

# Patient selection for corneal collagen cross-linking: an updated review

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**Abstract:** Corneal cross-linking (CXL) is an option that in the last decade has demonstrated its efficacy and safety in halting the progression of keratoconus (KCN) and other corneal ectasias. Its indication has been extended beyond the classic definition that required evidence of KCN progression, especially in the presence of some risk factors for a possible progression (particularly the younger age). However, the results can be still somewhat variable today. There are several protocols, each with its own advantages and disadvantages. Some predictors of CXL outcome have been identified. We will review the current knowledge on patient selection for CXL, its indications, and options in special cases (such as thin corneas).

**Keywords:** keratoconus, cornea, cross-linking, corneal collagen cross-linking, keratoectasia

## Introduction

Among corneal ectasias, primary forms include keratoconus (KCN) and pellucid marginal degeneration (PMD), while secondary ones occur as a late complication of Laser-Assisted In-Situ Keratomileusis (LASIK) or other refractive surgery procedures.<sup>1-6</sup>

Before the introduction of corneal cross-linking (CXL), there was no effective way in stopping corneal ectasias progression, which led to an important number of keratoplasties.<sup>7</sup> Since its introduction in the late 1990s, CXL has been established as a mainstream treatment for ectasias with signs of progression with many studies showing good short-term results and some reporting good long-term results between 7 and 10 years after the procedure.<sup>8-14</sup> However, Sykakis et al<sup>15</sup> in a recent Cochrane Review, which included three randomized clinical trials, concluded that the evidence for the use of CXL in the management of KCN is limited due to the lack of properly designed and conducted studies. The three studies comprised 119 eyes undergoing CXL according to the Dresden protocol and 100 keratoconic eyes as controls, but all the three clinical trials were found to be at high risk for detection bias (only in one of the studies an attempt to mask outcome assessment was done), performance bias (absence of masking), and attrition bias (incomplete follow-up). In addition, it was not possible to pool data because of differences in measuring and reporting outcomes.<sup>16-18</sup>

CXL has also shown mixed results in the treatment of nonectatic disorders such as corneal infections, chemical burns, and bullous keratopathy.<sup>19,20-23</sup> Those indications will not be included in this review.

## Principles of corneal collagen cross-linking

The main objective of CXL is to achieve strengthening of corneal tissue as a means to stop further progression of corneal ectasia. In order to induce cross-links within

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and between collagen fibers of corneal stroma, long-wave ultraviolet A (UVA) radiation (370 nm) is used combined with a chromophore (riboflavin, vitamin B2). Riboflavin acts as photosensitizer that when exposed to UVA is excited, producing oxygen free radicals that initiate the creation of those new covalent bonds bridging the amino groups of collagen fibrils and possibly other corneal macromolecules such as proteoglycans and nucleic acids.<sup>24</sup> This photopolymerization process results in the increased rigidity of corneal tissue.<sup>19,25,26</sup>

During late 1990s and early 2000s, several authors explored the use of riboflavin and UVA light in order to increase corneal stiffness in animal eyes, but a group of researchers from Dresden (Germany) were the ones who achieved the greatest breakthroughs at that moment.<sup>27,28</sup> Wollensak et al,<sup>29</sup> members of that group, in 2003 published the first *in vivo* study on 22 patients with progressive KCN, in which they documented a halted progression in all the cases and reduction of the maximal keratometry readings in around 70% of these cases.

Currently, CXL is an established procedure in managing progressive corneal ectatic disorders virtually all around the world; however, there is undoubtedly still much room for progress in improving the technique and the results of the procedure, in terms of reducing its invasiveness and increasing its safety profile and its stabilization effects.<sup>9,11,12,19</sup>

## Current protocols

### “Dresden protocol”: Epi-off CXL

It was named “Dresden protocol” because it was originally developed at the Technical University of Dresden (Dresden, Germany) by Wollensak et al.<sup>29</sup> It is still considered to be the standard CXL treatment protocol. “Dresden protocol” includes removal of central 8–9 mm of epithelium, application of 0.1% riboflavin solution every 5 minutes for 30 minutes, followed by exposure to UVA (370 nm, 3 mW/cm<sup>2</sup>) for 30 minutes with the application of riboflavin solution every 5 minutes during exposure.<sup>29–31</sup> Medium-term results (mean follow-up time between 6 and 26 months) have been shown to be good using this technique, in terms of safety and efficacy, as also shown in a recent meta-analysis.<sup>8,31–34</sup> However, long-term results are less defined. The group of Dresden published 10-year results in 2015 and concluded that CXL achieved long-term stabilization of the condition in 34 eyes.<sup>12</sup> Nevertheless, there are some concerns with regard to the quality of the data in that group of cases.<sup>35</sup> Poli et al<sup>36</sup> reported their results with 6 years of follow-up. CXL was

effective in stabilizing KCN and other ectatic disorders, but had a long-term failure rate, in terms of progression, in 11% of the eyes. O’Brart et al<sup>11</sup> also published their long-term (7 years) results in 2015 and concluded that improvements in both topographic and wavefront parameters found 1 year after the procedure continued to improve up to 5 years after the CXL and were maintained at 7 years. Not one of the 36 treated eyes progressed over the 7-year follow-up period.

Some groups have reported results using modifications, as partial deepithelization, suggesting also stabilization at short and medium term.<sup>37–39</sup> However, it remains to be determined whether this approach would have any real advantage over the traditional “Dresden protocol.”

Other modification of the “Dresden protocol” included mechanical compression of the tissue by suturing a semiscleral rigid contact lens with a flat curvature (back surface radius of 11.0 mm) to the cornea after applying the riboflavin. Then, riboflavin was injected every 5 minutes under the contact lens during UVA irradiation, using a blunt cannula. Since the UV filter in the contact lens absorbed 11% of the UVA light, the irradiation time was increased to 34 minutes. The contact lens was left in place for 1 hour after the procedure. However, in spite of improving the corneal flattening effect, the results of this technique at 6 months were inferior compared to the standard “Dresden protocol.” A possible explanation could be the differences in the precorneal riboflavin film with the contact lens in place.<sup>40</sup>

### Epi-on CXL technique

Initial studies showed limited diffusion of riboflavin (a large hydrophilic molecule with a molecular weight of 376.37 g/mol) through corneal epithelial tight junctions, thus reducing CXL effectiveness presented if this layer was not removed.<sup>30</sup> Nevertheless, epithelial debridement is considered to be the major source of complications secondary to CXL like keratitis, persistent epithelial defect, and most frequently reported severe postoperative pain.<sup>41–43</sup> Various techniques have been explored to solve this issue by adding chemical enhancers causing epithelial disruption of corneal epithelium, like the surfactant benzalkonium chloride (BAC) or ethylenediaminetetraacetic acid (EDTA). Wollensak and Iomdina<sup>44</sup> performed the first experimental study using iso-osmolar riboflavin (20% dextran) +0.005% BAC, and it increased the biomechanical changes after cross-linking in comparison to a control group, also without removing the epithelium but using no BAC. However, as discussed later, the CXL without epithelial removal using BAC-containing proparacaine eye drops led to a biomechanical stiffening

effect of only one-fifth of that induced by cross-linking according to the Dresden epi-off standard protocol.

Some providers of medical supplies offer different options of riboflavin for CXL. The company Avedro offers a particular formulation (ParaCel™, MedioCROSS TE, Avedro, Waltham, MA, USA [0.25% riboflavin, 1.2% hydroxypropyl methylcellulose, and 0.01% BAC]) for transepithelial epithelium-on technique. However, it is not clear if these transepithelial techniques, even with those special formulations of riboflavin, allow sufficient riboflavin absorption into the stroma to efficiently cause CXL. As mentioned, experimental studies with animal eyes by Wollensak and Iomdina<sup>44</sup> suggested that disruption of the epithelial tight junctions with BAC prior to CXL increased corneal stiffness only by around one-fifth of what regular epi-off CXL can achieve. A study in porcine eyes treated with an epi-off technique versus a transepithelial protocol (using 0.02% BAC and 0.44% NaCl as enhancers of the penetration) showed that the latter was around 70% less effective.<sup>45</sup> Some groups have reported good results, but efficacy of transepithelial techniques is still a matter of debate, with few studies showing increase in keratometric values and retreatment necessity in a significant percentage of cases.<sup>46-50</sup>

## Accelerated cross-linking

With the standard “Dresden protocol” using 3 mW/cm<sup>2</sup> of energy in a 9 mm treatment zone for 30 minutes, a total energy of 5.4 J/cm<sup>2</sup> is delivered.<sup>30</sup> Therefore, it can be inferred that by using higher intensity light the necessary, exposure time can be reduced to achieve the same total energy.

In an ex vivo study using porcine eyes, Wernli et al<sup>51</sup> examined their response to different levels of irradiances between 3 and 90 mW/cm<sup>2</sup> and found that irradiances above 50 mW/cm<sup>2</sup>, with illumination times of less than 2 minutes, failed to increase corneal stiffness.<sup>30</sup>

Experimental studies have shown that the use of UVA irradiance alone has a high cytotoxic activity, especially in endothelial cells. Cytotoxic level of UVA irradiance has been described as approximately 0.35 mW/cm<sup>2</sup>, which would be twice as much as it reaches in the use of the standard protocol (0.18 mW/cm<sup>2</sup>). The use of riboflavin aims to reduce the toxic effect of the use of UVA alone while still increasing corneal stiffness.<sup>52</sup> CXL effect on corneal endothelium has been a matter of debate, especially in the setting of accelerated cross-linking because of the higher levels of irradiance. Corneal thickness of a minimum of 400 microns has been described as the cut-off point to avoid endothelial damage, even though

there have been case reports of endothelial changes in corneas thicker than 400 μm before surgery.<sup>30</sup>

A study by Kanellopoulos with a mean follow-up of 46 months, in 21 patients treated with accelerated CXL (7 mW/cm<sup>2</sup> for 15 minutes) in one eye and with Dresden standard protocol CXL (3 mW/cm<sup>2</sup> for 30 minutes) in the fellow eye showed similar results in both eyes with no progression of KCN and equivalent improvement in visual acuity and keratometry. There was no evidence of endothelial damage.<sup>53</sup>

Shetty et al<sup>54</sup> compared four protocols of CXL in eyes with steep preoperative keratometry between 48.6 diopters (D) and 50.5 D. They found that over a follow-up period of 1 year, standard “Dresden protocol” CXL (3 mW/cm<sup>2</sup> for 30 minutes) showed greater flattening effect than accelerated CXL protocols of 9 mW/cm<sup>2</sup>, 18 mW/cm<sup>2</sup>, and 30 mW/cm<sup>2</sup>. Though the latter two groups did not show progression at 12 months, there was no significant corneal flattening in eyes in these two groups. No significant endothelial cell loss was detected.

Another comparative study between standard Dresden protocol CXL and accelerated CXL (18 mW/cm<sup>2</sup>, 365 nm UVA light, 5 minutes) also showed that the effect of the latter being lower on flattening of the cornea.<sup>52</sup>

A recent review concluded that with the short follow-up time of almost all the studies (usually 1 year or less), accelerated CXL seems to be a safe and effective method to stop the progression of ectasia, but the effect on flattening the cornea is most probably less than the standard “Dresden protocol.”<sup>55</sup>

Experimentally, studies have also found that the effect of accelerated CXL is less than the standard 30 minutes protocol. In porcine eyes, increased corneal enzymatic resistance was lower with accelerated CXL (9 mW/cm<sup>2</sup> for 10 minutes and 18 mW/cm<sup>2</sup> for 5 minutes).<sup>56</sup> Also, in porcine corneas exposed to riboflavin 0.1% and different protocols of UVA irradiation (3 mW/cm<sup>2</sup> for 30 minutes, 9 mW/cm<sup>2</sup> for 10 minutes, and 18 mW/cm<sup>2</sup> for 5 minutes), a decreased stiffening effect was found with increasing UVA intensity. In fact, using an irradiance of 18 mW/cm<sup>2</sup> for 5 minutes the stiffness of the corneas was not augmented compared to untreated controls. The authors suggested that using high irradiance with short irradiation time settings causes higher oxygen consumption, and due to limited intrastromal oxygen diffusion capacity, the treatment efficiency is reduced.<sup>57</sup>

For a few years now, it has been known that oxygen presence at an adequate concentration in the stroma is necessary for CXL to occur when applying UVA light in the presence of riboflavin.<sup>58</sup> According to a theoretical model of

photochemical kinetics of corneal cross-linking, the UVA illumination produced a rapid reduction of stromal available dissolved oxygen in a riboflavin-soaked cornea, secondary to the process of generation of reactive oxygen species (including singlet oxygen). However, turning the UV light off allowed recovery of the oxygen to its original level, from the environment, within 3–4 minutes.<sup>59</sup>

Therefore, an alternative used to increase the availability of oxygen in the corneal stroma is pulsing the UV light during cross-linking treatment, to permit the reoxygenation during pauses in exposure. Mazzotta et al<sup>60</sup> found that a modified protocol of pulsed accelerated cross-linking (pl-ACXL) with 8 minutes of exposure time (1 second on and 1 second off) and 30 mW/cm<sup>2</sup>, with a total energy dose of 7.2 J/cm<sup>2</sup> (ie, higher than in the “Dresden protocol”) had some better results than continuous light accelerated corneal collagen cross-linking (cl-ACXL) with the same instrument (UVA power setting at 30 mW/cm<sup>2</sup> for 4 minutes of continuous UVA light exposure, and energy dose of 7.2 J/cm<sup>2</sup>). With regard to “Topographic-derived apical curvature value”, no statistically significant differences were recorded after cl-ACXL, while a statistically significant decrease by a mean value –1.39 D at 1-year follow-up was found in pl-ACXL. With respect to topographic simulated K average value, a not statistically significant decrease by a mean value of –0.13 D was observed with cl-ACXL, while a statistically significant reduction by a mean value of –1.20 D was observed after pl-ACXL. It is striking that both groups exhibited a loss of corneal endothelial cells density: cl-ACXL: 3.9% and pl-ACXL: 6.6%. The demarcation line after cl-ACXL was uneven and at mean depth of 160 μm and after pl-ACXL, it showed a mean depth of 200 μm. The results on demarcation line depth were similar to those by Peyman et al<sup>61</sup> also using a total fluence of 7.2 J/cm<sup>2</sup> and a group of eyes with pulsed irradiation and the other with continuous light exposure. However, recently, Kymionis et al,<sup>62</sup> using another modified high-intensity CXL protocol for 7 minutes with 18 mW/cm<sup>2</sup> of UVA continuous irradiation with a total energy dose of 7.5 J/cm<sup>2</sup>, found a deeper demarcation line, with no differences with the standard “Dresden protocol” (313.4 microns versus 341.8 microns, respectively).

Another interesting approach is the oxygen enrichment of the environment to which the cornea is exposed during the procedure. Unfortunately, the ex vivo experimental results, recently published by Diakonis et al,<sup>63</sup> showed that in human corneas there was no significant effect of supplemental oxygen when accelerated cross-linking was performed (3 minutes exposure to UVA light at 30 mW/cm<sup>2</sup> after application of riboflavin). Further studies are required.

## Iontophoresis

This alternative has the purpose of enhancing riboflavin penetration through corneal epithelium, using a noninvasive system by means of a low-intensity electric field created by applying on the cornea two electrodes that are connected to a generator which delivers a small electric current. Iontophoresis transepithelial CXL avoids debridement of corneal epithelium and has been shown to provide better riboflavin saturation than epi-on approach. Clinical studies have shown good results in halting of KCN progression and improvement in topographic and visual parameters. However, the effect has shown to be inferior when compared to standard epi-off technique using the “Dresden protocol.”<sup>64,65</sup>

## Patient selection Indications

The most common indication for CXL is KCN. However, CXL is not primarily a refractive procedure but has the purpose of stopping the progression of corneal ectasia. Therefore, when the ectatic disorder is already stable, the procedure is not indicated. As the age increases, corneal collagen fibrils become thicker, and naturally occurring cross-linking increases stiffness of the tissue (determined by a parameter called the Young’s modulus).<sup>29,66,67</sup> These natural changes might explain that when KCN presents earlier in life, the patient has a higher risk of requiring keratoplasty and the classical finding that the condition usually progresses until the third to fourth decade of life, when it typically halts.<sup>2,68–74</sup> The Collaborative Longitudinal Evaluation of Keratoconus Study (CLEK study) found among 300 subjects, between 48 and 59 years of age, that there was a slow progression of KCN (0.24 D difference in steep K readings over a period of 3 years), which was statistically significant but clinically nonsignificant.<sup>75</sup> In fact, it has been suggested that KCN might regress in patients older than 60 years, because the number of patients in that age group seen by cornea specialists is very low.<sup>76</sup>

CXL has also been used in other ectatic diseases like PMD and in degenerative corneal diseases like Terrien Marginal Degeneration. However, a much smaller body of evidence supports the indication of CXL in these conditions.<sup>19,22,77,78</sup>

Therefore, one crucial requisite in order to determine the indication of CXL in KCN is to have definitive criteria for progression, but unfortunately there is no real global consensus. Parameters to consider are change in refraction (including sphere and astigmatism), uncorrected distance visual acuity (UCDVA), corrected distance visual acuity (CDVA), and measurements reflecting corneal shape (as



determined by keratometry, Placido disk reflection topography, or tomography using scanning slit with or without applying the Scheimpflug principle).<sup>18,29,31,79–81</sup> Clinical measurements, both refractive using manifest refraction and structural using diverse devices, are challenging and have lower repeatability in KCN due to the presence of irregular astigmatism and altered reflection of the corneal mires of the devices on the irregular corneal surface.<sup>82–86</sup> In addition, progression of KCN has been shown to be highly variable.<sup>72</sup> In the CLEK study, which gathered a very large sample of 1,988 eyes with KCN in adults, flat keratometry increased in average 1.60 D over a period of 8 years, but 24.1% of those eyes showed an increase greater than or equal to 3.00 D.<sup>87,88</sup>

The Global Delphi Panel of Keratoconus and Ectatic Disease published in 2015 recognized that there was no clear definition of ectasia progression, and so the experts suggested that it should be defined by a reliable change for the worse in two or three of the following parameters: radius of the anterior corneal curvature; radius of the posterior corneal curvature and central corneal thickness; or increase in the rate of change of pachymetry from the periphery to the thinnest point. The experts considered that although KCN progression frequently leads to a worsening in CDVA, a change in both UCDVA and CDVA was not required for documenting progression. In addition, they agreed that specific quantitative data were lacking to determine progression and that such data would most probably be specific to a given device. Also, that the interval between corneal examinations should be shorter among younger patients and that the same measuring equipment, when possible, should be used. However, they did not recommend a specific length of time between two consecutive examinations in order to define progression.<sup>89</sup> Moreover, the panel not only stated concerning the indication for CXL that it was essential in the management of KCN with documented clinical progression but also affirmed that it was useful for the treatment of KCN with a significant risk of progression even if the progression had not been documented, as it has been proposed by Chatzis and Hafezi.<sup>73</sup> This statement, which reflects the current reality, leaves the door open for the procedure to be performed when the clinician's judgment indicates it, even if the case does not meet the criteria for progression of the KCN. As we will discuss in the next section, the individualized evaluation of each patient will allow the clinician to make the best decision, taking into account other risk factors, such as age.<sup>89</sup>

In Table 1, we summarize diverse progression criteria that have been used in CXL studies by several authors.

## Age

KCN may appear very early in life. The youngest patients with KCN were 4 years of age: a girl with persistent eye rubbing and another one with Down syndrome (who in fact, underwent CXL).<sup>90–92</sup> Furthermore, modern ophthalmologists are more aware of KCN, and with the available diagnostic tools, it can be detected in very young children (as young as 4 years old, as mentioned), so the cornea specialist faces the dilemma of treating a child without evidence of progression or waiting until progression occurs. Now, as mentioned before, according to a panel of experts and other several authors, if there are risk factors that make progression very likely, CXL is indicated without an age limit. Those groups at risk include children and adolescents, and patients with advanced KCN. Some have suggested that unilateral KCN and CDVA 20/40 or worse are also indications of CXL.<sup>73,89</sup>

However, with regard to CXL in children, there is a scarcity of randomized control trials, so the majority of the information is extrapolated from the also limited data from clinical trials and case series with adults. So, it is not possible to affirm that there is a gold standard protocol to follow for making decisions in children. However, according to the results from the Siena CXL Pediatrics trial, it is the standard epi-off ("Dresden protocol") option that should be used when the available evidence is too weak to support the practice of other alternatives (like epi-on CXL). Topographic and functional improvement was obtained in 80% of children included in the study, while only 4.6% of the cases showed progression after CXL.<sup>87,93</sup> Nevertheless, according to some reports, CXL in children might not be as successful as in adults, and therefore the former need close postoperative follow-up, as the risk of progression is real and further CXL may be warranted.<sup>87,94–96</sup> In a recent report on long-term results, Godefrooij et al<sup>96</sup> found that among 54 eyes of 36 children, who underwent CXL with the "Dresden protocol" and had a follow-up time up to 5 years (18 eyes followed for 4 years and 9 eyes for 5 years), the maximum keratometry showed a significant improvement of 2.06 D on average, but in 12 eyes (22%) of nine children (25%), a progression in the keratometry values of 1.0 D or higher presented at the last follow-up visit.<sup>94</sup>

Recently, Frucht-Pery and Wajnsztajn<sup>97</sup> indicated that most of their patients undergoing CXL in the last 2 years have been diagnosed only several months earlier, because they do not wait for progression to happen if the patient is in a high-risk group, especially children and adolescents. However, they also stated that a case-by-case assessment is required to weigh the risks against the benefits of the surgery.

**Table 1** Progression definitions among literature

Study	Criteria for progression
Dresden protocol (Raiskup-Wolf et al, <sup>31</sup> 2008)	Any of the following three: <ul style="list-style-type: none"> <li>• Increase in <math>K_{max} \geq 1.00</math> diopter (D)</li> <li>• Patient's self-report of deteriorating of visual acuity</li> <li>• Need for new contact lens fitting more than once in 2 years</li> </ul>
Wittig-Silva et al, <sup>17</sup> 2008	One or more of the following: <ul style="list-style-type: none"> <li>• Increase of <math>\geq 1.00</math> D in the steepest simulated keratometry reading (<math>K_{max}</math>)</li> <li>• Increase of <math>\geq 1.00</math> D in manifest refraction's astigmatism</li> <li>• Increase of <math>\geq 0.50</math> D in manifest refraction SE</li> <li>• Decrease of <math>\geq 0.1</math> mm in back optic zone radius of the best fitting contact lens</li> </ul>
Vinciguerra et al, <sup>80</sup> 2009	Any of the following three: <ul style="list-style-type: none"> <li>• Change in myopia and/or astigmatism of <math>\geq 3.00</math> D in the previous 6 months</li> <li>• Mean central K-reading change of <math>\geq 1.50</math> D in 3 consecutive topographies during the previous 6 months</li> <li>• Mean central corneal thickness decrease of <math>\geq 5\%</math> in 3 consecutive tomographies performed in the previous 6 months</li> </ul>
O'Brart et al, <sup>16</sup> 2011	Any of the following two criteria met over the previous 18 months: <ul style="list-style-type: none"> <li>• Reduced UCDVA or CDVA by more than one line</li> <li>• Increase of refractive or corneal astigmatism, keratometry, or cone apex power by 0.75 D</li> </ul>
Hersh et al, <sup>18</sup> 2011	One or more of the following changes over a period of 24 months: <ul style="list-style-type: none"> <li>• Increase of <math>\geq 1.00</math> D in the steepest K measurement</li> <li>• Increase of <math>\geq 1.00</math> D in manifest cylinder</li> <li>• Increase of <math>\geq 0.50</math> D in manifest refraction SE</li> </ul>
Chatzis and Hafezi, <sup>73</sup> 2012	• Increase of $\geq 1.00$ D in $K_{max}$ over a maximum of 12 months
Hashemi et al, <sup>38</sup> 2013	Any of the following criteria met during the previous 12 months: <ul style="list-style-type: none"> <li>• Increase in <math>K_{max}</math>, manifest cylinder error, or manifest refraction SE <math>\geq 1.00</math> D</li> <li>• Loss of <math>\geq 2</math> lines of CDVA attributable just to keratoconus progression</li> </ul>
Mazzotta et al, <sup>60</sup> 2014	Variation of at least three of the following parameters (one clinical plus two instrumental) during a period of 4 months for patients under 18 years and 6 months for adults: <sup>a</sup> <ul style="list-style-type: none"> <li>• Worsening of UCDVA/CDVA <math>&gt;0.50</math> Snellen lines</li> <li>• Increase of SPH/CYL <math>&gt;0.50</math> D</li> <li>• Increase of topographic symmetry index SAI/SI <math>&gt;1.00</math> D</li> <li>• Increase of mean K reading <math>&gt;1.00</math> D</li> <li>• Reduction of the thinnest point at corneal optical coherence tomography pachymetry <math>\geq 10 \mu\text{m}</math></li> </ul>
Stojanovic et al, <sup>50</sup> 2014	During a period of 12 months: <ul style="list-style-type: none"> <li>• Increase of astigmatism or myopia <math>\geq 1.00</math> D or increase in average Sim K by 1.50 D</li> </ul>
Shetty et al, <sup>54</sup> 2015	• Increase in $K_{max}$ , corresponding change in the subjective refraction $\geq 1.0$ D or $\geq 5\%$ decrease in the thinnest pachymetry in the preceding 6 months
Poli et al, <sup>36</sup> 2015	• Deterioration of uncorrected (UDVA) and/or corrected (CDVA) distance visual acuities $>1$ Snellen line, SE refraction $>0.75$ D, cone apex keratometry $>0.75$ D, and/or reduction in corneal thickness (thinnest point) $>10$ microns lasting more than 6 months
Gomes et al, <sup>89</sup> 2015	Consistent change in at least two of the following parameters: <ul style="list-style-type: none"> <li>• Progressive steepening of the anterior corneal surface</li> <li>• Progressive steepening of the posterior corneal surface</li> <li>• Progressive thinning and/or an increase in the rate of corneal thickness change from the periphery to the thinnest point</li> <li>• No specific values were given because the panel considered them to be specific for each device</li> <li>• In addition, the panelists also indicated that CXL could be indicated for the treatment of KCN with a perceived risk of progression even if it had not been documented</li> </ul>
Godefrooij et al, <sup>110</sup> 2016	• Increase of $\geq 1.00$ D in the steepest K measurement within 6–12 months

**Note:** <sup>a</sup>The follow-up period was obtained from personal communication from the authors (Mazzotta et al, unpublished data, January 2, 2017).

**Abbreviations:**  $K_{max}$ , maximum keratometry; SE, spherical equivalent; UCDVA, uncorrected distance visual acuity; CDVA, corrected distance visual acuity; SAI, surface asymmetry index; SI, superior-inferior symmetry index.

We, as other clinicians and researchers, fully agree with their concepts and apply them in our clinical practice.<sup>94</sup>

In the case of children with mild disease, with good vision (CDVA of 20/20 or better), and few or undefined topographic signs, most probably will benefit from closer

observation with frequent examination (every 1–3 months). Now, if the child presents with a topographically evident KCN, in a relatively advanced stage, CXL most probably will be of benefit to the patient without waiting for the evidence of progression.<sup>87,91</sup>

When there is a fellow apparently healthy eye, it is important to remember that there is no such thing as a true “unilateral” KCN.<sup>86</sup> CXL is indicated in the eye with the progressive disease, and, unless another significant risk factor is present (very young age, persistent eye-rubbing, etc.), the less compromised eye with good vision can be maintained in close observation.

The longest reported follow-up time of CXL in children has been 3 years, and the effect of the procedure seemed to diminish after 2 years.<sup>89,98</sup> Therefore, the possibility of requiring an additional CXL should be considered when progression is found after the first procedure.<sup>99</sup> Some parameters have been suggested by Hamada et al<sup>90</sup> to determine the indication of a new CXL treatment: increase in the flattest K (K1), steepest K (K2), or  $K_{\max} > 1$  D, a change in the difference map between two consecutive topographies by 1 D, a deterioration of CDVA or any consistent change in the refractive astigmatism.<sup>87</sup>

## Corneal thickness

Before first trials in humans, animal models (specifically in rabbits) were used to establish that the currently used parameters usually affect the anterior 250–350 microns corneal stroma; therefore, a minimum of 400 microns stromal thickness was set as a safety margin in order to protect corneal endothelial cells.<sup>29</sup>

However, taking into account that KCN causes stromal thinning, in advanced cases the corneas frequently have less than 400 microns of thickness. Performing standard cross-linking on those cases implies the risk of endothelial cell loss, as shown by Kymionis et al<sup>100</sup> who treated 14 eyes (pachymetry after epithelial removal between 340 and 399 microns) and found a loss of endothelial cells of 10.7%.<sup>101</sup>

Several alternatives have been proposed in those cases.<sup>95</sup> In 2009, Hafezi et al<sup>102</sup> described a modified protocol for corneas thinner than 400 microns, swelling the stroma using hypoosmolar riboflavin solution. The standard iso-osmolar riboflavin 0.1% solution used in the “Dresden protocol” is prepared by diluting vitamin B2-riboflavin-5-phosphate 0.5% (Streuli Pharma, Uznach, Switzerland) with dextran T500 20% to reach an osmolarity of 402.7 mOsmol/L, while hypoosmolar riboflavin 0.1% solution is prepared by diluting vitamin B2-riboflavin-5-phosphate 0.5% with sodium chloride 0.9% solution, having 310 mOsmol/L, which causes corneal swelling. They used the treatment in corneas as thin as 323 microns after epithelial debridement and reached more than 400 microns after swelling in 20 cases.<sup>103</sup> However, a case that presented rapid progression after the procedure

was reported. Pachymetry after epithelial debridement was 268 microns, which reached 406 microns after swelling with hypoosmolar riboflavin solution and showed 2.3 D of progression 6 months after CXL.<sup>55,104,105</sup> Recently, accelerated (9 mW/cm<sup>2</sup> for 10 minutes) CXL with hypoosmolar riboflavin solution was shown to be effective in 49 eyes with thin corneas at short term (6 months of follow-up).<sup>98</sup> One advantage on the safety when using accelerated cross-linking in thin corneas is that the effect is most probably more superficial so that the corneal endothelium would be more protected. However, efficacy could be diminished.<sup>99</sup>

In corneas between 350 and 400 microns of thickness after epithelial abrasion, Jacob et al<sup>106</sup> used iso-osmolar solution of riboflavin 0.1% in dextran and an ultraviolet barrier-free hilafilcon soft contact lens (90 microns in thickness) soaked in iso-osmolar riboflavin 0.1% placed on the cornea to reach more than 400 microns of thickness of the complex contact lens-cornea. Then UVA irradiation was applied. In 14 eyes followed for around 6 months, no progression occurred and 28.5% showed a decrease in  $K_{\max}$  in 1.00 D or more.<sup>107</sup> Currently, in very thin corneas, which do not reach 400 microns even after the use of hypoosmolar riboflavin, we are performing a study with a different approach: protection of the 4 mm central cornea. This is thought to allow the CXL procedure to act at least in the periphery of the cornea, so that the receptor tissue is best prepared when a penetrating or lamellar anterior keratoplasty is performed in those advanced KCNs.

## CXL predictors of outcomes

Although some short-term and long-term studies have reported a rate of success of 100% in stopping the progression of KCN using CXL, failure rates between 7.6% and 11% have been found for other groups.<sup>11,41,36,108–110</sup>

In relation to corneal flattening, steeper pretreatment  $K_{\max}$  ( $\geq 54$  D), a more centrally located cone apex, and central pachymetry  $\geq 450$  microns have all been reported as predictive factors.<sup>109–112</sup>

Godefrooij et al<sup>110</sup> identified possible predictors for results of the effect of CXL in a prospective cohort, and recently in a different prospective cohort they performed a validation study.<sup>108</sup> They found by using univariate analysis that a predictor of higher improvement in  $K_{\max}$  was male sex, while atopia was a predictor of a slight (but significant) decrease in improvement in visual acuity.<sup>108</sup> Using a multivariable linear regression analysis, baseline visual acuity and cone eccentricity were the only two independent factors for predicting change in postoperative CDVA and  $K_{\max}$ ,

respectively. Patients with lower pretreatment visual acuity were more likely to have improved visual acuity after CXL, and patients with more central cones had more possibility of greater corneal flattening.<sup>108</sup> The latter finding was in concordance with those reported by Greenstein and Hersh.<sup>109</sup> Godefrooij et al<sup>110</sup> suggested, as Greenstein and Hersh,<sup>109</sup> that this might be related to the angle of exposure to UVA light: it is more perpendicular in central cones while peripheral ones receive light rays with an oblique incidence.<sup>108</sup> Therefore, they proposed that focusing the UVA light on the cone apex, instead of the geometrical center of the cornea, could improve results in eccentric cones.<sup>108</sup> In their validation set, Godefrooij et al<sup>110</sup> found similar results to their original prospective cohort.<sup>108</sup> Baseline preoperative CDVA was found to be the sole independent factor predicting an improvement in CDVA 1 year after the procedure, with patients with lower pretreatment visual acuity showing higher possibilities to benefit from CXL (with regard to visual acuity) and eyes with more central cones obtaining more benefit from the procedure in terms of corneal flattening.<sup>110</sup> In addition, unlike the findings in their first cohort, in the validation study they found that younger patients had significantly better results with respect to visual acuity.<sup>108,110</sup> Soeters et al<sup>113</sup> had also identified age as a prognostic factor.<sup>110</sup>

In another recent study, Godefrooij et al<sup>96</sup> also identified using a multivariable logistic regression analysis that cone eccentricity was the only independent factor significantly related to the progression of KCN in children who underwent CXL (mean age 14.8 years of age).

Greenstein and Hersh<sup>109</sup> studied 104 eyes of patients older than 13 years of age who had an axial corneal topography (Pentacam, Oculus Optikgeräte GmbH, Wetzlar, Germany) consistent with KCN or postrefractive surgery corneal ectasia and who underwent CXL. They found that in the multivariate regression analysis, the CDVA and maximum K value from the topography were the only significant predictors of the 1-year postoperative CDVA. However, although the multivariate analysis identified an association between the preoperative maximum K value and the postoperative CDVA, OR analyses failed to reach statistical significance. Their multivariate analysis also found that preoperative maximum K was the only significant predictor of the 1-year postoperative maximum K. In eyes with a maximum preoperative K value from the topography of 55.0 D or steeper, they calculated a probability 5.4 times higher to have a flattening of 2.0 D or more 1 year after CXL than eyes with a maximum K value of less than 55.0 D. While 45.4% of 44 eyes with a maximum K value

of 55.0 D or more showed a flattening by 2.0 D or more, 13.3% of 60 eyes had a preoperative maximum K of less than 55.0 D.<sup>109</sup>

With regard to topographic progression (1.0 D or more of topographic corneal steepening as determined using the maximum K) 1 year after the procedure, they found no difference between eyes with a maximum preoperative K value of 55.0 D or more, and eyes with a maximum K value less than 55.0 D. 10.0% of 44 eyes from the former group and 8.3% of 60 eyes in the latter group showed that level of postoperative progression.<sup>109</sup> On the other hand, Koller et al<sup>41</sup> had previously found that comparing a number of eyes undergoing CXL that showed progression 1 year after the surgery (8 eyes – 7.6% of a group of 105) with those without progression, maximum preoperative K over 58.0 D and female sex were identified as risk factors, with an OR of 5.32 for K and 3.11 for sex.

## CXL contraindications

### Pachymetry thinner than 400 microns

Traditionally, corneal thickness below 400 microns was considered a contraindication to CXL. However, as mentioned earlier, some alternatives exist in order to perform CXL in those corneas safely.<sup>95–101</sup> Therefore, a pachymetry thinner than 400 microns is now not an absolute contraindication but a relative one.

### Prior herpetic ocular infection

In a case series of infectious keratitis published by Price et al,<sup>114</sup> one patient having an apparently microbial keratitis, but with negative bacterial and fungal cultures, and who later turned out to have herpes simplex, developed severe dendritic lesions after receiving CXL.

Several other case reports of herpetic keratitis after CXL have been published in KCN, it being striking that patients denied a past history of the disease. Kymionis et al<sup>115</sup> reported the case of a young adult woman who 5 days after CXL presented with geographic epithelial herpetic keratitis and iritis. The etiology was confirmed by polymerase chain reaction of tear samples. Yuksel et al<sup>116</sup> also published the case of a 31-year-old woman who 4 days after CXL presented with a dendritic ulcer. The diagnosis was confirmed with polymerase chain reaction analysis of the corneal swab for herpes simplex.

More recently, Al-Qarni and AlHarbi<sup>117</sup> reported two cases of young adults who also had no past history of herpetic keratitis and presented dendritic ulcers in the early postoperative period.



Thus, past history of herpetic keratitis is a contraindication for CXL, but, as seen, herpetic keratitis may develop in a patient without past history of herpetic disease.

### Other contraindications

Concurrent ocular infection, severe corneal scarring, or opacification, neurotrophic keratopathy, past history of poor epithelial wound healing, severe dry eye, autoimmune disorders, and pregnancy are currently considered contraindication for CXL in corneal ectatic diseases.

### Conclusion

Experimental and clinical findings have demonstrated CXL effectiveness in the last 10 years, and the number of patients undergoing the procedure has increased significantly. Since the beginning of its use in the clinic by the group of researchers from Germany, almost 20 years ago, cross-linking had traditionally been indicated in cases of documented progression of corneal ectasia in a period of 6–12 months. However, since KCN is a disease that appears in the first 2 decades of life and usually has a more aggressive progression in young patients, it is the responsibility of ophthalmologists to treat these children and adolescents early, which is why the criterion of waiting for progression to be documented should be analyzed on a case-by-case basis. There are viable alternatives to offer treatment even in eyes with thin corneas (less than 400 microns after the deepithelization). Certain small changes in the technique (such as centering the treatment at the apex of the cone) may increase its effectiveness. Until there is a proven superior alternative treatment, CXL using UVA light and riboflavin will remain the backbone of KCN treatment in modern ophthalmology.

### Disclosure

The authors report no conflicts of interest in this work.

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