Autologous simple limbal epithelial transplantation for unilateral limbal stem cell deficiency: multicentre results

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ABSTRACT

Purpose To report outcomes of autologous simple limbal epithelial transplantation (SLET) performed for unilateral limbal stem cell deficiency (LSCD) at multiple centres worldwide.

Methods In this retrospective, multicentre, interventional case series, records of patients who had undergone autologous SLET for unilateral LSCD, with a minimum of 6 months of follow-up, were reviewed. The primary outcome measure was clinical success, defined as a completely epithelised, avascular corneal surface. Kaplan–Meier survival curves were constructed and survival probability was calculated. A Cox proportional hazards analysis was done to assess association of preoperative characteristics with risk of failure. Secondary outcome measures included the percentage of eyes achieving visual acuity of 20/200 or better, percentage of eyes gaining two or more Snellen lines and complications encountered.

Results 68 eyes of 68 patients underwent autologous SLET, performed across eight centres in three countries. Clinical success was achieved in 57 cases (83.8%). With a median follow-up of 12 months, survival probability exceeded 80%. Presence of symblepharon (HR 5.8) and simultaneous keratoplasty (HR 10.8) were found to be significantly associated with a risk of failure. 44 eyes (64.7%) achieved a visual acuity of 20/200 or better, and 44 eyes (64.7%) gained two or more Snellen lines. Focal recurrences of pannus were noted in 21 eyes (36.8%) with clinical success.

Conclusion Autologous SLET is an effective and safe modality for treatment of unilateral LSCD. Clinical success rates and visual acuity improvement are equal to or better than those reported with earlier techniques.

INTRODUCTION

The concept of limbal stem cells and limbal stem cell deficiency (LSCD) as a distinct entity gained acceptance in the last two decades of the 20th century.[1 2] Early approaches to treating unilateral LSCD were based on direct transplantation of donor limbal tissue from the fellow eye to the affected eye.[3 4] Conjunctival limbal autografting (CLAU) typically involved taking two pieces of limbal tissue from the donor eye, each extending up to 3 clock hours. The next advance in LSCD therapy was the advent of ex-vivo cultivated limbal epithelial transplantation (CLET), which reduced the amount of donor limbal tissue required and minimised the potential risk of iatrogenic LSCD at the donor site.[5] Three years ago, a novel surgical technique was described, which claimed to combine the advantages of both CLAU and CLET, while eliminating the major drawbacks of both earlier techniques. In simple limbal epithelial transplantation (SLET), a small piece of donor limbal tissue is cut into multiple pieces and placed on the recipient surface, using human amniotic membrane (hAM) to support in-vivo expansion of epithelial cells. It is a single-stage procedure, requires minimal donor limbal tissue and does not require an expensive laboratory setup for ex-vivo cell cultivation.[6] Subsequent to the original description of SLET, few more results have been reported in a small case series and individual case reports, mostly from the centre where the technique originated.[7–10] The authors of the original study had remarked that if the initial results were validated in a larger number of patients on a multicentre basis, SLET could significantly simplify the treatment of LSCD and benefit many more patients worldwide. In addition to validating or refuting the initial results, this would also indicate whether the technique is reproducible when performed by surgeons elsewhere. The prospect of such a study first materialised during the international SLET users' workshop and the first meeting of the Ocular Surface Interest Group at Hyderabad, India. Surgeons with experience in dealing with diseases of the cornea and ocular surface, who had started performing SLET, were invited to share their results. Here, we present data collated from surgeons performing SLET across multiple centres in different countries.

METHODS

This was a multicentre, retrospective interventional case series. Local ethics committee's approval for the study, as appropriate, as well as detailed informed consent for the surgical procedure, was obtained at each site. Investigators at each participating centre reviewed case records, using uniform definitions agreed on prior to starting data collection. Inclusion criteria included consecutive cases of unilateral LSCD with wet ocular surface for which autologous SLET was performed, with a minimum of 6 months of postoperative follow-up. All cases in which failure of SLET was documented were also included, irrespective of duration of follow-up. Cases of LSCD secondary to immune-

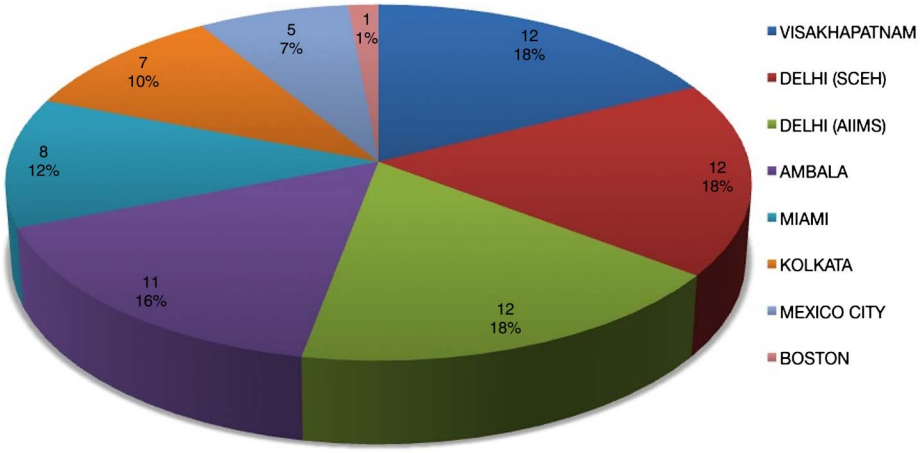

Figure 1 Pie chart showing number and percentage of cases from each participating centre. SCEH, Dr Shroff's Charity Eye Hospital; AIIMS, All India Institute of Medical Sciences.

mediated conditions such as Stevens–Johnson syndrome, mucous membrane pemphigoid and those with dry ocular surfaces were excluded. The diagnosis of LSCD was based on clinical signs such as absence of pigmented palisades of Vogt, irregular and lustreless corneal epithelium, persistent epithelial defects, fibrovascular pannus formation and conjunctivalisation of the corneal surface. Demographic details, aetiology of LSCD, prior surgery performed and clinical details including visual acuity at presentation, extent of LSCD (in clock hours), presence or absence of eyelid abnormalities, symblepharon and persistent epithelial defects were noted.

Surgical technique

The surgical technique of SLET has been described earlier.[6 10] In brief, a small piece of limbal tissue (1–2 clock hours) was harvested from the unaffected eye. Fibrovascular pannus was excised from the eye with LSCD, and hAM was spread over the bare surface, using fibrin glue as adhesive. Significant symblepharon, if present, was released and the bare area covered by placement of a conjunctival autograft. The limbal tissue was cut into multiple small pieces (typically 10–15), which were distributed over the hAM with application of more fibrin glue and covered with a bandage contact lens or another piece of hAM. Lamellar or penetrating keratoplasty was performed along with SLET in case of intraoperative corneal perforation or for logistical reasons in cases with significant corneal stromal opacities, if patients were unwilling or unable to undergo two separate staged surgical procedures. Postoperatively, topical antibiotic eye drops were prescribed till removal of the contact lens or complete epithelisation of the surface. Topical steroid eye drops were prescribed in tapering doses over 4–6 weeks.

Outcome measures

The primary outcome measure was clinical success, defined as a completely epithelised, avascular, stable corneal surface. Failure was defined as a recurrence of fibrovascular pannus encroaching on the central cornea, frequent epithelial breakdown or persistent epithelial defects. Kaplan–Meier survival curves were constructed and survival probability calculated using the R software environment for statistical computing (available freely at http://www.r-project.org). A Cox proportional hazards analysis was done to assess association of preoperative characteristics with risk of failure. Focal recurrences of pannus not progressing to the central cornea were not considered as failures and were evaluated separately. Secondary outcome measures included the percentage of eyes achieving a visual acuity of 20/200 or better, percentage of eyes gaining two or more Snellen lines of visual acuity and complications encountered.

RESULTS

The study includes data from eight participating centres across three countries (India, Mexico and USA). A total of 68 eyes of 68 patients underwent autologous SLET for unilateral LSCD. The number of cases from each centre is shown in figure 1. The median follow-up was 12 months, with a range of 6–59 months. Demographic and preoperative clinical data are shown in table 1. A majority of the patients were young men, with only three patients (4.4%) being older than 60 years. Ocular surface chemical and thermal burns (91.1%) were by far the most common cause of LSCD. The median duration from original injury to SLET was 16 months (range 3–540 months). More than two-thirds of eyes (67.6%) had total LSCD in this series. A lamellar or penetrating keratoplasty was performed simultaneously with SLET in five cases (7.35%).

A completely epithelised, avascular corneal surface (clinical success) was achieved in 57 cases (83.8%) (figure 2). The corresponding Kaplan–Meier survival curve is shown in figure 3. At the final follow-up visit, the survival probability remained over

Table 1 Demographic and clinical characteristics (n=71)

	n (% of total)
Age in years: median (range)	22 (3–75)
Male	51 (75)
Aetiology	
Ocular surface burns	62 (91.2)
Microbial keratitis	2 (2.9)
Post-surgery	2 (2.9)
Post-radiotherapy	1 (1.5)
Ocular surface squamous neoplasia	1 (1.5)
Prior ocular surgery	24 (35.3)
Prior amniotic membrane	38 (55.9)
Eyelid abnormalities	15 (22.1)
Symblepharon	34 (50)
Persistent epithelial defect	7 (10.3)
Extent of LSCD (clock hours)	
<6	4 (5.9)
6–8	13 (19.1)
9–11	5 (7.4)
12 (total LSCD)	46 (67.6)

LSCD, limbal stem cell deficiency.

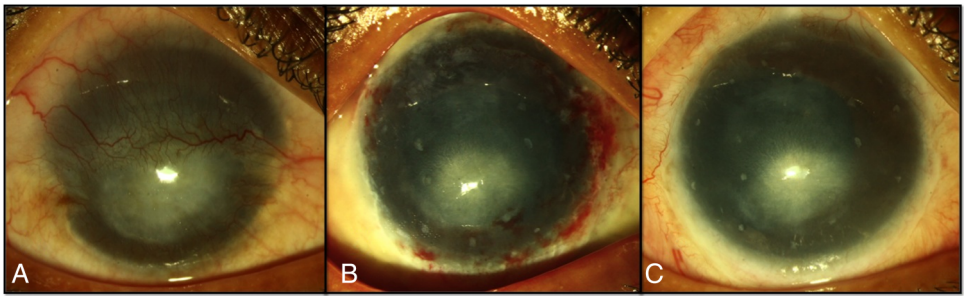

Figure 2 Panel of photographs showing ocular surface stabilisation after simple limbal epithelial transplantation (SLET). (A) An eye with fibrovascular pannus 360° and corneal scar post chemical burns is shown. (B) The same eye is shown, one-day post SLET—limbal transplants are visible as discrete opacities on the surface. (C) The same eye is shown, 1 month post SLET—a completely epithelised, avascular corneal surface with the original corneal scar is seen. Note that limbal transplants are still visible on the surface, but are less distinct.

80%. Apart from one case that failed at 9 months post-operatively, all other failures were recorded within the first 6 months following surgery. In the Cox proportional hazards analysis, presence of symblepharon (HR 5.8) and simultaneous keratoplasty (HR 10.8) were found to be significantly associated with a risk of failure of SLET. The survival curves with and without these risk factors are shown in figures 4 and 5. Difference in surgical technique, in terms of using either a bandage contact lens or a second piece of hAM, to cover the limbal transplants was not found to affect the risk of failure of SLET. Eight cases from one centre were performed using hAM instead of a bandage contact lens. The number is too small to draw meaningful conclusion from a subgroup analysis. However, clinical success was achieved in all eight cases, with no failure reported at last follow-up.

Visual acuity data were available in 67 cases. Preoperatively, 15 eyes (22.4%) had a visual acuity of 20/200 or better. This increased to 44 eyes (65.7%) after SLET (figure 6). Likewise, 44 eyes (65.7%) gained two or more lines of visual acuity. The most common complication was focal recurrences of pannus not progressing to the centre of the cornea in 21 eyes (30.9%) with clinical success. Other complications noted included microbial keratitis that resolved with appropriate antimicrobial therapy in five cases, ocular hypertension secondary to steroid use in one case, a pyogenic granuloma that resolved with topical steroid therapy in one case and focal iatrogenic LSCD at the site of the donor limbus, which remained restricted to within 2 mm of the peripheral cornea in one case (figure 7).

DISCUSSION

This study presents results of autologous SLET for cases of unilateral LSCD with a wet surface, when performed by different surgeons spread across eight centres and three countries. The largest number of cases from a single centre was 12, which still amounts to less than a fifth of all cases. Therefore, the possibility of the results being skewed by disproportionate weightage to any one centre is unlikely. A majority of failures of limbal stem cell transplantation are known to occur within the first 6 months after surgery.[11 12] We, therefore, believe that the follow-up duration reported in this study is adequate to reach conclusion about the efficacy of SLET.

The overall success rate of SLET in this study is 83.8% (95% CIs 72.6% to 92.6%), which remains above 80% at the final follow-up. This is similar to or better than most published results of CLAU or CLET. In their initial report of direct limbal autograft transplantation, Keivyon and Tseng reported 'stable epithelial adhesion' in 20 of 21 cases, arrest or regression of corneal neovascularisation in 15 cases and improvement in visual acuity in 17 cases.[3] Using the same technique,[13] could successfully reconstruct the ocular surface in 15 of 16 eyes, 9 of which achieved a visual acuity of 20/400 or better. Using a

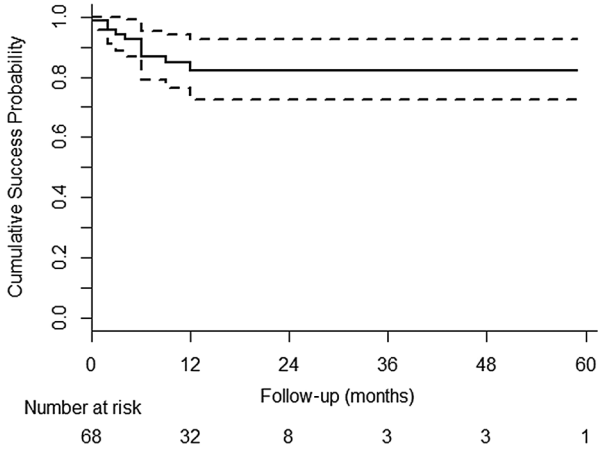

Figure 3 Kaplan–Meier curve showing survival probability after simple limbal epithelial transplantation—most transplantation failures occur within the first few months after surgery, and long-term survival probability exceeds 80%. Dashed lines represent 95% confidence intervals.

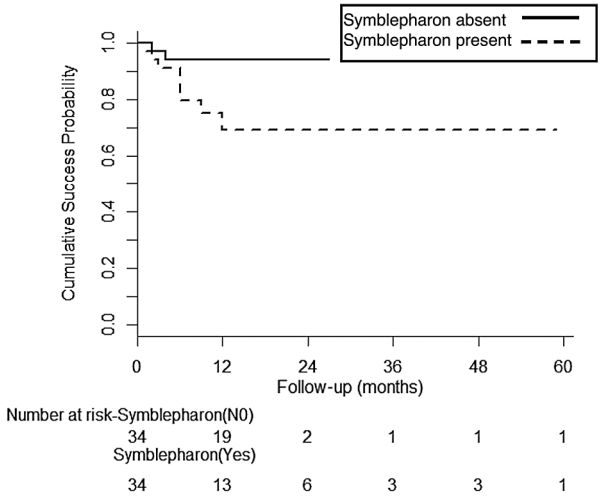

Figure 4 Kaplan–Meier curve showing survival probability after simple limbal epithelial transplantation in eyes with and without symblepharon. Presence of symblepharon reduces the long-term survival probability to less than 70%.

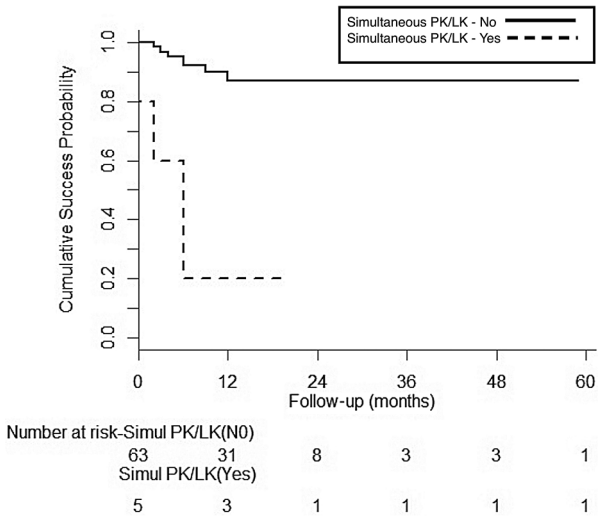

Figure 5 Kaplan–Meier curve showing survival probability after simple limbal epithelial transplantation with and without a simultaneous keratoplasty. Long-term survival probability in cases with a simultaneous keratoplasty is 20% (PK, penetrating keratoplasty; LK, lamellar keratoplasty).

combination of autolimbal and allolimbal sources, Shimazaki *et al*[14] and Miri *et al*[15] reported long-term success rates of 53.1% and 82%, respectively, with direct limbal transplantation. Despite the encouraging success rates with CLAU, the amount of donor limbal tissue required (10–20 mm or up to 6 clock hours) remains a concern. Using in vivo confocal microscopy to assess donor eyes used for harvesting limbal transplants, Miri *et al*[16] found the re-epithelised donor site to be covered by conjunctival epithelium in a large number of cases. By harvesting such a large amount of limbal tissue, there exists a potential for inducing iatrogenic LSCD at the donor site in CLAU. In contrast to this, SLET uses very little donor limbal tissue, minimising the area at risk in the donor eye. As most of the donor limbus is undisturbed, a repeat limbal biopsy can also be safely harvested

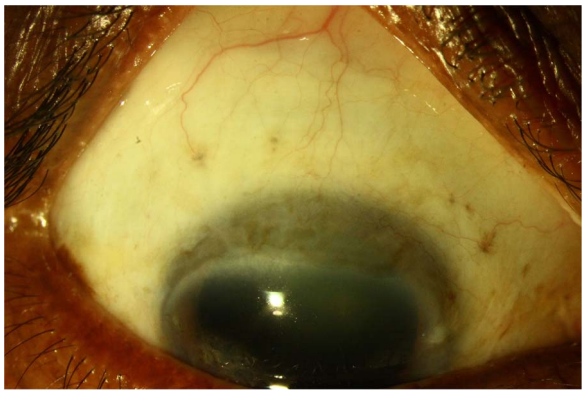

Figure 7 Photograph showing localised fibrovascular pannus formation at the donor site from where limbal biopsy was harvested.

from the donor eye for a repeat SLET procedure, if required.[7 17] This, to us, is the most significant advantage of SLET over CLAU.

Baylis *et al*[18] reviewed multiple studies and pooled 311 cases to derive a success rate of 76% for autologous CLET. More recently, a meta-analysis of 572 eyes found a success rate of 67% for CLET, with no difference between results of autologous and allogeneic transplants.[19] There are a few fundamental differences between SLET and CLET. As the need for ex-vivo expansion of cells is obviated, SLET can be offered virtually anywhere by a trained surgeon at a fraction of the cost of CLET, the availability of which is restricted to very few centres around the world. In SLET, the entire donor limbal tissue is directly transferred on to the recipient surface. This is in contrast to CLET, where ex-vivo culture is used to generate a confluent epithelial sheet that is eventually transplanted. The correlation between the percentage of stem cells in culture and eventual success rate of CLET has been described earlier. The success rate of CLET has been linked to the percentage of p-63-positive stem cells in culture, and cultures with less than 3% of such cells lead to drastically reduced success rates.[11] We hypothesise

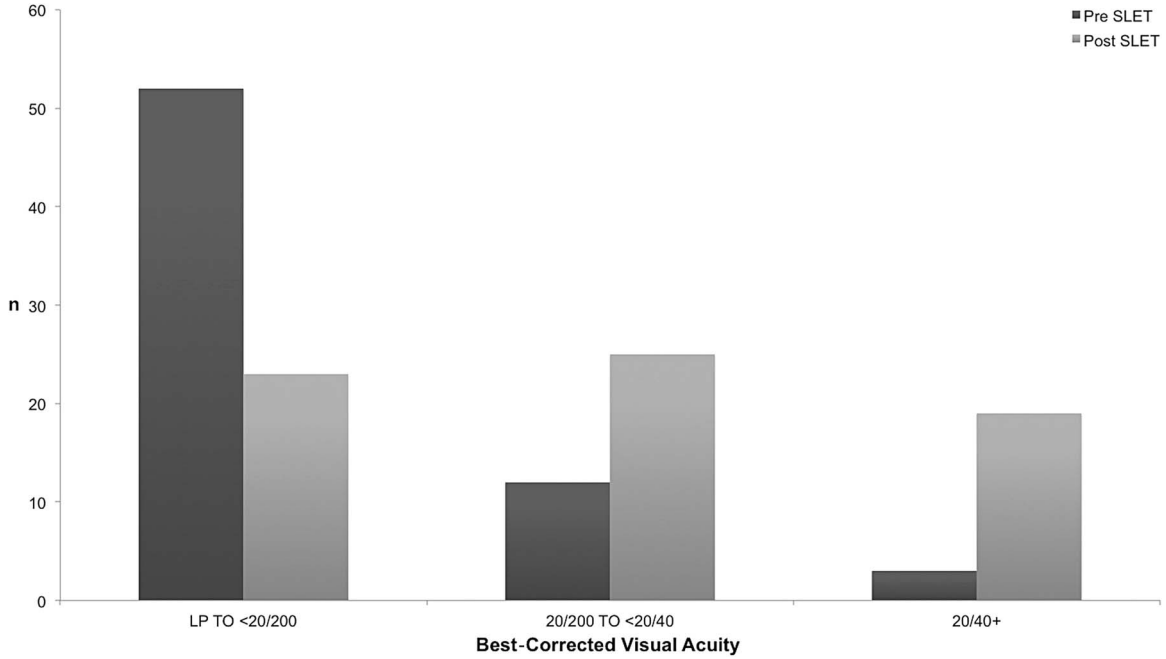

Figure 6 Bar graph showing distribution of best-corrected visual acuity before and after SLET. SLET, simple limbal epithelial transplantation.

that SLET may provide a greater number of stem cells to the stem cell-deficient recipient surface compared with CLET. A reliable method of tracking transplanted limbal stem cells in-vivo on the recipient surface would help test this hypothesis.

The interaction of limbal stem cells with limbal niche cells in different techniques of limbal transplantation is another interesting area of research. The limbal microvasculature and limbal stroma form a unique microenvironment which nurture limbal epithelial stem cells.[20] Anatomical and transcriptional profiling studies have suggested limbal epithelial crypts to be the structures functioning as the limbal stem cell niche.[21 22] In CLET, the stem cells and niche cells interact with each other during ex-vivo culture of donor tissue, possibly directing epithelial cell proliferation and migration.[23] In CLAU, the donor limbal tissue is placed in direct proximity to the recipient limbal niche, whereas in SLET the donor tissue is spread over the cornea. There is a theoretical concern that this could have a bearing on proliferation and migration of epithelial cells from the donor stem cells. Our results suggest that spreading donor limbal tissue over the cornea results in outcomes that are equally good or better than those achieved by placing it near the recipient limbus. Further laboratory and clinical studies would help elucidate the importance, if any, of the site of placement of donor tissue on the recipient surface.

We found presence of symblepharon and simultaneous keratoplasty to be associated with risk of failure of SLET. Simultaneous keratoplasty as a risk factor for failure of CLET has been described earlier.[12] In view of the limited number of failures in this study, these risk factors should be interpreted with caution. Presence of symblepharon and long-standing persistent epithelial defect, as well as the necessity for simultaneous keratoplasty, may indicate a greater severity of the original insult causing LSCD and may explain the higher risk of failure.

Rates of improvement in visual acuity in this study are comparable with those reported for CLET. Baylis *et al*[18] and Zhao *et al*[19] described an improvement of two or more lines of visual acuity in 51% and 67% of eyes, respectively, with CLET. The only complication encountered in this study in significant numbers was focal recurrence of LSCD. Such recurrences may be safely observed in case they are non-progressive and do not threaten the visual axis. In case intervention is required, a repeat SLET using a minimal amount of limbal tissue can be safely performed to specifically address the areas where pannus has recurred.[7 17]

The strengths of this study are the use of uniform definitions and protocols for data collection, as well as the pooling of results from multiple centres. These are the first few cases of SLET performed by each individual surgeon, and the high success rate indicates that the learning curve for SLET is not at all steep. We would like to stress that these were cases with wet surfaces without active inflammation, and surgeons adopting this technique would be well served by choosing cases of non-immune aetiology initially. The number of centres performing SLET is growing worldwide, and this can be attributed to the lack of need for sophisticated laboratory support as well as regulatory obstacles that make CLET an expensive proposition limited to very few centres.

In conclusion, multicentre results indicate that autologous SLET is an effective and safe modality for treatment of unilateral LSCD. Clinical success rates and visual acuity improvement are equal to or better than those reported with CLET. In addition, it is a single-stage procedure, does not require expensive laboratory facilities for ex-vivo cell cultivation and can be easily learnt by cornea surgeons. Considering these aspects, the moniker 'simple' is well deserved, and we believe autologous SLET should be the surgery of choice for treating unilateral LSCD of non-immune aetiology.

Contributors Concept and design of study—JV. Data collection and analysis—JV, MHA, NS, NG, VM, MA, GA, TC, AA-F, AR-M, AN, EOG-H and JC. Manuscript draft and review—JV, NG, GA, AA-F, AR-M, AN, EOG-H and JC. Final approval—JV, MHA, NS, NG, VM, MA, GA, TC, AA-F, AR-M, AN, EOG-H and JC.

Competing interests None declared.

Ethics approval Ethics Committee of the L V Prasad Eye Institute, Visakhapatnam, India.

Provenance and peer review Not commissioned; externally peer reviewed.

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