In Vivo Confocal Microscopic Evaluation of Previously Neglected Oval Cells in Corneal Nerve Vortex: An Inflammatory Indicator of Dry Eye Disease

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Research Article

Keywords: Dry eye disease (DED), Corneal vortex, Langerhans cells, In vivo confocal microscopy (IVCM), Artificial intelligence (AI)

Posted Date: December 29th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-1166279/v1>

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Abstract

To investigate association of a type of previously neglected oval cell that located in corneal vortex with dry eye disease (DED). Observational, prospective study of 168 patients with different degrees of DED. In vivo confocal microscopy was used to observe the corneal sub-basal nerves and Langerhans cells (LCs) in both corneal vortex and periphery. The bright and oval cells also be inspected in corneal vortex. An artificial intelligence (AI) technique generated the sub-basal nerve fibre parameters. Patients were divided into three different groups based on existence of inflammatory cells. Group 2 patients showed a significant increase in corneal peripheral nerve maximum length and corneal peripheral nerve average density. Patients in Group 3 had more LC numbers than others. A type of bright and oval cell was identified in the corneal vortex might be a type of immature LC and related to disease severity of DED.

Introduction

Dry eye disease (DED) is a worldwide epidemic disease, with a prevalence of 5–35% in different age groups.¹ Dry eye is a multifactorial origin, which is characterized by the loss of tear film homeostasis. The accompanying ocular symptom are various. Tear film instability and hyperosmolarity, ocular surface inflammation and damage, and abnormal neurosensory are the causes.² Investigation has traditionally required Ocular Surface Disease Index (OSDI), tear breakup time (TBUT), corneal fluorescein staining, Schirmer test and tear osmolality. These series are usually used to assess severity. The direct information of inflammatory activity provided by these methods is very poor. Inflammation is a recognized component of the pathophysiological mechanism of DED² and has been proposed to offer a stable indicator of DED severity.³ In vivo confocal microscopy (IVCM) is a new non-invasive technique for displaying the microscopic morphology of cornea at the cellular level, such as corneal epithelium, corneal nerve and inflammatory cells⁴, still achieving satisfactory resolution and allowing timely repeatable inspection.⁵ Sub-basal corneal nerve plexus are the most densely distributed corneal nerves, which is located between the basal layer and Bowman's membrane and are the most studied structure.⁶ The effects of dry eye in corneal sub-basal nerve plexus have been investigated controversially in diverse studies.^{7–9} Several explanations may account for these differences, such as severity of illness, classification of disease¹⁰, or examining location of cornea. Previous studies usually measured central cornea by IVCM, but due to less observed range and on positioning function. These result in each measurement was conducted in different position, increased variability, poor reproducibility, and decreased comparability. There for effective and reasonable examination location is needed. Corneal vortex is a special structure recognized in recent years, which is common in people.¹¹ Studies have shown radiating pattern of nerve fiber bundles converging toward an area approximately 1 to 2 mm inferior or nasal to the corneal apex in a whorl-like pattern.¹² Additionally, it is easy to recognize and has relatively fixed site compared with corneal center.¹³ Therefore the corneal nerves were divided in two parts, including corneal vortex and corneal periphery. Previous studies have shown that dry eye is a chronic CD4 (+) T cell-mediated autoimmune inflammatory disease on the ocular surface. Langerhans cells (LCS), as the first-line sentinel, play a role in the process of ocular surface inflammation.¹⁴ Lin et al¹⁵ found that central corneal LC density may serve as indicator of dry eye severity. Recently, a type of bright, oval cell was found at the vortex of the subepithelial nerve plexus in IVCM images that distributed in corneal vortex. Previous studies have shown this type of cell, but no further studies are indicated.^{11,16} It was considered as immature LCs and severity of DED is associated with them. To test this hypothesis, 169 patients were recruited and analyzed the effect of these cells on DED.

Materials And Methods

Subjects. This study was approved by the Ethics Committee of the Peking University Third Hospital and was performed in accordance with the principles of the Declaration of Helsinki (#M2019236). We obtained written informed consent from all patients before inspection.

Our prospective study included 168 patients (109 women and 59 men; average age, 69.00 ± 9.10 years; range, 60-89) with different degrees of DED that was diagnosed based on the 2017 Report of the Tear Film & Ocular Surface Society International Dry Eye Workshop (TFOS DEWS II).¹⁷ Patients with symptoms of dryness, foreign body sensation, burning, fatigue or blurred vision, and TBUT <10 s were recruited. The exclusion criteria were: any ocular surface and corneal abnormalities; history of contact lens wear within 1-month; recent eye surgery; nasolacrimal duct obstruction; glaucoma; ocular fundus diseases; diabetes and immunologic systemic disease. Right eye was routinely enrolled. If right eye did not meet inclusion criteria, the other eye was included.

Dry eye examinations. We conducted the clinical assessments of the enrolled patients in the following order: collection of demographic information, OSDI, Oculus, and IVCN. The OSDI questionnaire was used to describe subjective eye-related discomfort.¹⁸ The central tear meniscus height (TMH) of the lower eyelid, TBUT and the meibomian gland (MG) morphology of both the lower and upper eyelids were recorded by a Keratograph 5M (OCULUS, Wetzlar, Germany). The examination was performed by the same experimented technologist and the outcome of TMH, and MG morphology were further analyzed by the same ophthalmologist.

In Vivo Confocal Microscopy. In vivo confocal microscopy images were obtained by the IVCN (HRT II RCM Heidelberg Engineering Inc., Heidelberg, Germany, Rostock Cornea Module) had a definition of 384×384 pixels over an area of $400 \mu\text{m} \times 400 \mu\text{m}$, with lateral spatial resolution of $0.5 \mu\text{m}$ and a depth resolution of $1-2 \mu\text{m}$.¹⁹ In vivo confocal microscopy was performed on each eye in two different areas: corneal vortex and superior peripheral cornea. Periphery was determined as an outside one-sixth part of the cornea.²⁰ Approximately 30 pictures were captured, from the corneal epithelium to the endothelium, and images with good quality were selected for analysis.

Corneal Image Analysis. We selected each of 5 the high-quality images in corneal centre and periphery with no overlapping for analysis of the corneal nerve parameters by the CNS-Net.²¹ A mathematical model can repeatedly perform nerve tracing and evaluation, thus achieving relatively stable and consistent results.²¹ The corneal nerve parameters were obtained: the maximum length of the corneal vortical nerve, and average density of the corneal vortical nerve, the maximum length of the corneal peripheral nerve, and average density of the corneal peripheral nerve. Previously, bright irregular particles or undefined dendritic structures which appeared scattered among nerve fibres were LCs.²² But, quite apart from that, the bright, oval cells were included as LCs. We counted LCs of selected images and calculated their average for determining the LC number. Moreover, we counted five another selected images with more LCs and calculated their average for determining the average LC number of total cornea nerve.

And patients were divided into three groups based on no LCs (Group 1), both have LCs and bright, oval cells (Group 2), have LCs but no bright, oval cells (Group 3).

Statistical Analysis. All analyses were performed using SPSS version 23.0 software. We verified the normality of data distribution by the Kolmogorov-Smirnov test. Descriptive parameters were expressed as the number of patients (%) or mean $\pm$ standard deviation (SD)/median with interquartile range, depending on the distribution pattern.

Continuous variables were compared with ANOVA or Kruskal-Wallis tests among three groups. Continuous data among three groups were compared using independent t-tests or Mann-Whitney nonparametric U-tests. Categorical variables were compared with the Chi Square test. Differences in LC numbers, corneal nerve average density and corneal nerve maximum length between the vortex and the periphery of the cornea were analyzed using the paired Student's t-test. Correlation analyses between clinical variables were carried out with Spearman's index of linear correlation. P value <0.05 was considered significant for all comparisons.

Results

Patients Demographic and Ocular Characteristics. The mean OSDI score of all DED patients of this study was 34.87 ± 21.80 . Demographic and ocular characteristics of three groups are summarized in Table 1.

Table 1
Demographics and ocular characteristics of the subjects in three groups

Item	Group 1 (n=30)	Group 2 (n=78)	Group 3 (n=60)	p	Total
Sex					
Male (n)	8	23	28		59
Female (n)	22	55	32	0.06	109
Age (years)	66.90 ± 8.53	69.30 ± 9.00	69.67 ± 9.45	0.39	69.00 ± 9.10
OSDI score	33.80 ± 27.71	35.81 ± 22.09	33.69 ± 19.79	0.75	34.87 ± 21.80
TMH (mm)	0.18 ± 0.02	0.20 ± 0.07	0.18 ± 0.07	0.31	0.19 ± 0.06
TBUT (s)	4.59 ± 1.53	4.60 ± 2.03	4.69 ± 2.41	0.66	4.63 ± 2.09
MG score	3 (2,3)	3 (2,3)	3 (2,4)	0.22	3 (2,3)
OSDI: Ocular Surface Disease Index; TMH: tear meniscus height; TBUT: tear film break-up time. P values of less than 0.05 were considered statistically significant					

IVCM Examination Results. The results of the maximum length of the corneal vortical nerve, and average density of the corneal vortical nerve, the maximum length of the corneal peripheral nerve, and average density of the corneal peripheral nerve, as well as the average LC number of the corneal vortex, the average LC number of the corneal periphery and the average LC number of total corneas are shown in Table 2.

Table 2
Corneal sub-basal nerve parameters of the subjects

	Group 1 (n=30)	Group 2 (n=78)	Group 3 (n=60)	p
Corneal vortical nerve maximum length(mm)	2.71 ± 0.60	2.70 ± 0.73	2.47 ± 0.71	0.158
Corneal vortical nerve average density(mm/mm ²)	15.82 ± 3.47	15.89 ± 4.49	14.53 ± 4.07	0.146
Corneal peripheral nerve maximum length(mm)	3.20 ± 0.52	3.27 ± 0.62	3.02 ± 0.62	0.041*
Corneal peripheral nerve average density(mm/mm ²)	16.73 ± 2.89	17.54 ± 3.62	15.90 ± 3.65	0.036*
Average LC number of the corneal vortex (number)	0	1.90 ± 0.92	1.93 ± 0.90	0*
Average LC number of the corneal periphery (number)	0.90 ± 1.12	1.68 ± 0.93	2.22 ± 0.88	0*
Average LC number of total corneal (number)	5.56 ± 4.56	11.29 ± 10.62	21.12 ± 12.84	0*
LC: Langerhans cell				
P values of less than 0.05 were considered statistically significant				

Comparison of vortical and Peripheral Corneal Parameters. The corneal nerve average density and corneal nerve maximum length of peripheral area were significantly greater than that in vortical area. ($P < 0.01$) But there was no difference in LC number between the two area. ($P = 0.12$). In Table 3, more details were presented.

Table 3
Comparison of vortical and peripheral corneal parameters

	Peripheral area	Vortical area	P
Corneal nerve maximum length(mm)	3.17±0.61	2.62±0.71	0*
Corneal nerve average density (mm/mm ²)	16.86±3.53	15.39±4.21	0*
Average LC number (number)	1.73±1.05	1.66±1.12	0.12
LC: Langerhans cell			
P values of less than 0.05 were considered statistically significant			

Correlation Analysis of Sub-basal Nerve Parameters and Ocular Features of Each Group. Correlation coefficients of the nerve parameters and ocular features in DED are reported in Table 4. In Group 1, OSDI score, and poor vision were positively correlated with average LC number of the corneal periphery (CC=0.74, 0.82 respectively, all P<0.05). Besides, poor vision was positively correlated with average LC number of total corneas (CC=0.77, P<0.05). Blurred vision and TMH were positively correlated with the maximum length (CC=0.88, 0.88 respectively, all P<0.05) and average density (CC=0.54, 0.58 respectively, all P<0.05) of the corneal vortical nerve. While in Group 2, TBUT were negatively correlated with average LC number of the corneal vortex and the total cornea. (CC=0.33, 0.27 respectively, all P<0.05). In Group 3, OSDI score, and foreign body sensation were significantly correlated with average LC number of total corneas, with a correlation coefficient (CC) of 0.41 (P<0.05) and 0.54 (P<0.05) respectively. Moreover, foreign body sensation was positively correlated with average LC number of the corneal periphery (CC=0.42, P<0.05).

Table 4
Correlation of Corneal Nerve Parameters and Ocular Features

		OSDI score	Sensitivity to light	Foreign body sensation	Painful eye	Blurred vision	Poor vision	TMH	TBUT	MG score
Group 1	Corneal vortical nerve maximum length(mm)	NS	NS	NS	NS	.88*	NS	.54*	NS	NS
	Corneal vortical nerve average density(mm/mm ²)	NS	NS	NS	NS	.88*	NS	.58*	NS	NS
	Corneal peripheral nerve maximum length(mm)	NS	NS	NS	NS	NS	NS	NS	NS	NS
	Corneal peripheral nerve average density(mm/mm ²)	NS	NS	NS	NS	NS	NS	NS	NS	NS
	Average LC number of the corneal vortex (number)	NS	NS	NS	NS	NS	NS	NS	NS	NS
	Average LC number of the corneal periphery (number)	.74*	NS	NS	NS	NS	.82*	NS	NS	NS
	Average LC number of total cornea (number)	NS	NS	NS	NS	NS	.77*	NS	NS	NS
Group 2	Corneal vortical nerve maximum length(mm)	NS	NS	NS	NS	NS	NS	NS	NS	NS
	Corneal vortical nerve average density(mm/mm ²)	NS	NS	NS	NS	NS	NS	NS	NS	NS
	Corneal peripheral nerve maximum length(mm)	NS	NS	NS	NS	NS	NS	NS	NS	NS
	Corneal peripheral nerve average density(mm/mm ²)	NS	NS	NS	NS	NS	NS	NS	NS	NS
	Average LC number of the corneal vortex (number)	NS	NS	NS	NS	NS	NS	NS	-.33*	NS
	Average LC number of the corneal periphery (number)	NS	NS	NS	NS	NS	NS	NS	NS	NS

LC: Langerhans cell; The correlation coefficients (CC) are shown for all significant correlations (P <0.05); *Significant correlation (P <0.05); **Significant correlation (P <0.01); NS, no correlation was detected during correlation analysis.

		OSDI score	Sensitivity to light	Foreign body sensation	Painful eye	Blurred vision	Poor vision	TMH	TBUT	MG score
	Average LC number of total cornea (number)	NS	NS	NS	NS	NS	NS	NS	-.27*	NS
Group 3	Corneal vortical nerve maximum length(mm)	NS	NS	NS	NS	NS	NS	NS	NS	NS
	Corneal vortical nerve average density(mm/mm ²)	NS	NS	NS	NS	NS	NS	NS	NS	NS
	Corneal peripheral nerve maximum length(mm)	NS	NS	NS	NS	NS	NS	NS	NS	NS
	Corneal peripheral nerve average density(mm/mm ²)	NS	NS	NS	NS	NS	NS	NS	NS	NS
	Average LC number of the corneal vortex (number)	NS	NS	NS	NS	NS	NS	NS	NS	NS
	Average LC number of the corneal periphery (number)	NS	NS	.42*	NS	NS	NS	NS	NS	NS
	Average LC number of total cornea (number)	.41*	NS	.54**	NS	NS	NS	NS	NS	NS
LC: Langerhans cell; The correlation coefficients (CC) are shown for all significant correlations (P <0.05); *Significant correlation (P <0.05); **Significant correlation (P <0.01); NS, no correlation was detected during correlation analysis.										

Correlation Analysis among Sub-basal Nerve Parameters. We noticed that the maximum length and average density of the corneal vortical nerve, and average density of the corneal peripheral nerve were strongly associated to the peripheral LC number. For detailed information, see Table 5.

Table 5
Correlation Analysis among Sub-basal Nerve Parameters

	Corneal vortical nerve maximum length(mm)	Corneal vortical nerve average density(mm/mm ²)	Corneal peripheral nerve maximum length(mm)	Corneal peripheral nerve average density(mm/mm ²)
Average LC number of the corneal centre (number)	NS	NS	NS	NS
Average LC number of the corneal periphery (number)	-0.20**	-0.16*	-0.15*	NS
Average LC number of total cornea (number)	-0.16*	NS	NS	NS
LC: Langerhans cell; The correlation coefficients (CC) are shown for all significant correlations (P <0.05); *Significant correlation (P <0.05); **Significant correlation (P <0.01); NS, no correlation was detected during correlation analysis.				

Discussion

With the IVCM's assistance, more knowledge about the microenvironment of the cornea, nerves and cells under physiological and pathological conditions has been understood.²³ When using confocal microscope, it is lacking of immunohistochemical evidence for cell type identification. It is well known that the cornea is an immune-privileged site. Yet the privileged status is relative on account of susceptibility of immune-mediated inflammatory disease such as ocular infections and autoimmune diseases²⁴. Macrophages in stroma and dendritic cells (DCs) joined in this process and served as antigen presenting cells (APCs).²⁵ They can engage processing, and presenting antigens, resulting in the initiation of ocular surface immune inflammatory responses. LCs are unique subset of DCs which found at a depth of 35 to 60 μm in normal human central corneal epithelium and acting as the first-line sentinels and professional APCs in the ocular surface. It thought to be the only cells that constitutively express Ia molecules in the cornea. LCs are rarely observed in corneal center or present as immature phenotype which lacking dendrites under nonpathological conditions. However, those located in the periphery of the cornea shown long processes interdigitating in the corneal epithelium.²⁵ With the advancement of maturity, LCS experienced functional and morphological changes.²⁶ To date, few people understand the is location of LCs in the corneal center.

Recently when we browsed patient's IVCM images, a bright, oval cell at the vortex be noticed. Unlike slender normal immature LCs as used to believe, these cells appeared as round or ellipse with higher brightness. In the beginning, we excluded the possibility that it was a kind of macrophage. It is not only due to macrophages are rare, but also due to macrophages almost exclusively restricted to the lower stromal part of the cornea.²⁵ Hence, it hypothesized that the oval cell is a particular type of LC that may have related to the severity of inflammation. Experimental studies in vitro suggest the corneal center comprises almost only immature LCs, while the corneal periphery contains both mature and immature LCs.²⁵ The normal uninflamed cornea is gifted with many MHC class II-positive LCs that are in an immature state. While exposure to the proinflammatory cytokine, cells will harvest and lose their capacity to process antigens and instead gain the capacity to stimulate T cells in this process. Previous studies suggested that harmful stimulation such as the cauterization or transplantation of the cornea is associated with maturation of resident corneal LCs.²⁷ Morphologic differences in corneal LCs are associated with variations in their functional states.²⁸ In our study, this oval cell in corneal vortex of DED was found. To demonstrate the oval cell be associated with severity of DED, 168 patients with different degrees of DED were enrolled. Cases were grouped according to no LCs (Group 1), both have LCs and bright, oval cells (Group 2), have LCs but no bright, oval cells (Group 3). The IVCM approach seems less invasive, but as effective as impression cytology.²⁹

Through contrastive analysis, no significant differences were seen among the three groups in age, sex distribution, DED symptoms and signs. The evaluation of symptoms (may be associated with inflammation), as everyone knows, tears secretion and stability (may be reasons of inflammation), and superficial epithelial alters (may be brought on by inflammation) seems to provide complex information more suitable for disease classification rather than inflammatory activity assessment.²⁹ Individual symptoms and signs cannot well reflect the severity of disease that have given us a better understanding of inconsistent symptoms and signs of DED. And in our study, there were no differences in symptoms and signs among groups, while marked differences were found in corneal peripheral nerve maximum length, average density, and average LC numbers. Previous studies have shown that corneal sub-basal nerve plexus density and length tended to decrease, and tortuosity tended to increase in DED patients, indicating damaged nerve fibers.³⁰ These results have suggested that the changes of corneal nerve density and nerve number are related to the severity of the dry eye condition. We found that Group 2 that both have LCs and bright, oval cells have more corneal peripheral nerve maximum length and average density than other groups. It can be inferred that increased nerve length and number occur in mild or moderate dry eye condition. The results from current study consistent with previous reports suggest the regeneration of corneal nerves in dry eye patients.³¹ Besides increasing nerve number and density, nerve tortuosity increasing, and nerve sprouting were observed in dry eye, suggesting corneal nerve outgrowth.³² Nerve regeneration in DED may be attributable to ocular inflammatory responses. The long-lasting inflammation of the ocular surface stimulates the release of prostaglandins,

leukotrienes, and cytokines, such as IL-1 and IL-6, from the damaged corneal tissue and inflammatory cells.^{33–35} And leading to activation of keratocytes, which is followed by synthesis of nerve growth factor (NGF) or other neuronal growth factors such as ciliary neurotrophic factor (CNTF).³⁶ IL-6 itself can also contribute to neurotrophism.³⁷ As a result, early in the clinical course of DED, corneal nerve regeneration brings about increasing corneal length and density.

Meanwhile, we noticed that Group 3 which have LCs, but no bright, oval cells have less corneal peripheral nerve maximum length and average density than others, thus proving damaged corneal nerve fibers in DED. Probably the tear film is thinned, the mechanical stress generated by blinking becomes abnormally high, injuring terminal nerve branches that may contribute to nerve damage.³⁸ Another possible reason for corneal nerve damage is that it continued exposure to inflammatory agent.³⁹ Inflammation represents a major driving force in sensitization, damage, and regeneration of the peripheral sensory neurons.³⁹ The appearance of nerve damage shown that disease is severe enough so that the cornea cannot repair the damage. The maximum length and average density of the corneal vortical nerve, and average density of the corneal peripheral nerve were inversely related to peripheral LC number, which validated inflammation plays a key role in the pathogenesis and chronicity of DED.

Taken together, it may explain why previous studies reported controversial results of nerve quantitative change in DED, from a decreased nerve number to no change in the nerve number or an increased nerve number. This is because at different periods of diseases. The bright, oval cells may present patients are early or middle in the process of developing DED.

Recent studies have identified the density of DC significantly increased in some cases, for example, infectious keratitis, DED, and contact lens wear.^{15,40} Apart from density, the increasing of size is accompanied by altered morphology that is dendritic process lengthening.⁴¹ In our research, whether average LC number of the corneal vortex, the corneal periphery, or the total cornea, the number in turn were Group 3 > Group 2 > Group 1. DCs were activated in both animal models and patients of DED, and the corneal DC density increased.⁴² The severity of the disease can be inferred from the number of inflammatory cells. It was found that peripheral LC number is associated with symptoms and signs of DED, especially the blurred vision and TMH in Group 1; In Group 3, it only associated with foreign body sensation; While in Group 2, vortical LC number is associated with TBUT. The inconsistency of these correlation shown that patients in each group had different disease severity and was a good example of why some dry eye patients have inconsistent symptoms and signs. Group 2 that both have LCs and bright, oval cells have middle number of inflammatory cells, thus we suspected that patients with bright, oval cells might in the early or middle stage of DED. Not only it is an immature type of DC, but also an indicator of the severity of the disease. Additionally, we found these oval cells and LCs that have dendritic processes are simultaneously present in corneal vortex (Figure 1). It is to be assumed they are different stages of the same cells. Interestingly, we found the nerve maximum length, average density and average LC number of corneal peripheries are significantly greater than corneal vortex which don't conflict with previous studies. Because of corneal vortex is not located in the center of cornea. While the number of peripheral LCs were greater than that of central. We do not know that where the LCs come, although the traditional view is LCs are derived from corneal periphery and migrate to the center. It is quite possible that LCs are stored under the cornea vortex and tend to move from the vortical part to the peripheral part when the cornea is stimulated.⁴³ There are three reasons for this. Firstly, the immature LCs can be observed in the corneal vortex and the appearance of the oval cells is related to the severity of DED. Secondly, there are more LCs in the periphery than in vortex. Thirdly, area under the corneal vortex cannot be seen clearly by ICVM that suggest there may have special structures under the corneal vortex such as cell storage pool. LCs have been found to be contiguous to nerves of the basal epithelial plexus in histopathologic specimens.⁴⁴ Through further characterization of LCs using immunohistochemistry may be better validated our assumption, this is infeasible in vivo. Corneal nerves and corneal cells degenerate after few hours after death. These mean that we need to obtain isolated fresh human cornea of normal and patients.

Thin arrow shown a type of bright and oval cell; Thick arrow shown the differentiated LCs.

Limitations. Inevitably, this study has some limitations: First, we will follow up these patients and record the changes of oval cells before and after anti-inflammatory treatment. Second, because of fresh isolated human cornea is difficult to obtain, animal experiments may provide clues of oval cells.

Conclusions

Altogether, a type of bright and oval cell was identified in the corneal vortex might be an immature LC. Its appearance is related to the severity of DED. Moreover, it suggests that the origin of LC may come from the deep layer of corneal vortex. Further research should focus on corneal vortex which is a special and unique structure.

Declarations

Funding: The publication of this paper was supported by a grant from Beijing Natural Science Foundation (Grant no. 7202229). The sponsor or funding organisation had no role in the design or conduct of this research.

Conflicts of interest: The authors declare no conflicts of interest.

Availability of data and material: The analysis data used in this study are available from the corresponding author upon request.

Code availability: Not applicable.

Authors' contributions: All named authors meet the International Committee of Medical Journal Editors (ICMJE) criteria for authorship for this article and take responsibility for the integrity of the work as a whole. Dalan Jing: research design, data acquisition, data analysis, and manuscript preparation. Xiaodan Jiang: research design, manuscript modification. Yilin Chou: data acquisition, data analysis. Shanshan Wei: data acquisition. Ran Hao: data analysis. Jie Su: data acquisition. Xuemin Li: research design.

Ethics approval: The study was approved by the Ethics Committee of Peking University Third Hospital and the research was performed in accordance with the Declaration of Helsinki.

Consent to participate: Written informed consent was obtained from all participants. **Consent for publication:** All authors have given their approval for this version to be published.

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Figures

Figure 1

Example of different type of LC in corneal vortex of DED

Thin arrow shown a type of bright and oval cell; Thick arrow shown the differentiated LCs.