EVALUATION OF THE DEMARCATION LINE IN THE CORNEAL STROMA AFTER ACCELERATED CORNEAL CROSS-LINKING USING ANTERIOR SEGMENT OPTICAL COHERENCE TOMOGRAPHY

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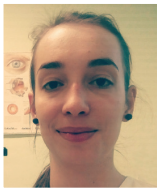

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SUMMARY
EVALUATION OF THE CORNEAL STROMAL DEMARCATION LINE AFTER THE ACCELERATED CORNEAL CROSS-LINKING USING ANTERIOR SEGMENT OCT

Objectives: Evaluation of the visibility and depth of the demarcation line in the corneal stroma in eyes with keratoconus 1 month and 3 months after epi-off accelerated corneal cross-linking (ACXL) using Anterior Segment Optical Coherence Tomography (AS OCT).
Material and
Methods: This study analyses a group of 34 eyes with keratoconus 1 month and 3 months after ACXL (9 mW/cm^2 for 10 min). The group was classified based on the ABCD clinical classification of keratoconus according to Belin and Duncan. AS OCT (Zeiss Cirrus 500, Anterior Segment Premier module) was used to assess the visibility and exact depth of the demarcation line in the corneal stroma.
Results: The demarcation line was visible 1 month after ACXL in 76.5 % of eyes with a mean depth of 238.13 ±20.36 µm and 3 months after ACXL in 100 % of eyes with a mean depth of 263.43 ±12.59 µm. Statistical analysis of the group did not show a significant relationship between the disease stage and the demarcation line visibility; however, there was a trend towards higher age (>30 years) in the group in those eyes where the demarcation line was visible vs. partially visible 3 months after ACXL. We found no difference in the mean and maximum line depth when comparing 1 month and 3 months after the procedure. There were no cases of disease progression 3 months after ACXL in the group.
Conclusion: Our study suggests that the assessment of the demarcation line in the corneal stroma is more reliable 3 months compared to 1 month after ACXL. We also observed a trend towards higher patient age in eyes where the demarcation line was clearly visible 3 months after ACXL. We did not confirm a relationship between the stage of keratoconus and the depth of the line, nor a difference in its mean and maximum depth 1 month and 3 months after the procedure.
Keywords: demarcation line, keratoconus, anterior segment optical coherence tomography, accelerated corneal cross-linking

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INTRODUCTION

Keratoconus is a progressive ectatic corneal disease, characterised by a gradual, bilateral, asymmetric, usually central increase in the corneal curvature and its thinning, leading to an irregular astigmatism. The resulting decrease in central visual acuity is often reluctant to spectacle correction. In some cases, scarring in the apex of the cone contributes to further decrease in visual acuity [1].

The disease typically begins in adolescence and, given its progressive nature and potential to substantially reduce visual acuity, the early prevention of its progression is essential in the management of these patients [2-4]. Currently, corneal cross-linking (CXL) is the only method known to slow down the progression or even to completely stabilise corneal findings in corneal ectasia. In this procedure, after initial epithelial abrasion, the corneal stroma is saturated with 0.1 % riboflavin solution, which

acts as a photosensitiser. With subsequent exposure to UV-A radiation, oxygen-free radicals are released, inducing the formation of covalent bonds between collagen molecules in the stroma, and collagen monomers are cross-linked with a stiffening of approximately 300 µm of the anterior stroma [5].

In recent years, the basic method of CXL, developed and published in 2003 under the still-used name of the Dresden Protocol, has been modified several times [6]. In particular, researchers have experimented with the intensity and duration of UV-A exposure, the need for corneal epithelial abrasion, or the adjustment of the composition of the riboflavin solution with respect to the pre-surgical corneal thickness [7-12]. The presence of what is called the demarcation line in the corneal stroma after CXL has been described in several studies [13,14]. Although its origin has not yet been precisely defined, it is considered as a line indicating the absolute depth of the cross-linking process of collagen fibrils in the stroma that occurred during CXL [15]. It can be visible as early as 2 weeks after the procedure, using Anterior Segment Optical Coherence Tomography (AS OCT) [16]. The line is more pronounced in the central part of the cornea, less pronounced and thinner paracentrally, or deeper centrally in corneas with continued thinning in the post-surgical period [17]. Using the Dresden Protocol, its depth reaches up to approximately 300 µm. In accelerated CXL (ACXL), the location of the demarcation line is shallower. It is likely that, with increasing radiation intensity, the depth of the demarcation line decreases and the variability of its location between individual patients increases [15]. However, the anterior stroma of the cornea contributes the most to its biomechanical strength; therefore, a shallower demarcation line is probably a sign of adequate CXL [16].

The objective of this study is to evaluate the visibility and to analyse the depth of the demarcation line in the corneal stroma in eyes with keratoconus after epi-off ACXL using AS OCT.

MATERIAL AND METHODS

We evaluated a group of 34 eyes of 27 patients (21 males, 6 females). The mean age of the patients was 24 years (15–37). These were eyes with keratoconus, and we classified the group based on the ABCD clinical classification of keratoconus, according to Belin and Duncan (Table 1,2) [18]. In all eyes, ACXL with epithelial abrasion was indicated after the confirmation of disease progression or, in the case of paediatric patients, after the confirmation of the presence of disease without the need to prove the progression. The treatment and observation of the patients was performed at UVEA Klinika s.r.o., Martin, in the period from June 2018 to June 2020. Considering the pre-surgical corneal thickness, after initial epithelial flap creation (using 20 % ethyl alcohol)

Table 1. ABCD keratoconus grading system

	A	B	C	D	
Stage	**ARC (3 mm)**	**PRC (3 mm)**	**Min. thickness**	**BCVA**	**Scarring**
0	7,25 mm (< 46,5 D)	5,90 mm (< 46,5 D)	490 µm	≥ 1,0	-
1	> 7,05 mm (< 48,0 D)	> 5,70 mm (< 59,25 D)	> 450 µm	< 1,0	-,+,++
2	6,35 mm (< 53,0 D)	5,15 mm (< 65,5 D)	> 400 µm	< 0,5	-,+,++
3	> 6,15 mm (< 55,0 D)	> 4,95 mm (< 68,5 D)	> 300 µm	< 0,2	-,+,++
4	< 6,15 mm (> 55,0 D)	< 4,95 mm (> 68,5 D)	< 300 µm	< 0,05	-,+,++

Explanations: ARC – anterior radii of curvature; PRC – posterior radii of curvature; BCVA – best corrected visual acuity; - no scarring/ + scar, iris visible/ ++ scar, iris not visible

Table 2. Analysed group of 34 keratoconic eyes classified with the ABCD keratoconus grading system

	A	B	C	D	
Stage	**ARC (3 mm)**	**PRC (3 mm)**	**Min. thickness**	**BCVA**	**Scarring**
0	8	0	11	1	34
1	6	31	11	22	0
2	11	3	10	10	0
3	4	0	2	1	0
4	5	0	0	0	0

Explanations: ARC – anterior radii of curvature, PRC – posterior radii of curvature, BCVA – best corrected visual acuity

with a diameter of 9 mm, the stroma was saturated with 0.1 % isoosmolar riboflavin solution with 20 % dextran (at a thickness of ≥400 µm after the epithelial abrasion), or 0.1 % hypoosmolar riboflavin solution (at a thickness of <400 µm after the epithelial abrasion) applied every 5 minutes for 30 minutes. This was followed by exposure to UV-A radiation with a wavelength of 370 nm and intensity of 9 mW/cm^2 for 10 minutes (LightLink – CXL-TM). Finally, the epithelial flap was returned on the treated corneal stroma, a topical antibiotic (levofloxacin), a topical corticosteroid (loteprednol etabonate), and a therapeutic soft contact lens were applied. The latter was retained until the follow-up on post-surgical Day 5. During the post-surgical Week 1, the patient applied an antibiotic (levofloxacin) 5 times daily, a corticosteroid (loteprednol etabonate) 5 times daily, and preservative-free artificial tears 5 times daily. In the post-surgical Weeks 2–4, a corticosteroid (loteprednol etabonate) was applied 5 times daily and preservative-free artificial tears 5 times daily. During the post-surgical Month 2, the topical ocular therapy included further application of a corticosteroid (loteprednol etabonate) 3 times daily for 2 weeks, followed by 2 times daily for 2 more weeks, as well as preservative-free artificial tears, according to the patient's subjective need.

The visualisation of the demarcation line in the corneal stroma was performed 2 times, at the patients' examinations 1 month and then again 3 months after ACXL. We used a Zeiss Cirrus 500 with the Anterior Segment Premier Module and the "HD Cornea" scanning mode (Carl Zeiss Meditec, Inc.). Three high-resolution horizontal corneal scans were evaluated: an image centred on the centre of the pupil, an image centred on the superior pupillary margin under scotopic conditions, and an image centred on the inferior pupillary margin under scotopic conditions. Scotopic conditions are naturally present when using the fixation light of the device, provided that the examination room is darkened. In each scan, the visibility of the demarcation line was analysed and, if reliably displayed, the exact depth of the demarcation line was determined at 9 landmarks.

For the visibility of the demarcation line, a subjective assessment of the demarcation line was quantified as follows: the demarcation line is not visible / the demarcation line is partially visible / the demarcation line is visible. If the line was reliably displayed, its exact depth relative to the corneal surface, including the epithelium, was also assessed at 9 points, using the provided software. These points were determined as follows: at the centre, in the nasal and temporal sections of the image centred on the pupil centre, at the centre, in the nasal and temporal section of the image centred on the superior pupillary margin under scotopic conditions, and at the centre, in the nasal and temporal sections of the image centred on the inferior pupillary margin under scotopic conditions.

Ancillary parameters, assessed based on the pre-surgical examination and then again 3 months after ACXL, included: uncorrected and best corrected central visual

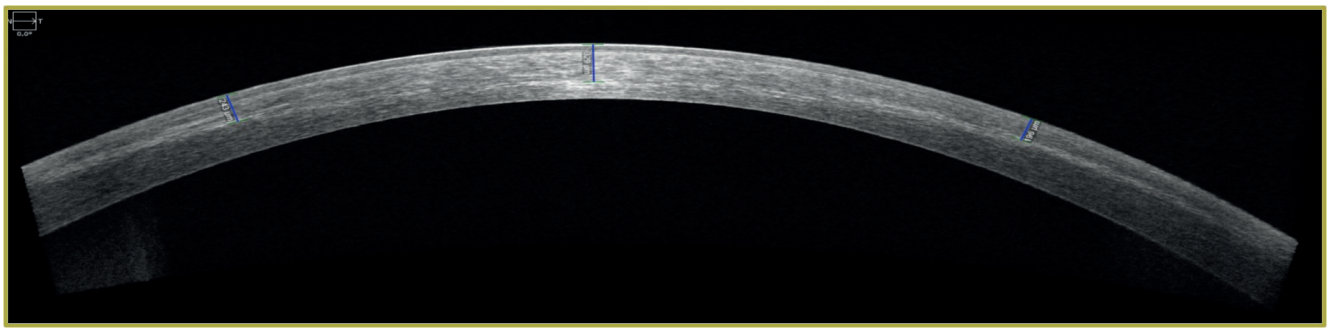

Image 1. Stromal demarcation line 1 month after accelerated corneal cross-linking treatment, depth nasally 243 µm, centrally 329 µm, temporally 196 µm

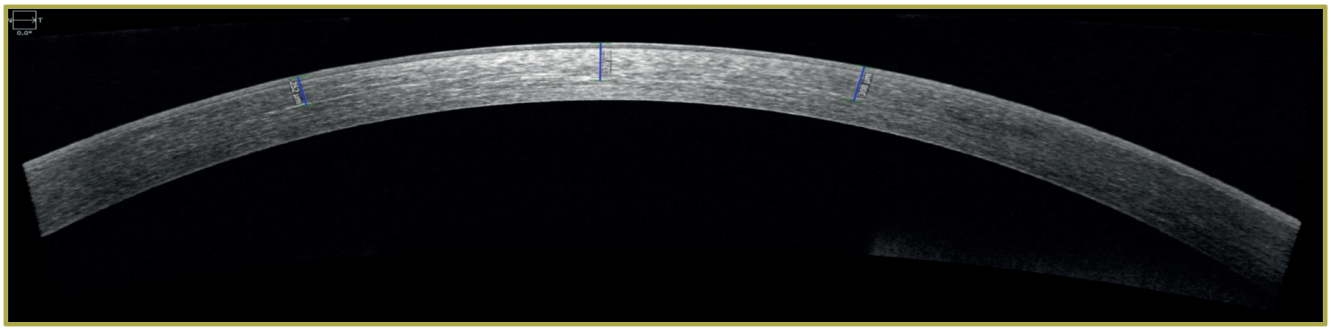

Image 2. Stromal demarcation line 1 month after accelerated corneal cross-linking treatment, depth nasally 252 µm, centrally 329 µm, temporally 308 µm

acuity (UCVA and BCVA), minimum corneal thickness, and central corneal thickness by topography (SCHWIND Sirius, SCHWIND eye-tech-solutions, GmbH). At the examination 3 months after ACXL, we also evaluated the parameters for possible ongoing disease progression (i.e. increase in Kmax, Kmean values and manifest spherical equivalent or a decrease in corneal thickness).

SYSTAT® statistical software was used for the statistical analysis of the results. For the specific statistical tests used, see the results below.

RESULTS

The demarcation line in the corneal stroma 1 month after ACXL was visible in 17 eyes (50 %, Figure 1,2), partially visible in 9 eyes (26.5 %), and not visible in 8 eyes (23.5 %). At scanning 3 months after ACXL, it was visible in 16 eyes (47 %, Figure 3,4) and partially visible in 18 eyes (53 %). The mean demarcation line depth values at the 9 points, analysed by AS OCT at 1 and 3 months after CXL, are shown in Table 3.

Analysis of the group revealed that there was no difference in the age of the patients when we divided them into groups according to the visibility of the line 1 month after ACXL (Kruskal-Wallis test, p = 0.477). The evaluation 3 months after ACXL showed a trend towards higher patient age in those eyes where the demarcation line was visible vs. partially visible (Mann-Whitney test, p = 0.067).

Evaluating the relationship between the visibility of the demarcation line and the stage of keratoconus using the Chi-square test, we found that in our group there was no significant difference in its visibility between the disease stages 1 month (p = 0.077) or 3 months after ACXL (p = 0.092).

Table 3. Depth of the demarcation line in the corneal stroma (epithelial thickness included) in µm

	Centrally	Nasally	Temporally	Centrally above	Nasally above	Temporally above	Centrally down	Nasally down	Temporally down	Average
1 month after ACXL	267,5	200,0	243,4	250,4	256,6	220,5	251,7	234,6	218,4	238,13 ±20,36
3 months after ACXL	264,7	268,75	251	284,7	255,5	284,45	256,6	253,8	251,4	263,43 ±12,59

Explanations: ACXL – accelerated corneal cross-linking

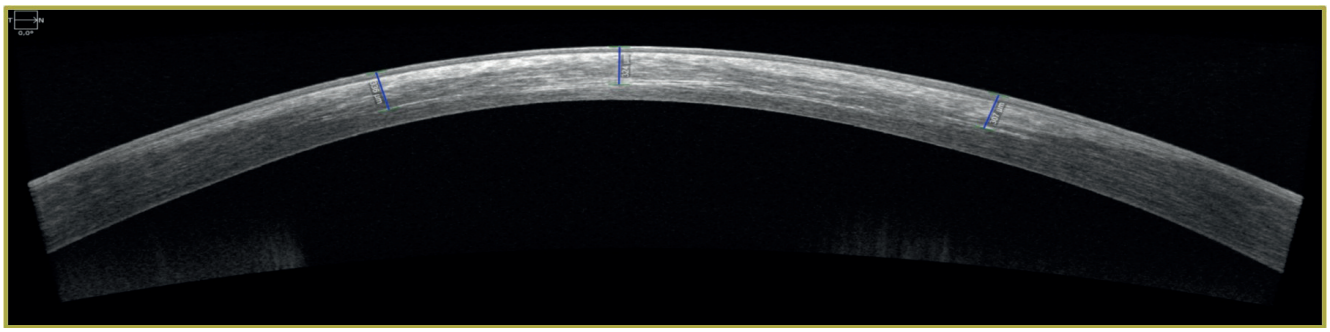

Image 3. Stromal demarcation line 3 months after accelerated corneal cross-linking treatment, depth nasally 307 µm, centrally 324 µm, temporally 336 µm

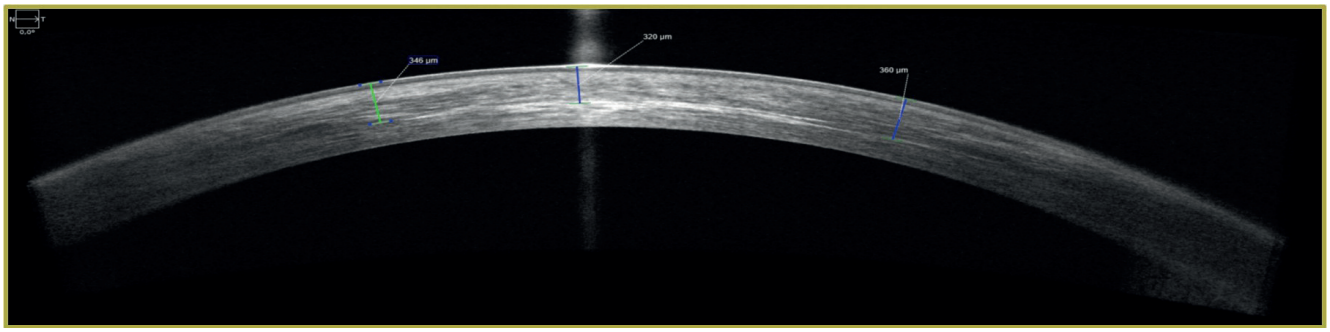

Image 4. Stromal demarcation line 3 months after accelerated corneal cross-linking treatment, depth nasally 346 µm, centrally 320 µm, temporally 360 µm

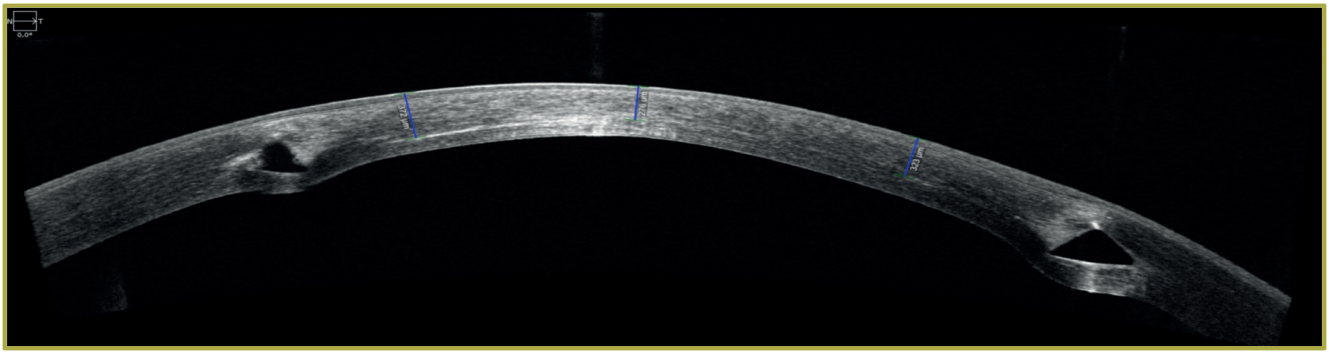

Image 5. Stromal demarcation line 1 month after accelerated corneal cross-linking treatment in eye with keraring intrastromal corneal ring segment, depth nasally 372 µm, centrally 276 µm, temporally 323 µm

Using the Wilcoxon test, we found that there was no difference in the mean depth of the demarcation line in the corneal stroma (mean of 9 points as described above) in our group, when measured 1 month vs. 3 months after ACXL ($p = 0.078$). Similarly, evaluating the maximum depth of the demarcation line (maximum of 9 points as described above), we found no difference in its depth 1 month and 3 months after ACXL (Wilcoxon test, $p = 0.999$).

We also evaluated ancillary parameters in the group of eyes studied, in terms of the difference between pre- and post-surgical (3 months after ACXL) UCVA and BCVA, minimal pachymetry, central pachymetry, and Kmax using the Wilcoxon test. We did not observe any difference in UCVA and BCVA values between pre- and post-surgical values (pUCVA = 0.278; pBCVA = 0.349). There was a significant decrease in both minimal and central corneal pachymetry values ($p < 0.001$ in both cases). There was no change in Kmax in the post-surgical period ($p = 0.376$). At the follow-up 3 months after ACXL, we did not observe disease progression in any case in the evaluated group of eyes. All post-ACXL patients have continued to be observed in the long-term, due to the potential detection of the possible progression of the disease later in the post-surgical period.

DISCUSSION

Currently, CXL is a standard surgical method to slow or halt the progression of ectatic corneal diseases by inducing structural changes in the corneal stroma in response to riboflavin and UV-A radiation, leading to corneal stiffening [19,20]. Its application in today's clinical practice is possible not only as an isolated surgical method, but also in combination with the implantation of intrastromal corneal segments (Figure 5) [21], or with other procedures that improve the refractive state of the cornea and can thus lead to improved visual acuity – called the "CXL plus" methods [18]. The determination of the effective depth of corneal treatment with CXL is possible, using in vivo confocal microscopy. In the stroma, the presence of vertical and lateral transition areas at a depth of 270 to 330 µm, separating the anterior (treated) from the posterior (untreated) corneal stroma, is evident [22]. In addition to this method, biomicroscopic changes can also be objectively assessed and visualised using AS OCT [23,24]. Seiler and Hafezi were the first to identify a demarcation line in the corneal stroma, at a depth of about 300 µm as early as 2 weeks after CXL. They defined it as the boundary between the CXL cross-linked stroma and the posterior, CXL-unaffected stroma [25]. As some studies published later have shown, its location depends strongly on the intensity and duration of UV-A radiation used in different CXL protocols. For example, the study by Kyminois et al., with identical ACXL parameters as used in our study (intensity 9 mW/cm^2 for 10 min), reports an average depth of the demarcation line of 288.46 ±42.37 µm [23]. The location of the demarcation line is significantly deeper when using the standard, Dresden Protocol (intensity 3.0 mW/cm^2 for 30 min) with a value up to 323.0 ±48.6 µm [26]. In contrast, CXL with pulsed UV-A radiation (intensity 30 mW/cm^2 for 8 min) is associated with a decrease in demarcation line depth to a mean value of 201.64 ±27.72 µm [27].

In our study, we used AS OCT to visualise and assess the depth of the demarcation line in the corneal stroma 1 month and 3 months after ACXL with an intensity of 9 mW/cm^2 for 10 min. In our group, the line could be visualised in 76.5 % and 100 % of eyes 1 month and 3 months after CXL, respectively. Its mean depth was 238.13 ±20.36 µm and 263.43 ±12.59 µm 1 month and 3 months after the procedure, respectively. Statistical analysis of the group revealed no clear relationship between the depth of the demarcation line and the age of the patients, although in the images taken 3 months after ACXL, there was a tendency toward a higher age of the patients (> 30 years) in those eyes where the demarcation line was visible vs. partially visible. We did not demonstrate an effect of keratoconus stage on the depth of the line. Neither mean nor maximum line depth was significantly different 1 month and 3 months after ACXL. Among the ancillary parameters evaluated, there was a significant decrease in both minimum and

central corneal pachymetry values in the group, which also correlates with other published studies [28].

CONCLUSION

The demarcation line in the corneal stroma, as a microscopic manifestation of the extent of CXL-treated stroma, can be effectively visualised using AS OCT. In our group, the examination of its presence by this method proved more reliable 3 months vs. 1 month after ACXL. A more detailed analysis of its location did not confirm a clear relationship between the depth of the demarcation line and the stage of keratoconus; in relation to the age of the patients, 3 months after ACXL, there was a tendency for a higher age of the patients in those eyes where the demarcation line was visible vs. partially visible. The values of the mean and maximum depth of the demarcation line did not differ between scans performed 1 month and 3 months after ACXL.

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