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Comparison of standard and accelerated corneal cross-linking for the treatment of keratoconus: a meta-analysis

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ABSTRACT.

Purpose: **To compare results between standard and accelerated corneal collagen cross-linking (CXL) for the treatment of progressive keratoconus.**

Methods: **We performed literature searches in PubMed, Cochrane Library, Web of Science, ISRCTN registry, ClinicalTrials.gov, and EMBASE for studies comparing conventional Dresden (C-CXL) and accelerated CXL (A-CXL). Outcomes were clinical results and changes in corneal properties. Weighted mean differences were used to evaluate the effects.**

Results: **Here, 22 studies with 1158 eyes (C-CXL: 577 eyes; A-CXL: 581 eyes) were included. At the last follow-up, C-CXL was superior regarding minimum keratometry ($p < 0.00001$) and demarcation line depth ($p < 0.00001$), whereas A-CXL should be favoured when considering minimum corneal thickness ($p = 0.0005$). No differences in uncorrected and corrected distance visual acuity ($p = 0.09$ and 0.98), spherical equivalent ($p = 0.11$), spherical and cylindrical error ($p = 0.29$ and 0.32), maximal and average keratometry ($p = 0.05$ and 0.65), central corneal thickness ($p = 0.15$), corneal biomechanical properties ($p \geq 0.21$ respectively), time of reepithelialization ($p = 0.76$), subbasal nerve density ($p = 0.69$), endothelial cell density ($p = 0.30$) and morphology ($p \geq 0.40$ respectively) were found among both groups.**

Conclusion: **Consideration of less corneal thinning favours A-CXL, whereas the deeper demarcation line and greater changes in minimum keratometric values in C-CXL may indicate a higher treatment efficacy. Altogether, C-CXL, as well as A-CXL, provides successful results in the strengthening of corneal tissue.**

Key words: accelerated cross-linking – corneal collagen cross-linking – different cross-linking protocols – progressive keratoconus – standard Dresden cross-linking

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Introduction

In recent years, corneal collagen cross-linking (CXL) has become a common and effective procedure in the treatment of keratoconus, being already reasonable in early stages of corneal ectasia (Cantemir et al. 2017). Characteristics of this progressive eye disease are corneal thinning combined with irregular steepening. Scarring of the apical part also contributes to a loss of visual acuity (Rabinowitz 1998). The purpose of CXL is to strengthen the corneal stability and stiffness, and thus to arrest the progression of keratoconus (Spoerl et al. 1998). A recent study stated further development of keratoconus during pregnancy and concluded that women who plan to become pregnant should consider CXL to halt the progression of keratoconus (Naderan & Jahanrad 2017). Since laser *in situ* keratomileusis (LASIK) is likely to induce iatrogenic corneal ectasia, a combination of LASIK and CXL may be a therapeutic option in order to prevent the cornea from developing keratoconus or to reduce progression of corneal thinning (Chan et al. 2017). However, to date, adjuvant CXL is not performed by default.

In corneal CXL, the interaction between the photosensitizer riboflavin and ultraviolet-A (UV-A) irradiation initiates a photochemical reaction. Inducing interfibrillar and intrafibrillar covalent cross-links within corneal collagen fibres, the procedure increases rigidity of the ectatic cornea (Spoerl et al. 1998; Wollensak et al. 2003).

The conventional Dresden protocol (C-CXL) was first reported by Wollensak et al. (2003). The standard Dresden protocol uses a 3 mW/cm² UV-A intensity for 30-min irradiation time. Many studies evaluated the short-term and long-term results and found it to

be safe and effective (Raiskup-Wolf et al. 2008; Wittig-Silva et al. 2014). Corneal wavefront aberrations did not change significantly at the 6-month follow-up (Baumeister et al. 2009). Long-term corneal stabilization could be achieved meaning the progression of keratoconus was halted (Raiskup et al. 2015).

Although showing promising results, there is one major drawback of the C-CXL protocol; the duration of half an hour for the treatment is arduous for both the patient and the surgeon. Therefore, the request for more effective time management led to the development of accelerated irradiation protocols (A-CXL) with higher UV-A intensities (Waszczykowska & Jurowski 2015). Reducing the time required for surgery should avoid corneal dehydration and intraoperative thinning (Holopainen & Krootila 2011). Furthermore, fewer complications, such as infections are expected on account of shorter exposure time.

Most of these new protocols are based on the Bunsen–Roscoe law of reciprocity (Bunsen & Roscoe 1857). It claims an inverse relationship between the applied intensity and illumination time. Equal photochemical effects on the cornea are expected using the same cumulative dose meaning higher intensities in a shorter time of treatment. Having investigated the validity of this law in tissue samples, it is known that the Bunsen–Roscoe law has only a restricted *in vivo* applicability (Schindl et al. 2001).

Comparing conventional and modified CXL, it should be considered that availability of oxygen is a limiting factor in CXL (Richoz et al. 2013). Reactive oxygen species are necessary for the catalysis of cross-linking (Kamaev et al. 2012). Therefore, treatment efficacy depends on intrastromal oxygen diffusion capacity and the proportion of provided and used oxygen. A lack of oxygen, and thus a decrease of oxygen-free radicals, may be responsible for a restricted treatment efficacy. Irradiation with higher intensities has a higher oxygen consumption rate and thus leads to less cross-linked bonds between corneal stromal collagen fibrils (Hammer et al. 2014).

To date, many studies have compared postoperative outcomes between the conventional and certain accelerated protocols. However, no protocol could prove its superiority. The aim of this meta-analysis is to summarize postsurgical outcomes of individual studies comparing C-CXL and A-CXL in terms of clinical outcomes and corneal properties.

Materials and Methods

Search strategy and data extraction

We searched PubMed, Cochrane Library, Web of Science, ISRCTN registry, ClinicalTrials.gov, and EMBASE for studies comparing C-CXL and A-CXL.

The following keywords and several combinations were used: 'corneal' and 'crosslinking', 'cross-linking', 'cross linking', 'crosslinkage', 'cross-linkage', 'cross linkage', and 'different', 'protocol', 'illumination', 'irradiation', 'intensity', and 'standard', 'conventional', 'Dresden', 'accelerated', 'modified', 'rapid'. No restrictions were made relating to the study design, publication date or language. Furthermore, manual searches were conducted by reviewing the reference lists of retrieved articles and reviews.

Two authors (CK, MS) independently performed the literature search from 22.05.2017 to 10.09.2017. Relevant studies were extracted reviewing the titles and abstracts of all studies under consideration. Any discrepancies were double checked and inconsistencies were resolved by discussion or by a third author (TK). Zotero (version 5.0.21, Roy Rosenzweig Center for History and New Media) and Cochrane Review Manager (RevMan [Computer program], version 5.3, Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2014) were used to manage the included studies. Data were extracted using a standard data-extraction form. The following aspects were recorded: authors, year of publication, location, study design, number of eyes, mean age, sex, follow-up time, details of treatment protocols and evaluated variables at different follow-up time points. The last search was conducted just before the final analysis.

Our meta-analysis was performed pursuant to the PRISMA statement (Liberati et al. 2009). The protocol was registered at the Prospective Register for Systematic Reviews (PROSPERO, registration number CRD42017076840). Our institutional ethics committee ruled that approval was not required for this study and the tenets of the Declaration of Helsinki were followed throughout the study.

Inclusion and exclusion criteria

We selected complete and published clinical studies comparing C-CXL and A-CXL. *Ex vivo* and paediatric studies were excluded as well as studies that combined corneal CXL with photorefractive keratectomy. Studies were only included if they reported at least 1 of the predetermined outcomes.

Outcome measurements

Endpoints of interest were changes in the following outcomes: uncorrected distance visual acuity (UDVA), corrected distance visual acuity (CDVA), spherical equivalent (SE), spherical and cylindrical error, central and minimum corneal thickness (CCT, MCT), corneal hysteresis (CH), corneal resistance factor (CRF), anterior stromal keratocyte density, subbasal nerve density, endothelial cell density (ECD), percentage of hexagonal endothelial cells, coefficient of variation of endothelial cells, as well as average, maximal and minimum keratometry values (K_{mean}, K_{max}, K_{min}). We also documented absolute values of demarcation line depth (DLD) and time of reepithelialization. If VA was reported in the decimal scale, it was converted to the logarithm of the minimum angle of resolution (logMAR). Absolute mean values or changes from baseline to different endpoints were recorded with correspondent standard deviations (SD). Not all studies evaluated all the parameters. Complications were assessed but not statistically analysed.

Data analysis and quality assessment

Included outcomes were extracted as mean values or changes and SD. Some studies did not report a SD of the change. Hence, we used the given p-values and changes in mean values to estimate the SD. In the cases where no p-value was reported, we used studies with a full dataset of mean values and SD to calculate an adequate estimation of SD for those studies without published SD (Borenstein et al. 2009). When no SD of baseline and postoperative values and no p-value were

reported, we could not calculate the SD of the change, resulting in the exclusion of the data.

Overall, changes were evaluated as weighted mean difference and 95% confidence interval. Heterogeneity between studies was determined using the chi-square test and computing the quantity I^2 statistic. An I^2 greater than 50% was considered to state significant heterogeneity. Random-effect models were used since studies were assumed to differ from each other regarding aspects of implementation (Borenstein et al. 2009). Funnel plots were analysed by an experienced statistician (EH) to evaluate publication bias and small study effects. A p-value less than 0.05 was considered to be statistically significant.

Results

Characteristics of included trials

Initially, a total of 622 studies were identified. Duplicates were rejected and remaining studies were screened by title and abstract. A full-text review was performed when necessary. After the removal of studies which did not fulfil our inclusion criteria or did not evaluate the predetermined variables, there were 23 trials remaining. Two studies (Viswanathan & Males 2015; Pircher et al. 2016) were only available as abstracts. One abstract (Viswanathan & Males 2015) did not present any utilisable values and thus was excluded. If only abstracts were available or some information was missing, we included the given information and asked the authors for the other values, but in most cases, we did not receive an answer. In the end, 22 studies qualified for the meta-analysis. A total of 577 eyes were treated with C-CXL and 581 eyes underwent A-CXL treatment. Characteristics of all the trials and details of the treatment protocols are presented in Table 1. Shetty et al. (2015) evaluated the outcomes of conventional Dresden protocol compared to 3 different protocols of A-CXL. We compared the standard group with one particular accelerated group, respectively, and treated the whole study as if it had been three single trials. For this reason, there are 24 studies mentioned in Table 1. However, when calculating the number of eyes in both groups, the eyes of the C-CXL group were only included once. Hashemi et al. published two studies (Hashemi et al. 2015a,b) reporting short-term and long-term results of C-CXL and A-CXL. Since the baseline values were exactly the same, we assumed the patients to be identical in both studies. Hence, we removed the duplicates of 6-month values and only considered their results once. Eyes were also included only once in the calculation of the total number of eyes. Corneal thickness less than 400 μm was an exclusion criterium in most trials, except for two studies (Brittingham et al. 2014; Hagem et al. 2017). However, all patients showed pachymetry values greater than this benchmark. Thus, only patients with a corneal thickness greater than 400 μm were included in our meta-analysis.

There was a lack of consistency between the given changes and the calculated differences between baseline and postoperative values in the study by Hashemian et al. (2014) and Tomita et al. (2014). We decided to include the given changes and their SD but we adjusted the algebraic signs. They were also adapted for the values of other studies (Sherif 2014; Hashemi et al. 2015a; Shetty et al. 2015) as we wanted to report the postoperative minus preoperative values. For Chow et al. (2015), the algebraic signs of the SE were adjusted as well as for the CDVA of Tomita et al. (2014). Mean changes and 95% confidence intervals are presented in all forest plots.

Visual acuity and manifest refraction

At 1-, 3-, and 6-month follow-up, similar changes in UDVA were reported for C-CXL and A-CXL ($p = 0.82$, 0.87, and 0.65, respectively) as provided in the forest plot (Fig. 1A). After 12 months, C-CXL resulted in greater improvement of UDVA, but this was not significant ($p = 0.09$).

Similar results were found when evaluating the CDVA. There was no difference between the two groups throughout the follow-up (Fig. 1B). At 1 month, C-CXL seemed to improve CDVA more than A-CXL, even though missing statistical significance ($p = 0.36$). C-CXL led to an equal improvement of CDVA compared to A-CXL at later follow-ups ($p = 0.99$, 0.75, and 0.98 respectively).

The change in SE did not differ between groups at the 6-month visit ($p = 1.00$) whereas at the last follow-up, SE was more inclined in the C-CXL group ($p = 0.11$).

At the early 1-month visit, the improvement in spherical error was comparable in both groups ($p = 0.65$). At 3 and 6 months, C-CXL showed better results ($p = 0.1$ and <0.0001 respectively), which was inverse to the end of the follow-up when greater decrease was stated with A-CXL ($p = 0.29$).

Conventional Dresden protocol (C-CXL) provided a greater decrease of cylindrical error after 1 month ($p = 0.39$) (Fig. 1C). At 3 months both techniques seemed to have the same effect ($p = 1.00$). Improvements at 6 months and at the final follow-up were found to be more pronounced using C-CXL ($p = 0.13$ and 0.32 respectively).

Keratometry

The short-term follow-up at 1 and 3 months showed similar changes in K_{max} in both groups ($p = 0.60$ and 0.41 respectively) (Fig. 2). At the 6-month visit, no significant differences in changes in maximal, average, and minimum keratometric parameters were ascertained comparing both procedures ($p = 0.57$, 0.61, and 0.15 respectively).

At the end of the follow-up, C-CXL was superior in terms of corneal flattening regarding K_{max} and K_{min} ($p = 0.05$ and <0.00001 respectively), with K_{max} barely missing statistical significance. In contrast, a similar reduction of corneal steepening was found for the average keratometry in both groups ($p = 0.65$).

At the final follow-up for K_{max}, we performed an analysis of subgroups according to the used A-CXL protocol. All studies measuring K_{max} at 12 or 18 months used a cumulative dose of 5.4 J/cm², except for 1 study (Sherif 2014) that was excluded from this subgroup analysis. Very high irradiation intensity for short treatment time (30 mW/cm² for 3 min) showed equal corneal flattening in both groups ($p = 0.67$) whereas a greater decrease of keratometry was found with C-CXL compared to A-CXL with an irradiance of 18 mW/cm² for 5 min ($p = 0.002$). Although the difference of changes in keratometry did not reach statistical significance ($p = 0.22$), slightly greater corneal flattening can be seen with

Table 1. Characteristics of included trials.

First author	Year	Study design*	Number of eyes*	Mean age (years)*	Follow-up (months)	A-CXL irradiation dose & duration	A-CXL cumulative dose (J/cm²)	0.1% Riboflavin before irradiation*	Riboflavin during irradiation*
Bouheraoua	2014	PNR	15/15	25.4/26.7	6	30 mW/cm² for 3 min	5.4	in 20% dextran every 2 min for 20 min/in 20% dextran every 1 min for 10 min	every 5 min/NR
Brittingham	2014	R	81/50	28.6/26.1	12	9 mW/cm² for 10 min	5.4	in 20% dextran every 5 min for 20 min	every 2 min
Choi	2017	PR	15/13	25.6/23.7	6	30 mW/cm² for 3:40 min	6.6	in 20% dextran every 3 min for 30 min/with HPMC every 2 min for 10 min	NR
Chow	2015	PNR	19/19	27.8/26.3	12	18 mW/cm² for 5 min	5.4	in 20% dextran every 2 min for 30 min	every 2 min
Cinar	2014	NR	13/13	17.0/18.8	6	9 mW/cm² for 10 min	5.4	in 20% dextran every 3 min for 30 min	every 5 min/ every 2 min
Cummings	2016	R/PNR	66/37	30.0/27.9	12	9 mW/cm² for 10 min	5.4	in 0–20% dextran every 1 min for 20–30 min	NR
Hagem	2017	PR	20/20	NR	12	9 mW/cm² for 10 min	5.4	with HPMC 1,1% for 20 min	every 2 min
Hashemi a	2015	PR	31/31	25.1	6	18 mW/cm² for 5 min	5.4	in 20% dextran every 3 min for 30 min	every 3 min
Hashemi b	2015	PR	31/31	25.1	18	18 mW/cm² for 5 min	5.4	in 20% dextran every 3 min for 30 min	every 3 min
Hashemian	2014	PR	76/77	22.3/22.6	15	30 mW/cm² for 3 min	5.4	in 20% dextran for 30 min	NR
Kymionis	2016	PR	16/16	27.6/25.1	1	18 mW/cm² for 7 min	7.56	in 20% dextran every 3 min for 30 min	every 3 min
Kymionis a	2014	PR	26/26	26.2/26.2	1	9 mW/cm² for 14 min	5.4	in 20% dextran every 3 min for 30 min	every 3 min
Kymionis b	2014	PR	9/12	22.3/23.2	1	9 mW/cm² for 10 min	7.56	in 20% dextran every 3 min for 30 min	every 3 min
Ng	2015	R	18/15	32.8/33.0	1	9 mW/cm² for 10 min	5.4	in 20% dextran every 2 min for 30 min	every 5 min
Ng	2016	R	14/12	36.1/32.6	mean: 13.9	9 mW/cm² for 10 min	5.4	in 20% dextran every 2 min for 25 min	every 5 min
Pircher	2016	R	50/36	NR	12	9 mW/cm² for 10 min	5.4	in 20% dextran for 30 min/with HPMC for 10 min	NR
Razmjoo	2017	PR	20/20	22.8/22.1	6	18 mW/cm² for 5 min	5.4	NR	NR
Sadoughi	2016	PR	15/15	19.4/19.4	mean: 12.1, minimum: 11	9 mW/cm² for 10 min	5.4	in 20% dextran every 2 min for 30 min	every 5 min
Sherif	2014	PR	11/14	23.6/21.6	12	30 mW/cm² for 4:20 min	7.8	in 20% dextran every 2 min for 30 min	every 2 min/none
Shetty a	2015	PR	36/36	22.8/24.2	mean: 15.3, minimum: 12	9 mW/cm² for 10 min	5.4	in 20% dextran every 2 min for 30 min	every 2 min
Shetty b	2015	PR	36/33	22.8/23.1	mean: 15.3, minimum: 12	18 mW/cm² for 5 min	5.4	in 20% dextran every 2 min for 30 min	every 2 min
Shetty c	2015	PR	36/33	22.8/19.9	mean: 15.3, minimum: 12	30 mW/cm² for 3 min	5.4	in 20% dextran every 2 min for 30 min	every 2 min
Tomita	2014	NR	18/30	30.8/31.1	12	30 mW/cm² for 3 min	5.4	in 20% dextran for 30 min/with HPMC for 15 min	NR
Touboul	2012	PNR	8/8	NR	6	30 mW/cm² for 3 min	5.4	in 20% dextran every 1 min for 30 min/in 20% dextran every 1 min for 10 min	every 5 min/NR

*Conventional cross-linking /accelerated cross-linking.
A-CXL = accelerated cross-linking, C-CXL = conventional cross-linking, HPMC = hydroxypropyl methylcellulose, min = minutes, NR = not reported, PNR = prospective non-randomized, PR = prospective randomized, R = retrospective.

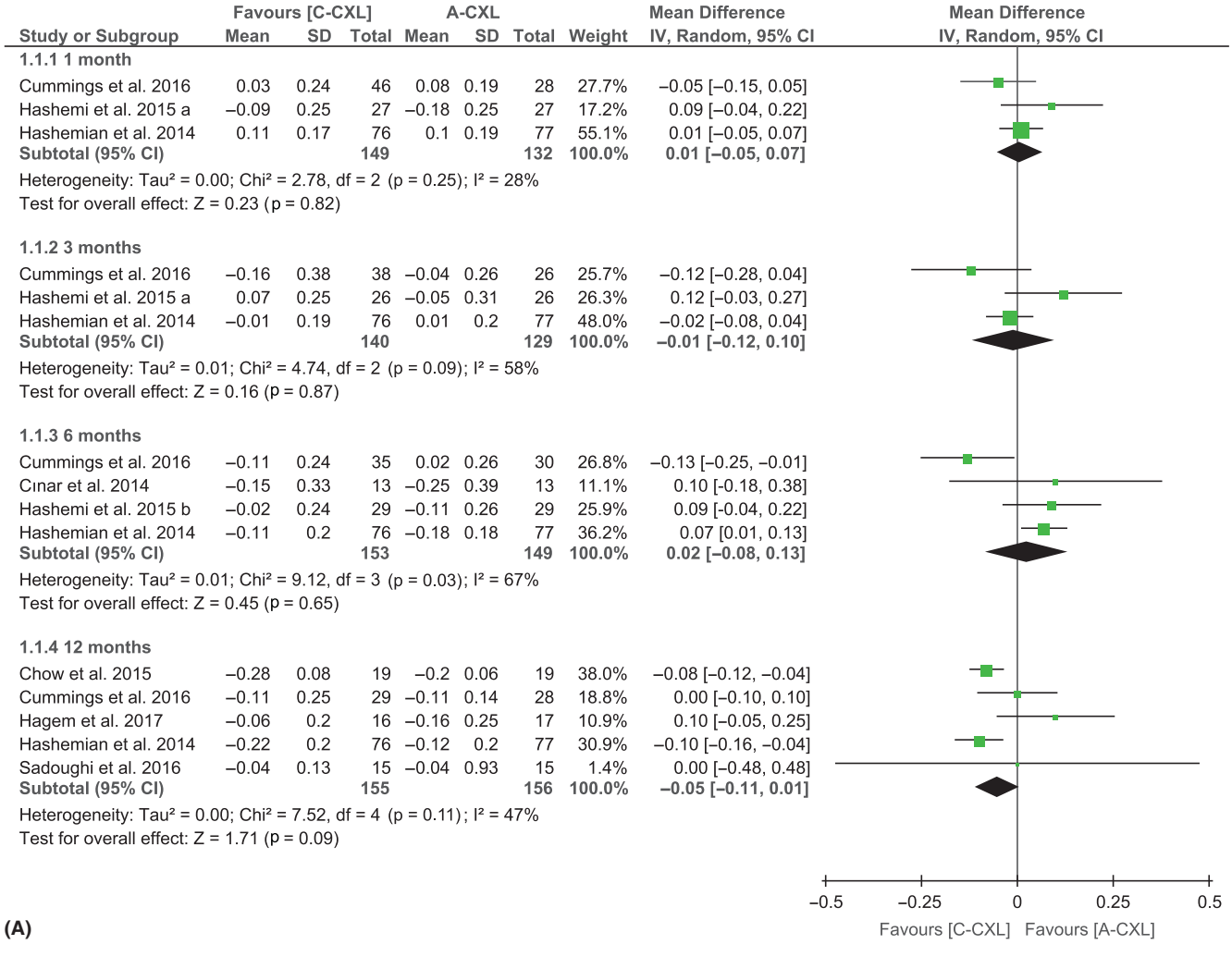


Fig. 1. Changes in visual acuity and refraction in conventional cross-linking (C-CXL) and accelerated cross-linking (A-CXL). CI = confidence interval, IV = inverse variance. (A) Uncorrected distance visual acuity (logMAR). (B) Corrected distance visual acuity (logMAR). (C) Cylindrical error (diopters).

C-CXL compared to A-CXL with a UV-A intensity of 9 mW/cm² for a treatment time of 10 min.

Corneal characteristics and structure

A higher early decrease of CCT was stated with C-CXL comparing both groups at 6 months (p = 0.14) (Fig. 3A). At 12 months, more declined CCT with C-CXL was found, without statistical significance between groups (p = 0.15). However, with A-CXL CCT seemed to remain more stable.

Changes in minimum corneal thickness were reported after 6 months (Cınar et al. 2014; Razmjoo et al. 2017) or after 12 months (Chow et al. 2015; Ng et al. 2016). Using C-CXL, a greater decrease in MCT was asserted (p = 0.0005) (Fig. 3B).

Corneal hysteresis (CH) and CRF were measured at 6 months (Hashemi et al. 2015b) or 12 months (Sherif 2014; Tomita et al. 2014; Sadoughi et al. 2016) postoperatively. Although C-CXL showed a smaller reduction in CH and CRF, statistical significance was missed comparing changes among both groups (p = 0.21 and 0.46 respectively) (Fig. 3C,D).

Throughout the follow-up (1, 3, and 6 months), a similar decrease in subbasal nerve density was discovered with both C-CXL and A-CXL (p = 0.63, 0.91, and 0.69 respectively).

Time of reepithelialization

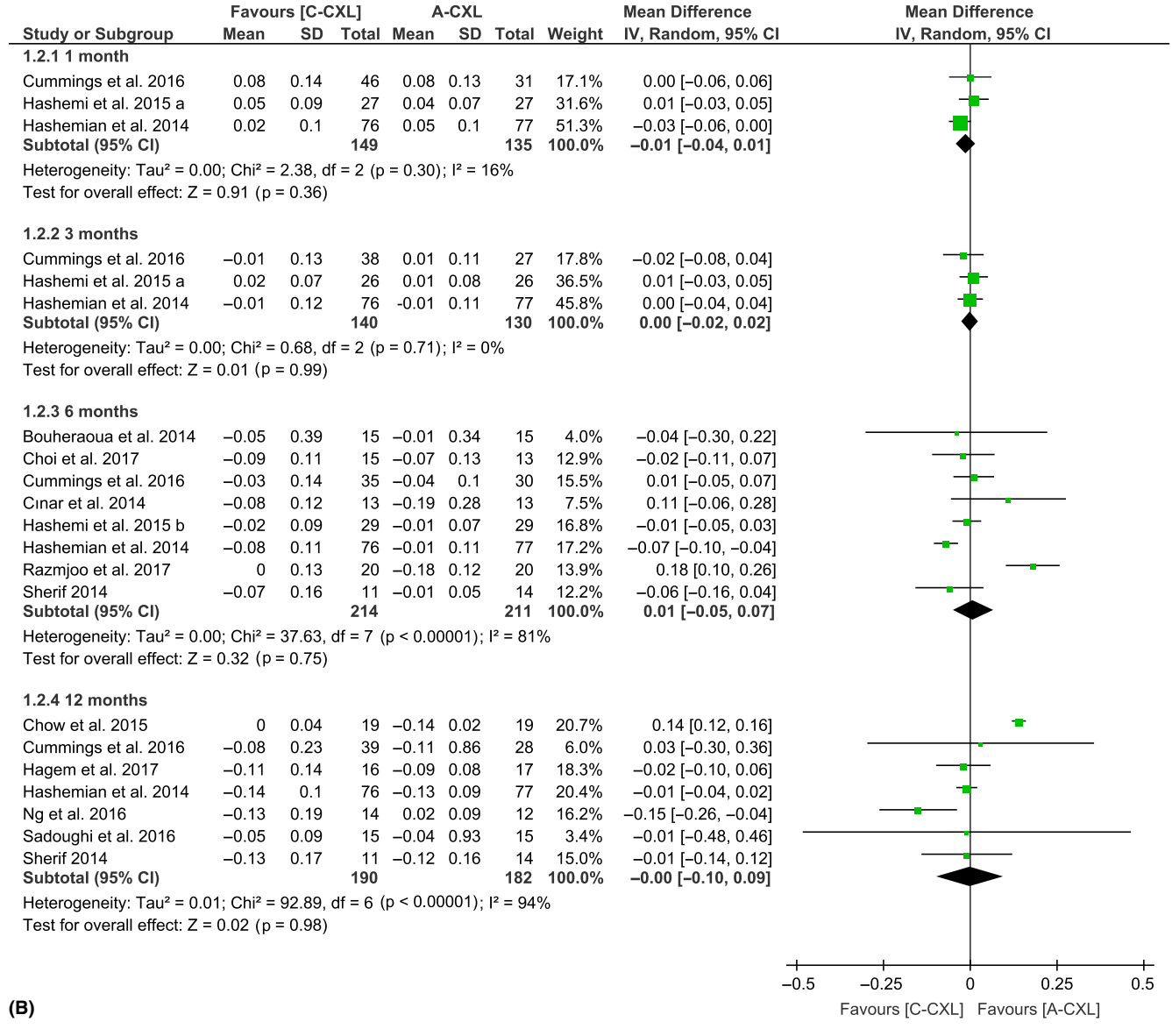
Summarizing three studies, the time of reepithelialization did not differ between both the C-CXL and A-CXL procedure (p = 0.76) (Fig. 4).

Endothelial cell profile

At the short-term follow-up of 1 month postoperatively, the change in endothelial cell density was greater with A-CXL (p = 0.25) (Fig. 5). Endothelial cell loss was greater with C-CXL at 6 and 12 months, just barely missing statistical significance at the 6-month follow-up (p = 0.06 and 0.30 respectively). Changes in percentage of hexagonal endothelial cells and coefficient of variation of endothelial cells, representing the cell morphology, were not significantly different in statistics in both groups (p = 0.6 and 0.4 respectively).

Demarcation line depth

In all the included studies, the DLD was provided by anterior segment optical coherence tomography after 1 month, except for 1 study (Shetty



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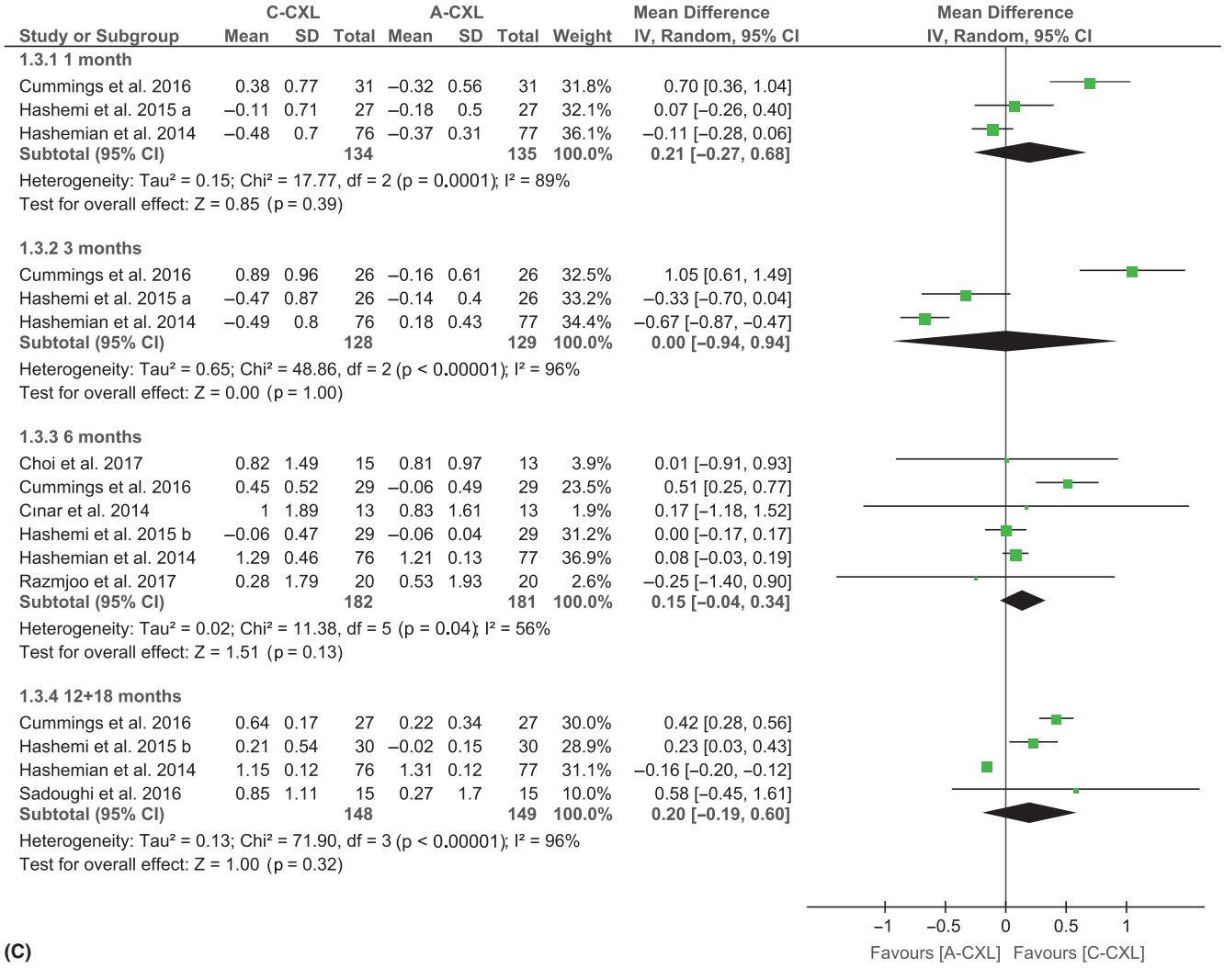
et al. 2015) that did not report the time of measurement. We performed a subgroup analysis of different A-CXL protocols (30 mW/cm² for 3 min, 9 mW/cm² for 10 min, remaining protocols). In the first two subgroups with intensities of 30 mW/cm² and 9 mW/cm², the demarcation line was significantly deeper with C-CXL ($p < 0.00001$ and 0.0007 respectively) (Fig. 6). The subgroup with all remaining protocols was composed of two trials (Kymionis et al. 2014a, 2016) using a higher cumulative dose of 7.56 J/cm² and 1 study (Shetty et al. 2015), using an intensity of 18 mW/cm² for 5 min. A deeper demarcation line was found with C-CXL in the third subgroup, just missing statistical significance ($p = 0.06$). Altogether, C-CXL revealed greater DLD by a significant margin ($p < 0.0001$).

Anterior stromal keratocyte density

Two trials (Bouheraoua et al. 2014; Hashemian et al. 2014) evaluated the change in anterior stromal keratocyte density. Since there were enormous differences in baseline parameters between the two studies (Bouheraoua: C-CXL 377, A-CXL 375; Hashemian: C-CXL 1249, A-CXL 1091), we did not perform a statistical analysis. At 1, 3, and 6 months postoperatively, a distinct greater reduction with C-CXL was stated by Hashemian et al. (2014) due to keratocyte apoptosis during CXL ($p = 0.00$ respectively). At the 12- and 15-month visit, the barely detectable differences between both procedures were not statistically significant ($p = 0.07$ and 0.06 respectively). Bouheraoua et al. (2014) reported the disappearance of keratocytes in the anterior stroma to be more distinctive with C-CXL at 1-, 3- and 6-month visits ($p = 0.042$, 0.006, and 0.089 respectively).

Complications

In all the included studies, only two trials (Sherif 2014; Shetty et al. 2015) documented any complications. Shetty et al. (2015) reported delayed epithelial healing in 2 cases of C-CXL, 3 cases of 9 mW/cm² A-CXL, and 1 case of 30 mW/cm² A-CXL. In only two eyes anterior



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stromal scarring was found after 9 mW/cm² A-CXL. At the 12-month follow-up, progression was stated in 1 case of 18 and 30 mW/cm² A-CXL respectively. Sherif (2014) documented trace or mild haze in 10 eyes of C-CXL and 10 eyes of 30 mW/cm² A-CXL. Only 1 case of severe central haze was reported in the C-CXL group after 1 month.

Eleven trials (Touboul et al. 2012; Bouheraoua et al. 2014; Cınar et al. 2014; Kymionis et al. 2014a,b, 2016; Chow et al. 2015; Hashemi et al. 2015a,b; Cummings et al. 2016; Ng et al. 2016) declared that no complications arose throughout the follow-up period. The other studies did not report whether complications occurred or not.

Discussion

Both minimal invasive procedures show great promise for slowing or halting keratoconus in ectatic corneas. However, the optimal treatment duration and dosage is still under investigation. We aimed to assemble the differences between the different protocols in a structured and objective manner. Our meta-analysis should facilitate the decision whether to use the standard or accelerated protocol for the treatment of progressive keratoconus.

Visual acuity and manifest refraction

Visual acuity (CDVA and UDVA) and subjective refraction (spherical equivalent, spherical and cylindrical refraction) are clinically important as these parameters may reflect the patients' satisfaction with the treatment. Comparing the final follow-up results, C-CXL showed a better improvement in UDVA whereas CDVA was equally improved with both techniques. Most patients still need visual aid. Therefore, we assume CDVA to be more important for a patients' quality of life, resulting in no preference for either protocol.

Our results with respect to the spherical equivalent, spherical and cylindrical error were found to be in conflict. Basically, the low repeatability of subjective refraction in keratoconus patients should be considered, by reason of optical irregularities of the distorted cornea causing blurring (Raasch et al. 2001). To calculate the spherical equivalent, measurements of spherical and cylindrical error are used. Thus, we expected similar results comparing these parameters. In contrast, at the last follow-up, improvements in spherical equivalent and cylindrical error were greater with C-CXL, whereas a higher decrease in spherical error was ascertained in the A-CXL group.

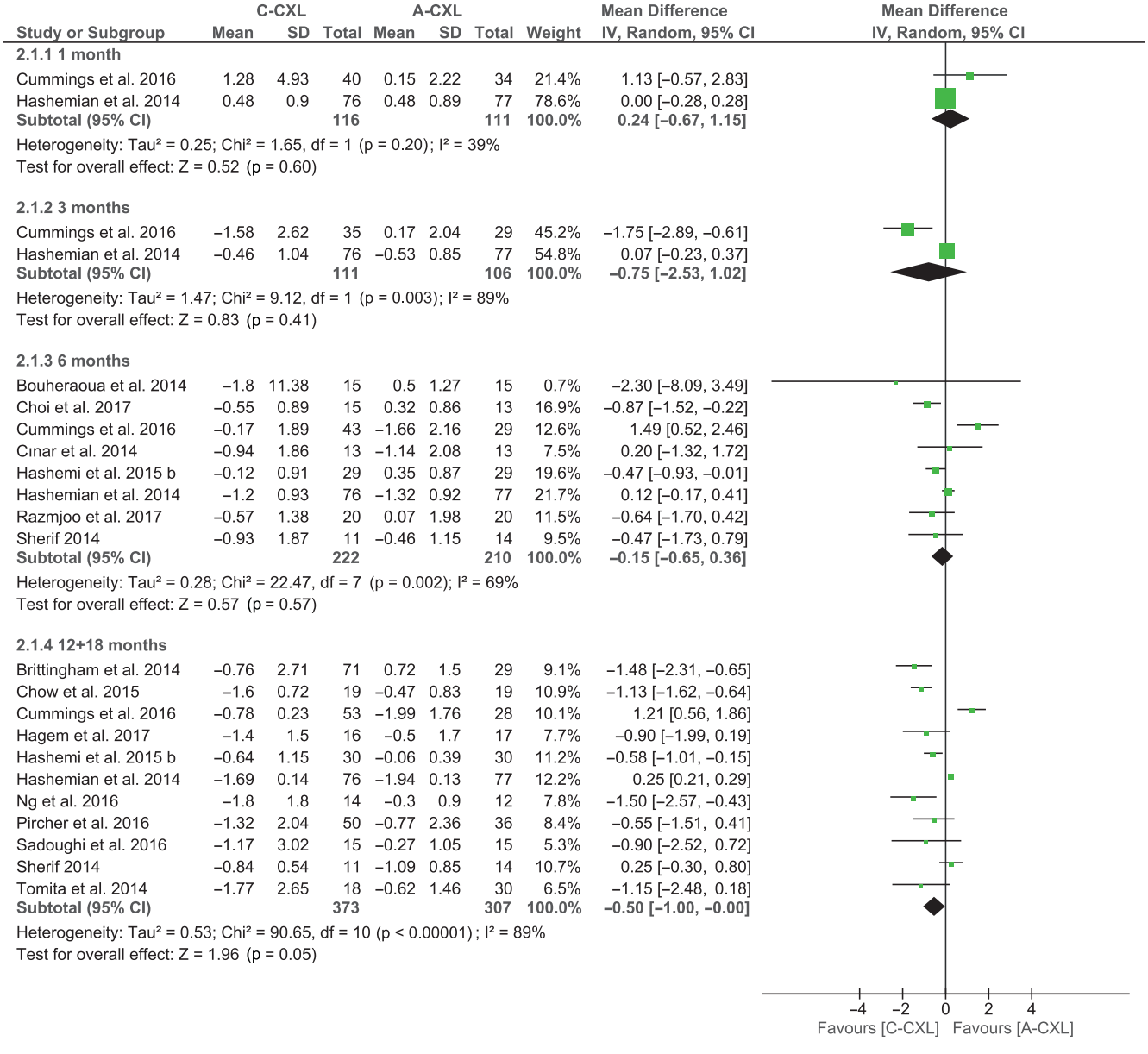


Fig. 2. Changes in maximal keratometry (diopters) in conventional cross-linking (C-CXL) and accelerated cross-linking (A-CXL). CI = confidence interval, IV = inverse variance.

Overall, we could not find any distinct differences of change in VA and refraction after C-CXL compared to after A-CXL.

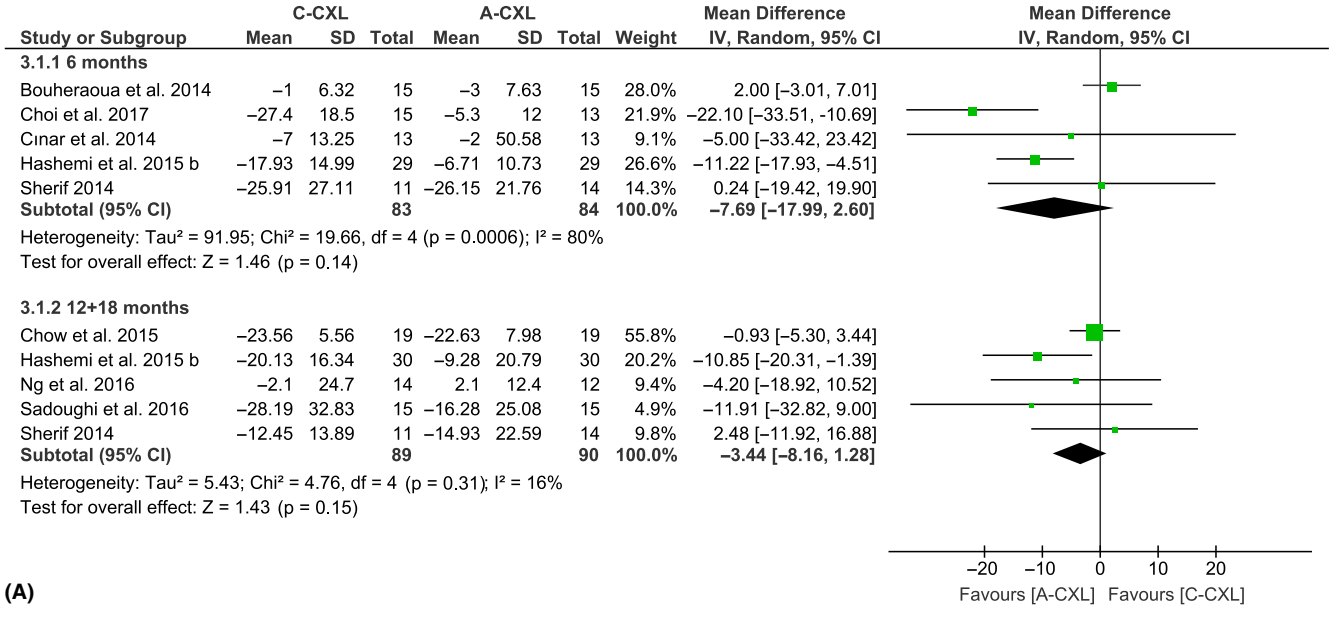
Keratometry

Despite using higher irradiances, A-CXL provided less corneal flattening than C-CXL regarding minimum, average, and maximal keratometry values at the final follow-up.

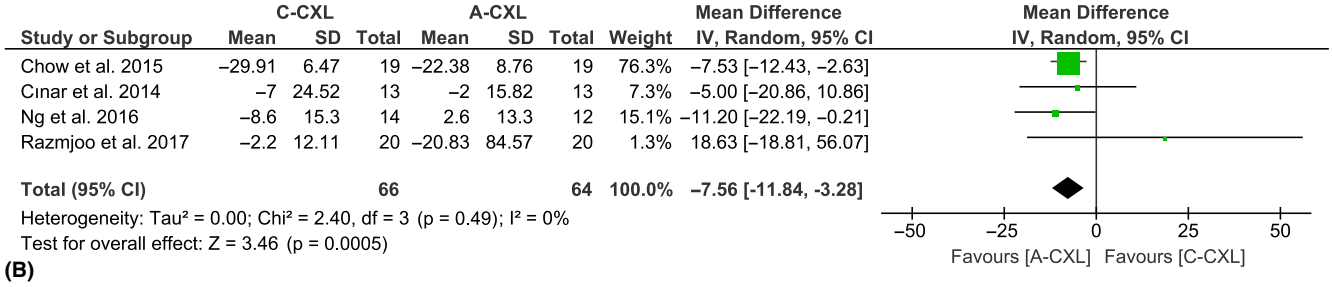
Keratometric change represents progression of keratoconus. However, in both studies (Hashemi et al. 2015b; Cummings et al. 2016) reporting 6 and 12 months results, K_{mean} decreased further from 6 to 12 months postoperatively. This was similar to 1 trial (Sherif 2014) measuring K_{min} and 4 studies (Hashemian et al. 2014; Sherif 2014; Hashemi et al. 2015b; Cummings et al. 2016) measuring K_{max}, except for 1 single case of increasing K_{max} with C-CXL (Sherif 2014). Altogether, both procedures provided promising results concerning the halt of keratoconus.

The greater the preoperative corneal steepness, the higher the flattening effect (Sloot et al. 2013). However, we do not expect any influence of baseline values since they were similar in both groups (p = 0.71 Mann–Whitney U-test).

Assessing K_{max} at the end of the follow-up, all studies found the 9 mW/cm² protocol to provide better corneal flattening with C-CXL except for 1 study (Cummings et al. 2016) that favours A-CXL. Statistical significance was missed. Using 18 mW/cm², two studies discovered significantly better results with C-CXL. Conventional Dresden protocol (C-CXL) resulted in a more pronounced decrease of keratometry when applying a very high UV-A intensity of 30 mW/cm², without reaching statistical significance. Overall, a general statement regarding different A-CXL protocols is difficult since changes during the



(A)



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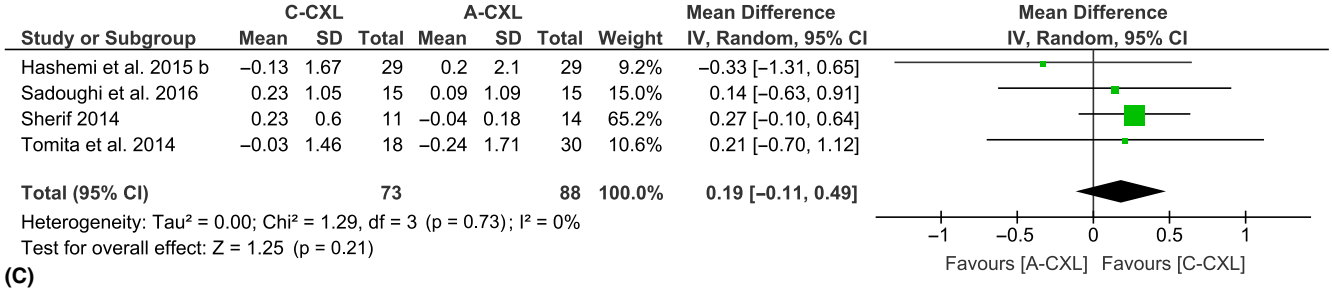

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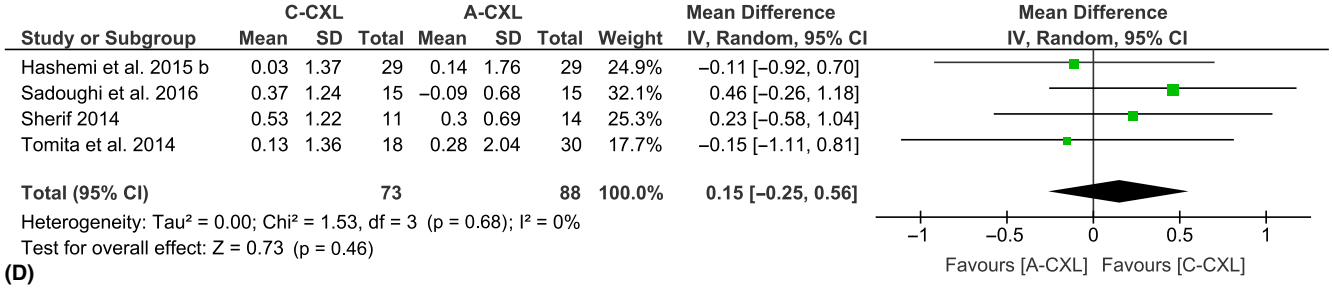

(D)

Fig. 3. Changes in corneal characteristics and structure in conventional cross-linking (C-CXL) and accelerated cross-linking (A-CXL). CI = confidence interval, IV = inverse variance. (A) Central corneal thickness (μm). (B) Minimum corneal thickness (μm). (C) Corneal hysteresis (mmHg). (D) Corneal resistance factor (mmHg).

longest and shortest protocols are similar to C-CXL.

Corneal characteristics and structure

Analysis of pachymetry included measurements of central and minimum corneal thickness. A difference in corneal thinning was observed at the final follow-up, with greater changes using C-CXL. A higher amount of corneal thinning with C-CXL may be the consequence of increased keratocyte apoptosis compared to A-CXL. Another explanation is greater corneal compactness after C-CXL due to a higher cross-linked volume (Greenstein et al. 2011).

Corneal hysteresis (CH) and CRF are measurements of corneal stability. Lower values stand for a biomechanically

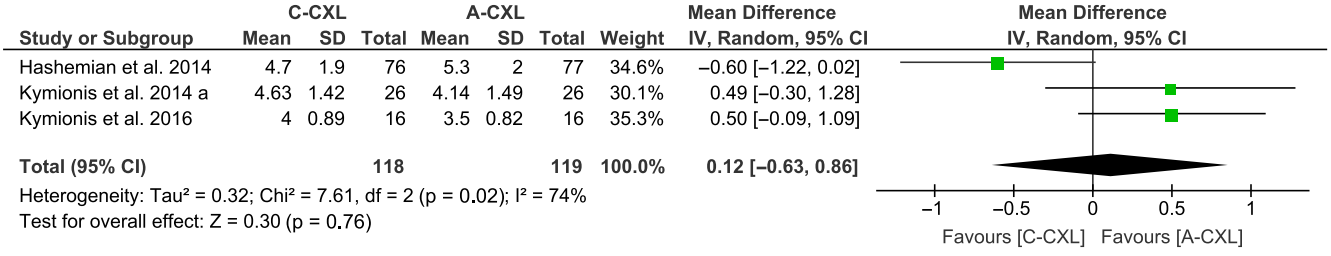

Fig. 4. Time of reepithelialization (days) in conventional cross-linking (C-CXL) and accelerated cross-linking (A-CXL). CI = confidence interval, IV = inverse variance.

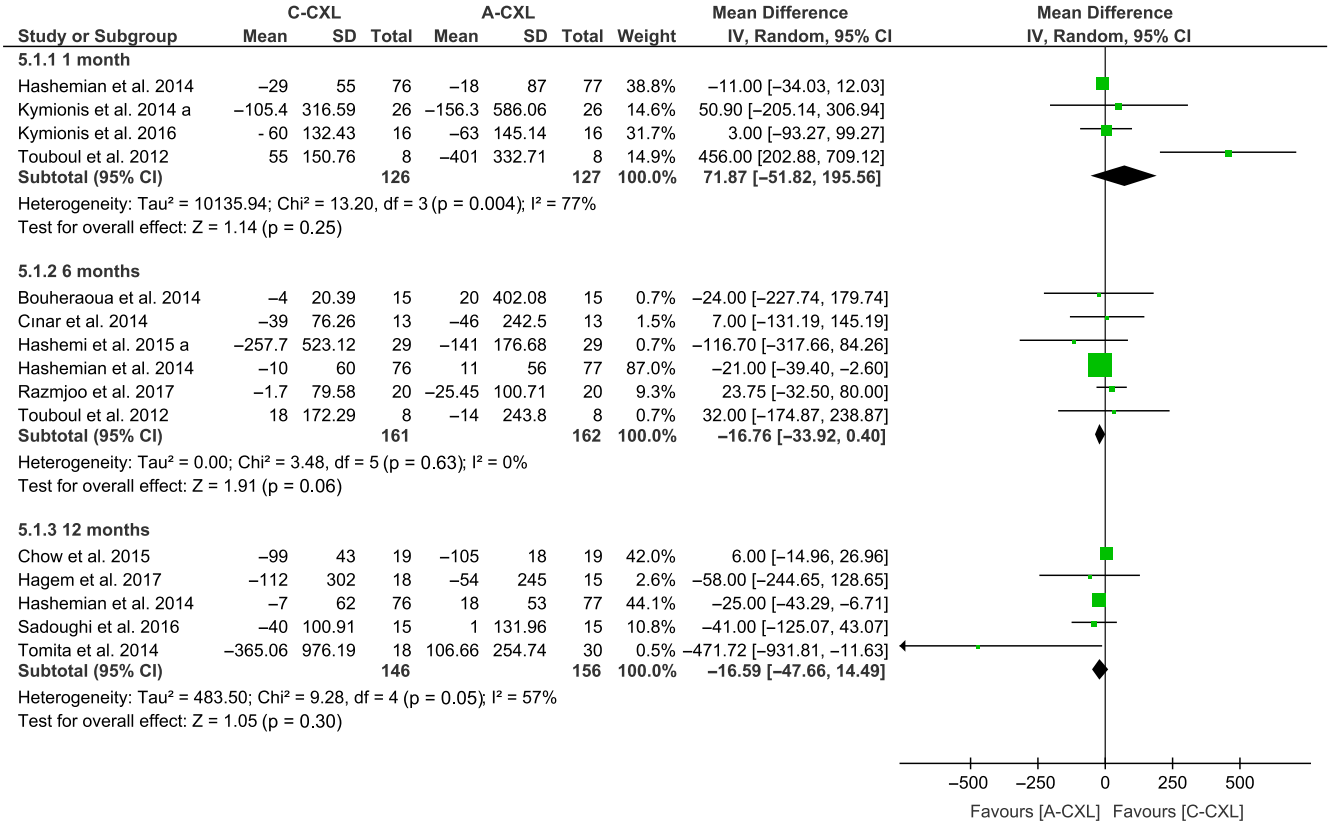

Fig. 5. Change in corneal endothelial cell density (cells/mm²) in conventional cross-linking (C-CXL) and accelerated cross-linking (A-CXL). CI = confidence interval, IV = inverse variance.

weaker cornea. Therefore, C-CXL is assumed to provide slightly better corneal stabilization than A-CXL.

With both techniques, brushing of the corneal epithelium leads to a loss of subbasal nerve fibres. Longer exposition time to UV-A irradiation may be the reason for the greater effect of C-CXL on the cornea, and thus differences in nerve population (Touboul et al. 2012). This was not confirmed by our finding of equal decrease in subbasal nerve density in both groups. However, only two studies were included in this comparison so our analysis cannot provide definitive information. One study (Hashemian et al. 2014) reported greater loss of subbasal nerves with C-CXL whereas another one (Bouheraoua et al. 2014) ascertained the contrary. Overall, the difference between both groups did not reach statistical significance. Touboul et al. (2012) did not indicate any quantitative values but also found nerve loss after both procedures.

Time of reepithelialization

The epithelial wound healing time is fundamental as the time of corneal reepithelialization determines the period of the cornea to be vulnerable to infectious keratitis (Hovakimyan et al. 2012). However, a similar behaviour was ascertained in both groups.

Endothelial cell profile

Due to the exposure of corneal structures to UV-A light and the induction of oxygen-free radicals, keratocyte cell destruction or apoptosis as well as endothelial cell damage are possible complications of CXL (Spoerl et al. 2007). Endothelial cell damage may lead to loss of VA on account of corneal edema. Therefore, endothelial cell count is a clinically important factor influencing patient satisfaction.

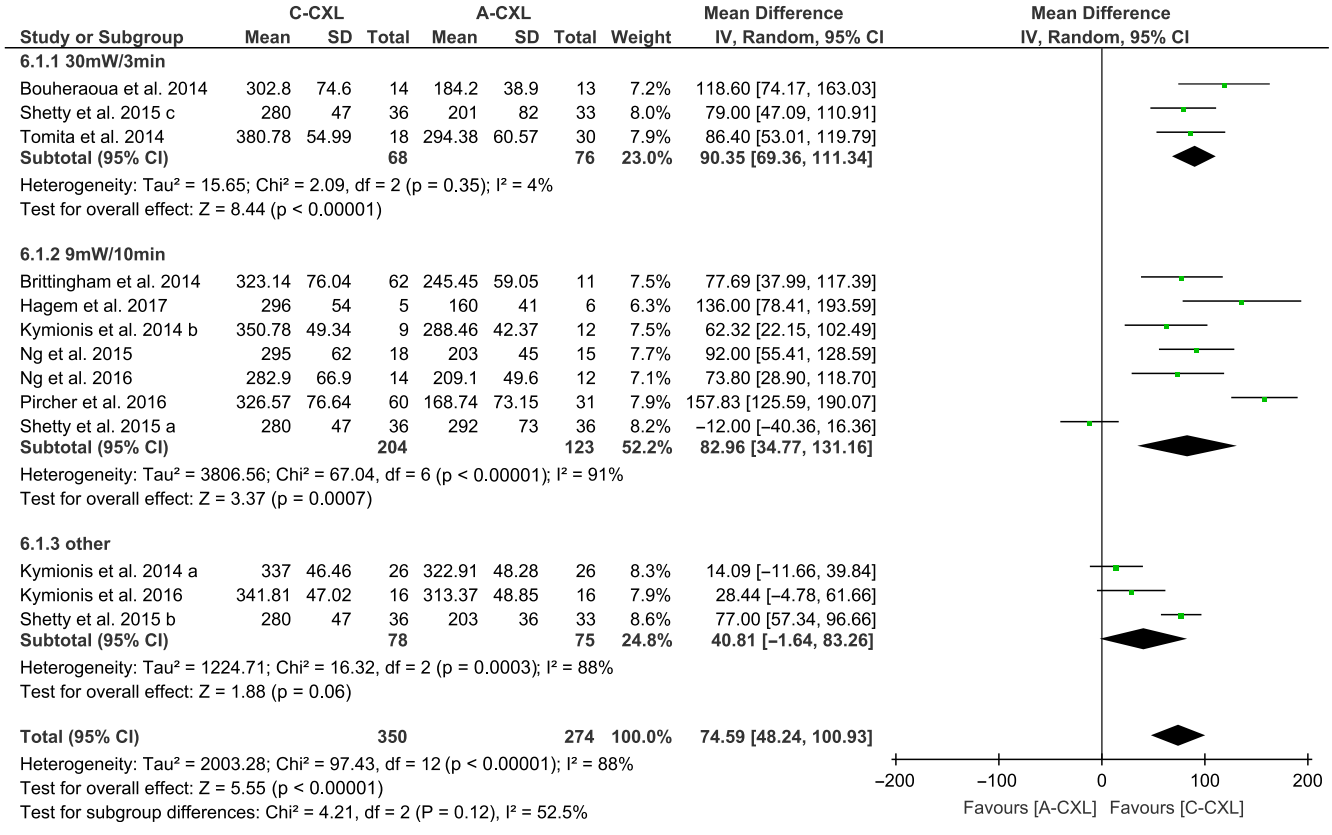

Fig. 6. Demarcation line depth (μm) in conventional cross-linking (C-CXL) and accelerated cross-linking (A-CXL). CI = confidence interval, IV = inverse variance.

In order to assess possible toxic effects on the corneal endothelium, we evaluated the endothelial cell profile consisting of endothelial cell density and endothelial cell indices as percentage of hexagonal endothelial cells and the coefficient of variation of endothelial cells. Endothelial cell loss seemed to be slightly more pronounced after C-CXL. The rather low number of eyes included in the analysis of hexagonality and coefficient of variation might not be able to provide sufficient information when comparing C-CXL and A-CXL. No significant differences of changes in ECD and endothelial cell indices were observed though. Hence, neither longer UV-A exposition nor higher UV-A irradiation intensity induced pronounced endothelial cell damage. This is similar to the findings by Touboul et al. (2012), discovering no damage of the endothelium with either procedure. Sadoughi et al. (2016) showed no significant correlation between changes in ECD and CCT in any of the groups. Thus, it is supposed that in practice, endothelial cell loss does not depend on whether the cornea is rather thin or thick. However, since CXL is likely to have toxic effects up to a depth of approximately 300–350 μm, it is not recommended to treat corneas thinner than 400 μm in order to prevent endothelium and lens damage (Spoerl et al. 2007; Dhawan et al. 2011).

Demarcation line depth

The DLD is useful to ascertain the corneal CXL treatment depth. It marks the area of elongated or apoptotic keratocytes due to exposure to riboflavin and UV-A light (Seiler & Hafezi 2006; Bouheraoua et al. 2014). Overall, all studies included in our meta-analysis reported A-CXL to result in shallower DLD, except for one study using the 9 mW/cm² for 10 min protocol (Shetty et al. 2015). Statistical significance was reached in 2 subgroups. Touboul et al. (2012) also noted a deeper demarcation line after C-CXL, unfortunately without reporting any absolute values. One reason for a shallower demarcation line after A-CXL may be insufficient availability of oxygen (Richoz et al. 2013; Ng et al. 2015).

Possibly due to reduced soak time, the occurrence of demarcation lines was less with A-CXL (Brittingham et al. 2014; Ng et al. 2015; Hagem et al. 2017). Demarcation line depth (DLD) is claimed to reliably monitor the treatment depth and efficacy (Doors et al. 2009; Kymionis et al. 2014b). However, Bouheraoua et al. (2014) did not discover any correlation between DLD and changes in CDVA, K_{max}, or CCT. Ng et al. (2016) also determined no correlation between DLD and change in K_{max} since the maximal keratometry value is often not measured at the corneal centre. In contrast, the DLD significantly correlated with K_{mean} (Ng et al. 2016). Assuming DLD to represent the efficacy level of CXL, it could be concluded that C-CXL results in higher volume of cross-linked stroma than A-CXL, and thus in greater treatment effect on the ectatic cornea.

Comparing the absolute values of DLD, we found a mean DLD of 224 μm in the 9 mW/cm² group and 227 μm in the 30 mW/cm² group. Hence, there was approximately no difference between the longest and shortest A-CXL protocol.

Kymionis et al. published two studies (Kymionis et al. 2014a, 2016) evaluating A-CXL protocols with a higher cumulative dose of 7.56 J/cm² and found similar DLD in both the C-CXL and A-CXL. This is the reason for the missing significance in our third subgroup. It should be remarked that both protocols with the higher cumulative dose resulted in DLD of more than 310 μm that is much deeper than with cumulative doses of 5.4 J/cm². This finding indicates higher treatment efficacy with high-intensity A-CXL protocols compared to standard A-CXL. Using identical cumulative doses in both groups lead to a deeper demarcation line in the C-CXL group (Kymionis et al. 2014b). Their results indicate that the Bunsen–Roscoe law is inapplicable for CXL in practice.

It should be considered that the deeper the demarcation line and thus the treatment area, the higher the risk of endothelial cell damage (Hagem et al. 2017). For this reason, it is of prime importance not to illuminate corneas thinner than 400 μm. Since A-CXL with a cumulative dose of 5.4 J/cm² shows a significant shallower treatment depth, it is supposed to be a safer procedure in rather thin corneas compared to C-CXL. This is similar to the recommendation by Kymionis et al. (2014b) and Bouheraoua et al. (2014). Overall, if corneas thinner than 400 μm require treatment with CXL, an A-CXL protocol with a standard cumulative dose of 5.4 J/cm² should be used. For corneas that are not remarkably thin, C-CXL can be performed as well as A-CXL with higher cumulative doses in an attempt to improve treatment efficacy.

Anterior stromal keratocyte density

Oxygen free radicals which are necessary for bonding collagen fibres are responsible for keratocyte apoptosis (Spoerl et al. 2007). Only two studies evaluated reduction of anterior keratocytes. The results by Bouheraoua et al. (2014) were congruent to those by Hashemian et al. (2014) that reported greater apoptosis of keratocytes in the anterior stroma using C-CXL compared to A-CXL. However, keratocyte density at 15 months postoperatively was similar to preoperative values in both groups which illustrates keratocyte repopulation (Hashemian et al. 2014). Due to a lack of appropriate studies reporting quantitative results, we cannot state any distinct effect. Touboul et al. (2012) stated obliteration of stromal keratocytes after both techniques, with greater morphologic change with A-CXL. Mazzotta et al. (2015) reported reduction of keratocyte density in both groups after the first 6 months, with no significant difference after 2 years which is consistent with the findings by Bouheraoua et al. (2014) and Hashemian et al. (2014). Even though greater keratocyte loss was observed at short-term follow-up with C-CXL compared to A-CXL, it seems like there is no difference of long-term effect on the anterior stromal keratocyte density among both techniques.

Complications

Several studies reported complications after both procedures. Sharma et al. (2012) found corneal oedema in 2.9% of 350 eyes treated with C-CXL after approximately 14 months. In contrast, Mazzotta et al. (2012) stated a disappearance of corneal edema 6 weeks after C-CXL. After A-CXL, they also described corneal edema to disappear after 6 months (Mazzotta et al. 2016). Hersh et al. (2017) reported 3 of 102 eyes having clinically significant corneal haze at 1 year after C-CXL. Bozkurt et al. (2017) did not state any corneal haze at 24 months after A-CXL. Overall, transient corneal haze is a common phenomenon after CXL, also reported in the study by Sherif (2014). A very rare complication after CXL is microbial keratitis. Shetty et al. (2014) found 4 eyes with keratitis in 1715 eyes undergoing C-CXL. In the A-CXL group, they did not state any case of this adverse finding. However, it should be noted that this group only consists of 325 eyes. Development of sterile corneal infiltrates is another complication that occurs more often after A-CXL (Cerman et al. 2017).

Since only two studies of our meta-analysis described any adverse events, we assume both procedures to be safe in the treatment of progressive keratoconus. If any serious complications had occurred, they would very probably have been reported. Thus, we do not expect any differences in long-term complications between both techniques.

Limitations

Our meta-analysis has several limitations

According to the Bunsen–Roscoe law, equal cumulative doses should provide the same treatment effect (Bunsen & Roscoe 1857). As mentioned before, this is not true for corneal CXL which was confirmed by a study on porcine corneas. Irradiating with higher intensities for shorter time duration, the biomechanical effect seemed to decrease, resulting in less stiffening (Hammer et al. 2014). Even if a cumulative dose of 5,4 J/cm² is applied, which is similar to the standard Dresden protocol, it does not mean that the effect on living corneal structures is comparable using different variations of irradiation time and dosage. Shetty et al. (2015) compared C-CXL to 3 different protocols of A-CXL with a cumulative dose of 5.4 J/cm². They found the C-CXL to provide the maximum flattening effect. With the highest UV-A intensity of 30 mW/cm² for 3 min, only a little stabilization of keratoconus was achieved. They conclude the A-CXL with 9 mW/cm² for 10 min and 18 mW/cm² for 5 min to have comparable effects in treatment of this eye disease compared to C-CXL. Effectiveness of the very high-intensity protocol was queried. The delivered cumulative dose seems to be not the only factor having an impact on the corneal properties. Nevertheless, we evaluated all protocols of A-CXL together, even though they were not uniform. Furthermore, there were some studies using a higher cumulative dose of 6.6 J/cm² (Choi et al. 2017), 7.56 J/cm² (Kymionis et al. 2014a, 2016), or 7.8 J/cm² (Sherif 2014). For K_{max} at the final follow-up and DLD, we generated subgroups according to the A-CXL protocol used. However, this was not possible for all parameters as too few studies exist comparing outcomes such as corneal biomechanical properties. If a differentiated statement in terms of effectiveness of different A-CXL protocols should be made, studies comparing different A-CXL protocols are to be included in such a meta-analysis in order to achieve reliable conclusions. It should be remarked that the duration and frequency of riboflavin instillation before and during UV-A irradiation also differed among the included studies. Longer application may result in

Table 2. Summary of evaluated comparisons.

Variable	Favoured protocol	p-value*
Demarcation line depth	C-CXL	<0.00001
Minimum keratometry	C-CXL	<0.00001
Minimum corneal thickness	A-CXL	0.0005
Maximal keratometry	C-CXL	0.05
Uncorrected distance visual acuity	C-CXL	0.09
Spherical equivalent	C-CXL	0.11
Central corneal thickness	A-CXL	0.15
Corneal hysteresis	C-CXL	0.21
Spherical error	A-CXL	0.29
Endothelial cell density	A-CXL	0.30
Cylindrical error	C-CXL	0.32
Coefficient of variation of endothelial cells	A-CXL	0.40
Corneal resistance factor	C-CXL	0.46
Percentage of hexagonal endothelial cells	C-CXL	0.60
Average keratometry	C-CXL	0.65
Subbasal nerve density	A-CXL	0.69
Time of reepithelialization	A-CXL	0.76
Corrected distance visual acuity	C-CXL	0.98

*p-value for the comparison between both protocols.
A-CXL = accelerated cross-linking, C-CXL = conventional cross-linking, min = minutes.

increased corneal penetration with riboflavin, and thus in different treatment effects.

Another limitation occurs since different compositions of riboflavin solutions were used, concerning different delivery vehicles. Riboflavin in 20-% dextran is used by default. Nevertheless, some studies reported the use of riboflavin with hydroxypropyl methylcellulose (HPMC) which is supposed to cause less corneal thinning during the procedure (Oltulu et al. 2014). Three studies (Tomita et al. 2014; Pircher et al. 2016; Choi et al. 2017) only used this composition in the A-CXL group, whereas Hagem et al. (2017) applied it to both groups. Hydroxypropyl methylcellulose (HPMC) was shown to increase the diffusion rate of fluorescein (Waltman & Patrowicz 1970). Hence, it is also supposed to allow for a better corneal penetration of riboflavin resulting in a deeper treatment area (Choi et al. 2017; Hagem et al. 2017). Reducing the surface tension, methylcellulose may be responsible for a lower risk of corneal desiccation as well as the shorter treatment in A-CXL (Choi et al. 2017). One drawback of HPMC is the cause of corneal swelling, and thus a decreased concentration of stromal collagen bundles which contribute to lower efficacy of CXL using riboflavin with HPMC (Mark et al. 2014). The composition and soak time may be influencing factors on the treatment efficacy and responsible for differences of changes in CCT (Choi et al. 2017). In our meta-analysis, comparison of CCT only included 1 study (Choi et al. 2017) using HPMC in the A-CXL group. For this reason, we do not expect different preparations of riboflavin to have a prominent influence on the results concerning CCT.

Furthermore, we selected all study designs and did not differentiate if they were prospective randomized, and thus more valuable, or not. All trials were weighted similarly. In some comparisons, heterogeneity was displayed, possibly due to variation at baseline or missing uniformity in conduct. However, all funnel plots were unremarkable and did not reveal any publication bias.

Conclusion

Our results are summed up in Table 2. The parameters were graded according to their p-value. In conclusion, C-CXL and A-CXL seem to provide comparable results in halting keratoconus. However, larger studies with longer follow-up times are necessary to evaluate the long-term results of different A-CXL protocols compared to C-CXL.

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