

BMJ Open Transepithelial versus epithelium-off corneal collagen cross-linking for corneal ectasia: protocol for a systematic review, meta-analysis and trial sequential analysis of randomised controlled trials

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ABSTRACT

Introduction Corneal ectasias are progressive, degenerative ocular diseases defined by abnormal structural changes in the cornea, leading to distortion of vision and substantial reduction in quality of life. Corneal collagen cross-linking (CXL) increases the biomechanical rigidity of the cornea and has been shown to halt ectatic processes. The established CXL protocol requires removal of the corneal epithelium. However, some surgeons have proposed transepithelial approaches to enhance patient recovery and minimise adverse events. Whether novel transepithelial approaches are as effective in arresting ectasia as the established epithelium-off protocol remains unclear. This study will systematically review the evidence on transepithelial CXL approaches and compare it to the epithelium-off protocol.

Methods and analysis We will include randomised controlled trials (RCTs) comparing transepithelial and epithelium-off CXL for any corneal ectasia. We will search 16 electronic databases including MEDLINE and Embase, as well as the grey literature. Two reviewers will independently screen search results to identify eligible studies, complete data abstraction and conduct quality assessment. We will assess the quality of individual RCTs using the Cochrane risk of bias assessment tool. Our primary outcome will be the change in maximal keratometry at 12 months after treatment, and we will examine 11 additional outcomes. We will summarise our analyses by measures of association (relative risk or odds ratio) and corresponding 95% confidence intervals (CIs) for dichotomous outcomes and weighted mean differences with 95% CIs for continuous outcomes. Prespecified subgroup analyses will be conducted to explore heterogeneity. The overall quality of evidence will be rated using the Grading of Recommendations Assessment, Development and Evaluation approach.

Ethics and dissemination Ethics approval is not required for this systematic review as it draws from previously published data. Results of the study will be submitted to a peer-reviewed journal for publication and discussed at conferences and seminars.

Strengths and limitations of this study

- This systematic review will examine the evidence on transepithelial cross-linking approaches and compare it to the established epithelium-off protocol for any corneal ectasia.
- A comprehensive and current literature search, conducted without language or time restrictions and developed in consultation with an academic librarian, will be implemented in order to capture all available evidence.
- Given the variation in protocols for loading riboflavin in transepithelial approaches, as well as the different techniques for removing the corneal epithelium, we expect to encounter heterogeneity. We will investigate heterogeneity through prespecified subgroup analyses.
- Our review will not provide a cost analysis investigating the economic impact of different cross-linking approaches.

PROSPERO registration number CRD42018102069

INTRODUCTION

Corneal ectasias are a group of progressive, degenerative ocular diseases characterised by thinning, bulging and structural changes in the central, paracentral or peripheral cornea, leading to distortion of vision.¹ The most common corneal ectasia is keratoconus, and recent estimates from the Netherlands find its prevalence to be as high as 1 in 375.² Despite considerable research into the genetics and molecular mechanisms underlying ectatic processes, the pathophysiology of these disorders remains poorly understood.³ While many patients with ectasia can be managed with spectacles or rigid gas permeable lenses,

disease-modifying treatments remain sparse, and a subset of patients have disease progression that necessitates a corneal graft.¹

Corneal collagen cross-linking (CXL) was first proposed in the late 20th century as a potential disease-modifying intervention for corneal ectasia.⁴ The aim of CXL is to strengthen the corneal stroma by increasing the number of covalent cross-links between collagen molecules, fibres and microfibrils. This is accomplished by using riboflavin as a photosensitiser activated by ultraviolet A (UVA) light to create free radicals and induce cross-link formation via the natural lysyl oxidase pathway.^{4,5} These increased cross-links function to enhance the biomechanical rigidity of the cornea and protect against further thinning and degeneration. Following successful animal and human studies, CXL was approved for treating keratoconus and other corneal ectasias in the early 2000s across Europe and in 2016 in the USA.^{6,7}

The standard approved CXL protocol involves removal of the central 7 mm of corneal epithelium prior to the addition of riboflavin, in order to facilitate sufficient penetration of the compound into the corneal stroma. The cornea is then exposed to UVA light for a predetermined time period to achieve a fluence of 5.4 J/cm² to allow cross-links to form.⁶ While removal of the corneal epithelium is routine in ophthalmic practice, it carries risks of postoperative infection, is associated with substantial patient discomfort and requires a prolonged recovery period. Recently, transepithelial approaches for loading riboflavin into the corneal stroma have been proposed.⁸ These protocols aim to sufficiently saturate the cornea with riboflavin in order to facilitate cross-linking without the need to remove the corneal epithelium, and thus, are associated with increased patient comfort and a faster recovery time. Transepithelial approaches also allow for safe treatment of corneas that have thinned beyond the threshold required for epithelium-off CXL, thereby expanding treatment availability to a greater number of patients.

While transepithelial approaches offer significant theoretical benefits, their safety and efficacy in comparison to established epithelium-off protocols remain unclear. Therefore, we will conduct a systematic review and meta-analysis in order to examine transepithelial CXL in comparison to epithelium-off CXL for corneal ectasia. This study protocol has been developed in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analysis Protocols (PRISMA-P)⁹ statement and is registered with the International Prospective Register of Systematic Reviews (PROSPERO).

METHODS AND ANALYSIS

We will conduct our systematic review and meta-analysis in agreement with the PRISMA^{10,11} statement and the *Cochrane Collaboration's Handbook for Systematic Reviews of Interventions* guidelines.¹² This protocol will be amended and updated as required in line with PRISMA-P criteria⁹

and updated versions will be made available on PROSPERO with catalogued version history.

Literature search

We will conduct a detailed search of the following electronic databases: MEDLINE, Embase, Web of Science, Cochrane CENTRAL, Cochrane DSR, CINAHL, OpenGrey, metaRegister of Controlled Trials, LILACS, ClinicalTrials.gov, WHO Clinical Trials Database, WangFangData, INSPEC, COMPENDEX, CQVIP and CNKI, from inception through to May 1, 2019. We will use keyword and medical subject heading terms related to corneal ectasia and the cross-linking protocol (transepithelial or epithelium-off) and clinical outcomes. Search strategies will be developed collaboratively by a multidisciplinary research team comprising clinicians, researchers and academic librarians. The search strategy proposed for the MEDLINE database is provided in [table 1](#). The electronic search will be supplemented by manually screening the references of relevant articles, reviewing the proceedings of pertinent meetings and contacting clinical experts in the field. Our search will be conducted without publication type, language or time restrictions.

Study selection

Search results will be collated and evaluated independently by two reviewers (SN and CS) against predefined eligibility criteria in order to identify relevant studies ([figure 1](#)). Results from all searched databases will be exported as .RIS, .CIW or .XML files containing the complete reference, and EndNote X9 (Clarivate Analytics, Philadelphia, Pennsylvania, USA) will be used for reference management. Reviewers will screen titles and abstracts (level I screening) against prespecified inclusion criteria and complete reports will be retrieved for all references that meet these criteria (level II screening), or where there is any ambiguity. In cases of ambiguity, the full text of the relevant study will be evaluated independently by both reviewers in order to reach a decision on inclusion. We will record the level of agreement between reviewers, and disagreements will be resolved collegially by discussion and consensus, and if needed, including an impartial reviewer (AK) or contacting the study authors. Our inclusion criteria will be as follows:

- ▶ *Study design*: randomised controlled trials (including cluster trials and pilot studies) comparing transepithelial and epithelium-off CXL, with no publication type (abstracts vs full articles), time or language restrictions. We will include both completely published reports and conference abstracts in the grey literature. Abstracts will be included only if they fulfil our eligibility criteria and if no follow-up study has been published.
- ▶ *Population*: patients of any demographic undergoing CXL for treatment of corneal ectasia following refractive surgery, keratoconus or pellucid marginal degeneration.
- ▶ *Intervention*: transepithelial CXL.

Table 1 Search strategy for the MEDLINE electronic database using the Ovid interface

Database	Search terms
Medline 1946–present	<ol style="list-style-type: none"> 1. Cross-Linking Reagents/ 2. CORNEA/ 3. cornea*.mp. 4. 1 and (2 or 3) 5. ((crosslink* or cross link* or xlink* or x link*) adj3 (cornea* or collagen)).mp. 6. CCL.mp. 7. CXL.mp. 8. C3R.mp. 9. 9 or/4–8 10. Dilatation, Pathologic/ 11. ectasia*.mp. 12. corneal diseases/ 13. cornea*.mp. 14. exp Corneal Dystrophies, Hereditary/ 15. 'pellucid marginal degeneration.mp. 16. (fuch* adj2 atroph*).mp. 17. ((fuch* or groenouw*) adj2 dystroph*).mp. 18. KERATOCONUS/ 19. keratoconus.mp. 20. Corneal Endothelial Cell Loss/ 21. Corneal Wavefront Aberration/ 22. keratopath*.mp. 23. or/10–22 24. 9 and 23 25. 'Transendothelial and Transepithelial Migration'/ 26. trans-epitheli*.mp. 27. transepitheli*.mp. 28. epitheli*.mp. 29. (dresden or phototherapeutic or phototherapeutic or iontophoresis or tight junctions).mp. 30. or/25–29 31. 24 and 30 32. remove duplicates from 31 33. (accelerat* or nonaccelerat* or conventional or regular or rapid or flash-linking or KXL).mp. 34. 24 and 33 35. remove duplicates from 34

- ▶ *Control*: epithelium-off CXL.
- ▶ *Outcomes*: clinical outcomes such as change in maximal keratometry (K_{\max}) at 12 months after treatment (primary outcome), incidence of serious adverse events, as well as incidence of disease progression, mean K_{\max} and change in the following: K_{\max} at longest follow-up, mean uncorrected distance visual acuity (UDVA), mean corrected distance visual acuity (CDVA), mean central corneal thickness, mean endothelial cell density, mean intraocular pressure, mean keratometry (K_{mean}) and mean spherical equivalent, at 12 months following treatment.

For studies published more than once (duplicates), we will include only the report with the most informative

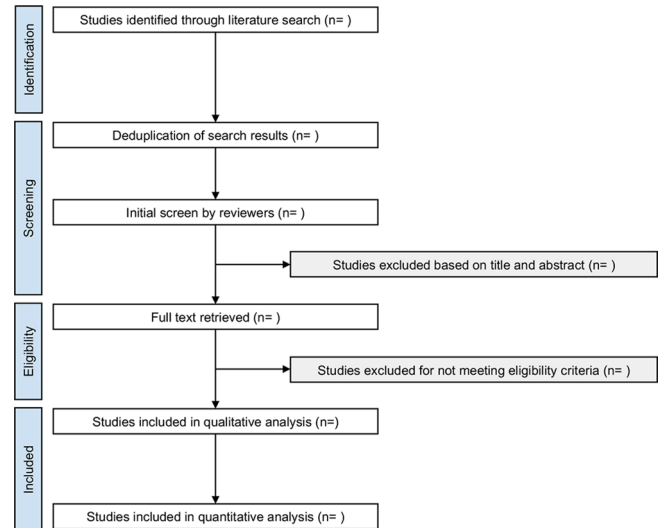


Figure 1 PRISMA flow diagram. PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-Analysis.

and complete data. We will exclude observational studies, narrative reviews, systematic reviews, animal studies, letters to the editor and correspondences, as well as randomised trials examining only one CXL modality (transepithelial or epithelium-off) without a comparator. Studies that examine different CXL modalities for indications other than corneal ectasia (eg, bacterial keratitis) will also be excluded. Reasons for excluding studies will be recorded.

Data management and collection

Data from eligible studies will be abstracted independently by two reviewers (SN and CS) and verified for accuracy by a third reviewer (AK). Prior to data collection, the complete articles of all studies meeting our inclusion criteria will be collated, and reviewers will develop and pilot data extraction forms. For studies not published in English, the full article will be translated into English and a clinical expert fluent in the original language of the article will be consulted. Discrepancies in data abstraction will be resolved collegially by deliberation among the two primary reviewers (SN and CS), consulting an independent third reviewer (AK) or contacting the original study authors. Where data in included studies are missing, ambiguous or incomplete, we will contact trial authors for further information and clarification.

We will collect data from eligible studies using forms with the following fields: study first author, year of publication, journal of publication, language, study design, included centres, included countries, number of patients, number of males, number of females, recruitment period, eligibility criteria, method of randomisation, indication for CXL, number of patients in transepithelial and epithelium-off groups, age of patients in transepithelial and epithelium-off groups, procedure for follow-up, number of patients with disease progression 12 months following CXL in transepithelial and epithelium-off groups, K_{\max} before CXL in transepithelial and epithelium-off groups, K_{\max} 12 months after CXL in

transepithelial and epithelium-off groups, K_{\max} at longest follow-up after CXL in transepithelial and epithelium-off groups, UDVA before CXL in transepithelial and epithelium-off groups, UDVA 12 months after CXL in transepithelial and epithelium-off groups, CDVA before CXL in transepithelial and epithelium-off groups, CDVA 12 months after CXL in transepithelial and epithelium-off groups, central corneal thickness before CXL in transepithelial and epithelium-off groups, central corneal thickness 12 months after CXL in transepithelial and epithelium-off groups, endothelial cell density before CXL in transepithelial and epithelium-off groups, endothelial cell density 12 months after CXL in transepithelial and epithelium-off groups, intraocular pressure before CXL in transepithelial and epithelium-off groups, intraocular pressure 12 months after CXL in transepithelial and epithelium-off groups, K_{mean} before CXL in transepithelial and epithelium-off groups, K_{mean} 12 months after CXL in transepithelial and epithelium-off groups, spherical equivalent before CXL in transepithelial and epithelium-off groups, spherical equivalent 12 months after CXL in transepithelial and epithelium-off groups, method for epithelium removal, method for transepithelial riboflavin application, postoperative pain following transepithelial and epithelium-off CXL, time to best CDVA following transepithelial and epithelium-off CXL, time to best UDVA following transepithelial and epithelium-off CXL, fluence of UVA light, incidence of significant complications (eg, corneal melt, persistent epithelial defects, scarring and persistent stromal haze) after transepithelial and epithelium-off CXL and preference of procedures as reported by authors.

Risk of bias in individual studies

All included trials will be assessed using the Cochrane Collaboration's risk of bias assessment tool by two independent authors (SN and CS).¹³ Studies will be evaluated to determine the risk of selection, performance, detection, attrition, reporting and other biases. Disagreements between reviewers will be resolved by discussion and consensus, and consultation with an impartial third reviewer (AK). If there is insufficient information available to make a judgement within an individual domain, we will categorise that domain as 'unclear' and the original study authors will be contacted for further information. Trials with one or more domains evaluated to be 'high risk' will be categorised as having an overall high risk of bias.

Definition of outcomes

Our primary outcome will be the change in K_{\max} (in dioptres, D) at 12 months after treatment, as measured by corneal topography. Additional outcomes will include incidence of ectatic disease progression (defined as an increase of the K_{\max} by ≥ 1.0 D) over 12 months, mean K_{\max} (D) at 12 months following treatment, change in K_{\max} (D) at longest follow-up after treatment, change in UDVA (in logMAR) at 12 months following treatment,

change in CDVA (in logMAR) at 12 months following treatment, change in central corneal thickness (in μM) at 12 months following treatment, change in endothelial cell density (in cells/ mm^2) at 12 months following treatment, change in intraocular pressure (in mmHg) at 12 months following treatment, change in K_{mean} (D) at 12 months following treatment, change in spherical equivalent (D) at 12 months following treatment and incidence of serious adverse events (eg, corneal melt, persistent epithelial defects, scarring and persistent stromal haze).

Data synthesis

All analyses will be conducted following the intention-to-treat principle using pooled study-level data. For dichotomous outcomes, such as the incidence of ectatic disease progression and the incidence of serious adverse events, we will summarise our analyses by calculating the relative risk (RR) or odds ratio (OR) with corresponding 95% confidence interval (CI). For continuous outcomes, such as the mean K_{\max} , change in K_{\max} , UDVA, CDVA, central corneal thickness, endothelial cell density, intraocular pressure, K_{mean} and spherical equivalent, we will determine the weighted mean difference with associated 95% CI. Moreover, we will calculate pooled estimates of all incidences across studies for the transepithelial group, and then separately for the epithelium-off group. The DerSimonian and Laird model will be used to conduct random-effects meta-analysis, and weights will be calculated using the inverse variance method.¹⁴ The threshold for type I error for statistical significance will be $\alpha=0.05$. Between-study heterogeneity will be evaluated using Cochran's Q test and quantified by the I^2 statistic, with I^2 values of 25%–49%, 50%–75% and $>75\%$ being graded as low, moderate and high heterogeneity, respectively.¹⁵ Values $<25\%$ will not be considered to be heterogeneous. Publication bias will be evaluated qualitatively by visual examination of funnel plot symmetry and quantified by Begg and Mazumdar's¹⁶ and Egger's tests.¹⁷

We will undertake prespecified subgroup analyses to explore heterogeneity for our primary outcome. Analyses will be performed for subgroups stratified by patient age and sex, CXL protocol (accelerated or non-accelerated) and severity of disease at time of treatment, as well as to compare low versus high risk of bias studies. We will conduct sensitivity analysis by performing trial sequential analysis (TSA) to evaluate whether the required information size has been met in order to make a determination of treatment benefit, harm or futility.^{18 19}

The overall evidence will be summarised using the Grading of Recommendations Assessment, Development and Evaluation approach.²⁰ Evidence will be assessed for the domains of risk of bias, consistency, precision, reporting bias, directness and other bias. The evidence for each outcome will be ranked as being of high, moderate, low or very low quality.

Statistical analyses will be conducted using Comprehensive Meta-analysis V.3.3.070 (Biostat, Englewood, New Jersey, USA), and TSA will be performed using Trial

Sequential Analysis V.0.9.5.10 Beta (Copenhagen Trial Unit, Copenhagen, Denmark).

Patient and public involvement

The outcomes selected for analysis were informed by examining surveys on corneal ectasia and patient experience. Our outcomes are directly linked to the quality of vision following CXL as well as the incidence of complications and adverse effects. While our review protocol did not have direct patient involvement, we expect the results of our review to substantially impact the lived experience of patients with corneal ectasia and their outcomes following CXL.

ETHICS AND DISSEMINATION

Research ethics board approval is not required for this study as it evaluates data from previously published trials. As such, there are no concerns of patient privacy or informed consent. The results of this review are expected to substantially inform clinical practice, future research and the management of patients with corneal ectasia. By comprehensively examining the evidence on CXL treatment approaches, this study is expected to influence how clinicians perform this evolving procedure, and potentially improve patient outcomes. Findings from this review will be submitted to a peer-reviewed journal for publication and will be presented widely at conferences and seminars.

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Contributors SN and CS conceived and designed the study. SN and LB developed the search strategy and SN, LB and CS piloted it in relevant databases. SN and CS developed the review protocol, selection criteria, risk of bias assessment strategy and data management procedures. SN, CS and AK established the data synthesis approach. SN, CS, AK, LB, MAF and WGH critically revised and commented on the intellectual content of the manuscript.

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