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

Running head: PACK-CXL for Infectious Keratitis

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ABSTRACT

Purpose: To examine the efficacy of adjuvant photoactivated chromophore for infectious keratitis–corneal 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 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 and protocol were registered with PROSPERO (registration number: CRD42019131290) and the Joanna Briggs Institute Database of Systematic Reviews and Implementation Reports.[20]

Data sources and search methods

Two authors (D.S.J.T and C.H.) searched MEDLINE (January 2003 to April 2019), EMBASE (January 2003 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”. The start date of January 2003 was selected because CXL was only introduced to clinical practice in 2003. 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.[21] 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 or perforation 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.[22] 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.[23] 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[24] 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.[23] 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.[23]

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.[25] 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.[26] 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 754 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 573 records were screened and 521 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,[27-32] 46 studies were included in the systematic review. These included four RCTs,[33-36] two NRS,[37, 38] 20 case series,[39-58] and 20 case reports,[59-78] 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[40] and Cristian et al. study.[41] 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,[33] Thailand,[34] Egypt,[35] and India.[36] Trials included participants aged between 15 and 84 years (mean age of 40 – 56 years), with slight male preponderance (59.1%). Two RCTs[34, 36] 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.[33] and Uddaraju et al.[36] included bacterial keratitis alone and fungal keratitis alone, respectively, whereas Kasetsuwan et al.[34] included both bacterial and fungal keratitis in their studies. Said et al.[35] 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[35] 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 an irradiance of 3 mW/cm^2 for 30 mins (total fluence 5.4mJ/cm^2), 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,[33, 34] within 48 hours in one study,[35] and after two weeks of non-improvement with SAT in one study.[36] 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),[33, 34] time to complete corneal healing or treatment duration (defined as complete re-epithelialization of the ulcer and disappearance of the infiltrate and hypopyon),[33, 35] adverse events[35,36] (defined by corneal perforation and/or endophthalmitis and/or increase in infiltrate size by ≥2mm), and visual-acuity.[34-36] The follow-up duration was between four and six weeks post-treatment, except for one study which the duration was not specified.[35] 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[33, 34] 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.[33, 35] Masking of participants was not possible but masking of assessors was possible in most studies. All trials had complete data except Uddaraju et al.[36] 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.[35] Other potential risk of bias included the lack of prospective registration of clinical trial in a publicly accessible database in two studies[33, 35] and the presence of conflict of interest in one study[35] 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.[37, 38] 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.[37]

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.[33, 35] 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.[35] 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).[33, 34] 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[34] 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).[33, 34]

C. Size of infiltrate

Two studies reported the size of infiltrate at 7 days and final follow-up (14 to 30 days).[33, 34] 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)[34, 35] (**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 worsening IK and/or corneal melt or perforation requiring tectonic / therapeutic keratoplasty or evisceration. Bamdad et al.[33] 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.[34-36] 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.[36] 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.[34, 35] 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.[34-36]

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,[33, 35, 37, 38] with one study of bacterial keratitis showing statistical significance.[33] 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.[34-38] 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),[38, 39, 41, 42, 44, 46, 47, 49, 51-53, 55, 57, 58] 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.[79] (consisting of 21 studies of 145 eyes) and Papaioannou et al.[80] (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.[79, 80] 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[33] included only participants with bacterial keratitis and the other study primarily included bacterial, fungal and mixed IK.[35] 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).[33, 34] 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.[36] 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.[52] 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.[35] In addition, potential confounding factors such as age and status of diabetes may affect the corneal wound healing time.[81-83] 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.[84] 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.[85] 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. It is also noteworthy to mention that the 400 μm limit is based on CXL studies on healthy corneas but not on inflamed or infected corneas. It is likely that the transmission of UV light behaves differently in infected corneas[59] but further research studies are required to elucidate this aspect. Corneal densitometry using Pentacam Scheimpflug imaging system may also serve as a useful adjuvant tool for monitoring the corneal response to IK.[86] 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.[87] 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[33, 34] and time taken to complete healing.[35] 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.[88, 89] Interestingly, when riboflavin was substituted with rose bengal, PACK-CXL was shown to demonstrate effective anti-amoebic activity.[88] Based on histopathologic examination, Hager et al.[28] 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.[52, 90]

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[33, 35] and the presence of conflict of interest.[35] 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)[35] 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.

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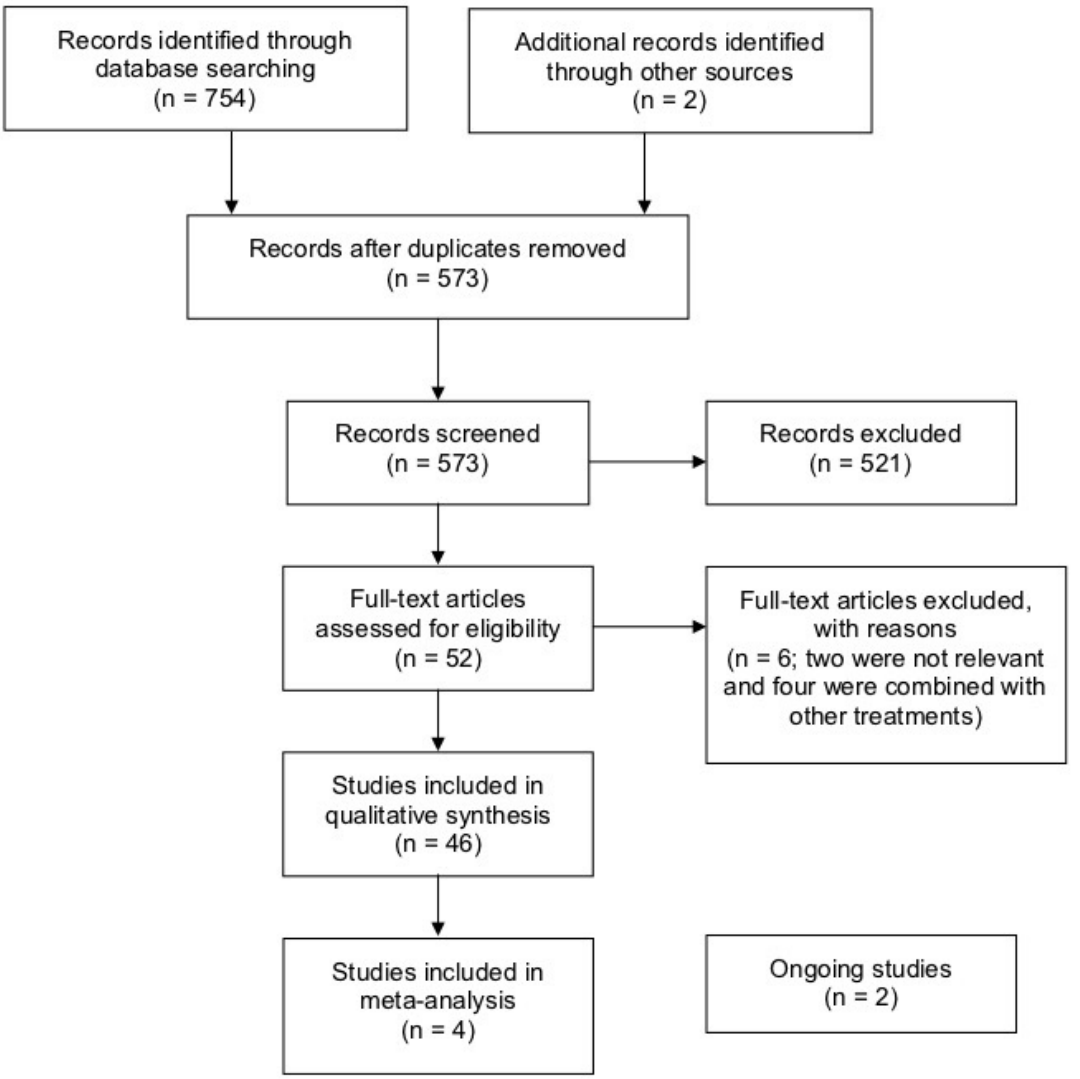
FIGURE LEGEND

Figure 1. PRISMA flow diagram of the literature search for “Photoactivated chromophore for infectious keratitis – corneal 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.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Additional records identified through other sources (n = 2)
Records after duplicates removed (n = 573)
Records screened (n = 573)
Records excluded (n = 521)
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)
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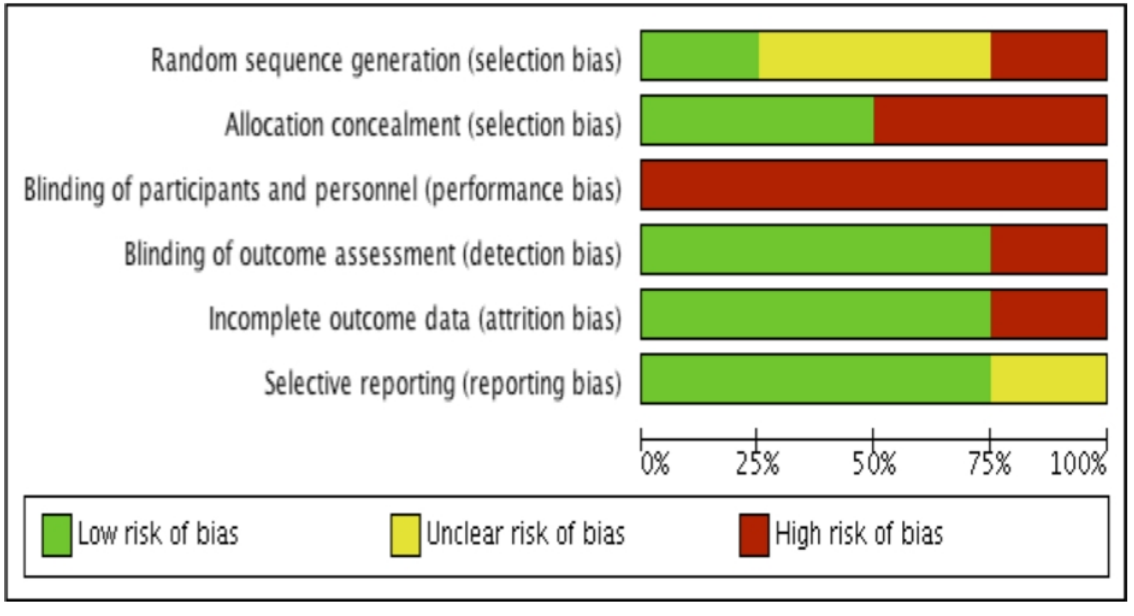
Studies included in meta-analysis (n = 4)
Ongoing studies (n = 2)


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)
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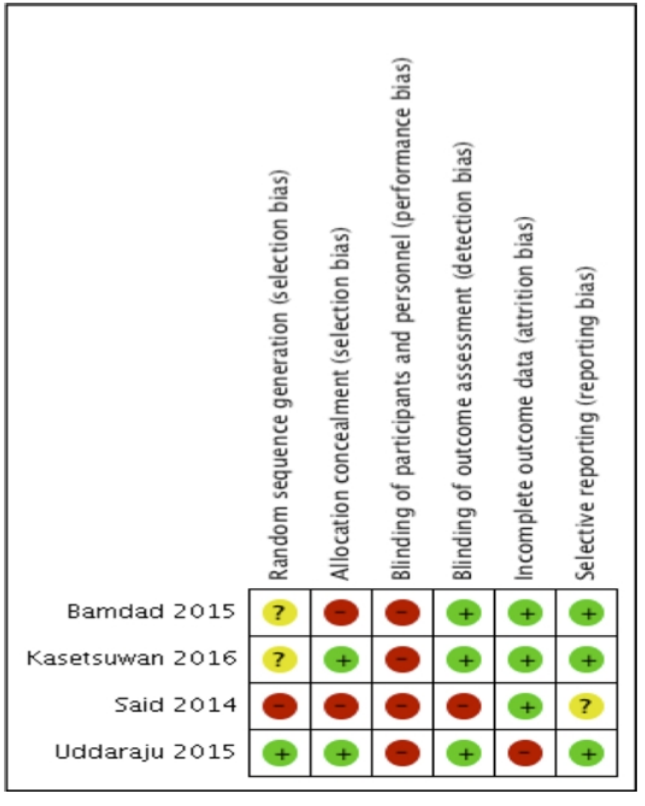
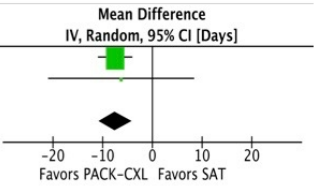
25%
50%
75%
100%
Low risk of bias
Unclear risk of bias
High risk of bias


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]**

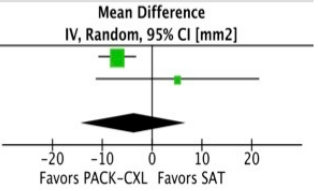
Heterogeneity: $Tau^2 = 0.00$; $Chi^2 = 0.03$, df = 1 (P = 0.87); $I^2 = 0\%$
Test for overall effect: Z = 4.45 (P < 0.00001)



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)

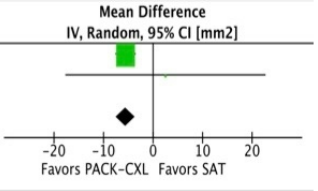


C. Size of infiltrate (mm^2)

(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]**

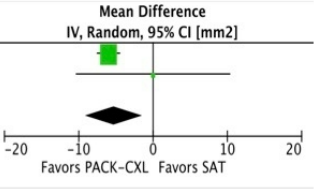
Heterogeneity: $Tau^2 = 0.00$; $Chi^2 = 0.61$, df = 1 (P = 0.43); $I^2 = 0\%$
Test for overall effect: Z = 5.52 (P < 0.00001)



(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]**

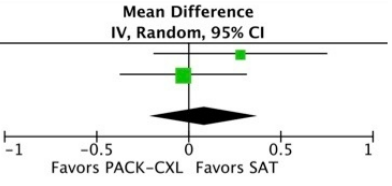
Heterogeneity: $Tau^2 = 3.81$; $Chi^2 = 1.27$, df = 1 (P = 0.26); $I^2 = 21\%$
Test for overall effect: Z = 2.68 (P = 0.007)



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]**

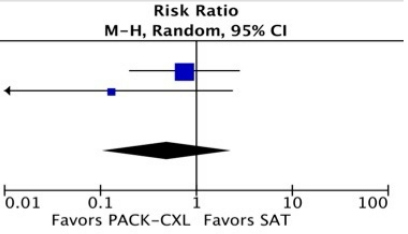
Heterogeneity: $Tau^2 = 0.00$; $Chi^2 = 1.10$, df = 1 (P = 0.30); $I^2 = 9\%$
Test for overall effect: Z = 0.55 (P = 0.58)



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)



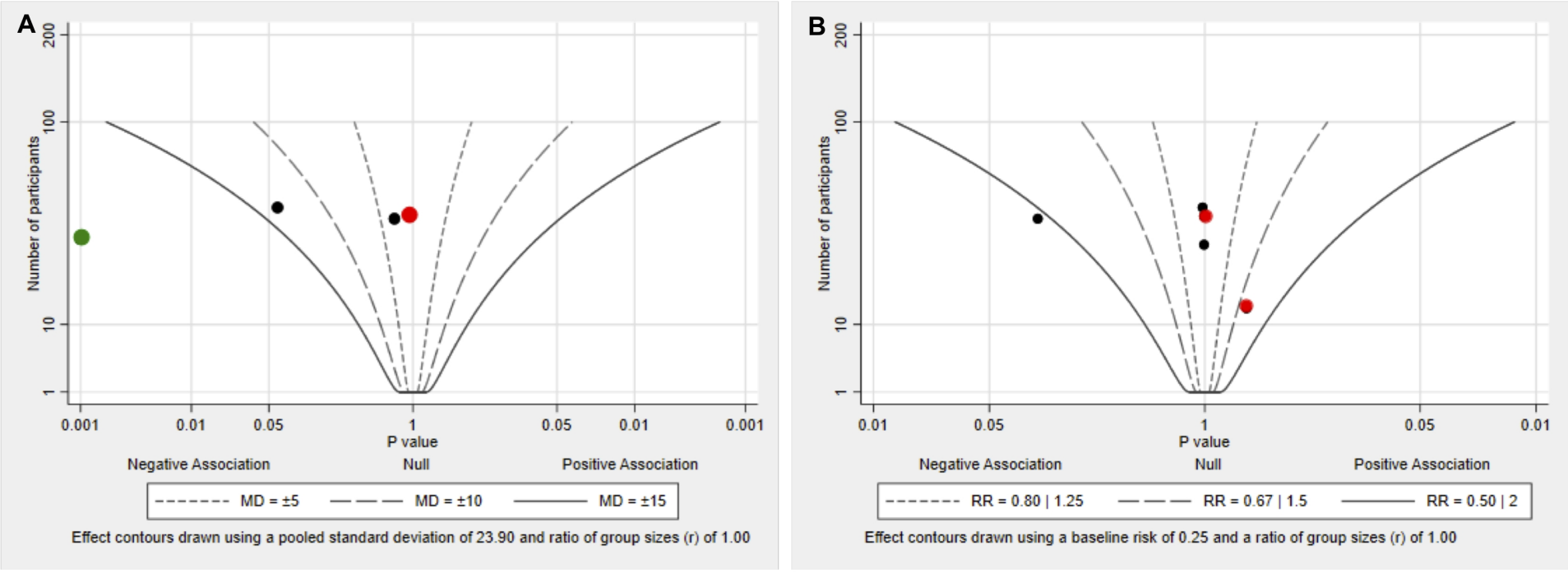

A
Number of participants
200
100
10
1
0.001
0.01
0.05
1
0.05
0.01
0.001
P value
Negative Association
Null
Positive Association
MD = ±5
MD = ±10
MD = ±15
Effect contours drawn using a pooled standard deviation of 23.90 and ratio of group sizes (r) of 1.00
B
Number of participants
200
100
10
1
0.01
0.05
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 1. Primary and secondary outcome measures used in randomized controlled trials evaluating the effect of photoactivated chromophore for infectious keratitis – corneal 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 (2) Treatment failure (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: (1) Size of epithelial defect (2) Size of stromal infiltrate (3) Grade of corneal ulcer (4) Duration of treatment (5) Treatment failure	
Said et al. (2014)	Not specified but following parameters were analyzed and reported: (1) Time to complete healing (defined by complete corneal epithelialization and clearance of infiltrate) (2) CDVA (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 3. 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] 1 NRS[27]	115	RR 0.57 (0.20 to 1.61)
Fungal	2	1 RCT[26] 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
18. Limit 17 to yr=”2003 – 2019’

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
18. Limit 17 to yr=”2003 – 2019”

Table S2. Summary of all randomized control trials evaluating the effectiveness and safety of photoactivated chromophore for infectious keratitis-corneal 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 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 <300μm 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-2nd 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