Original Article – Clinical Science

Nature and incidence of severe limbal stem cell deficiency in Australia and New Zealand

Samantha Bobba MD,[1] Nick Di Girolamo PhD,[2] Richard Mills PhD FRANZCO,[3] Mark Daniell FRANZCO,[4] Elsie Chan FRANZCO,[5] Damien G Harkin PhD,[6,7] Brendan G Cronin FRANZCO,[7] Geoffrey Crawford FRANZCO,[8,9] Charles McGhee DSc FRCS[10] and Stephanie Watson PhD FRANZCO[1,11,12]

1. Prince of Wales Clinical School, University of New South Wales, Australia
2. School of Medical Sciences, University of New South Wales, Australia
3. Flinders University of South Australia, Australia
4. University of Melbourne, Australia
5. Royal Victorian Ear and Eye Hospital, Melbourne, Australia
6. School of Biomedical Sciences, Queensland University of Technology, Brisbane, Australia
7. Queensland Eye Institute, South Brisbane, Australia
8. Centre for Ophthalmology and Visual Science, University of Western Australia
9. Lions Eye Institute, Perth, Australia
10. Department of Ophthalmology, New Zealand National Eye Centre, University of Auckland, New Zealand
11. Save Sight Institute, University of Sydney, Australia
12. Sydney Eye Hospital, Sydney, Australia

This is the author manuscript accepted for publication and has undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/ceo.12813

Correspondence: Dr Samantha Bobba, Prince of Wales Hospital, High Street, Randwick, New South Wales, 2031, Australia
Email: Samantha.bobba@gmail.com

Short running title: Incidence of limbal stem cell deficiency

Received 23 April 2016; accepted 2 August 2016
Conflict of interest: None
Funding sources: NHMRC Career Development Fellowship for Author SW (APP1050524)

ABSTRACT

Background: This study aimed to determine the nature and incidence of severe limbal stem cell deficiency in Australia and New Zealand.

Design: A one-year pilot surveillance study with a one-year follow-up period was conducted in association with the Australian and New Zealand Ophthalmic Surveillance Unit.

Participants: The study included patients reported by practising ophthalmologists on the Surveillance Unit's database.

Methods: Ophthalmologists were provided with a definition of severe limbal stem cell deficiency, contacted on a monthly basis by the Unit and asked to report newly diagnosed cases.

Main Outcome Measures: Severe LSCD was defined as at least 6 clock hours of whorl-like epitheliopathy, an opaque epithelium arising from the limbus, late fluorescein staining of the involved epithelium, and superficial corneal neovascularisation or conjunctivalisation.

Results: On average, 286 report cards were sent by the Surveillance Unit to practising ophthalmologists each month (total 3429 over 12 months) and the Unit received an average of 176 responses per month (total 2111; 62% response rate). During the 1-year study period from April 2013-March 2014, 14 positive cases were reported to the Unit. A range of underlying aetiologies were implicated, with contact lens over-wear and cicatrising conjunctivitis being the most common (n=3).

Conclusions: This surveillance study is the first worldwide to document the incidence of limbal stem cell deficiency, however due to study design limitations, it is likely to have been under-reported. It provides novel data on the demographics, clinical conditions and management of patients with limbal stem cell deficiency as reported by treating ophthalmologists.

Keywords: epidemiology, eye diseases, stem cells

INTRODUCTION

The corneal surface is continuously renewed by epithelial stem cells that reside in the limbus. Depletion of limbal epithelial stem cells (LESCs) or damage to their niche microenvironment can induce a condition known as limbal stem cell deficiency (LSCD). LSCD may present unilaterally or bilaterally, can be partial or total, and has a range of acquired and hereditary aetiologies[1]. The disease is characterised clinically by recurrent epithelial defects and chronic pain, secondary to an inability to renew the corneal epithelium[2]. Other features include conjunctivalisation, which involves corneal invasion by a vascularised pannus of conjunctival tissue leading to corneal opacity and loss of vision in severe cases[3].

The most commonly reported causes of LSCD are chemical and thermal burns (75%), followed by ocular surface inflammatory diseases (7.8%) such as Stevens Johnson syndrome and ocular cicatricial pemphigoid[4, 5, 6]. Other aetiologies include contact lens over-wear[7], chemotherapy, iatrogenic injury, ultraviolet irradiation and microbial infection[2]. Aniridia, a hereditary cause of LSCD due to oculogenic mutations, is typically characterised by iris hypoplasia, though cases with minimal iris change have been reported[8, 9].

Due to its various aetiologies and the lack of a clinical framework for diagnosis, the incidence of LSCD remains elusive. Extrapolating data from the most common causes, Shortt and colleagues estimated an incidence of

approximately 240 cases per annum in the United Kingdom (equivalent to 3.81 x 10^{-6} cases per million, per annum)[10]. Our surveillance study, however, is the first to directly investigate the incidence of LSCD. Determining the incidence and geographical distribution of this heterogenous disease may enable clinicians to establish specialist health networks relative to patient distribution, allowing efficient management strategies. Importantly, the surveillance study offers a comparison between how ophthalmologists diagnose and manage LSCD in practice and what the literature recommends. Furthermore there is potential to establish such a study internationally, allowing for geographical comparisons and analysis.

METHODS

Data collection

Initial and follow-up questionnaires to investigate the incidence of severe LSCD were developed for completion by participating ophthalmologists. Both questionnaires (Appendix I, Appendix II) received input from senior leading ophthalmologists and senior ocular scientists across five states in Australia and New Zealand. Severe LSCD was defined and characterised by; at least 6 clock hours of whorl-like epitheliopathy, an opaque epithelium arising from the limbus, late fluorescein staining of the involved epithelium, and superficial neovascularisation or conjunctivalisation[7, 8, 11, 12, 13]. The initial questionnaire (No. 1) requested patient demographics including; age, sex, and postcode, as well as date of diagnosis, how the diagnosis was made, investigations performed, other ocular disease, the underlying diagnosis, CL wear, previous limbal grafts, medical, drug and surgical history, and any treatment initiated after diagnosis. The second component of Questionnaire No. 1 focused on clinical signs at the most recent examination, and requested information on

best-corrected visual acuity, keratopathy, lids and lashes, conjunctival inflammation, symblepharon formation, tear film and ocular surface changes. Questionnaire No. 2 was developed as a follow-up document with the intention of sending it to ophthalmologists 12 months after the study onset. It included similar details as well as provision for entries on changes to the underlying diagnosis, new investigations performed and changes to treatment (topical and systemic).

Surveillance Study Design

The surveillance study was designed as a 1-year pilot study to determine the incidence of severe LSCD in Australia and New Zealand (population approximately 23 million and 4.5 million respectively), with follow-up data sought 12 months after the study onset. The project was conducted through the Australian and New Zealand Ophthalmic Surveillance Unit (ANZOSU) and commenced in April 2013 with approval from the South Eastern Sydney Local Health District Low/Negligible Risk Committee (SESLHD HREC-13/058). The ANZOSU was associated with the Royal Australian and New Zealand College of Ophthalmologists (RANZCO), and was based on the British Ophthalmic Surveillance Unit. Each month, the ANZOSU aimed to send all practising ophthalmologists in Australia and New Zealand (both private and public) a report card listing conditions being studied by the unit, which included severe LSCD from April 2013. Each month's report card was emailed to ophthalmologists at the end of the following month. Cases of severe LSCD were reported to the ANZOSU who then informed the authors and provided the contact details for the reporting ophthalmologists. Ophthalmologists were assigned a study number for each case and were contacted to complete the initial questionnaire. Questionnaire responses were entered on a proforma in

an electronic database; information was de-identified, with a random study protocol number assigned to each patient.

Newly diagnosed cases of severe LSCD were reported to the ANZOSU over a 21-month period from April 2013 – December 2014. However, the clinical and demographic data were obtained only from cases reported in the defined study period April 2013 – March 2014.

Statistical Analysis

The population incidence per annum was extrapolated from the number of cases reported during the 21-month period April 2013 – December 2014. Data from the defined study period April 2013 – March 2014 were used to calculate mean patient age, and frequencies (percentages) were used to represent other patient demographics, the underlying diagnosis, geographical distribution and diagnostic approach.

RESULTS

ANZOSU Response Rates

The study was conducted during the establishment phase of the Surveillance Unit. During this time, on average (mean), 283 report cards were sent to practising ophthalmologists each month (total 3393 over 12 months) and the Unit received an average of 174 responses per month (total 2089; 62% response rate). By 2015, the ANZOSU had records for 746 ophthalmologists, such that data was included from 38% of practising ophthalmologists within Australia and New Zealand.

In the 21-month period from April 2013 to December 2014, 26 newly diagnosed cases of severe LSCD were reported to the ANZOSU; this extrapolates to an incidence of 15 cases per year. Fourteen cases of severe LSCD were reported during the defined 1-year study period i.e. from April 2013 to March 2014, coinciding with a 100% return rate of both questionnaires. At the 1-year follow-up, 4 of these patients had been lost to follow-up. Of the 7 ophthalmologists who reported positive cases in the defined 1-year study period; 4 were cornea specialists and 3 were not. Of the 12 ophthalmologists in total that reported positive cases in the 21-month ANZOSU reporting period, 7 were cornea specialists and 5 were not. In terms of the number of cases reported, 12 of the 14 cases in the defined 1-year study period and 22 of the 26 cases in the 21-month reporting period were reported by cornea specialists. Ophthalmologists of other sub-specialities or generalists reported the remaining cases.

Patient Demographics

Patients resided in both metropolitan and regional cities, with most cases (*n=7*) reported in New South Wales, followed by Victoria (*n=5*) and Queensland (*n=2*). Although no cases from New Zealand were reported in the defined 1-year study period, 3 of the 26 cases in the 21-month study period were reported in New Zealand. The male to female ratio was 6:7 and the mean age at time of diagnosis was 51 ± 23 (range, 24 – 87) years.

Clinical features of patients with LSCD

In all subjects the diagnosis was made on the basis of clinical examination, however impression cytology was conducted on one patient (case 10), revealing goblet cells in the temporal corneal epithelium. Patients had a range of underlying aetiologies, with the most common being CL over-wear (*n=3*)

and cicatrising conjunctivitis (*n=3).* Patients diagnosed with CL over-wear reported greater than 10 years of CL-wear, for 8-10 hours per day and 6-7 days per week. Co-existing ocular disease included myopia, glaucoma and cataracts. In managing these patients, 71% of clinicians reported initiating a combination of topical lubricants and steroids post-diagnosis as first-line therapy, with 21% prescribing topical lubricants without steroids and 1 clinician reporting no medication history. One patient (case 2) received an autologous tissue graft, performed within 2 weeks of diagnosis. The ocular characteristics, results of clinical examination and management of reported cases are summarised in Tables 1-3.

DISCUSSION

To our knowledge, this surveillance of severe LSCD is the first worldwide to attempt to document its incidence, ocular characteristics and geographical distribution. The most common aetiologies of LSCD reported were CL-over-wear (21%), cicatrising conjunctivitis (21%) and chemical/thermal injury (14%). This is consistent with published reports which suggest chemical/thermal injury, Stevens-Johnson Syndrome, aniridia and ocular cicatricial pemphigoid are the most common causes[2-4]. Contact lens over-wear has also been recognised more recently as a cause of LSCD[7].

The diagnosis of patients in this study was primarily based on clinical examination. Interestingly only one ophthalmologist reported performing impression cytology, which has been considered the gold standard for diagnosing this disease[2, 4]. In this case the typical clinical features of LSCD were present. Overall however, this is consistent with other studies that suggest impression cytology is an unnecessary painful procedure associated

with the risk of epithelial defects without offering a significant diagnostic advantage[11, 12].

In terms of patient demographics, the wide range of ages (24 – 87yr) and similar proportion of males and females in our cohort suggests that LSCD could affect either sex at any age. Based on the distribution of their respective postcodes, subjects resided in both capital cities (33%) and non-capital cities (67%), and were distributed between states in a pattern consistent with the relative population size, for example, with most cases reported in New South Wales (population, 7.5 million) and no cases reported in Western Australia (population, 2.6 million). Whilst this suggests that referring to specialist centres in major cities remains a satisfactory approach, it is also possible that underreporting in non-metropolitan areas may have confounded these findings.

Based on responses received during the 21-month period April 2013 – December 2014, the incidence of severe LSCD was extrapolated to 15 cases per annum. Assuming a non-biased reporting cohort and given the 38% inclusion rate and 62% response rate to the ANZOSU, the incidence of severe LSCD can be further extrapolated from 15 cases per annum to 63 new cases expected in Australia and New Zealand. Notably however, ophthalmologists involved in the study had significantly higher reporting rates, with 46% of cases (12 of 26 cases from April 2013 – December 2014) reported by 2 key investigators, both corneal specialists, suggesting that a degree of reporter bias existed. Nonetheless, there is a tendency for LSCD to be managed in specialist centres.

Given the heterogenous nature of LSCD, its varying aetiologies and degrees of severity, Shortt and colleagues (2011) have been the only researchers to estimate the incidence of LSCD thus far[10]. Based on the relative incidences of its most common aetiologies, they estimated that 10% of these patients would develop LSCD and predicted an incidence of 240 cases in the United Kingdom. Assuming Australia and New Zealand have a similar distribution of LSCD, this equates to approximately 100 new cases per year adjusting for population size. Notably, our surveillance study focused only on severe LSCD, and thus a significantly lower incidence would be expected. This is consistent with an expected incidence of 63 new cases as extrapolated by our data. We limited the study responses to severe LSCD to reduce reporting bias given the heterogenous nature of LSCD and the difficulty diagnosing the condition, particularly in its early stages of mild vascularisation and surface irregularity. Moreover, it is the severe cases that importantly require intensive medical management and/or surgical therapies such as limbal stem cell transplantation (LSCT).

Vegumanti and colleagues (2015) suggested that 8.9 million people in India were blinded by corneal involvement, based on extrapolations from a population-based study conducted ten years earlier[14]. They predicted that 15-20% of these patients would benefit from LSCT, indicating a 1.25% prevalence of severe ocular surface disease. It is difficult to compare these figures with our surveillance study for a number of reasons. Firstly, Vegumanti and colleagues provide an indication of prevalence but not incidence, and the estimation of 15-20% developing ocular surface disease also appears to be based on subjective assessments rather than definitive data. Additionally, both incidence and prevalence of LSCD are likely increased

in India given higher rates of industrial/domestic accidents leading to chemical/thermal ocular injuries.

LSCD is unlikely to be treatable by medical management or conventional corneal transplants due to damage to LESCs and the niche microenvironment[3,15,16]. Interestingly, in the cases reported in our study, 93% of treating ophthalmologists managed patients conservatively with topical lubricants and/or steroids in the first year of treatment. This suggests that in practice, treating ophthalmologists prefer conservative approaches at least in the first year following diagnosis, despite evidence indicating that LSCD is likely to progress or remain stagnant without LSCT [2, 3, 5, 6, 17, 18]. This may also reflect the lack of access to LSCT or LESC therapies[17].

The study's key limitation was the 38% inclusion rate of practising ophthalmologists (246 report cards sent on average each month out of 746 potential) and the 62% response rate to the ANZOSU's monthly emails. In the preliminary years of the study, the ANZOSU was still obtaining accurate contact details for a number of ophthalmologists, explaining the low inclusion rate. Unfortunately, poor response rates are a limitation of many surveillance studies, given their non-compulsory and time-consuming nature. Notably, as a RANZCO-affiliated surveillance unit, the ANZOSU is likely to have had higher response rates than the alternative option of investigators directly contacting ophthalmologists. Another limitation of the study was delays in receiving the questionnaires mailed to ophthalmologists and occasional missing data as evidenced in Table 1. Repeating the study with a more established surveillance unit may yield higher response rates. Whilst the incidence of other eye-related diseases has been successfully documented (Leske et al.,

2001; Klein et al., 1997), this has typically been done via large-scale longitudinal studies, less feasible in the case of LSCD given its rarity.

Despite the challenges inherent in non-compulsory surveillance reporting, this study provides useful preliminary data on the incidence and nature of severe LSCD, and does so via direct reporting of cases. Given the relatively specialised field, recruiting more ophthalmologists that specialise in LSCD as investigators may increase reporting rates in future studies. A longer duration of surveillance could further improve reporting rates as familiarity with the study increases. Utilising electronic surveillance forms rather than paper versions may also increase clinician involvement by reducing the time delay between reporting and data collection. There is scope to also implement a similar study in other countries, allowing for comparative analysis.

Acknowledgements

Australian and New Zealand Ophthalmic Surveillance Unit

REFERENCES

1. Dua H, Miri A, Said D. Contemporary limbal stem cell transplantation – a review. *Clin Exp Ophthalmol* 2010;**38**:104-117.
2. Sejpal K, Bakhtiari P, Deng S. Presentation, diagnosis and management of limbal stem cell deficiency. *Middle East Afr J Ophthalmol* 2013;**20**:5.
3. Shortt A, Tuft S. Ocular surface reconstruction. *Br J Ophthalmol* 2011;7:901-902.
4. Baylis O, Figueiredo F, Henein C, Lako M, Ahmad S. 13 years of cultured limbal epithelial cell therapy: a review of the outcomes. *J Cell Biochem* 2011;**112**:993-1002.
5. Shortt A, Secker G, Munro P, Khaw P, Tuft S, Daniels J. Characterization of the limbal epithelial stem cell niche: novel imaging techniques permit in vivo observation and targeted biopsy of limbal epithelial stem cells. *Stem Cells* 2007;**25**:1402-1409.
6. Shortt A, Secker G, Notara M *et al*. Transplantation of ex vivo cultured limbal epithelial stem cells: a review of techniques and clinical results. *Surv Ophthalmol* 2007;**52**:483.
7. Chan C, Holland EJ: Severe limbal stem cell deficiency from contact lens wear: patient clinical features. *Am J Ophthalmol* 2013;**155**:544-549.
8. Dua HS, Azuara-Blanco A: Limbal stem cells of the corneal epithelium. *Surv Ophthalmol* 2000;**44**:415-425.
9. Skeens HM, Brooks BP, Holland EJ. Congenital aniridia variant: minimally abnormal irides with severe limbal stem cell deficiency. *Ophthalmology* 2011;**118**:1260-4.
10. Shortt A, Tuft S, Daniels J. Corneal stem cells in the eye clinic. *British medical bulletin* 2011;**100**:209-225.

11. Rama P, Matuska S, Paganoni G et al: Limbal stem-cell therapy and long-term corneal regeneration. *N Engl J Med* 2010;**363**:147-55.
12. Rama P, Bonini S, Lambiase A et al. Autologous fibrin- cultured limbal stem cells permanently restore the corneal surface of patients with total limbal stem cell deficiency. *Transplantation* 2001;**72**:1478-1485.
13. Bobba S, Chow S, Di Girolamo N, Watson S: Clinical outcomes of xeno-free expansion and transplantation of autologous ocular surface epithelial stem cells via contact lens delivery: a prospective case series. *Stem Cell Res Therapy* 2015; **6**:1-14.
14. Vemuganti GK, Sangwan VS. Interview: Affordability at the cutting edge: stem cell therapy for ocular surface reconstruction. *Regen Med*. 2010;**5**;337-34.
15. Kenyon K, Tseng S. Limbal autograft transplantation for ocular surface disorders. *Ophthalmology* 1989*;***96**:709.
16. Echevarria T, Di Girolamo N. Tissue-regenerating, vision-restoring corneal epithelial stem cells. *Stem Cell Rev Rep* 2011;**7**:256-268.
17. Harkin DG, Apel AJ, Di Girolamo N et al. Current status and future prospects for cultured limbal tissue transplants in Australia and New Zealand. *Clin Exp Ophthalmol* 2013;**41**:272-81.
18. Pellegrini G, Rama P, Rocco A et al. Concise Review: Hurdles in a Successful Example of Limbal Stem Cell□based Regenerative Medicine. *Stem Cells* 2014;**32**: 26-34.

TABLES

Table 1: Demographics and aetiology of reported cases of severe LSCD in Australia and New Zealand (April 2013 – March 2014)

Case	Geographical region of patient's residence	Sex (Male/ Female)	Age at diagnosis	Method of diagnosis	Duration of symptoms prior to diagnosis	Aetiology	Co-morbid ocular disease
1	Broadmeadows, Melbourne, VIC	F	35	clinical	5 years	cicatrising conjunctivitis, SJS	N/A
2	Townsville, QLD	M	30	clinical	2 weeks	chemical/thermal injury	N/A
3	Melbourne CBD, VIC	M	39	clinical	6 weeks	CL wear	myopia
4	Wandong, VIC	F	78	clinical	6 months	anti-metabolites (MMC), OSSN	OSSN
5	Port Macquarie, NSW	M	27	clinical	<1 week	chemical/thermal injury	N/A
6	Wollongong, NSW	M	79	clinical	4 weeks	severe pseudomonas keratitis	glaucoma, microbial keratitis
7	Rockhampton, QLD	M	86	clinical	>1 year	amiodarone-induced	cataract
8	Parramatta, Sydney, NSW	F	33	clinical	4 months	CL wear, cicatrising conjunctivitis (atopic keratoconjunctivitis)	N/A
9	Brighton East, Melbourne, VIC	F	61	clinical	3 years	†	N/A
10	Launceston, TAS (treating ophthalmologist Victoria-based)	F	57	clinical + impression cytology	1 year	CL wear, (family history of aniridia)	glaucoma, cataract
11	† (treating	F	89	clinical	3 years	glaucoma medication toxicity	glaucoma, ptosis,

	ophthalmologist NSW-based)						blepharitis, cataract
12	Woy Woy, Central Coast, NSW	†	†	clinical	>1 year	cicatrising conjunctivitis (NB. family history of ectodermal dysplasia)	N/A
13	North Ryde, Sydney, NSW	M	43	clinical	†	†	rosacea keratitis
14	Mt Druitt, Sydney, NSW	M	26	clinical	†	ectodermal dysplasia (with family history)	N/A

Legend: †:missing data; CL: contact lens; MMC: mitomycin C; OSSN: ocular surface squamous neoplasia; N/A: not applicable; SJS: Steven-Johnson syndrome

Table 2: Management of reported cases of severe LSCD in Australia and New Zealand (April 2013 – March 2014)

Case	Topical medications prior to diagnosis	Treatment initiated at diagnosis	Changes to management at the 1-year follow-up
1	topical lubricants and steroids, bimatoprost (Lumigan), brimonidine tartrate/timolol (Combigan)	S, L	azithioprine, MMC started and ceased due to compliance issues
2	lubricant eyedrops, topical antibiotics; NB. history of prior autologous graft	S, L#, lubricant gel, MMC, oral vitamin A	nil reported
3	olopatadine (may have precipitated disease)	S#, L#	nil reported
4	MMC (may have precipitated disease)	S#, L#, lubricant gel, lubricant ointment, MMC (2 cycles)	nil reported
5	nil reported	S#, L#, lubricant ointment, oral doxycycline	Amniotic membrane transplantation in the last 12 months
6	brinzolamide/timolol (Azarga), bimatoprost (Lumigan), brimonidine tartrate (Alphagan); NB. history of prior amniotic membrane transplant	L#, lubricant gel, lubricant ointment	FML, acetazolamide, dexamethasone topical for PED, predsol minims; left penetrating keratoplasty 2013
7	nil reported	L#	nil reported
8	nil reported	S#, L#, lubricant gel, acetylcysteine eyedrops, oral doxycycline	nil reported
9	nil reported	S#, L#, interferon 2beta	interferon ceased due to improvement, vitamin A started due to deterioriation
10	nil reported	nil reported	nil reported
11	brinzolamide (Azopt), lataoprost/timolol (Xalacom) (may have precipitated disease) chloramphenicol eyedrops (Chlorsig), FML, Lacri-lube	tafluprost (glaucoma therapy with preservatives ceased), L#	tafluprost ceased due to inadequate IOP control, bimatoprost/timolol started
12	lubricant eyedrops	S#, S (prednisolone 1%), L#, lubricant gel	nil reported

13	FML	S#, L#, oral doxycycline	nil reported
14	nil reported; NB, history of prior deep anterior lamellar keratoplasty	S#, L#, lubricant ointment	nil reported

Legend: †:missing data; CL: contact lens; L: lubricant eyedrops; L#: preservative-free lubricant eyedrops; MMC: mitomycin C; S: topical steroids; S#: preservative-free topical steroids

Table 3: Ocular examination findings in reported cases of severe LSCD in Australia and New Zealand (April 2013 – March 2014)

Case	Affected eye (RE/LE/BE)	BCVA (RE)	BCVA (LE)	Keratopathy (RE)	Keratopathy (LE)	Tear film (RE)	Tear film (LE)	Ocular changes at the 1-year follow-up
1	BE	LP	6/36	total conjunctivalisation	limbitis	reduced BUT	reduced BUT	N/A (no keratinisation reported)
2	LE	6/6	6/9	normal	punctate keratitis, limbitis +/- peripheral scarring(not axis)	reduced BUT	normal	similar ocular surface (partial keratinisation)
3	LE	6/5	6/18	normal	punctate keratitis, limbitis +/- peripheral scarring (not axis)	normal	normal	reduced opacity of ocular surface
4	BE	6/9	6/36	limbitis +/- peripheral scarring (not axis), PEDs	history of PED, limbitis +/- peripheral scarring(not axis)	reduced BUT	reduced BUT	improved ocular surface stability (from partial to no keratinisation reported)
5	BE	HM	LP	limbal ischemia	limbal ischemia - acute signs	dry eye	dry eye	N/A (no keratinisation reported)
6	LE	6/9	LP	normal	punctate microbial keratitis, history of PEDs, limbitis +/- peripheral scarring (not axis)	normal	reduced BUT	N/A (no keratinisation reported)
7	R	6/12	6/9	half conjunctivalisation	normal	reduced BUT	normal	N/A (no keratinisation reported)
8	LE	6/6	6/18	normal	history of PED, limbitis +/-	normal	normal	N/A (no keratinisation

					peripheral scarring (not axis)			reported)
9	BE	6/6	6/18	punctate keratitis	limbitis +/- peripheral scarring(not axis)	reduced BUT	reduced BUT	improved ocular surface stability (from partial to no keratinisation reported)
10	BE	6/60	HM	total conjunctivalisation	total conjunctivalisation	normal	normal	N/A (no keratinisation reported)
11	BE	6/36	6/24	punctate keratitis	punctate keratitis	reduced BUT	reduced BUT	reduced opacity/epitheliopathy of ocular surface
12	BE	6/12	6/45	punctate keratitis, limbitis +/- peripheral scarring (not axis)	punctate keratitis, limbitis +/- peripheral scarring (not axis)	reduced BUT	reduced BUT	N/A (no keratinisation reported)
13	BE	6/12	6/9	punctate keratitis, vascularisation	punctate keratitis, limbitis	reduced BUT	reduced BUT	N/A (no keratinisation reported)
14	LE	HM	6/6	normal	PEDs, history of microbial keratitis	reduced BUT	normal	N/A (no keratinisation reported)

Legend: †:missing data; BE: both; BUT; break-up time; LE: left; PED: punctate epithelial defect; RE: right