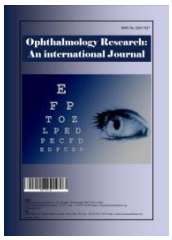

Ophthalmology Research: An International Journal

17(3): 33-41, 2022; Article no.OR.91311
ISSN: 2321-7227

Reassessment of the Clinical Significance of the Demarcation Line in the Corneal Stroma in Crosslinking

I. M. Kornilovskiy [a*]

[a] *National Medical and Surgical Center Named after N. I. Pirogov, Ministry of Health of Russia, Moscow, Russia.*

Authors' contributions

The sole author designed, analysed, interpreted and prepared the manuscript.

Article Information

DOI: 10.9734/OR/2022/v17i330260

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/91311

Original Research Article

Received 01 July 2022
Accepted 06 September 2022
Published 12 September 2022

ABSTRACT

Purpose: To consider the clinical significance and features of the formation of a demarcation line in the corneal stroma with various methods of corneal crosslinking.
Materials and Methods: Literature data on the evaluation of the effectiveness of various methods of crosslinking by the demarcation line in the corneal stroma were analyzed. The formation of a demarcation line during prophylactic and therapeutic excimer laser crosslinking was studied after various photorefractive operations, keratoconus and other pathologies of the cornea (168 operations).
Results: With various methods of crosslinking, including prophylactic and therapeutic excimer laser corneal crosslinking, the depth of the demarcation line in the stroma varied from 1/3 to 2/3 of the corneal thickness. It was noted that the saturation of the corneal stroma with a 0.25% solution of riboflavin is accompanied by a large increasing effect of the optical density in the stroma above the demarcation line at a shallower depth of its occurrence. The severity of the aseptic inflammatory reaction after corneal crosslinking affected the optical density, shape, intensity, and depth of the demarcation line in the stroma. In some cases, the formation of a demarcation line in the stroma was noted when the stroma was saturated with riboflavin immediately after refractive keratoablation without additional UV irradiation. The demarcation line in the stroma was revealed during inflammatory processes in the corneal stroma without the participation of riboflavin and its activation by UV radiation. The study showed that the assessment of corneal crosslinking by the depth of the

**Corresponding author: E-mail: Kornilovsky51@yandex.ru;*

demarcation line is not an indicator of the photochemical process and the number of crosslinks formed in the corneal stroma.
Conclusion: Based on the severity, shape and depth of the demarcation line, it is not possible to judge the density of crosslinks in the corneal stroma, which predetermine its strength properties after one or another method of corneal crosslinking.

Keywords: Cornea; crosslinking; demarcation line; excimer laser; keratoconus; keratoectasias; riboflavin.

1. INTRODUCTION

Corneal crosslinking has firmly entered the arsenal of modern methods for the treatment of keratoconus and secondary keratoectasias of various etiologies. When evaluating its effectiveness, special attention is paid to the data of OCT and corneal densitometry with an emphasis on the degree of expressiveness of the demarcation line in the corneal stroma, its depth, shape, time of appearance and reverse development [1-11]. This is due to the opinion firmly established among ophthalmologists, according to which the demarcation line is an indicator of the depth of the photochemical process of collagen crosslinking in the stroma and determines the increase in the strength properties of the cornea after crosslinking. However, it is difficult to agree with this on the basis of data from other instrumental studies and the results of remote clinical observations. According to some authors, the indicator “the deeper, the better” is a rather simplified approach to interpreting the clinical significance of the demarcation line in the corneal stroma when assessing the effect of crosslinking [12,13]. All of the above predetermined the necessity of writing this article.

1.1 Purpose

Consider the controversial issues of evaluating the effectiveness of corneal crosslinking by the depth of the demarcation line with various methods of its implementation.

2. MATERIALS AND METHODS

Studies by various authors were analyzed to assess the effect of corneal crosslinking in terms of severity, shape, depth, time of appearance and reverse development of the demarcation line in the stroma. The clinic analyzed 168 operations with an emphasis on OCT data and corneal densitometry. Particular emphasis in this work was placed on the analysis of 130 prophylactic laser-induced crosslinking operations for TransPRK, LASIK and FemtoLASIK operations and 38 therapeutic excimer laser crosslinking operations for keratoconus and other corneal pathologies. The age of patients ranged from 18 to 56 years, the average was 26±6.8 years. This material was selected from 710 prophylactic and therapeutic excimer laser corneal crosslinking operations, followed up from 1 month to 9 years. Preventive crosslinking in photorefractive surgery was implemented by saturating the stroma with riboflavin after epithelium ablation and its activation by secondary radiation of an argon-fluorine excimer laser induced during ablation. During therapeutic excimer laser crosslinking of the cornea after ablation of the epithelium, the stroma was saturated with riboflavin and switched to the argon-fluorine excimer laser radiation mode below the ablation threshold. In both cases, the corneal stroma was saturated with 0.25% isotonic riboflavin solution. For laser-induced crosslinking using subablative energy densities in a pulse, the Russian excimer laser ophthalmological device Microscan Visum was used. In this setup, a new technical solution was implemented for the first time, allowing a quick transition from ablative to subablative energy densities in a pulse, without any additional calibrations. A comprehensive ophthalmological examination was carried out using modern diagnostic devices. Particular emphasis was placed on OCT and corneal densitometry at various times after corneal crosslinking. Optical coherence tomography (OCT) of the cornea was performed using RTVue 100 and RTVue XR100 devices (Optovue, USA). Keratotopographic and densitometric studies were performed using a TMS-5 device (Topcon, Japan). This article focuses on debatable issues related to the interpretation of the clinical significance of the demarcation line in various corneal crosslinking techniques, with their confirmation by specific clinical examples and literature references in the discussion section. This approach, in our opinion, is important for practicing ophthalmologists. In subsequent publications, tables with static processing of a larger amount of clinical material will be presented.

All operations and diagnostic studies were carried out in compliance with the principles of the Declaration of Helsinki and with the permission of the ethical committee of the Federal State Budgetary Institution “N.I. Pirogov” of the Ministry of Health of Russia.

3. RESULTS

In our studies, the effect of therapeutic laser-induced crosslinking with stroma saturation with 0.25% isotonic riboflavin solution and its activation by secondary subablative radiation of an excimer laser was not inferior to traditional and accelerated technologies for its implementation with a similar time interval of formation, reverse development and the range of depth of the demarcation line in stroma. As confirmation, we present clinical observations of the dynamics of OCT of the cornea in the immediate and long-term follow-up period on the eye of an 18-year-old patient with progressive stage II keratoconus (Fig. 1). An important advantage of therapeutic laser-induced cross-linking of the cornea was the scanning of the cornea with a narrow beam (0.9 mm) of pulsed radiation of an argon-fluorine excimer laser in the subablative mode. This made it possible for the first time to carry out personalized crosslinking according to computed keratotopography data [14]. As an illustration, we present a clinical case of successful personalized topographically oriented crosslinking for progressive grade II keratoconus (Fig. 2). With this crosslinking technique, after 6 days, a twofold increase (from 0.3 to 0.6) in uncorrected visual acuity was noted. At the same time, a zone of increased optical density was clearly defined on the OCT of the cornea, the depth of which increased 6 days after crosslinking (Fig. 2).

We observed a similar increase in optical density during an aseptic inflammatory reaction in the corneal stroma. As an illustration, we present a clinical observation of an increase in the optical density in the stroma during long-term wearing of a soft contact lens. The latter provoked hypoxia and chronic subclinical aseptic inflammatory reaction in the corneal stroma. In this case, corneal OCT revealed changes in the stroma (Fig. 3) similar to those in the first days after crosslinking (Fig. 2). This indicated that it is not possible to judge the depth of the photochemical process of crosslink formation in the corneal stroma after crosslinking by increasing the optical density in the stroma layers. We have not noted the relationship between the depth of the demarcation line and the therapeutic effect of excimer laser crosslinking. The shape and severity of the demarcation line in the corneal stroma also did not affect the clinical effect of stabilizing optical refractive indices, the degree of flattening of the cornea, and the increase in uncorrected and corrected visual acuity. Moreover, in some cases, in the absence of the formation of a demarcation line in the stroma, the effect of flattening the cornea and increasing visual acuity without correction was observed. This indicated that the line of demarcation in the stroma does not fully reflect the effect of corneal crosslinking and its clinical significance needs to be reassessed.

In our studies, we observed the formation of a demarcation line in the corneal stroma after transepithelial PRK without additional UV irradiation. These were the cases when, after the completion of the ablation for the deactivation of peroxide radicals in the stroma, a 0.25% isotonic solution of riboflavin was instilled for 2-3 minutes. The demarcation line did not appear in all cases, which could be explained by varying degrees of severity of the corneal syndrome, aseptic inflammatory reaction, and instillation of dexamethasone after surgery. Sometimes the line of demarcation was combined with the phenomena of fibroplasia (Fig. 4). The development of fibroplasia was probably associated with a large accumulation of peroxide radicals in the stroma during its ablation without riboflavin, which plays a photoprotective protective role against secondary UV-B radiation induced during ablation [15-17].

Our clinical observations during prophylactic and therapeutic excimer laser corneal crosslinking did not reveal an increase in the depth of the demarcation line in the corneal stroma with an increase in the total energy dose of radiation. Suffice it to say that during prophylactic crosslinking, the dose of secondary radiation induced by ablation was several times lower than that in comparison with therapeutic excimer laser crosslinking. According to the demarcation line, it was not possible to judge the advantages and effectiveness of a particular crosslinking technique. Moreover, more often the line of demarcation in the stroma was located deeper than the primary zone of increased optical density visualized on OCT in the first days after corneal crosslinking.

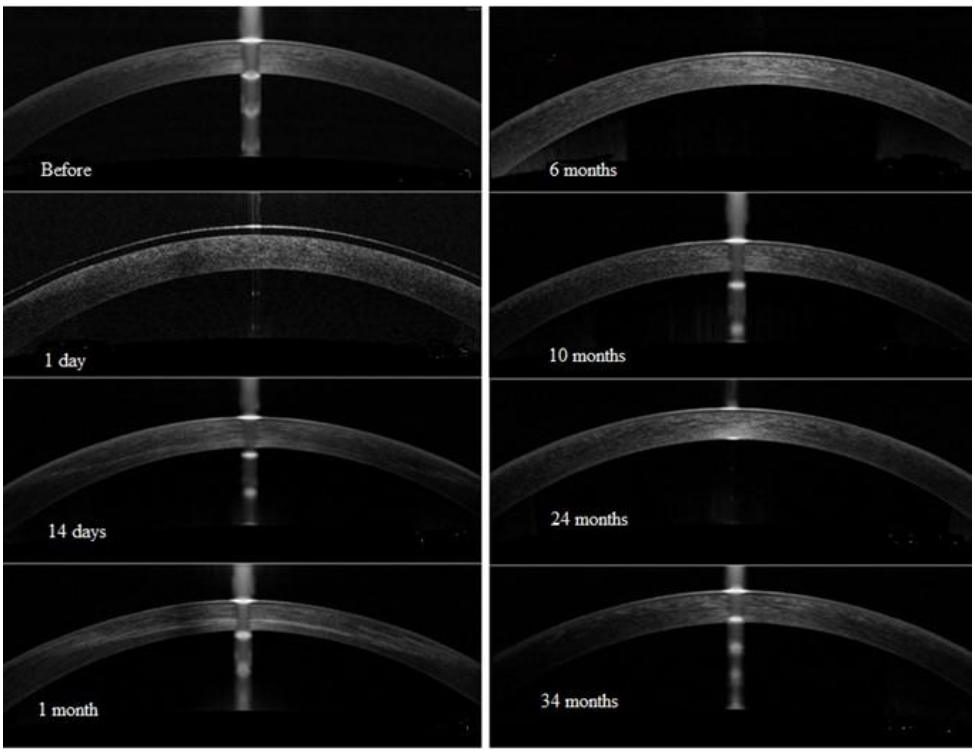

Fig. 1. OCT of the cornea at various times after corneal crosslinking with subablative argon-fluorine excimer laser radiation in progressive stage II keratoconus

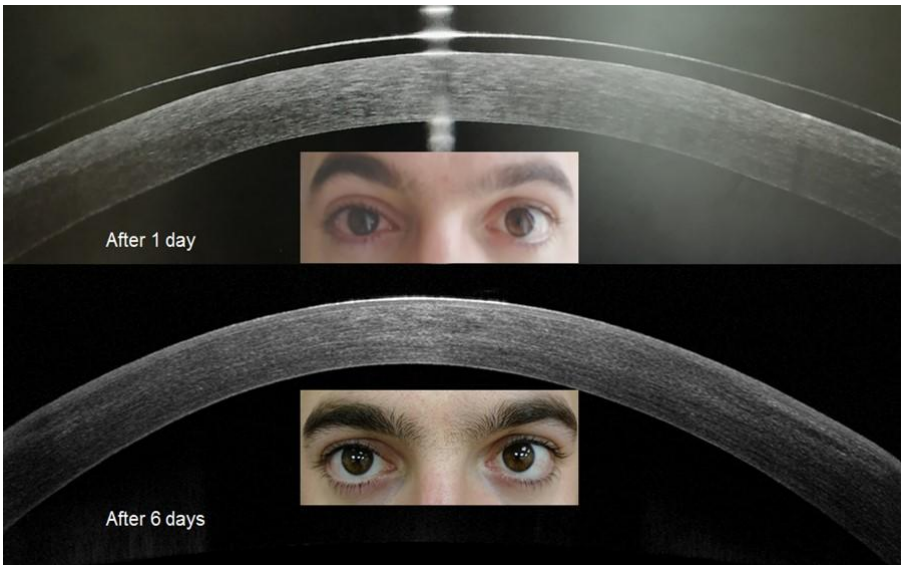

Fig. 2. OCT of the cornea with a zone of increased optical density and the state of the anterior segment of the right eye 1 and 6 days after topographically oriented crosslinking with subablative argon-fluorine excimer laser radiation in progressive keratoconus stage II

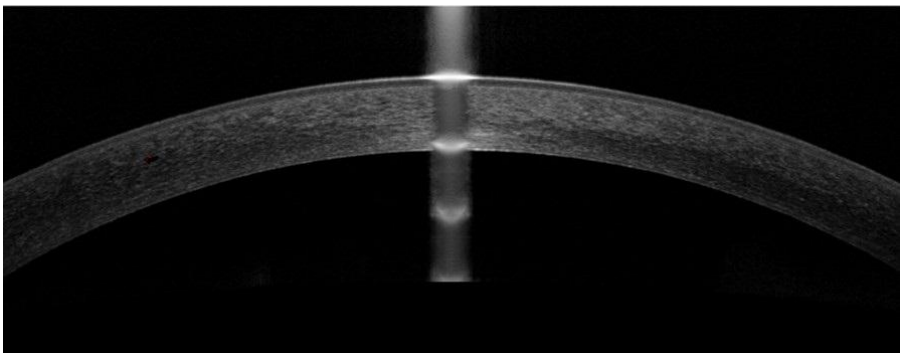

Fig. 3. OCT of the cornea with an increase in optical density in keratopathy with a subclinical aseptic inflammatory reaction against the background of long-term wearing of a soft contact lens

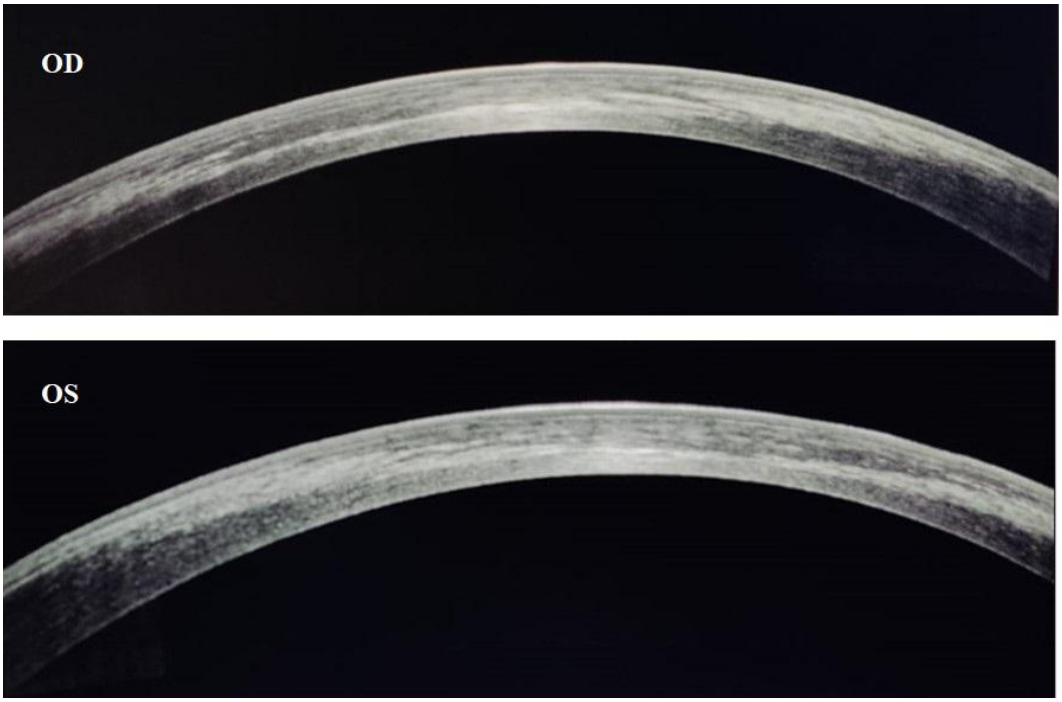

Fig. 4. OCT of the cornea of the right and left eyes with a demarcation line and fibroplastic changes in the layers of the corneal stroma 1 month after TransPRK. Immediately after completion of the ablation, the stroma was saturated with 0.25% isotonic riboflavin for 3 minutes. Additional UV irradiation was not carried out

During prophylactic and therapeutic excimer laser crosslinking of the cornea, we did not observe any dependence of the depth of the demarcation line in the stroma on the total dose of UV irradiation. As an illustration, we present a clinical case of the formation of a demarcation line in the stroma after therapeutic excimer laser circular peripheral crosslinking in secondary corneal keratoectasia. Peripheral keratoectasia developed after personalized LASIK for induced hypermetropia and irregular astigmatism. These refractive errors developed 15 years after radial keratotomy in an eye with moderate myopia. For circular crosslinking, profiling of the total dose of subablative excimer laser radiation with minimal impact on the central optical zone of the cornea was used. Two weeks later, an increase in refraction in the central optical zone by 2.5 diopters was noted. and an increase in uncorrected visual acuity from 0.4 to 0.7. According to OCT data, in the central zone of the corneal stroma, the demarcation line was formed at a greater depth, despite the minimum total energy dose of radiation (Fig. 5).

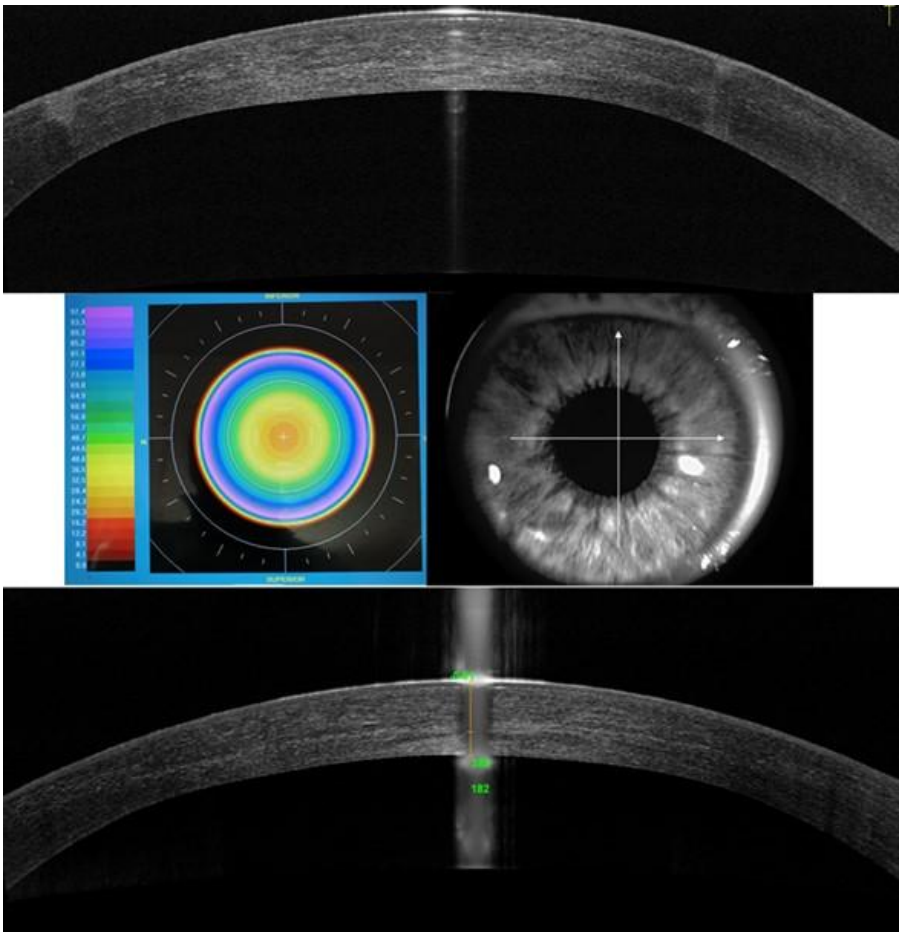

Fig.5. OCT of the cornea 14 days after therapeutic peripheral circular crosslinking with a complex distribution profile of the total energy dose of exposure. In the central optical zone, the demarcation line is at a greater depth, despite the minimum total energy dose of radiation

The conducted studies showed that the aseptic inflammatory response had a significant impact on the time of appearance, severity and depth of the demarcation line in the stroma. The appearance of a demarcation line during prophylactic excimer laser crosslinking was the exception rather than the rule. This could be explained by the appointment of steroid drugs immediately after the completion of epithelialization. The results of these studies will be presented in more detail in a separate publication.

4. DISCUSSION

An analysis of various methods of corneal crosslinking showed that when the stroma was saturated with a 0.1% solution of riboflavin and activated with UV radiation (365-370 nm), the demarcation line in the stroma was formed at a significantly greater depth with traditional than with accelerated methods of its implementation. During crosslinking, the range of fluctuations in the depth of the demarcation line in the stroma ranged from 1/3 to 2/3 of the corneal thickness [18-23]. At the same time, there was a large variability in the shape, severity and depth of the demarcation line in the corneal stroma, regardless of the method of corneal crosslinking. The same applied to the time of its appearance and reverse development. In addition, in a number of cases, with strict observance of the total energy dose, there was a different aseptic inflammatory response of the cornea to crosslinking. A similar nature of the formation and reverse development of the demarcation line and the aseptic inflammatory response was observed by us during prophylactic and therapeutic crosslinking of the cornea. In all cases, photoablation-induced oxidative stress was accompanied by the formation of peroxide radicals in the stroma and an aseptic inflammatory reaction [16-17]. The degree of expression of the latter depended on the

formation of additional peroxide radicals. Thus, the primary activator of riboflavin in prophylactic crosslinking was the ablation-induced secondary radiation of an argon-fluorine excimer laser. Peroxide radicals of oxidative stress were the secondary activator. That is why we noted the formation of a demarcation line in the stroma after instillation of riboflavin immediately after ablation without additional UV irradiation. It should be noted that an aseptic inflammatory reaction accompanies any technology of corneal crosslinking. Since all of these factors are interrelated with each other, the final effect of corneal crosslinking will depend on the severity of each of them. It should be noted that in some cases it is possible to form a line of demarcation in a chronic subclinical aseptic inflammatory reaction in the corneal stroma. At the same time, peroxide radicals in the stroma are able to initiate the effect of crosslinking without the presence of riboflavin in the stroma and its activation by UV radiation. This is confirmed by the cases of formation of a demarcation line in the stroma 12-15 days after a chemical burn. The depth of its occurrence confirmed the severity of the burn and had a prognostic value [24].

The formation of a demarcation line, even with the traditional method of corneal crosslinking, is observed only in 75-79% of cases [25,26]. In our experimental studies on biomechanical testing during keratoablation with saturation of the cornea with riboflavin, the effect of crosslinking without the formation of a demarcation line in the thinned stroma of the cornea was revealed [15,16].

All of the above makes it possible to understand why the line of demarcation in the stroma cannot serve as a criterion for assessing the strength properties of the cornea after crosslinking. We believe that such an indicator as the total number and density of crosslinks per unit volume of the stroma is of great importance in improving the strength characteristics of the cornea after crosslinking. This means that the same effect of increasing the strength properties of the cornea can be obtained with a smaller depth of the demarcation line in the stroma. The latter can be achieved by selecting the optimal combination of concentration and time of saturation with riboflavin, its activation by UV radiation in the range that overlaps at least one of the peaks of maximum absorption by riboflavin. The effect of crosslinking can be enhanced by additional aeration and local hypothermia of the cornea [17].

According to the goal, we considered it appropriate in this article to focus on debatable issues related to the interpretation of the clinical significance of the demarcation line in various corneal crosslinking techniques. Each of the discussed debatable issues was illustrated with clinical examples. This approach, in our opinion, is important for practicing ophthalmologists. We considered it impractical to carry out statistical processing of this clinical material due to the large scatter of data on the depth, shape and severity of the demarcation line in the corneal stroma with the same crosslinking technique.

An analysis of various protocols for crosslinking and our own clinical studies on preventive and therapeutic excimer laser crosslinking of the cornea made it possible to formulate the following conclusions given below.

5. CONCLUSIONS

1. The clinical significance of the shape, severity and depth of the demarcation line in the corneal stroma after crosslinking needs to be reassessed, since these indicators do not reflect the depth of the photochemical process of the formation of crosslinks and their density in the stroma layers, which predetermine the strength properties of the cornea. In addition, after crosslinking, the appearance of a demarcation line in the stroma is not observed in all cases.
2. The depth of the demarcation line in the stroma during corneal crosslinking is greatly influenced by the aseptic inflammatory reaction.
3. The formation of a demarcation line in the stroma is possible without the use of riboflavin and UV irradiation at certain stages of the inflammatory process in the cornea.

CONSENT

Written informed consent was obtained from all patients.

ETHICAL APPROVAL

All studies were carried out in compliance with the principles of the Declaration of Helsinki and with the permission of the ethical committee of the FSBI “National Medical and Surgical Center named after V.I. N.I. Pirogov ”of the Ministry of Health of Russia.

COMPETING INTERESTS

Author has declared that no competing interests exist.

REFERENCES

1. Seiler T., Hafezi F. Corneal Cross-Linking-Induced Stromal demarcation Line. Cornea 2006;25(9):1057–1059.
   DOI:10.1097/01.ico.0000225720.38748.588.
2. Kymionis G.D., Grentzelos M.A., Plaka A.D., et al. Evaluation of the corneal collagen crosslinking using anterior optical coherence tomography. Cornea. 2013; 32:907–910.
   DOI: 10.1097/ICO.0b013e31828733ea.
3. Koller T., Schumacher S., Fankhauser F II, et al. Riboflavin/ultraviolet a crosslinking of the paracentral cornea. Cornea. 2013;32:165–168.
   DOI: 10.1097/ ICO.0b013e318269059b.
4. Kymionis .D., Tsoulnaras K.I., Grentzelos M.A., et al. Corneal stromal demarcation line after standard and high-intensity collagen crosslinking determined with anterior segment optical coherence tomography. J. Cataract Refract. Surg 2014; 40:736–740.
   DOI.org/10.1016/j.jcrs.2013.10.029.
5. Kymionis, G.D.; Tsoulnaras, K.I.; Grentzelos, M.A.; Liakopoulos, D.A.; Tsakalis, N.G.; Blazaki, S.V.; Paraskevopoulos, T.A.; Tsilimbaris, M.K. Evaluation of corneal stromal demarcation line depth following standard and a modified-accelerated collagen cross-linking protocol. Am. J. Ophthalmol. 2014;158:671–675.e1.
6. Yam J.C., Cheng A.C. Reduced cross-linking demarcation line depth at the peripheral cornea after corneal collagen cross-linking. J Refract Surg. 2013; 29(1):49–53.
   DOI: 10.3928/1081597X-20121228-03 4.
7. Moramarco A., Iovieno A., Sartori A., Fontana L. Corneal stromal demarcation line after accelerated crosslinking using continuous and pulsed light. J Cataract Refract Surg. 2015;41:2546–2551.
   DOI.org/10.1016/j.jcrs.2015.04.033.
8. Malta J.B., Renesto A.C., Moscovici B.K., et al. Stromal demarcation line induced by corneal cross-linking in eyes with keratoconus and non-keratoconic asymmetric topography. Cornea 2015; 34:199–203.
   DOI: 10.1097/ICO.00000000. 00000305.
9. Samantha Bonnel, MD; Marouen Berguiga, MD; Benoit De Rivoyre, JD; Gabriel Bedubourg, MD; Damien Sendon, MD; Françoise Froussart-Maille, MD, MSc; Jean-Claude Rigal-Sastourne, MD, MSc Demarcation Line Evaluation of Iontophoresis-AssistedAssisted Transepithelial Corneal Collagen Cross-linking for Keratoconus. J Refract Surg. 2015;31(1):36-40.
10. Ng A.L.K., Chan T.C.Y., Lai J.S.M. and Cheng A.C.K. Comparison of the central and peripheral cornel stromal demarcation line depth in conventional versus accelerated collagen cross-linking. Cornea 2015;34(11):1432–1436.
    DOI: 10.1097/ ICO.0000000000000626.
11. Bikbova, G., Bikbov, M. Standard corneal collagen crosslinking versus transepithelial iontophoresis-assisted corneal crosslinking, 24 months follow-up: randomized control trial. Acta Ophthalmologica; 2016;94(7):e600-e606.
    DOI: 10.1111/aos.13032
12. Spadea l., Tonti T., Vingolo EM. Corneal stromal demarcation line after collagen cross-linking in corneal ectatic diseases: a review of the literature. Clinical Ophthalmology, 2016;10:1803-1810.
13. 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;44:596–602.
    DOI: 10.1016/j.jcrs. 2018.02.017.PMID: 29685772.
14. Kornilovskiy I.M., Gilya A.P., Khatataev R.R. Excimer laser topographically oriented corneal crooslinking. Modern problems of science and education. 2021;2.
    DOI 10.17513/spno.30613. URL: https://science education.ru/ru/article/view?id=3061.
15. Kornilovskiy I.M. Photorefractive Keratectomy with Protection from Ablation-Induced Secondary Radiation and Cross-linking Effect. EC Ophthalmology. 2019; 10 (70): 563-570.
16. Kornilovskiy I.M. Prophylactic and Therapeutic Laser-Induced Corneal Crosslinking. EC Ophthalmology. 2020; 11(12):74-82.

17. Kornilovskiy I.M. Application of Pulsed Laser Radiation of the Far Ultraviolet Range for Corneal Crosslinking. Acta Scientific Ophthalmology.2021; 4(4):51-55.
18. Mazzotta, C.; Ferrise, M.; Gabriele, G.; Gennaro, P.; Meduri, A. Chemically Boosted Corneal Crosslinking for the Treatment of Keratoconus through a Riboflavin 0.25% optimized solution with High Superoxide Anion Release. J. Clin. Med. 2021;10:1324.
Available: https://doi.org/10.3390/jcm10061324.
19. Tomita M., Mita M., Huseynova T. Accelerated versus conventional corneal collagen crosslinking. J. Cataract Rafract. Surg. 2014;40:1013–1020.
DOI: 10.1016/j. jcrs.2013.12.012.
20. Pircher, N.; Lammer, J.; Holzer, S.; Gschließer, A.; Donner, R.; Pieh, S.; Schmidinger, G. Correlation between central stromaldemarcation line depth and changes in K values after corneal cross-linking (CXL). Graefes Arch. Clin. Exp. Ophthalmol. 2018;256:759–764.
21. D'Oria F, Puzo P, Incandela C, Sborgia A, Gigliola S, Boscia F, Alessio G.J Evaluation of Demarcation Line after Epithelium-Off Iontophoresis Corneal Collagen Cross-Linking for Progressive Keratoconus. Clin Med. 2021;10(15): 3295.
DOI: 10.3390/jcm10153295
PMID: 34362077 Free PMC article.
22. Yehia Salah, Kholoud Omar, Ahmed Sherif, Sarah Azzam Study of Demarcation Line Depth in Transepithelial versus Epithelium-Off Accelerated Cross-Linking (AXL) in Keratoconus. J Ophthalmol., 2019;2019:3904565.
DOI: 10.1155/2019/3904565. eCollection 2019.
23. Kymionis GD, Tsoulnaras KI, Liakopoulos DA, Skatharoudi CA, Grentzelos Corneal Stromal Demarcation Line Depth Following Standard and a Modified High Intensity Corneal Cross-linking Protocol. MA, Tsakalis NG.J Refract Surg. 2016; 32(4):218-22.
DOI: 10.3928/1081597X-20160216-01.PMID: 27070227.
24. Brosh K., Rozenman Y. Chemical Burn-Induced Stromal Demarcation Line. Cornea, 2016; 35(2): 286-288.
DOI: 10.1097/ICO.0000000000000667.
25. Doors M., Tahzib N.G., Eggink F.A., et al. Use of anterior segment optical coherence tomography to study corneal changes after collagen crosslinking. Am. J. Ophthalmol.,2009;148:844–851.e2.
DOI: 10.1016/j.ajo.2009.06.031.
26. Ng A.L.K., Chan T.C.Y., Lai J.S.M. and Cheng A.C.K. Comparison of the central and peripheral cornel stromal demarcation line depth in conventional versus accelerated collagen cross- linking. Cornea 2015;34(11):1432–1436.
DOI: 10.1097/ICO.0000000000000626.

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/91311