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Corneal cross-linking for infectious keratitis of various causes: an umbrella review

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Abstract

Objective To explore the therapeutic role of corneal cross-linking (CXL) for infectious keratitis.

Methods This is an umbrella review of the systematic reviews and meta-analysis concerning the role of corneal CXL in treating infectious keratitis. Appropriate keywords were carefully selected following the identification of PICO (Population: People who have corneal cross-linking for infectious keratitis; Intervention: corneal cross-linking; Comparison: other treatments such as antibiotic therapy; Outcome: Primary outcome was considered as the efficacy of treatment using re-epithelization and heal rate, and secondary outcome was considered need to penetrating keratoplasty (PK)). The electronic search across various databases, including Cochrane, PubMed, MEDLINE, Embase, SCOPUS, CINAHL, Psychoinfo, and ProQuest, was performed until August 2024.

Results Five systematic reviews out of 53 identified records are included in the umbrella review. Due to the structure of the included studies, statistical analysis was not possible to be conducted. Four studies were included that mainly evaluated the role of adjuvant corneal CXL in bacterial keratitis, and the other study focused mainly on fungal keratitis. The studies reported heterogeneous results. Two systematic reviews reported a shorter period for corneal epithelium healing in the adjuvant CXL group compared to the standard antibiotic therapy (SAT), especially in fungal keratitis. However, two studies showed no significant change in re-epithelization duration. One meta-analysis reported a reduction in corneal infiltrate size 7 days after adjuvant corneal CXL compared to the SAT. None of the included studies reported a difference in corneal complications, such as perforation and the need for PK in the CXL group compared to SAT.

Conclusion The corneal CXL in infectious keratitis has no uniform protocol, especially regarding the de-epithelization procedure before CXL, leading to heterogeneity in the trial results. However, it seems the adjuvant corneal CXL next to SAT is not inferior to the unaccompanied SAT and may be superior in some cases, including fungal etiologies, regarding faster corneal healing.

Keywords Infectious keratitis, Corneal cross-linking, CXL, PACK-CXL, Re-epithelization, UVA

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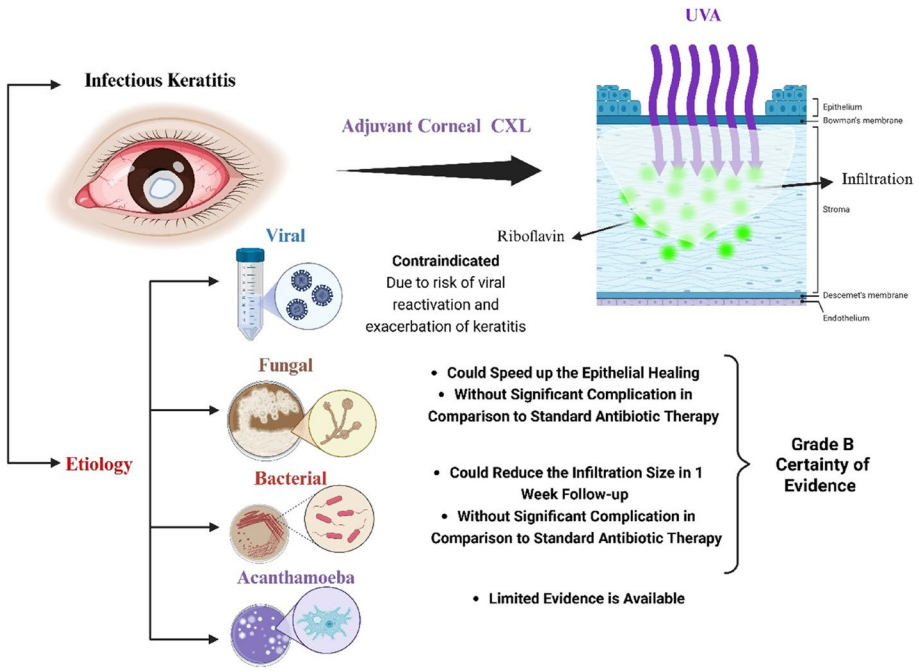
Graphical Abstract



Introduction

Infectious keratitis is an ocular condition characterized by diminished vision, photophobia, and red eye, potentially leading to corneal blindness. It is more prevalent in developing countries due to limited healthcare access and poor hygiene. The etiologies include bacteria, fungi, viruses, and parasites, with polymicrobial infections such as Staphylococcus, Pseudomonas aeruginosa, Fusarium, Candida, and Acanthamoeba playing a significant role. Predisposing factors for IK include contact lens use, eye trauma, ocular surface disorders, postoperative complications following corneal surgeries, and immunosuppressed states [1, 2].

The current treatment of IK involves accurate diagnosis, antimicrobial treatment, and surgical interventions [3]. However, managing this condition comes with significant challenges, such as treatment resistance due to undiagnosed or misdiagnosed predisposing factors, high antimicrobial resistance in bacteria like Staphylococcus aureus, and multidrug-resistant strains like Pseudomonas aeruginosa species. Moreover, excessive use of broad-spectrum antibiotics or a mixture of topical antibacterial, antiviral, and antifungal drugs may enhance drug toxicity and accelerate the development of resistance patterns. Additionally, Topical antimicrobials have limited penetration and can lead to complications such as corneal perforation, melting, and scarring. Ineffective treatment of IK may delay corneal epithelization and result in non-healing corneal ulcers [4–6]. Coexisting conditions in IK, including other corneal surface or systemic diseases, can significantly influence treatment outcomes. Corneal surface disorders, systemic illnesses, and co-infections involving multiple organisms in polymicrobial infections often overlap, obscuring accurate diagnosis and leading to treatment delays and exacerbated outcomes [7]. In severe cases that are resistant to conservative treatment, surgical interventions become imperative due to the potential risk of ocular perforation and extension of infection to the sclera or deeper ocular structures [8].

Corneal cross-linking (CXL) is a surgical intervention that facilitates the creation of covalent bonds within collagen fibers, enhancing their tensile strength and structural rigidity. The cross-linking process is performed by enzymes, notably lysyl oxidase (LOX) and advanced glycation end products (AGEs) [9]. Initiated by researchers at the Technical University of Dresden in the early 1990 s, Riboflavin-UVA CXL employs photochemical reactions to promote cross-linking in corneal collagen. This process involves UVA activating riboflavin to produce singlet oxygen, facilitating the linkage between collagen fibrils through several mechanisms, including imidazolone formation and carbonyl group activation [10].

Regarding infectious keratitis, CXL exhibits a diverse mechanism of action. UV rays and riboflavin were primarily used as photo-mediators to neutralize pathogens present in the blood, thereby diminishing the infectious load. Additionally, the specialized Photo Activated Chromophore for Keratitis-Corneal Cross-linking (PACK-CXL) variant alters collagen characteristics, enhancing the corneal stromal resistance to bacterial enzymes and preventing the progression of corneal melting. CXL shows more enhanced therapeutic outcomes in bacterial keratitis when used in conjunction with antimicrobial agents, compared to using antimicrobials alone, resulting in faster recovery and better ulcer healing. Concurrently, research highlights CXL's effectiveness in halting the progression of keratoconus and post-LASIK ectasia, with notable improvements in vision, keratometric measurements, and topographic data [11–13].

CXL demonstrates potential in the treatment of IK, particularly bacterial variants, but questions remain about its safety and effectiveness. The ideal protocol for PACK-CXL in such cases is still under investigation [14]. Research indicates that adjuvant PACK-CXL can accelerate corneal healing and infiltrate resolution. Additionally, the prolonged impacts of CXL in IK have not been extensively investigated [15]. This study aims to assess the effectiveness and safety of CXL in treating infectious keratitis. By combining the findings from systematic reviews and meta-analyses, this study provides a thorough understanding of the therapeutic advantages and possible risks associated with the procedure.

Methods

Search strategy and eligibility criteria

We conducted an umbrella review, wherein data from existing meta-analyses concerning the outcomes of corneal cross-linking in keratitis were systematically gathered and critically analyzed.

Although this investigation is classified as a secondary study, it comprises an analysis of previously conducted studies, thereby constituting an umbrella study of systematic reviews. The structural findings were explored according to established guidelines and predetermined criteria pertinent to the relevant evidence. The statistical population for this analysis encompasses all published and unpublished scientific literature up to August 2024. Following the identification of PICO (Population: People who have corneal cross-linking for infectious keratitis; Intervention: corneal cross-linking; Comparison: other treatments such as antibiotic therapy; Outcome: Primary outcome was considered as efficacy of treatment using re-epithelization and heal rate; and secondary outcome was considered need to PK), appropriate keywords were carefully selected. A search specialist performed the electronic search across various databases, including Cochrane, PubMed, MEDLINE, Embase, SCOPUS, CINAHL, Psychoinfo, and ProQuest. A manual search was conducted within relevant seminars, theses, and conferences to mitigate the potential for publication bias. The search strategy was delineated as follows:

A- The strategy for Persian sources involved searching through the title, abstract, and keywords of the articles.
B- The strategy for non-Iranian sources entailed selecting studies by a subject expert based on the title, abstract, and complete text across three distinct stages.

Supplementary file 1 delineates the search strategy comprehensively.

Two reviewers [Fereshteh Farhadi and Nazli Taheri] independently evaluated titles, abstracts, and full-text articles to ascertain study inclusion. The intervention of a third reviewer [Ali Mostafae] was employed to resolve any disagreements and uncertainties. The reference sections of studies selected for full-text retrieval were scrutinized to uncover additional potentially relevant research after excluding records that did not satisfy the inclusion criteria.

Eligibility criteria

Only systematic reviews, whether or not accompanied by meta-analysis, were considered. The studies were selected following the inclusion criteria, which comprised patients suffering from infectious keratitis, while those who conformed to the exclusion criteria were omitted from the study. No restrictions were imposed regarding the studies'date or country of origin.

Reviews that employ opinion polls or subjective texts as primary evidence were excluded. Other types of evaluations, such as narrative and comprehensive assessments, were also excluded.

Outcomes of interest include survival rates, complications, and other significant adverse effects.

Assessment of methodological quality

Subsequent to the selection of eligible studies by two evaluators utilizing the Joanna Briggs Institute (JBI) Critical Appraisal Checklist for systematic reviews and research syntheses, all forms of bias—including selection, performance, identification, attrition, and reporting—were examined.

Within the JBI Critical Appraisal Checklist System, scores ranging from 0–3 were deemed low quality, 4–7

were regarded as medium quality, and 8–11 were classified as high quality.

Data collection

Data extraction was conducted following the framework established by the investigator, summarized and documented utilizing the JBI Data Extraction Form for Review designated for Systematic Reviews and Research Syntheses. Two independent reviewers undertook the data extraction process separately. In instances of ambiguity regarding the inclusion and exclusion criteria for studies as well as the data extraction, both reviewers were consulted, and any discrepancies were resolved through collaborative discussion among the authors. The parameters collected encompassed the number and categories of primary studies, the aggregate number of participants, the duration of follow-up, and the variability of results within each synthesis.

In situations where multiple meta-analyses presented data concerning the same outcome, the most recent review that adhered to our inclusion criteria was selected, thereby excluding older meta-analyses to mitigate the potential for sample duplication. The quality of individual studies included in each systematic review was not evaluated. Among the systematic reviews included in our analysis, a limited number of studies investigated the sources of heterogeneity among the primary studies.

This study has been registered with PROSPERO. CRD42023441716. The Tabriz University of Medical Sciences research ethics committee has approved this study with the approval code of IR.TBZMED.REC.1402.331.

Certainty of evidence

JBI grades of recommendation were used in this study due to clinical appraisal of included systematic reviews [16]. Table 5 shows the results.

Statistical analysis

The outcomes evaluated in various systematic reviews were heterogeneous, with various definitions. On the other hand, only three included studies reported detailed meta-analysis results on outcomes. In addition, the included RCTs for meta-analysis had a notable overlap between different published systematic reviews, although they targeted various etiologies of infectious keratitis. Considering these issues, performing data pooling and meta-umbrella analysis was not feasible based on the included systematic reviews, so each study's outcomes were reported descriptively. All the figures and graphical abstracts are created in BioRender.com.

Results

Literature search

Figure 1 illustrates the methodological approach employed in the systematic identification of studies deemed suitable for inclusion in the umbrella review. The initial search yielded a total of 53 titles; after removing duplicate entries, 30 titles and corresponding abstracts underwent evaluation. Ultimately, 10 full texts were selected, and from this pool, five systematic reviews with or without meta-analyses were found to meet the eligibility criteria. The characteristics of 5 narrative reviews excluded from the umbrella review are provided in Supplementary file 2.

Main findings of systematic reviews with or without meta-analysis

The 5 included systematic reviews were published from 2016 to 2023 from 5 countries. In 2 of 5 studies, RCTs were only included [17, 18]; however, the Non-RCTs, case series, and case reports were included in the rest of the systematic reviews [19–21]. All of the systematic reviews were focused on the use of PACK-CXL and conventional CXL on infectious keratitis; however, the study of Marasini et al. was mainly focused on the use of various forms of UV radiation in infectious conditions, including acute corneal keratitis [21]. Iran, Thailand, Egypt, India, and Canada were the geographical contexts for the original research studies included in the aforementioned systematic reviews. The etiology of infectious keratitis was mixed and heterogeneous in all the included systematic reviews except for the study of Davis et al. [18]. Although the included studies in various systematic reviews had overlaps, each included systematic review had some unique studies discussed in the results. Tables 1 and 2 demonstrate the characteristics and results of the included systematic reviews. The research encompassed a variety of methodologies for cross-linking as a therapeutic intervention for keratitis. Among the outcomes analyzed within these studies, the duration of reepithelization, the healing rate of keratitis, and the necessity for ultimate penetrating keratoplasty emerged as the most frequently addressed metrics.

The average duration of reepithelization in included studies is delineated in Table 3. This table shows that in most studies, days of reepithelization are clinically high (more than 50 days overall). This duration has been elongated in more complex cases due to the challenging management of these kinds of keratitis.

Furthermore, although the cross-linking methodologies varied across these investigations, this outcome could be aggregated based on the time required for the reepithelization

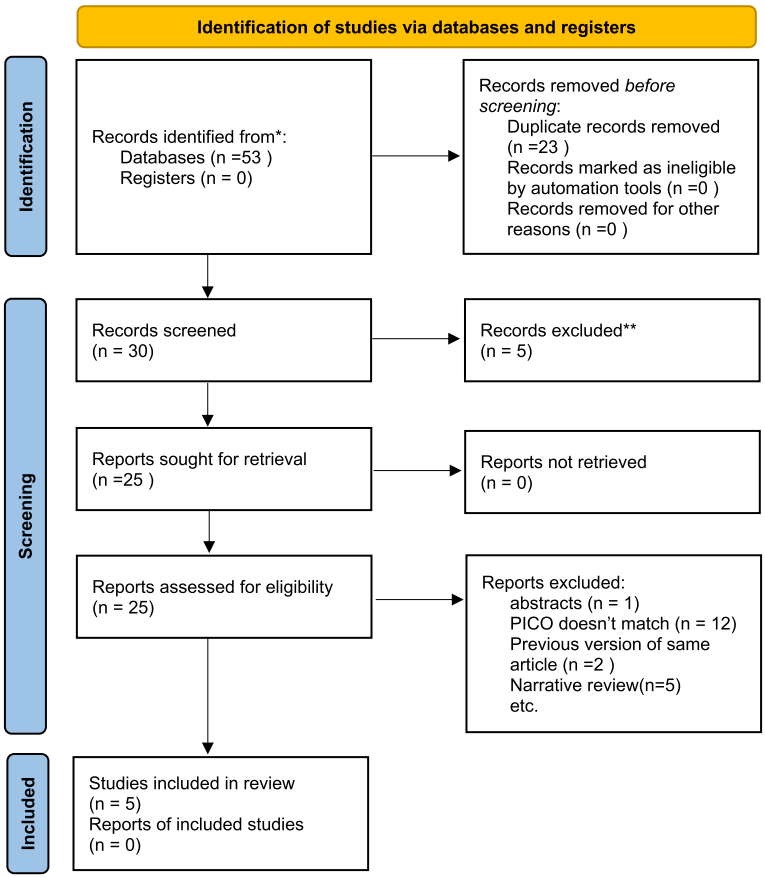


Fig. 1 PRISMA flow chart. *Consider, if feasible to do so, reporting the number of records identified from each database or register searched (rather than the total number across all databases/registers). **If automation tools were used, indicate how many records were excluded by a human and how many were excluded by automation tools

of the corneal surface. For all outcomes exhibiting statistically significant results, the robustness of the evidence was evaluated as weak. The rate of healing in case studies was quantifiable, demonstrating an efficacy of 86% following corneal cross-linking interventions in the case studies.

The results of descriptive analysis

Figure 2 represents the healing rate in the included studies. Despite differences in methodological aspects regarding cross-linking use, this healing rate seems clinically acceptable (varying from 43.09% to 86.45%). Ting 2019 and Liu 2023 did not measure this outcome. Figure 3 represents the cases that needed penetrating keratoplasty in the included studies, mostly in emergency conditions. This report, unfortunately, seems to be high (up to 15.71% of cases).

Assessment of risk of bias

Using the criteria suggested by the JBI checklist, all systematic reviews were rated critically high regarding methodological quality (Table 4). The most common reasons for potential bias were "not clear declaration of recommendations for practice" (question 10) and "missing information regarding the criteria for appraising studies " (question 5).

Certainty of evidence

The summarized recommendations of the included studies and the certainty of evidence for each recommendation are provided in Table 5.

Discussion

In this comprehensive umbrella review encompassing five systematic reviews incorporating meta-analyses, our findings indicate that the duration of re-epithelialization, the rate of keratitis healing, and the necessity for ultimate PK are the primary outcomes extensively examined concerning crosslinking for infectious keratitis.

PACK-CXL presents a novel and intriguing approach to managing infectious keratitis aimed at deferring or obviating the need for emergency keratoplasty. A

Table 1 Characteristics of the included studies in the umbrella review

Author (year)	Liu 2023 [17]	Ting 2019 [19]	Papaioannou 2016 [20]	Marasini 2021 [21]	Davis 2020 [18]
Country of Article	China	UK	Greece	New Zealand	USA
Individuals (Eyes)	283	435 (RCTs: 115)	210 (RCTs: 72)	346 (RCTs: 156)	59
Groups in RCTs	Treatment: 139 Control: 144	Treatment:58 Control: 57	Treatment: 37 Control: 35	Treatment: 79 Control: 77	Treatment: 30 Control: 29
Diagnosis in RCTs	Fungal Keratitis: 203 Bacterial Keratitis: 80	Fungal Keratitis: 30 Bacterial Keratitis: 53 Mixed (Fungal + Bacterial): 8 Acanthamoeba: 3 Culture Negative: 21	Fungal Keratitis: 5 Bacterial Keratitis: 47 Mixed (Fungal + Bacterial): 8 Acanthamoeba: 3 Inconclusive: 9	Fungal Keratitis: 54 Bacterial Keratitis: 32 Mixed (Fungal + Bacterial): 30 Unspecific: 40	Bacterial Keratitis: 59
Intervention	PACK-CXL	PACK-CXL	Conventional CXL and PACK-CXL	UVA-CXL	PACK-CXL
Search details	clinical condition ("keratitis,""corneal ulcer"), intervention ("crosslinking reagents,""riboflavin,""anti-infective agents","ultraviolet therapy,""photosensitizing agents,""ultraviolet rays,""collagen"), and study type (randomized clinical trial)	"cross-linking","PACKCXL", "riboflavin","Vitamin B", "keratitis", "corneal ulcer", and "corneal infection"	"corneal collagen cross-linking" or "photoactivated riboflavin" or "UVA light and riboflavin" and "infectious keratitis" or "corneal ulcer."	Keywords not mentioned	Keyword not mentioned Search strategy appendixes
Sources searched	Embase, MEDLINE with PubMed, Web of Science, and Cochrane Library	MEDLINE, EMBASE, and Cochrane Central	MEDLINE/PubMed and Cochrane Central Register of Controlled Trials	MEDLINE, Embase, Cochrane CENTRAL, ANZCTR, and US National Library of Medicine	Cochrane MEDLINE Ovid Embase.com PubMed LILACS US National Institutes of Health Ongoing Trials Register ICTRP
Range (years) of included studies	Up to April 5, 2022	January 2003 to April 2019	Up to March 19, 2015	Between 1946 and March 25, 2020:	up to July 8, 2019
Number of included study	7	46	25	53 (Ocular: 42)	3
Type of included study	7 RCTs	4 RCTs 2 Non-RCTs 20 Case series 20 Case reports	2 RCTs 13 Case series 10 Case reports	Ocular: 5 RCTs 4 non- RCTs 22 Case series 8 In vitro studies 3 Registred trials	2 RCTs 1 Quasi-RCTs
Country origin of included studies	Not mentioned	Egypt Iran Thailand India	Not mentioned	Egypt Iran Thailand India Canada	Iran Thailand
Appraisal	Was done	Was done	Was done	Was done	Was done
Appraisal instruments used	The Cochrane Handbook for Systematic Reviews of Interventions	The Cochrane Handbook for Systematic Reviews of Interventions	The Cochrane Collaboration tool for assessing risk of bias	The Cochrane risk of bias and the ROBINS-I tools	Cochrane method
Appraisal rating	Good	Good	Good	Good	Good
Analysis	RevMan	RevMan	OpenMeta Analyst	Microsoft Excel	RevMan 5
Method of analysis	Random-effects model	Random-effects model The Mantel–Haenszel method	Random-effects model	Random-effects model	Subgroup analysis Sensitivity analysis

Table 2 Outcomes and results of included studies in the umbrella review

Author (year)	Liu 2023 [17]	Ting 2019 [19]	Papaioannou 2016 [20]	Marasini 2021 [21]	Davis 2020 [18]
Outcome assessed	1) The duration of corneal healing 2) The size of corneal epithelial defect at 1 week 3) The size of corneal infiltrate at 1 week 4) The depth of corneal infiltrate at the final follow-up 5) Visual acuity at final follow-up 6) Adverse Events	1) Time to complete corneal healing 2) The size of the epithelial defect 3) The size of the infiltrate 4) Corrected-distance visual acuity (CDVA) 5) Adverse events	1) Healing of corneal ulcer is defined as complete re-epithelialization 2) Time (in days) to complete re-epithelialization 3) Proportion of complication	1) The time to wound resolution (days) 2) Change in wound area in the first four weeks 3) Adverse events	1) The proportion of participants with re-epithelization and complete healing with or without scar formation at four to eight weeks. (Complete healing was defined as the absence of infiltrate, epithelial healing, and no sign of inflammation or epithelial defect.) 2) The proportion of participants with best-corrected visual acuity (BCVA) of 20/100 or better at four to eight weeks based on measurements made on a logMAR chart 3) Mean change from baseline in BCVA at four to eight weeks, as measured on a logMAR chart 4) The proportion of participants with a reduction in corneal infiltrate, as defined by study investigators, at four to eight weeks 5) The proportion of participants with reduction in intraocular inflammation including, but not limited to, corneal keratic precipitates or anterior chamber cellular reaction at four to eight weeks 6) The proportion of participants with treatment failure, including, but not limited to, persistence or worsening of existing infectious keratitis, anterior chamber cellular reaction, corneal infiltrate, or epithelial defect, at four to eight weeks 7) Adverse events
Infiltration size	For fungal keratitis, all these trials found that adjuvant PACK-CXL could not reduce the size of corneal infiltrate at 1 week For bacterial keratitis, it was controversial	Size of infiltrate at 7 days follow-up: 14.88–28.1 mm2 MD 5.49 mm2 smaller (7.44 smaller to 3.54 smaller), p: < 0.00001, I^2:0%, p:0.43 Size of infiltrate at final follow-up (14–30 days): 9.3–9.63 mm2, MD 5.27 mm2 smaller (9.12 smaller to 1.41 smaller), p:0.007, I^2:21%, p:0.26	Not mentioned	Not mentioned	Not mentioned

Table 2 (continued)

Author (year)	Liu 2023 [17]	Ting 2019 [19]	Papaioannou 2016 [20]	Marasini 2021 [21]	Davis 2020 [18]
The mean re-epithelization time	Adjuvant PACK-CXL significantly reduced the time needed to perform corneal healing in fungal keratitis (MD =—1.13, 95% CI,—1.83 to—0.42, P < 0.05, I2:0%, p:0.33)	Mean time to healing 24.7–46.1 days MD 7.44 days shorter (10.71 shorter to 4.16 shorter), p: < 0.00001, I^2:0%, p:0.87	✓ 4 ✓ 31 ✓ 6 ✓ 7 ✓ 32 ✓ 37 ✓ 33 ✓ 37 ✓ 6 ✓ 13 ✓ 21 ✓ 8 ✓ 57 ✓ 21 ✓ 8 ✓ 5 ✓ 6 ✓ 11 ✓ 5 ✓ 7 ✓ 5 ✓ 39 ✓ 76 ✓ 46 ✓ 5 ✓ 21 ✓ 33 ✓ 3 ✓ 12 ✓ 17 ✓ 2 ✓ 24 ✓ 7	In RCTs Pooled data analysis shows no differences in the time to wound resolution (days) in the UVA-CXL group relative to the standard treatment group (mean difference [MD]: - 18.20 [95% CI:—39.04 to 2.65] days; p = 0.09) (I^2:87%, p:0.005)	The proportion of participants with re-epithelialization and complete healing with or without scar formation at 4 to 8 weeks RR = 1.53 (95%CI: 0.88–2.66)
Antibiotic usage	SAT in the control group	SAT in the control group in RCTs	Not mentioned	SAT in the control group in RCTs	SAT in the control group

Table 2 (continued)

Author (year)	Liu 2023 [17]	Ting 2019 [19]	Papaioannou 2016 [20]	Marasini 2021 [21]	Davis 2020 [18]
Result	Adjuvant PACK-CXL significantly reduced the time needed for corneal healing in fungal keratitis (*MD* = —1.13, 95% *CI*,—1.83 to—0.42, *P* < 0.05) All these trials found that adjuvant PACK-CXL could not reduce corneal epithelial defect size at 1 week in fungal and bacterial keratitis For fungal keratitis, all these trials found that adjuvant PACK-CXL could not reduce the size of corneal infiltrate at 1 week For bacterial keratitis, it was controversial	Adjuvant PACK-CXL resulted in a shorter mean time to complete corneal healing (– 7.44 days; 95% CI, – 10.71 to – 4.16) Quicker resolution of the infiltrate at 7 days (– 5.49 mm2; 95% CI, – 7.44 to – 3.54) and at 14–30 days (– 5.27 mm2; 95% CI, – 9.12 to – 1.41) There was no significant difference in the size of the epithelial defect (p:0.50), CDVA (p:0.58), and risk of adverse events (p:0.37) Evidence of the use of PACK-CXL in acanthamoeba and mixed IK was insufficient	The proportion of eyes healed with CXL was 87.2% (95% confidence interval (CI), 81.9%, 91.8%) For bacterial keratitis, the proportion of eyes healed was 85.7% (95% CI, 78.5%, 91.7%), whereas 10/11 and 25/32 eyes with acanthamoeba and fungal keratitis, respectively, were healed (available data not sufficient to provide a valid proportion analysis) The summary proportion of complications was 13.4% (95% CI, 8.9%–18.5%), whereas it was 14% (95% CI, 8%–22%) for bacterial cases	Pooled data analysis showed no difference in the time to wound resolution with UVA-CXL relative to standard treatment (mean difference [MD]: 18.20 [95% CI: 39.04 to 2.65] days; p = 0.09) (I^2:87%, p:0.005) Three studies evaluating UVA-CXL in acute corneal infection reported a change in wound size from baseline within four weeks. Data were not meta-analyzed because the methods of wound de-epithelisation prior to UV exposure differed between studies and could have confounded measurements of the rate of epithelial wound closure Pooled data analysis of five RCTs shows the risk of adverse events to be similar between the UVA-CXL group and standard treatment group (RR: 0.70 [95%CI: 0.32–1.79]; p = 0.35) (I2:30%, p:0.22)	It is very uncertain whether PACK-CXL with standard antibiotic therapy is more effective than standard antibiotic therapy alone for re-epithelialization and complete healing (risk ratio (RR) 1.53, 95% confidence interval (CI) 0.88 to 2.66; participants = 15) No participant had a best-corrected visual acuity of 20/100 or better at eight weeks (very low certainty evidence) There is also no evidence that using PACK-CXL with standard therapy results in fewer instances of treatment failure than standard therapy alone (RR 0.50, 95% CI 0.05 to 4.98; participants = 32)
Complications	Adjuvant PACK-CXL could not reduce adverse events in both fungal and bacterial keratitis (*RR* = 0.78, 95% *CI*, 0.38 to 1.60, *P* = 0.49, I^2:51%, p:0.11; *RR* = 0.36, 95% *CI*, 0.08 to 1.71, *P* = 0.20, I^2:0%, p:0.71, respectively)	Adverse events at the final follow-up (one to three months): RR 0.49 (0.11–2.29) 140 per 1000, (71 fewer per 1000 (125 fewer to 181 more)) (p:0.37)	Perforation, endophthalmitis, enucleation, or the need for tectonic keratoplasty or other interventions, such as amniotic membrane were considered as the adverse outcome or treatment failure • 1 (AMT) • 1 (PK) • 7 (6 PK, 1 SK) • 2 (PK) • 1 (PK) • 1 (PK) • 2 (1 PK, 1 enucleation) • 4 (3 perforations, 1 recurrence) • 3 (keratoplasties) • 1 (PK) • 1 (AMT) • 2 (1 AMT, 1 conjunctival flap)	In RCTs: 0/21 in the study treatment group; 4/19 in the standard treatment group (corneal perforation in 3, infection recurred in 1) • 5/6 in the study treatment group (corneal perforation in 4, increased infiltration • in 1); 4/7 in standard treatment group (increased infiltration in 1) • 1/16 in study treatment group (1 required AMT); 2/16 in standard treatment group (AMT in 1; conjunctival flap in 1) • 2/15 in the study treatment group (keratoplasty in 2); 4/15 in the standard treatment group (keratoplasty in 3, evisceration in 1) 3/21 in the study treatment group (corneal perforation in 3); 6/20 in the standard treatment group (corneal perforation in 6)	• Two cases of uncontrolled infection in the PACK-CXL with the standard therapy group at day 30, three cases of uncontrolled infection, and one case of endophthalmitis in the standard therapy-alone group • There were no complications in the PACK-CXL with the standard therapy group at the time of healing (14 to 120 days). In contrast, there were three cases of corneal perforation and one case of recurrent infection in the standard therapy-alone group
Heterogeneity	Low	Low	High	Moderate to High	Low

Table 3 Detailed data on re-epithelialization duration (days) in the included systematic reviews

Author/Year	Included Study type	Total Mean	Gram Negative	Gram Positive	Fungal	Acanthamoeba	Mixed	Culture Negative
Davis/2020	RCT	-	31	42 40 35 26 19 14	42 31 33 90	26	35 40 63 67 53 56	46 26 19
	Non-RCT	-	-	-	-	-	-	-
Marasini/2021	RCT	43.75 ± 10.30 39.76 ± 18.22 39.55 ± 5.12	-	-	-	-	-	-
	Non-RCT	7.23 ± 3.67 21.3 ± 6.14 30.85 ± 26.6 45.2 ± 44.43	30 60	45.2 ± 44.43 21.3 ± 6.14 7.23 ± 3.67 5 7 to 30	30.85 ± 26.6 6.0 ± 1.77 39.55 ± 5.12 (1.30 ± 0.93 months	32	-	8.57 ± 4.50 5
Papaioannou/2016	RCT	39.76 17.2	33 31	17.2 42 40 35 26 19 14	42 31 56 90	26	40 35 33 63 67 53	26 19 47
	Non-RCT	-	30 7 8 12 25 3 28 6	3 28 5 7 14 5 73 13 75 5 30 5 4 16	5 60 5 23 25 6 7 27 33 3	37 33 21 8 99 5 10	4 15 9 4	6 3 2 1 3
Ting/2019	RCT	24.7–46.1 days 17.2 + _ 4.1 days 39.76	-	-	-	-	-	-
	Non-RCT	-	-	-	-	-	-	-

recent randomized prospective trial conducted a comparative analysis between patients receiving PACK-CXL and medical treatment versus those administered antimicrobial therapy exclusively, revealing no statistically significant differences in corneal healing duration or final visual outcomes between the two cohorts; however, a notable complication rate of 21% was documented in the control group, contrasting with a 0% complication rate in the treatment group. It should be said that this good-quality RCT was also included in one of our included systematic reviews [22].

PACK-CXL has proven efficacious in halting the progression of corneal infiltration; however, it also poses a risk for the occurrence of corneal perforation when the infiltrate penetrates deeply, particularly in cases of fungal infection. Anterior segment optical coherence tomography (AS-OCT) has demonstrated utility as a diagnostic tool for monitoring patients with keratitis who have undergone PACK-CXL [23]. The pronounced inflammatory response within the anterior chamber may instigate proteolytic processes on the internal surface, resulting in thinning of the corneal tissue, thus indicating that reliance solely on pachymetric measurements is insufficient to deem the PACK-CXL procedure as safe; therefore, it is imperative to assess the cornea involved in the infectious process and the depth of the infiltrate. PACK-CXL may exhibit efficacy when the keratitis is localized to the anterior–middle stroma; however, it becomes ineffective and poses safety concerns in instances of deep infiltrations. In a study

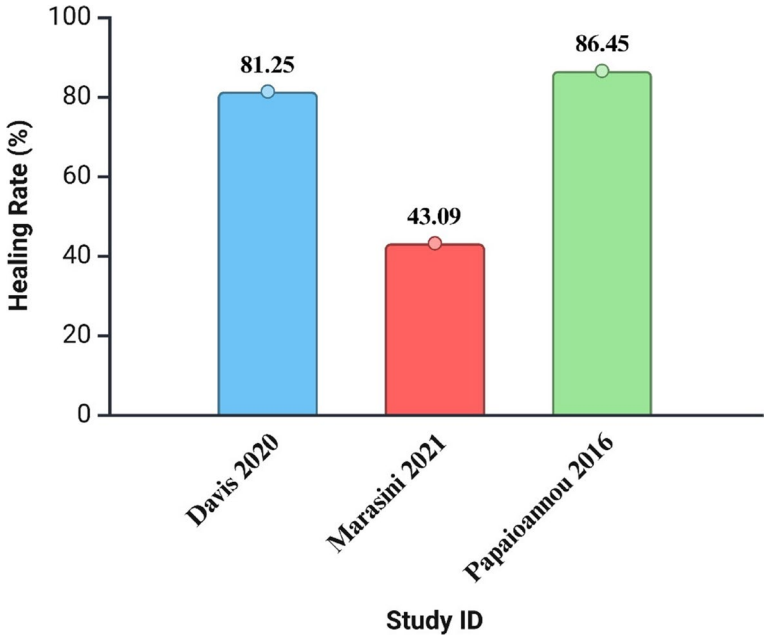


Fig. 2 Healing rate of infectious keratitis after adjuvant corneal cross-linking in various systematic reviews

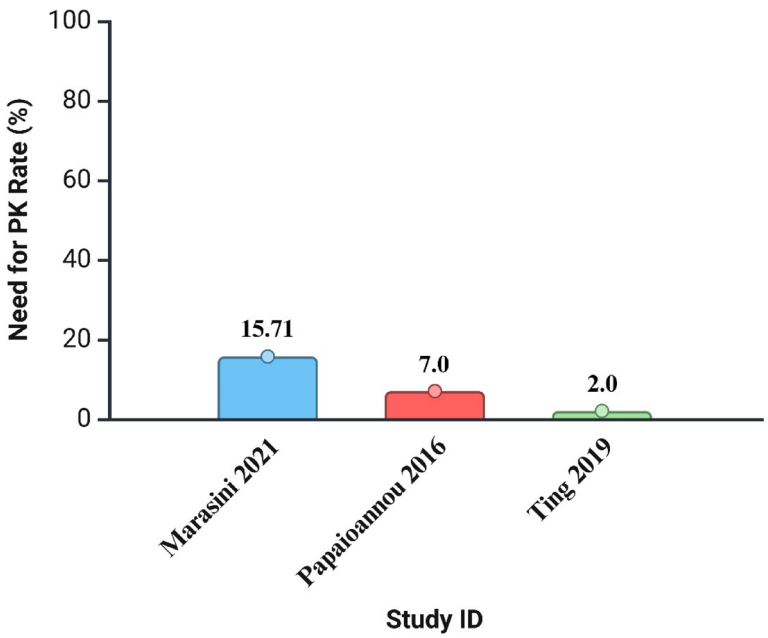


Fig. 3 The rate of need for PK in infectious keratitis cases treated with adjuvant corneal cross-linking. PK: Penetrating Keratoplasty

conducted by Sorkhabi et al., two cases exhibiting deep stromal infiltrates accompanied by hypopyon demonstrated a lack of response to corneal cross-linking. The deep localization of the infiltrate may not be adequately addressed by riboflavin photoactivation alone, potentially due to the limitations imposed by light penetration [24, 25].

Re-epithelialization emerged as the principal outcome highlighted across the majority of studies, with the protracted duration of re-epithelialization predominantly associated with fungal and mixed cases, averaging over 50 days. In the research conducted by Wie et al., the mean duration of re-epithelialization for fungal keratitis managed with crosslinking was recorded at 38.5 days,

Table 4 The included systematic reviews appraisal results based on the JBI checklist

Study ID	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Q9	Q10	Q11	Overall quality
Davis2020												High
Liu2023												High
Marasini2021												High
Papaioannou2016												High
Ting2019												High

Green: yes/red: no/yellow: unclear

Q1. Is the review question clearly and explicitly stated?

Q 2. Were the inclusion criteria appropriate for the review question?

Q 3. Was the search strategy appropriate?

Q 4. Were the sources and resources used for the study adequate?

Q 5. Were the criteria for appraising studies appropriate?

Q 6. Was critical appraisal conducted by two or more reviewers independently?

Q 7. Were there methods to minimize errors in data extraction?

Q 8. Were the methods used to combine studies appropriate?

Q 9. Was the likelihood of publication bias assessed?

Q 10. Were recommendations for policy and/or practice supported by the reported data?

Q 11. Were the specific directives for new research appropriate?

Table 5 GRADE: The summarized recommendations of the included studies and the certainty of evidence for each recommendation

Study	Recommendation	grade
Liu 2023	The present study suggests that adjuvant PACK-CXL accelerates corneal healing in fungal keratitis compared with standard antibiotic treatment (SAT) alone Ophthalmologists should pay more attention to the type and severity of infectious keratitis, drug regimens of SAT, and PACK-CXL protocol in clinical practice	B
Ting 2019	Adjuvant PACK-CXL may serve as a helpful addition to the therapeutic armamentarium for IK in reducing the time to complete healing and the size of the 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 PACK-CXL use in acanthamoeba keratitis remains elusive, with contradicting evidence from in vitro and clinical studies, whereas PACK-CXL is contraindicated in cases of viral keratitis	B
Papaioannou 2016	PACK-CXL seems promising in treating infectious keratitis, excluding herpetic keratitis, with increased expectations for bacterial and acanthamoeba cases compared with fungal keratitis	B
Marasini 2021	The studies in this review show that UV application can be safe and effective for treating localized infections. Firstly, UVA-CXL is effective for treating microbial keratitis caused by various infections, especially in severe cases. Secondly, UVC treatment improved outcomes in nearly 94% of chronic infection cases. UVB was studied only once, possibly due to concerns over cancer risk. UVC needs further research for specific dosing despite promising results in treating infected wounds, including those from antibiotic-resistant bacteria	B
Davis2020	There is insufficient evidence to establish whether Photo Activated Chromophore for Keratitis-Corneal Cross-linking (PACK-CXL) in combination with standard antibiotic therapy is safe and effective for bacterial keratitis	B

contrasting with a mean of 66.5 days within the control cohort. The authors indicated that, among patients achieving cure, crosslinking appeared to reduce the necessity for pharmacological interventions and surgical procedures, expedite the healing process of fungal ulcers, and diminish the overall treatment duration; furthermore, it appears that fungal infections within the cornea not only elicit an immune response that releases inflammatory mediators such as IL- 6, IL- 8, and MCP- 1, but also augment the activity of enzymes, including pepsin, trypsin, and collagenase, which degrade corneal collagen, potentially leading to corneal melting and perforation [26–28].

CXL can improve the resistance of collagen to enzymatic digestion. CXL increases the interlinking of chemical bonds to change the structure of corneal collagen fibers, thus blocking the interaction between enzymes and their target sites. CXL not only inactivates or eradicates pathogens by damaging ribonucleic acids but also has a direct cytotoxic effect on inflammatory cells, decreasing the inflammatory reaction associated with the immune response. CXL can also induce

keratocyte apoptosis in the anterior part of the cornea, reducing the transformation of activated keratocytes into fibroblasts, leading to less corneal opacity after CXL [29, 30]. The oxidant effect of UV-A combined with riboflavin is the major pathway of cellular damage, which is involved in pathogen eradication in corneal ulcers [31].

It is also worth mentioning that the CXL procedure could be safe for endothelial cells in infectious keratitis cases due to decreased penetration of UVA irradiation in infected corneas [32]. In addition, in-vitro studies have demonstrated a higher bacteriocidal effect for higher UVA intensity with higher energy irradiated on pathogens. This finding, besides the ex-vivo studies on porcine corneas, suggests a possible higher efficacy for accelerated PACK-CXL (30 mW/cm^2) for corneal ulcers, especially concerning the lower penetrance of UVA in infected corneal tissue [33, 34]. A pilot study on the clinical efficacy of accelerated PACK-CXL in infectious keratitis (20 cases) showed promising results with a mean re-epithelization time of 8.2 days without any significant change in endothelial cell density or need for tectonic keratoplasties [35].

The toxicity of antifungal eye drops may be one of the leading causes of delayed wound healing. When topical antifungal agents are frequently applied after wound healing, the corneal epithelium becomes thicker and more opaque, and the corneal surface becomes rougher and more irregular. A study by Wie et al. reported that CXL can be dangerous in refractory fungal lesions with deep-seated infiltrations and melting, especially in cases involving the posterior 1/3 of the corneal stroma [26].

Based on the included studies, adjuvant corneal cross-linking might be safe in bacterial or fungal keratitis treatments. It should be noted that, currently, the PACK-CXL has not been approved as a first-line treatment for infectious keratitis, but it is an adjunctive therapeutic intervention besides the standard antimicrobial therapy. However, the results regarding the efficacy of this method in healing keratitis and improving the visual outcome are heterogeneous, and more extensive clinical trials are needed to elaborate on this issue. The other issue that should be addressed in future studies is the unification of the CXL protocol for infectious keratitis, especially regarding the de-epithelization procedure before CXL. Also, the evidence regarding the therapeutic role of CXL in keratitis due to Acanthamoeba is too limited, and recommendations could not be given in this regard.

Limitations

The results of our investigation necessitate an interpretation that acknowledges its inherent limitations. Initially, the number of meta-analyses that fulfilled the selection criteria was confined to five studies encompassing five distinct outcomes, thereby underscoring the imperative for additional research in this domain. Also, there were some overlaps between the included studies. Furthermore, the notable correlations identified between the duration of reepithelization and various visual outcomes were influenced by certain potential biases, which ultimately culminated in a diminished strength of evidence for all such associations. In this regard, we posit that subsequent meta-analyses incorporating larger sample sizes may serve to address these deficiencies. Additionally, we observed a substantial level of heterogeneity (with I^2 exceeding 50%) in some studies, which likely reflects clinical variations, including disparate definitions of reepithelization, varying follow-up periods, and differing definitions of outcomes, among potentially other factors. On the other hand, the characteristics of included systematic reviews and their results prevented performing a meta-umbrella analysis, limiting our study's conclusion.

Supplementary Information

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Supplementary Material 1

Supplementary Material 2

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Authors' contributions

F.F., H.S., and A.M. contributed to the study's primary conception and design. F.F., H.S., and F.P. performed the systematic search. F.F., N.T., and A.M. performed the screening of the studies. F.F. and A.A. extracted the data from the included studies, performed the appraisal, and prepared the primary draft. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

This study adhered to the Declaration of Helsinki. The Tabriz University of Medical Sciences research ethics committee has approved this study with the approval code of IR.TBZMED.REC.1402.331.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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