Original research

Multipoint assessment of demarcation line depth after standard and accelerated cross-linking in central and inferior keratoconus

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Abstract

Purpose: To determine the changes in the depth of the demarcation line in the central to peripheral cornea following accelerated compared to standard corneal cross-linking (CXL).

Methods: In this prospective, non-randomized study, 60 eyes with progressive keratoconus underwent accelerated or standard CXL (30 in each group). Anterior segment optical coherence tomography (AS-OCT) was done one month later by two independent masked examiners to measure the depth of the demarcation line in the central cornea and on peripheral rings.

Results: The inter-examiner agreement (intra-class correlation coefficient) was >0.75 for all measured points, and average measurements were used in the analysis. The depth of the visualized demarcation line in the center was 223.4 ± 67.4 mm and 354.9 ± 79.0 mm in the accelerated and standard groups, respectively (P < 0.001). The depth significantly decreased from the center to the 7 mm ring in both groups (all P < 0.05). This change was 7.7 ± 26.1% and 2.2 ± 11.1% in the accelerated and standard groups, respectively. In the accelerated group, the demarcation line was deeper in the central cone sub-group compared to the inferior cone sub-group, but in the standard group, the demarcation line was deeper in the inferior cone sub-group (all P < 0.05). Cases with an inferior cone showed greater inter-group differences in all studied points.

Conclusions: The depth of the demarcation line with accelerated CXL is less than the standard protocol and decreases from the center towards the periphery. Demarcation lines are more homogenized with standard CXL. In cases with an inferior cone, demarcation line depth varies throughout the cornea.

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Keywords: Accelerated cross-linking; Standard cross-linking; Demarcation line; Peripheral cornea; Cone position

Introduction

Corneal cross-linking (CXL) is a procedure that can halt the progression of keratoconus by creating new covalent bands in the corneal stroma and biomechanical corneal strengthening.1 Short and long-term studies have shown the effectiveness of both the standard and accelerated protocols in stopping the
destructive effect of the disease, improving vision, and reducing the corneal curvature.\textsuperscript{2–5}

Another parameter assessed in the evaluation of CXL effectiveness is the corneal stromal demarcation line, which is the transition between the anterior treated stroma and the posterior untreated stroma.\textsuperscript{3} The line should be deep enough to ensure efficacy, but not too deep to risk safety by causing injury to endothelial cells. Several studies have used confoscan or anterior segment optical coherence tomography (AS-OCT), and they have reported depths up to 300 μm after the 30 min standard protocol.\textsuperscript{6–8} With the introduction of accelerated CXL protocols, studies began to address the line depth with this protocol. Some studies have reported a more superficial demarcation line after the 10-min, 9 mW/cm\textsuperscript{2} protocol compared to standard CXL.\textsuperscript{9,10} Some have studied it following 9-min\textsuperscript{11} and 14-min\textsuperscript{12} protocols, and Kymionis et al.\textsuperscript{13} have reported almost 244.3, 322.9, and 233.0 μm after the 5-min approach.

The present study is designed to compare the 5- and 30-min CXL protocols in terms of 1) the visibility and depth of the demarcation line; 2) line homogeneity in the center and at 3, 5, and 7 mm from the center; and 3) line depth in two subgroups with central and inferior cones.

Methods

In this non-randomized clinical trial, 60 eyes with progressive keratoconus were enrolled and underwent either standard (3 mW/cm\textsuperscript{2}, 30 min) or accelerated (18 mW/cm\textsuperscript{2}, 5 min) CXL (30 eyes in each group), consecutively. Enrollment was first completed for the accelerated group and then the standard group. Progression of keratoconus was defined as at least 1.0 diopter (D) increase in maximum keratometry (Kmax), manifest cylinder, or manifest refraction spherical equivalent (MRSE), or loss of two or more lines of corrected vision or other ocular disease were excluded from the study. The keratoconus cone was central (within the 3 mm zone) in 33.3% of cases and inferior in 66.7% (beyond the 3 mm zone). Posterior elevation map in Pentacam (Oculus Optikgeräte GmbH, Wetzlar, Germany) was used in the classification.

The study protocol was reviewed and approved by the local institutional review board, and it adhered to the tenets of the Declaration of Helsinki at all stages. All participants read and signed informed consents.

In the standard group, we used a method similar to that described by Wollensak et al.\textsuperscript{14} After administering local anesthesia, the central 9 mm of the corneal epithelium was manually removed. Then the lid speculum was removed, and riboflavin 0.1% drops in dextran 20% (Streuli Pharma, Uznach, Switzerland) were instilled onto the corneal surface at 3 min intervals for 30 min. After anterior chamber saturation with riboflavin, the cornea was irradiated for 30 min with 370 nm UV-A and an intensity of 3 mW/cm\textsuperscript{2} using the IROC UV-X System (Zürich, Switzerland) from a distance of 5 cm, and riboflavin instillation was repeated every 3 min. At the end of this stage, the corneal surface was rinsed with sterile balanced saline solution, one drop of levofloxacin was instilled, and a soft bandage contact lens (Night & Day, Ciba Vision, Duluth, GA, USA) was placed. The postoperative regimen included levofloxacin and betamethasone 0.1% eye drops four times daily and artificial tears (Hypermellose, preservative free) as needed. Patients were examined at one and three days after the procedure, and daily thereafter if necessary until complete epithelial healing was observed. When corneal re-epithelialization was complete, the bandage contact lens was removed, levofloxacin was discontinued, and betamethasone was continued 4 times daily for another week.

For the accelerated group, the same protocol was applied, but the cross-linking procedure was modified to a power of 18 mW/cm\textsuperscript{2} and irradiation time of 5 min using the CCL 365 (PESCHKE Meditgrade GmbH, Waldshut-Tiengen, Germany). The two devices are similar in terms of wavelength (365 nm), light emission (continuous wave), spot size (7–11 mm), and electric power (100–240 V).

All patients were examined with the Spectralis AS-OCT (Heidelberg Engineering GmbH, Heidelberg, Germany) at one week and one month after the procedure. Multiple images were acquired from each eye, and the one with the best line clarity was selected for depth measurement. To improve visualization, the reverse image was used during measurements, and line depth was measured from the back of the tear film to the front of the demarcation line. Line depth was measured at the corneal center and on 4 points on the superior, inferior, nasal, and temporal meridians at 3 mm, 5 mm, and 7 mm from the center. Measurements were done by two independent observers. The observers were masked to treatment groups. The agreement between their measurements was assessed using the Bland-Altman analysis. The averages of the two measurements were used in the main analyses. Statistical comparisons were made using the independent sample \( t \)-test.

Considering the limited number of samples in the subgroups, we used the G*Power software 3.1.9.2 to run a power analysis where the \( P \)-value was not significant with the two-group independent sample \( t \)-test.

Results

The mean age in the standard and accelerated groups was 25.2 ± 4.4 and 24.0 ± 4.5 years (\( P = 0.467 \)), respectively. No complication was observed either during or after the procedures. The baseline topographic data were compared between groups in Table 1. At one week after the procedure, the demarcation line was not visible in either group.

In the Bland-Altman analysis, the inter-examiner difference± standard deviation (SD) in line depth measurement in the center and at 3, 5, and 7 mm rings was, respectively, 0.29 ± 2.1, 1.97 ± 2.6, 0.1 ± 4.3, and 1.1 ± 10.7 in the accelerated group and 7.4 ± 8.7, 0.3 ± 16.7, 3.5 ± 16.6, and 0.7 ± 6.7 μm in the standard group. Except for the center in the standard group (\( P = 0.014 \)) and the 3 mm ring in the
accelerated group ($P < 0.001$), none of the inter-examiner differences were statistically significant (all $P > 0.050$).

At one month after the procedure, the depth of the demarcation line was not measurable in 5 eyes in the standard group and 1 eye in the accelerated group. The demarcation line in the central cornea was measured significantly deeper in the standard group ($354.9 \pm 79.0$ $\mu$m vs. $223.4 \pm 67.4$ $\mu$m, $P < 0.001$). Depth measurements at superior, inferior, nasal, and temporal points on 3, 5, and 7 mm rings, and the average of the 3, 5, and 7 mm rings are summarized in Table 2. At all 4 points, the demarcation line was significantly deeper in the standard group (all $P < 0.001$). Fig. 1 illustrates a sample AS-OCT image with each of the protocols at one month after the procedure.

Line depth significantly decreased from the center to the periphery in both groups (all $P < 0.05$). The decrease was 6.7% from the center to the 3 mm ring, 18.8% from the 3 mm to the 5 mm ring, and 26.1% from the 5 mm to the 7 mm ring in the accelerated group, and 2.2%, 7.2%, and 11.1% in the standard group, respectively (Fig. 2).

Table 2

| Demarcation line (microns) | Accelerated protocol (N = 29 eyes) | Standard protocol (N = 25 eyes) | Difference* |
|---------------------------|-----------------------------------|-------------------------------|-------------|
| Center                    | $223.4 \pm 67.4$                   | $354.9 \pm 79.0$              | $131.5 \pm 24.5$ |
| Superior $3$ mm           | $209.0 \pm 73.4$                  | $358.0 \pm 85.3$              | $149.0 \pm 22.1$ |
| Superior $5$ mm           | $159.4 \pm 73.0$                  | $325.4 \pm 121.1$             | $166.0 \pm 48.1$ |
| Superior $7$ mm           | $111.4 \pm 56.4$                  | $299.0 \pm 114.9$             | $187.6 \pm 34.8$ |
| Inerior $3$ mm            | $201.0 \pm 58.0$                  | $340.9 \pm 82.8$              | $139.9 \pm 23.2$ |
| Inerior $5$ mm            | $176.8 \pm 55.6$                  | $320.1 \pm 89.0$              | $143.2 \pm 24.8$ |
| Inerior $7$ mm            | $131.3 \pm 57.7$                  | $278.8 \pm 140.5$             | $147.5 \pm 54.5$ |
| Temporal $3$ mm           | $217.4 \pm 50.1$                  | $375.2 \pm 88.1$              | $157.8 \pm 21.7$ |
| Temporal $5$ mm           | $170.7 \pm 51.2$                  | $335.1 \pm 104.6$             | $164.4 \pm 25.8$ |
| Temporal $7$ mm           | $152.1 \pm 47.2$                  | $309.8 \pm 137.5$             | $157.7 \pm 46.8$ |
| Nasal $3$ mm              | $217.9 \pm 70.1$                  | $365.7 \pm 78.9$              | $147.8 \pm 25.0$ |
| Nasal $5$ mm              | $191.8 \pm 73.2$                  | $332.2 \pm 105.8$             | $140.4 \pm 30.6$ |
| Nasal $7$ mm              | $128.8 \pm 81.5$                  | $304.9 \pm 96.6$              | $176.0 \pm 55.6$ |
| Average                   | $209.3 \pm 58.9$                  | $363.0 \pm 82.5$              | $153.7 \pm 22.8$ |
| 3 mm                      | $176.2 \pm 55.7$                  | $338.5 \pm 97.2$              | $162.2 \pm 31.1$ |
| 5 mm                      | $139.7 \pm 49.9$                  | $304.8 \pm 109.2$             | $165.1 \pm 34.3$ |

* All $P$-value <0.001.

Table 1

Comparison of topographic data measured by Pentacam (Oculus Optikgeräte GmbH, Wetzlar, Germany) between the standard and accelerated (5 min, 18 mW/cm$^2$) cross-linking protocols.

|                         | Accelerated protocol (N = 29 eyes) | Standard protocol (N = 25 eyes) | $P$-value |
|-------------------------|-----------------------------------|-------------------------------|-----------|
| Maximum keratometry     | $47.0 \pm 4.0$                    | $47.4 \pm 2.5$                | 0.729     |
| Minimum keratometry     | $44.3 \pm 2.1$                    | $44.1 \pm 3.1$                | 0.837     |
| Minimum corneal thickness | $484.0 \pm 35.0$          | $492.0 \pm 31.8$              | 0.517     |
| Apical corneal thickness | $470.2 \pm 34.2$                  | $483.4 \pm 29.9$              | 0.269     |

Discussion

Various studied have demonstrated that the efficacy with the accelerated CXL approach, including 18 mW/cm$^2$ is comparable to the standard protocol.$^{2,15}$ Some studies have
reported a similar flattening effect with these two protocols, and some suggest the standard approach as the preferred method. In terms of corneal biomechanical changes after CXL, similar effectiveness has been observed. In a contralateral randomized clinical trial, we were able to show similar corneal biomechanics measured by Ocular Response Analyzer (ORA; Reichert Ophthalmic Instruments, Buffalo, USA) with standard CXL and the 18 mW/cm² alternative in the short-term (1, 3, and 6 months) and long-term (18 months).2,3

Despite several studies on this subject, the effective depth for arresting disease progression remains unknown, and there is still debate over the efficiency of cross-linking in the anterior part of the cornea. The depth of the demarcation line in the center of the cornea has been reported to be between 300 and 400 μm with the standard protocol,6 about 323 μm with the 14-min protocol,12 from 203 μm up to 288 μm with the 10-min approach,9,10,16 about 247 μm with the 9-min protocol,11 and 223 μm with the 5-min protocol.13 These results indicate reduced line depth with shorter exposure times, although there may be considerable variations in different populations. For example, Kymionis et al.13 used the 5 min + 18 mW/cm² protocol and reported a depth of 223 μm in the center of the cornea, similar to us.

Table 3

Comparison of the depth of the demarcation line (microns) between the standard and accelerated (5 min, 18 mW/cm²) cross-linking protocols regarding cone position.

|                    | Demarcation line at ring | Accelerated protocol | Standard protocol |
|--------------------|--------------------------|----------------------|-------------------|
| **Central keratoconus** |                         |                      |                   |
| Center             | 243.2 ± 75.4             | 327.2 ± 101.1        |                   |
| Superior           | 212.2 ± 76.5             | 353.8 ± 107.6        |                   |
| MCT: 478.4 ± 39.1 μm | 176.9 ± 108.5            | 293.0 ± 156.3        |                   |
| 5 mm               | 119.3 ± 76.5             | 342.2 ± 102.2        |                   |
| 7 mm               | 214.9 ± 55.3             | 334.5 ± 89.8         |                   |
| Inferior           | 5 mm                     | 192.1 ± 43.6         | 312.0 ± 99.5      |
| MCT: 470.8 ± 39.3 μm | 176.9 ± 108.5            | 293.0 ± 156.3        |                   |
| 5 mm               | 113.2 ± 54.4             | 270.7 ± 171.1        |                   |
| 7 mm               | 219.2 ± 37.4             | 346.8 ± 106.1        |                   |
| Temporal           | 5 mm                     | 175.7 ± 39.8         | 326.2 ± 153.1     |
| MCT: 53.3 ± 7.1 D  | 5 mm                     | 148.5 ± 44.6         | 293.5 ± 215.0     |
| 7 mm               | 232.7 ± 70.4             | 347.0 ± 105.2        |                   |
| Nasal              | 5 mm                     | 237.2 ± 89.7         | 316.8 ± 131.6     |
| MCT: 477.6 ± 31.3 μm | 237.2 ± 89.7             | 316.8 ± 131.6        |                   |
| 7 mm               | 232.7 ± 61.9             | 347.0 ± 105.2        |                   |
| Superior           | 3 mm                     | 194.9 ± 73.1         | 388.3 ± 20.8      |
| MCT: 493.2 ± 35.1 μm | 149.5 ± 44.4             | 393.1 ± 26.0         |                   |
| 5 mm               | 106.1 ± 43.0             | 330.5 ± 89.8         |                   |
| Inferior           | 5 mm                     | 193.7 ± 59.5         | 372.1 ± 31.3      |
| MCT: 477.6 ± 31.3 μm | 193.7 ± 59.5             | 372.1 ± 31.3         |                   |
| 5 mm               | 168.3 ± 60.7             | 360.4 ± 30.4         |                   |
| 7 mm               | 139.4 ± 59.0             | 341.7 ± 71.8         |                   |
| Temporal           | 3 mm                     | 216.4 ± 56.6         | 397.4 ± 24.4      |
| MCT: 51.4 ± 4.06 D | 5 mm                     | 167.9 ± 54.5         | 374.8 ± 34.2      |
| 7 mm               | 153.8 ± 49.8             | 356.4 ± 78.3         |                   |
| Nasal              | 3 mm                     | 210.1 ± 70.6         | 394.7 ± 32.4      |
| MCT: 51.4 ± 4.06 D | 5 mm                     | 169.0 ± 52.5         | 364.6 ± 86.3      |
| 7 mm               | 109.8 ± 63.5             | 364.6 ± 58.9         |                   |

CCT: Central corneal thickness; MCT: Minimal corneal thickness; Kmax: Maximum keratometry in 8 mm zone; D: Diopter.

Fig. 3. Comparison of demarcation line depths at different parts of the cornea between the standard and accelerated 5 min, 18 mW/cm² cross-linking protocols in patients with central keratoconus (A) and inferior keratoconus (B).
other studies by the same team, the line depth with the standard protocol was similar to that in our study (3517 and 34217 vs. 355 μm). On the other hand, Doors et al. reported a line depth of 314 μm after the standard method, which is more superficial compared to the other mentioned studies. This difference can be on account of differences in the CXL machine characteristics, minor changes in treatment protocols, and corneal structure or response to treatment in different populations. Nonetheless, there is yet no consensus on the optimal line depth that would maximize treatment effect without injuring underlying tissues and risking safety.

We also found that the depth of the demarcation line decreased from the center to the periphery, although to a lesser amount with the standard protocol compared to accelerated CXL. In other words, CXL was more homogenous with the standard protocol, and the accelerated protocol is shallower. When the laser is not vertical but oblique in the periphery, energy decrease and less absorption lead to more reflection and less penetration of UV. Therefore, in order to maintain the effect of CXL in the periphery, it is necessary to adjust device illumination and the focus of the irradiation beam in relation to the distance from the center to ensure homogeneous tissue changes throughout the cornea.

Our study showed that in cases with a central cone, covalent reactions occur deeper in the cornea in the center and the 3 mm and 5 mm zone; lack of a significant inter-protocol difference in the 7 mm zone, as indicated by the power analysis, was due to the small sample size and high variance in changes. The two protocols would show a significant difference at higher powers analysis if there were more sample sizes for this zone.

In cases with an inferior cone, the demarcation line was set deeper with the standard protocol from the center to the periphery. As displayed in Fig. 1, the inter-protocol difference was smaller in cases with a central cone compared to the inferior sub-group. CXL has a deeper effect in thinner areas of the cornea due to greater riboflavin diffusion, but in the accelerated method, there is less cross-linking due to oxygen depletion caused by faster oxygen consumption. Therefore, in central keratoconus where the central cornea is thinner, the two protocols have different results in the center, but in the periphery, lesser riboflavin diffusion causes the two protocols to have closer results. However, in cases with an inferior cone where the cornea is steep in the periphery, the depth of the line varies throughout the cornea. More detailed in vitro studies are needed on this subject.

In summary, the depth of the demarcation line with the 18 mW/cm² protocol is less than the standard protocol. The line depth decreases from the center to the periphery with both protocols. Demarcation line is more homogenized with the standard protocol than accelerated CXL. The location of the cone can influence line depth with each protocol; in cases with an inferior cone, the depth of the line varies throughout the cornea.

References

1. Wollensak G. Crosslinking treatment of progressive keratoconus: new hope. Curr Opin Ophthalmol. 2006;17(4):356–360.
2. 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.
3. Hashemi H, Miraftab M, Seyedian MA, et al. Long-term results of an accelerated corneal cross-linking protocol (18 mW/cm²) for the treatment of progressive keratoconus. Am J Ophthalmol. 2015;160(6):1164–1170.
4. Hashemi H, Seyedian MA, Miraftab M, Fotouhi A, Asgari S. Corneal collagen cross-linking with riboflavin and ultraviolet a irradiation for keratoconus: long-term results. Ophthalmol. J. 2013;120(8):1515–1520.
5. Henriquez MA, Izquierdo L, Bernilla C, Zakrzewski PA, Mannis M. Riboflavin/Ultraviolet A corneal collagen cross-linking for the treatment of keratoconus: visual outcomes and Scheimpflug analysis. Cornea. 2011;30(3):281–286.
6. Seiler T, Hafezi F. Corneal cross-linking-induced stromal demarcation line. Cornea. 2006;25(9):1057–1059.
7. Kymionis GD, Grentzelos MA, Plaka AD, et al. Evaluation of the corneal collagen cross-linking demarcation line profile using anterior segment optical coherence tomography. Cornea. 2013;32(7):907–910.
8. Yam JCS, Cheng ACK. Reduced cross-linking demarcation line depth at the peripheral cornea after corneal collagen cross-linking. J Refract Surg. 2013;29(1):49–53.
9. Brittingham S, Tappeiner C, Frueh BE. Corneal cross-linking in keratoconus using the standard and rapid treatment protocol: differences in demarcation line and 12-month outcomes. Invest Ophthalmol Vis Sci. 2014;55(12):8371–8376.
10. Kymionis GD, Tsoulanaras KI, Grentzelos MA, et al. Corneal stroma demarcation line after standard and high-intensity collagen crosslinking determined with anterior segment optical coherence tomography. J Refract Surg. 2014;40(5):736–740.
11. Bonnel S, Bergua M, De Rivoire B, et al. Demarcation line evaluation of iontophoresis-assisted transepithelial corneal collagen cross-linking for keratoconus. J Refract Surg. 2015;31(1):36–40.
12. Kymionis GD, Tsoulanaras KI, Grentzelos MA, et al. Evaluation of corneal stromal demarcation line depth following standard and a modified-accelerated collagen cross-linking protocol. Am J Ophthalmol. 2014;158(4):671–675.
13. Kymionis GD, Tsoulanaras KI, Liakopoulos DA, et al. Corneal stromal demarcation line determined with anterior segment optical coherence tomography following a very high intensity corneal collagen cross-linking protocol. Cornea. 2015;34(6):664–667.
14. Wollensak G, Spoerl E, Seiler T. Riboflavin/ultraviolet-a-induced collagen cross-linking for the treatment of keratoconus. Am J Ophthalmol. 2003;135(5):620–627.
15. Alnawaiseh M, Rosentreter A, Bohn MR, Eveslage M, Eter N, Zambagen L. Accelerated (18 mW/cm²) corneal collagen cross-linking for progressive keratoconus. Cornea. 2015;34(11):1427–1431.
16. Ng AL, Chan TC, Lai JS, Cheng AC. Comparison of the central and peripheral corneal stromal demarcation line depth in conventional versus accelerated collagen cross-linking. Cornea. 2015;34(11):1432–1436.
17. Kymionis GD, Tsoulanaras KI, Liakopoulos DA, Skatharoudi CA, Grentzelos MA, Tsakalis NG. Corneal stromal demarcation line depth following standard and a modified high intensity corneal collagen-cross-linking protocol. J Refract Surg. 2016;32(4):218–222.
18. Doors M, Talizib NG, Eegink FA, Berendschot TT, Webers CA, Nuijts RM. Use of anterior segment optical coherence tomography to study corneal changes after collagen cross-linking. Am J Ophthalmol. 2009;148(6):844–851.
19. Hammer A, Richoz O, Arba Mosquera S, Tabibian D, Hoogewoud F, Hafezi F. Corneal biomechanical properties at different corneal cross-linking (CXL) irradiances. Invest Ophthalmol Vis Sci. 2014;55(5):2881–2884.