.

In 2008, our group reported the results of 13 eyes of 8 patients with PMD after CXL procedures. We found stable keratometric results and good visual acuity in all except one eye, which had a decrease in visual acuity at 18-months followup.[59] We did not record any side effects of the treatment, and in particular, we did not observe any clinical signs of limbal stem cell insufficiency.

C. Iatrogenic Keratectasia

1. Ectasia after Laser In Situ Keratomileusis (LASIK)

Since the first description of LASIK in 1991, millions of patients have undergone this surgery. The intra- and postoperative complications were reduced with the improvement in mechanical keratomes and the introduction of new femtosecond laser systems. However, refractive surgery cuts and removes corneal tissue by creating a flap and removing tissue from the stromal bed. Therefore, all excimer laser procedures weaken the corneal biomechanics.

Corneal ectasia remains one of the most insidious complications after LASIK. Since the first reports by Seiler in 1998, only a few hundred cases have been reported in the literature, although this number is likely an underrepresentation of the actual incidence. The actual incidence remains undetermined, and no good data support firm predictions. However, previous estimates have ranged from 0.04% to 0.6%.[60-62] Post-LASIK ectasia can have dramatic consequences and, in many cases, can require further surgical interventions or even corneal transplantation for visual rehabilitation.[63]

a. Clinical Characteristics and Considerations

Keratectasia is characterized by a progressive corneal steepening, which can occur centrally or inferiorly, combined with severe refractive changes, loss of UCVA and BCVA, and stromal thinning of the cornea that can present days to years after LASIK. Risk factors for its development are thin corneas, a thin residual stromal bed, deep ablations, enhancement treatments, and preoperative abnormalities, such as forme fruste keratoconus or PMD.[64] Keratectasia can also occur in eyes treated according to the current guidelines without any risk factors and in patients after surface ablation.[65,66] Refractive surgeons should also very carefully explore the medical histories of candidates for refractive procedures to detect potential risk factors that would predispose them to development of ectasia as mentioned above: systemic connective tissue diseases, immunologic disorders, steroid medication or regular intake of other glucocorticoids, or pregnancy.[67]

The actual post-LASIK ectasia management includes, first and foremost, the prevention of its occurrence, which requires recognizing the problem, identifying and refining known risk factors, and utilizing alternative treatment strategies in high-risk patients. When ectasia occurs, prior to performing corneal transplantation, a variety of treatments

are currently available, including intraocular pressure reduction, rigid gas-permeable contact lenses, and intracorneal ring segments. Furthermore, as almost "causative" therapy, CXL should be the first choice for patients with post-LASIK progressing ectasia to surgically strengthen artificially weakened corneal stroma and stabilize the cornea.

b. Case Reports

The first (2005) case report of using the CXL procedure for iatrogenic ectasia described results in a female who developed keratectasia in both eyes 4 weeks after LASIK. Due to severe keratectasia 10 months after LASIK, treatment with riboflavin and UVA was performed. The biomechanical status of the cornea was stabilized, and further progression of the keratectasia was prevented. The postoperative refraction and corneal topography were stable at 18 months followup.[68]

In 2007, Hafezi et al published a report on a group of 10 patients with formerly undiagnosed forme fruste keratoconus or PMD who developed iatrogenic keratectasia following LASIK for myopic astigmatism. CXL induced by riboflavin and UVA arrested and/or partially reversed keratectasia over a postoperative followup period of up to 25 months, as demonstrated by postoperative corneal topographies and a reduction in maximum keratometric readings. No complications or side effects of the procedure were recorded.[69]

Vinciguerra analyzed 13 eyes from 9 patients who had undergone excimer laser refractive surgery (3 PRK, 10 LASIK) with resulting unstable corneal ectasia.[70] The treatment appeared to stabilize these iatrogenically ectatic eyes. Reduction in sphere, increase in BCVA, apparent central corneal flattening, and a tendency to decreased coma at the 6-month interval indicated a tendency toward improvement of the ectatic condition, with possible partial recentration of the optical zone.

The most recent report concerning CXL in post-LASIK keratectasias analyzed results of 22 eyes in 15 patients. The authors observed stabilization of UCVA, BCVA and K_{max} readings in their cohort postoperatively, and the procedure appeared safe and promising for stabilizing the refraction and the corneal topography. Thus, the ability to stop the progression of visual loss, thereby avoiding or delaying disease progression and keratoplasty, was promising.[71]

Kymionis et al investigated corneal tissue alterations after CXL in patients with post-LASIK ectasia and keratoconus in confocal microscopy records at a 1-year followup. Images of both keratoconic and post-LASIK corneal ectasia eyes revealed similar morphologic alterations. The subepithelial nerve plexus was absent immediately after treatment; regeneration of nerves was evident after the third postoperative month. Keratocytes were absent from the anterior 300 μm of the stroma in the first 3 months while the posterior stromal density of keratocytes was increased. Corneal collagen fibers in the anterior stroma were unevenly distributed in a net-like formation. Full-thickness keratocyte repopulation in the anterior and mid-corneal stroma was detected 6 months after treatment. The corneal endothelium did not undergo any significant changes.[72]

At the 2011 meeting of the Association for Research in Vision and Ophthalmology, our group presented a cohort of 10 eyes from 6 patients followed for up to 8 years. Each case was evaluated for stability and further progression.[74] In 6 eyes, K-values remained stable or even partially decreased; in 4 eyes from 2 patients, K-values progressed despite the CXL procedure. The patients with CXL failure had additional risk factors for ectasia progression, such as neurodermitis, allergy treated with systemic steroids, and pre-existing keratoconus. The modest results of our small group illustrate the need to be aware of patients with additional risk factors; additionally, when counseling patients, we should inform them that further progression is possible despite CXL that frequent followup is important, and that eventual retreatment may be necessary.[73]

Hormonal changes, especially during pregnancy, affect the biomechanical properties of the cornea.[24] Hafezi reported an exacerbation of iatrogenic keratectasia during pregnancy despite CXL.[25] We should counsel our refractive patients properly, especially fertile women, and discuss with them the possible risks of refractive surgery during this period. As previously mentioned, this type of ectasia occurs only rarely after LASIK treatment. Thus, only small groups of such patients have been treated with CXL, and studies did not include control groups. To further confirm the long-term stabilizing effect of CXL in these cases, randomized, controlled trials are needed. Clinical results of these case series are briefly presented in Table 2.

2. Ectasia after Radial Keratotomy

Peripheral corneal ectasia with hyperopic shift of 1 D or more is a complication occurring after radial keratotomy (**RK**). Hyperopic shift after RK has been recorded in several long-term prospective studies with statistical incidences that range between 31% and 43%.[74] Several techniques were proposed for the correction of secondary ectasia-related hyperopic refractive error,[75-78] and recently, a case report was published describing the use of CXL in the management of keratoconic corneal ectasia exacerbated by RK with hyperopic shift.[79] The authors analyzed the case of a 38-year-old male who underwent the RK procedure for keratoconus-related myopia and irregular astigmatism 10 years ago. CXL was performed according to the regular protocol with epithelium removal. The visual rehabilitation of the patient lasted several months, and 1-year postoperatively, UCVA had improved from 0.2 to 0.6 and BCVA from 0.3 to 0.8. K_{max} values changed from preoperative 61.11 D to 58.91 D postoperatively. The corneal thickness and endothelial cell count did not exhibit significant changes postoperatively. Several surgical strategies had already been attempted in the management of post-RK hyperopic shift with variable and unpredictable effects. CXL could be a new, less-invasive option for stiffening collagen in the anterior and mid-stroma and increasing interlamellar connections. A large cohort and a longer followup time

Table 2. Reports of CXL in secondary keratectasia

Author	Country	Study design	No. of eyes	Follow-up (months)	Topography	Visual acuity
Kohlhaas et al. 2005[68]	Germany	Case report	2	18	K_{max} stable	not recorded
Hafezi et al. 2007[69]	Switzerland, Greece	Retrospective, case series	10	12-25	K_{max} decreased in all cases	BCVA improved in 90%, stable in 10%
Vinciguerra et al. 2010[70]	Italy	Retrospective, case series	13	12	K_{max} decreased by 2.02D	BCVA improved by 0.1 logMAR
Kissner et al. 2011[73]	Germany	Retrospective, case series	10	96	K_{max} remained stable in 60%	BCVA stable in 60%
Salgado et al. 2011[71]	Germany	Prospective, nonrandomized	22	12	K_{max} changes were not statistically significant	BCVA improvement was not statistically significant
Hersh et al. 2011[18]	USA	Prospective, randomized, controlled trial	22	12	K_{max} decreased by 1.0D	CDVA improved significantly

are necessary to determine the real potential efficacy of this procedure in post-RK ectasia.

D. Infectious Keratitis and the Melting Process

Several basic studies note that riboflavin plus UV irradiation affects the viability of bacteria and fungi.[80-82] Swedish investigators initiated a prospective, nonrandomized study to investigate the effect of the CXL procedure with riboflavin and UVA irradiation as a primary therapy for bacterial keratitis.[83] The cohort consisted of 16 patients with a clinical diagnosis of bacterial keratitis. No patient had any prior antibiotic treatment for the current infection. Photography and microbial culturing of the infected cornea were performed. Isotonic riboflavin was topically administered into the cornea for 20 min, and UVA irradiation was executed for 30 min using irradiance and exposure times identical to those used to crosslink ectatic corneas. After irradiation, patients were examined once to several times daily, depending on the severity of the case, until obvious signs of improvement were observed. Any signs of progression of the ulcer or uncertainty regarding healing of the infection prompted treatment with antibiotics. Isolated ulcers were observed in 13 patients, and two infiltrates were identified in 3 patients. The sizes of the ulcers ranged between 0.1 and 2.5 mm in diameter, and hypopyon was seen in two eyes. The primary end point, healing of the corneal ulcer, was accomplished in all cases; nonetheless, antibiotic treatment during followup was necessary in 2 of the 16 patients. Corneal epithelialization without additional intervention was achieved in 15 patients. In one patient, epithelial recovery was achieved by amniotic membrane transplant. Thirteen patients had positive microbial culture results. The isolated bacterial strains included *Staphylococcus aureus, Staphylococcus epidermidis, Micrococcus species, Corynebacterium, Propionibacterium* and *Pseudomonas*.

The treatment responses of the infectious ulcers indicate that photochemically activated riboflavin could be used for future management of infectious keratitis, allowing for less frequent application of topical antibiotics and fewer patient visits. Use of the method might reduce the frequency of complications associated with corneal infections and increase the healing rate in the treatment of microbial keratitis. More research should be devoted to the photosensitization of riboflavin because it seems to show promise for treating corneal infections. As multi-drug antibiotic resistance is an emerging concern, this mode of therapy should be thoroughly investigated. Elimination of numerous pathogens has been demonstrated using the same principle and in the development of the device for pathogen inactivation in transfusion medicine. It is noteworthy that despite the response to the therapy, the majority of ulcers treated in this study were neither severe nor advanced stages of the disease. Thus, these findings must be further evaluated before this treatment can be recommended as a first-line treatment.[81-83]

Iseli and Al-Sabai also reported positive results of using CXL to treat patients who suffered from therapy-resistant infectious keratitis associated with corneal melting.[84,85] In all treated cases, the progression of the melting process was halted after CXL, and emergency keratoplasty was not necessary. The authors concluded, like Makdoumi et al, that CXL could be a valuable addition to our therapeutic options in the treatment of corneal melts.[84,85] When using CXL for this indication, we should withhold all topical antibiotics and fluorescein for at least 24 hours prior to the application of riboflavin and UVA irradiation, because antibiotics, eg, third-generation quinolones and fluorescein, are stronger absorbers of UVA irradiation at the 365 nm wavelength than riboflavin. Additionally, these substances compete with riboflavin for UVA, which could lead to failure of the procedure.[86]

E. Bullous Keratopathy

Wollensak has shown that CXL also has an anti-edematous effect. This observation was proven in a small interventional case series including 3 patients with pseudophakic bullous keratopathy, corneal transplant rejection, and Fuchs' endothelial dystrophy. In these patients, the thickness of the cornea 8 months after the procedure was reduced by 93.67 μm±14.22 μm compared with preoperative values, and the bullous changes were markedly improved, resulting in loss of pain and discomfort.[87]

Ghanem conducted a prospective study with 14 eyes from 14 patients with pseudophakic bullous keratopathy and examined the effect of CXL.[89] Corneal CXL significantly improved corneal transparency, corneal thickness, and ocular pain 1 month postopoperatively. However, 6 months after the procedure, the transparency, corneal thickness, and pain scores in all of these patients were similar to preoperative values.[88]

Danish investigators applied CXL for the same type of corneal pathology, and reductions in corneal thickness and improvements in visual acuity were observed in 10 of 11 patients.[89] The effect occurred over weeks and lasted for months. The results of these observations suggest that the edematous stroma has a prompt, positive reaction to CXL, but there is no evidence of a long-lasting effect. Thus, this procedure is not destined for indication as a possible routine treatment option; if indicated at all, it is only in palliative cases with poor visual prognoses.

III. COMPLICATIONS AFTER CXL

All above-mentioned studies and case reports showed CXL as an efficacious treatment option for stopping the progression of keratectasia and stabilizing the architecture of the corneal stroma. However, none of those studies evaluated the complication or failure rate of the procedure.

A. Success Rates and Risk Factors

Koller et al conducted a prospective study of 117 eyes in 99 patients with primary keratectasia and evaluated the complication and failure rate of CXL during the first postoperative year.[90] Progression of keratectasia was verified by repeated Scheimpflug imaging over at least 6 months (range, 6 months to 2 years). Progression was accepted if the increase in the K_{max} reading exceeded 1 D. The second eye was treated no earlier than 6 months after the first eye. Only eyes with mild to moderate keratoconus ($K_{max}<65$ D; corneal thickness >400 μm) were included. The complication rate was defined as the percentage of eyes with a loss of 2 or more Snellen lines of corrected distance visual acuity (**CDVA**) at 1-year followup compared to the preoperative visual acuity. The failure rate was defined as the percentage of eyes with an increase in the K_{max} of more than 1 D over the preoperative value. Approximately 90% of patients completed 1 year of followup. The complication rate was 2.9%, and the failure rate was 7.6%.

Age older than 35 years and a preoperative CDVA better than 20/25 were identified as significant risk factors for complications. Introducing an age limit of 35 years as an inclusion criterion would have reduced the complication rate to 1.04%. No morphologic or optical reason for the visual loss could be determined. A high preoperative K_{max} reading was a significant risk factor for failure. Changing the inclusion K_{max} from less than 65 D to less than 58 D would have reduced the failure rate to 2.8%. Sterile infiltrates were seen in 7.6% of eyes and central stromal scars in 2.8%. The results of Koller et al indicate that changing the inclusion criteria may significantly reduce the complications and failure of CXL. Patients should be realistically informed about chances for stabilization and improvement of corneal ectasia after the CXL procedure, risk factors for failure, and possible postoperative complications postoperatively according to individual preoperative values.

B. Infectious Disease

Australian authors reported a case of polymicrobial keratitis caused by *Streptococcus salivarius*, *Streptococcus oralis* and *coaugulase-negative Staphylococcus sp.* in a patient who underwent CXL for keratoconus and presented 1-day postoperatively with a painful red eye. This patient admitted to removing his bandage contact lens and cleaning it in his mouth before reapplying it in his eye.[38] Surgeons should thoroughly instruct each patient before surgery regarding the postoperative treatment regimen and behavior necessary to minimize the risk of developing microbial keratitis.

A case report by Kymionis et al showed that CXL can induce herpetic keratitis with iritis even in patients with no history of herpetic disease, and early diagnosis and proper treatment are essential for a favorable outcome.[35] Surgeons should be very cautious in treating patients with a medical history of corneal herpetic disease and coincidental progressive keratoconus, should inform patients about the possibility of this complication, and should pretreat these patients with systemic antiviral medications and give topical antiviral agents after the procedure. Kymionis et al also presented a case of a young man who, during the first post-treatment day after CXL for iatrogenic keratectasia, developed diffuse lamellar keratitis (**DLK**, stage III). With intensive treatment with topical steroids, the DLK resolved in 2 weeks.[91]

C. Corneal Haze

A typical corneal haze has generally been noted on clinical examination after CXL. Studies show that the depth of the CXL can be observed by following the demarcation line seen in the corneal stroma[92] or by grading the corneal haze with the slit lamp.[90] Herrmann et al described a temporary subepithelial haze following CXL in a keratoconus patient who was treated with topical steroids; the haze disappeared several months after the procedure.[93]

Mazzotta et al analyzed cases of stromal haze after CXL for keratoconus with in vivo confocal microscopy.[94] The stromal haze developed 2-3 months after the procedure and was resistant to topical steroids. An increased extracellular fibrillar matrix density was seen, which was greater in

patients with more advanced keratoconus. Dark Vogt microstriae were also present, a finding not detectable in patients with early-stage disease. The opacities, if well-managed with topical preservative-free steroids, last only 30-40 days. Preoperative confocal analysis in patients younger than 20 years old revealed hyperactivated keratocyte nuclei in the anterior stroma to a depth of 80 μm and dark, reticular-patterned microstriae in patients older than 20 years. The latter also had preoperative slit-lamp evidence of strong Vogt striae. These could be risk factors for corneal opacity after CXL.[94,95]

Evaluation of patients enrolled as part of a multicenter, prospective, randomized, controlled clinical trial conducted according to the guidelines of the U.S. Food and Drug Administration determined the natural history of CXL-associated corneal haze measured by Scheimpflug imagery (densitometry) and slit-lamp biomicroscopy in patients with keratoconus or iatrogenic keratectasia. The time course of corneal haze after CXL was objectively quantified; it was greatest at 1 month, plateaued at 3 months, and significantly decreased between 3 and 12 months. Changes in haze did not correlate with postoperative clinical outcomes.[96]

D. Stromal Scarring

In a retrospective study, our group analyzed the development of corneal stromal scarring after CXL.[97] The cohort consisted of 163 eyes from 127 patients with stage 1-3 keratoconus according to Krumeich's classification. At 1 year after CXL, 8.6% of the eyes developed significant stromal scarring. The eyes that developed this scarring had high K_{max} values at the apices (mean 71.1±13.2 D) and thin corneas (mean 420.0±33.9 μm). Based on these results, we postulated that advanced keratoconus should be considered at higher risk of corneal scarring after CXL due to low corneal thickness and high corneal curvature.

E. Cell Density Loss

Another group of complications after CXL is endothelial cell density loss. Kymionis et al reported the outcomes after CXL in patients with thin corneas (<400 μm).[98] They treated 14 eyes of 12 patients with a mean corneal thickness of 373.92 ± 22.92 μm after epithelial removal. In 1 year of followup time, they detected a significant decrease in endothelial cell density from 2733 ± 180 cells/mm^2 to 2441 ± 400 cells/mm^2. They described treatment using isotonic 0.1% riboflavin and 20% dextran solution, which could be a source of deswelling of the corneal stroma intraoperatively and even more accelerated thinning of the already thin cornea; thus, this treatment could be the reason for the endothelial cell damage.

Other case reports describing findings of corneal endothelial damage could have a common denominator similar to the previous report. The patients had "threshold" corneal thicknesses that were not measured or proved intraoperatively after the epithelium removal and were treated with iso-osmolar 0.1% riboflavin with 20% dextran solution.[99,100]

F. Corneal Melt

Several case reports describe the melting process after collagen CXL for keratoconus. Labiris et al described an otherwise healthy young man with contact lens intolerance whose residual corneal thickness after epithelial debridement was 407 μm and who was treated with 0.1% riboflavin with 20% dextran solution.[101] On the first postoperative day, severe corneal haze, endothelial precipitates, and inflammatory cells in the anterior chamber were present. The cornea re-epithelialized very slowly, and progressive thinning resulted in a descemetocele that finally perforated in the second postoperative month. This case highlights the need to be very careful in the postoperative period, to follow the patient very closely for prolonged epithelialization, and to promote epithelialization by, eg, performing amnionic membrane transplant or applying epithelialization-promoting pharmacological agents (drops of autologous serum) to prevent corneal perforation.

Gokhale and Vemuganti reported a case of acute corneal melt with perforation in a patient with keratoconus 1-week postoperatively after uncontrolled use of topical diclofenac and proparacaine eyedrops.[102] Faschinger et al described a patient with Down syndrome and keratoconus with thin corneas and no objective records of progression who underwent CXL in both eyes simultaneously.[103] The patient developed a corneal melt with perforation in one eye 1 week postoperatively and in the second eye 4 weeks postoperatively, and emergency corneal grafts were performed. Very critical evaluation of this case raises the question of whether the corneal findings actually indicated the CXL procedure: proof of progression was missing, and the corneas were very thin. The authors did not comment on any limitations this patient may have had related to Down syndrome (compliance with postoperative topical treatment, eye rubbing, etc.).

Eberwein et al described a 45-year-old patient with severe atopic disease and keratoconus who had corneal melting following CXL and deep anterior lamellar keratoplasty due to subclinical infection with *Herpes simplex* virus. Penetrating keratoplasty and intensive antiviral and immunosuppressive systemic treatment were necessary to control the infection.[104] According to our personal experience, patients with atopic disease are high-risk patients with regard to the postoperative healing process and prolonged epithelialization. Additionally, they are more susceptible to infection and at a higher risk of procedure failure. These cases of irreversible damage to the corneal stroma after CXL should cause us to be extremely cautious during preoperative examination of patients. We should ensure that patients meet all necessary criteria to be indicated for the procedure, and we should follow our patients very closely postoperatively. An overview of published papers commenting on complications after CXL is depicted in Table 3.[35,38,90,91,93,95,97-99,101-103]

Table 3. Reported complications after CXL procedure

Author	Country	Study design	No. of eyes	Complication
Kymionis et al. 2007[35]	Greece	Case report	1	Induced herpetic keratitis with iritis
Kymionis et al. 2007[91]	Greece	Case report	1	Diffuse lamellar keratitis
Mazzotta et al. 2007[95]	Italy	Case report	2	Stromal haze
Herrmann et al. 2008[93]	Germany	Case report	1	Diffuse subepithelial opacification
Koller et al. 2009[90]	Switzerland	Prospective	117	2.9% lost => 2 lines 7.6% failure
Zamora et al. 2009[38]	Australia	Case report	1	Polymicrobial keratitis
Raiskup et al. 2009[97]	Germany	Retrospective	163	Stromal scar in 8.6%
Gokhale et al. 2010[102]	India	Case report	1	Acute corneal melt
Faschinger et al. 2010[103]	Austria	Case report	2	Corneal melting in both eyes of 1 patient
Gokhale 2011[99]	India	Case report	1	Endothelial cell loss
Labiris et al. 2011[101]	Greece	Case report	1	Acute corneal melt with perforation
Kymionis et al. 2012[98]	Greece	Prospective case series	14	Endothelial cell loss in thin corneas

IV. COMBINATION OF CXL WITH REFRACTIVE VISION-IMPROVING INTERVENTIONS

Collagen CXL retards, aborts, or, in many cases, partially reverses the ectatic process in eyes with keratoconus and ectasia after LASIK, as measured by visual acuity and corneal curvature. CXL is minimally effective as a refractive procedure per se; corneal curvature irregularity must be significantly reduced and regularized before improved visual acuity can be achieved. There are several means to regularize the ectatic cornea in combination with CXL, as described below.

A. Topography-Guided Custom Ablation

Stojanovic et al treated 12 eyes of 12 patients with keratectasia with transepithelial topography-guided custom ablation followed by CXL according to the standard protocol.[105] Mean UCVA increased from 20/100 preoperatively to 20/125 12 months postoperatively, and mean BCVA improved from 20/57 to 20/35, with no loss of lines of visual acuity. Mean astigmatism was reduced from 5.40±2.13 D to 2.7±1.44 D, and keratometric asymmetry decreased from 6.38±1.02 D to 2.76±0.73 D.

In treating post-LASIK ectasia, Kanellopoulos and Binder used the topography-guided transepithelial PRK technique to normalize the cornea by reducing irregular astigmatism while treating part of the refractive error.[106] To remove the smallest amount of tissue possible, the effective optical zone was decreased to 5.5 mm, and the treatment was planned to reduce up to 70% of cylinder and sphere so as not to exceed 50 μm in planned stromal removal. After PRK treatment, 0.02% Mitomycin C solution was applied for 20 sec and irrigated with 10 ml of a balanced salt solution. For the next 10 min, hypotonic 0.1% riboflavin solution was applied every 2 min and was followed by 30 min of UVA irradiation. The mean followup time for 32 eyes in 22 patients was 27 months. UDVA improved in 27 eyes, was unchanged in 4 eyes, and worsened in 1 eye. CDVA was 20/40 or better in 27 of 32 eyes and 20/25 or better in 14 eyes. The authors believe it may be counterintuitive to remove the crosslinked tissue with topography-guided PRK at a later time, as it would potentially remove a beneficial layer of the stiffer, crosslinked cornea, which helps to maintain the normalized corneal shape. Additionally, they think that removing the Bowman layer with topography-guided PRK may facilitate riboflavin solution penetration in the corneal stroma and cause less "shielding" of UVA light in its passage through the cornea, resulting in more effective CXL.[106]

Kymionis et al reported their results in a followup of at most 25 months after simultaneous topography-guided photorefractive keratectomy followed by CXL for keratoconus.[107] The epithelium was removed by transepithelial PTK, where the ablation was performed in an 8.0-mm zone at an intended depth of 50 μm. A solid-state laser with a wavelength of 213 nm was used for the PRK procedure. The customization was performed based on the topographic data, the relevant attempted correction was up to 60% of sphere and cylinder of patient refractive error, and the upper limit of the depth of tissue removed was 50 μm. The postoperative mean spherical equivalent, mean steep and flat keratometry readings, and logarithm of the minimal angle of resolution UCVA and BCVA decreased significantly from preoperative values.[107]

B. Implantation of Intracorneal Ring Segments

Another possibility for regularizing an ectatic cornea together with the CXL procedure is with implantation of

intracorneal ring segments. Vincente and Boxer Wachler analyzed results from 14 eyes that underwent transepithelial CXL and same-day Intacs implantation in a 3-year retrospective study.[108] CXL followed the Intacs insertion, and the patients were pretreated with tetracaine. Mean BCVA improved from 20/32 preoperatively to 20/25 postoperatively at the third-year followup, and all keratometry values improved significantly. The authors showed that patients with advanced keratoconus had a greater improvement in BCVA, but final BCVA tended not to be as good as it was for less-advanced keratoconus.[108]

Transepithelial CXL can provide additional improvement after prior Intacs implantation. Ertan et al reported an additive effect of transepithelial CXL in eyes following Intacs implantation. Their study noted improvements in UCVA, BCVA, cylinder, sphere and keratometry values when transepithelial CXL was performed an average of four months after Intacs.[109]

Saelens et al reported results of seven eyes from seven patients.[110] They implanted Ferrara intracorneal ring segments in patients with progressive keratoconus. After ring segments were implanted, 0.1% riboflavin in 20% dextran was applied every 3 min for 25 min on the cornea and injected into the intrastromal canals. Riboflavin penetration of the stroma was confirmed at the slit lamp. The central 8.5-mm cornea was then irradiated with UVA light. At 1-year followup, one inferior ring from one patient was explanted because of partial extrusion. Mean UCVA improved from 0.10±0.07 to 0.6±0.24, and BCVA improved from 0.56±0.08 to 0.82±0.25. Three of these patients could wear contact lenses, one patient was still contact lens-intolerant, and two patients did not need any correction because of excellent UCVA.[110]

Coskunseven et al compared two sequences of combined intrastromal corneal ring segment implantation and CXL in progressive keratoconus: CXL was followed by ring implantation (group 1), or ring implantation was followed by CXL (group 2). The implantation of corneal rings followed by CXL resulted in greater improvement of postoperative keratoconus parameters (BCVA, SE, mean K-values).[111]

C. Intrastromal Pocket for Riboflavin Infusion

Alió's group compared results of CXL in progressive keratoconus using the standard technique (with epithelial removal) with results from intrastromal pocket creation after previous corneal ring segment implantation.[112] In all cases, KeraRing segments had been implanted using a femtosecond laser 3-12 months before the CXL procedure. An intrastromal pocket was created with a femtosecond laser at a corneal depth ranging from 70−90 μm, riboflavin was injected directly into the corneal pocket, and UVA was applied to the cornea for 20 min. The standard procedure was performed in 16 eyes from 13 patients, and the pocket technique was performed on 11 eyes from 8 patients. In this study, similar improvements in UDVA and CDVA were noted in both groups. However, statistical significance for the global change between the preoperative and 12-month postoperative data was only found for the change in UDVA in the classic group. One factor that could have accounted for this finding was the larger variability present in the pocket group, with patients having different levels of visual limitation. The sample size was limited in both groups, which could have also limited the power of the statistical tests.

Alió believes that CXL surgery with creation of an intrastromal pocket for riboflavin infusion seems to be as effective for corneal and refractive changes in keratoconic corneas as classic CXL surgery with previous epithelial debridement, with potentially less postoperative pain.[112] Risks of infection and scarring are present with both CXL techniques, but with the possibility of deeper stromal infection or scarring with the pocket technique. Although not observed in this series, these risks should be evaluated and analyzed in a larger sample of patients. In addition, the pocket technique may be restricted in some patients due to the higher costs of femtosecond lasers.[112]

D. Implantation of Intraocular Lens

Implantation of Artiflex foldable anterior iris-claw phakic intraocular lens (**PIOL**) following CXL is another possible option to improve visual rehabilitation in keratoconic eyes. Izquierdo et al implanted PIOL 6 months after CXL in 11 eyes in patients with stage I (18.2%) and stage II (81.8%) keratoconus.[113] Mean UDVA changed from 1.4±0.4 logMAR to 0.16±0.06 logMAR 6 months after PIOL implantation. Mean CDVA changed from 0.14±0.06 logMAR to 0.04±0.05 logMAR 6 months after implantation. Mean keratometry was reduced by 1.27 D 6 months after CXL and by 2.14 D 6 months after Artiflex implantation. No progression of cone was noted in any eyes. The endothelial cell count 6 months after CXL was not significantly lower, but was significantly reduced 6 months after PIOL implantation. Based on the available data and results, the authors suggest that PIOL implantation after CXL in patients with keratoconus would have a similar refractive effect on nonkeratoconic eyes. An additional benefit is provided by CXL in reducing corneal steepening and limiting the progression of keratoconus, thereby improving the two principal problems of refractive error and progression of the disease.[113]

Another kind of PIOL, the Visian implantable collamer lens (**ICL**), has been indicated for use in patients with stable keratoconus and patients with keratoconus whose corneal condition has been stabilized by CXL.[114,115] In contrast, the clinical course of keratoconus is often unpredictable, with periods of progression alternating with periods during which the ectatic process appears to have been arrested. Many patients who received an ICL during a period of disease stability may need CXL at a later stage should ectasia progress.

For this reason, Hafezi et al conducted an experiment testing whether UVA irradiation used during CXL might have unwanted effects on the optical and material characteristics of the Visian ICL.[116] After instillation of riboflavin, an

Table 4. Reports of CXL in combination with other refractive procedures — "CXL plus"

Author	Country	Study design	No. of eyes	Follow-up (months)	Procedure
Kanellopoulos et al. 2007,[118] 2009,[119,120] 2011[106]	Greece	Case reports, prospective studies	1, 325, 2, 32	18, 24-68, 30, 6-59	Topography-guided PRK, topography-guided partial transepithelial PRK
Kymionis et al. 2009,[120] 2011[115]	Greece	Case report, prospective studies	1, 31, 14	12, 12-25, 3-16	Simultaneous topography-guided PRK
Ertan et al. 2009[109]	Turkey	Retrospective	25	3	Intacs
Coskunseven et al. 2009[111]	Turkey	Prospective, consecutive, comparative	48	6	Intrastromal corneal ring segment (ICRS)
Vicente et al. 2010[108]	USA	Retrospective	14	36	Intacs
Stojanovic et al. 2010[105]	Norway	Prospective	12	12	Topography-guided trans-epithelial surface ablation
Alió et al. 2011[112]	Spain	Retrospective	27	12	KeraRing
Izquierdo et al. 2011[113]	Peru	Prospective, comparative	11	12	Artiflex foldable anterior iris-claw phakic lens

intense yellow staining of ICL was observed; this staining completely resolved within 6 hours. Analysis of the dioptric power before and after CXL revealed a shift that stayed within the ISO (International Standard on ophthalmic implants) limits of 0.30 D. Analysis of the transmission spectrum showed similar curves before and after CXL for all experimental conditions. From these results, Hafezi concluded that performing CXL in eyes that had undergone previous ICL implantation can be considered safe.[116]

When analyzing results published on the combination of the CXL procedure with additional refractive treatment (excimer laser ablation, implantation of intrastromal corneal rings or implantation of phakic IOLs), it should be taken into consideration that with the exception of one study that included 325 eyes, these studies were case reports or small studies that included a maximum of 48 eyes (Table 4).[96,105,108,109,112,113,115,117-120] To determine which refractive procedure and which sequence are most suitable in combination with CXL treatment depending on the stage of corneal ectasia, controlled studies with larger cohorts and longer followup periods are necessary.

V. SUMMARY AND CONCLUSIONS

Fifteen years ago, corneal collagen CXL by means of riboflavin and ultraviolet light was proposed as a therapeutic approach to improve the biomechanical and biochemical properties of the cornea. The advent of this procedure is one of the more promising discoveries in the management of corneal ectatic disorders. Currently available conservative and surgical therapeutic options can only temporarily correct the refractive effect but do not stop the progression of the ectatic process. In the long term, radical surgical intervention, such as keratoplasty, is the only choice, although potential intraoperative and postoperative complications limit good long-term results. Several of the clinical studies cited in this review demonstrate that by increasing the biomechanical stability of the cornea using riboflavin and UVA-induced collagen CXL, it is possible to stop the progression of corneal ectasia, with very low incidence of complications or side effects.

The CXL procedure also offers economic and psychosocial benefits. It could be easily provided as an outpatient service, and it is a minimally invasive, cost-effective treatment with minimal stress for patients. At present, none of the corneal ectatic pathologies are curable. However, CXL can stop their progression. Thus, it is important to crosslink corneas with progressive ectasia as early as possible. Moreover, the antimicrobial, antiproteolytic and antiedematous properties of CXL have been demonstrated in a series of studies, suggesting its therapeutic indications in infectious keratitis, corneal melting processes, and bullous keratopathy as an adjuvant treatment to conventional therapeutic modalities. In addition, newer applications of this procedure, including combinations with refractive procedures, such as topography-guided surface corneal photoablation or implantation of corneal ring segments or phakic intraocular lenses, to improve and to shorten the visual rehabilitation time in patients with progressive ectasia will gain popularity and will require strict evaluation of the risks and benefits.

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