Comparison of mini-simple limbal epithelial transplantation and conjunctival–limbal autograft for the treatment of primary pterygium: a randomised controlled trial


ABSTRACT
Purpose The purpose of this double-masked, parallel randomised controlled trial was to compare the recurrence rate and other outcomes between conjunctival–limbal autograft (CLAu) and mini-simple limbal epithelial transplantation (mini-SLET) after excision of pterygium.

Methods Eligibility criteria for participants was the presence of a primary nasal pterygium extending equally to or greater than two millimetres on the cornea on its horizontal axis from the nasal limbus. The participants were allocated into two groups (CLAu and mini-SLET) using simple randomisation with a table of random numbers. Participants and the outcome assessor were masked to the intervention. The study protocol is listed and available on https://clinicaltrials.gov (Identifier: NCT03363282).

Results A total of 61 eyes were enrolled in the study, 33 underwent CLAu (group 1) and 28 mini-SLET (group 2), all eyes were analysed in each group. At 2, 3, 6 and 12 months the CLAu group exhibited a recurrence of 0%, 6.1%, 8.1% and 8.1%, while the mini-SLET exhibited a recurrence of 0%, 17.9%, 50% and 53.5% (p<0.05). There were no intraoperative or postoperative complications in either of the two groups.

Conclusion The findings of this study suggest that mini-SLET has a higher recurrence rate and provides no advantage over CLAu in the treatment of primary pterygium.

WHAT IS ALREADY KNOWN ON THIS TOPIC?
⇒ Conjunctival–limbal autograft (CLAu) and conjunctival autograft are considered the gold standard treatment for primary pterygium. However, it may be inadequate in patients who may require glaucoma filtration surgery in the future. Mini-simple limbal epithelial transplantation (mini-SLET) requires less tissue than the CLAu. The purpose of this randomised controlled trial was to compare the recurrence rate of CLAu with mini-SLET in a reference centre in Mexico City.

WHAT DOES THIS STUDY ADD?
⇒ Primary pterygium excision followed by mini-SLET showed a higher rate of recurrence compared with CLAu. The recurrence results in the mini-SLET group are comparable to those obtained with the use of amniotic membrane alone.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY?
⇒ mini-SLET should not be the preferred option for the treatment of primary nasal pterygium, in view of the unacceptably high recurrence rates. It should only be considered in exceptional cases where donor conjunctival is not available.

INTRODUCTION
Pterygium is considered an aberrant wound healing process, characterised by centripetal growth of a leading edge of altered limbal epithelial cells, followed by a squamous metaplastic epithelium with goblet cell hyperplasia and an underlying stroma of activated, proliferating fibroblasts, neovascularisation, inflammatory cells and extracellular matrix remodelling.1 It is a non-cancerous elastic degeneration usually located nasally but can grow temporally or in both directions. The prevalence of pterygium ranges from 7% to 15%, being higher in countries closer to the equator and in environments of high reflectance.2–4 Recurrence following simple surgical removal is common.5 Conjunctival–limbal autograft (CLAu) over bare sclera is used to arrest future invasion of the fibrovascular tissue after excision.6 CLAu and conjunctival autograft (CAG) are considered the gold standard treatment with a recurrence rate between 3.5% and 21%.6–15 However, it may be difficult or inadequate in cases with large excisions and in patients who may require glaucoma filtration surgery in the future. Mini-simple limbal epithelial transplantation (mini-SLET) requires less tissue than the conventional CLAu. The purpose of this randomised controlled trial was to compare the recurrence rate CLAu with mini-SLET in a reference centre in Mexico City.

MATERIALS AND METHODS
Study design The study complied with the requirements and tenets of the Declaration of Helsinki, the General
Health Regulations on Health Research and the procedural rules of the Institutional Review Board of the Instituto de Oftalmología Fundación Conde de Valenciana, with internal reference number CEI: 2015/05/10. Pterygium excision with either mini-SLET or CLAu was performed by two surgeons (EOG-H, AN-C), the study was done from September 2015 to November 2017. The study protocol is listed and available on https://clinicaltrials.gov (Identifier: NCT03363282).

Patients with primary nasal pterygium extending equally to or greater than 2 mm on the cornea on its horizontal axis from the nasal limbus were enrolled in the study. Patients with rheumatoid arthritis, collagenopathies, pregnancy, ocular surface disorders, trauma, glaucoma or previous ocular surgeries were excluded from the study. Preoperatively, all trial participants underwent a comprehensive ophthalmic evaluation.

The participants were assigned into two balanced groups using simple randomisation with a table of random numbers: group I (control group) underwent pterygium excision with CLAu and group II underwent pterygium excision with mini-SLET. A single author (AJ-C) generated the random allocation sequence and assigned participants to intervention groups (figure 1). Allocation concealment was done with sealed envelopes. A proportion formula with an alpha value of 0.05 and beta of 0.2 was used to calculate the sample size considering the difference between the proportion of recurrence observed in a previous study.16 The number of eyes in each group was calculated as 28 per group. A 20% loss was considered, and the total number of patients was 34 per group.

Surgical technique
In both groups, conventional resection of the pterygium was performed as follows: blunt resection of the head, neck and body of the pterygium, polishing of the surgical site with a surgical diamond burr, when necessary and haemostasis with bipolar cautery. For CLAu, an autograft was obtained from the superotemporal bulbar conjunctiva. The autograft was adhered to the surgical site using fibrin glue (Tissucol; Baxter, Vienna, Austria). For mini-SLET, a layer of amniotic membrane (AM) with surgical adhesive was adhered to the surgical site, then 2×2 mm limbus cells were resected, cut up into six pieces and placed bordering the limbus on the AM, and covered with another layer of AM (figure 2). Tobramycin and dexamethasone drops (Trazidex, Sophix, Mexico) were placed at the end of the surgery, covering the eye with an eye patch. The patch was removed the next day and both groups were managed with drops of tobramycin and dexamethasone (Trazidex, Sophia, Mexico) and carboxymethylcellulose (Refresh Tears, Allergan, USA) applied every 4 hours in the eye for 60 days. All surgeries were performed by two surgeons EOG-H and AN-C.

Follow-up visits
All patients were reviewed at day 1, weeks 2 and months 1, 3, 6 and 12 after the surgical procedure. Postoperatively, the patients were reviewed for recurrence rate and surgical results using the Tseng scale.17 This outcome was measured from the first month after the surgical event. At each visit the clinical findings were recorded using a camera (Nikon 9000; Nikon, Japan) and coupled to a slit lamp, using 6× and 16× magnifications. A single author (VO-B) assessed the outcomes in a masked fashion.

Statistical analysis
Data analysis was performed with the Statistical Package for the Social Sciences (SPSS; IBM, USA) V.17. The χ² test was used
to compare nominal variables. Mann-Whitney U test (for non-parametric data) was used to compare quantitative data variables. Using the Kaplan-Meier method, we calculated the recurrence rate, from surgery to the end of the study.

Safety analysis of the mini-SLET was evaluated through the complication rate, with a cut-off point less than or equal to the CLAu. Analysis of effectiveness was carried out at the end of the follow-up with the same cut-off point.

RESULTS

A total of 61 eyes were enrolled in the study, 33 underwent CLAu (group 1) and 28 mini-SLET (group 2). The baseline demographic characteristics are depicted in table 1. In the CLAu group, the mean age was 57.4 years (range: 31–89 years), in group 2 the mean age was 59.6 years (range: 32–89 years). CLAu group included 18 women and 11 men, and the mini-SLET group was comprised of 20 women and 8 men. The mean pterygium size in CLAu group was 3.69 mm (±1.36 mm) and 3.66 mm (±1.13 mm) in the mini-SLET group. Among these variables, no statistically significant differences were found between both groups (table 1).

Recurrence rate

Kaplan-Meier analysis showed a recurrence rate at 2, 3, 6 and 12 months of 0%, 6.1%, 8.1% and 8.1% in the CLAu group and 0%, 17.9%, 50% and 53.5% in the mini-SLET group, respectively (figure 3). Due to the higher incidence of recurrence observed in mini-SLET, it was decided not to perform surgery in the remaining patients (five participants). A χ² test was performed to assess pterygium recurrence, which showed a statistically significant difference between the two groups, with a value of p<0.005.

Surgical site

The appearance of the surgical site did not present statistically significant differences in the CLAu group at months 1, 3, 6 and 12 (p=0.441). The mini-SLET group had a statistically significant difference at months 1, 3, 6 and 12 (p<0.005). There were no intraoperative or postoperative complications in either of the two groups. Figures 4 and 5 show the anatomical outcomes of the two groups.

DISCUSSION

We observed a higher recurrence rate in the mini-SLET group compared with the CLAu group (53% vs 9%, respectively) in patients with primary nasal pterygium. The main purpose of pterygium surgery is to remove the pterygium and restore the ocular surface. Despite the available surgical techniques and associated medications, the recurrence rate varies from 50% to 80% when the simple excision technique is used, and it drops to around 15% when more advanced surgical techniques are used. Among these, AM transplantation and CLAu are commonly used techniques to treat pterygium. Despite the modifications in the surgical techniques used in the management of pterygium, recurrence continues to be a factor that limits its success. The rate of recurrence obtained in our study for the CLAu group was comparable with the results previously reported. The mini-SLET recurrence rate observed in our study was higher than that reported in an AM graft group followed for 1 year. We decided...
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Figure 3  Kaplan-Meier plot of the probability of survival. The Kaplan-Meier plot displayed a difference in the survival of both groups ($p<0.05$). mini-SLET, mini-simple limbal epithelial transplantation. LCA, limbal conjunctival autograft.

Due to the higher number of recurrences observed in the mini-SLET group, we decided not to perform surgery on the five remaining patients in the group.

The best opportunity to cure this disease is at the first procedure and recurrence rates are likely to be higher for second procedures and sometimes the choice is limited by previous surgery. In this case, mini-SLET has not precluded a second procedure using a conjunctival–limbal graft. The surgical technique performed to use CLAu instead of CAG to compare surgical techniques that involve the limbus in the graft.

When analysing the survival curves of the graft, the surgical technique of mini-SLET has a shorter survival time and a higher cumulative risk of presenting recurrence in the third and sixth postoperative months. When jointly analysing the survival curves for each technique, a statistically significant difference between the two was observed.

Figure 4  Outcomes of conjunctival–limbal autograft in three eyes with primary pterygium. Each row shows a case documented preoperatively, 1 and 6 months after surgery.

in CLAu showed superiority in the success rate compared with Mini-SLET. (90% vs 53%, respectively).

The appearance of the surgical site did not show statistically significant differences in the CLAu group during the months of evaluation. This is attributed to the fact that the graft improved its appearance over time, compared with the recent postoperative period. In the mini-SLET group, statistically significant differences were observed at months 1, 3 and 6, this is due to the recurrence that occurred mainly between the third and sixth postoperative month. Cosmesis presented statistically significant differences between the two groups, which correlates with the greater recurrence rate in the Mini-SLET group. Both techniques were shown to be safe, since there were no postoperative complications in either of the two groups.

Compared with the reports in the literature, the recurrence for CLAu was mainly within the first three postoperative months, and for group 2 between the third and sixth postoperative months. These results are similar to those reported in the literature for primary pterygium excision followed by AM transplantation without adjuvant therapy.17

Shanbhag et al.25 compared the clinical outcomes of CAG and SLET in unilateral partial limbal stem cell deficiency and found the CAG to be superior to SLET. These findings are mirrored by the ones observed in our study, eyes with most of the limbus intact might be able to compensate for the function of the non-functional part and thus, may not benefit from additional stem cells.

The higher recurrence rate in our study may have different explanations. Recurrence has been associated with environmental factors such as UV exposure and seasonal effects,22 both of which were not controlled in our study. Surgeon factor may also play a role in recurrence, in our study each group was operated on by a different surgeon which may have been better analysed if both procedures were performed by either a single surgeon or multiple surgeons. Therefore, surgeon factor bias may have been introduced and should be accounted for in the interpretation of our results. Our results contrast those published in similar randomised controlled trial with a 6-month follow-up, in which mini-SLET was equivalent or superior to CLAu,23 however the authors of the study used a single layer of AM. Early in the mini-SLET procedure evolution, a modified technique including a second layer of AM theoretically was placed to protect the limbal grafts.16 This second layer may act against limbal graft survival and thus increase the risk of recurrence.

The strength of the study is that, to the best of our knowledge, it is the randomised controlled trial comparing mini-SLET and CLAu with the longest follow-up time. The lack of serial documentation with fluorescein staining is a limitation of the study, it might have helped us understand the speed at which the epithelium is growing from the transplants. The lack of the full trial protocol published in a scientific journal is a methodology limitation of the present study. Nevertheless, a copy of the original protocol can be accessed as supplemental material to reduce the risk of publication bias.

The continuous search for the best surgical technique with the lowest recurrence rate idea gave rise to this clinical trial in which the recurrence rate of CLAu and mini-SLET were compared. Our study contributes to the current knowledge of SLET and its applications in ocular surface pathology, but future clinical trials comparing three groups: (1) AM layer, (2) AM layers and (3) CLAu are necessary to assess the efficacy of mini-SLET.

In conclusion, the surgical results observed in this study of primary pterygium excision followed by CLAu or mini-SLET showed a higher rate of recurrence with the mini-SLET technique, these results are comparable to those obtained in the literature with the use of AM alone.

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Contributors VO-B, AN-C, AJ-C, AN, AR-M and EOG-H designed the study, AN-C and EOG-H performed the surgical procedures. Data acquisition, analysis and interpretation were performed by NK-L and VO-B. NK-L wrote the manuscript with input from all authors. VO-B, AN-C, AJ-C, AN, AR-M and EOG-H reviewed the manuscript. EOG-H is guarantor.
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**REFERENCES**