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

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/cm² CXL, 20 eyes treated with 30 mW/cm² ACXL with continuous (10 eyes) and pulsed (10 eyes) UV-A exposure (1 sec on, 1 sec off), 20 eyes treated with 15 mW/cm² pulsed light ACXL and 20 eyes using the 9 mW/cm² ACXL protocol. IVCM was performed by the HRT II Rostock Corneal 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/cm² protocol as a benchmark.

Results: The average demarcation depths were 350 ± 50 µm for the 3 mW/cm² conventional protocol, 200 ± 50 µm for the 30 mW/cm² continuous light ACXL, 250 ± 50 µm for the 30 mW/cm² pulsed light ACXL and 280 ± 30 µm for the 15 mW/cm² pulsed light ACXL. There was a very high correlation between the depth of the demarcation line and the calculated data with a slope of m = 1.03 and an R² 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. 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. 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 reducing the need of corneal transplants in 30% up to 50%.

Beyond ectasia stabilization, the standard irradiance of 3 mW/cm² 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. 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 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.

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. 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/cm² × 10 minutes, 18 mW/cm² × 5 minutes, 15 mW/cm² × 6 minutes 30 mW/cm² × 3 minutes, 45 mW/cm² × 2 minutes while maintaining a constant E of 5.4 J/cm² we can achieve...
the same biomechanical and biochemical (biological) effect as the standard CXL protocol at 3 mW/cm² for 30 minutes. In reality, this is only a theoretical principle of photochemistry because, according to Brillouin microscopy biomechanical studies 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, has a biomechanical efficacy of 70% less than standard (or only a 30% efficacy) epithelium-off Dresden protocol assumed as CXL benchmark.

If an ACXL treatment penetrates at 200 µm of corneal stroma depth, the proportion according to Brillouin data 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. 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. 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.

The preclinical laboratory study conducted by Krueger et al. 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/cm² (the Dresden protocol), 9 mW/cm² and 15 mW/cm² of continuous or pulsed light UV-A exposure while maintaining the standard energy dose of 5.4 J/cm².

Kamaev et al. 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.

Mazzotta et al. 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/cm² ACXL protocol and afterward confirmed by Moramarco et al. and Peyman et al.

Jiang et al. 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 allowed for the first time at international level a precise qualitative and quantitative analysis of the cornea after CXL. In vivo analysis included the evaluation of 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
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J/cm² 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/cm² continuous light accelerated treatment (Avedro's continuous light ACXL protocol)²⁸,⁴¹,⁴² 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/cm² pulsed light treatment (Avedro's pulsed light ACXL protocol)²⁸,⁴² 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/cm² with continuous light UV 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.²⁹ 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/cm² 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/cm² 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/cm² for 10 minutes of UV-A exposure¹⁵-²⁰ is also included in the "M nomogram". IVCM and OCT comparative studies after 9 mW/cm² showed almost the same CXL penetration of 3 mW/cm² (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/cm² 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/cm² CXL in the mid-term follow-up.¹⁵,¹⁸,²⁰

The 18 mW/cm² ACXL protocol with standard 5.4 J/cm² 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/cm² protocol and being not previously validated in the “biomechanical equivalence” laboratory preclinical study by Krueger³⁴ 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 et 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 crosslinking 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 Mazzotta²⁹ 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/cm² CXL, 20 eyes treated with 30 mW/cm² ACXL with continuous (10 eyes) and pulsed (10 eyes) UV-A exposure (1 sec on 1 sec off), 20 eyes treated with 15 mW/cm² pulsed light ACXL and 20 eyes using the 9 mW/cm² ACXL protocol. The measured lines of demarcation depth²⁹ 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.
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>
<thead>
<tr>
<th>Riboflavin soaking time (minutes)</th>
<th>UVA irradiation (mW/cm²)</th>
<th>UVA irradiation time (minutes) and modality</th>
<th>Energy dose (E) (Joule/cm²)</th>
<th>Measured demarcation line depth (µm)</th>
<th>Calculated demarcation line depth (µm)</th>
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thinest 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/cm² set in the Dresden protocol.

A lot of methods were proposed for the treatment of thin corneas, such as a stromal expansion with hypotonic riboflavin solutions, transepithelial CXL, epithelial island technique leaving epithelium on the area unreached by the effect riboflavin + UV-A + oxygen interaction, regularly populated 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. 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. 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. 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 of oxygen in the stromal tissue and as recently demonstrated by Mazzotta et al. 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. 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”.

REFERENCES


