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Simple Limbal Epithelial Transplantation

Long-Term Clinical Outcomes in 125 Cases of Unilateral Chronic Ocular Surface Burns

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Purpose: This study describes the long-term clinical outcomes of autologous simple limbal epithelial transplantation (SLET), a relatively new technique of limbal stem cell transplantation.

Design: This was a single-center prospective interventional cases series.

Participants: This study included 125 patients, 65 adults and 60 children who developed unilateral limbal stem cell deficiency (LSCD) after suffering with ocular surface burns and underwent SLET between 2010 and 2014.

Methods: A 1-clock hour limbal biopsy sample was obtained from the unaffected eye. At the same sitting, the recipient eye was surgically prepared and the donor tissue was divided into small pieces and transplanted using an amniotic membrane scaffold with fibrin glue.

Main Outcome Measures: The diagnosis and outcome in every case was validated by 5 independent masked assessors. The primary outcome measure was restoration of a completely epithelized, stable, and avascular corneal surface. The secondary outcome measure was improvement in visual acuity. Complications, risk factors for failure, and immunohistochemistry analysis of corneas that underwent SLET also were described.

Results: At a median postoperative follow-up of 1.5 years (range, 1−4 years), 95 of 125 eyes (76%; 95% confidence interval, 68.5%−83.5%) maintained a successful outcome. Kaplan-Meier analysis revealed a comparable survival probability at 1 year of 80% in adults and 72% in children ($P = 0.304$). Two-line improvement in visual acuity was seen in 75.2%, and 67% of successful cases attained 20/60 or better vision ($P < 0.0001$). Progressive conjunctivalization occurred in 18.4% of eyes. The clinical factors associated with failure were identified as acid injury, severe symblepharon, SLET combined with keratoplasty, and postoperative loss of transplants ($P \leq 0.0075$). Success rates were comparable among faculty and trainees ($P = 0.71$). Immunohistochemistry revealed successful regeneration of normal corneal epithelium ($CK3^+/12^+$) without admixture of conjunctiva cells ($Muc5AC^-/CK19^-$) and replenishment of limbal stem cell ($\Delta Np63\alpha^+/ABCG2^+$) reserve.

Conclusions: Autologous SLET is an effective, reliable and replicable technique for long-lasting corneal regeneration and vision restoration in unilateral chronic ocular surface burns. Simple limbal epithelial transplantation is probably preferable to other techniques of limbal stem cell transplantation, particularly where cell cultivation facilities are unavailable. *Ophthalmology 2016;■:1−11 © 2016 by the American Academy of Ophthalmology. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).*

Supplemental material is available at www.aaojournal.org.

A delicately thin layer of stratified but nonkeratinized squamous epithelium covers the corneal surface. This epithelial cover is renewed continuously as younger cells migrate inward from the periphery and older cells are lost from the surface.[1] The constant source of corneal epithelial cells is believed to be the limbus, which is the annular transitional area between the cornea and the sclera.[1] Corneal epithelial stem cells have been identified deep within a protected microenvironment or niche at the limbal palisades of Vogt.[2,3] When the limbus is intact, corneal epithelial defects heal promptly. But when the limbus is damaged, either because of injury or inflammation, the normal corneal epithelial physiologic features are disrupted. Delay or failure in corneal epithelialization leads to conjunctival encroachment over the cornea, vascularization, and nonhealing epithelial defects.[4] The consequent clinical condition, termed *limbal stem cell deficiency* (LSCD), is a rare but severe cause of corneal blindness.

Fortunately, transplantation of healthy limbal tissue can reverse LSCD and restore a normal corneal surface.[5,6] In the last 3 decades, both the understanding of limbal biology and the techniques of limbal transplantation have evolved considerably.[7,8] Although conjunctival-limbal or keratolimbal grafting continues to be practiced,[9] transplantation of ex vivo−cultivated limbal epithelial sheets has become popular in many centers worldwide.[7,8] Regulatory issues

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and the expenses of maintaining a clinical-grade laboratory limit the use of ex vivo cultivation, whereas conventional limbal grafting requires no special infrastructure, but is technically demanding and carries some risk to the donor eye.[10] No head-to-head trials have been conducted, and it is unclear whether one technique is more effective than the other. Therefore, availability of resources or individual preference, rather than scientific evidence, usually determines which technique a particular surgeon or center adopts.

Having performed more than 1000 ex vivo–cultivated limbal epithelial transplantation (CLET) procedures and having reported long-term outcomes comparable with those of other groups,[10–13] we adopted a novel technique called simple limbal epithelial transplantation (SLET) in 2010.[14] Simple limbal epithelial transplantation essentially showed that direct transplantation of a tiny limbal fragment could reverse LSCD without needing ex vivo expansion.[14] After that initial report, 2 other groups independently replicated the successful outcomes in varied indications using slight modifications of the original technique.[15,16] However, for wider acceptance of any new technique, the results need to be validated in larger numbers and with longer follow-up. Therefore, in this study, we report the outcomes of autologous SLET in a large cohort of patients with unilateral LSCD after sustaining ocular surface burns.

Methods

Study Approval, Design, and Subjects

The Ethics Committee of the L. V. Prasad Eye Institute, Hyderabad, India, prospectively approved this study. After evaluating the results of the initial pilot trial involving 6 patients,[14] the committee approved SLET as an alternative option to ex vivo CLET for the treatment of LSCD. This study was conducted in strict adherence to the tenets of the Declaration of Helsinki. All adults and legal guardians of children who underwent SLET gave informed written consent for all procedures described in this study.

All 163 consecutive patients who underwent SLET between October 1, 2010, and March 31, 2014, were considered for analysis. Of 163 patients, 125 patients met the following inclusion criteria: (1) a documented history of chemical or thermal burns, and (2) presence of unilateral (defined as no history or clinical signs of ocular surface disease in the other eye) LSCD (defined as total or partial superficial corneal vascularization, punctate fluorescein staining of the corneal surface with or without persistent epithelial defects, conjunctivalization of the corneal surface, and absence of limbal palisades of Vogt). The 39 cases that were excluded from this study included 12 cases with unknown cause of LSCD, 11 cases of bilateral LSCD, 5 cases of primary or recurrent pterygium, 5 cases of LSCD occurring after ocular surface tumor excision, 3 cases of LSCD occurring after radiotherapy for intraocular tumors, and 3 cases of LSCD without visual potential in which SLET was performed for cosmetic correction. Patients with untreated concurrent ocular problems, such as severe dry eye disease (Schirmer's test I measure of less than 10 mm of wetting at 5 minutes), entropion, trichiasis, lagophthalmos, glaucoma, and infection, were not considered for surgery.

Outcome Measures of Efficacy

In recipient eyes, the primary outcome measure was the success of SLET, defined clinically as a completely epithelized, clinically stable, and avascular corneal surface (Fig 1A–J). Failure was defined as the occurrence of progressive conjunctivalization of the cornea encroaching onto the central 8 mm, occurrence of persistent epithelial defects, or both (Fig 1P–T). Occurrence of microbial keratitis and need for repeat surgery were additional criteria for failure. Survival time was calculated in months from the date of SLET to the date of failure or the date of last follow-up, depending on the clinical outcome. The secondary outcome measure of efficacy was the change in best-corrected visual acuity (BCVA) at each postoperative follow-up visit.

Outcome Measures of Safety

The outcome measures of safety were intraoperative and postoperative complications of both limbal biopsy and SLET in the donor and recipient eye.

Surgical Technique of Simple Limbal Epithelial Transplantation

We followed the surgical technique that has been described previously for total LSCD[14] with certain modifications for partial LSCD cases (Supplemental Appendix 1 and Supplemental Fig 1, available at www.aaojournal.org). All tissue samples excised during SLET or keratoplasty during or after SLET were processed in a standardized fashion for histopathologic and immunohistochemical analysis, as described in Supplemental Appendix 2 and Supplemental Table 1 (available at www.aaojournal.org).

Postoperative Care and Follow-up Schedule

All patients underwent comprehensive ophthalmic examinations of both eyes at every follow-up visit. Patients were seen on days 1, 7, 30 (at 1 month) or day 42 (at 6 weeks), 90, and at 3-month intervals thereafter. For the entire duration of the first year after surgery, patients were contacted by telephone if they missed a scheduled visit, and the next earliest possible appointment was arranged for them. This was done proactively to ensure that all patients completed at least 1 year of follow-up after the procedure. Patients were prescribed ciprofloxacin 0.3% eye drops (Cipla India, Mumbai, India) 4 times daily for 1 week and prednisolone acetate 1% eye drops (Alcon Laboratories Pvt. Ltd, Bangalore, India) 6 times daily tapered weekly over 6 weeks in both eyes. The bandage contact lens (BCL) was removed from the recipient eye on day 7 and carboxymethyl cellulose 0.5% eye drops (Allergan India Pvt. Ltd, Bangalore, India) were added in the recipient eye.

Data Collection

Data were collected at every visit in a predesigned format and the completed form was filed in the medical record. These data included patient age and gender, type and date of injury, details of prior ocular procedures, Snellen BCVA, intraocular pressure, presence or absence of lid abnormalities, dry eye disease, symblepharon, degree of limbal involvement, intraoperative surgical details, postoperative complications, duration of follow-up, and status of the ocular surface at each visit (slit-lamp findings including fluorescein staining).

Validation of Diagnosis and Outcome by Independent Masked Assessors

Five fellowship-trained cornea specialists (3 ocular surface disease specialists and 2 refractive surgeons) volunteered as assessors to validate the investigator's assessment of the diagnosis of LSCD and the outcome of treatment in every case based on 5 objective

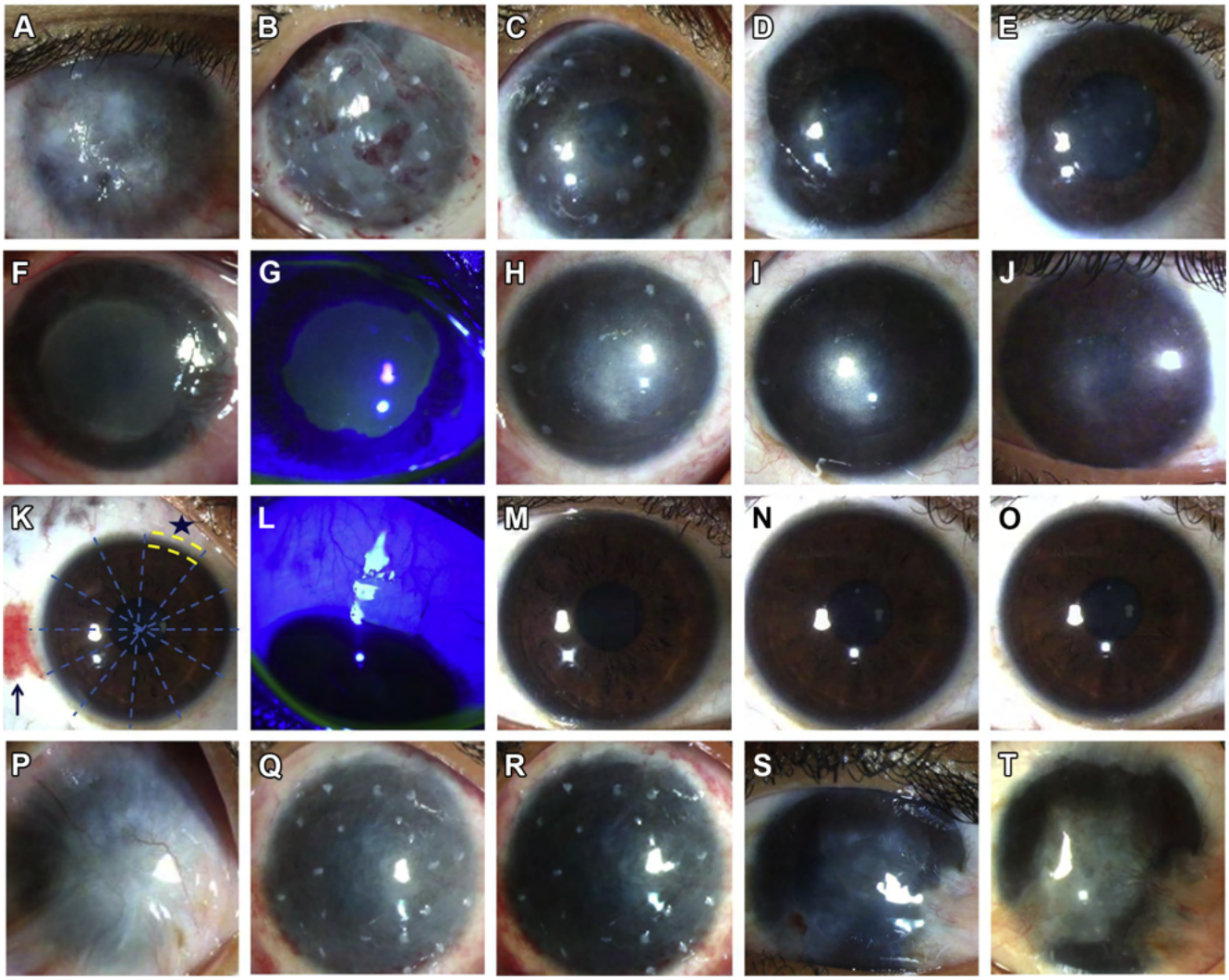

Figure 1. Slit-lamp photographs showing the typical clinical course after simple limbal epithelial transplantation (SLET). **A**, Total limbal stem cell deficiency in the right eye of a 6-year-old child with lime burns. **B**, Same eye 1 day after SLET showing fibrin glue and SLET transplants under the contact lens. **C**, Same eye 1 month after SLET showing intact transplants with an epithelized and avascular corneal surface. **D**, Same eye 6 months after SLET showing peripheral conjunctivalization from 4 to 5 o'clock and 7 to 10 o'clock. **E**, Same eye 2 years after SLET showing no progression in conjunctivalization and maintenance of a stable epithelized corneal surface. **F**, **G**, Left eye of a 15-year old boy with total limbal stem cell deficiency (LSCD) and persistent epithelial defect (PED) after lime burns. He had previously undergone 3 amniotic membrane grafts without resolution. **H**, Same eye 3 months after SLET showing a quiet and stable ocular surface with anterior stromal haze in the area of the PED. **I**, **J**, At 1 and 3 years after SLET, the surface remained stable and there was significant reduction in the stromal scarring and improvement in vision over time. **K**, **L**, Right eye of the same patient showing the area of the 1-clock-hour (cornea shown divided into 12 clock hours by bluedashed lines) biopsy site (yellow dashed lines with blue asterisks). **M**–**O**, Same eye **(M)** 1 year, **(N)** 2 years, and **(O)** 3 years after SLET showing a stable surface without donor site LSCD. **P**, Total LSCD in the left eye of a 5-year-old girl after lime burns. **Q**, **R**, Same eye at **(Q)** 1 week and **(R)** 1 month after SLET showing the presence of SLET transplants and an epithelized corneal surface. **S**, Same eye 6 months after SLET showing recurrence of conjunctivalization superiorly, inferonasally, and inferotemporally. **T**, Same eye 1 year after SLET showing progression of conjunctivalization and failure of SLET.

criteria: visual acuity, grade of symblepharon, grade of conjunctivalization, grade of corneal vascularization, and clock hours of limbal involvement. The grading system was adapted from Sotozono et al[17] (Supplemental Fig 2, available at www.aaojournal.org). They were masked to the identity of the patient and the nature of the procedure performed. In cases where there was disagreement between the assessors or investigators, the majority opinion of the independent assessors was taken as the final diagnosis or outcome of treatment. The details of the assessment procedure are provided in Supplemental Appendix 3 (available at www.aaojournal.org).

Statistical Analysis

MedCalc software version 11.4.3.0 (MedCalc Software, Mariakerke, Belgium) was used for data analysis. Continuous parametric data were reported as mean (±standard deviation) and nonparametric data were reported as median with range. Agreement

between different assessors was analyzed using Light's κ statistic.[18] Success rates were reported as percentages with 95% confidence intervals (CI) or Kaplan-Meier survival probability rates. The Fisher exact test was used to compare categorical data. A multivariate Cox proportional hazards model with stepwise elimination using Akaike information criterion was designed (after checking the assumptions and interactions of the model) to test the association between graft failure and clinical variables. Patients younger than 16 years were considered children because 16 years is the legal age for valid informed consent in India. A 2-tailed *P* value less than 0.05 was considered statistically significant.

Results

The study included 125 patients, 65 adults and 60 children, with unilateral LSCD occurring after ocular burns. Total LSCD was seen in the affected eye in 55 adults and 52 children, and the remaining 18 eyes had partial LSCD ranging from 6 to 9 clock hours of limbal involvement. Table 1 summarizes the baseline features of the cohort and compares each characteristic between adults and children. Males were significantly more common among adults than children ($P = 0.0234$). This is explained by industrial trauma being more common among adults, compared with domestic accidents being the major cause among children. In urban and semiurban India, the industrial workforce comprises mainly adult men, who therefore are more likely to become injured than women. Accidental edible lime–related trauma was the most common cause of LSCD in children, whereas in adults other causes, including industrial alkali and thermal burns and vitriolage, were more common ($P = 0.0001$). The proportion of eyes having advanced symblepharon (51.6% vs. 27.7%; $P = 0.029$) and those needing keratoplasty along with SLET (8.3% vs. 3%; $P = 0.256$) was greater in children than in adults, respectively, indicating the greater seriousness of injury in children. The minimum follow-up after SLET in both groups was 1 year, ranging from 1 to 3.5 years in children and 1 to 4 years in adults ($P = 0.7$). Twenty-two patients were followed up for 2 years, 6 patients were followed up for 3 years, and 2 patients were followed up for 4 years.

Table 1. Baseline Characteristics of Patients Undergoing Simple Limbal Epithelial Transplantation for Limbal Stem Cell Deficiency Occurring after Ocular Burns

Characteristic	Children	Adults	*P* Value
Sex			
Female	27 (45)	16 (24.6)	**0.0234**
Male	33 (55)	49 (75.4)	
Laterality			
Right	31 (51.7)	32 (49.2)	0.855
Left	29 (48.3)	33 (50.8)	
Cause of ocular burn			
Alkali	50 (83.3)	37 (56.9)	**0.006**
Acid	6 (10)	8 (12.3)	
Blast	0 (0)	8 (12.3)	
Cement	3 (5)	7 (10.8)	
Unknown chemical	1 (1.7)	5 (7.7)	
Prior ocular surgery			
AMG	38 (63.3)	33 (50.8)	0.254
LSCT	5 (8.3)	3 (4.6)	
Others	6 (10)	8 (12.3)	
None	11 (18.4)	21 (32.3)	
BCVA at presentation			
20/200 or worse (blindness)	56 (93.3)	60 (92.3)	0.873
20/70 to 20/160 (low vision)	3 (5)	3 (4.6)	
20/60 or better	1 (1.7)	2 (3.1)	
Symblepharon			
Limited to conjunctiva	10 (16.7)	10 (15.4)	**0.029**
Extending to limbus	9 (15)	17 (26.1)	
Extending to cornea	31 (51.6)	18 (27.7)	
Absent	10 (16.7)	20 (30.8)	
Combined SLET and keratoplasty			
Yes	5 (8.3)	2 (3)	0.256
No	55 (91.7)	63 (97)	
Extent of LSCD (clock hours)			
12 (total)	52 (86.7)	55 (84.6)	0.744
6–11 (partial)	8 (13.3)	10 (15.4)	
<6	0 (0)	0 (0)	
Duration between ocular burn and SLET			
3–5 mos	13 (21.7)	9 (13.8)	0.063
6 mos–1 yr	29 (48.3)	21 (32.4)	
>1 yr	18 (30)	35 (53.8)	
Follow-up after SLET (mos)			
12–17	43 (71.7)	53 (81.5)	0.47
18–23	4 (6.7)	4 (6.2)	
24–35	11 (18.3)	6 (9.2)	
36–48	2 (3.3)	2 (3.1)	

AMG = amniotic membrane grafting; BCVA = best-corrected visual acuity; LSCD = limbal stem cell deficiency; SLET = simple limbal epithelial transplantation.
Data are no. (%) unless otherwise indicated. Boldface values indicate statistically significant *P* values.

Agreement between Investigators and Independent Masked Assessors

The overall agreement between individual assessors and the investigators both for diagnosis (Light's κ, 0.93; 95% CI, 0.86–0.99) and treatment outcome (Light's κ, 0.89; 95% CI, 0.83–0.96) were excellent. There was 100% agreement between the initial impression of the investigators and the final consensus opinion of the assessors.

Efficacy of Simple Limbal Epithelial Transplantation

At the final follow-up visit after SLET, 95 of 125 eyes (76%; 95% CI, 68.51–83.49%) maintained a successfully regenerated stable corneal surface without progressive conjunctivalization, development of persistent epithelial defect, infection, or need for repeat SLET (Fig 2). Among patients with total LSCD, successful outcomes were observed in 44 of 55 adult eyes (80%; 95% CI, 69.43–90.5%) and in 37 of 52 pediatric eyes (71.2%; 95% CI, 58.8–83.44%; $P = 0.69$). Among patients with partial LSCD, successful outcomes were observed in 8 of 10 adult eyes (80%; 95% CI, 55.21–104.79%) and in 6 of 8 pediatric eyes (75%; 95% CI, 44.99–105.01%; $P = 0.79$). Kaplan-Meier analysis revealed a survival probability of 80% at 1 year and beyond in adults and of 72% at 1 year and 66% subsequently in children (Fig 3; $P = 0.304$). The success rates of SLET performed by the senior surgeon with more than 10 years of experience in ocular surface procedures (V.S.S.) was comparable with that of a less experienced surgeon with 5 years of experience (S.B.) and also with that of cornea fellows in training ($P = 0.71$; Fig 4A).

There was significant improvement in BCVA after SLET compared with baseline (Fig 3). Among the 95 cases with successful outcome, 64 (67%), or more than two thirds of eyes, recovered a BCVA of 20/60 or better. Among the remaining 31

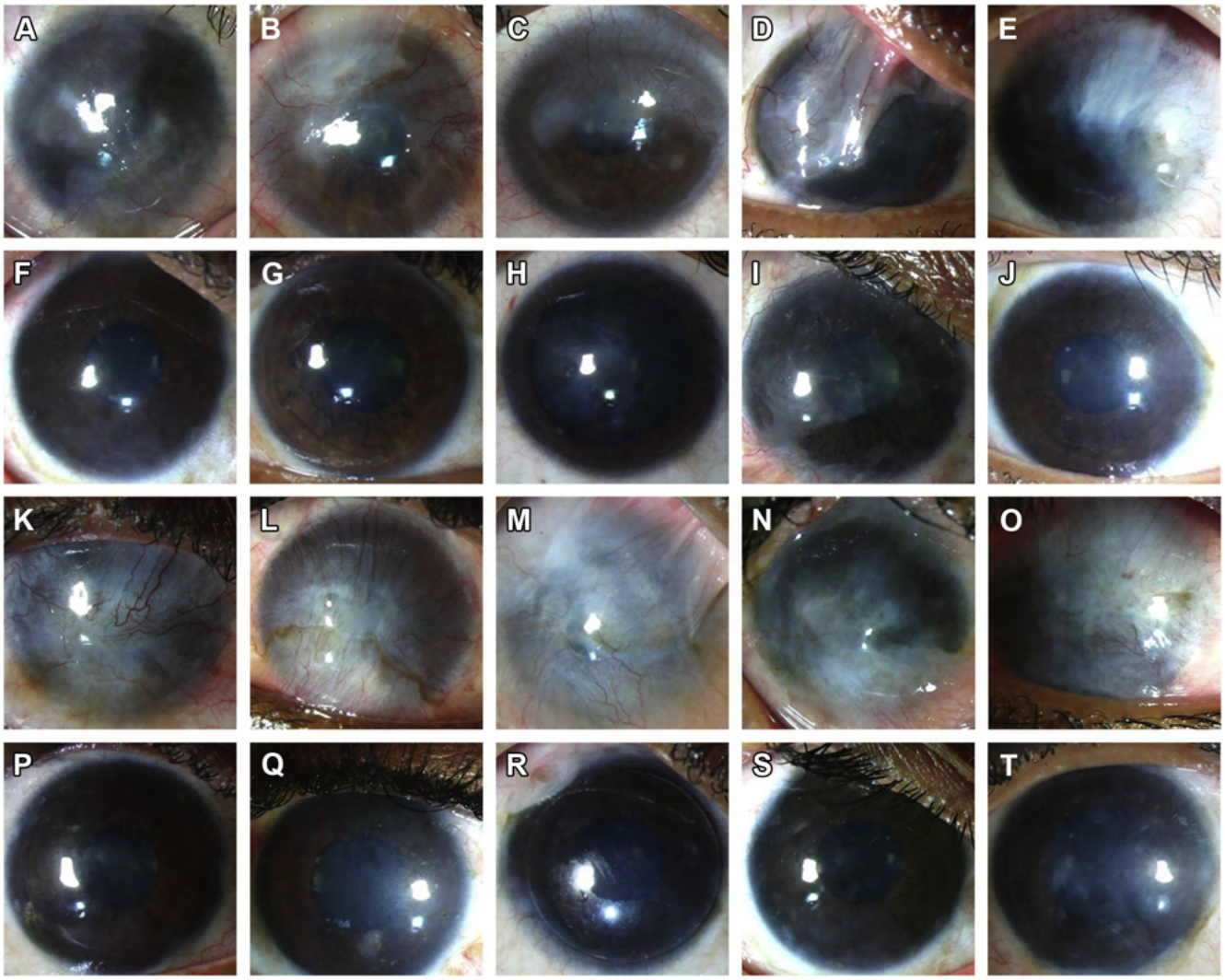

Figure 2. Slit-lamp photographs showing 2-year clinical outcomes of simple limbal epithelial transplantation (SLET). **A**–**J**, Patients with partial limbal stem cell deficiency (LSCD) after ocular burns: **(A**–**F)** preoperative photographs and **(F**–**J)** their corresponding 2-year postoperative photographs showing a completely epithelized and stable corneal surface. **K**–**U**, Eyes with total LSCD: **(K**–**O)** preoperative clinical photographs and **(P**–**T)** corresponding 2-year postoperative photographs after SLET showing excellent anatomic outcomes.

cases, dense amblyopia (n = 12; 38.7%), stromal scarring (n = 14; 45.2%), and cataract (n = 5; 16%) were noted to be the causes of suboptimal visual improvement. Penetrating keratoplasty and cataract surgery were performed in 10 and 5 eyes, respectively, for further visual gain. A 2-line improvement in BCVA compared with baseline was seen in 94 of 125 eyes (75.2%) at the final follow-up visit.

Risk Factors of Failure

A Cox proportional hazards model predicted the following preoperative, intraoperative, and postoperative risk factors for SLET failure: history of acid burns (hazard ratio [HR], 3.6; 95% CI, 1.2−10.4; $P = 0.0075$), presence of symblepharon extending onto the cornea (HR, 7.8; 95% CI, 3.2−18.9; $P < 0.0001$; Fig 4B), keratoplasty combined with SLET (HR, 11.6; 95% CI, 4.2−32.2; $P = 0.0001$; Fig 4C), and postoperative loss of SLET transplants (HR, 22.8; 95% CI, 8.1−64.2; $P < 0.0001$; Fig 4D).

Safety of Limbal Biopsy in the Donor Eye

None (95% CI, 0−3.6%) of the donor or fellow eyes demonstrated any donor site LSCD or other complications. Typically, the donor site epithelial defect had completely healed by 1 week (Fig 1K−M). The most common observation in the donor eye was subconjunctival hemorrhage in 35 of 125 eyes (28%), which resolved by 1 month (Fig 1K−M).

Safety of Simple Limbal Epithelial Transplantation in the Recipient Eye

Complications of SLET are summarized in Table 2. Recurrence of progressive conjunctivalization was the most common complication in 23 of 125 recipient eyes (18.4%). Progressive symblepharon was seen in 21 of 125 eyes (16.8%). Hemorrhage under the human amniotic membrane (hAM) graft was observed in 10 of 125 eyes (8%); 9 cases resolved spontaneously, and in 1 case, the blood had to be drained by puncturing the hAM with a 26-gauge needle.

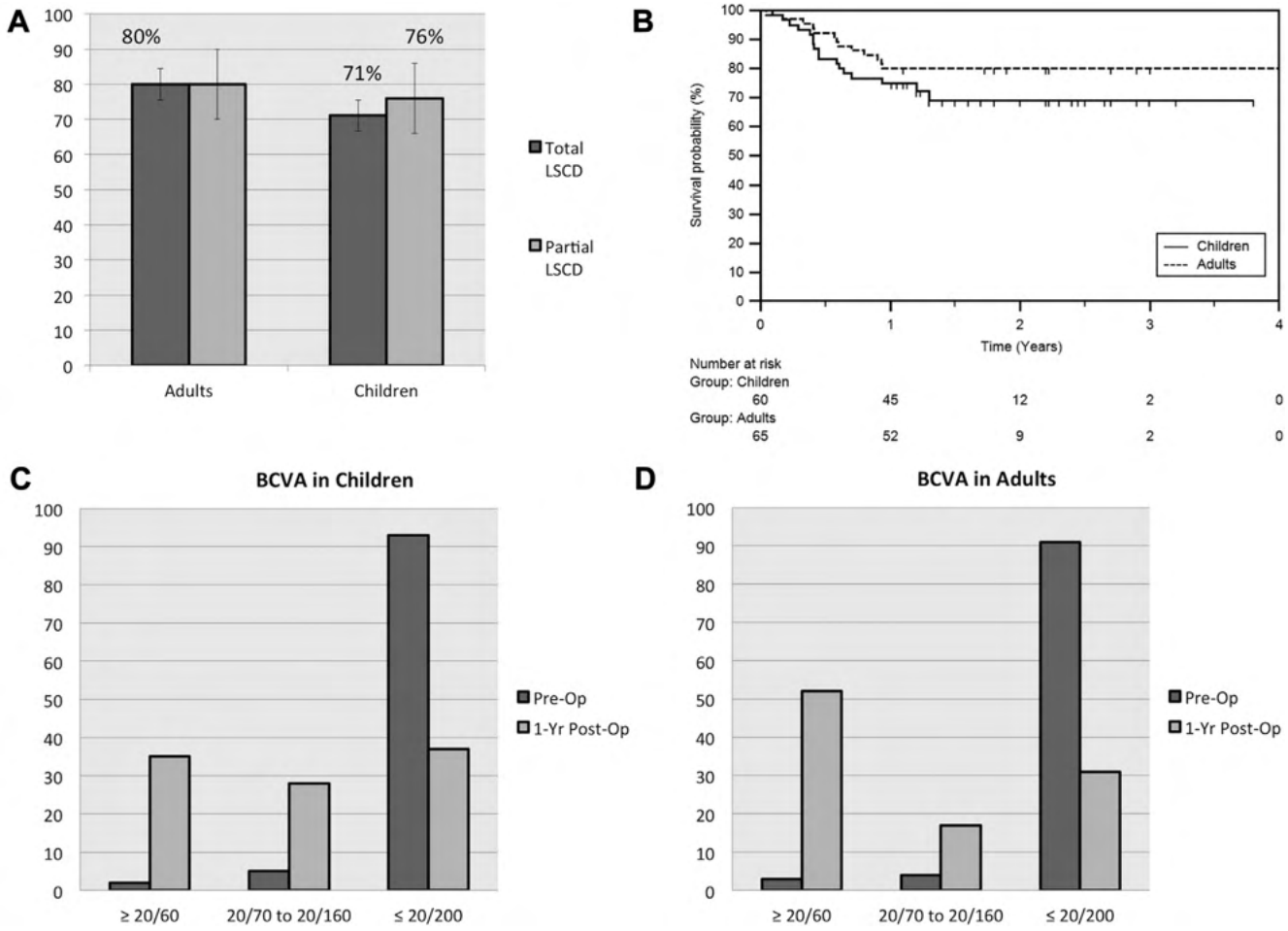

Figure 3. Graphs showing anatomic and visual outcomes of simple limbal epithelial transplantation (SLET). **A**, Successful regeneration of the corneal surface described as maintenance of a completely epithelized, avascular, and stable corneal surface without progressive conjunctivalization was observed in 80% eyes of adults and 71.2% eyes of children ($P = 0.69$) with total limbal stem cell deficiency (LSCD). Among patients with partial LSCD, successful outcomes were observed in 80% eyes of adults and 76% eyes of children ($P = 0.79$). **B**, Kaplan-Meier analysis showing a survival probability of 80% at 1 year and beyond in adults and 72% at 1 year and 66% subsequently in children ($P = 0.304$). **C, D**, Best-corrected visual acuity (BCVA) improved significantly after SLET both in **(C)** children and **(D)** adults ($P < 0.0001$).

Partial loss of SLET transplants was noted in 7 of 125 eyes (5.6%). In all of these cases, the patient had lost the contact lens within the first week and showed recurrence of LSCD over varying lengths of time. Sterile or microbial keratitis developed in 8 of 125 eyes (6.4%). Corneal melting with perforation was seen in 2 eyes (1.6%).

Histopathologic and Immunofluorescence Analysis

Histopathologic and immunofluorescence analysis showed the following results. In all cases, the excised pannus showed the presence of variably thick, stratified squamous epithelium with occasional goblet cells and underlying fibrocollagenous matrix laden with plasma–lymphocytic infiltrate (Fig 5A). This confirmed the clinical diagnosis of LSCD. In 10 cases, areas of absent epithelium with underlying necrosis suggestive of persistent epithelial defect were seen. In 7 cases, basophilic deposits suggestive of calcification were seen in the subepithelial tissue. Corneas that underwent SLET showed the presence of a smooth stratified squamous epithelium without goblet cells identical to normal corneal tissue over a thick basement membrane with positive periodic acid–Schiff staining suggestive of persistent hAM (Fig 5A). Immunohistochemistry examination confirmed the presence of corneal markers CK3 and CK12 in corneas that underwent SLET, which were absent in the excised pannus. Similarly, conjunctival markers MuC5AC and CK19 were present in the excised pannus and absent in corneas that underwent SLET (Fig 5A). Immunohistochemistry examination of corneas that underwent SLET also showed positive results for epithelial progenitor cell marker p63 (Fig 5B) and focal basal cell positive results for putative limbal epithelial stem cell markers ΔNp63α and ABCG2 (Fig 5C). In summary, immunohistochemistry analysis showed regeneration of a normal corneal epithelium with focal retention of limbal epithelial stem cells in the basal epithelial layers after SLET.

Discussion

This study showed that, as indicated by initial reports,[14–16,19,20] SLET was successful in the long-term regeneration of the corneal surface in a large cohort of

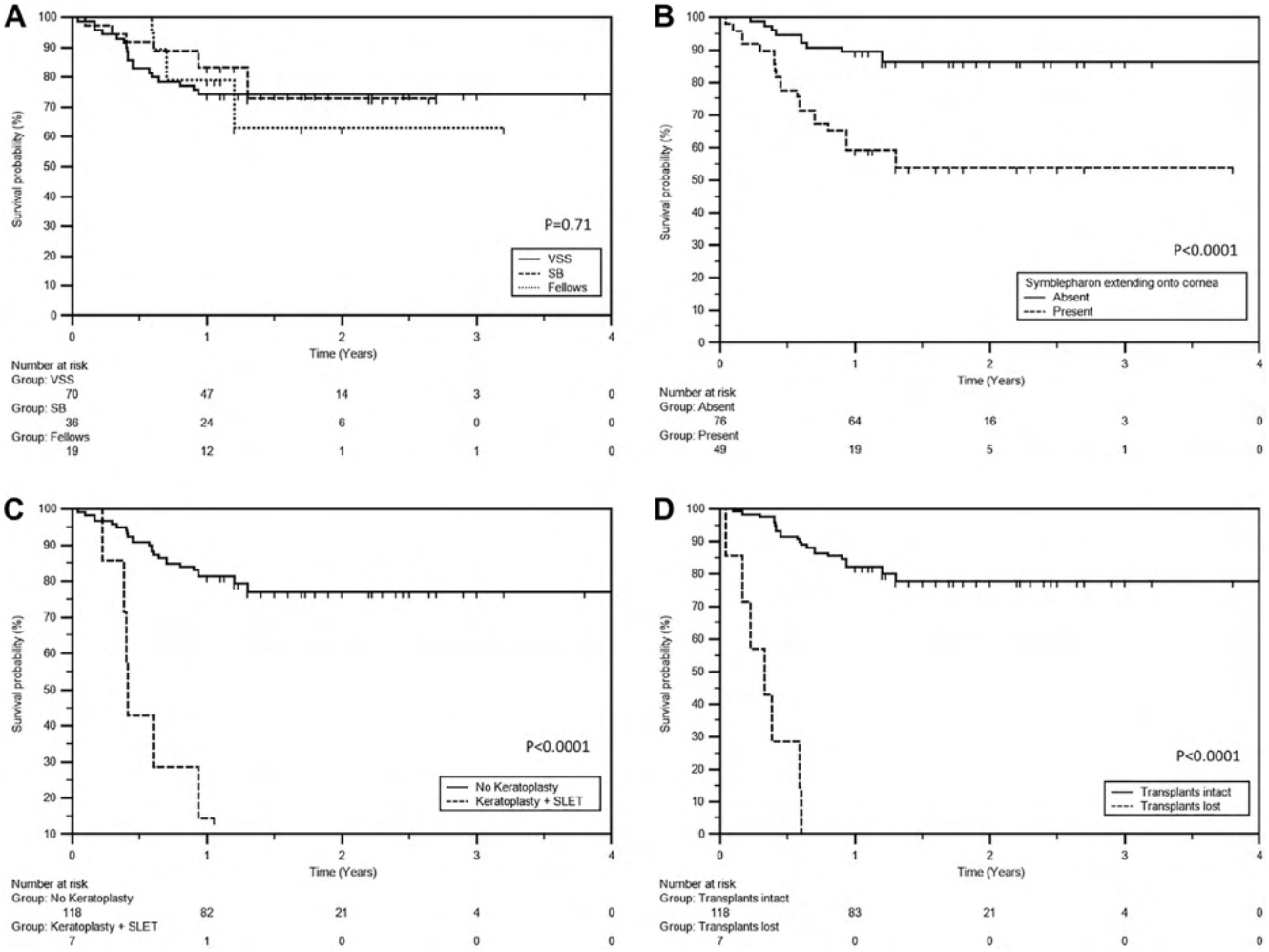

Figure 4. Kaplan-Meier survival curves comparing the success rates of simple limbal epithelial transplantation (SLET) in different subgroups. **A**, The survival rate was comparable between the senior ocular surface surgeon with more than 10 years of experience (V.S.S.), a comparatively less experienced surgeon with 5 years of experience (S.B.), and cornea fellows in training. The survival rate was affected adversely when **(B)** symblepharon extending onto the cornea was present before surgery, **(C)** SLET was combined with keratoplasty during surgery, and **(D)** the SLET transplants were lost after surgery.

patients with unilateral LSCD occurring after ocular burns. Simple limbal epithelial transplantation was equally effective in children and adults as well as in total and partial LSCD. In addition to surface restoration, most patients undergoing SLET reported a significant improvement in visual acuity. We also showed that the corneal surface after SLET was identical to that of the native cornea, comprising uniform nonkeratinized stratified squamous epithelium without goblet cells or vascularization. Because SLET requires only minimal donor tissue and does not require any clinical-grade laboratory support, these results make SLET an attractive alternative to conventional limbal grafting or ex vivo CLET for the treatment of LSCD.

Table 3 provides an overall comparison of the results of this study with that of other large series (more than 20 cases) of autologous CLET and conjunctival–limbal autografting for the treatment of unilateral LSCD.[5,11,21–23] Because the inclusion criteria and definition of success vary across studies, superficial comparisons sometimes can lead to deeply flawed conclusions. Therefore, it may be worthwhile to limit the comparison with our own experience with autologous CLET. We have reported an overall success rate of 71.4% in total LSCD[11] and 75% in partial LSCD,[24] rates that are comparable with the outcomes of SLET in this study. In children, however, SLET seemed to have a much better success rate than CLET (71% vs. 37%).[25] Although one would intuitively expect better results in pediatric patients owing to the greater regenerative potential of younger donor tissue, this effect may have been dampened by the greater severity of injury in children.

The findings of this study question the paradigm regarding the minimum amount of limbal tissue that is necessary to regenerate the entire corneal surface. It seems that the conventional assumption of needing 3 to 6 clock hours of donor tissue based on animal studies may not necessarily hold true in humans.[5] Even the pioneers of limbal transplantation themselves have questioned this and

Table 2. Postoperative Complications of Simple Limbal Epithelial Transplantation for Limbal Stem Cell Deficiency Occurring after Ocular Burns

Complication	Children	Adults	*P* Value
Donor eye			
Subconjunctival hemorrhage	17 (28.3)	18 (27.7)	0.93
Pyogenic granuloma	2 (3.3)	0 (0)	0.44
LSCD	0 (0)	0 (0)	1
Recipient eye			
Conjunctivalization	14 (23.3)	9 (13.8)	0.17
Symblepharon	12 (20)	9 (13.8)	0.47
Hemorrhage under hAM	4 (6.7)	6 (9.2)	0.84
Loss of transplants	4 (6.7)	3 (4.6)	0.91
Detached hAM	3 (5)	1 (1.5)	0.55
Keratitis	5 (8.3)	3 (4.6)	0.63
Corneal perforation	2 (3.3)	0 (0)	0.44
Lignocaine allergy	0 (0)	1 (1.5)	0.97

hAM = human amniotic membrane graft; LSCD = limbal stem cell deficiency.
Data are no. (%) unless otherwise indicated.

have tried using lesser amounts of limbal tissue, with successful results.[26] Simple limbal epithelial transplantation also challenges the usefulness of ex vivo cultivation, considering the additional costs involved. These results suggest that, at least in terms of clinical efficacy, it does not seem to matter whether a 1-clock-hour limbal biopsy sample is cultured ex vivo on a Petri dish with laboratory reagents or in vivo on the corneal surface itself. The visual outcomes after SLET are particularly impressive. It is known that limbal biopsies yield both epithelial and mesenchymal stem cells and that the mesenchymal cells can modulate corneal wound healing and can ameliorate scarring.[27] It may be possible that keeping the epithelial-mesenchymal microenvironment intact in SLET has a beneficial effect on the corneal stroma and results in less scarring, and hence better visual outcomes.

Mittal et al[16] recently described the epithelization pattern after SLET in a clinical setting. They showed that epithelization starts from the limbal transplants over the hAM on the second postoperative day and that ocular surface epithelialization is completed within 2 weeks, which is similar to our experience. Interestingly, they also noted variations in the epithelization rate depending on the size of the transplants and the age of the donor.[16] Using ultra high-resolution optical coherence tomography, Amescua et al[15] showed persistence of the hAM while epithelial cells stratified over it, which correlates well with the histopathologic findings of this study. It is important to note that hAM plays a critical role in promoting and preserving the stemness of the limbal epithelial stem cells,[28,29] and the membrane's persistence after SLET may contribute to the prolonged success of the procedure. Both epithelial progenitor cells ($p63^+$) and limbal epithelial stem cells ($\Delta Np63\alpha^+/ABCG2^+$) were observed in the basal layers of the regenerated epithelium, next to the retained hAM, many months after SLET.

This study also sheds new light on the possible factors responsible for recurrence of LSCD and failure of SLET. The presence of symblepharon extending up to the cornea before surgery could indicate some form of conjunctival deficiency, and outcomes may improve further if the symblepharon is addressed before or at the time of SLET. Inadvertent corneal perforation at the time of dissection should be avoided because it necessitates penetrating keratoplasty, and this in turn adversely impacts the outcome of SLET. We previously showed a similar detrimental impact of combining penetrating keratoplasty with CLET.[30] Therefore, it may be advisable to identify those eyes with extremely thin corneas by performing optical coherence tomography or ultrasound biomicroscopy before surgery. We speculate that such cases may fare better if a lamellar corneal graft is planned along with SLET. Early loss of SLET transplants is another factor that may result in recurrence of LSCD. This finding confirms the observation made by Konomi et al[31] that hAM alone, without limbal transplantation, may not be sufficient for corneal surface regeneration, even in cases with partial LSCD. To prevent this rare complication, a temporary tarsorrhaphy or an additional layer of hAM[15] may be considered in cases where the contact lens is unstable or is likely to be lost early (e.g., in very young children).

The major strengths of this study are that it was planned prospectively, it had a large sample, and the diagnosis we made and outcomes we assessed in every case were validated by 5 independent masked assessors. Although LSCD was defined clinically in this study, the surface pannus excised from all eyes was subjected to histopathologic examination to confirm the diagnosis. Other more objective means, such as confocal microscopy or impression cytologic analysis, could have been used. However, considering that almost half of the cohort constituted children, it would have meant extra procedures under anesthesia at each follow-up visit, which was not feasible. We compared our results with historical controls instead of conducting a comparative clinical trial. However, for a noninferiority trial of SLET compared with CLET or conjunctival–limbal autografting, assuming a 2-tailed α error of 5%, power of 80%, and a 75% success rate for CLET or conjunctival–limbal autografting per the published literature, a sample size of 800 eyes in each group is needed. Because LSCD occurring after ocular burns is a rare condition, achieving this number, even if a multicenter trial is attempted, is impractical. In our institute, approximately 100 new cases of chronic ocular burns are seen every year, and therefore, it would take us 16 years just to enroll enough patients for such a study.

Transplantation of ex vivo–cultivated limbal epithelial sheets is currently in vogue and is accepted as standard of care, particularly in Europe and Japan. Although we have published the largest clinical series of this technique with excellent outcomes over the past decade in more than 1000 cases, we realized that this technique was prohibitively expensive and therefore was out of reach of most corneablind individuals, most of whom live in the developing world. Because of regulatory restrictions, it is not practiced in many countries, including the United States. To circumvent these limitations, we developed the technique of SLET, drawing from our experience with ex vivo cultivation. This study showed that the results of SLET are extremely

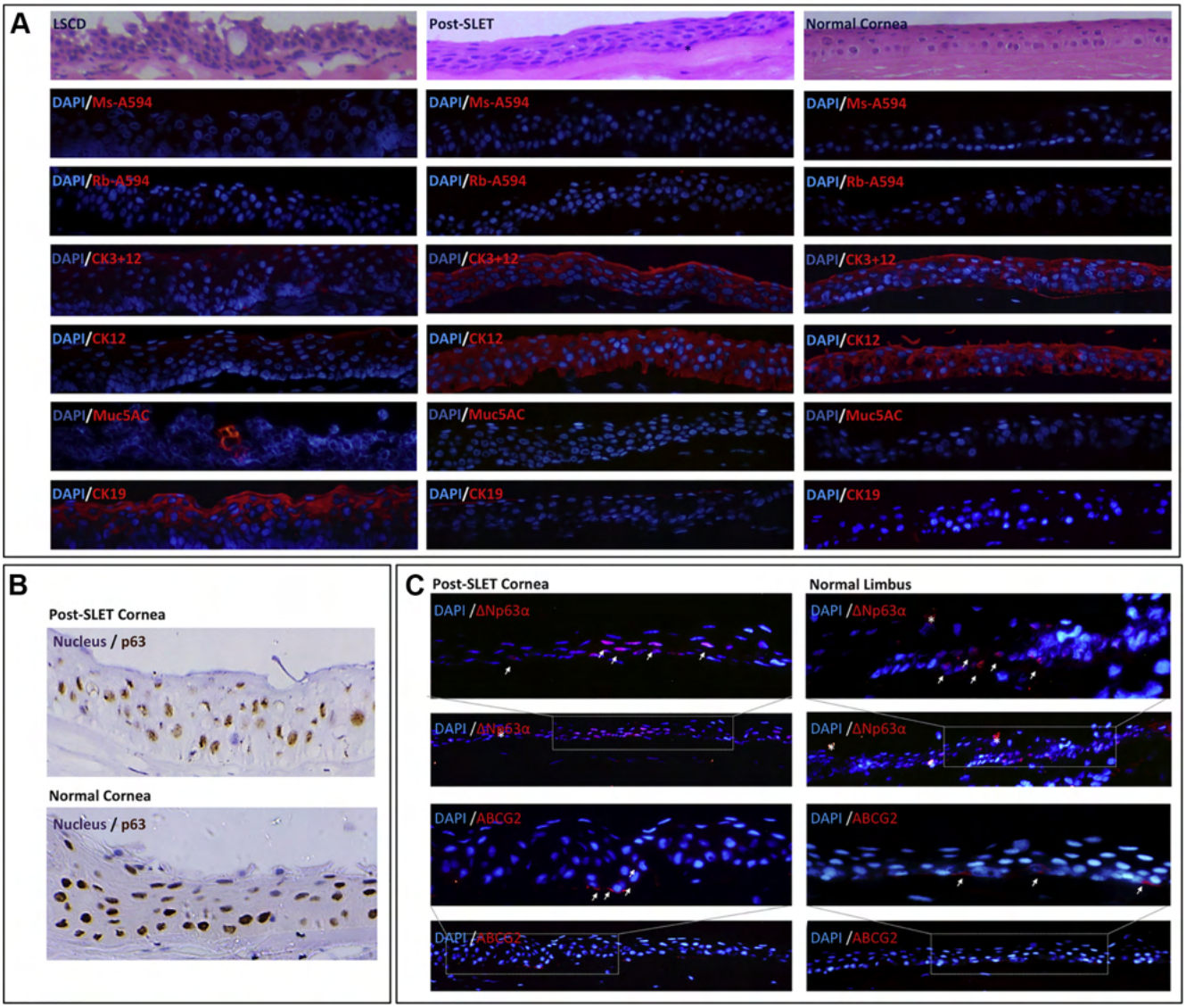

Figure 5. Photomicrographs showing immunohistochemistry analysis results using fluorescence-labeled antibodies. **A**, Left column represents sections of the ocular surface pannus excised from eyes with clinically diagnosed limbal stem cell deficiency (LSCD) occurring after ocular burns. Middle column represents sections of corneas excised during penetrating keratoplasty from eyes of patients who had previously undergone successful simple limbal epithelial transplantation (SLET). Right column represents sections of a normal cadaveric cornea as controls. Immunohistochemistry analysis confirmed the diagnosis of LSCD in the excised pannus, which showed negative results for corneal cytokeratin markers (CK3/12) and positive results for conjunctival markers (CK19, Mu5Ac). Corneal sections obtained after SLET showed stratified squamous epithelium without goblet cells with cytokeratin expression identical to that of normal control corneas ($CK3^+/12^+$, $CK19^-/Mu5Ac^-$) over a thick basement membrane with positive periodic acid–Schiff results suggestive of persistent human amniotic membrane (blue asterisks). **B**, Top row represents sections of corneas that underwent SLET and the bottom row represents sections of normal cadaveric human corneas. Positive nuclear expression of epithelial progenitor cell marker p63 is seen in both corneas that underwent SLET and normal corneas. **C**, Left column represents sections of corneas that underwent SLET and the right column represents sections of normal human cadaveric limbus. Positive expression (white arrows) of putative limbal epithelial stem cell markers ΔNp63α and ABCG2 is seen focally in the basal epithelial layers in corneas that underwent SLET, similar to that seen in the normal limbus. This indicates the persistence of limbal epithelial stem cells on the cornea after SLET. White asterisks indicate artifacts.

promising and that SLET potentially could make ex vivo cultivation redundant and limbal transplantation accessible to hundreds of thousands of people with corneal blindness worldwide.

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Table 3. Outcomes of Different Techniques of Autologous Limbal Stem Cell Transplantation for Treatment of Unilateral Limbal Stem Cell Deficiency

Authors	Year	Technique	No. of Eyes	Anatomic Success Rate (%)	2-Line Visual Acuity Gain (%)	Follow-up Range (yrs)
Kenyon and Tseng[5]	1998	CLAu	26	77	65	0.2–3.75
Rama et al[21]	2010	CLET	107	68	54	1–10
Pauklin et al[22]	2010	CLET	30	77	73	0.8–6
Sangwan et al[11]	2011	CLET	200	71.4	60.3	1–7.6
Barreiro TP et al[23]	2014	CLAu	23	87	80	0.9–3
Basu et al	2015	SLET	125	75.2	75	1–4

CLAu = conjunctival–limbal autografting; CLET = cultivated limbal epithelial transplantation; SLET = simple limbal epithelial transplantation.

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Abbreviations and Acronyms:
BCVA = best-corrected visual acuity; **CI** = confidence interval; **CLET** = cultivated limbal epithelial transplantation; **hAM** = human amniotic membrane; **HR** = hazard ratio; **LSCD** = limbal stem cell deficiency; **SLET** = simple limbal epithelial transplantation.

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Autologous limbal stem cell transplantation: a systematic review of clinical outcomes with different surgical techniques

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ABSTRACT

Purpose To conduct a systematic review on outcomes of three different techniques of autologous limbal stem cell transplantation (LSCT): conjunctival-limbal autografting (CLAu), cultivated limbal epithelial transplantation (CLET) and simple limbal epithelial transplantation (SLET), in unilateral limbal stem cell deficiency (LSCD).

Methods Literature searches were conducted in MEDLINE (Ovid), Embase, Web of Science and Cochrane Central Register. Standard systematic review methodology was followed using Meta-analysis of Observational Studies in Epidemiology guidelines. Studies with a sample size of more than 10 eyes were included. The primary outcome measure of efficacy was restoration of a completely epithelised, stable and avascular corneal surface (anatomical success). The secondary outcome measure of efficacy was improvement in best-corrected visual acuity of two-lines or greater (functional success).

Results The review identified 22 non-comparative case series, which included 1023 eyes. Ocular burns were the major (88%) indication for surgery. Overall, at a median postoperative follow-up of 1.75 years, autologous LSCT for unilateral LSCD showed anatomical and functional success rates of 69% and 60%, respectively, without any serious adverse events in the donor eye. The follow-up duration and indications for surgery were comparable across all groups (p>0.05). The anatomical and functional success rates of SLET (78%; 68.6%) and CLAu (81%; 74.4%) were comparable, and significantly better than those of CLET (61.4%; 53%; p=0.0048).

Conclusion Autologous LSCT is a safe and effective treatment for unilateral LSCD. In the absence of randomised controlled trials, existing evidence clearly suggests that clinical outcomes are better with SLET and CLAu as compared with CLET.

INTRODUCTION

The limbus contains stem cells which continually replenish the corneal epithelium in the physiological state.[1] Limbal stem cell deficiency (LSCD) arises in eyes where these epithelial stem cells are damaged.[2 3] In LSCD, the corneal epithelium is replaced by the conjunctiva, leading to loss of corneal clarity and impairment of vision. Corneal transplantation alone is ineffective in LSCD and invariably results in repeated surface breakdowns and epithelial healing issues, ultimately leading to graft failure.[4] Fortunately, limbal stem cell transplantation (LSCT) can restore the normal epithelial phenotype and re-establish a stable corneal surface.[5] In patients with unilateral LSCD, the healthy fellow eye can serve as the source for donor limbal tissue, which makes the procedure autologous and obviates the need for any systemic immunosuppression.

While the basic concept of autologous LSCT has remained unchanged over the last three decades, not only have three different surgical approaches evolved with time, but they also continue to be practised concurrently. The original technique of LSCT, conjunctival-limbal autografting (CLAu), was described by Kenyon and Tseng in 1989 and involves direct transplantation of two 3-clock-hour long conjunctival-limbal lenticules from the healthy eye onto the diseased limbal bed.[5] Subsequently in 1997, Pellegrini *et al* described cultivated limbal epithelial transplantation (CLET), where a 2 mm×2 mm limbal biopsy from the donor eye is expanded ex-vivo in the laboratory for 10–14 days into a sheet of epithelial cells and then transplanted onto the surface of the affected eye.[6] Finally, Sangwan *et al* in 2012, described the technique of simple limbal epithelial transplantation (SLET) where a strip of donor limbal tissue from the superior limbus, similar to CLET, is obtained from the healthy eye, but instead of ex-vivo expansion it is divided into small pieces and transplanted directly onto the affected cornea for in-vivo expansion over a human amniotic membrane graft.[7] Although the long-term clinical outcomes of all three LSCT-techniques have been reported, there is no clear consensus on whether one technique is superior in any way to the others. Currently this choice is based on the surgeon's individual preference and familiarity, rather than evidence. To address this issue, the authors conducted a systematic review on the clinical outcomes of the three techniques of autologous LSCT, namely CLAu, CLET and SLET, in eyes with unilateral LSCD.

METHODS

Search strategy

This systematic review was conducted as per the Meta-analysis of Observational Studies in Epidemiology guidelines.[8] The systematic review protocol was registered at the Prospective Register for Systematic Reviews (PROSPERO; https://www.crd.york.ac.uk/prospero/display_record.php?RecordID=89913). The search strategy for this review is reported in online supplementary appendix A. The search was conducted during July

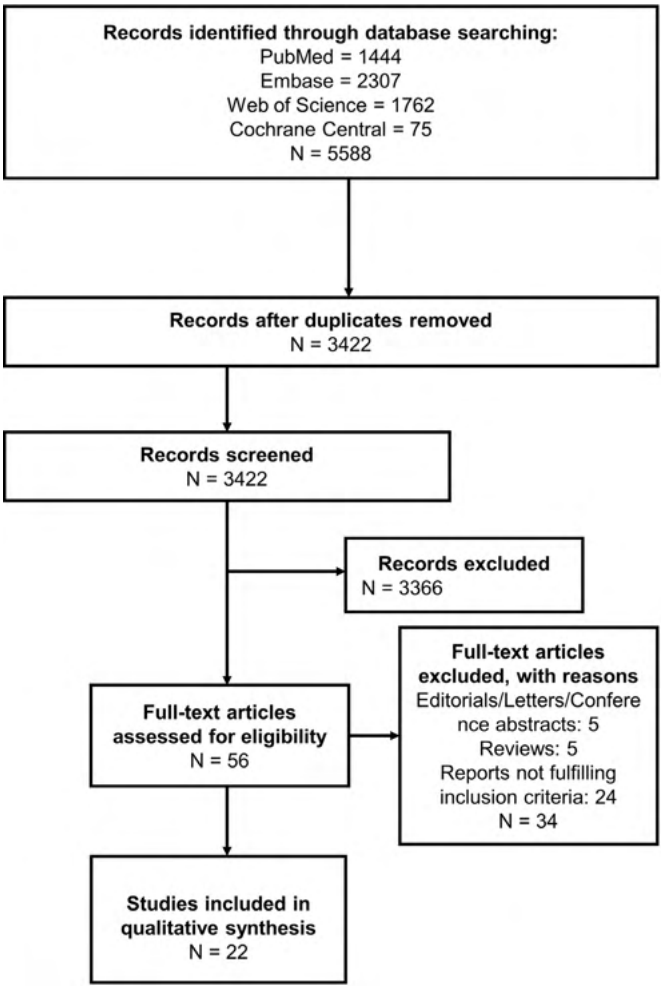

Figure 1 Flowchart detailing the literature search approach for studies on outcomes of autologous limbal stem cell transplantation in patients with unilateral limbal stem cell deficiency.

2018 in the following electronic databases: MEDLINE (Ovid), Embase, Web of Science, and the Cochrane Central Register. PROSPERO was searched for ongoing or recently completed systematic reviews. All reports published before 1 July 2018 were screened. No specific dates or years were used as time limits. No language restrictions were used. Reference lists were scanned to identify additional applicable studies.

Inclusion criteria

Reports in English language were considered for analysis. Reports where outcomes of the following interventions: CLAu, CLET and SLET could be discerned in >10 patients with total unilateral LSCD were included. Reports where repeat LSCT was performed for eyes in which previously performed LSCT had failed were also included.

Exclusion criteria

Letters, conference abstracts, case reports, review articles, editorials and animal studies were excluded. Other excluded studies were: (1) those which focused on the outcomes of penetrating keratoplasty after LSCT without describing the outcomes of the initially performed LSCT; (2) those focusing on outcomes of LSCT in partial LSCD alone; (3) those that included both partial LSCD (where limbal biopsy was obtained from the same eye) and total LSCD, but ≤10 eyes had total LSCD; (4) reports that included both autologous and allogeneic LSCT, but where outcomes specific to the autografts could not be discerned; (5) reports where at least one of the two primary outcome measures could not be discerned; (6) those with a mean follow-up duration of <6 months.

Special considerations

Some studies classified outcomes as success, partial success and failures. However, the criteria used to define partial success were consistent with that of failure in other studies. For the sake of consistency, partial successes were therefore counted as failures. In studies which reported the outcomes of the same procedure being repeated, only the outcomes of the primary procedure were considered for analysis. Measures were taken to avoid including multiple studies reporting outcomes of the same cohort of patients at different time intervals.

Article selection and data extraction

Of all the studies that were suitable for inclusion, the titles and abstracts were screened by two authors (SSS and NN). Disagreements were resolved by consensus of all authors. Selected full-text articles were retrieved and assessed for eligibility. From the full-text studies that were suitable for inclusion, information was recorded on a data extraction form. Data recorded included study design, study population, aetiology of LSCD, interventions, clinical outcomes, mean follow-up period and complications. A descriptive synthesis was undertaken. The primary outcome measure of efficacy was the restoration of a completely epithelised, stable and avascular corneal surface (anatomical success). The secondary outcome measure of efficacy was a two-line or greater improvement in best-corrected visual acuity (BCVA) after intervention (functional success).

Quality assessment

To rate individual studies based on study design, a scale based on the Scottish Intercollegiate Guidelines Network (https://www.sign.ac.uk/assets/qrg50.pdf, last accessed on 31 December, 2018) was used. Two reviewers (SSS, PRD) independently assessed the quality of all the included studies using a validated checklist consisting of 14 questions (online supplemental table). Any disagreements were resolved by consensus or arbitration by the senior author (SB). This checklist was developed by using a combination of two quality appraisal checklists specifically devised for interventional case series.[9 10] Items that were applicable to this study were used. A score of 1 was assigned when the answer to a quality question was clearly affirmative. The score for all studies were collated.

Statistical analysis

MedCalc (V.11.4.3.0, MedCalc Software, Mariakerke, Belgium) statistical software was used for data analysis. The anatomical and functional success rates were reported as proportions (percentages) with 95% CIs. The study quality, sample size, indication for surgery and follow-up duration between the groups were compared using the one-way-analysis of variance and Student-Newman-Keuls test. The comparisons in success rates between the different techniques were performed using the Pearson's χ^2 test. A p value <0.05 was considered statistically significant.

RESULTS

Selection of studies for review

A total of 5588 articles of potential interest were identified by the original literature search (figure 1). No similar ongoing or

Table 1 Descriptive information of studies on autologous limbal stem cell transplantation included in the review

Author	Year	Country	Design	SIGN	N	Main indication (n)*	Surgery	Size of donor limbal tissue	Anatomical success† (%)	Functional success (%)	LK/PK (%)	Mean follow-up in years (range)	Complications (n)
Kenyon and Tseng[5]	1989	USA	U	3	26	Chemical burns (20)	CLAu	8 clock hours (2 grafts × 4 each)	77	65	35	1.56 (0.2–3.75)	Perforation (1), PED (1), PK rejection (1), failed graft (1)
Rao *et al*[11]	1999	India	R	3	16	Chemical burns (14)	CLAu	6–8 clock hours (2 grafts × 2–3 each)	94	86	44	1.6 (0.3–3.75)	PK rejection (2)
Shimazaki *et al*[12]	2004	Japan	R	3	11	Chemical burns (7)	CLAu	5 mm×5 mm (2 each)	91	NA	27	1.3‡	PK rejection (1), PED (1), glaucoma (2)
Özdemir *et al*[13]	2004	Turkey	U	3	15	Chemical burns (14)	CLAu	NA	87	80	0	1.2 (0.3–2)	PED (1)
Wylegala *et al*[14]	2008	Poland	U	3	21	Chemical burns (16)	CLAu	NA	NA	71	0	2.6‡	NA
Miri *et al*[15]	2010	UK	R	3	12	NA	CLAu	4 clock hours (2 grafts × 2 each)	100	NA	NA	3.9 (1–9.9)	NA
Baradaran-Rafii *et al*[16]	2012	Iran	R	3	34	Chemical burns (25)	CLAu	Two 60° arcs of limbal tissue	88	NA	53	1.4 (0.5–2.75)	Graft dislodgement (4), thick graft (4), progressive LSCD (2), PK rejection (7)
Burcu *et al*[17]	2013	Turkey	R	3	16	Chemical burns (16)	CLAu	100°–120° (1 or 2, 60° each)	69	69	44	7.6 (4.3–15.5)	LSCD recurrence (3), corneal melt (1), PK rejection (2), glaucoma (1), graft failure (2)
Barreiro *et al*[18]	2014	Brazil	R	3	23	Chemical burns (23)	CLAu	5 mm (2 grafts each)	87	79	22	1.7 (0.7–3)	NA
Moreira *et al*[19]	2015	Brazil	R	3	15	NA	CLAu	≤120°	33	NA	NA	1.5	PED (2), corneal melt (1)
Pauklin *et al*[20]	2010	Germany	U	3	30	Chemical burns (16)	CLET	1–2 mm^2 (2 pieces)	77	70	NA	2.4 (0.8–6)	NA
Rama *et al*[21]	2010	Italy	U	3	107	Chemical burns (103)	CLET	1–2 mm^2	68	50	43	2.9 (1–9.4)	Haemorrhage (12), epithelial defect (35), inflammation (59), graft-transportation issues (14), PK rejection (22), PK failure (1), keratitis (3)
Sangwan *et al*[22]	2011	India	R	3	200	Chemical burns (179)	CLET	2 mm×2 mm (1 piece)	71	60	5	3 (1–7.6)	Haemorrhage (56), PED (13), sterile melt (5), bacterial keratitis (3)
Prabhasawat *et al*[23]	2012	Thailand	P	3	12	Chemical burns (10)	CLET	2 mm×2 mm or 3 mm×1 mm	67	50	33	2.4 (0.5–3.8)	PED (3), infectious keratitis (1), symblepharon (1)
Sejpal *et al*[24]	2012	India	R	3	107	Chemical burns (107)	CLET	NA	37	NA	18	3.4 (1–9.8)	Microbial keratitis (7), PK rejection (4), inflammatory granuloma (2), corneal thinning (2)
Zakaria *et al*[25]	2014	Belgium	P	3	12	Chemical burns (6)	CLET	1 mm×2 mm (1 piece)	58	17	25	1.6 (0.3–3.6)	PED (1), glaucoma (1), perforation (1)
Ganger *et al*[26]	2015	India	R	3	54	Chemical burns (52)	CLET	2 mm×2 mm (1 piece)	72	23	NA	1.8 (0.3–3.3)	Corneal melt (1), recurrent symblepharon (1), failed LK (1), vascularised granulation tissue (2)
Fasolo *et al*[27]	2017	Italy	R	3	59	Chemical burns (48)	CLET	1–3 mm×2 mm	42	NA	10	6 (1–13)	Haemorrhage (11), microperforation (2), inflammation (16), symblepharon (4), epithelial defect (16), keratitis (6), descemetocele/melt (9)
Basu *et al*[28]	2016	India	P	3	125	Chemical burns (117)	SLET	1 clock hour	76	75	8	1.5 (1–4)	Keratitis (8), perforation (1)
Vazirani *et al*[29]	2016	Multicentre	R	3	68	Chemical burns (62)	SLET	1–2 clock hours	84	65	7	1 (0.5–4.9)	Focal recurrence of LSCD (21), microbial keratitis (5), glaucoma (1), pyogenic granuloma (1)
Gupta *et al*[30]	2018	India	P	3	30	Chemical burns (26)	SLET	1–2 clock hours	70	50	10	1.1 (0.5–3.4)	LSCD recurrence (9)

Continued

Table 1 Continued

Author	Year	Country	Design	SIGN	N	Main indication (n)*	Surgery	Size of donor limbal tissue	Anatomical success† (%)	Functional success (%)	LK/PK (%)	Mean follow-up in years (range)	Complications (n)
Basu *et al*[31]	2018	India	P	3	30	Chemical burns (30)	SLET	1 clock hour	80	NA	13	2.3 (0.8–3.8)	LSCD recurrence (6), haemorrhage (2), PED (1)

*Only chemical burns were included, thermal burns were not included.
†Eyes with partial success were not considered.
‡Mean follow-up is not specific to eyes which underwent autograft, mean follow-up is mentioned across all eyes in this study.
CLAu, conjunctival limbal autograft; CLET, cultivated limbal epithelial transplantation; LK, lamellar keratoplasty; LSCD, limbal stem cell deficiency; NA, Data not available; P, prospective; PED, persistent epithelial defect; PK, penetrating keratoplasty; R, retrospective; SIGN, Scottish Intercollegiate Guidelines Network rating; SLET, simple limbal epithelial transplantation; U, unclear.

recently completed systematic reviews were found on PROSPERO. After removal of duplicated records, 3422 records were screened. After reviewing the titles and abstract, 3366 articles were excluded, and 56 full-text articles were assessed for eligibility. The eligibility criteria were met by a total of 22 reports and these were included in this systematic review.[5 11–31] Full-text articles that did not fulfil the eligibility criteria included: (1) multiple studies on CLAu in cases of pterygium; (2) studies with a sample size of ≤10 eyes with total LSCD[32–34]; (3) a study where the outcomes of autografts and allografts could not be differentiated[35]; (4) one study which reported outcomes of autologous LSCT at a mean follow-up of <6 months.[36] Several groups reported outcomes in the same cohort of patients at different time intervals, and hence, only the studies with longest duration of follow-up were included.[37–41]

Study designs and quality assessment

There were no randomised controlled trials; all studies were non-comparative interventional case series. Descriptive information for each study is presented in table 1. All 22 studies included in this systematic review were rated and the quality assessed (table 2 and figure 2). When a quality assessment question was graded as "yes", it represented a positive measure of quality. Of the 22, five (23%) studies collected data prospectively, one (5%) study was multicentric, 13 (59%) studies clearly mentioned the recruitment period, 18 (82%) studies mentioned the characteristics of the patients. The inclusion and exclusion criteria were clearly mentioned in 14 (64%) studies. None of the 22 studies had participants at a similar stage of disease preoperatively. The intervention of interest (along with the number of clock hours of limbal biopsy) was clearly described in 17 (77%) studies. Three (14%) studies clearly mentioned that the surgeries were performed by an experienced surgeon. Additional postoperative surgical interventions were clearly described in 18 (82%). Relevant outcomes measures were clearly mentioned in 18 studies (82%). Only one (5%) study clearly mentioned that the outcome assessors were blinded regarding the intervention. All-important outcome measures were considered and evaluated in 15 (68%) studies. The outcomes were evaluated at a follow-up of 6 months or more in 16 (73%) studies (in six studies, there were some individual patients with follow-up of <6 months, but the overall mean follow-up was ≥6 months). Adverse events were reported in 19 (86%) studies. The 10 studies where CLAu was performed had an average quality score of 5.6/14 (40%; 95% CI 32% to 49%). The eight studies where CLET was performed had an average quality score of 7.6/14 (54.4%; 95% CI 45% to 64%) while the four studies where SLET was performed had an average quality score of 10/14 (71.4%; 95% CI 58% to 83%).

Efficacy of CLAu

The outcomes of CLAu were analysed in 189 eyes across 10 studies.[5 11–19] Eight studies with 162 eyes provided data regarding aetiology, and 83% (135/162) eyes had LSCD secondary to chemical burns. The primary outcome measure of a stable corneal surface at the end of the follow-up period was mentioned in 168 eyes across nine studies. At a mean follow-up period of 1.56 years, 81% (136/168, 95% CI 74.2% to 86.6%) eyes had a stable, fully epithelised and avascular surface. The secondary outcome measure of a two-line improvement in BCVA could be discerned in 117 eyes across six studies. At a mean follow-up period of 1.65 years, 74.4% (87/117, 95% CI 65.5% to 82%) eyes had a two-line improvement in BCVA.

Table 2 Quality scores of studies on autologous limbal stem cell transplantation included in the review

	Quality questions (see online supplementary table 1)														
Authors	1	2	3	4	5	6	7	8	9	10	11	12	13	14	Score
CLAu															
Kenyon *et al*[5]	U	U	N	Y	Y	N	N	U	Y	N	U	Y	N	Y	5
Rao *et al*[11]	N	N	Y	Y	Y	N	Y	U	Y	Y	U	Y	N	Y	8
Shimazaki *et al*[12]	N	U	N	Y	Y	N	Y	U	Y	Y	U	N	Y	Y	7
Ozdemir *et al*[13]	U	U	N	Y	Y	N	N	U	Y	N	U	Y	N	Y	5
Wylegala *et al*[14]	U	U	N	N	N	U	N	U	N	N	U	N	Y	N	1
Miri *et al*[15]	N	N	Y	N	Y	U	Y	U	U	Y	U	N	Y	N	5
Baradaran-Rafii *et al*[16]	N	N	N	Y	Y	U	Y	U	Y	Y	U	N	Y	Y	7
Burcu *et al*[17]	N	U	Y	Y	N	U	Y	U	Y	Y	U	Y	Y	Y	8
Barreiro *et al*[18]	N	N	N	Y	Y	N	Y	U	Y	Y	U	Y	Y	Y	8
Moreira *et al*[19]	N	N	Y	N	N	N	N	N	N	N	U	N	N	Y	2
CLET															
Pauklin *et al*[20]	U	N	Y	Y	N	N	Y	Y	N	Y	U	Y	Y	N	7
Rama *et al*[21]	U	U	Y	Y	N	N	Y	U	Y	Y	N	Y	Y	Y	8
Sangwan *et al*[22]	N	N	Y	Y	Y	U	Y	U	Y	Y	U	Y	Y	Y	9
Prabhasawat *et al*[23]	Y	N	N	Y	Y	N	Y	U	Y	Y	U	Y	Y	Y	9
Sejpal *et al*[24]	N	N	Y	Y	Y	N	N	U	Y	Y	U	Y	Y	Y	8
Zakaria *et al*[25]	Y	N	N	Y	N	U	Y	U	Y	Y	U	Y	N	Y	7
Ganger *et al*[26]	N	N	Y	N	N	N	Y	Y	N	Y	U	Y	N	Y	6
Fasolo *et al*[27]	N	N	Y	Y	N	N	Y	U	Y	Y	U	N	Y	Y	7
SLET															
Basu *et al*[28]	Y	N	Y	Y	Y	N	Y	N	Y	Y	Y	Y	Y	Y	11
Vazirani *et al*[29]	N	Y	N	Y	Y	N	Y	U	Y	Y	U	Y	Y	Y	9
Gupta *et al*[30]	Y	N	Y	Y	Y	N	Y	Y	Y	Y	N	Y	Y	Y	11
Basu *et al*[31]	Y	N	Y	Y	Y	N	Y	U	Y	Y	U	N	Y	Y	9

CLAu, conjunctival limbal autograft; CLET, cultivated limbal epithelial transplantation; N, No;SLET, simple limbal epithelial transplantation; U, Unclear;Y, Yes.

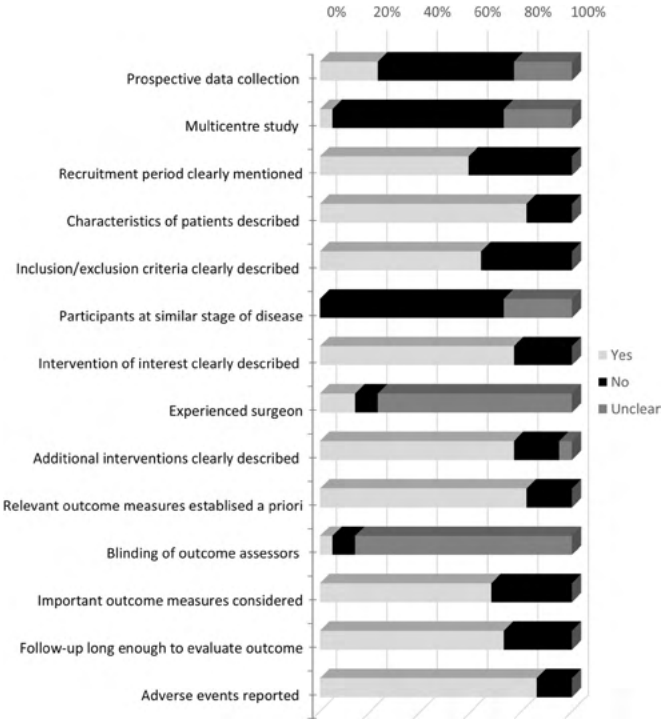

Figure 2 Bar graph showing the proportion of studies meeting specific quality measures for systematic review on outcomes of autologous limbal stem cell transplantation in patients with unilateral limbal stem cell deficiency.

Efficacy of CLET

The outcomes of CLET were analysed in 581 eyes across eight studies.[20–27] All eight studies provided data regarding aetiology, and 90% (521/581) eyes had LSCD secondary to chemical burns. The primary outcome measure of a stable corneal surface at the end of the follow-up period was mentioned in all eight studies. At a mean follow-up period of 2.9 years, 61.4% (357/581; 95% CI 57.4% to 65.4%) eyes had a stable, epithelised, avascular surface. The secondary outcome measure of a two-line improvement in BCVA was mentioned in 425 eyes across six studies. At a mean follow-up period of 2.4 years, 51.5% (219/425; 95% CI 46.7% to 56.4%) eyes had a two-line improvement in BCVA.

Efficacy of SLET

The outcomes of SLET were analysed in 253 eyes across four studies.[28–31] All four studies provided data regarding aetiology, and 93% (235/253) eyes had LSCD secondary to chemical burns. The primary outcome measure of a stable surface at the end of the follow-up period was mentioned in all four studies. At a mean follow-up period of 1.48 years, 78% (197/253, 95% CI 72.2% to 82.8%) eyes had a stable, epithelised, avascular surface. The secondary outcome measure of a two-line improvement in BCVA was mentioned in 223 eyes across three studies. At a mean follow-up period of 1.2 years, 68.6% (153/223; 95% CI 62.1% to 74.6%) eyes had a two-line improvement in BCVA.

Comparison of primary and secondary measures (CLAu vs CLET vs SLET)

Overall, at a median postoperative follow-up of 1.75 years (range, 1–7.6 years), 68.9% (690/1002, 95% CI 65.9% to

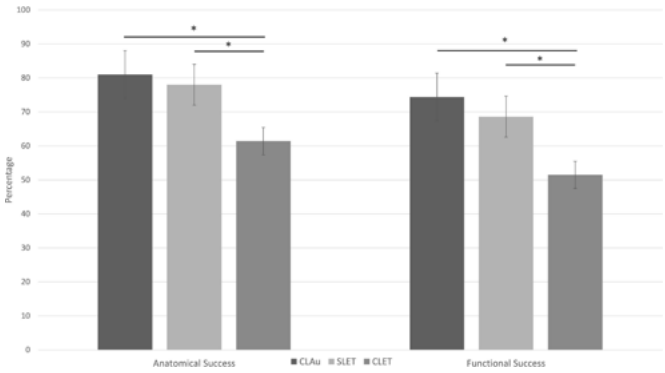

Figure 3 Bar graph comparing the anatomical and functional success rates of conjunctival-limbal autografting (CLAu), simple limbal epithelial transplantation (SLET) and cultivated limbal epithelial transplantation (CLET). *Statistically significant difference (Pearson's χ^2 test).

71.7%) eyes maintained a stable, epithelised, avascular cornea, and 60.8% (459/755, 95% CI 57.2% to 64.3%) eyes gained at least two-lines of BCVA. The overall follow-up (p=0.358), and indications for surgery (p=0.089) were comparable across all three groups. However, study quality (p=0.003) and sample size (p=0.039) were significantly lesser in the CLAu group. Statistically, SLET and CLAu had significantly better outcomes compared with CLET, both in terms of anatomical (p=0.0048) and functional success (p≤0.0001). The anatomical success rates were almost identical between SLET and CLAu, while the functional success rates were marginally better with CLAu, although not statistically significant (p=0.27). The results are summarised in table 1 and figure 3.

Safety in the recipient eye

The reported complications of autologous LSCT are listed in table 1. In most studies, the most common early postoperative complication was haemorrhage under the amniotic membrane graft. Late postoperative complications were either recurrence of LSCD or persistent epithelial defects. The latter rarely led to secondary complications such as perforation or microbial keratitis.

Safety in the donor eye

In 6/10 studies on CLAu, donor-site complications were looked for but none were noted. In 3/8 studies on CLET, donor-site complications were looked for and again none were noted. In all four studies on SLET, the donor-eye was evaluated postoperatively and findings were noted, but no serious adverse events were reported.

DISCUSSION

In the absence of randomised controlled trials, systematic reviews of non-comparative case series form the strongest pillars of evidence-based medicine. This systematic review compared the anatomical and functional outcomes of three different techniques of autologous LSCT for unilateral LSCD. After a thorough literature review, the authors identified 22 non-comparative case series that included more than 10 cases with at least 6 months of mean postoperative follow-up. Most of the cases covered in this systematic review had LSCD secondary to chemical burns. All cases underwent autologous grafts from the unaffected fellow eye without any long-term topical or systemic immunosuppressive medications. Similarities in indication for surgery and follow-up duration across the three groups provided for a uniform platform to compare the individual techniques. The findings of this systematic review clearly suggest that: (1) irrespective of the technique used, autologous LSCT is effective in treating unilateral LSCD and safe for the donor eye; (ii) SLET and CLAu have significantly better anatomical and functional success rates compared with CLET.

The strengths of this study include a robust methodology, with rigorous inclusion and exclusion criteria, adopted to select for reliable studies within each group with adequate numbers and follow-up. However, there are caveats. Since ocular burns in general and chemical burns in particular, were the major indications for surgery across all studies, the results of this review should not be extrapolated to other indications of autologous LSCT. With regards to both CLAu and SLET, the studies within each group used similar techniques and therefore minor variations in the surgical methods were unlikely to affect the validity of the comparisons. For CLET, however, varying cultivation protocols were used by different groups, which may have influenced the overall outcomes. Nevertheless, no individual study on CLET, irrespective of the laboratory protocols, reported success rates that were better than those noted for CLAu or SLET. Finally, the success rates observed with CLAu should be placed in the context of the fact that this group had the smallest sample size and lowest study quality.

The overarching goal of this systematic review was to help the treating surgeon decide which technique of autologous LSCT to use when confronted with a patient with unilateral LSCD. Although this review does not answer the question directly, it does provide direction. The results indicate that those practising CLAu or SLET can continue to do so and do not need to "upgrade" or shift to a different technique. However, the high laboratory costs for CLET may be difficult to justify considering the finding that it does not provide any proven clinical benefit over CLAu or SLET. Intuitively, SLET has the advantage over CLAu of needing less tissue from the donor eye. Although there is no data regarding efficacy based on the severity of injury, the authors recommend SLET for LSCD cases with milder conjunctival involvement (lesser grades of symblepharon) and CLAu for LSCD with more severe conjunctival involvement.[42] Also, in circumstances where the cost or availability of the amniotic membrane and/or fibrin glue may be limiting factors, CLAu may be preferred over SLET. However, despite the poorer clinical results with ex-vivo cultivation, it will continue to have relevant research and clinical applications. For example, CLET can be combined with gene therapy for congenital causes of LSCD such as epidermolysis bullosa, which cannot be treated by SLET or CLAu.[43 44] Therefore, each of the three procedures may be preferred in specific situations, rather than one procedure being the recommended standard of care.

In conclusion, this systematic review of 22 studies including 1023 eyes aimed to compare the clinical outcomes of three different techniques of autologous LSCT for the treatment of unilateral LSCD. The findings of the review clearly indicate that autologous LSCT, irrespective of the technique used is a safe and effective therapy for this condition; and that SLET and CLAu have better long-term anatomical and functional success rates in comparison to CLET.

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