# Photoactivated Chromophore for Infectious Keratitis – Corneal Collagen Cross-Linking (PACK-CXL): A Systematic Review and Meta-Analysis

**Running head:** PACK-CXL for Infectious Keratitis

Darren Shu Jeng Ting*, FRCOphth[1]

Christin Henein*, MBBS, MRes[2,3]

Dalia G. Said, FRCS(Ed)[1]

Harminder S. Dua, FRCOphth, PhD[1]

[1]Academic Ophthalmology, Division of Clinical Neuroscience, School of Medicine, University of Nottingham, Nottingham, UK.

[2]Newcastle University, Newcastle upon Tyne, UK.

[3]National Institute for Health Research (NIHR) Biomedical Research Centre at Moorfields Eye Hospital NHS Foundation Trust and UCL Institute of Ophthalmology, London, UK.

*DSJT and CH are the joint first authors of this paper with equal contribution of work.

**Corresponding author:** Darren Shu Jeng Ting

**Email:** ting.darren@gmail.com

**Tel:** +44 (0) 115 951 5151

**Corresponding address:** Academic Ophthalmology, Division of Clinical Neuroscience, School of Medicine, University of Nottingham, Nottingham, NG7 2RD UK.

**Financial Support / Funding:** D.S.J.T is supported by the Fight for Sight / John Lee, Royal College of Ophthalmologists Primer Fellowship.

**Conflict of Interest:** No conflicting relationship exists for any author.

**ABSTRACT**

**Purpose:** To examine the efficacy of adjuvant photoactivated chromophore for infectious keratitis–corneal collagen cross-linking (PACK-CXL) for the treatment of infectious keratitis (IK).

**Methods:** Electronic databases, including MEDLINE, EMBASE and Cochrane Central, were searched for articles related to PACK-CXL. All clinical studies, including randomized controlled trials (RCTs), non-randomized controlled studies, case series and case reports, were included. A meta-analysis was further performed when there were sufficient similarities in the included RCTs. Primary outcome measure was time to complete corneal healing and secondary outcome measures included size of epithelial defect and infiltrate, corrected-distance-visual-acuity (CDVA), and adverse events.

**Results:** Forty-six eligible studies (including four RCTs) with 435 patients were included. When compared to standard antimicrobial treatment (SAT) alone, adjuvant PACK-CXL resulted in shorter mean time to complete corneal healing (-7.44 days; 95% CI, -10.71 to -4.16) and quicker resolution of the infiltrate at 7 days (-5.49mm$^2$; 95% CI, -7.44 to -3.54) and at 14 to 30 days (-5.27mm$^2$; 95% CI, -9.12 to -1.41). There was no significant difference in the size of epithelial defect, CDVA and risk of adverse events. Evidence on the use of PACK-CXL in acanthamoeba and mixed IK was insufficient.

**Conclusions:** Our study demonstrates that adjuvant PACK-CXL expedites the healing of IK when compared to SAT alone (low-quality evidence). Further adequately powered, high-quality RCTs are required to fully ascertain the therapeutic effect of PACK-CXL.

**Keywords:** Antibiotic; Antimicrobial; Corneal infection; Corneal ulcer; Cross-linking; CXL; Microbial keratitis; Infectious keratitis; PACK-CXL

## INTRODUCTION

Infectious keratitis (IK) represents the leading cause for corneal blindness in the world.[1, 2] It is a common, yet potentially sight-threatening, ophthalmic emergency that often warrants hospital admission for intensive antibiotic treatment and monitoring.[3, 4] It can be caused by a wide array of microorganisms, including bacteria, fungi, viruses and parasites. Broad-spectrum antimicrobial therapy is currently the mainstay of treatment for IK; however there is a decline in efficacy of antibiotic treatment due to an emerging trend of antimicrobial resistance in ocular infection.[5-7] Furthermore complications such as corneal melt, perforation and endophthalmitis, may ensue despite timely and intensive topical antibiotic treatment, necessitating further surgical interventions such as tectonic or therapeutic keratoplasty in a trial to preserve the eye and vision.[3, 4, 8, 9] However performing tectonic / therapeutic keratoplasty in a “hot eye” is associated with an increased incidence of recurrence of the disease, uncontrolled intraocular pressure, and graft rejection / failure.[3, 10] These issues highlight the need for alternative or adjuvant antimicrobial treatment to supplement the current therapeutic armamentarium for IK.

Corneal collagen cross-linking (CXL) was first introduced in 2003 by Wollensak et al.[11] to stabilize the progression of keratoconus. It utilizes a combination of ultraviolet-A (UVA) light of 370 nm and photosensitizing agent “riboflavin” to increase the corneal biomechanical stability and rigidity. The long-term efficacy and safety of CXL for corneal ectatic disorders have been well established by many long-term studies.[12-14] In addition to the stiffening effect on the cornea, CXL has been increasingly used for IK in the recent years. The rationale for using CXL for infection is based on the strong inherent antimicrobial activity of the UV light, which can directly damage the DNA and RNA of various types of microorganisms. Furthermore, the reactive oxygen species released from photoactivated riboflavin can directly affect the DNA and cell membranes of the microorganisms, culminating in a powerful synergistic antimicrobial action.[15-17] These effects together with the increased corneal rigidity

and hence resistance to proteolytic enzymatic digestion of stromal collagen has made CXL an attractive adjuvant in the management of IK.[18]

In view of the emerging evidence of CXL for infectious keratitis, a new terminology – Photo-Activated Chromophore for Keratitis – Corneal Cross-Linking (PACK-CXL) – was coined in 2013 at the ninth CXL congress in Dublin, Ireland, to help distinguish its use from CXL for corneal ectasia and to avoid scientific confusion.[19] However PACK-CXL is not routinely used in clinical practice due to the uncertainty of its efficacy and safety. This is primarily attributed to the wide heterogeneity of the literature in relation to the patient cohort, causative microorganisms, characteristics and severity of the ulcers, and treatment protocol, and the lack of large randomized control trials (RCTs). The aim of this quantitative systematic review is to examine the efficacy and safety of PACK-CXL based on the current literature.

## METHODS

### Protocol and registration

The systematic review title was registered with The Joanna Briggs Institute Database of Systematic Reviews and Implementation Reports (http://joannabriggs.org/research/registered_titles.aspx) and the protocol was registered with PROSPERO (registration number: CRD42019131290).

### Data sources and search methods

Two authors (D.S.J.T and C.H.) searched MEDLINE (January 1950 to April 2019), EMBASE (January 1980 to April 2019), Cochrane Central Register of Controlled Trials (CENTRAL), ISRCTN registry (www.isrctn.com/editAdvancedSearch), US National Institutes of Health Ongoing Trials Register ClinicalTrials.gov (http://clinicaltrials.gov) and World Health Organization (WHO) International Clinical Trials Registry Platform (ICTRP) (www.who.int/ictrp) for primary research related to CXL for infectious keratitis or “PACK-CXL”. There was no date restriction in the search for trials, however the search was

restricted to English articles. Electronic databases were first searched on 05 August 2018, followed by a final update on 15 April 2019. Key words used were “cross-linking”, “PACK-CXL”, “riboflavin”, “Vitamin B”, “keratitis”, “corneal ulcer”, and “corneal infection”. The bibliographies of included articles were independently and manually screened by two authors (D.S.J.T and C.H.) to identify further relevant studies. Search strategies for MEDLINE and EMBASE are provided in the **Supplemental Table S1**.

**Study selection**

All clinical studies, encompassing randomized controlled trials (RCTs), non-randomized controlled studies (NRS), case series and case reports, related to PACK-CXL were included as few RCTs were anticipated. The analysis was conducted at two levels; (1) a meta-analysis of all eligible RCTs and (2) a systematic review of all clinical studies, including NRS, case series and case reports. All types of IK, including bacterial, fungal, viral, parasitic or mixed infection, were included in this review. Studies related to suspected non-infectious causes of keratitis or CXL used for non-antimicrobial purpose were excluded from this study. For the meta-analysis, the intervention group included cases of IK that were treated by PACK-CXL with standard antimicrobial therapy (SAT) whereas the control group included cases of IK that were treated with SAT alone. Restriction was made to publications in English but no restriction was applied to the location or setting of the study, or patients’ demographic factors. This study conformed to the Preferred Reporting Items for Systematic reviews and Meta-Analysis (PRISMA) guideline.

**Data extraction**

A web application designed for systematic reviews, Rayyan (Qatar), was used to help collate the potential studies and expedite the initial screening of abstracts and titles.[20] The titles and abstracts obtained from the searches were independently screened by two authors (D.S.J.T and C.H.) to include studies that fulfilled the eligibility criteria. The authors then independently assessed the full-text version of all the selected articles and extracted data

onto a standardized data collection form for qualitative review. The extracted data included the authors and study title, year of publication, sample size, types of interventions, types of causative microorganisms, results and complications. Discrepancies were resolved by group consensus and independent adjudication (H.S.D) if consensus could not be reached.

For the meta-analysis, the following information were extracted from the included RCTs and entered into RevMan (Review Manager 5.3) software:

(1) Study characteristics: Year of publication, country of study, prospective registration of clinical trials in a publicly accessible database, sample size, eligibility criteria, demographic factors, diagnostic criteria, method of randomization, method of masking, number of study arms, number of participants, types of interventions, types of comparators, use of antimicrobial therapy in the intervention arm, source of funding, and any potential conflict of interest.

(2) Outcomes: Primary and secondary outcomes, risk of adverse events, complications during the procedure, post-procedure complications or secondary surgery, duration of follow-up, loss to follow-up and intervals at which outcomes were assessed.

**Outcome measures**

For the meta-analysis, the primary outcome measure was the time to complete corneal healing (defined as complete corneal re-epithelialization and clearance of infiltrate and hypopyon; days) and the secondary outcome measures included the size of epithelial defect ($mm^2$) and size of infiltrate ($mm^2$) at 7 days and at final follow-up (14 to 30 days), visual acuity (LogMAR) at final follow-up (one to three months), and risk of adverse events (defined as worsening IK and/or corneal melt requiring tectonic / therapeutic keratoplasty or evisceration) at final follow-up (one to three months). A summary of the available data of all included studies was also performed and reported.

Continuous variables such as time to complete corneal healing, size of corneal epithelial defect and infiltrate, and corrected-distance-visual-acuity (CDVA) were presented as mean with standard deviation (SD). In studies that reported median and interquartile range, the means and SDs were estimated using formulas reported by Wan et al.[21] and the Cochrane Handbook estimator.[22] Dichotomous variable such as risk of adverse events was defined by the number of participants with adverse events.

**Assessment of risk of bias**

Risk of bias was assessed by two authors (D.S.J.T and C.H) independently and any disagreement was adjudicated by H.S.D. Included RCTs were assessed for sources of systematic bias according to the guidelines in Chapter 8 of the Cochrane Handbook for Systematic Reviews of Interventions.[22] The review authors were not masked to the authors of the studies during this assessment. A judgement of 'high', 'low', or 'unclear' risk of bias was made for the following domains: (1) selection; (2) performance; (3) detection; (4) attrition; and (5) selective outcome reporting biases. NRS were assessed for risk of bias using the ROBINS-I tool[23] against seven domains; the worst judgement in any of the domains was used as the overall risk of bias.

**Measure of treatment effect**

Dichotomous data were measured as risk ratios (RRs) with 95% confidence intervals (CI) and continuous data as mean differences (MDs) with 95% CI. The unit of analysis was the participant and there was no issue with the unit of analysis in the included RCTs. The review was conducted based on the available data from the trials. When data were unavailable but the level of missing data and reasons for missing data in each group were similar, data were analyzed even when intention-to-treat (ITT) analysis was not performed.

**Assessment of heterogeneity**

The heterogeneity of the RCTs and NRS was checked by careful review of the full-text, assessment of forest plots and examination of the $I^2$ value with its confidence interval. The overall characteristics of the studies, in particular the types of participants and types of interventions were examined to assess the extent to which the studies were similar enough to make pooling study results sensible. The results of forest plots were reviewed for consistency of the size and direction of effects. $I^2$ values greater than 50% were considered indicative of substantial heterogeneity and meta-analysis could not be conducted due to inconsistency of effect estimates.[22] It was anticipated that some degree of heterogeneity will always exist due to clinical and methodological differences of the studies; therefore a random-effects model was used for the meta-analysis. The Chi² p-value was also considered as this has a low power when the number of studies were few. A p-value of <0.1 was considered statistically significant.[22]

**Data synthesis and analysis**

A meta-analysis was undertaken when there were sufficient similarities in the reporting of outcome measures. A random-effects model in RevMan 5.3 was used in view of the expected heterogeneity across different studies. The Mantel-Haenszel method was employed for analyzing the risk ratio of adverse events in view of the small expected number of events. If there was inconsistency between the results of individual studies such that a pooled result might not be a good summary of the individual trial results – for example, the effects were in different directions or $I^2$ >50% and P <0.1 – the data were not pooled but described in narrative format. Where there was statistical heterogeneity the data were pooled when all the effect estimates were in the same direction, such that a pooled estimate would seem to provide a good summary of the individual trial results. Sensitivity analysis was performed by assessing the impact of including studies at high risk of bias for an outcome in one or more key domains. This was conducted by omitting each study in turn to examine the influence of individual studies (with high risk of bias) on the overall pooled

estimate. A summary of findings is presented below including the assessment of the quality of the evidence for outcomes using the GRADE approach with GRADE Pro/GDT software.[24] All RCTs were started with a rating of 'high-quality' evidence and were downgraded by one level for serious concerns (or by two levels for very serious concerns) regarding the risk of bias, inconsistency, indirectness, imprecision or publication bias. The quality of evidence of studies was graded by two assessors (D.S.J.T and C.H.) independently and any disagreement was adjudicated by H.S.D.

**Assessment of adverse events**

In addition to the meta-analysis, a comprehensive review of adverse events was conducted where both experimental and quasi-experimental study designs including RCTs, NRS, and analytical observational studies such as prospective and retrospective cohort studies and case-control studies were reviewed. Descriptive case series and case reports were also reviewed for rare or uncommon adverse events. The risk of adverse events was graphically represented on albatross plots generated by the module installed on STATA 15.1 statistical software.[25] Pooled estimates of the risk of adverse events across comparative studies, including RCTs and NRS, were calculated. The risk of adverse events of PACK-CXL was summarized according to the type of IK. Studies were categorized as bacterial, fungal, acanthamoeba, viral, mixed or culture-negative presumed IK cohort. Mixed IK cohort referred to studies that included more than one group of causative microorganism.

**RESULTS**

**Literature search and study characteristics**

The electronic searches last conducted on 15 April 2019 retrieved a total of 780 titles and abstracts (see **Figure 1** for the PRISMA flow chart). After removing 181 duplicates and including two additional records identified through other sources, the remaining 599 records were screened and 547 references that were not relevant to the scope of the review were excluded. A total of 52 full-text copies of papers were assessed for eligibility. After excluding

6 ineligible articles,[26-31] 46 studies were included in the systematic review. These included four RCTs,[32-35] two NRS,[36, 37] 20 case series,[38-57] and 20 case reports,[58-77] examining the efficacy and safety of PACK-CXL in 435 participants (438 eyes) with IK, of which 311 eyes received PACK-CXL with SAT, 15 eyes received PACK-CXL alone, and 112 eyes received SAT alone. All studies included one eye per participant except for Chan et al. study[39] and Cristian et al. study.[40] Within the group that received PACK-CXL with/without SAT, the causative microorganisms included 152 (46.6%) bacteria, 89 (27.3%) fungi, 20 (6.1%) acanthamoeba, 4 (1.2%) viruses, 20 (6.1%) mixed, and 41 (12.6%) culture-negative presumed IK. The main characteristics of all RCTs and non-RCTs, including the authors' name, year of publication, number of treated participants, treatment protocol used, types of causative microorganisms, severity of IK, main results, adverse events, and visual outcome (if available), are summarized in **Supplemental Tables S2** and **S3**, respectively. Outcomes of RCTs are analyzed and summarized under the meta-analysis section. In addition, two ongoing RCTs were identified from the searches of clinical trial registries (https://clinicaltrials.gov/ct2/show/NCT02570321 and https://clinicaltrials.gov/ct2/show/NCT02717871).

**Meta-analysis of eligible RCTs**

*Overall description*

The study characteristics of all RCTs are summarized in **Supplemental Table S2**. The four RCTs included a total of 115 participants, with 58 participants receiving PACK-CXL plus SAT (intervention group) and 57 participants receiving SAT alone (control group). These consisted of four single-centered RCTs, which was conducted separately in Iran,[32] Thailand,[33] Egypt,[34] and India.[35] Trials included participants aged between 15 and 84 years (mean age of 40 – 56 years), with slight male preponderance (59.1%). Two RCTs[33, 35] were prospectively registered with the clinicaltrials.gov (NCT01831206 and NCT02328053).

*Types of microbes and severity of IK*

The RCTs were heterogeneous in terms of the types of infection. Bamdad et al.[32] and Uddaraju et al.[35] included bacterial keratitis alone and fungal keratitis alone, respectively, whereas Kasetsuwan et al.[33] included both bacterial and fungal keratitis in their studies. Said et al.[34] included bacterial, fungal, acanthamoeba and mixed infection. None of the studies included viral infection. The collective microbiological profiles included 53 (46.1%) bacteria, 30 (26.1%) fungi, 3 (2.6%) acanthamoeba, 8 (7.0%) mixed bacteria/fungi, and 21 (18.3%) culture-negative presumed IK. The proportion of the types of organisms was similar between intervention and control arms in all RCTs, except for one RCT[34] where there was a significantly higher proportion of mixed bacteria/fungi infection in the intervention arm (7, 33.3%) compared to the control arm (1, 5.3%; p=0.027).

*Treatment protocols and outcome measures*

All four trials compared PACK-CXL plus SAT with SAT alone. Dresden CXL protocol, using UVA of 3 mW/cm$^2$ for 30 mins, was employed in all intervention arms. After enrolment the participants were treated with PACK-CXL immediately on the first day of presentation in two studies,[32, 33] within 48 hours in one study,[34] and after two weeks of non-improvement with SAT in one study.[35] The primary and secondary outcome measures used in these RCTs are summarized in **Table 1**. They included size of stromal infiltrate and epithelial defect at 7 days and final follow-up (14 to 30 days),[32, 33] time to complete corneal healing or treatment duration (defined as complete re-epithelialization of the ulcer and disappearance of the infiltrate and hypopyon),[32, 34] adverse events[34,35] (defined by corneal perforation and/or endophthalmitis and/or increase in infiltrate size by ≥2mm), and visual-acuity.[33-35] The follow-up duration was between four and six weeks post-treatment, except for one study which the duration was not specified.[34] The lack of similarity in outcome measures limited the possibility for combining all data from individual RCTs.

*Risk of bias*

Risk of bias was for RCTs determined by using the “risk of bias” assessment tool. Sequence generation, allocation concealment, masking of participants, personnel and outcome assessors, incomplete outcome data, and selective outcome reporting were considered (**Figure 2**). Sequence generation was unclear in two of the studies[32, 33] where simple randomization was performed but the method was not clearly stated, potentially increasing the risk of bias. No details of attempts to conceal allocation of intervention assignment were given in two trials.[32, 34] Masking of participants was not possible but masking of assessors was possible in most studies. All trials had complete data except Uddaraju et al.[35] which reported outcomes for 13 participants of the intended 31 participants with fungal keratitis, as the trial was terminated early due to the concern of corneal perforation rate in the PACK-CXL group. Selective reporting was not considered to be a problem in the included trials but it was not always possible to assess this risk of bias adequately for one trial.[34] Other potential risk of bias included the lack of prospective registration of clinical trial in a publicly accessible database in two studies[32, 34] and the presence of conflict of interest in one study[34] where one of the lead authors was a co-inventor for the CXL technology (UV light source).

Risk of bias for NRS was determined by using the ROBINS-I grading criteria. Two NRS identified from database search were graded as having critical risk of bias (score 4), as both studies showed selection bias of participants and lacked balance of unknown confounding factors.[36, 37] Both studies had post-intervention bias in terms of the care provided and measurement of outcomes. One study had bias due to missing follow-up data.[36]

*Effects of interventions*

The effects of interventions were categorized into: (A) time to complete corneal healing; (B) size of epithelial defect; (C) size of infiltrate; (D) corrected-distance-visual-acuity (CDVA) in LogMAR; and (E) risk of adverse events. The GRADE summary of findings for each treatment outcome is summarized in **Table 2**.

A. Time to complete corneal healing

Two RCTs reported the time to complete corneal healing (or treatment duration), which was defined as complete re-epithelialization and disappearance of infiltrate and hypopyon.[32, 34] There is very low-quality evidence that adjuvant PACK-CXL shortened the time to complete healing compared to SAT alone (MD -7.44 days; 95% CI -10.71 to -4.16; $I^2$=0%; p<0.0001) (**Figure 3A**). Notably, the size of corneal ulcer in the adjuvant PACK-CXL group was significantly larger than the control group at baseline in one study.[34] Quality of evidence was downgraded due risk of bias and imprecision. There was a lack of allocation concealment and blinding. The total number of participants pooled in the meta-analysis were less than the number generated by a conventional sample size calculation.

B. Size of epithelial defect

Two studies reported the size of epithelial defect at 7 days and final follow-up (14 to 30 days).[32, 33] There is very low-quality evidence that adjuvant PACK-CXL was equally effective as SAT alone in reducing the size of corneal epithelial defect at 7 days follow up (MD -3.66 $mm^2$; 95% CI -14.26 to 6.94; $I^2$=50%; p=0.50) (**Figure 3B**). The quality of evidence was downgraded due to risk of bias, inconsistency and imprecision. In terms of the size of epithelial defect at final follow-up (14 to 30 days), there was heterogeneity between the two studies so meta-analysis was not performed ($I^2$=58%). There is very low-quality evidence that adjuvant PACK-CXL was equally effective as SAT alone in reducing the size of corneal epithelial defect at final follow up. The quality of evidence was downgraded due to risk of bias, inconsistency, indirectness and imprecision. Final follow-up differed between 14 days and 30 days. The observed heterogeneity could be due to the mixed cohort of bacterial and fungal keratitis cases included in Kasetsuwan et al. study[33] where 60% of the cases were fungal. When analysis of only bacteria keratitis cases was performed, the size of epithelial defect at final follow-up (14 to 30 days) favored PACK-CXL (MD -6.60; 95% CI -9.64 to -3.57; p<0.0001).[32, 33]

C. Size of infiltrate

Two studies reported the size of infiltrate at 7 days and final follow-up (14 to 30 days).[32, 33] There is very low-quality evidence that adjuvant PACK-CXL was more effective than SAT alone at reducing the size of infiltrate at 7 days' (MD -5.49mm$^2$; 95% CI -7.44 to -3.54; $I^2$=0%; p<0.0001) and at final follow-up (MD -5.27mm$^2$; 95% CI -9.12 to -1.41; $I^2$=21%; p=0.007) (**Figure 3C**). The quality of the evidence was downgraded due to high risk of bias and imprecision.

D. Visual acuity

There is very low-quality evidence that there was no difference in the mean CDVA at the final follow-up (one to three months) between the adjuvant PACK-CXL group and the SAT group (0.08; 95% CI -0.21 to 0.37; $I^2$=9%; two RCTs with 70 participants)[33, 34] (**Figure 3D**). The quality of the evidence was downgraded due to high risk of bias and imprecision.

E. Adverse events

Adverse events were defined as corneal perforation and/or uncontrolled IK requiring tectonic / therapeutic keratoplasty or evisceration. Bamdad et al.[32] reported no event of corneal perforation in both treatment arms though three participants required secondary surgeries such as amniotic membrane transplant (AMT) or conjunctival flap (one in PACK-CXL group and two in standard care group). For the other three RCTs, a total of eight participants randomized to PACK-CXL required therapeutic keratoplasty or evisceration for uncontrolled IK compared to 11 participants that received SAT.[33-35] There is very low-quality evidence that adjuvant PACK-CXL patients did not reduce the rate of adverse events at final follow-up (one to three months) when compared to SAT alone [risk ratio (RR) 0.84; 95% CI 0.26 to 2.71; p=0.77; four studies with 115 participants]. A sensitivity analysis was performed with the exclusion of Uddaraju et al. study because early trial termination might bias the effect estimate.[35] When excluded, there was no statistically significant change in the effect estimate on the risk of adverse events between adjuvant PACK-CXL and SAT alone (RR

0.49; 95% CI 0.11 to 2.29; $I^2$=22%; p=0.37; three RCTs with 102 participants) (**Figure 3E**). The quality of evidence was downgraded due to high risk of bias and imprecision. There are few events and the confidence interval included appreciable benefit and harm. Two studies did not report the type of microorganism involved in cases that were complicated by corneal perforation.[33, 34] According to consort harms reporting guidance, we found the quality of harms reporting inadequate in three included studies where the severity of adverse events and clinical sequalae were not clearly described.[33-35]

*Subgroup analysis*

There were insufficient RCTs to perform subgroup analysis between bacterial and fungal keratitis outcomes.

As effectiveness RCTs are lacking, NRS were included in the assessment of time to complete healing and adverse events. The mean time to complete corneal healing and number of adverse events reported in all comparative interventional studies, including RCTs and NRS, are summarized in albatross plots (**Figure 4**). All comparative studies investigating time to complete healing favored adjuvant PACK-CXL,[32, 34, 36, 37] with one study of bacterial keratitis showing statistical significance.[32] In terms of the risk of adverse events, one study showed negative association favoring adjuvant PACK-CXL in mixed IK cohort and one study showed a positive association favoring SAT alone in fungal keratitis cohort; however none of the comparative studies showed statistical significance.[33-37] Pooled risk estimates of adjuvant PACK-CXL on the risk of adverse events in comparative studies were grouped and analyzed according to type of IK cohorts (**Table 3**). It was found that adjuvant PACK-CXL did not significantly influence the risk of adverse events in mixed IK (RR 0.57; 95% CI 0.20 to 1.61) and fungal keratitis cohorts (RR 1.30; 95% CI 0.66 to 2.54).

Case series and case reports were reviewed for uncommon or unexpected adverse events. Analysis of the outcomes of the PACK-CXL, based on different types of causative

microorganisms, are summarized in **Supplemental Table S4**. Studies that reported pooled results with no distinction among the causative microorganisms were excluded from the subgroup analysis. Based on large NRS (≥ 5 eyes for a particular type of microorganism),[37, 38, 40, 41, 43, 45, 46, 48, 50-52, 54, 56, 57] the overall rate of complete corneal epithelial healing was 84.8% (78/92) for bacterial keratitis, 73.5% (36/49) fungal keratitis, 66.7% (4/6) acanthamoeba keratitis, and 92.0% (23/25) culture-negative presumed IK. There was insufficient evidence on the use of PACK-CXL in acanthamoeba and mixed infection whereas viral keratitis was considered a contraindication for PACK-CXL in the majority of the studies. Based on small case series and case reports, the complete healing rate was reported to be 75% (9/12) for acanthamoeba keratitis, 33% (1/3) for mixed bacterial / fungal keratitis, 75% (3/4) for mixed acanthamoeba / fungal keratitis, 100% (1/1) for mixed bacterial / acanthamoeba keratitis, and 25% (1/4) for viral keratitis.

**Characteristics of ongoing studies**

Two ongoing RCTs, comparing PACK-CXL and/or SAT with SAT alone, were identified from the searches of clinical trial registries. One RCT, named Cross-linking for Corneal Ulcers Treatment Trial (CLAIR), is recruiting 266 participants with bacterial or fungal keratitis (https://clinicaltrials.gov/ct2/show/NCT02570321). The primary outcome measure is the microbiological cure on repeat culture after adjuvant PACK-CXL or SAT alone. The other RCT, named Swiss PACK-CXL Multicenter Trial for the Treatment of Infectious Keratitis, is enrolling 252 participants with bacterial, fungal or mixed bacterial / fungal keratitis (https://clinicaltrials.gov/ct2/show/NCT02717871). This trial is investigating PACK-CXL as a primary treatment alone in early IK. The time to re-epithelialization of the cornea and time from treatment to discharge of patient are set as the primary and secondary outcome measures, respectively.

**DISCUSSION**

To the best of our knowledge, this study represents the most up-to-date and comprehensive systematic review and meta-analysis examining the effectiveness and safety of PACK-CXL in 435 participants (438 eyes), of which 325 participants received PACK-CXL with / without SAT. Since the last systematic review on PACK-CXL conducted by Abbouda et al.[78] (consisting of 21 studies of 145 eyes) and Papaioannou et al.[79] (consisting of 25 studies of 210 eyes) in 2016, a further 21 studies (including two additional RCTs) of 215 participants have been published. The doubling amount of literature on PACK-CXL in the recent years highlights a growing demand for innovative antimicrobial treatment for refractory IK, and the increasingly challenge faced by the clinicians in managing advanced and complex cases of IK. Both previous systematic reviews highlighted the potential utility of PACK-CXL but further high-quality RCTs were required.[78, 79] However the conclusion was not based on the findings of meta-analysis, primarily due to the limited number of published RCTs during the conduct of the systematic reviews.

**Summary of main findings**

In this systematic review, we included four RCTs with 58 participants in the intervention group (PACK-CXL with SAT) and 57 participants in the control group (SAT alone). The majority of the studies focused on either bacterial or fungal keratitis or a combination of both, with only one RCT including acanthamoeba keratitis (three participants). None of the RCTs examined the utility of PACK-CXL in viral keratitis. Based on the meta-analysis of two RCTs with 72 participants, we demonstrated that adjuvant PACK-CXL shortened the time to complete corneal healing by 7 days when compared to SAT alone. One study[32] included only participants with bacterial keratitis and the other study primarily included bacterial, fungal and mixed IK.[34] In addition adjuvant PACK-CXL was superior to SAT alone in terms of the resolution of the size of infiltrate at 7 days and at final follow-up (14-30 days).[32, 33] These two RCTs examined a total of 62 participants with 44 culture-proven and presumed bacterial keratitis and 18 culture-proven and presumed fungal keratitis. Both studies included

primarily moderate IK (2-6 mm ulcer size and involved up to the anterior two third of the stroma) and severe IK (>6 mm ulcer size and involved the posterior one third of the stroma).

In terms of other outcomes, adjuvant PACK-CXL did not reduce the risk of adverse events such as corneal perforation when compared to SAT alone group. While three RCTs showed a similar or lower risk of adverse events in the PACK-CXL group, Uddaraju et al.[35] reported an opposite trend in their study. The latter study included cases of severe and refractory fungal keratitis that involved the posterior two third of the cornea and did not respond to the standard anti-fungal treatment for at least two weeks. Their findings might be confounded by the difference in the baseline severity of the fungal keratitis where the PACK-CXL group was considerably worse than the SAT group. Based on their findings, they have cautioned the use of PACK-CXL in patients with severe deep fungal infection. When Uddaraju et al. study was excluded from the analysis (due to early termination and significant difference in the baseline severity of the ulcer), there was an increase in direction favoring towards the use of adjuvant PACK-CXL in reducing the risk of adverse events (RR 0.49) but the difference was not statistically significant. There was also no significant difference between adjuvant PACK-CXL and SAT alone for other outcomes such as the size of epithelial defect and corrected-distance-visual-acuity (CDVA).

Based on NRS and case series ($\geq$ 5 participants for a particular type of microorganism), we observed a complete healing rate of 84.8% (78/92) for bacterial keratitis, 73.5% (36/49) fungal keratitis, 66.7% (4/6) acanthamoeba keratitis, and 92.0% (23/25) culture-negative presumed IK. Only one participant (1.1%) in the bacterial keratitis group and three participants (6.1%) in the fungal keratitis group required additional AMT to help achieve complete re-epithelialization. These findings suggest that PACK-CXL may serve as a useful adjuvant therapy for bacterial and fungal keratitis. However, there were insufficient data to support the adjuvant use of PACK-CXL in acanthamoeba, mixed and viral IK.

**Overall completeness and applicability of evidence**

Overall the evidence was very low-quality with only four RCTs reporting small sample sizes. Price et al.[51] and Hafezi and Kling[19] had previously performed sample size power calculation for evaluating the effectiveness of PACK-CXL and had highlighted that around 200-250 participants were required. However, the sample size in all four included RCTs was substantially underpowered in efficacy and safety outcomes. Small sample sizes precluded any subgroup analysis of different causative microorganisms.

The other limiting issue with the meta-analysis was the inconsistency in measurement and reporting of the treatment outcomes among the four included RCTs. There was a total of eight different outcomes being reported and risk of adverse events was the only outcome that was consistently examined in all four RCTs. Two studies reported time to complete healing (defined by complete corneal re-epithelialization with clearance of infiltrate and hypopyon). Although this serves as a good outcome measure, it is important to bear in mind that some eyes with IK may develop delayed corneal epithelial wound healing or persistent epithelial defect after complete sterilization of the ulcer in relation to neurotrophic keratopathy. Interestingly a transient increase in hypopyon may be observed within 24 hours after PACK-CXL, likely attributed to the significant death of microorganisms and release of endotoxins, similar to a Jarisch-Herxheimer reaction.[34] In addition, potential confounding factors such as age and status of diabetes may affect the corneal wound healing time.[80-82] Visual outcome was examined in three RCTs; however, this parameter is often not the best outcome measure for IK because it can be confounded by the location (e.g. whether the visual axis is affected) and the severity of the corneal ulcer, which may vary significantly between cases.

Other important issue related to the evaluation of the efficacy of PACK-CXL was that some studies included deep corneal ulcers/infiltrates which were outside the Dresden protocol therapeutic window of 400 μm of the cornea. Spoerl et al.[83] reported that 94% of the UVA

was absorbed in the anterior 400 μm of the cornea, therefore deeper ulcers are unlikely to benefit from adjuvant PACK-CXL. In addition, some studies included eyes with corneal thickness of <400 μm, which could potentially increase the risk of endothelial dysfunction following PACK-CXL.[84] Anterior segment optical coherence tomography would better quantify the depth of ulcer and the corneal thickness than slit lamp examination or ultrasound pachymetry for eyes with IK as these two parameters can be highly variable. Corneal densitometry using Pentacam Scheimpflug imaging system may also serve as a useful adjuvant tool for monitoring the corneal response to IK.[85] Many studies reported the application of fluorescein stain prior to PACK-CXL to measure the size of the epithelial defect. This has an important clinical implication because the presence of fluorescein could compete with riboflavin for energy at 365 nm and reduce the effectiveness of PACK-CXL.[86] However, it is not always possible to ascertain the interval between the application of fluorescein drop and the initiation of PACK-CXL.

Based on the available evidence, three RCTs demonstrated that adjuvant PACK-CXL could expedite the resolution of IK in bacterial keratitis and potentially fungal keratitis, in terms of size of infiltrates[32, 33] and time taken to complete healing.[34] In contrast there were insufficient data to perform any meaningful analysis for acanthamoeba, viral and mixed IK. Several *in vitro* studies have shown that PACK-CXL did not confer any positive anti-amoebic effect on acanthamoeba cyst or trophozoites.[87, 88] Interestingly, when riboflavin was substituted with rose bengal, PACK-CXL was shown to demonstrate effective anti-amoebic activity.[87] Based on histopathologic examination, Hager et al.[27] similarly reported the persistence of acanthamoeba cyst and trophozoites in the acanthamoeba-infected corneal buttons after combined PACK-CXL and cryotherapy. PACK-CXL should not be employed to treat viral keratitis as UV radiation may exacerbate or activate herpes simplex infection.[51, 89]

**Quality of evidence and potential biases in the review**

All four RCTs were associated with a high risk of performance bias (the participants and investigators were not masked from the procedure) and at least one or more high / unclear risk of bias in other domains. The overall quality of the evidence was judged to be very low (see **Table 2**). As such, further research is very likely to have an important impact on the confidence in the estimate of effect and is likely to change the estimate. The main reasons for downgrading the evidence included risk of bias in the included studies, inconsistency, imprecision and indirectness. Other important risk of bias included the lack of prospective registration of the clinical trial in a publicly accessible database[32, 34] and the presence of conflict of interest.[34] As fewer than 10 studies were eligible for inclusion, we were unable to use a funnel plot to identify possible publication bias. Due to the potential bias, D.G.S. (who is the first author in Said et al. study)[34] was not involved in the data analysis of this systematic review.

In summary, adjuvant PACK-CXL may serve as a useful addition to the therapeutic armamentarium for IK in reducing the time to complete healing and size of infiltrate. There remains uncertainty regarding the effectiveness and safety of adjuvant PACK-CXL in the treatment of fungal keratitis and its use was cautioned in severe deep fungal cases. The use of PACK-CXL in acanthamoeba keratitis remains elusive, with contradicting evidence from in vitro and clinical studies, whereas PACK-CXL is contraindicated in cases of viral keratitis. Further adequately powered, high-quality RCTs are required to provide a true evaluation of the effectiveness and safety of PACK-CXL. Standardization of the reporting of outcome measures will enable better applicability of the evidence and allow easier comparison of the results across different studies related to PACK-CXL.

**REFERENCES**


1. http://atlas.iapb.org/global-burden-vision-impairment/gbvi-global-cause-estimates/
2. Whitcher JP, Srinivasan M, Upadhyay MP. Corneal blindness: a global perspective. Bull World Health Organ 2001;79:214-21.
3. Khor WB, Prajna VN, Garg P, et al. The Asia Cornea Society Infectious Keratitis Study: A Prospective Multicenter Study of Infectious Keratitis in Asia. Am J Ophthalmol 2018;195:161-70.
4. Keay L, Edwards K, Naduvilath T, et al. Microbial keratitis predisposing factors and morbidity. Ophthalmology 2006;113:109-16.
5. Asbell PA, Sanfilippo CM, Pillar CM, DeCory HH, Sahm DF, Morris TW. Antibiotic Resistance Among Ocular Pathogens in the United States: Five-Year Results From the Antibiotic Resistance Monitoring in Ocular Microorganisms (ARMOR) Surveillance Study. JAMA Ophthalmol 2015;133:1445-54.
6. Lalitha P, Manoharan G, Karpagam R, et al. Trends in antibiotic resistance in bacterial keratitis isolates from South India. Br J Ophthalmol 2017;101:108-13.
7. Ung L, Bispo PJ, Shanbhag SS, Gilmore MS, Chodosh J. The Persistent Dilemma of Microbial Keratitis: Global Burden, Diagnosis, and Antimicrobial Resistance. Surv Ophthalmol 2018.
8. Henry CR, Flynn HW, Jr., Miller D, Forster RK, Alfonso EC. Infectious keratitis progressing to endophthalmitis: a 15-year study of microbiology, associated factors, and clinical outcomes. Ophthalmology 2012;119:2443-9.
9. Hossain P, Tourkmani AK, Kazakos D, Jones M, Anderson D. Emergency corneal grafting in the UK: a 6-year analysis of the UK Transplant Registry. Br J Ophthalmol 2018;102:26-30.
10. Anshu A, Lim LS, Htoon HM, Tan DT. Postoperative risk factors influencing corneal graft survival in the Singapore Corneal Transplant Study. Am J Ophthalmol 2011;151:442-8.e1.

11. Wollensak G, Spoerl E, Seiler T. Riboflavin/ultraviolet-a-induced collagen crosslinking for the treatment of keratoconus. Am J Ophthalmol 2003;135:620-7.
12. Caporossi A, Mazzotta C, Baiocchi S, Caporossi T. Long-term results of riboflavin ultraviolet a corneal collagen cross-linking for keratoconus in Italy: the Siena eye cross study. Am J Ophthalmol 2010;149:585-93.
13. Hashemi H, Seyedian MA, Miraftab M, Fotouhi A, Asgari S. Corneal collagen cross-linking with riboflavin and ultraviolet a irradiation for keratoconus: long-term results. Ophthalmology 2013;120:1515-20.
14. Mazzotta C, Traversi C, Baiocchi S, et al. Corneal Collagen Cross-Linking With Riboflavin and Ultraviolet A Light for Pediatric Keratoconus: Ten-Year Results. Cornea 2018;37:560-6.
15. Kumar V, Lockerbie O, Keil SD, et al. Riboflavin and UV-light based pathogen reduction: extent and consequence of DNA damage at the molecular level. Photochem Photobiol 2004;80:15-21.
16. Naseem I, Ahmad M, Hadi SM. Effect of alkylated and intercalated DNA on the generation of superoxide anion by riboflavin. Biosci Rep 1988;8:485-92.
17. Tsugita A, Okada Y, Uehara K. Photosensitized inactivation of ribonucleic acids in the presence of riboflavin. Biochim Biophys Acta 1965;103:360-3.
18. Garg P, Das S, Roy A. Collagen Cross-linking for Microbial Keratitis. Middle East Afr J Ophthalmol 2017;24:18-23.
19. Hafezi F, Randleman JB. PACK-CXL: defining CXL for infectious keratitis. J Refract Surg 2014;30:438-9.
20. Ouzzani M, Hammady H, Fedorowicz Z, Elmagarmid A. Rayyan-a web and mobile app for systematic reviews. Syst Rev 2016;5:210.
21. Wan X, Wang W, Liu J, Tong T. Estimating the sample mean and standard deviation from the sample size, median, range and/or interquartile range. BMC Med Res Methodol 2014;14:135.

22. Higgins JPT, Green S. Cochrane handbook for systematic reviews of interventions. Version 5.1.0 (updated March 2011). The Cochrane Collaboration (2011). Available from http://handbook.cochrane.org/.
23. Sterne JA, Hernan MA, Reeves BC, et al. ROBINS-I: a tool for assessing risk of bias in non-randomised studies of interventions. BMJ 2016;355:i4919.
24. Schunemann H, Brozek J, Guyatt G, Oxman A, editors. GRADE handbook for grading quality of evidence and strength of recommendations. Updated October 2013. The GRADE Working Group 2013.
25. Harrison S, Jones HE, Martin RM, Lewis SJ, Higgins JPT. The albatross plot: A novel graphical tool for presenting results of diversely reported studies in a systematic review. Res Synth Methods 2017;8:281-9.
26. Ammermann C, Cursiefen C, Hermann M. Corneal cross-linking in microbial keratitis to prevent a chaud keratoplasty: A retrospective case series. [German]. Klinische Monatsblatter fur Augenheilkunde 2014;231:619-25.
27. Hager T, Hasenfus A, Stachon T, Seitz B, Szentmary N. Crosslinking and corneal cryotherapy in acanthamoeba keratitis - a histological study. Graefes Arch Clin Exp Ophthalmol 2016;254:149-53.
28. Idrus EA, Utti EM, Mattila JS, Krootila K. Photoactivated chromophore corneal cross-linking (PACK-CXL) for treatment of severe keratitis. Acta Ophthalmol 2018; doi:10.1111/aos.14001.
29. Mattila JS, Korsback A, Krootila K, Holopainen JM. Treatment of Pseudomonas aeruginosa keratitis with combined corneal cross-linking and human amniotic membrane transplantation. Acta Ophthalmol 2013;91:e410-1.
30. Schnitzler E, Sporl E, Seiler T. Crosslinking of the corneal collagen by UV-radiation with riboflavin for the mode of treatment melting ulcera of the cornea, first results of four patients. [German]. Klinische Monatsblatter fur Augenheilkunde 2000;217:190-3.
31. Wu HP, Luo SR, Dong N, Liu ZS, Shang XM, Yan L. [Corneal collagen crosslinking in the treatment of infectious keratitis]. Zhonghua Yan Ke Za Zhi 2013;49:890-5.

32. Bamdad S, Malekhosseini H, Khosravi A. Ultraviolet A/riboflavin collagen cross-linking for treatment of moderate bacterial corneal ulcers. Cornea 2015;34:402-6.
33. Kasetsuwan N, Reinprayoon U, Satitpitakul V. Photoactivated Chromophore for Moderate to Severe Infectious Keratitis as an Adjunct Therapy: A Randomized Controlled Trial. Am J Ophthalmol 2016;165:94-9.
34. Said DG, Elalfy MS, Gatzioufas Z, et al. Collagen cross-linking with photoactivated riboflavin (PACK-CXL) for the treatment of advanced infectious keratitis with corneal melting. Ophthalmology 2014;121:1377-82.
35. Uddaraju M, Mascarenhas J, Das MR, et al. Corneal Cross-linking as an Adjuvant Therapy in the Management of Recalcitrant Deep Stromal Fungal Keratitis: A Randomized Trial. Am J Ophthalmol 2015;160:131-4.e5.
36. Basaiawmoit P, Selvin SST, Korah S. PACK-CXL in Reducing the Time to Heal in Suppurative Corneal Ulcers: Observations of a Pilot Study From South India. Cornea 2018;37:1376-80.
37. Vajpayee RB, Shafi SN, Maharana PK, Sharma N, Jhanji V. Evaluation of corneal collagen cross-linking as an additional therapy in mycotic keratitis. Clin Exp Ophthalmol 2015;43:103-7.
38. Agrawal N, Singh SK. Collagen cross-linking in recalcitrant corneal ulcers: A case series. Nepal J Ophthalmol 2016;8:47-53.
39. Chan E, Snibson GR, Sullivan L. Treatment of infectious keratitis with riboflavin and ultraviolet-A irradiation. J Cataract Refract Surg 2014;40:1919-25.
40. Cristian C, Marco CDV, Arturo K, et al. Accelerated collagen cross-linking in the management of advanced Acanthamoeba keratitis. Arq Bras Oftalmol 2019;82:103-6.
41. Erdem E, Harbiyeli II, Boral H, Ilkit M, Yagmur M, Ersoz R. Corneal Collagen Cross-Linking for the Management of Mycotic Keratitis. Mycopathologia 2018;183:521-7.

42. Iseli HP, Thiel MA, Hafezi F, Kampmeier J, Seiler T. Ultraviolet A/riboflavin corneal cross-linking for infectious keratitis associated with corneal melts. Cornea 2008;27:590-4.
43. Khalili MR, Jahadi HR, Karimiarimi M, Yasemi M. Corneal collagen cross-linking for treatment of bacterial and herpetic keratitis. J Clin Diagn Res 2017;11:NC12-NC6.
44. Khan YA, Kashiwabuchi RT, Martins SA, et al. Riboflavin and ultraviolet light a therapy as an adjuvant treatment for medically refractive acanthamoeba keratitis: Report of 3 cases. Ophthalmology 2011;118:324-31.
45. Knyazer B, Krakauer Y, Baumfeld Y, Lifshitz T, Kling S, Hafezi F. Accelerated Corneal Cross-Linking With Photoactivated Chromophore for Moderate Therapy-Resistant Infectious Keratitis. Cornea 2018;37:528-31.
46. Li Z, Jhanji V, Tao X, Yu H, Chen W, Mu G. Riboflavin/ultravoilet light-mediated crosslinking for fungal keratitis. Br J Ophthalmol 2013;97:669-71.
47. Makdoumi K, Mortensen J, Crafoord S. Infectious keratitis treated with corneal crosslinking. Cornea 2010;29:1353-8.
48. Makdoumi K, Mortensen J, Sorkhabi O, Malmvall BE, Crafoord S. UVA-riboflavin photochemical therapy of bacterial keratitis: A pilot study. Graefes Arch Clin Exp Ophthalmol 2012;250:95-102.
49. Muller L, Thiel MA, Kipfer-Kauer AI, Kaufmann C. Corneal Cross-Linking as Supplementary Treatment Option in Melting Keratitis: A Case Series. Klinische Monatsblatter fur Augenheilkunde 2012;229:411-5.
50. Panda A, Krishna SN, Kumar S. Photo-activated riboflavin therapy of refractory corneal ulcers. Cornea 2012;31:1210-3.
51. Price MO, Tenkman LR, Schrier A, Fairchild KM, Trokel SL, Price Jr FW. Photoactivated riboflavin treatment of infectious keratitis using collagen cross-linking technology. J Refract Surg 2012;28:706-13.
52. Ramona BI, Catalina C, Andrei M, Daciana S, Calin T. Collagen crosslinking in the management of microbial keratitis. Rom J Ophthalmol 2016;60:28-30.

53. Rosetta P, Vinciguerra R, Romano MR, Vinciguerra P. Corneal collagen cross-linking window absorption. Cornea 2013;32:550-4.
54. Shetty R, Nagaraja H, Jayadev C, Shivanna Y, Kugar T. Collagen crosslinking in the management of advanced non-resolving microbial keratitis. Br J Ophthalmol 2014;98:1033-5.
55. Skaat A, Zadok D, Goldich Y, et al. Riboflavin/UVA photochemical therapy for severe infectious keratitis. Eur J Ophthalmol 2014;24:21-8.
56. Sorkhabi R, Sedgipoor M, Mahdavifard A. Collagen cross-linking for resistant corneal ulcer. Int Ophthalmol 2013;33:61-6.
57. Zloto O, Barequet IS, Weissman A, Nimni OE, Berger Y, Avni-Zauberman N. Does PACK-CXL change the prognosis of resistant infectious keratitis? J Refract Surg 2018;34:559-63.
58. Abbouda A, Estrada AV, Rodriguez AE, Alio JL. Anterior segment optical coherence tomography in evaluation of severe fungal keratitis infections treated by corneal crosslinking. Eur J Ophthalmol 2014;24:320-4.
59. Anwar HM, El-Danasoury AM, Hashem AN. Corneal collagen crosslinking in the treatment of infectious keratitis. Clin Ophthalmol 2011;5:1277-80.
60. Arance-Gil A, Gutierrez-Ortega AR, Villa-Collar C, Nieto-Bona A, Lopes-Ferreira D, Gonzalez-Meijome JM. Corneal cross-linking for Acanthamoeba keratitis in an orthokeratology patient after swimming in contaminated water. Cont Lens Anterior Eye;37:224-7.
61. Casagrande MK, Frings A, Katz T, Steinberg J, Linke SJ. Corneal crosslinking in Pasteurella multocida-induced severe keratitis. JCRS Online Case Reports 2014;2:50-3.
62. Chan TCY, Chow VWS, Jhanji V. Collagen cross-linking with photoactivated riboflavin (PACK-CXL) for bacterial keratitis after small incision lenticule extraction (SMILE). J Refract Surg 2017;33:278-80.

63. Demirci G, Ozdamar A. A case of medication-resistant acanthamoeba keratitis treated by corneal crosslinking in Turkey. Case Rep Ophthalmol Med 2013;2013:608253.
64. Ferrari G, Iuliano L, Vigano M, Rama P. Impending corneal perforation after collagen cross-linking for herpetic keratitis. J Cataract Refract Surg 2013;39:638-41.
65. Ferrari TM, Leozappa M, Lorusso M, Epifani E, Ferrari LM. Escherichia coli keratitis treated with ultraviolet A/riboflavin corneal cross-linking: A case report. Eur J Ophthalmol 2009;19:295-7.
66. Garduno-Vieyra L, Gonzalez-Sanchez CR, Hernandez-Da Mota SE. Ultraviolet-a light and riboflavin therapy for acanthamoeba keratitis: a case report. Case Rep Ophthalmol 2011;2:291-5.
67. Igal V, Pikkel Igal YS, Pikkel YY. Corneal Cross-Linking as a Treatment for Fungal Keratitis Associated with Corneal Melting. Case Rep Ophthalmol 2017;8:148-51.
68. Kozobolis V, Labiris G, Gkika M, et al. UV-A Collagen Cross-Linking Treatment of Bullous Keratopathy Combined With Corneal Ulcer. Cornea 2010;29:235-8.
69. Kymionis GD, Kouroupaki AI, Liakopoulos DA,  A, jelovic IR, Tsoulnaras KI. Multiorganism, drug-resistant keratitis treated by corneal crosslinking. Eur J Ophthalmol 2016;26.
70. Labiris G, Giarmoukakis A, Larin R, Sideroudi H, Kozobolis VP. Corneal collagen cross-linking in a late-onset graft infectious ulcer: A case report. J Med Case Rep 2014;8.
71. Moren H, Malmsjo M, Mortensen J, Ohrstrom A. Riboflavin and ultraviolet a collagen crosslinking of the cornea for the treatment of keratitis. Cornea 2010;29:102-4.
72. Oflaz AB, Bozkurt B, Kamis U, Koktekir BE. Corneal collagen crosslinking treatment in a case with pneumococcal keratitis. Turk Oftalmoloiji Dergisi 2017;47:161-4.
73. Passilongo M, Pedrotti E, Talli PM, et al. Accelerated corneal crosslinking to treat Acanthamoeba and Fusarium coinfection of the cornea. JCRS Online Case Reports;6:19-21.

74. Saglk A, Ucakhan OO, Kanpolat A. Ultraviolet A and riboflavin therapy as an adjunct in corneal ulcer refractory to medical treatment. Eye Contact Lens 2013;39:413-5.
75. Tabibian D, Richoz O, Riat A, Schrenzel J, Hafezi F. Accelerated photoactivated chromophore for keratitis-corneal collagen cross-linking as a first-line and sole treatment in early fungal keratitis. Journal of Refractive Surgery 2014;30:855-7.
76. Yagci A, Palamar M, Hilmioglu SP, Irkec M. Cross-linking treatment and corneal transplant in refractory acremonium keratitis: Case report. Exp Clin Transplant 2016;14:580-3.
77. Zarei-Ghanavati S, Irandoost F. Treatment of Refractory Keratitis after a Boston Type i Keratoprosthesis with Corneal Collagen Cross-Linking. Cornea 2015;34:1161-3.
78. Abbouda A, Abicca I, Alio JL. Current and Future Applications of Photoactivated Chromophore for Keratitis-Corneal Collagen Cross-Linking (PACK-CXL): An Overview of the Different Treatments Proposed. Semin Ophthalmol 2018;33:293-9.
79. Papaioannou L, Miligkos M, Papathanassiou M. Corneal Collagen Cross-Linking for Infectious Keratitis: A Systematic Review and Meta-Analysis. Cornea 2016;35:62-71.
80. Bikbova G, Oshitari T, Baba T, Bikbov M, Yamamoto S. Diabetic corneal neuropathy: clinical perspectives. Clin Ophthalmol 2018;12:981-7.
81. Chen WL, Lin CT, Ko PS, et al. In vivo confocal microscopic findings of corneal wound healing after corneal epithelial debridement in diabetic vitrectomy. Ophthalmology 2009;116:1038-47.
82. Dua HS, Gomes JA, Singh A. Corneal epithelial wound healing. Br J Ophthalmol 1994;78:401-8.
83. Spoerl E, Mrochen M, Sliney D, Trokel S, Seiler T. Safety of UVA-riboflavin cross-linking of the cornea. Cornea 2007;26:385-9.
84. Chen X, Stojanovic A, Eidet JR, Utheim TP. Corneal collagen cross-linking (CXL) in thin corneas. Eye Vis (Lond) 2015;2:15.

85. Otri AM, Fares U, Al-Aqaba MA, Dua HS. Corneal densitometry as an indicator of corneal health. Ophthalmology 2012;119:501-8.
86. Richoz O, Gatzioufas Z, Francois P, Schrenzel J, Hafezi F. Impact of fluorescein on the antimicrobial efficacy of photoactivated riboflavin in corneal collagen cross-linking. J Refract Surg 2013;29:842-5.
87. Atalay HT, Dogruman-Al F, Sarzhanov F, et al. Effect of Riboflavin/Rose Bengal-Mediated PACK-CXL on Acanthamoeba Trophozoites and Cysts in Vitro. Curr Eye Res 2018;43:1322-5.
88. del Buey MA, Cristobal JA, Casas P, et al. Evaluation of in vitro efficacy of combined riboflavin and ultraviolet a for Acanthamoeba isolates. Am J Ophthalmol 2012;153:399-404.
89. Kymionis GD, Portaliou DM, Bouzoukis DI, et al. Herpetic keratitis with iritis after corneal crosslinking with riboflavin and ultraviolet A for keratoconus. J Cataract Refract Surg 2007;33:1982-4.

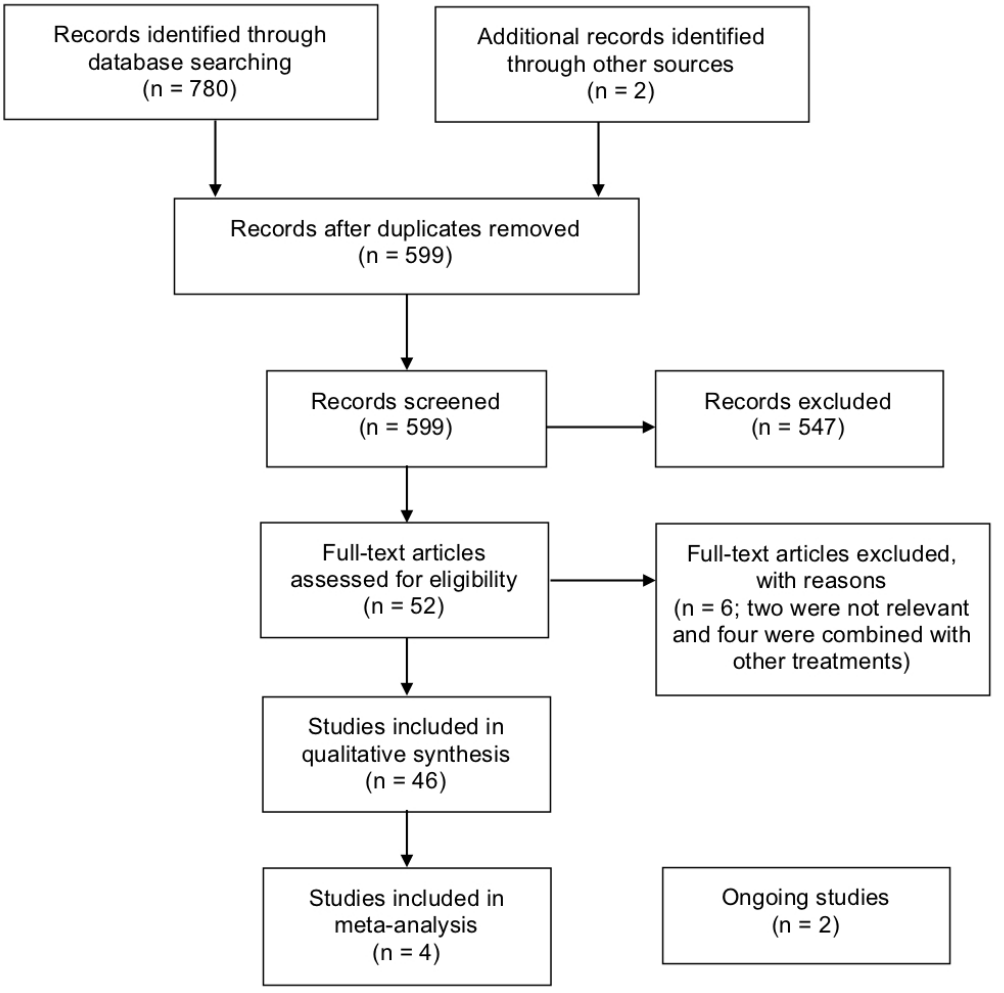
**FIGURE LEGEND**

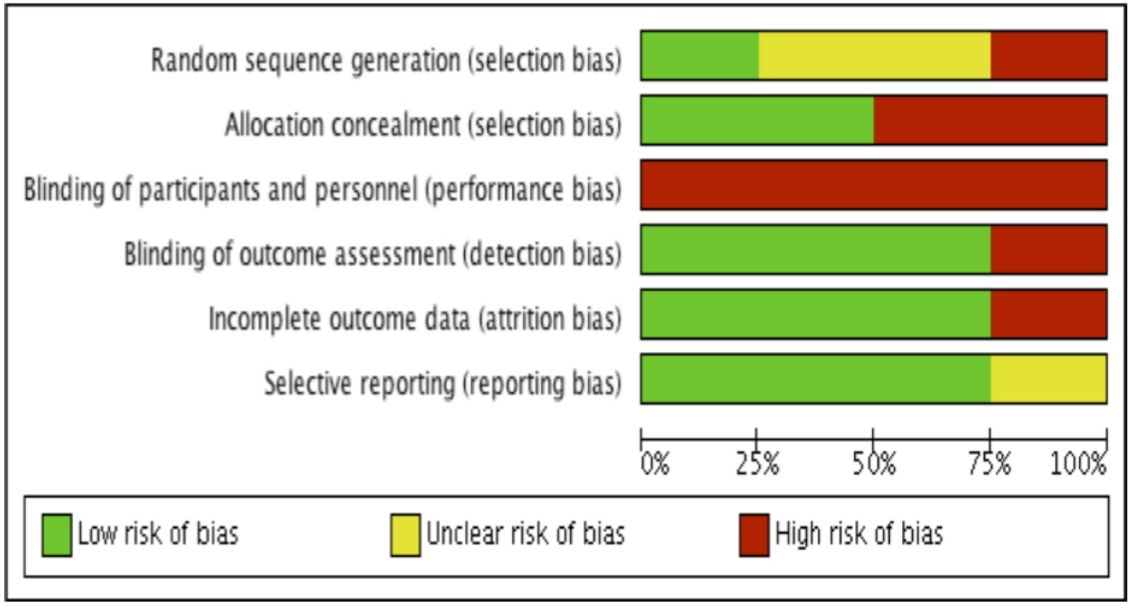
**Figure 1.** PRISMA flow diagram of the literature search for “Photoactivated chromophore for infectious keratitis – corneal collagen cross-linking (PACK-CXL).

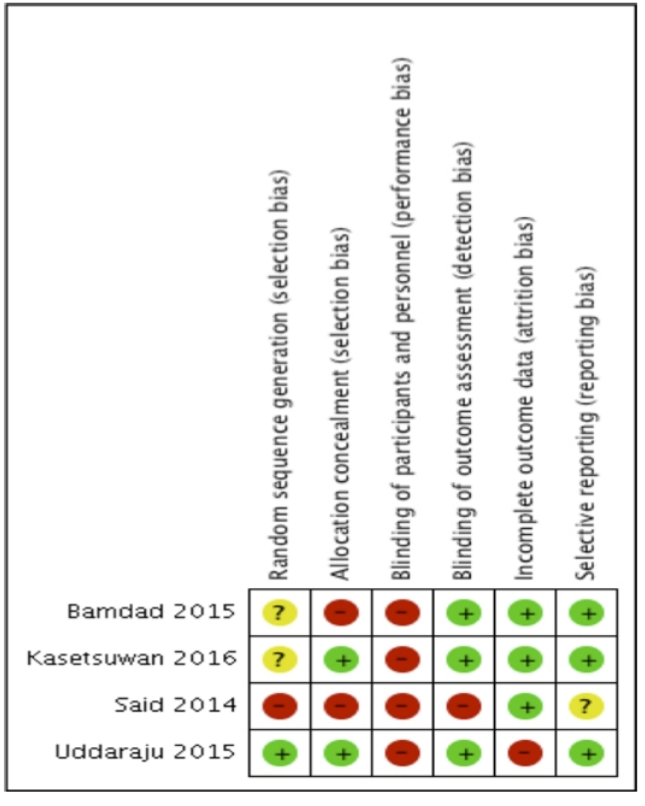
**Figure 2.** Risk of bias graph: Review authors' judgements about each risk of bias item presented as percentages across all included studies.

**Figure 3.** Summary of the meta-analysis (forest plot) comparing the efficacy between PACK-CXL plus standard antimicrobial treatment (SAT) and SAT alone in eligible randomized controlled trials, in terms of: **(A)** time to complete corneal healing; **(B)** size of epithelial defect; **(C)** size of stromal infiltrate; **(D)** corrected-distance-visual-acuity; and **(E)** risk of adverse events.

**Figure 4. (A)** Albatross plot for studies comparing the mean time to healing of adjuvant PACK-CXL with standard antimicrobial treatment (SAT) alone, with contours for mean differences (MDs). Red points represent comparative studies of fungal keratitis, black points represent mixed infectious keratitis cohorts, and green point represents study that included bacterial keratitis. Negative association favors the use of adjuvant PACK-CXL and positive association favors the use of SAT alone. Points lying outside the p-value of 0.05 were considered statistically significant. **(B)** Albatross plot for studies comparing adverse events of adjuvant PACK-CXL with SAT alone for infectious keratitis with contours for relative risk (RR). Red points represent comparative studies of fungal keratitis and black points represent mixed infectious keratitis cohorts. Negative association favors the use of adjuvant PACK-CXL and positive association favors the use of SAT alone. Points lying outside the p-value of 0.05 were considered statistically significant.


Records identified through database searching (n = 780)
Additional records identified through other sources (n = 2)
Records after duplicates removed (n = 599)
Records screened (n = 599)
Records excluded (n = 547)
Full-text articles assessed for eligibility (n = 52)
Full-text articles excluded, with reasons (n = 6; two were not relevant and four were combined with other treatments)
Studies included in qualitative synthesis (n = 46)
Studies included in meta-analysis (n = 4)
Ongoing studies (n = 2)

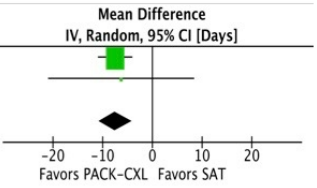

Random sequence generation (selection bias)
Allocation concealment (selection bias)
Blinding of participants and personnel (performance bias)
Blinding of outcome assessment (detection bias)
Incomplete outcome data (attrition bias)
Selective reporting (reporting bias)
0%
25%
50%
75%
100%
Low risk of bias
Unclear risk of bias
High risk of bias


Random sequence generation (selection bias)
Allocation concealment (selection bias)
Blinding of participants and personnel (performance bias)
Blinding of outcome assessment (detection bias)
Incomplete outcome data (attrition bias)
Selective reporting (reporting bias)
Bamdad 2015
Kasetsuwan 2016
Said 2014
Uddaraju 2015

**A. Time to complete corneal healing**

| Study or Subgroup | PACK-CXL Mean [Days] | PACK-CXL SD [Days] | PACK-CXL Total | SAT Mean [Days] | SAT SD [Days] | SAT Total | Weight | Mean Difference IV, Random, 95% CI [Days] |
|---|---|---|---|---|---|---|---|---|
| Bamdad 2015 | 17.2 | 4.1 | 16 | 24.7 | 5.5 | 16 | 95.0% | -7.50 [-10.86, -4.14] |
| Said 2014 | 39.76 | 18.22 | 21 | 46.05 | 27.44 | 19 | 5.0% | -6.29 [-20.88, 8.30] |
| **Total (95% CI)** | | | **37** | | | **35** | **100.0%** | **-7.44 [-10.71, -4.16]** |

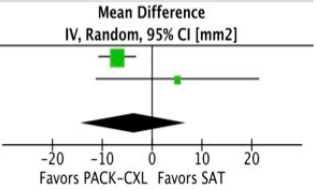
Heterogeneity: $Tau^2 = 0.00$; $Chi^2 = 0.03$, df = 1 (P = 0.87); $I^2 = 0\%$
Test for overall effect: Z = 4.45 (P < 0.00001)




**B. Size of epithelial defect ($mm^2$) at 7 days**

| Study or Subgroup | PACK-CXL Mean [mm2] | PACK-CXL SD [mm2] | PACK-CXL Total | SAT Mean [mm2] | SAT SD [mm2] | SAT Total | Weight | Mean Difference IV, Random, 95% CI [mm2] |
|---|---|---|---|---|---|---|---|---|
| Bamdad 2015 | 7.94 | 4.47 | 16 | 14.94 | 6.05 | 16 | 72.4% | -7.00 [-10.69, -3.31] |
| Kasetsuwan 2016 | 26 | 20.8 | 15 | 20.9 | 24.6 | 15 | 27.6% | 5.10 [-11.20, 21.40] |
| **Total (95% CI)** | | | **31** | | | **31** | **100.0%** | **-3.66 [-14.26, 6.94]** |

Heterogeneity: $Tau^2 = 36.84$; $Chi^2 = 2.01$, df = 1 (P = 0.16); $I^2 = 50\%$
Test for overall effect: Z = 0.68 (P = 0.50)

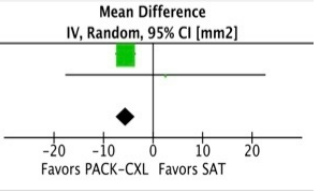



**C. Size of infiltrate ($mm^2$)**

(i) At 7 days

| Study or Subgroup | PACK-CXL Mean [mm2] | PACK-CXL SD [mm2] | PACK-CXL Total | SAT Mean [mm2] | SAT SD [mm2] | SAT Total | Weight | Mean Difference IV, Random, 95% CI [mm2] |
|---|---|---|---|---|---|---|---|---|
| Bamdad 2015 | 9.31 | 3.84 | 16 | 14.88 | 1.11 | 16 | 99.1% | -5.57 [-7.53, -3.61] |
| Kasetsuwan 2016 | 30.6 | 27.8 | 15 | 28.1 | 28.4 | 15 | 0.9% | 2.50 [-17.61, 22.61] |
| **Total (95% CI)** | | | **31** | | | **31** | **100.0%** | **-5.49 [-7.44, -3.54]** |

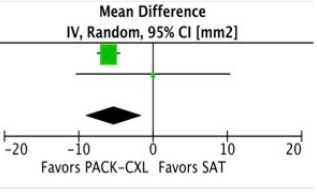
Heterogeneity: $Tau^2 = 0.00$; $Chi^2 = 0.61$, df = 1 (P = 0.43); $I^2 = 0\%$
Test for overall effect: Z = 5.52 (P < 0.00001)




(ii) At final follow-up (14 to 30 days)

| Study or Subgroup | PACK-CXL Mean [mm2] | PACK-CXL SD [mm2] | PACK-CXL Total | SAT Mean [mm2] | SAT SD [mm2] | SAT Total | Weight | Mean Difference IV, Random, 95% CI [mm2] |
|---|---|---|---|---|---|---|---|---|
| Bamdad 2015 | 3.63 | 2.83 | 16 | 9.63 | 1.21 | 16 | 87.8% | -6.00 [-7.51, -4.49] |
| Kasetsuwan 2016 | 9.3 | 17 | 15 | 9.3 | 11.3 | 15 | 12.2% | 0.00 [-10.33, 10.33] |
| **Total (95% CI)** | | | **31** | | | **31** | **100.0%** | **-5.27 [-9.12, -1.41]** |

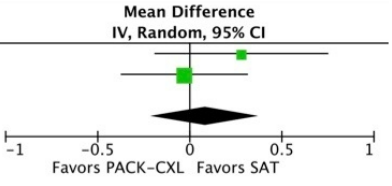
Heterogeneity: $Tau^2 = 3.81$; $Chi^2 = 1.27$, df = 1 (P = 0.26); $I^2 = 21\%$
Test for overall effect: Z = 2.68 (P = 0.007)




**D. Visual acuity (mean LogMAR) at final follow-up (one to three months)**

| Study or Subgroup | PACK-CXL Mean | PACK-CXL SD | PACK-CXL Total | SAT Mean | SAT SD | SAT Total | Weight | Mean Difference IV, Random, 95% CI |
|---|---|---|---|---|---|---|---|---|
| Kasetsuwan 2016 | 1.48 | 0.64 | 15 | 1.2 | 0.67 | 15 | 36.1% | 0.28 [-0.19, 0.75] |
| Said 2014 | 1.64 | 0.62 | 21 | 1.67 | 0.48 | 19 | 63.9% | -0.03 [-0.37, 0.31] |
| **Total (95% CI)** | | | **36** | | | **34** | **100.0%** | **0.08 [-0.21, 0.37]** |

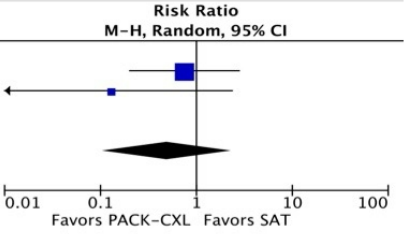
Heterogeneity: $Tau^2 = 0.00$; $Chi^2 = 1.10$, df = 1 (P = 0.30); $I^2 = 9\%$
Test for overall effect: Z = 0.55 (P = 0.58)

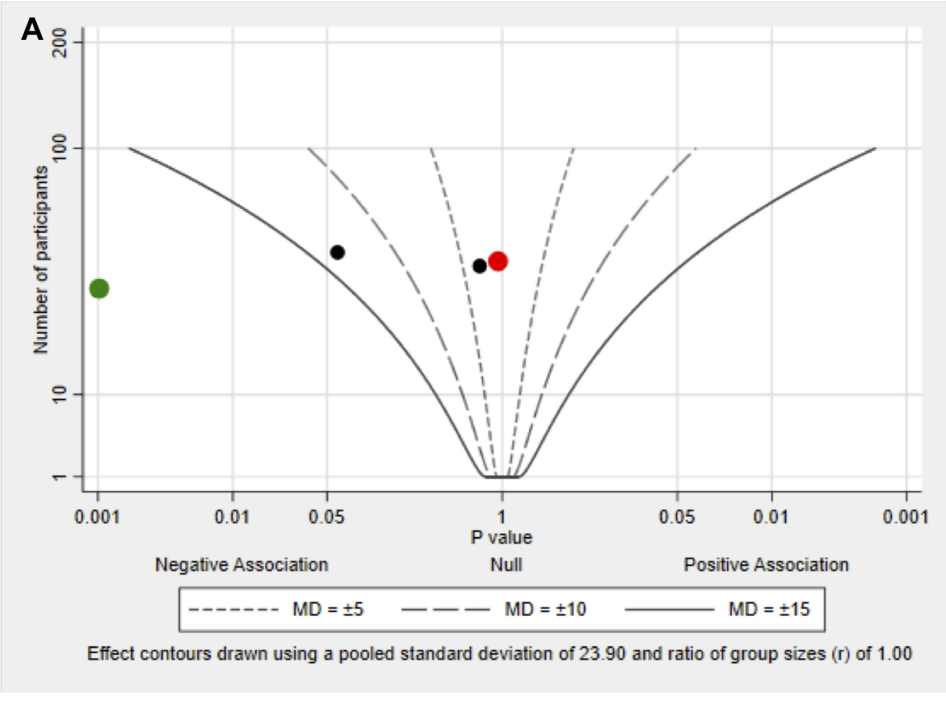



**E. Adverse events at final follow-up (one to three months)**

| Study or Subgroup | PACK-CXL Events | PACK-CXL Total | SAT Events | SAT Total | Weight | Risk Ratio M-H, Random, 95% CI |
|---|---|---|---|---|---|---|
| Bamdad 2015 | 0 | 16 | 0 | 16 | | Not estimable |
| Kasetsuwan 2016 | 3 | 15 | 4 | 15 | 75.8% | 0.75 [0.20, 2.79] |
| Said 2014 | 0 | 21 | 3 | 19 | 24.2% | 0.13 [0.01, 2.36] |
| Uddaraju 2015 | 5 | 6 | 4 | 7 | | Not estimable |
| **Total (95% CI)** | | **52** | | **50** | **100.0%** | **0.49 [0.11, 2.29]** |
| Total events | 3 | | 7 | | | |

Heterogeneity: $Tau^2 = 0.37$; $Chi^2 = 1.28$, df = 1 (P = 0.26); $I^2 = 22\%$
Test for overall effect: Z = 0.91 (P = 0.37)




A
Number of participants
200
100
10
1
0.001
0.01
0.05
1
0.05
0.01
0.001
P value
Negative Association
Null
Positive Association
MD = ±5
MD = ±10
MD = ±15
Effect contours drawn using a pooled standard deviation of 23.90 and ratio of group sizes (r) of 1.00

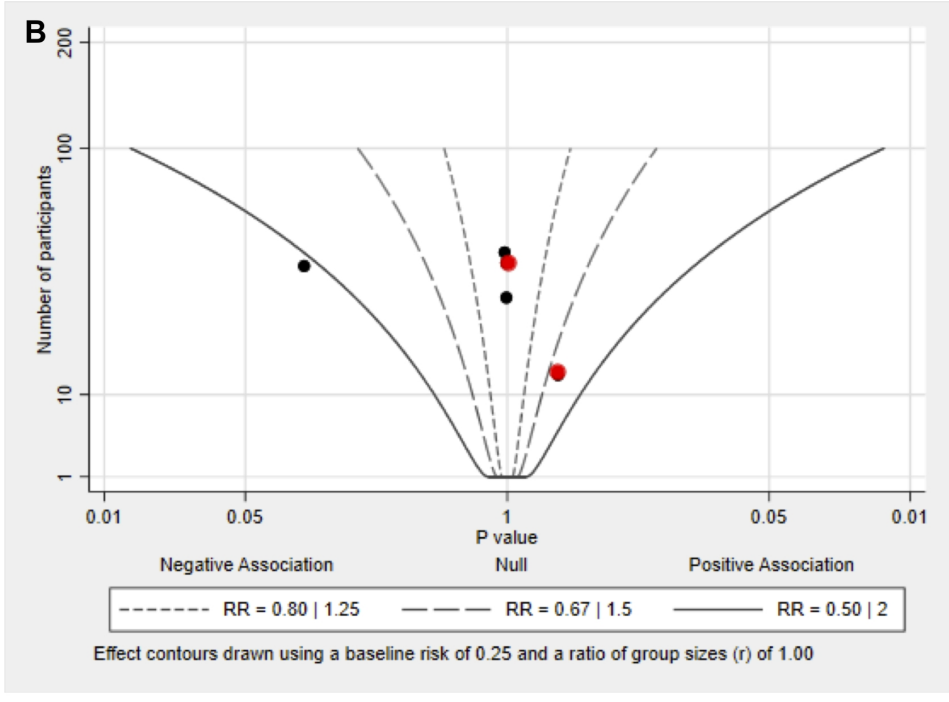

B
Number of participants
200
100
10
1
0.01
0.05
1
0.05
0.01
P value
Negative Association
Null
Positive Association
RR = 0.80 | 1.25
RR = 0.67 | 1.5
RR = 0.50 | 2
Effect contours drawn using a baseline risk of 0.25 and a ratio of group sizes (r) of 1.00

**Table 3.** Primary and secondary outcome measures used in randomized controlled trials evaluating the effect of photoactivated chromophore for infectious keratitis – corneal collagen cross-linking (PACK-CXL)**.**

| Authors (year) | Primary Outcome Measures | Secondary Outcome Measures |
|---|---|---|
| Kasetsuwan et al. (2016) | (1) Size of stromal infiltrate | (1) Size of epithelial defect<br>(2) Treatment failure<br>(3) BPVA |
| Uddaraju et al. (2015) | (1) Treatment failure at 6 weeks | (1) UDVA |
| Bamdad et al. (2015) | Not specified but following parameters were analyzed and reported:<br>(1) Size of epithelial defect<br>(2) Size of stromal infiltrate<br>(3) Grade of corneal ulcer<br>(4) Duration of treatment<br>(5) Treatment failure | |
| Said et al. (2014) | Not specified but following parameters were analyzed and reported:<br>(1) Time to complete healing (defined by complete corneal epithelialization and clearance of infiltrate)<br>(2) CDVA<br>(3) Treatment failure | |

BPVA = Best-corrected-pinhole-visual-acuity; UDVA = Uncorrected distance visual acuity; CDVA = Corrected distance visual acuity

**PACK-CXL compared to standard antimicrobial treatment for Infectious Keratitis**

**Patient or population**: Infectious Keratitis
**Intervention**: PACK-CXL
**Comparison**: Standard antimicrobial treatment

| Outcomes | № of participants (studies) Follow-up | Certainty of the evidence (GRADE) | Relative effect (95% CI) | Anticipated absolute effects | |
|---|---|---|---|---|---|
| | | | | Risk with standard antimicrobial treatment | Risk difference with PACK-CXL* |
| Mean time to healing | 72 (2 RCTs) | ⊕◯◯◯ VERY LOW [a,b] | - | 24.7 to 46.1 days | MD 7.44 days shorter (10.71 shorter to 4.16 shorter) |
| Size of epithelial defect at 7 days | 62 (2 RCTs) | ⊕◯◯◯ VERY LOW [a,b] | - | 14.94 to 20.9 $mm^2$ | MD 3.66 $mm^2$ smaller (14.26 smaller to 6.94 smaller) |
| Size of epithelial defect at final follow-up (14 to 30 days) | 62 (2 RCTs) | ⊕◯◯◯ VERY LOW [a,b,c,d] | - | 4.9 to 8.31 $mm^2$ | MD 5.09 $mm^2$ smaller (9.43 smaller to 0.74 smaller) |
| Size of Infiltrates at 7 days | 62 (2 RCTs) | ⊕◯◯◯ VERY LOW [a,b] | - | 14.88 to 28.1 $mm^2$ | MD 5.49 $mm^2$ smaller (7.44 smaller to 3.54 smaller) |
| Size of infiltrate at final follow-up (14 to 30 days) | 62 (2 RCTs) | ⊕◯◯◯ VERY LOW [a,b,d] | - | 9.3 to 9.63 $mm^2$ | MD 5.27 $mm^2$ smaller (9.12 smaller to 1.41 smaller) |
| CDVA (LogMAR) at final follow-up (one to three months) | 70 (2 RCTs) | ⊕◯◯◯ VERY LOW [a,b,d] | - | 1.2 to 1.67 | MD 0.08 LogMar worse (0.21 better to 0.37 worse) |
| Adverse events at final follow-up (one to three months) | 102 (3 RCTs) | ⊕◯◯◯ VERY LOW [a,b,d,e] | RR 0.49 (0.11 to 2.29) | 140 per 1,000 | 71 fewer per 1,000 (125 fewer to 181 more) |

*The risk in the intervention group (and its 95% confidence interval) is based on the assumed risk in the comparison group and the relative effect of the intervention (and its 95% CI).

**CI:** Confidence interval; **MD:** Mean difference; **RR:** Risk ratio

**GRADE Working Group grades of evidence**
**High certainty:** We are very confident that the true effect lies close to that of the estimate of the effect
**Moderate certainty:** We are moderately confident in the effect estimate: The true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different
**Low certainty:** Our confidence in the effect estimate is limited: The true effect may be substantially different from the estimate of the effect
**Very low certainty:** We have very little confidence in the effect estimate: The true effect is likely to be substantially different from the estimate of effect

## Explanations

a. High risk of bias due to lack of allocation concealment and blinding.
b. Total number of participants is less than the number generated by a conventional sample size calculation.
c. There is significant heterogeneity.
d. Differences in final follow-up
e. There are few events and the confidence interval includes appreciable benefit and harm.

**Table 5.** Pooled risk estimates of PACK-CXL on risk of adverse events in comparative studies grouped and analyzed according to type of infectious keratitis (IK) cohorts.

| IK cohorts | Number of Studies | Types of studies | Number of participants | Estimate of magnitude (95% CI) |
|---|---|---|---|---|
| **Bacterial** | 1 | 1 RCT[23] | 32 | NK |
| **Mixed*** | 4 | 2 RCTs[24,25]<br>1 NRS[27] | 115 | RR 0.57 (0.20 to 1.61) |
| **Fungal** | 2 | 1 RCT[26]<br>1NRS[28] | 54 | RR 1.30 (0.66 to 2.54) |

RCT = Randomized controlled trial; NRS = Non-randomized controlled studies; CI = Confidence interval; RR = Risk ratio; NK = Not known

*Mixed IK cohorts refer to cohorts consisting of two or more types of IK, which could be either bacterial, fungal, acanthamoeba or mixed IK.

**Search strategy for PACK-CXL in MEDLINE**

1. Cross-link*.mp
2. Crosslink*.mp
3. CXL.mp
4. KXL.mp
5. Cross-Linking Reagents/
6. Riboflavin*.mp
7. Vitamin B.mp
8. Photosensiti*.mp
9. Keratitis.mp
10. Corneal infect*.mp
11. Corneal ulcer*.mp
12. Exp Keratitis/
13. Exp Corneal Ulcer/
14. 1 or 2 or 3 or 4 or 5 or 6 or 7 or 8
15. 9 or 10 or 11 or 12 or 13
16. 14 and 15
17. Limit 16 to humans

**Search strategy for PACK-CXL in EMBASE**

1. Cross-link*.mp
2. Crosslink*.mp
3. CXL.mp
4. KXL.mp
5. Riboflavin*.mp
6. Vitamin B.mp
7. Photosensiti*.mp
8. Exp cross linking/
9. Keratitis.mp
10. Corneal infect*.mp
11. Corneal ulcer*.mp
12. Exp keratitis/
13. Exp corneal ulcer/
14. 1 or 2 or 3 or 4 or 5 or 6 or 7 or 8
15. 9 or 10 or 11 or 12 or 13
16. 14 and 15
17. Limit 16 to humans

**Table S2.** Summary of all randomized control trials evaluating the effectiveness and safety of photoactivated chromophore for infectious keratitis-corneal collagen cross-linking (PACK-CXL).

| Authors | Year | Protocol registration* | Age, years | Male gender, % | Total eyes (PACK-CXL) | Total eyes (control) | Causative organisms (in CXL group) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | B | F | A | V | M | CN |
| Bamdad et al. | 2015 | N | Mean = 39.6 (CXL) vs. 40.3 (control) | 21 (66%) | 16 | 16 | 16 | | | | | |
| Kasetsuwan et al. | 2016 | Y | Mean = 45 (CXL) vs. 54 (control) | 21 (70%) | 15 | 15 | 7 | 8 | | | | |
| Said et al. | 2014 | N | Mean = 37 (CXL) vs. 50 (control) | 18 (45%) | 21 | 19 | 7 | 3 | 1 | 0 | 7 | 3 |
| Uddaraju et al. | 2015 | Y | Median = 40 (CXL) vs. 56 (control) | 8 (61%) | 6 | 7 | | 6 | | | | |

| Authors | Pre-CXL vision (LogMAR) | Time from first presentation to PACK-CXL | CXL treatment protocol | Combined with SAT (for CXL group)? | Severity of ulcer[$] | Follow-up | COI |
|---|---|---|---|---|---|---|---|
| Bamdad et al. | - | Same day | 3mW/cm$^2$ for 30mins | Y | Mean = 17mm$^2$ (CXL) vs. 20mm$^2$ (control) | 1 month | N |
| Kasetsuwan et al. | Mean = 1.8 (CXL) vs. 1.7 (control) | Same day | 3mW/cm$^2$ for 30mins | Y | Median = 31mm$^2$ (CXL) vs. 31mm$^2$ (control) | 1 month | N |
| Said et al. | Mean = 2.2 (CXL) vs. 2.0 (control) | Within 48 hours | 3mW/cm$^2$ for 30mins | Y | Mean = 30mm$^2$ (CXL) vs. 16mm$^2$) | 2 weeks – 4 months | Y |
| Uddaraju et al. | Median = HM (CXL) vs. HM (control) | After 2 weeks | 3mW/cm$^2$ for 30mins | Y | Deep stromal; median = 7mm (CXL) vs. 5mm (control) | - | N |

*Prospective registration of the clinical trial protocol in a publicly accessible database.

B = Bacteria; F = Fungi; A = Acanthamoeba; V = Viruses; M = Mixed infection; CN = Culture-negative presumed infectious keratitis; SAT = Standard antimicrobial treatment; COI = Conflict of interest; IQR = Interquartile range; HM = Hand movement

**Table S3.** Summary of all clinical studies (excluding randomized control trials) evaluating the effectiveness and safety of photoactivated chromophore for infectious keratitis-corneal collagen cross-linking (PACK-CXL).

| Authors | Year | Study design | Total eyes (PACK-CXL) | Total eyes (control) | Causative organisms (in CXL group) | | | | | | Severity of ulcer[$] |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | B | F | A | V | M | CN | |
| Basaiawmoit et al. | 2018 | CCT | 13* | 32 | 4 | 9 | | | | | Mean = 15mm$^2$ (CXL) vs. 13mm$^2$ (control) |
| Vajpayee et al. | 2015 | CCT | 20 | 21 | | 20 | | | | | Mean = 16mm$^2$ (CXL) vs. 17mm$^2$ (control) |
| Agrawal et al. | 2016 | Case series | 6 | - | 1 | | | | | 5 | 4-9mm |
| Chan et al. | 2014 | Case series | 4 | 2 | 2 | | 1 | | | 1 | 11-64mm$^2$ |
| Cristian et al. | 2019 | Case series | 6 | - | | | 6 | | | | 5-9mm |
| Erdem et al. | 2018 | Case series | 13 | - | | 9 | | | | 4 | 6-25mm$^2$ |
| Iseli et al. | 2008 | Case series | 5 | - | 3 | 2 | | | | | - |
| Khalili et al. | 2017 | Case series | 8 | - | 6 | | | 2 | | | Severe (>6mm and involved posterior 1/3) |
| Khan et al. | 2011 | Case series | 3 | - | | | 3 | | | | 3-6mm |
| Knyazer et al. | 2018 | Case series | 20 | - | 9 | 1 | | | 1 | 9 | 2-7mm and <300$\mu$m depth |
| Li et al. | 2013 | Case series | 8 | - | | 8 | | | | | 1-6mm |
| Makdoumi et al. | 2012 | Case series | 16 | - | 12 | | | | | 4 | Median = 1mm |
| Makdoumi et al. | 2010 | Case series | 7 | - | 4 | | | | | 3 | 0.25mm$^2$ – total cornea |
| Muller et al. | 2012 | Case series | 6 | - | 3 | 2 | 1 | | | | - |
| Panda et al. | 2012 | Case series | 7 | - | | 1 | 1 | | 5 | | Grade 3-5 |
| Price et al. | 2012 | Case series | 40 | - | 24 | 7 | 2 | 1 | | 6 | 0.3-104mm$^2$ |
| Ramona et al. | 2016 | Case series | 10 | - | 10 | | | | | | - |
| Rosetta et al. | 2013 | Case series | 4 | - | 3 | | 1 | | | | Severe ulcer +/- melt |
| Shetty et al. | 2014 | Case series | 15 | - | 9 | 6 | | | | | Mild (superficial) to severe (full-thickness) |
| Skaat et al. | 2014 | Case series | 6 | - | 3 | | | | | 3 | 2-5mm |
| Sorkhabi et al. | 2013 | Case series | 10 | - | 9 | 1 | | | | | 12mm$^2$ – total cornea |
| Zloto et al. | 2018 | Case series | 18 | - | 13 | | | | | 5 | Mean = 10mm$^2$ |
| Abbouda et al. | 2014 | Case report | 2 | - | | 2 | | | | | >6mm |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Anwar et al. | 2011 | Case report | 2 | - | 1 | 1 | | | | 4-6mm |
| Arance-Gil et al. | 2014 | Case report | 1 | - | | | 1 | | | - |
| Casagrande et al. | 2014 | Case report | 1 | - | 1 | | | | | Severe ulcer with thinning |
| Chan et al. | 2017 | Case report | 1 | - | 1 | | | | | 1.5mm |
| Demirci et al. | 2013 | Case report | 1 | - | | | 1 | | | Ring ulcer with melt |
| Ferrari et al. | 2013 | Case report | 1 | - | | | | 1 | | - |
| Ferrari et al. | 2009 | Case report | 1 | - | 1 | | | | | - |
| Garduno-Vieyra et al. | 2011 | Case report | 1 | - | | | 1 | | | 8mm, anterior stroma |
| Igal et al. | 2017 | Case report | 1 | - | | 1 | | | | 3mm |
| Kozobolis et al. | 2010 | Case report | 2 | - | 1 | | | | | - |
| Kymionis et al. | 2016 | Case report | 1 | - | | | | 1 | | Severe deep infiltrate with perineuritis |
| Labiris et al. | 2014 | Case report | 1 | - | 1 | | | | | 3.5 mm with severe thinning |
| Moren et al. | 2010 | Case report | 1 | - | | | 1 | | | - |
| Oflaz et al. | 2017 | Case report | 1 | - | 1 | | | | | $20mm^2$ |
| Passilongo et al. | 2018 | Case report | 1 | - | | | | 1 | | Deep stromal |
| Saglk et al. | 2013 | Case report | 1 | - | | 1 | | | | Deep stromal |
| Tabibian et al. | 2014 | Case report | 1 | - | | 1 | | | | 1mm, anterior third of the stroma |
| Yagci et al. | 2016 | Case report | 1 | - | | 1 | | | | $6mm^2$ |
| Zarei-Ghanavati et al. | 2015 | Case report | 1 | - | | | | | 1 | - |

[$]Severity of the corneal ulcer is presented either in maximum linear diameter (mm) or in area ($mm^2$).

*Two patients lost to follow-up.

**Table S3 (Continue).** Summary of all clinical studies (excluding randomized control trials) evaluating the effectiveness and safety of photoactivated chromophore for infectious keratitis-corneal collagen cross-linking (PACK-CXL).

| Authors | Pre-CXL vision (LogMAR) | Time from first presentation to PACK-CXL | CXL treatment protocol | Combined with SAT | Outcomes (i.e. healing time and size of ED or infiltrate) | Treatment failure* | Post-CXL vision | Follow-up |
|---|---|---|---|---|---|---|---|---|
| Basaiawmoit et al. | - | Within 48 hours | 3mW/cm$^2$ for 30mins | Y | Mean healing time = 22 days (CXL) vs. 49 days (control) | 1 (CXL) vs. 4 (control) | - | - |
| Vajpayee et al. | 1.4 (CXL) vs. 1.5 (control) | Same day | 3mW/cm$^2$ for 30mins | Y | Complete healing = 98% (CXL) and 86% (control); Mean healing time = 31 days (CXL) and 31 days (control); Corneal perforation in 2. (CXL) and 3 (control) | 2 (CXL) vs. 3 (control) | Mean = 1.1 (CXL) vs. 1.3 (control) | 3 months |
| Agrawal et al. | CF – PL | >10 days | 3mW/cm$^2$ for 30mins | Y | Completely healing = 4 (67%) | 2 (33%) | HM – PL | 3 months |
| Chan et al. | Mean = 2.30 (CXL) vs. 3.0 (control) | Median = 6.5 days | 3mW/cm$^2$ for 30mins | Y | Resolution of infiltrate in all 6 cases; Complete ED healing = 2 (CXL) vs. 1 (control) | None | Mean VA = 1.23 (CXL) vs. 1.8 (control) | 3 months |
| Cristian et al. | 0.05 - NPL | Mean = 57 days | 4.5mW/cm$^2$ for 20mins or 9mW/cm$^2$ for 10mins | Y | Complete healing = 4 (67%); Mean healing time=109 days | None | 0.05 - NPL | Mean = 30.4 months |
| Erdem et al. | 0.05 – HM | Mean = 8 days | 3mW/cm$^2$ for 30mins | Y | 7 (54%) healed completely, 6 worsened; pain score improved from mean of 8 to 3.5 (Those that improved were mainly mild-moderate ulcer; all severe ulcer had progressive melting) | 6 (46%) | 0.05 - HM | Mean = 5.3 months |
| Iseli et al. | 20/30 - PL | - | 3mW/cm$^2$ for 30mins | Y | Halting of infection after CXL. | None | Vision improved; | 1-9 months |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Khalili et al. | 20/200 – PL | Mean = 15 days | 3mW/cm$^2$ for 30mins | Y | 67% bacterial keratitis healed; 50% viral keratitis healed | 2 (25%) | 20/30 – 20/400 - | - |
| Khan et al. | - | 1-2 months | 3mW/cm$^2$ for 30mins (x2) | Y | Mean healing time = 5.3 weeks | None | - | 4-12 months |
| Knyazer et al. | - | 3 days | 30mW/cm$^2$ for 3mins | Y | Mean healing time = 6.5 days | 1 (5%) | - | - |
| Li et al. | - | 5 - 30 days | 3mW/cm$^2$ for 30mins | Y | Complete healing time = 3-8 days | None | Improved vision in 6 | - |
| Makdoumi et al. | Median = 0.15 | Same day | 3mW/cm$^2$ for 30mins | N (2 needed SAT due to progression) | Complete healing = All (100%) cases (1 needed AMT); Mean healing time = 7 days | None | Median = 0.0 | 1-14 days |
| Makdoumi et al. | 0.05 - PL | 0-7 days | 3mW/cm$^2$ for 30mins | Y | Complete healing = All (100%) cases | None | 0.08 – PL | 1-6 months |
| Muller et al. | Mean = 1.3 | Median = 3 days | 3mW/cm$^2$ for 30mins | Y | Complete healing = 4 (67%) cases | 1 (17%) | Mean = 1.0 | 2-9 months |
| Panda et al. | - | 30 – 120 days | 3mW/cm$^2$ for 30mins | Y | Complete healing = All (100%) cases; Mean healing time = 8.6 days | None | - | - |
| Price et al. | - | 0-45 days (90% within 10 days) | 3mW/cm$^2$ for 15-45mins (x2 in 7 eyes) | Y | Complete healing = 25 (62.5%); Healing rate corresponds well with the size of infiltrate | 7 (18%) | - | - |
| Ramona et al. | - | 14 days or longer | 3mW/cm$^2$ for 30mins | Y | Complete healing = 8 (80%) | None | Visual improved in 8 eyes | 12 months |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Rosetta et al. | - | Mean = 6 days | 3mW/cm$^2$ for 30mins | Y | Mean ED healing time = 7.0 days (bacteria); 10 days (acanthamoeba) | None | Mean = 0.4 | Mean = 3.5 months |
| Shetty et al. | - | 14 days or longer | 3mW/cm$^2$ for 30mins | Y | Complete healing = 9 (67%); Mean ED healing time = 21.3 days, Mean infiltrate resolution time = 33.4 days. Improvement in pain score. | 3 (20%) | - | - |
| Skaat et al. | Mean = 0.7 | Mean = 12 days | 3mW/cm$^2$ for 30mins | Y | Complete healing = 5 (83%); Mean healing time = 17.2 days | 1 (17%) | Mean = 0.22 | Mean = 1.5 months |
| Sorkhabi et al. | Mean = 1.9 | - | 3mW/cm$^2$ for 30mins | Y | Complete healing = 8 (80%) | 2 (20%) | Mean = 1.5 | - |
| Zloto et al. | Mean = 1.5 | Mean = 10 days | 9mW/cm$^2$ for 10mins | Y | Not specified | 1 (6%) | CDVA maintained | - |
| Abbouda et al. | - | - | 3mW/cm$^2$ for 30mins | Y | Complete healing = 0% but melting was stopped in one case | 1 (50%) | - | - |
| Anwar et al. | CF – HM | 21 days | 3mW/cm$^2$ for 30mins | Y | Completely healed in 2 cases (at 2 weeks – 2 months) | None | 20/40-20/50 | Mean = 4 months |
| Arance-Gil et al. | - | 1 year | 3mW/cm$^2$ for 30mins | Y | Infection not controlled and cornea melted 8 months post-CXL requiring TK | 1 (100%) | 20/60 | - |
| Casagrande et al. | PL | - | 3mW/cm$^2$ for 30mins | Y | Complete healing at 2 months (had AMT at 1 month) | None | 20/400 | 2 months |
| Chan et al. | 20/50 | 2 days | 18mW/cm$^2$ for 5mins | Y | Complete healing in 2 weeks | None | 20/20 | 2 weeks |
| Demirci et al. | - | >2 months | 3mW/cm$^2$ for 30mins | Y | Complete healing at 10 days | None | - | 2 months |
| Ferrari et al. | - | 12 days | - | | | | | |
| Ferrari et al. | - | - | - | - | Complete healing at 30 days | None | - | 1 month |
| Garduno-Vieyra et al. | 20/400 | ~60 days | 3mW/cm$^2$ for 30mins | Y | Complete healing at 21 days | None | 20/20 | 5 months |
| Igal et al. | - | | Not specified | Y | Complete healing | None | 20/80 | 2 years |
| Kozobolis et al. | 20/400 – PL | | 3mW/cm$^2$ for 30mins | Y | Complete healing in all cases at 7 days. | None | 20/100 | 2 months |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Kymionis et al. | HM | 3 months | 3mW/cm$^2$ for 30mins | Y | Complete healing at 2 months | None | - | 2 months |
| Labiris et al. | HM | 3 days | 3mW/cm$^2$ for 30mins | Y | Complete healing at 5 days | None | - | 12 months |
| Moren et al. | 1.8 | 29 days | 3mW/cm$^2$ for 30mins | Y | Complete healing at 32 days | None | 0.2 | 9 months |
| Oflaz et al. | HM | 30 days | 3mW/cm$^2$ for 30mins | Y | Complete healing at 30 days | None | 20/400 | 6 weeks |
| Passilongo et al. | - | ~40 days | 9mW/cm$^2$ for 9mins | Y | Perforation at 3 days post-CXL | 1 (100%) | 20/40 | 16 months |
| Saglk et al. | - | 17 days | 3mW/cm$^2$ for 30mins (x2) | Y | Complete healing at 7 days (post-2$^{nd}$ CXL) | None | CF | 6 months |
| Tabibian et al. | 20/25 | Same day | 9mW/cm$^2$ for 10mins | N | Complete healing at 3 days | None | 0.0 | 1 month |
| Yagci et al. | - | - | 3mW/cm$^2$ for 30mins | Y | No improvement post-CXL | 1 (100%) | 0.2 | 14 months |
| Zarei-Ghanavati et al. | - | 60 days | 3mW/cm$^2$ for 30mins | Y | Complete healing at 2 weeks | None | - | 3 months |

*Treatment failure was defined as uncontrolled / worsening infectious keratitis requiring tectonic keratoplasty or evisceration.

SAT = Standard antimicrobial treatment; CF = Counting fingers; HM = Hand movement; PL = Perception of light; NPL = No perception of light; ED = Epithelial defect; CDVA = Corrected-distance-visual-acuity

**Table S4.** Summary of the healing rate and treatment failure of PACK-CXL in non-randomized controlled studies (NRS), case series and case reports based on the types of causative microorganisms.

| Authors | Year | Numbers | Complete healing | Additional treatment | Healing time (days) | Treatment failure |
|---|---|---|---|---|---|---|
| *Bacterial keratitis* | | | | | | |
| Agrawal et al. | 2016 | 1 | 1 (100%) | | - | 0 (0%) |
| Anwar et al. | 2011 | 1 | 1 (100%) | | 14 | 0 (0%) |
| Casagrande et al. | 2014 | 1 | 1 (100%) | AMT | 60 | 0 (0%) |
| Chan et al. | 2014 | 2 | 1 (50%) | | 90 | 0 (0%) |
| Chan et al. | 2017 | 1 | 1 (100%) | | 14 | 0 (0%) |
| Ferrari et al. | 2009 | 1 | 1 (100%) | | 30 | 0 (0%) |
| Iseli et al. | 2008 | 3 | 3 (100%) | | 28 – 120 | 0 (0%) |
| Khalili et al. | 2017 | 6 | 4 (67%) | | - | 2 (33%) |
| Knyazer et al. | 2018 | 9 | 9 (100%) | | 4 – 30 | 0 (0%) |
| Kozobolis et al. | 2010 | 1 | 1 (100%) | | 7 | 0 (0%) |
| Labiris et al. | 2014 | 1 | 1 (100%) | | 5 | 0 (0%) |
| Makdoumi et al. | 2012 | 12 | 12 (100%) | 1 AMT | 1 – 14 | 0 (0%) |
| Makdoumi et al. | 2010 | 4 | 4 (100%) | | - | 0 (0%) |
| Muller et al. | 2012 | 3 | 2 (67%) | | 120 | 1 (33%) |
| Oflaz et al. | 2017 | 1 | 1 (100%) | | 30 | 0 (0%) |
| Price et al. | 2012 | 24 | 20 (83%) | | 4 – 40 | 3 (17%)* |
| Ramona et al. | 2016 | 10 | 8 (80%) | | 30 | 2 (20%) |
| Rosetta et al. | 2013 | 3 | 3 (100%) | | 3 – 45 | 0 (0%) |
| Shetty et al. | 2014 | 9 | 6 (67%) | | 14 – 28 | 3 (33%) |
| Skaat et al. | 2014 | 3 | 2 (67%) | | - | 1 (33%) |
| Sorkhabi et al. | 2013 | 9 | 7 (78%) | | - | 2 (22%) |
| Zloto et al. | 2018 | 13 | 12 (92%) | | - | 1 (8%) |
| *Fungal keratitis* | | | | | | |
| Abbouda et al. | 2014 | 2 | 0 (0%) | | - | 1 (50%) |
| Anwar et al. | 2011 | 1 | 1 (100%) | | 60 | 0 (0%) |
| Erdem et al. | 2018 | 9 | 4 (44%) | 3 AMT | 15 – 33 | 4 (44%) |
| Igal et al. | 2017 | 1 | 1 (100%) | | - | 0 (0%) |
| Iseli et al. | 2008 | 2 | 1 (50%) | | - | 1 (50%) |
| Knyazer et al. | 2018 | 1 | 1 (100%) | | 16 | 0 (0%) |
| Li et al. | 2013 | 8 | 8 (100%) | | 3 – 8 | 0 (0%) |
| Muller et al. | 2012 | 2 | 1 (50%) | | 120 | 1 (50%) |

| | | | | | | |
|---|---|---|---|---|---|---|
| Price et al. | 2012 | 6 | 3 (50%) | | 26 – 28 | 3 (50%) |
| Saglk et al. | 2013 | 1 | 1 (100%) | | 7 (after 2nd CXL) | 0 (0%) |
| Shetty et al. | 2014 | 6 | 3 (50%) | | 15 – 90 | 3 (50%) |
| Sorkhabi et al. | 2013 | 1 | 1 (100%) | | - | 0 (0%) |
| Tabibian et al. | 2014 | 1 | 1 (100%) | | 3 | 0 (0%) |
| Vajpayee et al. | 2015 | 20 | 18 (90%) | | 15 – 90 | 2 (10%) |
| Yagci et al. | 2016 | 1 | 0 (0%) | | - | 0 (0%) |
| *Acanthamoeba keratitis* | | | | | | |
| Arance-Gil et al. | 2014 | 1 | 0 (0%) | | - | 1 (100%) |
| Chan et al. | 2014 | 1 | 1 (100%) | | 60 | 0 (0%) |
| Cristian et al. | 2019 | 6 | 4 (67%) | | 59 – 217 | 0 (0%) |
| Demirci et al. | 2013 | 1 | 1 (100%) | | 10 | 0 (0%) |
| Garduno-Vieyra et al. | 2011 | 1 | 1 (100%) | | 21 | 0 (0%) |
| Khan et al. | 2011 | 3 | 3 (100%) | | 21 – 42 | 0 (0%) |
| Moren et al. | 2010 | 1 | 1 (100%) | | 31 | 0 (0%) |
| Muller et al. | 2012 | 1 | 1 (100%) | | - | 0 (0%) |
| Price et al. | 2012 | 2 | 0 (0%) | | 54 – 145 | |
| Rosetta et al. | 2013 | 1 | 1 (100%) | | 10 | 0 (0%) |
| *Viral keratitis* | | | | | | |
| Ferrari et al. | 2013 | 1 | 0 (0%) | | - | 1 (100%) |
| Khalili et al. | 2017 | 2 | 1 (50%) | | 25 | 1 (50%) |
| Price et al. | 2012 | 1 | 0 (0%) | SK | - | 0 (0%) |
| *Mixed bacterial / fungal keratitis* | | | | | | |
| Erdem et al. | 2018 | 2 | 1 (50%) | 1 AMT | - | 1 (50%) |
| Knyazer et al. | 2018 | 1 | 0 (0%) | | - | 1 (100%) |
| *Mixed acanthamoeba / fungal keratitis* | | | | | | |
| Erdem et al. | 2018 | 2 | 2 (100%) | | 15 – 35 | 0 (0%) |
| Kymionis et al. | 2016 | 1 | 1 (100%) | | 60 | 0 (0%) |
| Passilongo et al. | 2018 | 1 | 0 (0%) | | - | 1 (100%) |
| *Mixed acanthamoeba / bacterial keratitis* | | | | | | |
| Panda et al. | 2012 | 1 | 1 (100%) | | 8 | 0 (0%) |

| | | | | | |
|---|---|---|---|---|---|
| *Culture negative* | | | | | |
| Agrawal et al. | 2016 | 5 | 3 (60%) | - | 2 (40%) |
| Chan et al. | 2014 | 1 | 1 (100%) | 120 | 0 (0%) |
| Knyazer et al. | 2018 | 9 | 9 (100%) | - | 0 (0%) |
| Kozobolis et al. | 2010 | 1 | 1 (100%) | 7 | 0 (0%) |
| Makdoumi et al. | 2012 | 4 | 4 (100%) | 4 – 12 | 0 (0%) |
| Makdoumi et al. | 2010 | 3 | 3 (100%) | - | 0 (0%) |
| Panda et al. | 2012 | 6 | 6 (100%) | 5 – 18 | 0 (0%) |
| Skaat et al. | 2014 | 3 | 3 (100%) | 19 – 47 | 0 (0%) |
| Zarei-Ghanavati et al. | 2015 | 1 | 1 (100%) | 14 | 0 (0%) |
| Zloto et al. | 2018 | 5 | 5 (100%) | - | 0 (0%) |

*One patient lost to follow-up.

PACK-CXL = Photoactivated chromophore for infectious keratitis-corneal collagen cross-linking; AMT = Amniotic membrane transplant