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SURGICAL TECHNIQUE

Pachymetry-based Accelerated Cross-linking: The "M Nomogram" for Standardized Treatment of All-thickness Progressive Ectatic Corneas

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ABSTRACT

Purpose: To assess the safety and efficacy of a new customized epithelium-off accelerated crosslinking (ACXL) nomogram "M nomogram" based on preoperative corneal optical thinnest point for progressive keratoconus and iatrogenic corneal ectasia.

Methods: Comparative analysis including the measured depths of the demarcation lines by in vivo confocal microscopy (IVCM) and corneal OCT in 20 eyes treated with conventional 3 mW/cm^2 CXL, 20 eyes treated with 30 mW/cm^2 ACXL with continuous (10 eyes) and pulsed (10 eyes) UV-A exposure (1 sec on, 1 sec off), 20 eyes treated with 15 mW/cm^2 pulsed light ACXL and 20 eyes using the 9 mW/cm^2 ACXL protocol. IVCM was performed by the HRT II Rostock Cornea Module (Heidelberg, Germany) and corneal OCT by the OptoVue (Freemont, Irvine, USA). The mathematical cross-linking profile was determined according to a calculated depth of the demarcation line and the threshold cross-link concentration adopting the conventional 3 mW/cm^2 protocol as a benchmark.

Results: The average demarcation depths were 350 ± 50 µm for the 3 mW/cm^2 conventional protocol, 200 ± 50 µm for the 30 mW/cm^2 continuous light ACXL, 250 ± 50 µm for the 30 mW/cm^2 pulsed light ACXL and 280 ± 30 µm for the 15 mW/cm^2 pulsed light ACXL. There was a very high correlation between the depth of the demarcation line between the measured and calculated data with a slope of m = 1.03 and an R^2 value 0.73.

Conclusion: ACXL M nomogram allows safe and efficacious CXL parameters setting based on preoperative minimum corneal thickness also including a more standardized treatment of thin ectatic corneas between 250 µm and 400 µm.

Keywords: Crosslinking, Keratoconus, M nomogram, Pachymetry, Thin corneas.

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INTRODUCTION

Corneal collagen crosslinking (CXL) has revolutionized the conservative management of primary and secondary progressive corneal ectatic disorders.[1-8] Prior to the advent of the CXL procedure, no conservative treatment for corneal ectasia existed, with 20% of KC patients progressing to eventually require a lamellar or penetrating keratoplasty.[9] The conventional riboflavin UV-A induced corneal CXL with epithelium removal (epi-off) represents an evidence-based treatment with documented clinical-instrumental efficacy in stabilizing progressive keratoconus and secondary ectasia in pediatric and adult patients as clearly documented in a series of long-term follow-up non-randomized and randomized clinical trials,[1-8] reducing the need of corneal transplants in 30% up to 50%.[10,11] Beyond ectasia stabilization, the standard irradiance of 3 mW/cm^2 for 30 minutes demonstrated its utility in the management of antibiotic-resistant infectious keratitis due to the cytotoxic effect of the reactive singlet oxygen generated during the CXL photo-oxidative process.[12] The conventional CXL procedure was time-consuming requiring almost 1-hour treatment time and a cornea with a minimum stromal corneal thickness of 400 µm[13] thus new developing accelerated or high-irradiance crosslinking (ACXL) protocols have been proposed to shorten the whole CXL treatment time from 1 hour to 20–25 minutes on balance thus improving patient's comfort while maintaining a good safety and efficacy profile.[14-23]

Microstructural Background of the Accelerated Pachymetry-guided CXL "M nomogram"

ACXL protocols are based on the so-called *"equal dose"* principle stated in the photochemical Bunsen-Roscoe's law of reciprocity.[24] This law states that the biological effect is proportional to the total energy dose delivered regardless of the applied irradiance and time. During CXL treatment what we transfer to the corneal stroma is energy (E) or fluence (F), not UV-A power. UV power is energy × time, so by setting the UV-A power at 9 mW/cm^2 × 10 minutes, 18 mW/cm^2 × 5 minutes, 15 mW/cm^2 x 6 minutes 30 mW/cm^2 × 3 minutes, 45 mW/cm^2 × 2 minutes while maintaining a constant E of 5.4 J/cm^2 we can achieve

the same biomechanical and biochemical (biological) effect as the standard CXL protocol at 3 mW/cm^2 for 30 minutes. In reality, this is only a theoretical principle of photochemistry because, according to Brillouin microscopy biomechanical studies[25] demonstrating that epithelium-on treatments penetrating at 100 µm instead of 300 µm of stromal depth (a proportion of one third), as confirmed by corneal OCT and in vivo Confocal Microscopy (IVCM) evidence of demarcation lines depth,[26-29] has a biomechanical efficacy of 70% less than standard (or only a 30% efficacy) epithelium-off Dresden protocol assumed as CXL benchmark.[1]

If an ACXL treatment penetrates at 200 µm of corneal stroma depth,[30] the proportion according to Brillouin data[25] is 200 µm *vs* 300 µm with an estimated biomechanical efficiency of 60% compared with the standard considered as a point of reference, having the maximum (100%) in a 400 µm corneal stromal thickness.[13] However, despite these proportions there is nonlinear relationships between depth and biomechanical efficacy that depends from multiple factors such as chemical crosslinks amount and stromal "saturation effect", patients age, eye rubbing, genetic and environmental factors.[31] According to theoretical models and laboratory studies, long-lasting stability of ectasia can be achievable if at least the two third of the baseline corneal thickness is saturated by crosslinks, thus allowing a sufficient amount and volume of cross-linked corneal stroma.[32,33]

The preclinical laboratory study conducted by Krueger et al.[34] on high-irradiance CXL demonstrated a substantial "biomechanical equivalence" in terms of stress-strain behavior between treated corneas with standard riboflavin 0,1% solution and 3 mW/cm^2 (the Dresden protocol), 9 mW/cm^2 and 15 mW/cm^2 of continuous or pulsed light UV-A exposure while maintaining the standard energy dose of 5.4 J/cm^2.

Kamaev et al.[35] documented that when fractionating the UV light exposure by pulsing the light (1 second on and 1 second off), the efficiency of high irradiance cross-linking improved by allowing a partial intraoperative oxygen re-diffusion during UV-A light exposure pauses also known as "dark phase amplification". This physical laboratory study established that pulsed UV-A delivery should improve the degree of cross-links during CXL procedure where oxygen is consumed more quickly.[36]

Mazzotta et al.[30] proved for the first time in humans, by means of *in vivo* confocal microscopy (IVCM) and corneal optical coherence tomography (OCT), that one of the main advantages of pulsing the light during CXL inducing intraoperative partial re-oxygenation of corneal stroma consisted in a higher penetration of the photo-oxidative effect (at least 50 µm over the continuous UV-light exposure) as demonstrated by IVCM and OCT thus increasing the depth of optical demarcation line compared with continuous light UV-light exposure. This data was firstly demonstrated by using the 30 mW/cm^2 ACXL protocol and afterward confirmed by Moramarco et al.[37] and Peyman et al.[38]

Jiang et al.[39]confirmed these data also showing another advantage of pulsed light CXL consisting of less microstructural damage and reduced wound healing stromal stimulation, very useful in the prevention of postoperative haze.

Mazzotta et al. pilot IVCM studies by IVCM[22,26-30,40,41] allowed for the first time at international level a precise qualitative and quantitative analysis of the cornea after CXL. In vivo analysis included the evaluationof the optical demarcation line depth by anterior segment corneal OCT and the overall time-dependent corneal changes at cellular level and 1 µm axial resolution by means of scanning laser IVCM comprising: progressive corneal epithelial stratification, nerves disappearance, regeneration and interconnections, keratocytes loss (apoptosis) and progressive stromal repopulation, wound healing response and changes in the collagen-proteoglycans extracellular matrix complex (ECM-CPC), analysis of corneal endothelium.

Corneal OCT scans provided a simple noninvasive clinical examination with a "perfect match" with the most powerful diagnostic tool of IVCM by documenting the optical reflection line (demarcation line) correlating with CXL-induced photo-oxidative damage thus estimating treatment penetration.

The combination of these methodologies, by documenting in vivo the corneal cellular modifications, endothelial safety and demarcation line boundary between cross-linked and noncross-linked stroma, generated the possibility for the creation of a customized "pachymetry-based" CXL nomogram, called "M nomogram", matching the simulated CXL-depth mathematical models with *in vivo* IVCM and OCT measurements. The "M nomogram" allows the possibility to preoperatively estimate the depth of the treatment according to baseline optical thinnest point pachymetry data, thus maintaining endothelial safety (a safety endothelial margin of +50 µm is used in the M nomogram offset) and including also ectatic thin corneas under 400 µm (range 250–400 µm with epithelium), (Fig. 1).

Figure 2 illustrates the micromorphological comparative basis of the "M nomogram" showing the IVCM keratocytes apoptosis and contemporary OCT optical demarcation lines depth, corresponding to the average standard CXL stromal penetration at 300 µm (350 measured from the epithelial surface) as CXL benchmark, the "M nomogram" includes the following UV-A power settings always maintaining the standard energy of 5.4

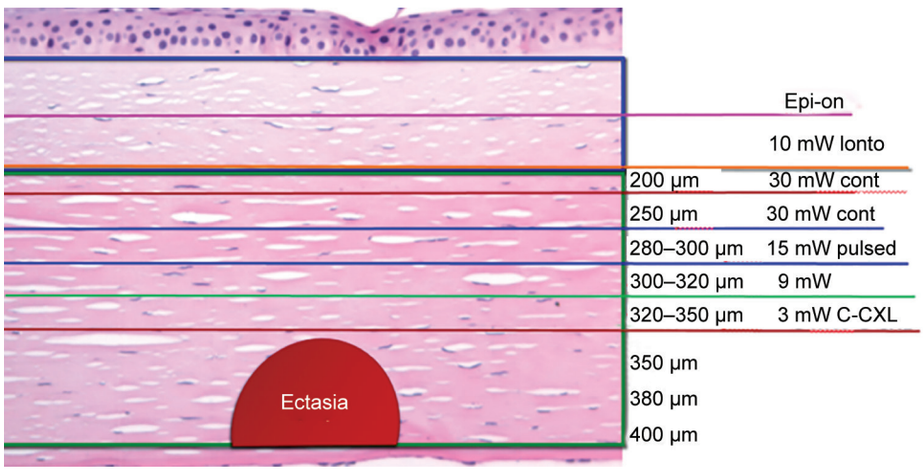


Fig. 1: Schematic illustration of the CXL penetration in different UV-Apower settings and consequent continuous and pulsed UV-light mode of exposure and times indicating the relative demarcation lines depth according to in vivo IVCM and OCT morphological studies

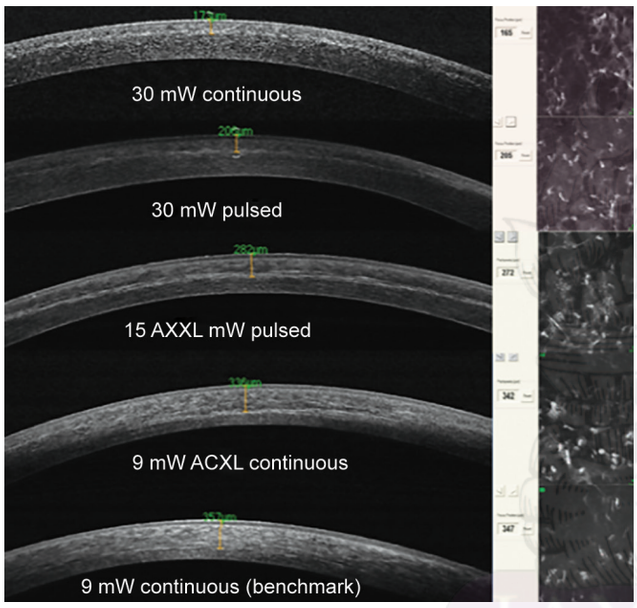


Fig. 2: "M nomogram" depth of demarcation lines compared with 3 mW/cm^2 protocol (benchmark) and relative cell viability match (keratocytes apoptosis) recorded 1 month after treatments showing that the penetration of photooxidative damage can be calibrated according to baseline pachymetry needing (Mazzotta post-CXL imaging archives)

J/cm^2 set in the epithelium-off CXL (Dresden protocol)[1] and including an offset endothelial safety margin of + 50 µm for the minimum corneal thickness at inclusion (e.g., the minimum corneal thickness included is 250 µm with epithelium): 30 mW/cm^2 continuous light accelerated treatment (Avedro'scontinuous light ACXL protocol)[29,30,41,42] generating a photo-oxidative apoptotic effect documented by IVCM and an average boundary optical demarcation line documented by corneal OCT at 150 µm on average (range 100–200 µm with epithelium), approximately 50 µm less than pulsed-light setting at 30 mW/cm^2 pulsed light treatment (Avedro'spulsed light ACXL protocol)[29,30] generating keratocytes apoptosis (CXL photo-oxidative effect) at an average stromal depth of 200 µm (range 150 µm max 250 µm measured from epithelial surface). The finding on 30 mW/cm^2 with continuous light UV irradiation reaching an average depth of 150 µm were documented for the first time by Touboul et al.[42] and confirmed by Mazzotta et al. The first demonstration concerning the pulsed light effect and penetration documented in vivo by Mazzotta et al.[30] was confirmed afterward by Moramarco et al.,[37] Peyman et al.[38] and more recently by Jiang et al.[39]

The "M nomogram" includes also the 15 mW/cm^2 pulsed light ACXL (called Siena accelerated CXL protocol) recently published by Mazzotta et al.,[43] based on Krueger et al.[34] preclinical laboratory studies of "biomechanical equivalence" between standard and high-irradiance (9 and 15 mW/cm^2 UV power) revealing a distinct demarcation line at 280 µm of stromal depth on average (range 250–310 µm) as clearly documented by IVCM and comparative OCT scans.

The accelerated 9 mW/cm^2 for 10 minutes of UV-A exposure[15-20] is also included in the "M nomogram". IVCM and OCT comparative studies after 9 mW/cm^2 showed almost the same CXL penetration of 3 mW/cm^2 (benchmark) as reported in Figure 1 and demonstrated to be effective in stabilizing topographic parameters over 24-month of follow-up in mild-moderate keratoconus-affected corneas. Moreover, the accelerated 9 mW/cm^2 protocol improved UDVA and CDVA, was safe for corneal endothelium, stabilized the progression of keratoconus and iatrogenic ectasia with a significant reduction in topographic keratometric values comparable with conventional 3 mW/cm^2 CXL in the mid-term follow-up.[15,18,20]

The 18 mW/cm^2 ACXL protocol with standard 5.4 J/cm^2 Energy dose reported by Hashemi et al.[44] reaching an average demarcation line depth at 220 µm (range 170–280 µm) was not included in the nomogram due to less K_{max}

flattening demonstrated in the literature and showing penetration data closer but slightly inferior to the 15 mW/cm^2 protocol and being not previously validated in the "biomechanical equivalence" laboratory preclinical study by Krueger[34] that represented the preclinical laboratory setting inspiring the M nomogram.

The data concerning endothelial safety were reported in the IVCM review by Mazzotta et al.[29] As per biomechanical studies performed by Shumacher et al.[33] on optimization model for UV-riboflavin corneal CXL and the experimental results provided by Kohlaas at al.[32] assessing that CXL treatment should cover at least two third of the corneal stroma according to baseline the thinnest pachymetry. The "M nomogram" allows to set the desired depth of treatment sparing corneal endothelium and including also ectatic corneas between 250 µm and 400 µm of minimum corneal thickness (epithelium included) thus definitively solving the problem of the "thin" ectatic corneas that can be safely and more efficaciously managed with standardized epithelium-off A-CXL.[45-52]

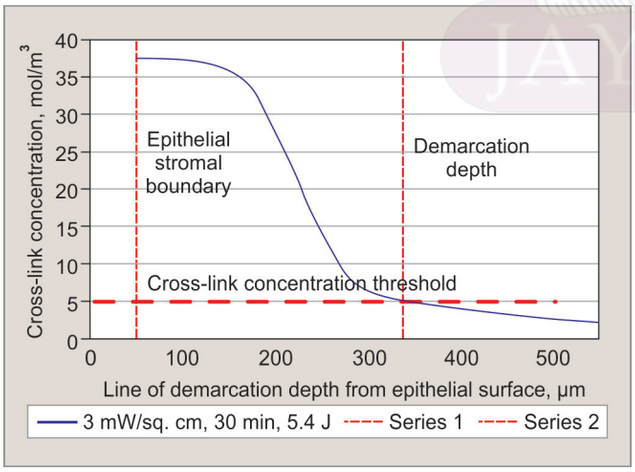
The physical and mathematical background of the "M nomogram".

Experimental model counteracting oxygen and irradiance dynamics during CXL can be modulated to reach different intrastromal crosslinks amounts and volumetric distributions.

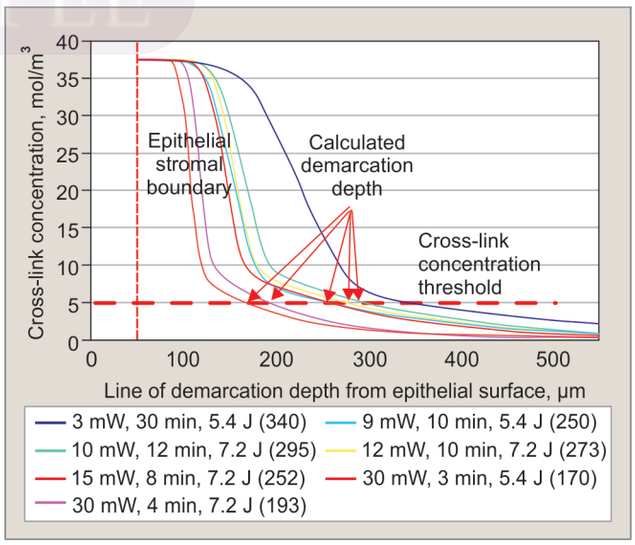
The demarcation line represents the depth of cross-linking photo-oxidative impact and the tissues healing response to some threshold.[26,53,54] This threshold is related to singlet oxygen minimum concentration that causes enough photo-oxidative damage to the tissue thus eliciting a tissue response as the line of demarcation is most visible approximately 1–3 months post-CXL treatment. A study by Friedman et al., titled "Photochemical reactions during CXL" and presented at the International CXL Congress (Boston MA, USA, 2015) showed the theoretical cross-linking profile determined as a function of depth and a potential threshold crosslink concentration representative for the demarcation line assuming the conventional Dresden CXL protocol as benchmark (Graph 1). Different accelerated epithelium-off crosslinking protocols according to literature, were calculated for their crosslinking demarcation depths and distribution profiles (Graph 2).

The report of the measured and calculated demarcation lines depth (Table 1) including epithelium shows a high correlation between the measured and calculated lines of demarcation (slope of m = 1.03 and an R^2 value 0.73). The green circles showed in Graph 3 are that of Mazzotta[29] determining the in vivo average demarcation line depth measured from the epithelial surface.

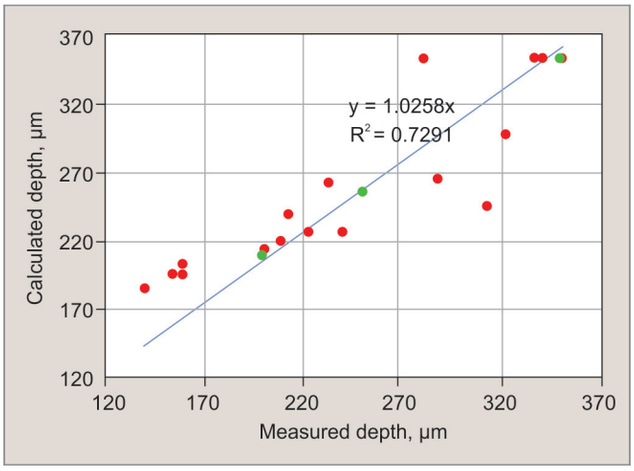
Comparative analysis included the measured depths of the demarcation lines by in vivo confocal microscopy (IVCM) and corneal OCT in 20 eyes treated with conventional 3 mW/cm^2 CXL, 20 eyes treated with 30 mW/cm^2 ACXL with continuous (10 eyes) and pulsed (10 eyes) UV-A exposure (1 sec on 1 sec off), 20 eyes treated with 15 mW/cm^2 pulsed light ACXL and 20 eyes using the 9 mW/cm^2 ACXL protocol. The measured lines of demarcation depth[29] showed a high correlation to the model presented by Friedman et al. (photochemical reactions during CXL) at the CXL Congress, Boston MA 2015 in which the theoretical cross-linking profile was determined as a function of depth and a potential threshold crosslink concentration representative for the demarcation line was chosen using the Dresden protocol as the standard.



Graph 1: The mathematical assessment of the cross-links concentration threshold according to the measured demarcation line assuming the Dresden protocol as benchmark demonstrated that the maximum interaction between UV-A, riboflavin, oxygen, and collagen–proteoglycans complex is in the first 200 µm were the 70% of riboflavin-UV-A interactions occur, while the remaining 30% of CXL photooxidative reaction is dissipated in the deep stroma between 200 µm and 300 µm



Graph 2: Cross-links concentration threshold in different high-irradiance epithelium-off protocols calculated for their cross-linking distribution profiles and predicted demarcation line depths



Graph 3: Calculated (red circles) versus measured line of demarcation depth for various protocols for the treatment of keratoconus. Green circles are the results demonstrated by Mazzotta et al. in the literature

As seen in Table 1, the reported standard deviations for this measurement are large, revealing the variability of as much as 40% in depth of demarcation line depth for nominally equivalent clinical protocols. A lot of factors such as UV-A irradiance, exposure time, mode of exposure (pulsed or not), riboflavin solutions, diffusion and drops administration, illumination beams, beam focus and environmental conditions might contribute to this variability in clinical outcome. Riboflavin can vary from 0.1–0.15% to 0.25% in concentration and the impact of an increased concentration from 0.1 to 0.15% on the depth of a demarcation line depth is –22 µm. On the contrary by using hypotonic saline impacted the line of demarcation depth by +22 µm. Individual device calibration and focusing may change the depth of the demarcation line by ± 34 µm and center to edge variance according to beam profiles impacted the line by ± 51 µm. The 40% variability of demarcation line depth while using the same high irradiance protocol may explain the lack of consistency and repeatability in the CXL surgical procedures application, thus leading to potential variability in clinical outcomes. Although the correlation to CXL clinical outcome especially for keratoconus has yet to be determined, achieving consistent and repeatable demarcation line depths require attention and consistency, standardizing the surgical procedure.[35,40,]

DISCUSSION

The pachymetry guided M nomogram developed by Mazzotta was based on *in vivo* demarcation lines international reports by means of IVCM and corneal OCT analysis in different high-irradiance CXL protocols matching the clinical and instrumental observation with the mathematical models calculated by Friedman et al., thus allowing the treatment of every baseline corneal thickness, including thin corneas with a minimum optical

Table 1: Measured and calculated demarcation lines in different cross-linking protocols

Riboflavin soaking time (minutes)	*UVA irradiation (mW/cm²)*	*UVA irradiation time (minutes) and modality*	*Energy dose (E) (Joule/cm²)*	*Measured demarcation line depth (µm)*	*Calculated demarcation line depth (µm)*
30	3	30 continuous	5.4	294.2 ± 51.2	352
30	3	30 continuous	5.4	350 ± 20	352
30	3	30 continuous	5.4	341.8 ± 47.02	352
30	3	30 continuous	5.4	350.78 ± 49.34	352
30	3	30 continuous	5.4	337 ± 46.46	352
15	30	3 continuous	5.4	140.4 ± 39.1	185
30	30	4 continuous	7.2	200 ± 20	209
30	30	8 pulsed 1:1 sec	7.2	250 ± 20	255
15	30	4 continuous	7.2	153.85 ± 33.11	195
10	30	8 pulsed 1:1 sec	7.2	213 ± 47	239
10	20	12 pulsed 1:1 sec	7.2	233 ± 92	262
20	30	4 continuous	7.2	160 ± 20	202
20	9	10 continuous	5.4	288.46 ± 42.37	265
20	18	5 continuous	5.4	208.64 ± 18.41	219
30	18	5 continuous	5.4	240.37 ± 18.89	225
30	18	7 continuous	7.56	313.37 ± 48.85	245
30	18	5 continuous	5.4	223 ± 32	225
30	9	14 continuous	7.56	322.91 ± 48.28	296
10	30	4 continuous	7.2	159.88	195
10	30	8 pulsed 1:1 sec	7.2	201.11	213

thinnest point between 250 µm and 400 µm measured with epithelium (minimum stromal pachymetry range between 200 µm and 350 µm) without affecting corneal endothelium (a safety endothelial margin of +50 µm was used in the nomogram offset) thus respecting the standard energy dose of 5.4 J/cm^2 set in the Dresden protocol.

A lot of methods were proposed for the treatment of thin corneas[45-52] such as a stromal expansion with hypotonic riboflavin solutions,[45,46] transepithelial CXL,[47] epithelial island technique[48,49] leaving epithelium on the pachymetry thinnest point area, contact lens assisted crosslinking,[50,51] SMILE-lenticule assisted CXL[52] with contradictory results, photochemical and technical limitations. However, no standardized methods were available for treating thin corneas.

The "M nomogram" finally offered the advantage of a uniform CXL method for the treatment of all-thickness progressive ectatic corneas, sparing endothelium, ensuring a sufficient CXL penetration, presetting the depth of CXL photo-oxidative effect penetration (i.e., demarcation line depth) and allowing a standardized management of thin corneas 400 µm and under that are often encountered in the ophthalmological clinical practice, not only in about 5–10% of KC eyes, but frequently after secondary iatrogenic ectasia (post LASIK, SMILE, PRK), pellucid marginal degeneration (PMD) and post radial keratotomy hyperopic drift. The "M nomogram" allows to set the desired depth of treatment sparing corneal endothelium and including also ectatic corneas between 250 µm and 400 µm of minimum corneal thickness (epithelium included) thus facilitating the problem of the "thin" ectatic corneas that can be safely and efficaciously managed with a standardized protocol. A safety margin of + 50 µm from endothelium is covered in the preoperative planning for thin corneas due to the variability and standard deviation of the demarcation line depth.[29,35,40]

The demarcation line[26,29,53,54] observed after epithelium-off standard CXL and ACXL, represents an expression of the light-scattering (reflectivity changes) through different tissue densities, underlying the transition from an early edematous area devoid of cells (stromal oedema and apoptosis spreading at IVCM ± 50 microns) to an area unreached by the effect riboflavin + UV-A + oxygen interaction, regularly populated of cells.[43] The deep corneal stroma beyond 350 (measured from the epithelial surface) in conventional CXL, 200 µm in continuous light ACXL, 250 µm in pulsed light ACXL, did not undergo tissue changes beyond vertical demarcation lines.[29] The depth of demarcation lines reassumed in Table 1 and Figure 2 are the direct expression of CXL induced photo-oxidative damage (i.e., penetration) correlating, even not linearly, with CXL biochemical and biomechanical impact (cross-links stromal saturation). The ACXL protocols, by reducing the treatment time at 20–25 minutes, offer great advantages in clinical applications where 1-hour treatment time is too long for most patients and clinicians.

Moreover, the high-irradiance CXL protocols allow a better "customization" of CXL treatment according to baseline optical minimum corneal thickness, thus solving the problem of treating the thin corneas without affecting endothelium.[40] However, in the case of corneal CXL, there are many other factors beyond the dose that contribute to the total amount and 3-dimensional distribution of cross-linking obtained in the cornea. Factors related to the clinical procedure include the beam profile, the concentration and diffusion rate of the formulation of the riboflavin used, the length of the riboflavin presoaking time, the viscosity of the riboflavin film, the riboflavin administration timing, as well as the presence and concentration of oxygen in the stromal tissue and as recently demonstrated by Mazzotta et al.[55,56] Individual patient variability, including the corneal structure and baseline corneal biomechanics may also influence the outcome of the CXL procedures and the presence of comorbidities such as eye rubbing and allergy.[31] The strong connection between the depth of the demarcation line and the increase of the CXL biomechanical efficacy can be actually explained by means of UV-A CXL chemical investigations demonstrating that only a limited amount of free reactive collagen residues is involved in the short wave UV-mediated CXL. Thus, the CXL density can rise only up to an upper boundary value, i.e., the "saturation value".[56]

REFERENCES

1. Wollensak G, Spoerl E, Seiler Th. Riboflavin/ultraviolet-A-induced collagen crosslinking for the treatment of keratoconus. Am J Ophtalmol 2003;135(5):620-627.
2. Caporossi A, Baiocchi S, Mazzotta C, et al. Parasurgical therapy for keratoconus by riboflavin-ultraviolet type A-induced cross-linking of corneal collagen: preliminary refractive results in an Italian study. J Cataract Refract Surg 2006;32(5):837-845.
3. Hafezi F, Kanellopoulos J, Wiltfang R, et al. Corneal collagen crosslinking with riboflavin and ultraviolet A to treat induced keratectasia after laser in situ keratomileusis. J Cataract Refract Surg 2007;33(12):2035-2040.
4. Raiskup F, Theuring A, Pillunat LE, et al. Corneal collagen crosslinking with riboflavin and ultraviolet-A light in progressive keratoconus: ten-year results. J Cataract Refract Surg 2015;41(1):41-46.
5. Mazzotta C, Traversi C, Baiocchi S, et al. Corneal Collagen Cross-Linking with Riboflavin and Ultraviolet A Light for Pediatric Keratoconus: Ten-Year Results. Cornea 2018;37(5):560-566.
6. O'Brart DP, Patel P, Lascaratos G, et al. Corneal Cross-linking to Halt the Progression of Keratoconus and Corneal Ectasia: Seven-Year Follow-up. Am J Ophthalmol 2015;160(6): 1154-1163.

7. Caporossi A, Mazzotta C, Baiocchi S, et al. Long-term results of riboflavin ultraviolet a corneal collagen cross-linking for keratoconus in Italy: the Siena eye cross study. Am J Ophthalmol 2010;149(4):585-593.
8. Wittig-Silva C, Chan E, Islam FM, et al. A randomized, controlled trial of corneal collagen cross-linking in progressive keratoconus: three-year results. Ophthalmology 2014;121(4):812-821.
9. Rabinowitz YS. Keratoconus. Surv Ophthalmol 1998;42(4):297-319.
10. Godefrooij DA, Gans R, Imhof SM, et al. Nationwide reduction in the number of corneal transplantations for keratoconus following the implementation of cross-linking. Acta Ophthalmol 2016;94(7):675-678.
11. Sandvik GF, Thorsrud A, Råen M, et al. Does Corneal Collagen Cross-linking Reduce the Need for Keratoplasties in Patients With Keratoconus? Cornea 2015;34(9):991-995.
12. Tabibian D, Mazzotta C, Hafezi F. PACK-CXL: Corneal cross-linking in infectious keratitis. Eye Vis (Lond) 2016;3:11.
13. Spoerl E, Mrochen M, Sliney D, et al. Safety of UVA-riboflavin cross-linking of the cornea. Cornea 2007;26(4):385-389.
14. Hagem AM, Thorsrud A, Sandvik GF, et al. Randomized Study of Collagen Cross-Linking With Conventional Versus Accelerated UVA Irradiation Using Riboflavin With Hydroxypropyl Methylcellulose: Two-Year Results. Cornea 2019;38(2):203-209.
15. Elbaz U, Shen C, Lichtinger A, et al. Accelerated (9-mW/cm2) corneal collagen crosslinking for keratoconus-A 1-year follow-up. Cornea 2014;33(8):769-773.
16. Ulusoy DM, Göktaş E, Duru N, et al. Accelerated corneal crosslinking for treatment of progressive keratoconus in pediatric patients. Eur J Ophthalmol 2017;27(3):319-325.
17. Marino GK, Torricelli AA, Giacomin N, et al. Accelerated corneal collagen crosslinking for postoperative LASIK ectasia: two-year outcomes. J Refract Surg 2015;31(6):380-384.
18. Chow VW, Chan TC, Yu M, et al. One year outcomes of conventional and accelerated collagen crosslinking in progressive keratoconus. Sci Rep 2015;5:14425.
19. Hashemian H, Jabbarvand M, Khodaparast M, et al. Evaluation of corneal changes after conventional versus accelerated corneal cross-linking: a randomized controlled trial. J Refract Surg 2014;30(12):837-842.
20. Shetty R, Pahuja NK, Nuijts RM, et al. Current protocols of corneal collagen crosslinking - visual, refractive and tomographic outcomes. Am J Ophthalmol 2015;160(2):243-249.
21. Hashemi H, Miraftab M, Seyedian MA, et al. Long-term Results of an Accelerated Corneal Cross-linking Protocol ($18mW/cm^2$) for the Treatment of Progressive Keratoconus. Am J Ophthalmol 2015;160(6):1164-1170.
22. Mazzotta C, Traversi C, Paradiso AL, et al. Pulsed light accelerated crosslinking versus continuous light accelerated crosslinking one-year results. J Ophthalmol 2014;2014:1-6.
23. Sadoughi MM, Einollahi B, Baradaran-Rafii A, et al. Accelerated versus conventional corneal collagen cross-linking in patients with keratoconus: an intrapatient comparative study. Int Ophthalmol 2016:1-8.
24. Brindley GS. The Bunsen-Roscoe law for the human eye at very short durations. J Physiol 1952;118(1):135-139.
25. Scarcelli G, Kling S, Quijano E, et al. Brillouin microscopy of collagen crosslinking: non-contact depth-dependent analysis of corneal elastic modulus. Invest Ophthalmol Vis Sci. 2013;54(2):1418-1425.
26. Mazzotta C, Balestrazzi A, Traversi C, et al. Treatment of progressive keratoconus by riboflavin-UVA-induced cross-linking of corneal collagen: ultrastructural analysis by Heidelberg Retinal Tomograph II *in vivo* confocal microscopy in humans. Cornea 2007;26(4):390-397.
27. Mazzotta C, Traversi C, Baiocchi S, et al. Corneal healing after riboflavin ultraviolet-A collagen cross-linking determined by confocal laser scanning microscopy *in vivo*: early and late modifications. Am J Ophthalmol 2008;146(4):527-533.
28. Mazzotta C, Traversi C, Baiocchi S, et al. Conservative treatment of keratoconus by riboflavin-UVA-induced cross-linking of corneal collagen: qualitative investigation. Eur J Ophthalmol 2006;16(4):530-535.
29. Mazzotta C, Hafezi F, Kymionis G, et al. *In Vivo* Confocal Microscopy after Corneal Collagen Crosslinking. Ocul Surf. 2015 Oct;13(4):298-314.
30. Mazzotta C, Traversi C, Caragiuli S, et al. Pulsed vs continuous light accelerated corneal collagen crosslinking: in vivo qualitative investigation by confocal microscopy and corneal OCT. Eye (Lond) 2014;28(10):1179-1183.
31. Mazzotta C, Traversi C, Mellace P, et al. Keratoconus Progression in Patients with Allergy and Elevated Surface Matrix Metalloproteinase 9 Point-of-Care Test. Eye Contact Lens 2018;44 Suppl 2:S48-S53.
32. Kohlhaas M, Spoerl E, Schilde T, et al. Biomechanical evidence of the distribution of cross-links in corneas treated with riboflavin and ultraviolet A light. J Cataract Refract Surg 2006;32(2):279-283.
33. Schumacher S, Mrochen M, Wernli J, et al. Optimization model for UV-riboflavin corneal cross-linking. Invest Ophthalmol Vis Sci 2012;53(2):762-769.
34. Krueger R, Herekar MS, Spoerl E. First Proposed Efficacy Study of High Versus Standard Irradiance and Fractionated Riboflavin/Ultraviolet A Cross-Linking with Equivalent Energy Exposure. Eye & Contact Lens 2014;40:353-357.
35. Kamaev P, Friedman MD, Sherr E, et al. Photochemical kinetics of corneal cross-linking with riboflavin. Invest Ophthalmol Vis Sci 2012;53(4):2360-2367.
36. Kling S, Richoz O, Hammer A, et al. Increased Biomechanical Efficacy of Corneal Cross-linking in Thin Corneas Due to Higher Oxygen Availability. J Refract Surg 2015;31(12):840-846.
37. Moramarco A, Iovieno A, Sartori A, et al. Corneal stromal demarcation line after accelerated crosslinking using continuous and pulsed light. J Cataract Refract Surg 2015;41(11):2546-2551.
38. Peyman A, Nouralishahi A, Hafezi F, et al. Stromal Demarcation Line in Pulsed Versus Continuous Light Accelerated Corneal Cross-linking for Keratoconus. J Refract Surg 2016;32(3):206-208.
39. Jiang LZ, Jiang W, Qiu SY. Conventional vs. pulsed-light accelerated corneal collagen cross-linking for the treatment of progressive keratoconus: 12-month results from a prospective study. ExpTher Med 2017;14(5):4238-4244.
40. Friedman MD, Kamaev P, Smirnov M, et al. Can we safely cross-link thinner corneas? Pathways for optimized CXL treatment planning, ESCRS 2015, Barcelona, Spain.
41. Mazzotta C, Paradiso AL, Baiocchi S, et al. Qualitative Investigation of Corneal Changes after Accelerated Corneal Collagen Cross-linking (A-CXL) by *in vivo* Confocal Microscopy and Corneal OCT. J Clin Exp Ophthalmol 2013;4:6.

42. Touboul D, Efron N, Smadja D, Praud D, et al. Corneal confocal microscopy following conventional, transepithelial, and accelerated corneal collagen cross-linking procedures for keratoconus. J Refract Surg 2012;28(11): 769-776.
43. Mazzotta C, Baiocchi S, Bagaglia SA, et al. Accelerated 15 mW pulsed-light crosslinking to treat progressive keratoconus: Two-year clinical results. J Cataract Refract Surg 2017;43(8):1081-1088.
44. Hashemi H, Fotouhi A, Miraftab M, et al. Short-term comparison of accelerated and standard methods of corneal collagen crosslinking.J Cataract Refract Surg 2015;41(3):533-540.
45. Hafezi F, Mrochen M, Iseli HP, et al. Collagen crosslinking with ultraviolet-A and hypoosmolar riboflavin solution in thin corneas. J Cataract Refract Surg 2009;35(4):621-624.
46. Raiskup F, Spoerl E. Corneal cross-linking with hypo-osmolar riboflavin solution in thin keratoconic corneas. Am J Ophthalmol 2011;152(1):28-32.e1.
47. Caporossi A, Mazzotta C, Paradiso AL, Baiocchi S, Marigliani D, Caporossi T. Transepithelialcornealcollagencrosslinking for progressive keratoconus: 24-month clinicalresults. J Cataract Refract Surg 2013 Aug;39(8):1157-1163.
48. Mazzotta C, Ramovecchi V. Customized epithelial debridement for thin ectatic corneas undergoing corneal crosslinking: epithelial island cross-linking technique. Clin Ophthalmol 2014;8:1337-1343.
49. Kymionis GD, Portaliou DM, Diakonis VF, et al. Corneal collagen cross-linking with riboflavin and ultraviolet-A irradiation in patients with thin corneas. Am J Ophthalmol 2012; 153(1):24-28.
50. Jacob S, Kumar DA, Agarwal A, et al. Contact lens-assisted collagen cross-linking (CACXL): A new technique for cross-linking thin corneas. J Refract Surg 2014;30(6):366-372.
51. Mazzotta C, Jacob S, Agarwal A, et al. *In Vivo* Confocal Microscopy After Contact Lens-Assisted Corneal Collagen Cross-linking for Thin Keratoconic Corneas.J Refract Surg. 2016;32(5):326-331.
52. Sachdev MS, Gupta D, Sachdev G, et al. Tailored stromal expansion with a refractive lenticule for crosslinking the ultrathin cornea. J Cataract Refract Surg 2015;41(5):918-923.
53. Seiler T, Hafezi F. Corneal cross-linking-induced stromal demarcation line. Cornea 2006;25(9):1057-1059.
54. Spadea L, Di Genova L, Tonti E. Corneal stromal demarcation line after 4 protocols of corneal crosslinking in keratoconus determined with anterior segment optical coherence tomography. J Cataract Refract Surg 2018;44(5):596-602.
55. Richoz O, Hammer A, Tabibian D, et al. The biomechanical effect of corneal collagen cross-linking (CXL) with riboflavin and UV-A is oxygen dependent. Trans Vis Sci Tech 2013;2(7):6.
56. Mazzotta C, Wollensak G, Raiskup F, et al. The meaning of the demarcation line after riboflavin-UVA corneal collagen cross-linking. Expert Review of Ophthalmology 2019;14(2):115-131.