Effectiveness and Safety of Adjuvant Amniotic Membrane Transplant Versus Standard Antimicrobial Treatment for Infectious Keratitis: A Systematic Review Protocol

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Review title

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Abstract

Objective: To systematically examine the effectiveness of adjuvant amniotic membrane transplant (AMT) versus standard antimicrobial therapy (SAT) for infectious keratitis (IK).

Introduction: IK is a major cause of corneal blindness worldwide. Broad-spectrum topical antimicrobial therapy is currently the gold standard for managing IK. AMT has been employed as an adjuvant therapy for IK to promote corneal healing; however high-quality evidence is limited.

Inclusion criteria: This review will consider studies that include patients of all age groups with all types of IK, including bacterial, fungal, viral, acanthamoeba, mixed and culture-negative presumed IK. We will exclude patients who had undergone other types of primary surgery other than AMT during the initial management and those who had less than 7 days' follow-up from the commencement of the treatment.

Methods: Electronic databases, including MEDLINE, EMBASE, Cochrane CENTRAL and relevant registries, will be searched for relevant studies. Titles, abstract and full text of the relevant studies will be independently assessed by two reviewers. Extracted data will include authors, year of publication, sample size, types of AMT techniques, types of causative microorganisms, main outcomes, visual acuity, and adverse events. No restriction will be applied to the date or language of the publication. Bibliographies of the included articles will be independently and manually screened by two authors to identify further relevant studies. Eligible studies will be critically appraised by two independent reviewers at the outcome level for methodological quality. A meta-analysis will be performed for the included randomized controlled trials when there are sufficient similarities.

Systematic review registration number: This systematic review will be registered on PROSPERO registration after the completion of review of this JBI systematic review protocol.

Keywords: Amnion; Amniotic membrane; Antimicrobial; Corneal infection; Keratitis

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Introduction

Infectious keratitis (IK) is the leading cause of corneal blindness worldwide.[1] IK is a painful and potentially sight-threatening ophthalmic condition that often requires early and intensive treatment to prevent irreversible visual impairment or blindness. Moreover, it can be associated with lengthy duration of treatment and hospitalization, posing significant impact on the affected patients and healthcare systems.[2, 3] Depending on the geographical locations and population-based risk factors (e.g. agricultural practice, trauma, use of contact lens, etc.), bacteria and fungi are shown to be the main causative microorganisms for IK, followed by viruses and parasites (particularly acanthamoeba).[4-6] Broad-spectrum topical antimicrobial therapy is currently the gold standard for managing IK in routine clinical practice.[6] In severe IK, with or without impending/actual corneal perforation, adjuvant therapies such as tarsorrhaphy,[7] fibrin glue,[7] photoactivated chromophore-corneal cross-linking (PACK-CXL),[8] and amniotic membrane transplant (AMT)[9] can be employed to augment the therapeutic effect of antimicrobial therapy and expedite the process of corneal healing and visual rehabilitation.

Amniotic membrane (AM) is the innermost layer of the fetal membrane, comprising a monolayer of metabolically active epithelium, a thick basement membrane, and an avascular stromal matrix.[10] AM has been shown to possess a plethora of beneficial biological properties, including anti-inflammatory, anti-angiogenic, antimicrobial, wound healing, and anti-fibrotic functions.[9] The role of amniotic membrane transplant (AMT) in ocular diseases was first documented in 1940.[11] However, it was not until the early 1990s that Batlle and Perdomo had reinvigorated the interest of employing AMT for various ocular surface diseases, for which they noted that the process of corneal re-epithelialization could be achieved within 72 hours of surgery.[9] Subsequently, Kim and Tseng[12] popularized the use of AMT for ocular surface reconstruction using glycerin-preserved AM. By using an *in vivo* rabbit model of limbal stem cell deficiency and corneal epithelial defect, they demonstrated that AMT could inhibit corneal neovascularization, promote corneal re-epithelialization and restoration of cornea-like epithelial phenotype.[12] Although the benefit of AMT for treating IK has been demonstrated in some studies,[13,14] this surgical treatment is usually reserved as a second-line therapy when IK did not improve after the standard antimicrobial therapy (SAT). Therefore, the value of employing AMT in addition to SAT in the early stage of IK remains uncertain.

In view of the easy accessibility to AM donor tissues (due to relative availability of placenta), refinement of preservation and storage methods, and lack of donor tissue-related immunogenicity, AMT is now widely deployed as part of the management of many ocular surface diseases, including persistent epithelial defect / non-healing corneal ulcer, infectious keratitis, corneal perforation, severe ocular surface chemical burn, limbal stem cell deficiency, cicatricial conjunctivitis, symptomatic bullous keratopathy, and pterygium surgery, amongst others.[9, 15] Depending on the clinical need and circumstances, AMT can be performed using three different surgical techniques; (a) overlay / patch technique: an AM that is larger than the epithelial defect (epithelial side-up or side-down) is transplanted to cover the ocular surface, allowing the host corneal epithelium to regenerate under the

AM; (b) inlay / graft technique: an AM of similar size to the epithelial defect (epithelial side-up) is transplanted to act as a substrate for the host epithelium to grow over; and (c) sandwich technique: a combined overlay and inlay technique that facilitates the regeneration of host epithelium between the AMs.[15] In addition, AM can be used in fresh form or preserved for longer-term storage purpose using several methods, including cryopreservation, lyophilization (freeze-drying) and air-drying methods,[15] with comparable clinical efficacy observed amongst these methods.[16]

A preliminary search of PROSPERO, MEDLINE, the Cochrane Database of Systematic Reviews and the *JBI Database of Systematic Reviews and Implementation Reports* was conducted and no current or underway systematic reviews on the topic were identified. Despite the widespread clinical applications of AMT for ocular surface reconstruction, the majority of clinical studies related to the use of AMT in IK are of small case series and case reports. Liu et al.[17] recently conducted a meta-analysis of 17 studies on the use of AMT for infective and non-infective corneal ulcers (with a last search date in December 2017). Although the review provided a detailed meta-analysis on the corneal healing rate and visual improvement rate associated with the use of AMT, it did not compare adjuvant AMT with standard antimicrobial therapy. Furthermore, as the previous study was a meta-analysis focused on the corneal epithelium healing rate and visual improvement rate, some key studies were missed, including two randomized controlled trials (RCTs)[13, 14] and two non-randomized controlled studies (NRCS),[18, 19] rendering the robustness of evidence uncertain. The reason for this might be due to the difference in the scope of the systematic review and the search strategy that was employed. In light of these limitations, we aim to conduct a systematic review to critically examine the effectiveness of adjuvant AMT versus SAT on corneal healing in patients with IK.

Review question(s)

The question of this review is: what is the effectiveness of adjuvant AMT versus SAT on corneal healing in patients with IK?

Additional specific review sub-questions are:

1) What are the effects of the intervention on other outcomes such as unaided or best-corrected visual acuity and corneal neovascularization?
2) What are the effects of the intervention on adverse events?

Inclusion criteria

Participants

This review will consider studies that include patients of all age groups with all types of IK, including bacterial, fungal, viral, acanthamoeba, mixed and culture-negative presumed IK. We will exclude patients who had undergone other types of primary surgery other than AMT during the initial management and those who had less than 7 days' follow-up from the commencement of the treatment.

Intervention(s)

This review will consider studies that evaluate the effectiveness of adjuvant AMT for IK. There is no restriction on the treatment protocol, including the surgical technique and the number of layers of AM being transplanted during the surgery. AM prepared using freeze drying, cryopreservation and low temperature vacuum evaporation methods as well as fresh AM will be included.

Comparator(s)

This review will consider studies that compare adjuvant AMT to SAT alone. There is no restriction on the treatment regime of SAT as it will vary significantly across different studies. For studies with no comparator, the effect of intervention will be presented in a narrative manner and will not be included in the meta-analysis.

Outcomes

This review will consider studies that include the following outcomes:

Primary outcome measure:

i. Time to complete corneal healing (defined by complete corneal re-epithelialization and resolution of infiltrate / infection)

Secondary outcome measures:

i. Unaided or best-corrected visual acuity at 1-3 months
ii. Corneal neovascularization at final follow-up
iii. Treatment failure defined by worsening IK, endophthalmitis or corneal perforation requiring corneal gluing, tectonic keratoplasty or evisceration at final follow-up
iv. Adverse events, including recurrence of IK, dislocation of AM, and glaucoma, at final follow-up

Types of studies

This review will consider both experimental and quasi-experimental study designs, including RCTs, NRCS, and before and after studies,. Only RCTs will be included in meta-analysis. In addition, analytical observational studies including prospective and retrospective cohort studies, case-control studies and analytical cross-sectional studies will be considered for inclusion. This review will also consider descriptive observational study designs including case series, individual case reports and descriptive cross-sectional studies for inclusion. There will be no restriction on the date or language of the publication.

Methods

The proposed systematic review will be conducted in accordance with the Joanna Briggs Institute methodology for systematic reviews of effectiveness evidence.[20] The review title was registered with the Joanna Briggs Institute (https://joannabriggs.org/research/registered_titles.aspx)

Search strategy

The search strategy will aim to locate both published and unpublished studies. An initial limited search of MEDLINE (OVID) and EMBASE (OVID) was undertaken to identify articles on the topic. The text words contained in the titles and abstracts of relevant articles, and the index terms used to describe the articles were used to develop a full search strategy for MEDLINE (see **Appendix 1**). The search strategy, including all identified keywords and index terms, will be adapted for each included information source. The reference list of all studies selected for critical appraisal will be screened for additional studies.

Information sources

The databases to be searched include: MEDLINE, EMBASE, 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), World Health Organization (WHO) International Clinical Trials Registry Platform (ICTRP) (www.who.int/ictrp), and EU Clinical Trials Register (https://www.clinicaltrialsregister.eu).

Study selection

Following the search, all identified citations will be collated and uploaded into EndNote X9 (Clarivate Analytics, PA, USA) and duplicates removed. Titles and abstracts will then be screened by two independent reviewers for assessment against the inclusion criteria for the review. Potentially relevant studies will be retrieved in full and their citation details imported into Rayyan (Qatar) for title and abstract screening.[21] The full text of selected citations will be assessed in detail against the inclusion criteria by two independent reviewers. Reasons for exclusion of full text studies that do not meet the inclusion criteria will be recorded and reported in the systematic review. Any disagreements that arise between the reviewers at each stage of the study selection process will be resolved through discussion, or with a third reviewer. The results of the search will be reported in full in the final systematic review and presented in a Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) flow diagram.[22]

Assessment of methodological quality

Eligible studies will be critically appraised by two independent reviewers at the outcome level for methodological quality in the review using standardized critical appraisal instruments from the Joanna Briggs Institute for experimental and quasi-experimental studies.[20] Authors of papers will be contacted to request missing or additional data for clarification, where required. Any disagreements that arise between the reviewers will be resolved through discussion, or with a third reviewer. All studies,

regardless of the results of their methodological quality, will undergo data extraction and synthesis (where possible). As case series and case reports are subjected to high risk of bias, critical appraisal will be limited to randomized and non-randomized controlled trials. The results of critical appraisal such as risk of bias assessment will be reported in narrative and tabular form.

Data extraction

Data will be extracted from studies included in the review by two independent reviewers using the standardized templates in RevMan 5.3.[23] The data extracted will include specific details about the authors, year of publication, study design, sample size, types of causative microorganisms, types of AMT surgical techniques, main outcomes, and risk of adverse events. The proportion of patients with adverse events, with 95% confidence intervals (CIs), will be derived for each study. When studies report zero events in a treatment or control arm, we will use a classic half-integer continuity correction for the calculation of relative risk. We will assess if adverse events were pre-defined, actively searched or patient reported, and whether an assessment of possible causality was performed.[24] Any disagreements that arise between the reviewers will be resolved through discussion, or with a third reviewer. Authors of papers will be contacted to request missing or additional data, where required.

Data synthesis

Studies will, where possible, be pooled with statistical meta-analysis using RevMan 5.3. Effect sizes will be expressed as either odds ratios (for dichotomous data) or post-intervention mean differences (for continuous data) and their 95% confidence intervals will be calculated for analysis. Heterogeneity will be assessed statistically using the standard chi squared and I^2 tests.[25] Statistical analyses will be performed using random effect.[26] Subgroup analyses will be conducted where there is sufficient data to investigate the therapeutic effect on different causative microorganisms and the influence of different surgical techniques (overlay vs. inlay vs. sandwich) on the therapeutic outcomes. Sensitivity analyses will be conducted to test decisions made regarding studies at high risk of bias for an outcome in one or more key domains, including selection, performance, detection, attrition and reporting biases.[25, 27] Where statistical pooling is not possible the findings will be presented in narrative form including tables and figures to aid in data presentation, where appropriate. A funnel plot will be generated using RevMan 5.3 to assess publication bias if there are 10 or more studies included in a meta-analysis. Statistical tests for funnel plot asymmetry (Egger test, Begg test, Harbord test) will be performed where appropriate.

Assessing certainty in the findings

The Grading of Recommendations, Assessment, Development and Evaluation (GRADE) approach for grading the certainty of evidence will be followed and a Summary of Findings (SoF) will be created using GRADEPro GDT software 2013 (McMaster University, ON, Canada).[28] The SoF will present the following information where appropriate: absolute risks for the treatment and control, estimates of

relative risk, and a ranking of the quality of the evidence based on the risk of bias, directness, heterogeneity, precision and risk of publication bias of the review results. The outcomes reported in the SoF will be: time to complete healing, unaided or best-corrected visual acuity, corneal neovascularization and adverse events.

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Conflicts of interest

There is no conflict of interest in this project.

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Appendix I: Search strategy for MEDLINE

Last search conducted on 24 January 2020.

#	**Searches**	**Records retrieved**
1	Keratitis.mp.	18699
2	Corneal infection*.mp.	1017
3	Corneal ulcer*.mp.	6711
4	Exp Keratitis/	20438
5	Exp Corneal Ulcer/	5106
6	1 OR 2 OR 3 OR 4 OR 5	26207
7	Exp Amnion/	8913
8	Amnion.mp.	10914
9	Amniotic membrane.mp.	3133
10	7 OR 8 OR 9	12044
11	6 AND 10	386
12	exp animals/ not humans.sh.	4666856
13	11 NOT 12	368