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Miguel Pereira Correia Natal

Crosslinking de colagénio corneano transepitelial acelerado em pacientes com queratocone progressivo: resultados a longo prazo

Transepithelial accelerated corneal collagen crosslinking in patients with progressive keratoconus: long term follow-up results

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Oftalmologia

TÍTULO DISSERTAÇÃO/~~MONOGRAFIA~~ (riscar o que não interessa)

Transepithelial Accelerated Corneal Collagen Crosslinking in Patients with Progressive Keratoconus: Long Term Follow-up Results

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Dedicatória

Aos meus pais, por estarem presentes em cada etapa do caminho, e por não ter dúvidas que, sem eles, nada disto seria possível.

À Inês, melhor irmã do mundo, minha parceira de todos os momentos e para sempre melhor amiga.

Aos meus Avós, que sempre acreditaram em mim e me deram força. Sei que todos eles me estão a ver e espero que os deixe orgulhosos.

A todos os meus amigos, parte integrante e essencial deste percurso, que não seria o mesmo sem eles.

Obrigado e até já.

“Only a life lived for others is a life worthwhile.”

Albert Einstein

Transepithelial accelerated corneal collagen crosslinking in patients with progressive keratoconus: long term follow-up results

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Abstract

Purpose: to systematically evaluate the long-term efficacy of transepithelial accelerated corneal collagen crosslinking (TE-ACXL) in the treatment of eyes with progressive keratoconus by reporting its visual and morphological outcomes throughout a 4-year follow-up.

Methods: eyes of patients who underwent TE-ACXL (6mW/cm2 for 15 minutes) for progressive keratoconus were included in this retrospective cohort study. Best-corrected visual acuity (BCVA), keratometry measurements, thinnest corneal thickness (PachyMin), and topographic indexes were analyzed preoperatively and every 6 months after TE-ACXL, up to a maximum of 48 months. Disease progression was defined as an increase ≥ 1.00 D in corneal astigmatism, an increase ≥ 1.00 D in maximum keratometry (Kmax), a decrease ≥ 2% in PachyMin, or an increase ≥ 0.42 units in D-index.

Results: the study enrolled 39 eyes from 30 patients. No significant differences were observed in BCVA, corneal astigmatism, Kmax, index of surface variance (ISV), index of height decentration (IHD), and keratoconus index (KI) between baseline and subsequent follow-up evaluations (p>0.05). There was a significant increase at 12-, 24- and 36-months follow-up in mean keratometry (Km) (0.66 ± 1.07 D, p=0.001; 0.94 ± 1,42 D, p=0.001; 1.48 ± 1.19 D, p=0.002) and D-index (0.50 ± 1.05 units, p=0.011; 0.53 ± 1.19 units, p=0.024; 1.29 ± 1.11 units, p=0.003). There were significant decreases in PachyMin at 36 months (-10.45 ± 15.20 µm, p=0.046) and in index of vertical asymmetry (IVA) at 24 months (-0.07 ± 0.16 units, p=0.024). 28 (71.8%) eyes maintained progression by at least one criterion. 2 (5.1%) eyes fulfilled all 4 progression criteria. Surgery and follow-up were uneventful in all subjects.

Conclusion: TE-ACXL seems to be a safe and effective treatment for progressive keratoconus. Definition of new specific and significant progression criteria and further prospective studies with larger cohorts are recommended.

Keywords: cornea, keratoconus, transepithelial accelerated corneal collagen crosslinking, disease progression.

Introduction

Keratoconus is a progressive, bilateral and asymmetric ectatic disease of the cornea, first presenting in the second to third decades of life, with features initially in only one eye.[1,2] Central or paracentral corneal stromal thinning occurs, accompanied by apical protrusion (ectasia), resulting in irregular myopic astigmatism and late corneal scarring with consequent mild to significant visual impairment.[2-4] It is the most frequent form of corneal ectasia, with an estimated incidence of up to 1 in 2000[5] in the general population, with recent studies suggesting an even higher incidence.[6]

Though its etiology is still not fully understood, recent studies highlight its multifactorial nature, with genetic predisposition and environmental factors contributing to its occurrence. Classically, keratoconus was considered a noninflammatory disease, due to the absence of corneal cellular infiltration and neovascularization.[4,7] Nevertheless, recent studies also demonstrate the presence of local inflammatory processes with increased local inflammatory mediators and oxidative stress, such as proteolytic enzymes, cytokines and free radicals, which together contribute to the progressive stromal tissue loss and biomechanical changes that ultimately lead to corneal curvature defects and protrusion.[8] There is also a positive and significant correlation with eye rubbing, atopy, repeated ocular microtrauma and hypoxia due to prolonged contact lenses wear and ocular allergic diseases. Furthermore, keratoconus prevalence is increased in patients with connective tissue disorders (Ehlers-Danlos or Marfan syndrome, Down syndrome and mitral valve prolapse).[1,4,7-11]

Keratoconus should always be suspected and excluded in a patient with irregular astigmatism, especially if unstable, difficult to correct and progressing over time[1]. Currently, corneal tomography and pachymetry are the most useful tools in the early diagnosis, evaluation and follow-up of keratoconus since they can produce anterior and posterior corneal elevation maps and document corneal thickness.[1,9,12]

Keratoconus treatment has evolved greatly in recent years. Previously, initial treatment involved refractive correction with spectacles, rigid contact lenses and corneal rings to improve

visual acuity. However, patients frequently advanced to severe disease with corneal damage not further amenable to optical correction, eventually requiring corneal transplantation, since none of these treatments can change the natural course of the disease;[13,14] in fact, no treatment option focused on limiting disease progression, with some authors estimating that approximately 10 to 20% keratoconus patients would go on to need keratoplasty.[5,15] Recently, corneal collagen crosslinking (CXL) has emerged as the most effective treatment in decreasing or even aborting disease progression, possibly reducing the need for keratoplasty. It is a safe and minimally invasive procedure that increases the corneal biomechanical rigidity and is the only form of treatment that specifically targets the disease pathophysiology.[14,16]

The first established CXL protocol – Dresden Protocol – was described by Wollensack *et al* in a prospective non-randomized clinical trial, and to this day has been considered the standard (or conventional, C-CXL) procedure. It involves epithelial debridement to facilitate stromal riboflavin absorption, application of a 0.10% riboflavin 5-phosphate solution for 30 minutes, followed by exposure to UVA radiation (365 nm, 3 mW/cm^2) for 30 minutes, enabling a total fluence of 5.4 J/cm^2.[17] The interaction of riboflavin and UVA leads to the generation of riboflavin radicals and oxygen free radicals that together increase the formation of covalent bonds between collagen fibrils, establishing crosslinks between collagen molecules in the corneal stroma. The whole process results in increased corneal rigidity and stability.[5,14,16,18]Nonetheless, corneal epithelium debridement and prolonged corneal exposure (a total of 60 minutes) are responsible for the main adverse effects and disadvantages of C-CXL, such as the risk of infection, sub-epithelial haze, sterile corneal infiltrates, corneal scarring, endothelial damage and postoperative pain, delaying visual rehabilitation.[13,14,18,19] To reduce these negative outcomes, new protocols have emerged. In an attempt to reduce the time of corneal exposure and improve the comfort of the procedure for the patient, accelerated protocols (A-CXL) were designed. These are based on the Bunsen-Roscoe law of photochemical reciprocity, which states that the same photochemical effect can be achieved with a reduction in the illumination time and a corresponding increase in the radiation intensity, therefore maintaining the cumulative dose of radiation administered. On the other hand, to avoid the possible complications associated with epithelial debridement, the

so-called transepithelial protocols (TE-CXL) have evolved, in which the epithelium is retained throughout the whole procedure.[14,18,20]

Thus, transepithelial accelerated corneal crosslinking (TE-ACXL) emerged as a more appealing and promising alternative to traditional protocols, combining the transepithelial and accelerated protocols to surpass some disadvantages of the original protocol. Notwithstanding, TE-ACXL effectiveness may be limited by the epithelial barrier to riboflavin diffusion and UVA and oxygen absorption by corneal stroma and the assumption that increasing irradiation intensity during a reduced period has the same biological effect in the cornea.[21,22] Therefore, long-term efficacy and safety TE-ACXL studies are required to fully determine the role of this technique in keratoconus treatment.

The purpose of the present study is to evaluate the long-term efficacy of the TE-ACXL protocol in the treatment of patients with a diagnosis of progressive keratoconus by reporting its 4-year outcomes, using a similar but increased cohort that was analyzed previously for 2-year outcomes of the same technique.[23]

Patients and methods

The authors conducted a retrospective observational cohort study of 39 eyes with progressive keratoconus who underwent TE-ACXL (6 mW/cm² for 15 minutes) and were followed at the Ophthalmology Corneal Department of Centro Hospitalar Universitário de São João from January 2016 to January 2021. This study was performed in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of Centro Hospitalar Universitário de São João. Written informed consent was obtained from all patients or legal guardians (in patients under the age of consent) before surgical interventions. Medical records of all patients who underwent TE-ACXL were analyzed between December 2020 and January 2021. The present cohort is based on a preexisting one that we used in a previous study of the same protocol.

This study's inclusion criteria were defined as: age between 14 and 32, pachymetry at its thinnest point (PachyMin) ≥ 380 µm and previously documented progression of keratoconus.

Keratoconus was deemed to be progressive if 1 or more of the following changes were present in the previous 6 months: an increase ≥ 1.00 diopter (D) in maximum keratometry (Kmax), a 2% decrease in PachyMin or an increase ≥ 1.00 D in corneal cylinder. The exclusion criteria were apical corneal scarring, severe dry eye, delayed epithelial healing, active ocular infections, connective tissue disease, pregnancy or lactation and previous history of cornea surgery.

All clinical, visual, corneal topographic and tomographic and pachymetric parameters from the eyes included in the study were evaluated preoperatively and every 6 months postoperatively to a maximum of 48 months. Best-corrected visual acuity (BCVA) was recorded via a Snellen chart and converted to the logarithm of minimal angle of resolution (logMAR) units to allow statistical analysis. Corneal astigmatism (Astg, K2-K1) mean keratometry (Km), Kmax, PachyMin, index of height decentration (IHD), index of vertical asymmetry (IVA), index of surface variance (ISV), keratoconus index (KI) and Belin/Ambrósio D-index were recorded using Oculus Pentacam (Pentacam HR®, Oculus Optikgeräte GmbH, Wetzlar, Germany). Keratoconus Classification (KC) is in accordance with the Pentacam HR® Ktc Scoring System.

The baseline score for all parameters was defined as the preoperative measurement closest to the date of the procedure; the maintenance of keratoconus' progression represented treatment failure. Disease progression was assessed at 12, 24, 36 and 48 months after TE-ACXL and defined as the presence of one or more of the following: an increase ≥ 1.00 D in corneal astigmatism, an increase ≥ 1.00 D in Kmax, a decrease ≥ 2% in thinnest pachymetry or an increase ≥ 0.42 units in D-index.[24-26]

SURGICAL TECHNIQUE AND POSTOPERATIVE CARE

All operations were conducted under sterile conditions in an operative room. Oxybuprocaine hydrochloride 4mg/L eyedrops were used for preoperative local anesthesia. TE-ACXL was carried out through an intact epithelium; to do so, a TE riboflavin preparation (0,25% riboflavin, ethylenediamine tetra-acetic acid [EDTA], trometamol [Tris], benzalkonium chloride [BAC]) and a 0.45% phosphate buffer saline preparation were instilled in the cornea every 3 minutes for 30 minutes preoperatively. UV-A irradiation began after corneal stromal saturation was confirmed through slit-lamp assessment of the anterior chamber flare. The cornea was

exposed to a UV-A light beam at an intensity of 6mW/h for 15 minutes to achieve a total dose intensity of 5,4J/cm^2. During this period, a riboflavin solution and a sterile balance sodium solution were administered alternatively every 3 minutes to avoid corneal dehydration.

After the procedure, antibiotic eye drops (ofloxacin 0.30%) were prescribed for a week, as well as topical steroids eye drops (fluorometholone 0,10%) for 2 weeks and sodium hyaluronate 0.20% as needed. Regarding the follow-up, visits were scheduled for day 1 postoperatively, at 3 months and every 6 months afterward.

STATISTICAL ANALYSIS

Categorical variables were reported as frequencies and proportions, while continuous variables were presented as the mean ± standard deviation. The postoperative variation in visual, keratometric, pachymetric, topographic and tomographic parameters was calculated by subtracting their baseline values from the subsequent readings at each follow-up visit (therefore, positive delta values denote an increase in that parameter, whilst negative delta values represent a decrease). Paired *t-tests* were used to compare preoperative and postoperative outcomes; multiple related samples were compared via within-subjects ANOVA test. Comparisons between groups that had progression throughout our study and those who did not present progression were conducted with independent samples t-tests. A *p* value less than 0.05 was considered statistically significant. Statistical analysis was performed using SPSS Statistics software (version 26, SPSS, Inc., Chicago IL., USA).

Results

39 eyes (22 right eyes and 17 left eyes) of 30 patients fulfilled the inclusion criteria and were included in this study. Our cohort included 22 male and 8 female patients; 9 patients received TE-ACXL in both eyes (7 male and 2 female patients). The mean age was 20.59 ± 4.43 years (ranging from 14 to 32). Further baseline characteristics are shown in Table 1. Regarding

KC, its preoperative mean was 2,69 ± 0,65 and its preoperative distribution is displayed in Figure 1. The most frequent keratoconus grade was 3 (N=18, 46.1%), the second being 2 (N=6, 15.4%).

All 39 eyes completed the 6 months follow-up, while 33, 31, 30, 17, 11, 9 and 3 eyes completed the 12, 18, 24, 30, 36, 42 and 48-month follow-up, respectively. Variation (Δ) between baseline visual, keratometric, pachymetric, topographic and tomographic corneal parameters at 6, 12, 18, 24, 30 36, 42 and 48 months postoperatively are presented in Table 2. All surgical procedures were carried out uneventfully and no complications were registered throughout follow-up.

VISUAL ACUITY

Figure 2 shows BCVA variation over time (in logMAR values). Mean BCVA preoperatively was 0.49 ± 0.36 logMAR units. Mean variation was -0.03 ± 0.26 logMAR units at 12 moths, -0.01 ± 0,26 logMAR units at 24 months, 0.11 ± 0.22 logMAR units at 36 months and 0.13 ± 0.28 logMAR units at 48 months postoperatively. No statistically significant differences were determined throughout follow-up (p = 0.503, p = 0.844, p = 0. 260, p = 0,500 at 12, 24, 36 and 48 months respectively).

KERATOMETRY

Baseline mean Kmax was 58.15 ± 5.58 D. No significant changes were found during follow-up. The mean variation was 0.42 ± 2.12 D (p = 0.269) at 12 months, 0.38 ± 1.94 D (p = 0.297) at 24 months, 0.75 ± 1,95 D (p = 0.13) at 36 months and 2.17 ± 2.64 D (p = 0,290) at 48 months postoperatively. Baseline mean Km was 48.85 ± 3.95, and it increased significantly throughout follow-up. Its mean variation was 0.66 ± 1.07 D at 12 months (p = 0.001), 0.94 ± 1,42 D at 24 months (p = 0.001) and 1.48 ± 1.19 D at 36 months (p = 0.002). At 48 months follow-up, mean variation was 1.50 ± 1.49 D (p = 0.244), thus not statistically significant. As for Astg, no statistically significant differences were found, with a mean variation of -0.003 ± 1.08 D (p = 0.987) at 12 months, -0.007 ± 1.18 D (p = 0.975) at 24 months, -0.05 ± 0.76 D (p = 0.817) at 36 months and 1.43 ± 1.83 D (p = 0.309) at 48 months follow-up. Variation of Kmax and Km throughout follow-up can be visualized in Figures 3 and 4, respectively.

PACHYMETRY

Figure 5 represents the evolution of mean PachyMin over time. The preoperative mean PachyMin value was 453.33 ± 36.82 µm. There were no statistically significant variations in the first 24 months of follow-up (-1.06 ± 12.54 µm at 12 months, p = 0.630; -3.07 ± 15.65 µm at 24 months, p = 0.292). A significant decrease of PachyMin was determined at 36 months follow-up (-10.45 ± 15.20 µm; p = 0.046). No significant variations were registered at 48 months postoperatively (-24.33 ± 26.16 µm, p = 0.248).

TOPOGRAPHIC INDICES

Mean values were statistically similar to baseline throughout the whole follow-up period for all of the topographic indices studied, except for the D-index. Indeed, there was a significant increase in this parameter at 12 (0.50 ± 1.05 units, p = 0.011), 24 (0.53 ± 1.19 units, p = 0.024) and 36 months (1.29 ± 1.11 units, p = 0.003), but not at 48 months 1.36 ± 1.35 units, p = 0.222).

PROGRESSION

Table 3 discriminates the number of patients that had progression of ectatic disease at 12, 24, 36 and 48-months follow-up utilizing each of the aforementioned progression parameters. There was an increase ≥ 1.0 D in Kmax in 24.2% (8/33) of the studied eyes at 12 months, 30.0% (9/30) at 24 months, 36.4% (4/11) at 36 months and 66.7% (2/3) at 48 months. Regarding corneal astigmatism, 6.1% (2/33) of the studied eyes registered an increase ≥ 1.0 D at 12 months, 6.7% (2/30) at 24 months, 9.1% (1/11) at 36 months and 33.3% (1/3) at 48 months. PachyMin showed a decrease ≥ 2% in 30.3% (10/33) of the studied eyes at 12 months, 33.3% (10/30) at 24 months, 54.5% (6/11) at 36 months and 66.7% (2/3) at 48 months. Finally, an increase ≥ 0.42 units in D-index was recorded in 53.1% (17/32), 55.2% (16/29), 81.8% (9/11) and 66.7% (2/3) of the studied eyes at 12, 24, 36 and 48 months, respectively.

A comparison of baseline characteristics was made between the groups that had progression by each parameter and the groups that did not progress by that same parameter, and the results can be visualized in Tables 4 and 5. Table 4 shows the comparison of visual, keratometric, pachymetric, topographic and tomographic parameters. Eyes that had progression

utilizing PachyMin and D-index revealed no statistically significant differences from the ones that did not show progression. Eyes that had an increase ≥ 1.0 D in Kmax had significantly higher baseline Astg values than those that did not have this increase during follow-up ($p = 0.045$). Furthermore, the group that had an increase ≥ 1.0 D in Astg showed significantly higher baseline ISV ($p = 0.026$), IHD ($p = 0.008$) and KC ($p = 0.005$) compared to the group with no such increase. Regarding the demographic and clinical baseline characteristics (Table 5), no statistically significant differences were reported in the comparison between the groups that had progression using Kmax, Astg, PachyMin and D-index and the groups that showed no progression in each of these paramenters. An additional comparison of baseline characteristics was made between eyes that had progression by any of the four criteria (a total of 28 eyes) and those which did not fill any of those criteria (a total of 11 eyes) (Table 6). Once more, this comparison found no statistically significant differences.

2 (5.1%) eyes fulfilled all 4 progression criteria. Comparing baseline visual, keratometric, pachymetric, topographic and tomographic parameters between those eyes and the remaining sample (37 eyes), only the baseline keratoconus grade was significantly different (3.00 ± 0.00 vs. 2.68 ± 0.66; $p = 0.005$).

Of the 17 (43.6%) eyes that had an increase ≥ 1.0 D in Kmax throughout follow-up, mean Kmax variation was 1.42 ± 2.08 D at 12 months, 1.39 ± 2.00 D at 24 months, 1.73 ± 1.67 D at 36 months and 3.25 ± 2.62 D at 48 months. Of the 4 (10.3%) eyes that had an increase ≥ 1.0 D in Astg throughout follow-up, mean Astg variation was 5.05 ± 4.91 D at 12 months, 5.05 ± 4.13 D at 24 months, 4.05 ± 0.64 D at 36 months and 5.90 ± 0.00 D at 48 months. Of the 18 (46.2%) eyes that had a decrease ≥ 2% in PachyMin throughout follow-up, mean PachyMin variation was -9.30 ± 7.10 µm at 12 months, -12.47 ± 8.93 µm at 24 months, -19.57 ± 9.68 µm at 36 months and -24.33 ± 26.16 µm at 48 months. Of the 24 (61.5%) eyes that had an increase ≥ 0.42 units in D-index throughout follow-up, mean D-index variation was 0.96 ± 0.70 units at 12 months, 0.99 ± 0.96 units at 24 months, 1.66 ± 0.82 units at 36 months and 2.11 ± 0.59 units at 48 months.

Discussion

Keratoconus is a multifactorial, highly prevalent disease of the cornea that begins early in life and tends to progress over time and impair the patients' quality of vision, making it one of the most common indications for keratoplasty worldwide. From the various treatment approaches to this condition, only CXL focuses on the disease's pathophysiology and natural history, aiming to slow down or cease its progression.[2,14,15,27]

Since its first description by Wollensak et al in 2003, CXL has been widely used and proved to be a safe and effective method of corneal stabilization in keratoconus patients.[17,28] However, CXL is not devoid of disadvantages. As so, several modifications to the conventional protocol have been made to improve this procedure, with overall favorable results. The TE-CXL procedure emerged as a solution to the problems related to the removal of the corneal epithelium, with several studies highlighting its faster healing, improved patient comfort, lower risk of corneal haze or infectious keratitis and a better safety profile in advanced cases in which low corneal thickness would preclude treatment.[14,29,30] After the realization that the epithelium would act as barrier and reduce the effects of riboflavin and UVA on the cornea, it became clear that maintaining it throughout the whole procedure would require methods to facilitate riboflavin diffusion, such as chemical enhancers (EDTA, Tris and BAC, used in the present study, are examples of these substances) or iontophoresis.[19,31,32] Posteriorly, the A-CXL protocols were introduced to reduce illumination time by increasing intensity while maintaining the overall fluence, following the aforementioned Bunsen-Roscoe law[33-36]. Nonetheless, this law may not directly apply to CXL in living corneal tissue [37] and the higher irradiance required may lead to excessive oxygen consumption and therefore less availability.[18,38] Combining these two modified protocols into TE-ACXL brings about both their advantages and disadvantages, whereby studying its effect during the longest possible follow-up period is essential to determine if such a combination is effective in the treatment of keratoconus. Published evidence on TE-ACXL has increased significantly since its inception, reflecting the increasing interest in this method. Most studies acknowledge its safety and efficacy, albeit the majority of them agree that more long-term studies

290 are required.[39-48] Madeira et al compared TE-ACXL and C-CXL and deemed them similarly
291 effective.[49]

292 Our study did not find statistically significant variations in the BCVA. Other studies
293 demonstrated conflicting results in this matter; while Aixinjueluo et al reported a significant
294 improvement in BCVA using a TE-ACXL protocol at 30 mW/cm2[48], no change was seen by Akbar
295 et al 12 months after treatment at 9mW/cm2[41]. These inconsistencies are somewhat expected
296 since the main goal of CXL is not vision correction, but stabilization of disease. That is why BCVA
297 is not considered a primary outcome in CXL studies and does not solely reflect disease severity
298 and progression.

299 Apart from Km (which suffered a significant increase and 12-, 24- and 36- months follow-
300 up) and an isolated significant decrease in PachyMin at 36 months, the keratometric and
301 pachymetric parameters did not present significant variations. This stabilization in Kmax and Astg
302 and is in accordance with the studies by Sun et al[39] and Huang et al.[42] Regarding the PachyMin,
303 Tian et al[46] and Zhang et al[43] found no significant changes after 36- and 12-months follow-up,
304 respectively, whilst Akbar et al reported a significant decrease after 12 months.[41] The different
305 protocols used in TE-ACXL studies (especially in terms of irradiance and duration of treatment)
306 and different mean baseline parameters may explain these variable results. The topographic
307 indices are less commonly studied in other papers since their value in evaluating progression is
308 still being debated. The present study showed an isolated significant decrease of IHA at 24
309 months and no significant variation in ISV, IHD and KI throughout the whole follow-up period,
310 similar to the findings of Ziaei et al.[47] Nonetheless, there was a significant increase in D-index at
311 12, 24 and 36 months.

312 To evaluate keratoconus progression herein, we used 4 criteria: an increase ≥ 1.00 D in
313 corneal astigmatism, an increase ≥ 1.00 D in Kmax, a decrease ≥ 2% in thinnest pachymetry or
314 an increase ≥ 0.42 units in D-index. Fulfillment of one of these during the follow-up period would
315 indicate the presence of progression. As so, a total of 28 (71.8%) eyes showed progression.
316 These results raised the question of whether they represent treatment failure and TE-ACXL

inefficacy in controlling progression or the utilization of parameters that may be too sensitive to evaluate progression, leading to its overestimation.

Analyzing progression by Kmax, we found that 17 (43.6%) eyes had an increase ≥ 1.00 D throughout follow-up, whereas 18 (46.2%) eyes had progression by the PachyMin criterium. Kmax is one of the most widely used parameters in the documentation of the progression of keratoconus.[24] However, by ignoring the contribution of the posterior cornea to keratoconus progression and failing to reflect the degree of ectasia, Kmax has been considered by some authors as a poor parameter for both progression and crosslinking efficacy,[50,51] even stating that ectasia progression may occur without changes in Kmax, especially in earlier stages. PachyMin is a less used parameter, whose usefulness has also been subject to doubt.[9] Kanellopoulos et al have suggested that the thinnest pachymetry alone may be misleading to evaluate severity or progression.[52]

Given the fact that our cohort included mainly young patients, the mean age being 20.59 ± 4.43 years, changes in isolated keratometric and topographic parameters are far from unexpected. Indeed, young age at diagnosis is an important risk factor for progression and the eyes of younger patients tend to progress more rapidly than their adult counterparts.[25,51,53] Moreover, included patients already exhibited high Kmax and Km values at baseline (58.15 ± 5.58 D and 48.85 ± 3.95, respectively), as well as a mean KC value of 2.69 ± 0.65, which would also make them especially prone to progression and so to substantial variations in keratometry and topography.[15,25,42] Taking this into account, we analyzed the mean variation in Kmax and PachyMin among the patients that progressed by each of these parameters. We found that, despite fulfilling the defined criteria, the magnitude of the changes was not as high as expected (mean ΔKmax was 1.42 ± 2.08 D at 12 months, 1.39 ± 2.00 D at 24 months, 1.73 ± 1.67 D at 36 months and 3.25 ± 2.62 D at 48 months and mean ΔPachyMin was -9.30 ± 7.10 µm at 12 months, -12.47 ± 8.93 µm at 24 months, -19.57 ± 9.68 µm at 36 months and -24.33 ± 26.16 µm at 48 months). These findings may suggest that some eyes did not present major changes in their keratometric and topographic parameters but were considered progressive due in part to the oversensitive criteria used.

In a recent study by Shajari et al, the utilization of at least two simultaneous parameters affected by keratoconus was suggested to evaluate progression; this statement is supported by the 2014 Global Consensus on Keratoconus and Ectatic Diseases, which stated that ectasia progression is defined by at least two of the following: steepening of the anterior corneal surface, steepening of the posterior corneal surface or thinning and/or an increase in the rate of corneal thickness change from the periphery to the thinnest point).[9,26] Alternatively, the authors suggest using parameters that include several variables. One such example is the D-index, a multimetric parameter that takes into account pachymetric, anterior and posterior elevation parameters. It has proven to be useful in the early diagnosis of keratoconus by highlighting changes in the corneal surface earlier than, for example, Kmax.[54] Nevertheless, its applicability as a sole parameter in indicating progression is still being debated. In the present study, 24 (61.5%) eyes suffered an increase ≥ 0.42 units in D-index, with 17 of them fulfilling this criterium in the first 12 months of follow-up, which shows its higher sensitivity for detecting early changes.

Kanellopoulos et al also described ISV and IHD as crucial parameters in the diagnosis and probably progression.[55] No significant changes during follow-up were evident in our study regarding these parameters, IVA and/or KI.

In short, many factors might predict keratoconus progression, but to date, none have been validated.[24] The probable best approach is to not use a single parameter given this is a multifactorial pathology and should be evaluated as an interaction of factors. In our study, we sought to describe variation throughout follow-up of some of the most used progression predictors. Though when considered together they might accurately reflect disease progression, when considered individually they may overestimate it. This is further sustained by the fact that only 2 of the 39 eyes studied (5.1%) fulfilled all 4 progression criteria simultaneously. Moreover, the literature reports the utilization of these variables mostly as a means to detect early progression in order to act therapeutically as soon as it is required, but not to evaluate further progression in patients already diagnosed with progressive keratoconus and submitted to CXL.

When we compared the baseline parameters of the 2 eyes that fulfilled the 4 criteria with the remaining 37 eyes, the former showed a statistically significant higher KC (3.00 ± 0.00 vs.

2.68 ± 0.66), which is in line with the idea that more advanced keratoconus cases at presentation tend to progress more. Furthermore, since CXL acts through increased formation of collagen crosslinks in the stroma to enhance corneal biomechanical stiffness, the effects of TE-ACXL may be limited in cases of severe keratoconus, in which there is greater stromal degeneration and fewer fibrils able to establish crosslinks during the procedure.[14,42]

Overall, the patients' eyes remained statistically stable during follow-up after TE-ACXL, which is demonstrated by the absence of significant variations in almost all of the visual, keratometric, pachymetric, topographic and tomographic parameters evaluated, despite young age and relatively thin corneas with high Kmax and high KC preoperatively. In addition, no complications or adverse events were recorded, which attests to the good safety profile of the TE-ACXL. Although most eyes did maintain progression, various limitations may be pointed out to the criteria used. The lack of established criteria of keratoconus progression in the literature makes it difficult to systematically evaluate progression. Hence, future efforts should be made to establish not only the most accurate criteria but also the magnitude of its variation that best reflects keratoconus progression both before and after treatment.

There were several limitations in this study, most of them inherent to its retrospective nature. The sample size was relatively small, the patients' baselines were not similar and there was no control group. Additionally, the follow-up time was not uniform among the eyes studied, with only 3 eyes reaching the 48-month mark and 6 eyes not even completing 12 months of follow-up. All of these limit the statistical efficiency and external validity of the study results and highlight the necessity of randomized prospective studies with a large sample size and control group.

Conclusion

In conclusion, our study demonstrates that TE-ACXL (6 mW/cm^2 for 15 minutes, 5,4J/cm^2) seems to be a safe and effective treatment in the stabilization of progressive keratoconus, especially if detected in earlier stages. This procedure should yield better comfort during surgery and reduce complications for keratoconus patients. Further studies are required

to clearly define accurate progression criteria and shed light on the role of TE-ACXL in today's clinical practice.

Abbreviations

A-CXL, accelerated corneal collagen crosslinking; Astg, corneal astigmatism; BCVA, Best-corrected visual acuity; CXL, Crosslinking; C-CXL, Conventional crosslinking; LogMAR, logarithm of minimal angle of resolution; IHD, Index of height decentration; ISV, Index of surface variance; IVA, Index of vertical asymmetry; K1, Flat Keratometry; K2, Steep Keratometry; KC, Keratoconus classification; Kmax, Maximum keratometry; Km, Mean keratometry; PachyMin, pachymetry at the thinnest pointSD, Standard-deviation; TE-ACXL, Transepithelial accelerated corneal collagen crosslinking; TE-CXL, transepithelial corneal collagen crosslinking; UVA, Ultraviolet A.

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Disclosure

The authors report no conflicts of interest in this work.

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Figure 1. Distribution of baseline keratoconus classification.

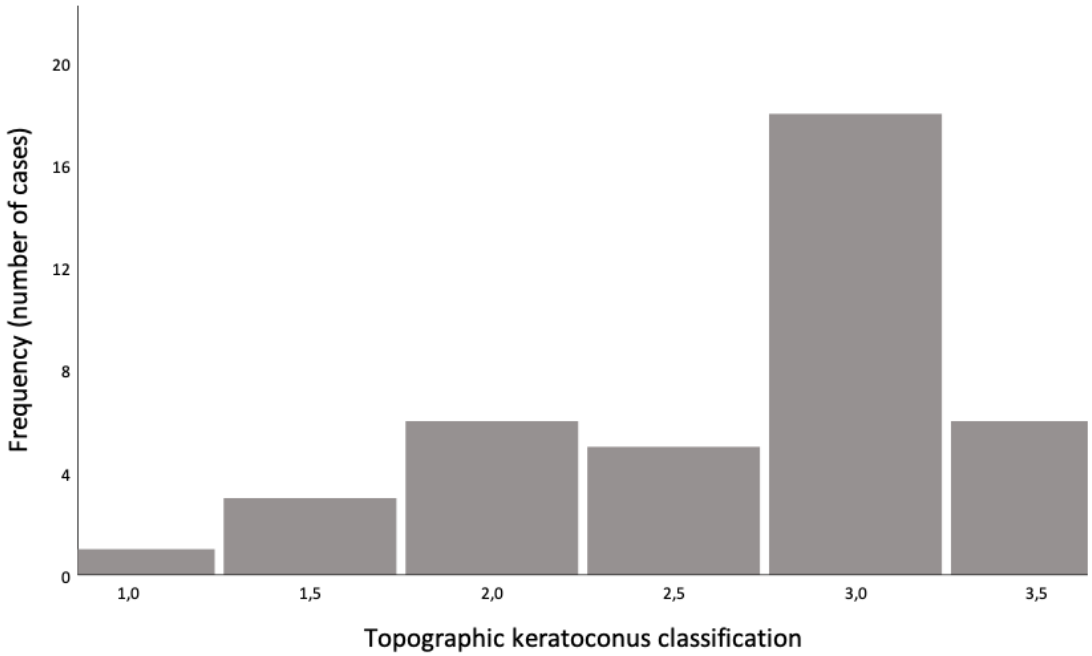

Figure 2 – Best-corrected visual acuity (BCVA) in LogMAR compared with baseline at 6, 12, 18, 24, 30, 36, 42 and 48 months after trans-epithelial accelerated CXL.

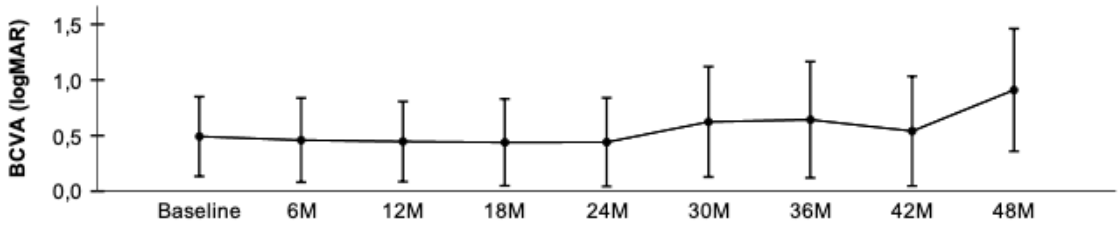

Figure 3 – Maximum keratometry (Kmax) in dioptres (D) compared with baseline at 6, 12, 18, 24, 30, 36, 42 and 48 months after trans-epithelial accelerated CXL.

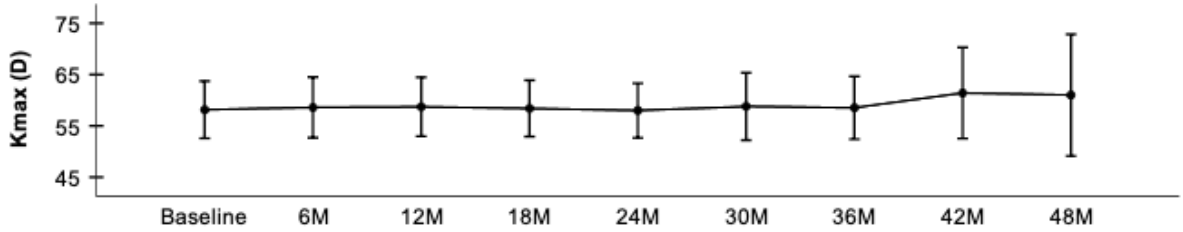

Figure 4 – Mean Keratometry (Km) in dioptres (D) compared with baseline at 6, 12, 18, 24, 30, 36, 42 and 48 months after trans-epithelial accelerated CXL.

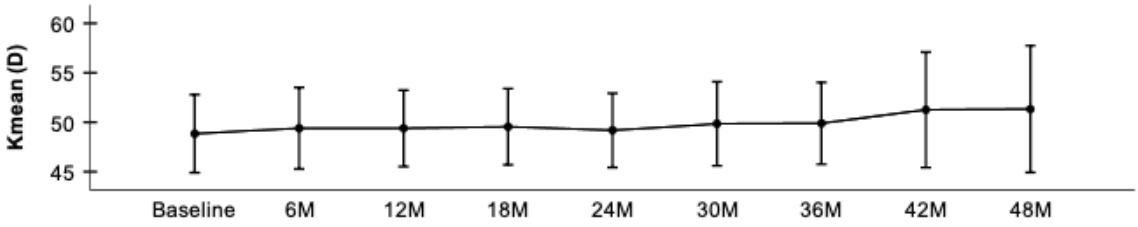

Figure 5 – Minimum pachymetry (PachyMin) in micrometres (µm) compared with baseline at 6, 12, 18, 24, 30, 36, 42 and 48 months after transepithelial accelerated CXL.

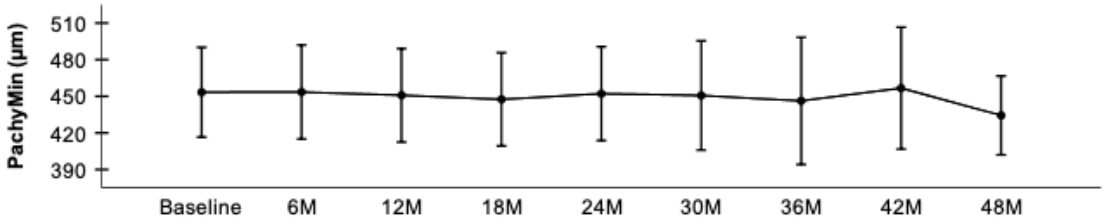

Table 1. Baseline demographic, clinical, visual, corneal topographic, tomographic and pachymetric characteristics of patients undergoing transepithelial accelerated crosslinking.

Variables	**N (%)**	**Pre-Operation N = 39 Mean ± SD**
Patients [male:female]	30 [22:8]	
Age*		20.59 ± 4.43
Eyes	39	
Right	22 (56.4)	
Left	17 (43.6)	
Eye rubber	11 (28.2)	
Allergic conjunctivitis	14 (35.9)	
Atopy	14 (35.9)	
Asthma	2 (5.1)	
BCVA (logMAR)		0.49 ± 0.36
K1 (D)		47.16 ± 3.71
K2 (D)		50.70 ± 4.44
Astg (D)		3.54 ± 1.94
Kmax (D)		58.15 ± 5.58
Km (D)		48.85 ± 3.95
PachyMin (µm)		453.33 ± 36.82
ISV		103.05 ± 30.41
IVA		1.10 ± 0.39
IHD		0.16 ± 0.06
KI		1.28 ± 0.12
D-Index		10.15 ± 3.22
KC		2.69 ± 0.65

Note: *Age at surgery.
Abbreviations: %, percentage; SD, standard deviation; µm, micrometre; Astg, astigmatism (K2-K1); BCVA, best-corrected visual acuity; D, dioptre; IHD, index of height decentration; ISV, index of surface variance; IVA, index of vertical asym- metry; K1, flat keratometry; K2, steep keratometry; KI, keratoconus index; KC, keratoconus classification; Kmax, maximum keratometry; Km, mean keratometry; logMAR, logarithm of minimal angle of resolution; PachyMin, minimum pachymetry; SD, standard deviation.

Table 2. Mean Changes in Visual, Corneal Tomographic, Topographic and Pachymetric Parameters Between 6, 12, 18, 24, 30, 36, 42 and 48 Months and Baseline Values

Variables	**Post-Operation**					
	Δ 6-months	**Δ 12-months**		**Δ 18-months**	**Δ 24-months**	
	N = 39	**N = 33**		**N = 31**	**N = 30**	
	Mean ± SD	**Mean ± SD**	***p* value**	**Mean ± SD**	**Mean ± SD**	***p* value**
BCVA (logMAR)	-0.02 ± 0.21	-0.03 ± 0.26	0.503	-0.03 ± 0.26	-0.01 ± 0.26	0.844
Astg (D)	0.13 ± 1.07	0.003 ± 1.08	0.987	0.29 ± 1.97	-0.007 ± 1.18	0.975
Kmax (D)	0.44 ± 2.18	0.42 ± 2.12	0.269	0.08 ± 2.15	0.38 ±1.94	0.297
Km (D)	0.55 ±0.85	0.66 ± 1.07	0.001	0.68 ± 1.25	0.94 ±1.42	0.001
PachyMin (µm)	0.15 ± 11.27	-1.06 ± 12.54	0.630	-4.29 ± 11.34	-3.07 ± 15.65	0.292
ISV	-0.21 ± 8.90	-0.09 ± 11.90	0.965	-2.17 ± 13.31	-1.14 ± 11.19	0.588
IVA	-0.03 ± 0.12	-0.03 ± 0.16	0.321	-0.06 ± 0.18	-0.07 ± 0.16	0.024
IHD	-0.002 ± 0.02	-0.004 ± 0.03	0.425	-0.006 ± 0.04	-0.005 ± 0.02	0.269
KI	0.002 ± 0.03	0.004 ± 0.05	0.687	-0.005 ± 0.05	-0.05 ± 0.04	0.525
D-index	0.35 ± 0.99	0.50 ± 1.05	0.011	0.61 ± 0.89	0.53 ± 1.19	0.024

Variables	**Post-Operation**					
	Δ 30-months	**Δ 36-months**		**Δ 42-months**	**Δ 48-months**	
	N = 17	**N = 11**		**N = 9**	**N = 3**	
	Mean ± SD	**Mean ± SD**	***p* value**	**Mean ± SD**	**Mean ± SD**	***p* value**
BCVA (logMAR)	0.06 ± 0.37	0.11 ± 0.22	0.260	0.13 ± 0.20	0.20 ± 0.28	0.500
Astg (D)	-0.26 ± 0.93	-0.05 ± 0.76	0.817	-0.12 ± 0.80	1.43 ± 1.83	0.309
Kmax (D)	0.75 ± 2.04	0.75 ±1.95	0.233	2.62 ± 5.94	2.17 ± 2.64	0.290
Km (D)	1.36 ± 1.23	1.48 ± 1.19	0.002	2.43 ± 4.56	1.50 ± 1.49	0.224
PachyMin (µm)	-5.76 ± 13.37	-10.45 ± 15.20	0.046	-15.22 ± 28.63	-24.33 ± 26.16	0.248
ISV	1.12 ± 16.09	4.27 ± 20.34	0.502	10.33 ± 30.37	9.00 ± 27.87	0.632
IVA	-0.05 ± 0.23	-0.01 ± 0.30	0.853	0.02 ± 0.30	0.06 ± 0.46	0.849
IHD	-0.0001 ± 0.03	-0.002 ± 0.04	0.883	0.001 ± 0.07	-0.01 ± 0.07	0.788
KI	0.006 ± 0.05	0.03 ± 0.09	0.232	0.02 ± 0.10	0.04 ± 0.07	0.401
D-index	1.09 ± 1.46	1.29 ± 1.11	0.003	2.43 ± 3.93	1.36 ± 1.35	0.222

Abbreviations: µm, micrometre; Astg, astigmatism (K2-K1) BCVA, best-corrected visual acuity; D, dioptre; IHD, index of height decentration; ISV, index of surface variance; IVA, index of vertical asymmetry; K1, flat keratometry, K2, steep keratometry; KI, keratoconus index; Kmax, maximum keratometry; Km, mean keratometry; logMAR, logarithm of minimal angle of resolution; PachyMin, minimum pachymetry; SD, standard deviation.

Table 3. Evaluation of Parameters Used to Determine Progression of Ectatic Disease

Variables	Post-Operation			
	Δ 12-months	**Δ 24-months**	**Δ 36-months**	**Δ 48-months**
	N = 33	**N = 30**	**N = 11**	**N = 3**
	N (%)	**N (%)**	**N (%)**	**N (%)**
Kmax + 1 (D)[a]	8 (24.2)	9 (30.0)	4 (36.4)	2 (66.7)
Astg + 1 (D)[b]	2 (6.1)	2 (6.7)	1 (9.1)	1 (33.3)
PachyMin – 2% (µm)[c]	10 (30.3)	10 (33.3)	6 (54.5)	2 (66.7)
D-index + 0.42[d]	17 (53.1)	16 (55.2)	9 (81.8)	2 (66.7)

Notes: [a]Increase of at least 1 D in Kmax. [b]Increase of at least 1 D in astigmatism (K2- K1). [c]Decrease of at least 2% in PachyMin. [d]Increase of at least 0.42 in D-index. **Abbreviations:** µm, micrometre; Astg, astigmatism; D, dioptre; K1, flat kerato- metry; K2, steep keratometry; Kmax, maximum keratometry; PachyMin, minimum pachymetry.

Table 4. Comparison of Visual, Corneal Tomographic, Topographic and Pachymetric Baseline Parameters Between the Group That Had Progression and the Group That Had No Progression in the Kmax, D-Index and Thinnest Pachymetry at any point throughout follow-up.

Variable	Kmax + 1 (D)[a]		*p* value	Astg + 1 (D) [b]		*p* value
	Progression	No Progression		Progression	No Progression	
	N = 17	N = 22		N = 4	N = 35	
	Mean ± SD	Mean ± SD		Mean ± SD	Mean ± SD	
BCVA (logMAR)	0.54 ± 0.31	0.45 ± 0.39	0.447	0.60 ± 0.53	0.48 ± 0.34	0.529
K1 (D)	46.75 ± 2.91	47.49 ± 4.26	0.524	48.30 ± 2.26	47.03 ± 3.84	0.525
K2 (D)	50.99 ± 4.11	50.48 ± 4.77	0.729	52.00 ± 3.52	50.55 ± 4.56	0.545
Astg (D)	4.24 ± 2.43	3.00 ± 1.26	0.045	3.70 ± 2.83	3.52 ± 1.87	0.863
Kmax (D)	58.34 ± 4.92	58.01 ± 6.15	0.857	59.90 ± 3.37	57.95 ± 5.77	0.516
Km (D)	48.74 ± 3.29	48.93 ± 4.47	0.886	50.05 ± 2.54	48.70 ± 4.09	0.527
PachyMin (µm)	444.06 ± 36.74	460.50 ± 36.07	0.170	436.00 ± 44.91	455.31 ± 36.02	0.327
ISV	106.44 ± 22.60	100.59 ± 35.36	0.566	116.00 ± 5.77	101.53 ± 31.80	0.026
IVA	1.11 ± 0.30	1.10 ± 0.46	0.919	1.25 ± 0.14	1.09 ± 0.41	0.442
IHD	0.16 ± 0.04	0.16 ± 0.07	0.968	0.18 ± 0.01	0.15 ± 0.06	0.008
KI	1.29 ± 0.12	1.28 ± 0.13	0.822	1.35 ± 0.06	1.27 ± 0.12	0.239
D-index	10.53 ± 2.65	9.87 ± 3.61	0.540	11.29 ± 1.43	10.01 ± 3.35	0.202
KC	2.79 ± 0.47	2.61 ± 0.75	0.366	3.00 ± 0.00	2.657 ± 0.67	0.005

Variable	PachyMin – 2% (µm)[c]		*p* value	D-index + 0.42		*p* value
	Progression	No Progression		Progression	No Progression	
	N = 18	N = 21		N = 24	N = 15	
	Mean ± SD	Mean ± SD		Mean ± SD	Mean ± SD	
BCVA (logMAR)	0.57 ± 0.40	0.43 ± 0.32	0.235	0.51 ± 0.32	0.45 ± 0.43	0.650
K1 (D)	47.43 ± 2.76	46.93 ± 4.42	0.670	47.11 ± 2.92	47.25 ± 4.82	0.917
K2 (D)	51.33 ± 3.99	50.16 ± 4.83	0.419	50.80 ± 3.83	50.54 ± 5.43	0.859
Astg (D)	3.90 ± 2.36	3.23 ± 1.48	0.286	3.70 ± 2.25	3.29 ± 1.31	0.528
Kmax (D)	58.82 ± 5.00	57.59 ± 6.09	0.499	58.38 ± 4.56	57.79 ± 7.06	0.750
Km (D)	49.27 ± 3.17	48.49 ± 4.57	0.546	48.85 ± 3.18	48.84 ± 5.08	0.995
PachyMin (µm)	454.50 ± 36.48	452.33 ± 37.97	0.857	453.0 ± 38.74	453.87 ± 34.84	0.944
ISV	108.76 ± 27.98	98.43 ± 32.17	0.304	109.34 ± 24.94	92.29 ± 36.54	0.096
IVA	1.14 ± 0.32	1.08 ± 0.45	0.614	1.18 ± 0.33	0.98 ± 0.47	0.146
IHD	0.17 ± 0.05	0.15 ± 0.07	0.393	0.17 ± 0.05	0.14 ± 0.07	0.167
KI	1.30 ± 0.12	1.26 ± 0.12	0.304	1.30 ± 0.12	1.25 ± 0.12	0.228
D-index	10.47 ± 3.08	9.88 ± 3.38	0.581	10.59 ± 2.67	9.38 ± 3.98	0.267
KC	2.86 ± 0.51	2.55 ± 0.72	0.122	2.85 ± 0.48	2.43 ± 0.80	0.080

Notes: [a]Increase of at least 1 D in Kmax. [b]Increase of at least 1 D in astigmatism (K2- K1). [c]Decrease of at least 2% in PachyMin. [d]Increase of at least 0.42 in D-index.

Abbreviations: µm, micrometre; BCVA, best-corrected visual acuity; D, dioptre; IHD, index of height decentration; ISV, index of surface variance; IVA, index of vertical asymmetry; K1, flat keratometry, K2, steep keratometry; KC, topographic keratoconus classification; KI, keratoconus index; Kmax, maximum keratometry; Km, mean keratometry; logMAR, logarithm of minimal angle of resolution; PachyMin, minimum pachymetry; SD, standard-deviation.

Table 5. Comparison of Demographic and Clinical Baseline Parameters Between the Group That Had Progression and the Group That Had No Progression in the Kmax, D-Index and Thinnest Pachymetry at any point throughout follow-up.

Variable	Kmax + 1 (D)[a]		*p* value	Astg + 1 (D)[b]		*p* value
	Progression	No Progression		Progression	No Progression	
	N = 17	N = 22		N = 4	N = 35	
	N (%)	N (%)		N (%)	N (%)	
Gender [male:female]	14:3	15:7	0.464	4:0	25:10	0.556
Age at surgery	20.41 ±3.61	20.73 ± 5.05	0.829	17.50 ± 2.38	20.94 ± 4.49	0.143
Eye			0.754			0.618
Right	9 (52.9)	13 (59.1)		3 (75.0)	19 (54.3)	
Left	8 (47.1)	9 (40.9)		1 (25.0	16 (45.7)	
Eye rubber	2 (11.8)	9 (40.9)	0.073	0 (0)	11 (31.4)	0.309
Allergic conjunctivitis	7 (41.2)	7 (31.8)	0.546	2 (50.0)	12 (34.3)	0.609
Atopy	8 (47.1)	6 (27.3)	0.201	1 (25.0)	13 (37.1)	0.632
Asthma	0 (0)	2 (9.1)	0.495	1 (25.0)	1 (2.9)	0.197

Variable	PachyMin – 2% (µm)[c]		*p* value	D-index + 0.42[d]		*p* value
	Progression	No Progression		Progression	No Progression	
	N = 18	N = 21		N = 24	N = 15	
	N (%)	N (%)		N (%)	N (%)	
Gender [male:female]	15:3	14:7	0.290	19:5	10:5	0.463
Age at surgery	19.56 ± 3.73	21.48 ± 4.86	0.180	20.42 ± 4.26	20.87 ± 4.82	0.762
Eye			0.163			0.721
Right	8 (44.4)	14 (66.7)		13 (54.2)	9 (60.0)	
Left	10 (55.6)	7 (33.3)		11 (43.6)	6 (40.0)	
Eye rubber	4 (22.2)	7 (33.3)	0.442	6 (25.0)	5 (33.3)	0.718
Allergic conjunctivitis	8 (44.4)	6 (28.6)	0.303	8 (33.3)	6 (40.0)	0.673
Atopy	8 (44.4)	6 (28.6)	0.303	11 (45.8)	3 (20.0)	0.102
Asthma	1 (5.6)	1 (4.8)	1.000	0 (0)	2 (13.3)	0.142

Notes: [a]Increase of at least 1 D in Kmax. [b]Increase of at least 1 D in astigmatism (K2- K1). [c]Decrease of at least 2% in PachyMin. [d]Increase of at least 0.42 in D-index.

Abbreviations: µm, micrometre; BCVA, best-corrected visual acuity; D, dioptre; IHD, index of height decentration; ISV, index of surface variance; IVA, index of vertical asymmetry; K1, flat keratometry, K2, steep keratometry; KC, topographic keratoconus classification; KI, keratoconus index; Kmax, maximum keratometry; Km, mean keratometry; logMAR, logarithm of minimal angle of resolution; PachyMin, minimum pachymetry; SD, standard-deviation.

Table 6. Comparison of Demographic, Clinical, Visual, Corneal Tomographic, Topographic and Pachymetric Baseline parameters between the group that filled at least one criterium of progression and the grouped that did not fill any criteria of progression in the Kmax, D-Index and Thinnest Pachymetry at at any point throughout follow-up.

Variable	Any criterium of progression[a]		*p* value
	Progression	**No Progression**	
	N = 28	**N = 11**	
	Mean ± SD	**Mean ± SD**	
Gender [male:female]	22:6	7:4	0.424
Age at surgery	20.32 ± 4.20	21.27 ± 5.12	0.553
Eye			0.725
Right	15 (53.6)	7 (63.6)	
Left	13 (46.4)	9 (36.4)	
Eye rubber	7 (25.0)	4 (36.4)	0.694
Allergic conjunctivitis	10 (35.7)	4 (36.4)	0.970
Atopy	12 (42.9)	2 (18.2)	0.148
Asthma	1 (3.6)	1 (9.1)	0.490
BCVA (logMAR)	0.52 ± 0.35	0.41 ± 0.38	0.400
K1 (D)	46.96 ± 3.06	47.69 ± 5.15	0.665
K2 (D)	50.46 ± 4.03	51.33 ± 5.53	0.589
Astg (D)	3.40 ± 2.19	3.63 ± 1.14	0.846
Kmax (D)	57.86 ± 4.80	58.86 ± 7.43	0.625
Km (D)	48.61 ± 3.37	49.44 ± 5.30	0.636
PachyMin (µm)	455.04 ± 38.21	449.00 ± 34.35	0.651
ISV	106.78 ± 26.38	93.91 ± 38.52	0.242
IVA	1.16 ± 0.33	0.97 ± 0.51	0.184
IHD	0.16 ± 0.05	0.14 ± 0.08	0.273
KI	1.29 ± 0.12	1.25 ± 0.13	0.369
D-index	10.28 ± 2.80	9.81 ± 4.21	0.739
KC	2.80 ± 0.52	2.41 ± 0.86	0.178

Notes: [a]Increase of at least 1 D in Kmax OR Increase of at least 1 D in astigmatism (K2- K1) OR Decrease of at least 2% in PachyMin OR Increase of at least 0.42 in D-index.

Abbreviations: µm, micrometre; BCVA, best-corrected visual acuity; D, dioptre; IHD, index of height decentration; ISV, index of surface variance; IVA, index of vertical asymmetry; K1, flat keratometry, K2, steep keratometry; KC, topographic keratoconus classification; KI, keratoconus index; Kmax, maximum keratometry; Km, mean keratometry; logMAR, logarithm of minimal angle of resolution; PachyMin, minimum pachymetry; SD, standard-deviation.

Anexos

Anexo 1: reporting guidelines

STROBE Statement—Checklist of items that should be included in reports of ***cohort studies***

	Item No	Recommendation	Page No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract "were included in this retrospective cohort study" (*b*) Provide in the abstract an informative and balanced summary of what was done and what was found "to systematically evaluate the long-term efficacy of transepithelial accelerated corneal collagen crosslinking (TE-ACXL) in the treatment of eyes with progressive keratoconus (…) TE-ACXL seems to be a safe and effective treatment for progressive keratoconus."	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported "Recently, corneal collagen crosslinking (CXL) has emerged as the most effective treatment in decreasing or even aborting disease progression, possibly reducing the need for keratoplasty. (…) Thus, transepithelial accelerated corneal crosslinking (TE-ACXL) emerged as a more appealing and promising alternative to traditional protocols, combining the transepithelial and accelerated protocols to surpass some disadvantages of the original protocol. (…) Therefore, long-term efficacy and safety TE-ACXL studies are required to fully determine the role of this technique in keratoconus treatment."	4, 5
Objectives	3	State specific objectives, including any prespecified hypotheses "The purpose of the present study is to evaluate the long-term efficacy of the TE-ACXL protocol in the treatment of patients with a diagnosis of progressive keratoconus by reporting its 4-year outcomes, using a similar but increased cohort that was analyzed previously for 2-year outcomes of the same technique."	5
Methods			
Study design	4	Present key elements of study design early in the paper "The authors conducted a retrospective observational cohort study of 39 eyes with progressive keratoconus who underwent TE-ACXL (6 mW/cm² for 15 minutes)"	5
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection "were followed at the Ophthalmology Corneal Department of Centro Hospitalar Universitário de São João from January 2016 to January 2021.	5, 6

		(…) Medical records of all patients who underwent TE-ACXL were analyzed between December 2020 and January 2021. (…) All clinical, visual, corneal topographic and tomographic and pachymetric parameters from the eyes included in the study were evaluated preoperatively and every 6 months postoperatively to a maximum of 48 months."	
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up "This study's inclusion criteria were defined as: age between 14 and 32, pachymetry at its thinnest point (PachyMin) ≥ 380 µm and previously documented progression of keratoconus. Keratoconus was deemed to be progressive if 1 or more of the following changes were present in the previous 6 months: an increase ≥ 1.00 diopter (D) in maximum keratometry (Kmax), a 2% decrease in PachyMin or an increase ≥ 1.00 D in corneal cylinder. The exclusion criteria were apical corneal scarring, severe dry eye, delayed epithelial healing, active ocular infections, connective tissue disease, pregnancy or lactation and previous history of cornea surgery." (*b*) For matched studies, give matching criteria and number of exposed and unexposed NA	5,6
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable The exposure is described in the "Surgical technique and Postoperative Care" section. "Disease progression was assessed at 12, 24, 36 and 48 months after TE-ACXL and defined as the presence of one or more of the following: an increase ≥ 1.00 D in corneal astigmatism, an increase ≥ 1.00 D in Kmax, a decrease ≥ 2% in thinnest pachymetry or an increase ≥ 0.42 units in D-index."	6, 7
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group "Best-corrected visual acuity (BCVA) was recorded via a Snellen chart and converted to the logarithm of minimal angle of resolution (logMAR) units to allow statistical analysis. Corneal astigmatism (Astg, K2-K1) mean keratometry (Km), Kmax, PachyMin, index of height decentration (IHD), index of vertical asymmetry (IVA), index of surface variance (ISV), keratoconus index (KI) and Belin/Ambrósio D-index were recorded using Oculus Pentacam (Pentacam HR®, Oculus Optikgeräte GmbH, Wetzlar, Germany). Keratoconus Classification (KC) is in accordance with the Pentacam HR® Ktc Scoring System."	6
Bias	9	Describe any efforts to address potential sources of bias "Medical records of all patients who underwent TE-ACXL were analyzed (…) Comparisons between groups that had progression throughout our	5, 7

		study and those who did not present progression were conducted with independent samples t-tests."	
Study size	10	Explain how the study size was arrived at "39 eyes with progressive keratoconus who underwent TE-ACXL (6 mW/cm² for 15 minutes) and were followed at the Ophthalmology Corneal Department of Centro Hospitalar Universitário de São João from January 2016 to January 2021" (these are the eyes that matched the inclusion criteria)	5
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why "Best-corrected visual acuity (BCVA) was recorded via a Snellen chart and converted to the logarithm of minimal angle of resolution (logMAR) units to allow statistical analysis. Corneal astigmatism (Astg, K2-K1) mean keratometry (Km), Kmax, PachyMin, index of height decentration (IHD), index of vertical asymmetry (IVA), index of surface variance (ISV), keratoconus index (KI) and Belin/Ambrósio D-index were recorded using Oculus Pentacam (Pentacam HR®, Oculus Optikgeräte GmbH, Wetzlar, Germany). Keratoconus Classification (KC) is in accordance with the Pentacam HR® Ktc Scoring System. (…) Categorical variables were reported as frequencies and proportions, while continuous variables were presented as the mean ± standard deviation."	6,7
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding "Categorical variables were reported as frequencies and proportions, while continuous variables were presented as the mean ± standard deviation. The postoperative variation in visual, keratometric, pachymetric, topographic and tomographic parameters was calculated by subtracting their baseline values from the subsequent readings at each follow-up visit (therefore, positive delta values denote an increase in that parameter, whilst negative delta values represent a decrease). Paired *t-tests* were used to compare preoperative and postoperative outcomes; multiple related samples were compared via within-subjects ANOVA test." (*b*) Describe any methods used to examine subgroups and interactions "Comparisons between groups that had progression throughout our study and those who did not present progression were conducted with independent samples t-tests." (*c*) Explain how missing data were addressed NA (*d*) If applicable, explain how loss to follow-up was addressed "Disease progression was assessed at 12, 24, 36 and 48 months after TE-ACXL". In our study, the entirety of patients studied were followed via regular visits to the doctor every six months after the date of the procedure. While many of them maintained this regularity, others stopped showing to the visits. Still, we included the latter in our analysis and evaluated the outcomes of interest until their last visit since they were still relevant for our study's objectives (follow-up time discriminated on the "Results" section). (*e*) Describe any sensitivity analyses NA	6. 7

Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed "All 39 eyes completed the 6 months follow-up, while 33, 31, 30, 17, 11, 9 and 3 eyes completed the 12, 18, 24, 30, 36, 42 and 48-month follow-up, respectively." (b) Give reasons for non-participation at each stage Please read point 12d. (c) Consider use of a flow diagram NA	8
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders "Our cohort included 22 male and 8 female patients; 9 patients received TE-ACXL in both eyes (7 male and 2 female patients). The mean age was 20.59 ± 4.43 years (ranging from 14 to 32). Further baseline characteristics are shown in Table 1." (b) Indicate number of participants with missing data for each variable of interest NA (c) Summarise follow-up time (eg, average and total amount) "All 39 eyes completed the 6 months follow-up, while 33, 31, 30, 17, 11, 9 and 3 eyes completed the 12, 18, 24, 30, 36, 42 and 48-month follow-up, respectively."	7, 8
Outcome data	15*	Report numbers of outcome events or summary measures over time "Variation (Δ) between baseline visual, keratometric, pachymetric, topographic and tomographic corneal parameters at 6, 12, 18, 24, 30 36, 42 and 48 months postoperatively are presented in Table 2. (…) Table 3 discriminates the number of patients that had progression of ectatic disease at 12, 24, 36 and 48-months follow-up utilizing each of the aforementioned progression parameters."	
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included NA (*b*) Report category boundaries when continuous variables were categorized NA (*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period NA	
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses "A comparison of baseline characteristics was made between the groups that had progression by each parameter and the groups that did not progress by that same parameter, and the results can be visualized in Tables 4 and 5. (…) An additional comparison of baseline characteristics was made between eyes that had progression by any of the four criteria (a total of 28 eyes) and those which did not fill any of those criteria (a total of 11 eyes) (Table 6)."	9, 10
Discussion			
Key results	18	Summarise key results with reference to study objectives "Our study did not find statistically significant variations in the BCVA. (…) Apart from Kmean (which suffered a significant increase and 12-, 24- and 36- months	12

		follow-up) and an isolated significant decrease in PachyMin at 36 months, the keratometric and pachymetric parameters did not present significant variations. (…) To evaluate keratoconus progression herein, we used 4 criteria: an increase ≥ 1.00 D in corneal astigmatism, an increase ≥ 1.00 D in Kmax, a decrease ≥ 2% in thinnest pachymetry or an increase ≥ 0.42 units in D-index. Fulfillment of one of these during the follow-up period would indicate the presence of progression. As so, a total of 28 (71.8%) eyes showed progression."	
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias "These findings may suggest that some eyes did not present major changes in their keratometric and topographic parameters but were considered progressive due in part to the oversensitive criteria used. (…) There were several limitations in this study, most of them inherent to its retrospective nature. The sample size was relatively small, the patients' baselines were not similar and there was no control group. Additionally, the follow-up time was not uniform among the eyes studied, with only 3 eyes reaching the 48-month mark and 6 eyes not even completing 12 months of follow-up."	13, 15
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence "Overall, the patients' eyes remained statistically stable during follow-up after TE-ACXL, which is demonstrated by the absence of significant variations in almost all of the visual, keratometric, pachymetric, topographic and tomographic parameters evaluated, despite young age and relatively thin corneas with high Kmax and high KC preoperatively. In addition, no complications or adverse events were recorded, which attests to the good safety profile of the TE-ACXL. Although most eyes did maintain progression, various limitations may be pointed out to the criteria used. The lack of established criteria of keratoconus progression in the literature makes it difficult to systematically evaluate progression. Hence, future efforts should be made to establish not only the most accurate criteria but also the magnitude of its variation that best reflects keratoconus progression both before and after treatment."	15
Generalisability	21	Discuss the generalisability (external validity) of the study results "All of these limit the statistical efficiency and external validity of the study results and highlight the necessity of randomized prospective studies with a large sample size and control group."	15
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based NA	

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.

Anexo 2: Normas para publicação da revista Clinical Ophtalmology

Manuscript preparation

- While the editors fully understand the extra challenges posed to authors whose native language is not English, we must ask that all manuscripts be reviewed and edited by a native speaker of English with expertise in that area prior to submission
- Double-spacing
- 3-cm margins
- Page numbers
- Line numbers
- Clear concise language
- American spelling (all components of a manuscript must be in English)
- Ensure tables and figures are cited
- The preferred electronic format for text is Microsoft Word
- Manuscripts will be accepted in LaTeX as long as the native LaTeX and a PDF is also supplied
- Use International Systems of Units (SI) symbols and recognized abbreviations for units of measurement
- Do not punctuate abbreviations eg, et al, ie
- Spell out acronyms in the first instance in the abstract and paper
- Word counts are not specified. In general, shorter items range from 1000 to 3000 words and reviews from 3000 to 7,500
- Generic drug names are used in title, text, tables, and figures
- Suppliers of drugs, equipment, and other brand-name material are credited in parentheses (company, name, city, state, country)
- If molecular sequences are used, provide a statement that the data have been deposited in a publicly accessible database, eg, GenBank, and indicate the database accession number
- Depositing laboratory protocols on protocols.io is encouraged, where a DOI can be assigned to the protocol. To include a link to a protocol in your manuscript:
  1) Describe your step-by-step protocol on protocols.io
  2) Select "Get DOI" to issue your protocol with a unique DOI (digital object identifier)
  3) Include the DOI link in the Methods section of your manuscript using the format provided by protocols.io: http://dx.doi.org/10.17504/protocols.io.xxxxxxx

(where xxxxxxx is the unique DOI)
At this stage, your protocol is only visible to those with the link. This allows editors and reviewers to consult your protocol when evaluating the manuscript. You can make your protocols public at any time by selecting "Publish" on the protocols.io website. Any referenced protocols will automatically be made public when your article is published.

Updated 7 January 2019

Manuscript templates

We have prepared two manuscript templates to help authors when submitting their manuscript to one of our journals. The first template is for all journals except *Core Evidence*, and the second is only for the journal *Core Evidence*.
Please click on the appropriate link below and 'Save As' the Word document onto your local computer.

- Template for all journals (except *Core Evidence*)
- *Core Evidence template*

When you are ready to submit your paper please go to our **online submission form**, which is designed to be as quick and painless as possible.
If you have any questions about submitting your manuscript please email our **Editorial team** or use the green/red **'Live Support'** button on the website.

Updated 7 April 2020

Manuscript structure

Title page

- First name/given name(s) and last name/family name of authors (see Authorship section below)
- Author affiliations: department, institution, city, state, country
- ORCID number(s) for all authors whenever available

Abstract

There are two types of abstracts - structured and unstructured. Original research papers require a structured abstract. Both types of abstracts should be no more than 300 words.

Plain Language Summary (optional)

It is useful for researchers to write plain language summaries of their articles to make them

accessible to a wider audience but also to make research accessible to professionals in nearby disciplines. Crucially, plain language summaries are beneficial to improve public engagement with science and medical research. By helping the public to understand biomedical research, researchers can contribute to raising awareness of its value and attracting further public support and involvement.

As an author, promoting your work in an engaging way to a wider audience can help you:

- Attract more readers
- Potentially increase the number of citations to your articles
- Get noticed
- Build a strong reputation
- Connect with patients, carers, politicians, policy-makers and other decision-makers
- Attract more funding opportunities
- Expand your professional network

The plain language summary should have between 150 and 250 words, be written in plain English, and be placed after the Abstract and before the Introduction. The plain language summary should be distinct from the abstract and should be written in an accessible, interesting way without spinning or exaggerating the story.

- The plain language summary should not be a "dumbed down" version of your work. You must not treat your audience as stupid or patronise the reader.
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3–6 keywords

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Results

Discussion

Conclusions

Abbreviations (if any)

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See Reference Style Guidelines

Updated 4 March 2021

Figures and tables

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- Font size is consistent
- Lines are a minimum of 0.3pt
- Images do not contain any layers, or transparent objects
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Preparation and Submission

Recommended image resolutions:

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Tables should present new information rather than duplicating what is in the text. Readers should be able to interpret the table even if presented separately from the text. Ensure that each table is cited within the text of the manuscript.

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Consider the size of each table and whether it will fit on a single journal page. If the table is cramped in a Microsoft Word document, where the default setting represents an A4 page (210 x 297 mm), it will be difficult to represent it clearly on a B5 journal page (176 x 250 mm). If this is the case, please consider splitting the data into two or more tables.

Updated 15 October 2020

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Updated 3 October 2019

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