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Correlation between the existence of the palisades of Vogt and limbal epithelial thickness in limbal stem cell deficiency

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Abstract

Background—To investigate limbal epithelial thickness (LET) in subjects with limbal stem cell deficiency (LSCD) and to evaluate the correlation between the palisades of Vogt (POV) and LET.

Design—Cross-sectional observational study.

Participants—Twenty-four subjects (39 eyes) with LSCD and 20 normal controls (20 eyes).

Methods—Anterior segment optical coherence tomography (AS-OCT) and laser scanning confocal microscopy (LSCM) were performed to assess each quadrant of the limbus.

Main Outcomes Measures—LET and POV morphology in each quadrant were characterized. The correlation between LET and POV was analyzed.

Results—The average LET in eyes with LSCD were 19.9%, 23.4%, 13.8%, and 13.5% less than normal controls at superior, inferior, nasal and temporal limbus (P=0.008, 0.006, 0.014, and 0.011, respectively). LET within limbal quadrants with POV were similar to those measured in the same quadrants in normal controls, whereas LET in the superior, inferior, nasal, and temporal quadrants without POV were 27.8%, 29.8%, 14.7%, and 15.6% less than the LET in corresponding regions of normal eyes (superior and inferior: P<0.001; nasal and temporal: P=0.005). LET in the nasal and temporal quadrants was significantly less than that in the superior and inferior quadrants, both in normal controls and in LSCD subjects(P<0.001 and P=0.019). Regression analysis showed that LET had a significant correlation with the presence of POV in each quadrant (superior, P=0.002; inferior, P=0.001; nasal, P=0.047; temporal, P=0.030).

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Conflict of interest: None

Conclusions—A significant correlation was found between LET and the presence of POV. Limbal epithelial thinning as observed with AS-OCT is a sign of LSCD.

Keywords

limbal stem cell deficiency; limbal epithelial thickness; palisades of Vogt; anterior segment optical coherence tomography; in vivo confocal microscopy

INTRODUCTION

Healthy limbal stem cells (LSCs), which are located in the transitional zone between the cornea and the bulbar conjunctiva,[1] are essential for maintaining transparency of the corneal epithelium. Many factors, such as chemical and thermal injury, autoimmune diseases (e.g., Stevens-Johnson syndrome), genetic disorders (e.g., aniridia), and contact lens wear can deplete the LSC population and impact the normal function of LSCs. Partial or total limbal stem cell deficiency (LSCD) may develop and, depending on the extent of damage, lead to different degrees of conjunctivalization, which can affect the entire corneal surface or only specific sectors adjacent to the affected limbus.

The diagnosis of LSCD is usually made on the basis of the patient's medical history and clinical presentation. However, inherent limitations are associated with the interpretation of clinical signs.[2] Sectoral or partial LSCD can be easily missed by clinical examination alone because the clinical signs are very subtle. Impression cytology, the standard diagnostic test for LSCD,[3] is time-consuming and could cause trauma to the ocular surface. Therefore, new non-invasive diagnostic tools for LSCD are needed.

In vivo laser scanning confocal microscopy (LSCM), a non-invasive, real-time, three-dimensional technique that provides high-quality images of ocular surface tissues, has proved to be a promising tool in the diagnosis of LSCD.[4–8] LSCM is capable of not only defining the phenotype of the epithelium that covers the corneal surface, but also visualizing the palisades of Vogt (POV), a widely accepted clinical marker to indicate the presence of limbal stem cells (LSCs). It has been confirmed that LSCM has a high degree of concordance with impression cytology in the diagnosis of LSCD.[4, 7] Nevertheless, the examination procedure of LSCM, despite its noninvasiveness, requires physical contact between the LSCM imaging lens and the corneal epithelium or conjunctiva. It is also technically more challenging to perform than impression cytology is.

Anterior segment optical coherence tomography (AS-OCT), a noninvasive and noncontact technique that allows cross-sectional tomographic imaging and high-resolution analysis of ocular anterior segment tissue, enables quantitative and qualitative evaluation of the cornea, limbus, anterior chamber, and angle without the need of ocular anesthesia. With high consistency, AS-OCT can measure the corneal epithelium thickness not only in healthy subjects, but also in subjects with various ocular surface disorders. [9–11] Moreover, AS-OCT has recently been shown to be a promising method in visualizing the structural details of the limbus and POV.[12, 13] However, its application in the diagnosis of LSCD has not been evaluated.

Our previous study revealed the atrophy or the absence of POV and age-related limbal epithelial thinning in the older population.[9, 14] In addition, the limbal epithelium became progressively thinner in subjects with more severe LSCD.[15] These findings suggest that limbal epithelial thickness (LET) is associated with the function of LSCs. Because POV is believed to harbor a high density of LSCs, we investigated the thickness of limbal epithelium in subjects with LSCD and explored the relationship between POV and LET. Based on our findings that are presented here, we further discuss the potential of AS-OCT as a diagnostic tool for LSCD.

METHODS

Subjects

This study received the approval of the Ethics Committee of the Eye & Ear, Nose, Throat Hospital of Fudan University, and followed the tenets of the Declaration of Helsinki. Written informed consent was obtained from all the subjects.

A total of 24 subjects (17 men and 7 women) referred to the Eye & Ear, Nose, Throat Hospital from June 2012 to December 2014 were enrolled in the study. The mean age of enrolled subjects was 44.7 ± 13.0 years (range, 24–75 years). History of injury and surgical treatment, as well as other demographic data were collected. Slit lamp examination was performed, and a photograph of the anterior segment was taken to determine the extent of LSCD. A total of 39 eyes were diagnosed as having total or partial LSCD; detailed demographic data for these subjects were summarized in Table 1. Twenty age-matched and gender-matched healthy subjects were also included, with their right eyes serving as normal controls.

AS-OCT and measurement of LET

To avoid potential artifacts or interference from other ocular examinations, AS-OCT was performed immediately following slit lamp examination. Fourier-domain OCT system (RTVue-100; Optovue Inc., Fremont, CA, USA) with a cornea anterior module long adapter lens (1.96-mm scan depth and 8-mm scan width) was used. Cross-line scan mode was selected to obtain images of the limbal area in each quadrant (superior, inferior, nasal, and temporal). Each subject was asked to fixate at a peripheral target to maintain the perpendicularity of the OCT beam at the surface of the targeted tissue; this perpendicularity was essential for obtaining accurate thickness values. All tests were performed by one trained operator.

The measurement of LET was performed by an experienced ophthalmologist who was masked to the subjects' demographic features. Before the measurement of LET, a corneoscleral transitional zone was first determined, which was usually 1.0-mm wide and extended centripetally from the scleral spur according to the anatomical definition (Figure 1A). If the anatomical definition could not be identified in eyes with severe LSCD because of increased reflectivity of overlying fibrovascular tissue, the limbal area was defined as the 1-mm-wide zone with the proximal edge 5 mm away from the cornea center (Figure 1B). LET in this area was measured with software (Image-Pro Plus; Media Cybernetics, Inc.,

Rockville, MD, USA) as we previously described. [9] For each image of the limbal area, LET was calculated as the average of three independent measurements.

In vivo LSCM and the classification of POV

The Heidelberg retina tomograph (HRTII)/Rostock cornea module (RCM) (Heidelberg Engineering GmbH, Dossenheim, Germany) with a 60× water-immersion objective lens (Olympus Europa GmbH, Hamburg, Germany) was used for in vivo LSCM. Before the examination, one drop of 0.4% oxybuprocaine hydrochloride (Benoxil; Santen Pharmaceutical, Japan) was applied to the lower conjunctival sac. Images of the central cornea of the enrolled eye were obtained first. Subjects were then instructed to look downward, upward, to the left side, and to the right side to permit examination of the superior, inferior, nasal, and temporal limbus, respectively. More than 100 Z-scan images of each eye were taken at every examination point. Images of limbal area were examined by one experienced ophthalmologist. Images of POV were classified as typical, atypical, or absent. The typical POV was characterized as a wavy epithelium–stromal boundary or alternating epithelium–stromal cords, with a bright fringe of hyperreflective basal epithelial cells and a slender blood vessel along each stromal papilla (Figure 2A). The atypical POV was defined as hyperreflective stromal cords without a bright fringe of hyperreflective basal epithelial cells, which appeared to be double contoured or parallel lines (Figure 2B). If neither alternating epithelium–stromal cords nor hyperreflective stromal cords were found in a quadrant, the region was classified as having no POV (Figure 2C and 2D). The examiner was masked to the subjects' diagnosis and LET measurements.

Statistical analysis

Data were analyzed by the SPSS program statistical package V13.0 (SPSS Inc, Chicago, IL, USA). Basic descriptive statistics were calculated for all data, and values were reported as the mean ± standard deviation (SD). Depending on the data distribution, comparisons between groups were conducted with Student's *t* test, Mann-Whitney U test, or ANOVA. The incidence of POV presence among different quadrants was compared with the Chi-square test. Logistic regression analysis was performed to explore the relationship between LET and the presence of POV. A P value less than 0.05 indicated statistical significance.

RESULTS

The etiology of LSCD included chemical injury (19 eyes), congenital aniridia (10 eyes), thermal injury (5 eyes), vernal keratoconjunctivitis (4 eyes), and multiple surgery and drug toxicity (1 eye). Partial LSCD was identified in 16 eyes, and the other 23 eyes were diagnosed as total LSCD. The detailed information of LSCD in each subject is presented in Table 1.

LSCM examination of subject with LSCD showed that typical POV were only identified in the superior quadrant in 5 eyes (12.8%), the inferior quadrant in 1 eye (2.5%), the nasal quadrant in 1 eye (2.5%), and the temporal quadrant in 2 eyes (5.1%). Atypical POV were found in the superior quadrant in 7 eyes (18.0%), the inferior quadrant in 8 eyes (20.6%), the nasal quadrant in 2 eyes (5.1%), and the temporal quadrant in 4 eyes (10.3%). Incidence of

POV detection was significantly higher in the superior or inferior limbus than in the nasal or temporal quadrants (X^2=7.794, P=0.049).

The epithelium of cornea and conjunctiva could be identified in images taken by AS-OCT (Figure 3). Epithelium and its underlying substantia propria were distinguished by the difference in reflectivity. The gradual transition from the more transparent corneal epithelium to the more optically scattering conjunctival epithelium was seen at the limbus of normal subjects (Figure 3A). In subjects with LSCD, similar images were found at the quadrants with POV present, although the interface between epithelium and underlying substantia propria was not as distinct as that seen in normal eyes (Figure 3B). In contrast, conjunctival epithelium was visible in quadrants without POV, with the absence of the transition to corneal epithelium (Figure 3C).

The average LET in eyes with LSCD were 55.9±11.6 μm in the superior limbus, 54.8±13.7 μm in the inferior limbus, 49.2±7.3 μm in the nasal limbus, and 51.1±9.4 μm in the temporal limbus. They were significantly less than those measured in normal controls (Table 2). Mean LET in subjects with LSCD but without POV were significantly less than those of LSCD subjects with POV at the superior and inferior limbus (superior: P=0.009, inferior: P=0.013). LET in quadrants with POV were similar to those of normal controls, whereas those in quadrants without POV were all significantly less than those of normal eyes (superior and inferior: both P<0.001, nasal and temporal: both P=0.005).

Comparison of mean LET in different quadrants showed that limbal epithelium in the nasal and temporal quadrants were significantly less than those at the superior and inferior quadrants, both in normal controls and in subjects with LSCD (P<0.001 and P=0.019, respectively). Further analysis revealed that the difference in LET in different quadrants persisted in eyes with POV, but was not apparent in eyes with LSCD but without POV (Table 2).

Logistic regression analysis showed that LET had a significant correlation with the presence of POV in each quadrant (superior: P=0.002, inferior: P=0.001, nasal: P=0.047, temporal: P=0.030).

DISCUSSION

With the improvement of AS-OCT resolution, studies of LET in normal subjects have become feasible in the recent years.[9, 15–17] However, the alterations of LET under pathological conditions, such as LSCD, has been recently addressed using LSCM, but not with AS-OCT.[18]

The present study found that in subjects with LSCD, mean LET values in eyes or quadrants with POV were similar to those in normal controls. However, LET was significantly less in eyes or quadrants without POV than in those with POV or in the normal controls. POV, which projects upward from the stroma deep into the epithelium and has an increasing area of interface between the limbal epithelium and stroma, is believed to be one site of the LSC niche.[19–22] A healthy niche environment is necessary for the survival of LSCs and is essential in maintaining their “stemness.” The destruction of the POV has been shown to

correlate with LSCD.[4] LSCs play a critical role in homeostasis and normal wound healing of corneal epithelium.[23] Atrophy or disappearance of the POV could indicate damage to the niche microenvironment, loss of protection for LSCs from trauma and environmental insults, and loss of the normal epithelial-mesenchymal interactions in this area, all of which threaten LSC survival. Therefore, the decreased epithelium thickness in the limbus without POV may be an indicator of a reduced LSC population or a lack of functional LSCs in this area. Epithelial thinning may represent the impairment of LSC function. This hypothesis is consistent with previous observations that LET decreases in eyes with LSCD and the degree of reduction positively correlates with the severity of the clinical presentation.[18]

Replacement of corneal epithelium by conjunctival epithelium is a hallmark of LSCD. Corneal epithelium consists of 5 or 6 layers of stratified squamous epithelium; in contrast, bulbar conjunctival epithelium is a stratified epithelium made up of 2 or 3 cell layers. [24] The thickness of bulbar conjunctival epithelium is reported to be between 42.0 to 44.9 μm (measured by AS-OCT), [15, 17, 25, 26] which is significantly thinner than the central corneal epithelium and limbal epithelium. The replacement of corneal epithelium by conjunctival epithelium at the limbal area may also explain reduced LET in subjects with LSCD.

A previous histopathological study found significant regional heterogeneity in the limbus, with a larger number of stem cells in the superior and inferior limbus than in the nasal and temporal areas.[27] A recent study indicated that the superior and inferior limbal regions have the highest density of limbal crypts, where p63α+ cells were detected.[28] LSCM findings also confirmed that the POV was more likely to be identified in superior and inferior quadrants in normal subjects.[14, 29] Accordingly, limbal epithelium in the superior and inferior quadrants was thicker than that in the nasal and temporal quadrants of normal subjects.[9] The current study found that in eyes with sectoral LSCD, POV was predominantly seen in the superior and inferior limbus. Compared with the superior and inferior limbus, the nasal and temporal limbus has less protection by eyelids and is more susceptible to extrinsic environmental and traumatic factors, such as ultraviolet radiation and chemical agents. Therefore, superior and inferior limbus have protective advantageous for LSCs. This possibility may explain the disparities in LET among different quadrants.

There were at least three limitations of the current study. First, in subjects with severe LSCD, the limbal area was covered by light-scattering tissue (e.g., fibrovascular tissue). The short wavelength light of Fourier-domain OCT could not penetrate this thick tissue and could fail to obtain an image of the underlying sclera spur and other structures. Because of individual differences in anatomy, some bias might exist in the interpretation of the exact location of the limbus on the sole basis of the distance from the corneal center. Second, the impact of tear film cannot be completely eliminated because the OCT system was unable to discriminate tear film. In this study, subjects with LSCD caused by chemical burn or thermal injury had severe tear abnormalities. Other subjects did not have or had only mild tear abnormalities, such as those associated with aniridia. The differences in tear film thickness among subjects with LSCD might have led to bias in the measurement of LET to some extent. These two limitations may be resolved by future imaging technologies with a longer wavelength light source and higher resolution. Third, our study was unable to evaluate possible correlations between the POV and limbal crypts and LSCD severity. The clinical

stages of LSCD could be divided into early, intermediate, and late stages. Microstructural changes in the limbal area have a significant correlation with the clinical stage.[8] Further investigation using high-resolution imaging techniques is needed to evaluate the POV and limbal crypts in correlation to LSCD severity. The relationship between POV and LET should also be validated in subjects with successful restoration of LSCs by keratolimbal allograft transplantation, which has been shown to be accompanied by the reconstruction of POV. [5]

In conclusion, we found a significant correlation between the thickness of limbal epithelium and the presence of POV. Limbal epithelial thinning as observed with AS-OCT is a sign of LSCD. The non-contact imaging technique of AS-OCT could be used to correlate LET with LSC density and function.

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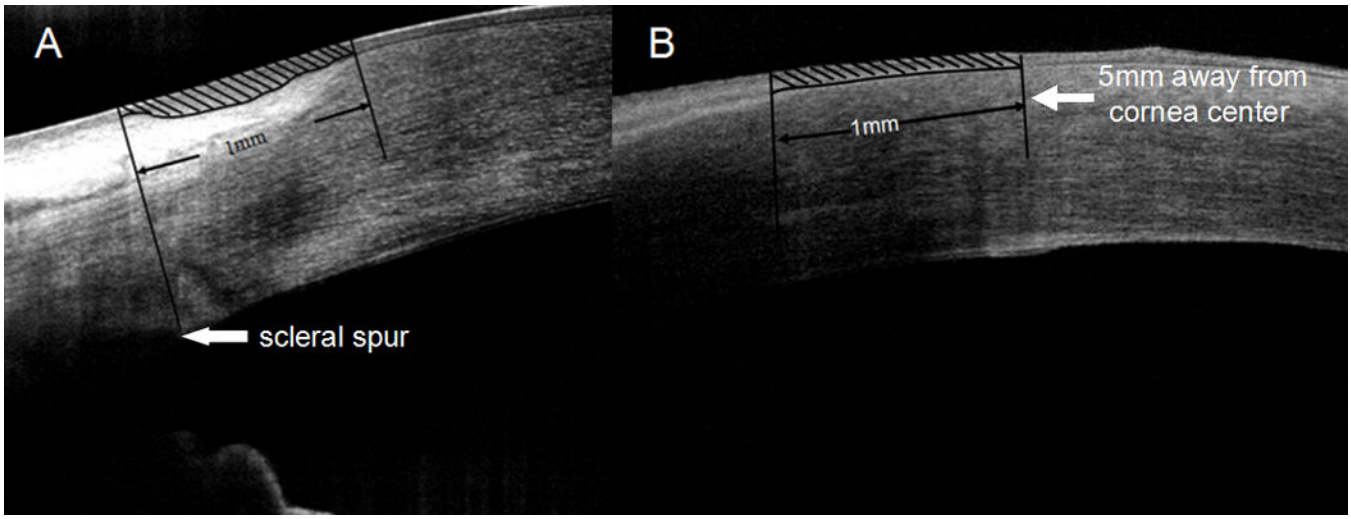

Figure 1.

The definition of limbal area in normal and subjects with LSCD in an AS-OCT cross-line image. A: In subjects whose scleral spur could be identified, a 1.0-mm-wide area extending centripetally from the scleral spur was defined as the limbus. The shaded area indicates the target limbal epithelium. B: In subjects whose scleral spur could not be identified because of the increased reflectivity of overlying fibrovascular tissue, the anterior margin of the limbus was defined as the boundary 5 mm away from the cornea center. The limbal area was then defined as the 1-mm-wide zone posterior to the anterior margin. The target limbal epithelium is also shown as the shaded area. The mean LET was the average distance between the anterior and posterior epithelial surface.

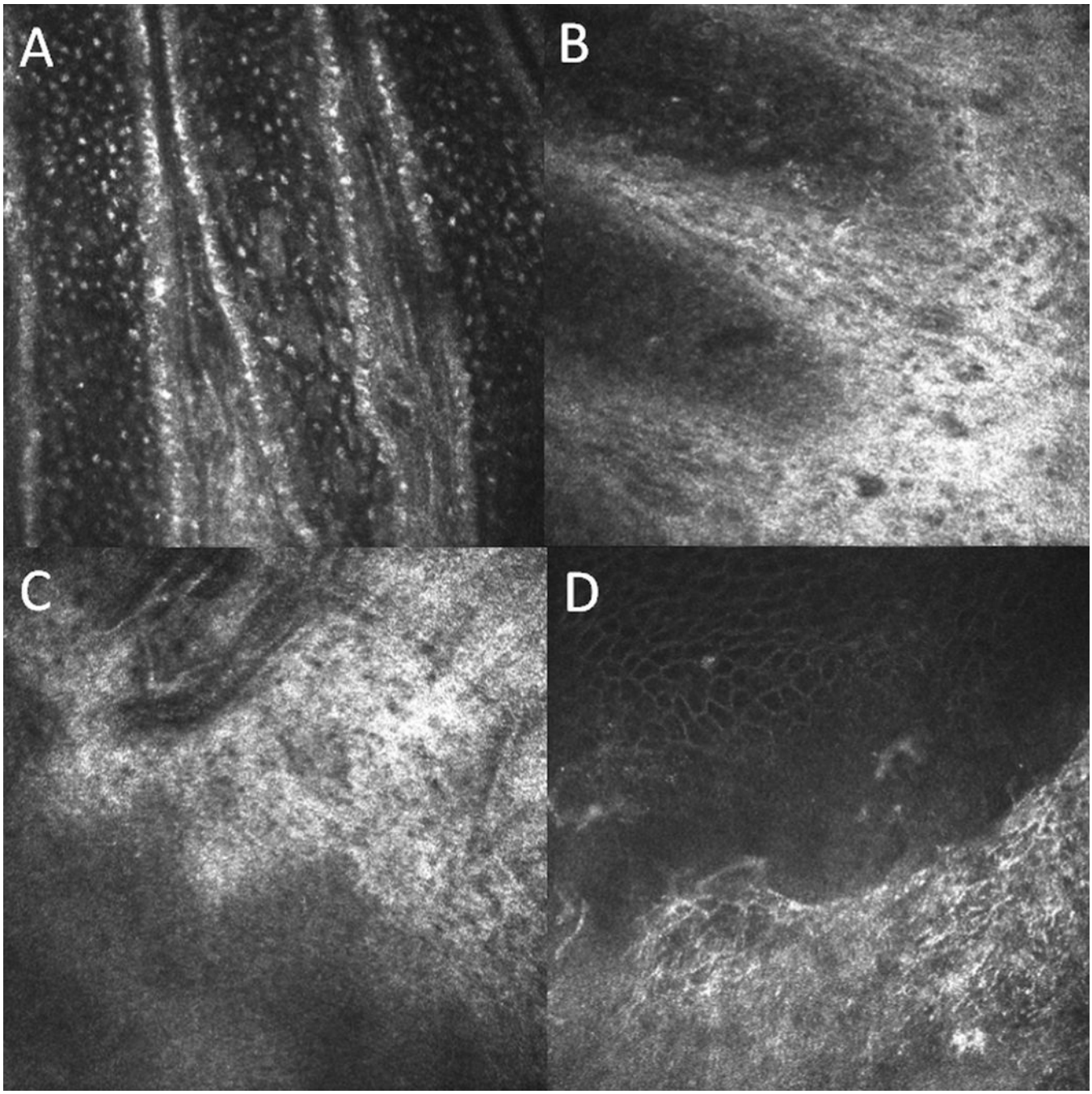

Figure 2.

Classification of POV by LSCM. A: A wavy epithelium–stromal boundary or alternating epithelium–stromal cords with a bright fringe of hyperreflective basal epithelial cells were defined as typical POV, along with a slender blood vessel along each stromal papilla. B: Hyperreflective stromal cords without a bright fringe of hyperreflective basal epithelial cells were classified as atypical POV, which appeared to be double contoured or parallel lines. No long epithelial-stromal cords were present. C: The absence of POV refers to the absence of

alternating epithelium–stromal cords or hyperreflective stromal cords. D: Influx of dendritic cells was observed at the limbus without the POV.

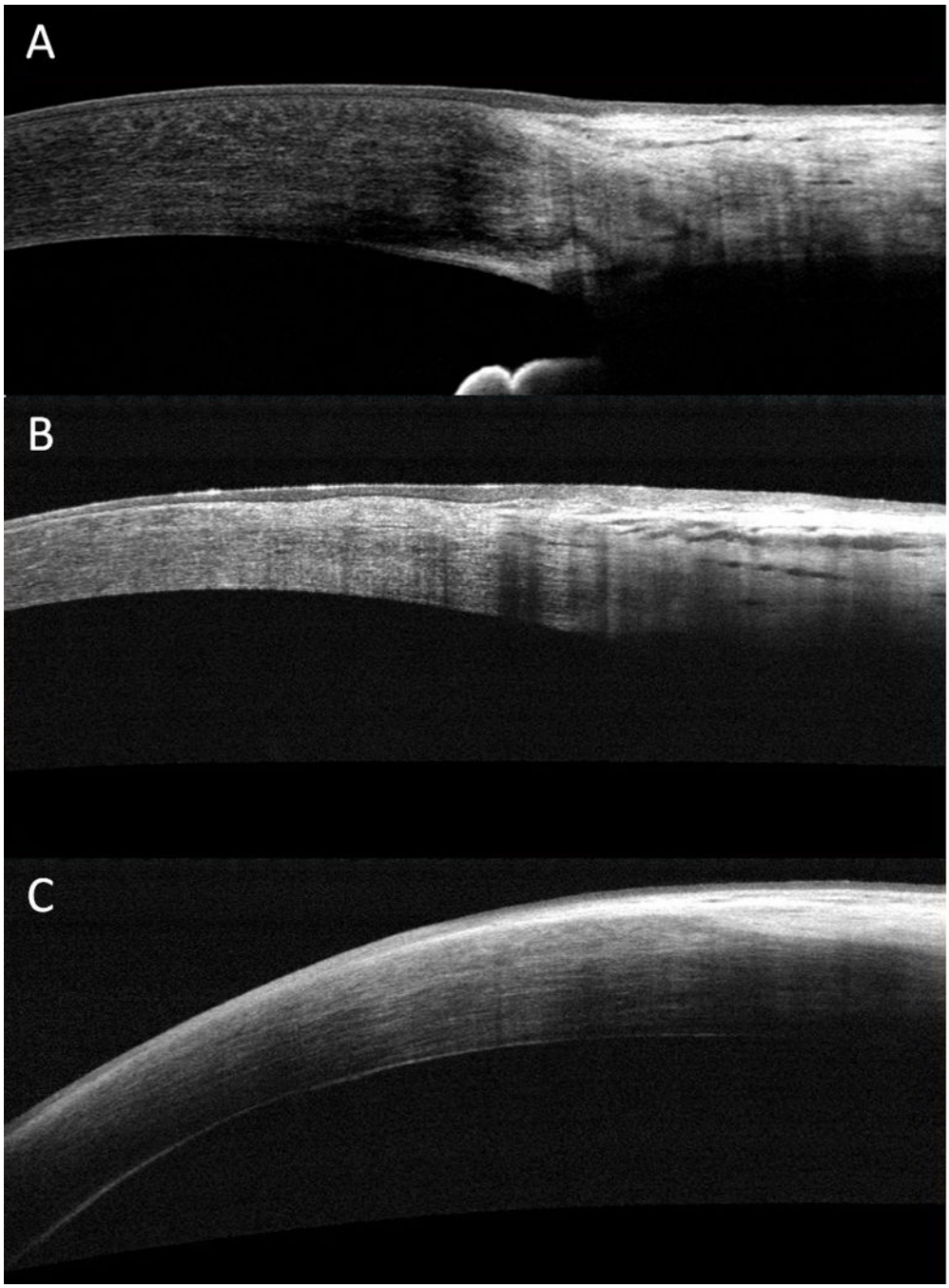

Figure 3.

The images of limbal area taken by AS-OCT. A: In normal eyes, the gradual transition from more transparent corneal epithelium to more optically scattering conjunctival epithelium was identified. B: In subjects with LSCD, similar images were seen in quadrants with POV present, although the border between the epithelium and underlying substantia propria was not as distinct as that seen in normal eyes. C: Only hyperreflective conjunctival epithelium

was visible at quadrants without POV and was not accompanied by the transition to corneal epithelium. Hyperreflectivity beneath the epithelium was found.

Table 1

Demographic data of study subjects

No.	Gender	Age	Diagnosis	Eye	History of the diagnosis	History of surgery	Total/partial LSCD
1	female	58	congenital aniridia	OU	from birth	OU phaco+IOL implantation	total
2	male	43	acid chemical burn	OD	1Y4M	OD AMT	total
3	male	53	congenital aniridia	OU	from birth	OD phaco+IOL, OD anti-glaucoma surgery	total
4	male	51	acid chemical burn	OD	1Y	-	inferior, temporal & nasal
5	male	30	alkaline chemical burn	OD	1Y	OD AMT	superior, inferior & nasal
6	male	50	acid chemical burn	OU	9M	-	OD:superior, inferior & nasal OS:total
7	male	60	acid chemical burn	OU	9M	OU AMT	OD:total OS:temporal & nasal
8	male	36	alkaline chemical burn	OU	2Y	OU AMT	total
9	male	27	congenital aniridia	OU	from birth	-	OU:temporal & nasal
10	female	54	congenital aniridia	OU	from birth	-	total
11	female	29	acid chemical burn	OD	1Y	OD AMT	superior & nasal
12	female	42	alkaline chemical burn	OU	40Y	-	total
13	female	75	multiple surgery & drug toxic keratopathy	OS	7Y	OS phaco+IOL, OS anti-glaucoma surgery	total
14	male	67	thermal injury	OU	9M	-	OD:inferior & nasal OS:inferior, temporal & nasal
15	male	36	alkaline chemical burn	OU	1Y	-	OD:inferior, temporal & nasal OS:superior & nasal
16	male	50	acid chemical burn	OU	1Y6M	-	OD:inferior, temporal & nasal OS:total
17	female	43	thermal injury	OD	1Y6M	-	inferior, temporal & nasal

No.	Gender	Age	Diagnosis	Eye	History of the diagnosis	History of surgery	Total/partial LSCD
18	male	48	acid chemical burn	OU	1Y	-	OD:total OS: nasal
19	female	44	alkaline chemical burn	OS	1Y6M	-	inferior, temporal & nasal
20	male	52	congenital aniridia	OU	from birth	OU phaco+IOL implantation	total
21	male	24	vernal keratoconjunc-tivitis(mix form)	OU	15Y	-	total
22	male	29	vernal keratoconjunc-tivitis(limbus form)	OU	17Y	-	OD: total OS: superior, temporal & nasal
23	male	28	thermal injury	OD	2Y	OD AMT	total
24	male	44	thermal injury	OD	1Y	-	total

Y: year, M: month, AMT: amniotic membrane transplantation, phaco: phacoemulsification, IOL: intraocular lens

Table 2

Average thicknesses of limbal epithelium in four quadrants

	superior	inferior	nasal	temporal	F	P
LSCD	55.9±11.6	54.8±13.7	49.2±7.3	51.1±9.4	3.704	0.019*
normal controls	69.8±16.7	71.5±15.6	57.1±14.3	59.1±9.7	15.229	<0.001≠
t value	7.994	9.158	5.916	6.624		
P value	0.008	0.006	0.01	0.01		
with POV	68.12±4.5	70.1±8.3	55.9±3.4	57.9±3.2	13.381	<0.001≠
without POV	50.4±9.6	50.2±11.6	48.7±7.3	49.9±9.8	0.221	0.881
t value	7.741	6.082	2.588	2.902		
P value	0.009	0.01	0.09	0.06		

LSCD: limbal stem cell deficiency; POV: palisades of Vogt

*P<0.05,

†P<0.01,

≠P<0.001