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Application of hypotonic riboflavin in corneal crosslinking in patients with keratoconus

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低渗核黄素在圆锥角膜患者角膜交联术中的应用

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摘要
目的：比较圆锥角膜患者角膜交联(CXL)、等渗(IR)和低渗核黄素(HR)的结果。
方法：回顾性研究,包括 29 例 29 眼圆锥角膜患者行角膜交联术,IR 组 15 眼,HR 组 14 眼。参数分析包括(随访1a)：最佳矫正视力(BCVA),球体和柱体,中央和较薄处角膜厚度,平均和最大角膜曲率(分别为 Km 和 Kmax),并发症及进展。
结果：观察到两组的 BCVA 提高：治疗前 IR 组为 0.26±0.57,HR 组为 0.47±0.72。术后 1a,IR 组为 0.13±0.79,HR 组为 0.29±1.52,IR 组 BCVA 更高(P=0.018)。术后1mo,两组中央角膜厚度(μm)均减少,但在接下来的数月中增加：术前 IR 组为 497±28 μm,HR 组为 432±14 μm;术后 1a,IR 组为 480±31 μm,HR 组为 424±15 μm。较薄处角膜厚度也是如此,术前 IR 组为 487±29 μm,HR 组为410±20 μm;术后 1a,IR 组为 468±33 μm,HR 组为 413±13μm。两组中 Km 和 Kmax 均降低。每组 6 眼呈现短暂的混浊,无持续性混浊。
结论：在中央角膜厚度<400μm 的患者中,HR 的使用似乎是一个有效的替代执行传统 CXL 的技术。
关键词：圆锥角膜;交联术;核黄素;薄角膜

Abstract

• AIM：To compare the results of corneal cross - linking (CXL) with isotonic (IR) and hypotonic riboflavin (HR) in patients with keratoconus.
• METHODS：Retrospective study of 29 eyes/keratoconus patients submitted to CXL, 15 eyes with application of IR (IR group) and 14 eyes with application of HR (HR group). The parameters analysed included (1 - year follow-up)：best corrected visual acuity (BCVA), sphere and cylinder, central and finer pachymetry, mean and maximum keratometry (Km and Kmax respectively), complications and progression.
• RESULTS：An increase on the BCVA scale (LogMAR, logarithm of the minimal angle of resolution) was observed in the two groups：0.26±0.57 (IR) and 0.47±0.72 (HR) before treatment, and 0.13±0.79 (IR) and 0.29±1.52 (HR) at the 1y. Only at 1y, the difference was statistically significant (P=0.018, group IR with higher BCVA). The central pachymetry (μm) decreased at 1mo in both groups, and increased in the following months：497±28 μm (IR) and 432±14 μm (HR) before treatment, and 480±31 μm (IR) and 424±15 μm (HR) to the 1y. The thinner pachymetry (μm) presented the same evolution：487±29 μm (IR) and 410±20 μm (HR) before treatment, and 468±33 μm (IR) and 413±13μm (HR) at 1y. Km and Kmax decreased in both groups (P>0.05). 6 eyes from each group presented transitory haze. No eye progressed to the 1y.
• CONCLUSION：The use of hypotonic riboflavin seems to be a valid alternative for performing the traditional corneal cross - linking technique in eyes with a central corneal thickness of <400 μm.
• KEYWORDS：keratoconus; cross - linking; riboflavin, thin cornea

INTRODUCTION

K eratoconus is a disorder that is characterized by conical shape ectasia and progressive thinning of the cornea, which cause irregular astigmatism and myopia, resulting in a significant decrease in visual acuity[1-3]. Its pathophysiology is not completely known, but seems to consist mainly of a

reduced number of connections between collagen fibres and increased activity of proteolytic stroma enzymes, compared to normal corneas. These changes result in greater biochemical and biomechanical fragility of the corneal stroma with keratoconus[1-3].

Currently, corneal cross-linking (CXL) is the only treatment option that allows for the stabilisation or even decrease of keratoconus progression, as it acts precisely on the biochemical and biomechanical characteristics of the stroma, attenuating or reverting the aforementioned changes and strengthening the corneas with keratoconus (action mainly evident in the previous 300 μm of the stroma)[4-8]. With the application of ultraviolet A (UV-A, 370 nm) and riboflavin (photosensitiser) in the presence of oxygen, an oxidative photopolymerization reaction occurs, resulting in the creation of new covalent bonds between collagen fibrils[5-8]. Riboflavin also has a " buffering" effect that protects the incidence of UV-A radiation in deeper ocular structures, such as the corneal endothelium, lens, and retina[5-9]. Therefore, the main limitation of the application of CXL in thinner corneas is that their reduced thickness increases the risk of absorption of UV-A by the endothelium, with subsequent injury of same, as demonstrated in the study by Kymionis et al[5-9]. In this context, Wollensak et al[11], pioneers in 2003 of CXL treatment to halt the progression of keratoconus, have demonstrated that the application of the parameters of the conventional protocol (Dresden protocol, epithelium-off, with isotonic riboflavin, IR-0.1% riboflavin in 20% dextran-and 3 mW/cm^2 of UV-A for 30min) in corneas with a central thickness of < 400 μm achieved the cytotoxicity limit of 0.35 mW/cm^2 for endothelial cell damage. This is why 400 μm was the central corneal thickness limit identified for the application of the conventional CXL protocol[9-12].

However, because, in practice, this aspect limits the application of CXL in more advanced cases of keratoconus, Hafezi et al[13] created a modified CXL technique in 2009 in order to increase the safety of the procedure. His technique involves the use of hypotonic riboflavin (HR, 0.1% riboflavin in 0.9% sodium chloride instead of dextran), replacing the isotonic solution. HR is applied after corneal de-epithelialization and until its saturation reaches 400 μm of central thickness. Thus, the CXL procedure in thinner corneas acquires greater safety by reducing the referred risk[13]. This effect of the HR solution is due to its lower oncotic pressure due to the absence of dextran in its constitution (relative to the IR solution), causing corneal intumescence associated with the hydrophilic properties of the stromal proteoglycans and, consequently, a transient increase of its thickness[14-18]. Despite the study by Hafezi et al[13] and others that have subsequently demonstrated safety and efficacy of the procedure, its effectiveness compared with the use of IR is not yet clear. This fact is related to its possible lower

biomechanical effect, as its lower oncotic pressure can result in a lower concentration and a greater distance from the collagen fibres, making it difficult to establish connections between them[14-18]. On the other hand, the same authors suggested a central corneal thickness limit of 330 μm for CXL with HR, to allow an effective CXL procedure and prevent UV-A toxicity to the endothelium[14-18].

Although promising, the various modified CXL techniques are relatively recent, and only a few studies on them have been conducted to date, which mainly include a relatively small sample, a short follow-up, and some documented limitations and/or disadvantages.

SUBJECTS AND METHODS

Retrospective study of 29 eyes of 29 patients with keratoconus from the Cornea Clinic of the Ophthalmology Department at the Hospital Professor Doutor Fernando Fonseca EPE (HFF, Lisbon, Portugal), submitted to CXL between 2012 and 2018, 15 eyes with IR application (IR group) and 14 eyes with HR application (HR group). Inclusion criteria consisted of: keratoconus in stages 1 to 3, according to Amsler-Krumeich classification; keratoconus with documented progression in the 12mo prior to the start of treatment, through refraction and/or corneal topography. Exclusion criteria consisted of: previous corneal surgery; history of herpetic keratitis; recurrent corneal infections; severe dry eye, and stable keratoconus in stage 4. All consecutive patients meeting the criteria during the period considered were included. The study followed the principles of the Helsinki Declaration and was approved by the Hospital Ethical Committee. Data was collected for the study through consultation of patients' clinical processes and topographic parameters obtained by Pentacam© . The clinical parameters studied were: best corrected visual acuity (BCVA, in logarithm of the minimal angle of resolution scale–LogMAR), manifest value of the sphere and cylinder (in dioptres–D), and complications. The topographic parameters of Pentacam© studied were: central and thinner pachymetry (in μm) and mean (Km) and maximum (Kmax) keratometry parameters in D. Clinical (BCVA and cylinder value) and topographic parameters (Kmax) were used to identify the presence of progression, this being defined in previous studies as the loss of ≥2 lines of the BCVA or an increase of ≥1 D in the manifested cylinder or in the Kmax, in the 1y[1-4]. The aforementioned parameters were analysed during a 1-year follow-up: prior to the CXL and, in the 1 and 6mo and in the 1y, after the CXL. The results of the study parameters were compared between the 2 groups.

The division of eyes/patients between the IR and HR groups was based on the value of the central corneal thickness as measured by ultrasonic pachymetry following the de-epithelialization procedure: IR group if ≥400 μm and HR group if <400 μm. The CXL technique applied to corneas with a central thickness of ≥400 μm followed the Dresden protocol

and the technique applied to corneas with a central thickness of <400 μm followed the modified technique described by Hafezi et al[13]. The procedure commenced with pachymetry corneal ultrasonography，followed by corneal de－epithelialization in the central 7 mm，using a scalpel and methylcellulose sponge，and subsequent repeated determination of pachymetry. One drop of the riboflavin solution was applied every 3min for 30min：isotonic solution of 0.1% riboflavin－5－phosphate with 20% dextran if pachymetry was ≥400 μm，and hypotonic solution of 0.1% riboflavin－5－phosphate without dextran if pachymetry was <400 μm. In the cases with pachymetry >400 μm，the irradiation of the cornea with the source of UV－A radiation（370 nm）was initiated，whereas in the cases with pachymetry <400 μm，distilled water was applied before a corneal thickness ≥400 μm. Irradiation of the cornea was carried out for 30min，with the source of UV－A radiation placed at 45 mm from same，with a power of 3 mW/cm^2，maintaining the application of riboflavin solution every 3min，interspersed with the application of distilled water. To ensure the safety of the technique in the HR group，the pachymetry was repeated intraoperatively after application of riboflavin. At the end of the procedure，a silicone－hydrogel contact lens was placed in each patient's eye for better pain relief，to aid in the epithelial healing and to accelerate recovery in the eyes with corneal haze. Patients were medicated with ofloxacin and dexamethasone/neomycin eye drops.

A statistical analysis was performed with the SPSS program（Statistical Package for Social Sciences，Version 22.0），involving descriptive statistical measures；Chi－square and Fisher tests to analyse the differences between groups in qualitative variables and the Student's t－test to test the differences in quantitative variables；the assumptions of normal distribution and variance homogeneity were analysed with the Kolmogorov－Smirnov tests and Levene's test，and in those cases where these assumptions were not satisfied，the aforementioned tests were replaced by the Mann－Whitney test. The results were considered statistically significant when P value was less than 0.05（power of the study：95%）.

RESULTS

The study sample included 29 eyes from 29 CXL－treated keratoconus patients（16 males and 13 females），with an average age of 21.4±6.0（20.1±4.0 in the IR group and 21.3±5.0 in the HR group）years old. CXL was performed with the application of IR on 15 of the 29 eyes and with HR on the remaining 14. The results are expressed as mean±standard deviation，in absolute values，and are shown in Tables 1 and 2 and in Figures（graphics）1 to 7. Those tables also include the statistical significance（P value）of the differences between the two groups. For each group，the statistical significance difference（P value）of all the parameters between the preoperative evaluation and each moment of the follow－up（Table 3）.

Table 1　Comparison of BCVA and refraction between groups

Groups	Preoperative	1mo	6mo	1y
BCVA（LogMAR）				
IR	0.26±0.57	0.30±0.0	0.15±0.58	0.13±0.79
HR	0.47±0.72	0.30±0.0	0.27±1.30	0.29±1.52
P	0.327	1.001	0.400	0.018[a]
Sphere（dioptres）				
IR	2.1±0.68	2.3±0.55	2.4±0.54	2.6±0.50
HR	3.4±2.5	2.6±1.9	1.5±1.6	2.5±1.6
P	0.393	0.408	0.398	0.569
Cylinder（dioptres）				
IR	6.0±2.2	5.8±0.0	5.4±1.1	4.3±1.0
HR	6.2±2.1	6.5±1.8	6.6±1.7	5.2±0.2
P	1.000	0.400	0.401	0.251

BCVA：Best corrected visual acuity. [a]P value <0.05.

1）BCVA：there was an improvement in BCVA in both groups，without satistical significance between the preoperative evaluation and each moment of the follow-up. In the first year alone，the difference between groups was statistically significant（P = 0.019），with the IR group showing a better BCVA.

2）Manifest value of the sphere and the cylinder：the sphere increased slightly in the IR group，while in the HR group it decreased. The cylinder decreased in both groups. In each group，the results were not statistically significant between the preoperative evaluation and each moment of the follow－up. Both parameters did not present statistically significant differences between the groups during follow-up.

3）Central and thinner pachymetry：a reduction in central pachymetry was observed at the 1st month，with a progressive increase up to the 1st year of follow－up in both groups（IR group with higher values）. In the IR group，both central and thinner pachymetry showed statistically significant differences at all the follow-up moments，while in the HR group only the thinner pachymetry showed a statistically significant difference between the preoperative evaluation and the evaluations of the 1st and the 6th month. Differences between the groups were statistically significant in all follow-up moments except for the 1st month.

4）Km and Kmax：a Km reduction was observed throughout the follow-up in both groups，with only the IR group showing a statistically significant result between the preoperative evaluation and the 1st year evaluation. There were no statistically significant differences between the groups.

5）Complications：6 eyes of each group（total of 12 eyes）presented a transient haze，with spontaneous resolution until the 3rd week after treatment. All epithelial defects were resolved within 1wk after treatment and no cases of corneal or permanent stromal scarring were observed.

6）Progression：no eye presented progression in the 1st year in either group，according to the criteria used to define progression.

Table 2　Comparison of queratometry and pachymetry between groups

| Groups	Preoperative	1mo	6mo	1y
Mean keratometry（dioptres）				
IR	50.2±2.9	49.6±0.1	48.9±1.9	48.5±2.5
HR	48.9±3.5	48.6±2.6	48.2±2.9	47.9±2.5
P	0.444	1.000	0.327	0.915
Maximum keratometry（dioptres）				
IR	60.6±5.2	59.6±0.1	58.7±4.3	58.2±5.0
HR	59.4±7.2	58.8±5.9	58.3±5.1	57.6±4.6
P	0.833	1.000	0.428	1.001
Central pachymetry（μm）				
IR	497±28	397±0.1	471±43	480±31
HR	432±14	427±8	413±18	424±15
P	0.002[a]	0.501	0.030[a]	0.011[a]
Thinner pachymetry（μm）				
IR	487±29	375±0	454±42	468±33
HR	410±20	406±26	388±21	413±13
P	0.002[a]	0.501	0.018[a]	0.019[a]

[a]P value <0.05.

Table 3　Statistical significance between the preoperative evaluation and each moment of the follow-up, for each group

| Groups	1mo preoperative	6mo preoperative	1y preoperative
BCVA			
IR	0.77	0.75	0.66
HR	0.25	0.194	0.194
Sphere			
IR	0.40	0.317	1.0
HR	0.266	0.29	0.285
Cylinder			
IR	0.45	0.655	0.273
HR	0.281	0.109	0.593
Mean keratometry			
IR	0.068	0.075	0.028[a]
HR	0.178	0.177	0.715
Maximum keratometry			
IR	0.275	0.251	0.045[a]
HR	0.412	0.344	0.715
Central pachymetry			
IR	0.045[a]	0.046[a]	0.026[a]
HR	0.061	0.077	0.46
Thinner pachymetry			
IR	0.026[a]	0.028[a]	0.028[a]
HR	0.038[a]	0.042[a]	0.47

[a]P value <0.05.

DISCUSSION

The study showed an improvement in the studied parameters （BCVA, refraction, Km and Kmax）, without relevant complications and without progression, in both groups, which is in agreement with the literature and other studies[4,16]. These results in both groups corroborate the objectives of the CXL itself, culminating in the stability or even reduction of the progression of keratoconus by strengthening the covalent
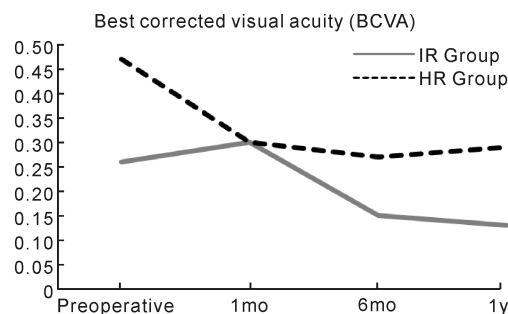
Figure 1　Best corrected visual acuity throughout follow-up.
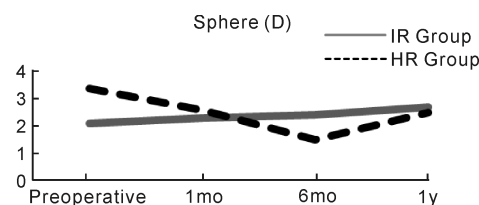

Figure 2　Value of the sphere（in dioptres）throughout follow-up.
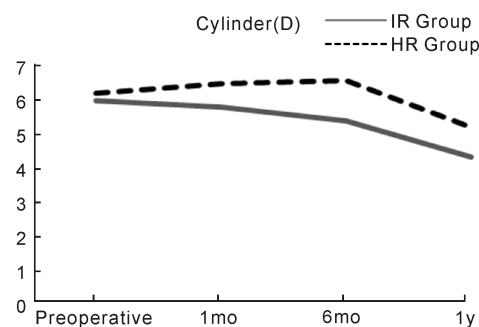
Figure 3　Value of the cylinder（in dioptres）throughout follow-up.

bonds between the collagen fibres of the cornea. Thus, the primary objective of CXL was achieved through the application of both IR and HR.
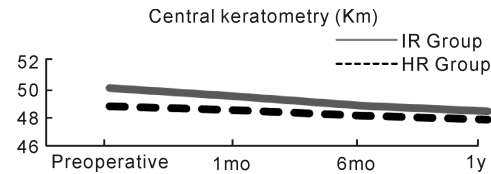
According to the results obtained, the application of HR seems to be an option to consider in thinner corneas. Its effect
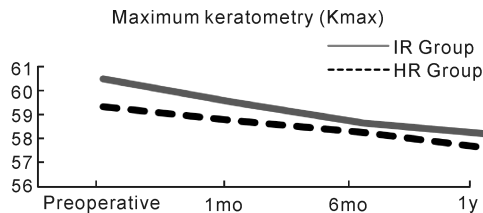
Central pachymetry (μm)
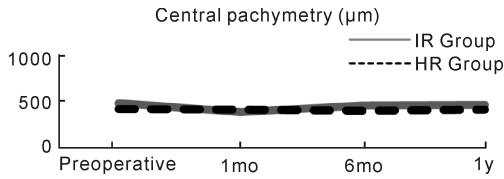
Figure 4　Central pachymetry（μm）throughout follow-up.

Thinner pachymetry (μm)
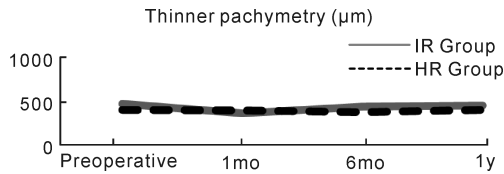
Figure 5　Thinner pachymetry（μm）throughout follow-up.

Central keratometry (Km)

Figure 6　Central keratometry（Km）through the follow-up.

Maximum keratometry (Kmax)

Figure 7　Maximum keratometry（Kmax）throughout follow-up.

can be explained by its lower oncotic pressure，leading to an increase in corneal swelling until the corneal thickness threshold of 400 μm is reached for the procedure showing a lower risk of endothelial toxicity associated with UV - A radiation. The study by Nassaralla et al[16] confirmed the efficacy of HR application in corneas of ＜400 μm，with a statistically significant reduction of keratometry and BCVA in the first year of follow-up. The study by Raiskup et al[15] demonstrated no statistically significant stabilization of keratometry and of BCVA with the same follow-up period.

Although our results are consistent with the literature available，there are still few comparative studies between CXL with IR and HR. Similar to our study，those conducted by Raiskup et al[15] and Nassaralla et al[16]，mentioned above，also revealed more discrete results with HR，contrary to previous studies with corneas of ≥ 400 μm treated with IR[4,10-12]. On the other hand，the study by Rosenblat et al[14]，showed that there were no statistically significant differences between IR and HR groups regarding improvement of Kmax，uncorrected visual acuity and the thickness of the cornea. The study by Chen et al[19] also showed the absence of a statistically significant difference between the IR and HR groups in relation to keratometry，BCVA and a spherical equivalent.

A theory discussed regarding the possible lower efficacy of HR is the eventual lower biomechanical effect with greater difficulty in establishing connections between collagen fibres，due to the lower oncotic pressure associated with corneal

intumescence，resulting in lower concentration and greater distance from collagen fibres[14-18]. However，even if HR is not as effective as IR，showing no statistically significant change in the parameters under study，and/or showing stabilization of parameters rather than improvement，its application is still promising because by stabilizing the parameters，it is already delaying progression without any apparent increased risk for the endothelium.

Regarding the modifications in BCVA，cylinder and sphere，some studies demonstrate results similar to those obtained in our study，with varying significance；others show no relevant changes in these parameters[15-16]. The study conducted by Hashemi et al[4] indicates as a possible justification the differences in sample size and base refractive error.

The changes in pachymetry in our study，with a decrease in the comparison between the initial value and the first month and with a later increase up to the first year，are in agreement with other studies[11,17-18,20-21]. Gu et al[17] Hassan et al[20] suggest that the initial change is due to measurement errors due to the initial swelling of the cornea caused by the procedure. Also Wollensak et al[11] demonstrated that the swelling of the cornea depended on the degree of CXL：the higher the CXL，the smaller the swelling，and vice versa. On the other hand，de-epithelialization was initially performed，which alone can increase the rate of evaporation of stromal water and，as it is not resistant to dehydration，makes the cornea vulnerable to a reduction in its thickness，as indicated by Nassaralla et al[16] Furthermore，the effect of CXL may probably decrease corneal thickness due to the increase in endothelial cell activity caused by the initial exposure to UV- A radiation，as indicated by several authors，such as Holopainen et al[21]，with subsequent stabilization and increased thickness. Still in relation to pachymetry，we can highlight the study of the recent study by Han et al[18] on the comparison of CXL results in 4 groups of patients with different corneal thicknesses（including one group with a corneal thickness of ＜ 400 μm submitted to HR，as opposed to another 3 groups with a corneal thickness of ≥ 400 μm submitted to IR）[18]. In this study，the authors not only confirm the success of CXL in delaying the progression of keratoconus but also emphasize the existence of a negative linear correlation between corneal thickness and Kmax，meaning that more advanced cases of keratoconus（therefore with thinner corneas）seem to obtain a greater benefit from the flatting effect of CXL[18].

Regarding complications，the results are also in agreement with the literature and other studies，including the presence of corneal haze[21]. In our study，the absence of complications affecting the final outcome of CXL supports the safety of applying HR. This is also a controversial topic given that some studies，as one study of Kymionis et al[23]，documented no complications after standard CXL with the standard IR in patients with corneal thickness ＜ 400 μm. In that study，the

author showed that the corneal endothelium (evaluated postoperatively by corneal confocal microscopy) did not undergo any significant changes.

The limitations included the retrospective profile of the study, a small sample and a short follow-up. These limitations are considered relative, since they are resolved with the continuity of the study. It is a type of study that may have an impact on clinical practice, as it allows more effective and safer treatment of more advanced cases of keratoconus, associated with thinner corneas, where current treatment options are still limited.

To conclude, the application of HR is promising in the treatment of thinner corneas, stopping the progression of keratoconus without seeming to compromise the safety of the procedure. Further studies on this type of procedure, including an increase in the number of cases and greater follow-up, will contribute to a greater and better knowledge of its long-term efficacy and safety in clinical practice.

REFERENCES

1 Ahn SJ, Kim MK, Wee WR. Topographic progression of keratoconus in the Korean population. *Korean J Ophthalmol* 2013;27(3):162-166

2 Choi JÁ, Kim MS. Progression of keratoconus by longitudinal assessment with corneal topography. *Invest Ophthalmol Vis Sci* 2012;53(2):927-935

3 Gomes JA, Tan D, Rapuano CJ, Belin MW, Ambrósio R Jr, Guell JL, Malecaze F, Nishida K, Sangwan VS. Global consensus on keratoconus and ectatic diseases. *Cornea* 2015;34(4):359-369

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. *Ophthalmology* 2013;120(8):1515-1520

5 Sorkin N, Varssano D. Corneal collagen crosslinking: a systematic review. *Ophthalmologica* 2014;232(1):10-27

6 Galvis V, Tello A, Ortiz AI, Escaf LC. Patient selection for corneal collagen cross-linking: an updated review. *Clin Ophthalmol* 2017;11:657-668

7 Padmanabhan P, Dave A. Collagen cross-linking in thin corneas. *Indian J Ophthalmol* 2013;61(8):422-424

8 Chen XJ, Stojanovic A, Eidet JR, Utheim TP. Corneal collagen cross-linking (CXL) in thin corneas. *Eye Vis (Lond)* 2015;2:15

9 Kymionis GD, Portaliou DM, Diakonis VF, Kounis GA, Panagopoulou SI, Grentzelos MA. Corneal collagen cross-linking with riboflavin and ultraviolet-A irradiation in patients with thin corneas. *Am J Ophthalmol* 2012;153(1):24-28

10 Wollensak G, Spoerl E, Seiler T. Riboflavin/ultraviolet-a-induced collagen crosslinking for the treatment of keratoconus. *Am J Ophthalmol* 2003;135(5):620-627

11 Wollensak G, Aurich H, Pham DT, Wirbelauer C. Hydration behavior of porcine cornea crosslinked with riboflavin and ultraviolet A. *J Cataract Refract Surg* 2007;33(3):516-521

12 Caporossi A, Mazzotta C, Baiocchi S, Caporossi T. Long-term results of riboflavin ultraviolet a corneal collagen cross-linking for keratoconus in Italy: the Siena eye cross study. *Am J Ophthalmol* 2010;149(4):585-593

13 Hafezi F, Mrochen M, Iseli HP, Seiler T. Collagen crosslinking with ultraviolet-A and hypoosmolar riboflavin solution in thin corneas. *J Cataract Refract Surg* 2009;35(4):621-624

14 Rosenblat E, Hersh PS. Intraoperative corneal thickness change and clinical outcomes after corneal collagen crosslinking: Standard crosslinking versus hypotonic riboflavin. *J Cataract Refract Surg* 2016;42(4):596-605

15 Raiskup F, Spoerl E. Corneal cross-linking with hypo-osmolar riboflavin solution in thin keratoconic corneas. *Am J Ophthalmol* 2011;152(1):28-32

16 Nassaralla BA, Vieira DM, Machado ML, Figueiredo MN, Nassaralla JJ Jr. Corneal thickness changes during corneal collagen cross-linking with UV-A irradiation and hypo-osmolar riboflavin in thin corneas. *Arq Bras Oftalmol* 2013;76(3):155-158

17 Gu SF, Fan ZS, Wang LH, Tao XC, Zhang Y, Mu GY. Corneal collagen cross-linking with hypoosmolar riboflavin solution in keratoconic corneas. *Biomed Res Int* 2014;2014:754182

18 Han YF, Xu YY, Zhu W, Liu YL, Liu Z, Dou XX, Mu GY. Thinner corneas appear to have more striking effects of corneal collagen crosslinking in patients with progressive keratoconus. *J Ophthalmol* 2017;2017:6490915

19 Chen W, Li ZW, Zhao XM, Xu WW, Mu GY. Evaluation of corneal endothelium after UVA/riboflavin cross-linking in thin keratoconic corneas. *Int J Ophthalmol* 2016;9(2):321-322

20 Hassan Z, Modis L Jr, Szalai E, Berta A, Nemeth G. Intraoperative and postoperative corneal thickness change after collagen crosslinking therapy. *Eur J Ophthalmol* 2014;24(2):179-185

21 Holopainen JM, Krootila K. Transient corneal thinning in eyes undergoing corneal cross-linking. *Am J Ophthalmol* 2011;152(4):533-536

22 Raiskup F, Hoyer A, Spoerl E. Permanent corneal haze after riboflavin-UVA-induced cross-linking in keratoconus. *J Refract Surg* 2009;25(9):S824-S828

23 Kymionis GD, Diakonis VF, Kalyvianaki M, Portaliou D, Siganos C, Kozobolis VP, Pallikaris AI. One-year follow-up of corneal confocal microscopy after corneal cross-linking in patients with post laser *in situ* keratosmileusis ectasia and keratoconus. *Am J Ophthalmol* 2009;147(5):774-778.e1