

# The formation of perifoveal cysts in idiopathic macular holes and their relationship with retinal blood flow

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## Research article

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# Abstract

**Background :** To study the formation and distribution of perifoveal cysts in an idiopathic macular hole and their correlation with retinal blood flow.

**Method:** There were 16 patients in the small hole group, and 23 patients in the large hole group. In the preoperative procedure, we measured the number, the total area and the average size of the cysts in the inner nuclear layer, outer plexiform and Henle fiber layer complex around the hole; and the vascular density of the retinal capillary plexuses of all cases. Postoperatively, we measured the retinal capillary plexuses vascular density in 17 eyes.

**Results:** The number and the total area of the cysts in the inner nuclear layer of the large hole group were greater than those of the small hole group ( $t=-2.882$ ,  $P =0.007$ ,  $t= -3.412$   $P =0.002$ ). And we came to the same conclusion in the outer plexiform and Henle fiber layer complex ( $t=-3.935$   $P =0.000$ ,  $t=-4.335$   $P =0.000$ ). The average cystic area of the inner nuclear layer and outer plexiform and Henle fiber layer complex had no significant difference between the two groups ( $t=0.178$   $P =0.860$ ,  $t=-1.767$   $P =0.085$ ). There was a significant correlation among the idiopathic macular hole diameter, the number and the total area of cysts in the outer plexiform and Henle fiber layer complex, the vascular density of the retinal superficial capillary plexuses and deep capillary plexuses; the respective coefficients were -0.725, -0.474, -0.314, -0.768, and +0.624.

**Conclusions:** 1. The diameter of the idiopathic macular hole was related to the formation of cysts around the hole. 2. Multiple mechanisms are involved in the interaction between the cystic space around the idiopathic macular hole and the retinal capillary plexuses.

## Background

Idiopathic macular hole (IMH) is more common in the female population over 50 years old, with slow progress of the disease. Patients with advanced-stage disease can experience vision deformation and reduced visual acuity<sup>[1]</sup>. Recently, with the aggravation of aging trends in China, more attention has been paid to the study of IMH.

After many studies on IMH by researchers, it is generally recognized that the key formation mechanism of IMH is the traction of posterior vitreous on the tangential direction of retina in the central area of macula<sup>[2]</sup>. During macular hole formation, cystic patterns can occur in the middle retina around the hole, which have been studied by some researchers<sup>[3,4,5,6,7]</sup>, but the formation mechanism of cystic patterns is not clear at present. The perifoveal cystoid cavities are mainly distributed in the inner nuclear layer and in the complex consisting of the outer plexiform layer and Henle fiber layer. Such en face OCT images have recently been used to characterize full-thickness macular holes as well as the perifoveal hyporeflexive intraretinal spaces observed surrounding them. OCTA is a novel imaging platform that utilizes motion contrast to visualize macular microvascular perfusion in a rapid, noninvasive, and depth-resolved

fashion. Therefore, patients can be repeatedly examined to observe the retinal and choroid blood flow [8,9]. The purpose of this report was to observe features of cystoid cavities and retinal capillaries in eyes with a full-thickness macular hole and further discuss the correlation between the diameter of the macular hole and the number and area of cavities, as well as the relationship between cavity and blood flow of capillary capillaries.

## Methods

### 1. Study subjects

This prospective clinical study evaluated 39 eyes of 37 patients with IMH. These eyes had been imaged between March 2018 and December 2018 at the Second Hospital of Hebei Medical University. There were 16 cases with IMH diameter  $\leq 400 \mu\text{m}$  (known as the small hole group) and 23 cases with IMH diameter  $>400 \mu\text{m}$  (known as the large hole group).

Patients were included in the study if they had an IMH diagnosed by FFA and OCT at the ophthalmology center of the Second Hospital of Hebei Medical University and were cooperative. Patients with poor image quality, refractive stroma not clear, any other concurrent macular disorder (such as epiretinal membrane, choroidal neovascularization, macular atrophy, macular edema), or with systemic diseases that affect eyes (such as diabetes and kidney failure) were excluded. This study, data collection, analysis, and presentation adhered to the tenets of the Declaration of Helsinki and the Health Insurance Portability and Accountability Act of 1996 (HIPAA).

### 2. Study methods

All patients underwent a baseline ophthalmic examination, including medical and ocular history, family medical history, best-corrected visual acuity (BCVA), slit-lamp examination of the anterior and posterior segments, measurement of intraocular pressure, dilated fundus examination, and axial length measurement with noncontact partial coherence laser interferometry (Carl Zeiss Shanghai Co., Ltd).

Spectral-domain OCT (SD-OCT, Heidelberg) was used to acquire the minimum macular hole diameter and observe the closure of the hole after surgery. OCT angiography (Cirrus 5000, ZEISS) was used to acquire the number, the total area and the average area of cystic cavities in the inner nuclear layer and the outer plexiform and Henle fiber layer complex. The vascular density (VD) of superficial capillary plexuses (SCP) and deep capillary plexuses (DCP) was also measured by OCT angiography preoperatively and postoperatively. All surgeries were performed with the 3-port pars plana vitrectomy + indocyanine green assisted internal limiting membrane (ILM) peeling+intraocular air filling, and postoperative face-down positioning.

Measurement method of minimum diameter in IMH

We obtained OCT images using SD-OCT, and all operations were performed with multiple scans by two experienced clinical technicians to obtain the best images. The minimum diameter of the macular hole was defined as the smallest diameter of the hole parallel to the retina. The obtained images were measured by the same clinician using the built-in caliper of the OCT device, and the minimum diameter of the macular hole was obtained.

#### Measurement method of the cystoid cavities of IMH

In the application of OCTA, en face OCT scanning was selected to scan the macular area and a 3 mm × 3 mm scanning pattern was performed. All examinations were performed by the same clinician. Because previous studies have found that the perifoveal cystoid cavities were mainly concentrated within the INL and OPL+HFL complex, en face OCT images from these two regions were selected for analysis in our study. For each layer, the scan with the highest quality was selected among those located centrally in the stack. This scan was used to measure the number, the total area and the average area of the hyporeflective spaces in the frontal plane<sup>[7]</sup>. All the images were analyzed using the public domain ImageJ software (Version 1.48q, Wayne Rasband; National Institutes of Health, Bethesda, Maryland, USA). First, after conversion to 8-bit grayscale images, hyporeflective spaces were identified via the auto threshold v1.15 function using the “minimum thresholding” method. As shown in Figure 1, A1 is the B-scan image of IMH, and the red line is located in the INL. A2 shows the en face image of INL, corresponding to the red line position in A1. A3 is the image processed by ImageJ software, where the black area represents the cystic cavities in INL. Similarly, B1-B3 respectively show the B-scan image of IMH, the en face image of OPL+HFL complex, and the marked cystic cavities in OPL+HFL. Finally, the number of cystic cavities and their areas were calculated by particle analysis. To reduce noise, an area of greater than 0.001 mm<sup>2</sup> was set as the threshold for inclusion in particle analysis calculations.

#### Measurement of retinal capillary vascular density

In the application of OCTA, angio-OCT scanning was selected to scan the macular area, and a 3 mm × 3 mm scanning pattern was performed. All examinations were performed by the same clinician. The VD ratio was defined as the ratio of the blood vessel area represented by white pixels to the total pixel area of the 3 mm×3 mm scanning area. The SCP and the DCP of the retina were automatically divided from the whole retinal scanning images (Figure 2). The SCP of the retina included the vascular system located in the retinal nerve fiber layer and the optic nerve ganglion cell layer. The DCP of the retina includes the vascular system located in the inner plexus layer, the inner nuclear layer and the outer plexus layer. To calculate the retinal capillary vascular density, the adjustment threshold tool in the ImageJ software was used to transform the OCTA image. The specific operation was the same as the previous research report<sup>[10]</sup>, except that the threshold we set was different. To more accurately display capillaries in each layer, we selected different thresholds for SCP and DCP. When measuring the VD of SCP, the threshold was set at 80, while when measuring the VD of DCP, the threshold was set at 50.

#### Operating procedure

All surgeries were performed by two experienced surgeons from the Second Hospital of Hebei Medical University. Surgeries were performed using 3-port pars plana vitrectomy (PPV). After the vitreous was completely removed, gas-liquid exchange was conducted, and then perfusion fluid was extracted from the vitreous. The exposed retinal pigment epithelium at the hole was covered with viscoelastic agent and injected with indocyanine green (ICG) for 10 seconds; the viscoelastic agent and ICG were cleaned up, and the ILM was stained with indocyanine green. The ILM was peeled from the macula and around the MH, and then silicone oil or aseptic air was injected into the vitreous cavity. The surgical incision was closed, and the patient was kept in a face-down position after surgery.

### 3. Statistical analyses

All analyses were conducted with SPSS version 20.0 for Windows. Data are expressed as the means  $\pm$  standard deviations for continuous variables. A paired sample t test was used to compare the number, the total area and the average area of cystic cavities between the INL and OPL+HFL complex. A *P* value  $<0.05$  was set for significance. The number, the total area and the average area of cystic cavities in the two groups were compared by an independent sample t test; *P* $<0.05$  was considered significant. Canonical correlation analysis was used to observe the correlation coefficient and analyze the correlation among the duration of patients, the BCVA, the minimum IMH diameter, the capillary vascular density and number, the total area and the average area of cystic cavities in the two layers. A paired sample t test was used to compare the retinal capillary VD preoperative and postoperative. *P* $<0.05$  was considered significant. The number and the total area of cystic cavities with the postoperative retinal capillary VD were analyzed by bivariate correlation analysis, and *P* $<0.05$  indicated the correlation between the two sets.

## Results

### 1. Basic information of patients

According to the inclusion and exclusion criteria, 39 eyes (13 right eyes and 26 left eyes) of 37 patients diagnosed with IMH by FFA and OCT were included in this study (Table 1). There were 13 (33.3%) males and 26 (66.7%) females, with an average age of  $64 \pm 9$  years. Among them, there were 16 patients with IMH hole diameter  $\leq 400 \mu\text{m}$  (the small group) and 23 patients with IMH hole diameter  $> 400 \mu\text{m}$  (the large group). For 32 eyes, simultaneous cataract extraction was performed. Four eyes retained the original lens, 2 eyes had an intraocular lens, and 1 eye was not treated with surgery. One patient with IMH underwent vitrectomy + ILM peeling + aseptic air filling for the first time, but experienced failure. Then, after the second operation, the eye was injected with silicone oil and the hole was closed. The other eyes were all closed after surgery; 6 eyes were injected with silicone oil, and the other 31 eyes were injected with aseptic air. Seventeen eyes with macular hole closure were observed postoperatively, and the average time of OCTA examination was 55 days after surgery.

Table 1  
General information about IMH patients

<b>The total number of cases</b>	<b>39 eyes of 37 patients</b>
Gender (male/female)	13/26
Age (years)	64 ± 9
Course of illness (month)	4.93 ± 7.13
BCVA (Log MAR)	4.07 ± 0.36
Intraocular tension (mmHg)	15.69 ± 2.95
IMH hole diameter ≤ 400 μm (the small group)	
Number of cases	16
Diameter (μm)	288 ± 92
IMH hole diameter > 400 μm (the large group)	
Number of cases	23
Diameter (μm)	608 ± 163

## 2. Comparison of the number, the total area and the average area of cystic cavities in different layers

On OCT B-scan images, we found that the perifoveal cystoid cavities were mainly concentrated in the INL and OPL + HFL complex. In the INL, there were small and circular cystoid cavities, similar to sponges. The number, the total area and the average area of cavities in the INL were  $67 \pm 76$ ,  $0.643 \pm 0.529 \text{ mm}^2$  and  $0.015 \pm 0.020 \text{ mm}^2$ , respectively. The OPL + HFL complex contained elongated radial regular cystoid cavities, similar to petals. The number, the total area and the average area of cavities in the OPL + HFL complex were  $26 \pm 15$ ,  $1.109 \pm 0.746 \text{ mm}^2$  and  $0.048 \pm 0.037 \text{ mm}^2$ , respectively. As shown in Fig. 1 and Table 2, the number ( $t = 3.820$   $P = 0.000$ ), the total area ( $t = -6.140$   $P = 0.000$ ) and the average area ( $t = -5.720$   $P = 0.000$ ) were significantly different between the two layers. The number of cysts in the INL was larger than that in the OPL + HFL complex, and the total area and average area of the INL were smaller than those in the OPL + HFL complex.

Table 2  
Comparison of the parameters of cysts in retinal INL and OPL + HFL complex

	INL	OPL + HFL complex	t	P
Number of cysts	67 ± 75	26 ± 15	3.820	0.000
Total area of cysts (mm <sup>2</sup> )	0.643 ± 0.529	1.109 ± 0.746	-6.140	0.000
Average area of cysts (mm <sup>2</sup> )	0.015 ± 0.020	0.047 ± 0.037	-5.720	0.000

### 3. The effect of different hole sizes on the number of cysts, the total area and the average area of the cysts

In the small hole group, the number, the total area and average area of cysts in the INL were  $29 \pm 31$ ,  $0.337 \pm 0.333 \text{ mm}^2$  and  $0.015 \pm 0.027 \text{ mm}^2$ , respectively, while those in the OPL + HFL complex were  $17 \pm 11$ ,  $0.597 \pm 0.490 \text{ mm}^2$  and  $0.035 \pm 0.026 \text{ mm}^2$ , respectively. In the large hole group, the number, the total area and average area of cysts in the INL were  $94 \pm 86$ ,  $0.856 \pm 0.541 \text{ mm}^2$  and  $0.014 \pm 0.016 \text{ mm}^2$ , respectively, while those in the OPL + HFL complex were  $33 \pm 14$ ,  $1.466 \pm 0.689 \text{ mm}^2$  and  $0.056 \pm 0.042 \text{ mm}^2$ , respectively. The number and the total area of cysts in the INL and the OPL + HFL complex were significantly different between the two groups ( $P < 0.05$ ), and the number and the total area of cysts in the small hole group were lower than those in the large hole group. The average area did not differ significantly between the two groups ( $P > 0.05$ ) (Table 3). The scatter diagrams (Fig. 3) for the hole diameter and the number and the total area of perifoveal cysts also showed that the number and the total area of cysts in the INL and the OPL + HFL complex increased with the diameter of the holes. At the same time, we found that in the small hole group, no cyst was found in the INL and the OPL + HFL complex in 2 eyes, and in the other 3 eyes no cyst was found in the INL, but there were cysts in the OPL + HFL complex. As shown in Fig. 4, the minimum macular hole diameter in G1 and G2 was  $314 \mu\text{m}$  and  $257 \mu\text{m}$ , respectively. In both cases, no cystic cavity was found in the INL (indicated by the green arrow), but only in the OPL + HFL complex (indicated by the yellow arrow). However, no such phenomenon was found in the large group.

**Table 3 Comparison of INL and OPL + HFL complex cavities in two groups**

	IMH hole diameter $\leq$ 400 $\mu\text{m}$ (the small group)	IMH hole diameter $\leq$ 400 $\mu\text{m}$ (the small group)	t	P
The number of cysts in INL	$29 \pm 31$	$94 \pm 86$	-2.882	0.007
The total area of cysts in INL ( $\text{mm}^2$ )	$0.337 \pm 0.333$	$0.856 \pm 0.541$	-3.412	0.002
The average area of cysts in INL ( $\text{mm}^2$ )	$0.015 \pm 0.027$	$0.014 \pm 0.016$	0.178	0.860
The number of cysts in OPL + HFL complex	$17 \pm 11$	$33 \pm 14$	-3.935	0.000
The total area of cysts in OPL + HFL complex ( $\text{mm}^2$ )	$0.597 \pm 0.490$	$1.466 \pm 0.689$	-4.335	0.000
The average area of cysts in OPL + HFL complex ( $\text{mm}^2$ )	$0.035 \pm 0.026$	$0.056 \pm 0.042$	-1.767	0.085

### 4. The correlation among the number, the total area, the average area of cysts and the retinal vascular density preoperatively

Canonical correlation analysis was used to observe the correlation coefficient and analyze the correlation among the duration of patients, the BCVA, the minimum IMH diameter, the capillary vascular density and the number, the total area and the average area of cystic cavities in the two layers. The results are shown in Table 4, and the value represents the correlation coefficient of each parameter. The higher the correlation coefficient, the higher the correlation, and “-” indicates a negative correlation. Among them, the coefficients of the hole diameter (X3), the VD of the preoperative SCP (X4), the VD of the preoperative DCP (X5), the number of cysts in OPL + HFL complex (Y4), and the total area of cysts in OPL + HFL complex (Y5) were relatively large, at 0.725, -0.768, + 0.624, -0.474, and - 0.314, respectively. The results indicated that the hole diameter and the number and the total area of cysts in OPL + HFL complex were positively correlated (X3 and Y4 Y5); the VD of the preoperative SCP and the number and the total area of cysts in OPL + HFL complex were positively correlated (X4 and Y4 Y5); and the VD of the preoperative DCP and the number and the total area of cysts in OPL + HFL complex were negatively correlated (X5 and Y4 Y5).

Table 4  
Canonical correlation analysis result

<b>X1</b>	<b>0.082</b>	<b>Y1</b>	<b>-0.173</b>
X2	0.132	Y2	-0.184
X3	-0.725	Y3	0.013
X4	-0.768	Y4	-0.474
X5	0.624	Y5	-0.314
		Y6	-0.190
(X1 medical duration, X2 BCVA, X3 macular hole diameter, X4 the VD of the preoperative SCP, X5 the VD of the preoperative DCP, Y1 the number of cysts in INL, Y2 the total area of cysts in INL, Y3 the average cystic area in INL, Y4 the number of cysts in the OPL + HFL complex, Y5 the total area of cysts in the OPL + HFL complex, Y6 the average cystic area in the OPL + HFL complex)			

In comparisons within group X (X1, X2, X3, X4, X5) (not listed in the table), there was no significant correlation between the hole diameter (X3) and the VD of the preoperative SCP (X4) and the VD of the preoperative DCP (X5) (0.2600, -0.0153, respectively), while the duration of patients (X1) correlated with the hole diameter (X3) (0.5772). Therefore, the IMH diameter increased with the extension of duration. Correlation between the hole diameter and the preoperative VD correlation was not obvious when analyzed, but when considering the various parameters of the surrounding cysts, comprehensive analysis yielded a relatively obvious correlation, suggested that the diameter of IMH may affect the retinal VD of each layer by affecting the formation of cysts in the OPL + HFL complex.

##### 5. Correlation analysis between the number, the total area of cysts and postoperative retinal VD

The number and the total area of cysts in the INL were not correlated with the postoperative retinal VD of SCP and DCP ( $P > 0.05$ ). Similarly, the number and the total area of cysts in the OPL + HFL complex were

not correlated with the postoperative retinal VD of SCP and DCP ( $P > 0.05$ ). Thus, changes in the retinal capillary VD in IMH were not affected by the single factor of the perifoveal cysts.

## 6. Comparison of pre- and postoperative VD

Table 5  
Comparison of preoperative and postoperative vascular density in the two retinal capillary plexuses

	Preoperative	Postoperative	t	P
VD of SCP (%)	38.92 ± 13.64	34.43 ± 11.94	2.007	0.062
VD of DCP (%)	38.46 ± 12.14	41.06 ± 14.35	-1.003	0.331

The VD of retinal SCP was 38.92 ± 13.64% preoperatively and 34.43 ± 11.94% postoperatively, showing no significant difference ( $P = 0.062 > 0.05$ ). The pre- and postoperative VD of retinal DCP were 38.46 ± 12.14% and 41.06 ± 14.35%, respectively, again having no significant difference ( $P = 0.331 > 0.05$ ) (Table 5). In our study, there was no significant difference in retinal capillary VD before and after surgery.

## Discussion

Recently, the emergence of en face OCT and angio-OCT have provided us with a new perspective of observation. En face OCT can help us understand the structural changes of the retina from different layers parallel to the retina, realizing the breakthrough of observing the retina and choroid membrane from point to surface. Through multiple scans of the same cross section, OCTA processes multiple images to form images representing blood flow. Since it does not require contrast agent injection, it is a safe and noninvasive examination method, and patients can be repeatedly examined to observe retinal and choroid blood flow. In this study, we used en face OCT to observe the features of cystoid cavities around IMH and OCTA to observe the VD of retinal capillaries and analyzed the results.

In this study, we found that the perifoveal cystoid cavities around the holes were mainly concentrated in the INL and OPL+HFL complex, and the morphology was completely different between the two layers. In the INL, there were smaller scattered, spongy cystoid cavities. In the OPL + HFL complex, the cystoid cavities presented a larger regular radial petal-like structure (as shown in Figure 1). This finding was consistent with the research results of Matet et al. [7]. Einar [3] has proposed in the literature that the causes of edema may be cytotoxicity and vascular factors. Cytotoxicity or ischemia and hypoxia mainly cause intracellular edema, while vascular factors (such as increased hydrostatic pressure gradient or decreased osmotic pressure gradient) mostly cause intercellular edema. The deep capillary plexuses of the retina are located between the INL and the OPL [11]. Therefore, we believe that the formation of cystoid cavities may be caused by the vitreous traction to the fovea, which leads to the decrease of hydrostatic pressure in retinal tissue, thereby increasing the pressure gradient between vessel and tissue, and causing fluid leakage from vessel to tissue. At present, it is believed that the distribution of cystoid cavities around

the holes is related to the distribution of Müller cells in the retina [7], and the different arrangement of cysts in the two layers is consistent with the "Z-shape structure" of Müller cells [12]. The "Z-shape structure" of Müller cells was observed in both animal and human models, which are arranged in three consecutive directions in the retina [13,14]. Starting from the ILM, Müller cells around the fovea go vertically in the INL, and then in the OPL+HFL complex turn to the direction of the central fovea, being inclined and almost horizontal; at the junction with the outer nuclear layer, the cells reach the outer limiting membrane vertically again. In the INL, the tissue is relatively dense, and Müller cells are vertically distributed, corresponding to the small and scattered "spongy" distribution structure of the cystoid cavities. In the OPL+HFL complex, the tissue is relatively loose and Müller cells are distributed almost horizontally, corresponding to the large, regular and radial "petal-like" structure of the cystoid cavities. Therefore, we believe that the distribution of the cystic cavity may be related to the distribution of Müller cells. Moreover, Müller cells are the only type of retinal cells that span the INL and the OPL, which further proves that the formation of cystic space in different layers of the retina may be mainly related to Müller cells. More research is needed to confirm the specific relationship between cystoid cavities in the retina and Müller cells.

In this study, we found that the number and the total area of cysts in the small hole group were smaller than those in the large hole group, but there was no difference in the average area of the cysts. A study of 13 patients with IMH suggested that the diameter of IMH was not correlated with the total area of cavities in INL and OPL+HFL complex [15], which was inconsistent with our conclusion. In the small hole group, 2 eyes had no cysts in the INL and the OPL+HFL complex, and in the other 3 eyes, no cyst was found in the INL, but there were cysts in the OPL+HFL complex. The diameter of IMH is correlated with the formation of cysts. We also found that the diameter of the hole correlated with the medical duration of the disease. Therefore, we speculated that with the continuous extension of time, the diameter of the hole was continuously enlarged, and the fluid in the retinal layers was also continuously accumulated, leading to the formation of a larger number of cysts with a larger total area in the large hole group. The OPL is the synaptic site where the end spheres of the cones and rods are connected with the dendrites of bipolar cells and horizontal cell protuberances. It is a porous network structure, which can explain why the cysts only appear in the OPL in eyes with the smaller macular hole. After that, the fluid in the tissue continued to increase and then flowed into the INL along with the gap of Müller cells, thus forming a double-layer cystic change consistent with the "Z-shape structure" of Müller cells. Paul & Tornambe [16] proposed that in the early stage of IMH, the vitreous body pulled and tore the inner layer of retina in the fovea, and the fluid in the vitreous body penetrated into the retinal layer, first forming a cavity in the inner retina, then progressing to a deeper layer, and finally accumulating in the OPL, which was different from the phenomenon observed by us. There was no significant difference in the average cystic area, that is, the cystic size, in either the INL or OPL+HFL complex, between the small hole group and the large hole group. We believe that an interstitial space exists between cells that is used to communicate with each other; when a space accumulates fluid to a certain degree, after clearance increases, the tension of the surrounding tissue and permeability increase, and the liquid will flow from one gap to other gap, which can lead to increasing numbers of cysts and increase of the total area, and does not cause the increase

of average size. Therefore, the size of the cysts have no difference between the small hole group and the large hole group. However, the formation mechanism of cysts around the hole is still unclear, and more relevant studies are needed.

We also found that preoperative VD of retinal DCP was negatively correlated with the number and total area of cysts in the OPL+HFL complex, while the VD of SCP was positively correlated with the number and total area of cysts in the OPL+HFL complex. The effect of the perifoveal cysts on the capillaries in the deep retina is currently assumed as follows: 1. Rizzo et al. [16] proposed the "vascular sliding" theory. They believed that during the disease process, the microvasculature did not disappear, but was hidden in the border of the cystoid cavities; after the absorption of intraretinal fluid postoperatively, retinal VD increased. 2. When studying retinal vein occlusion, Coscas et al. [17] found that the damage of the deep retinal capillaries was more serious. Therefore, they believed that during the formation of IMH and retinal cystoid cavities, mechanical traction was generated on the capillaries, resulting in decreased blood flow density [18]. Both hypotheses can explain the negative correlation between VD of retinal DCP and the number and total area of cysts in the OPL+HFL complex. However, we cannot give a reasonable explanation for the phenomenon that the VD of SCP is positively correlated with the number and total area of cysts in the OPL+HFL complex. In one study, Pierro et al. [19] proposed the hypothesis that tangential traction could cause vasodilation and congestion in the formation of macular hole. It has also been reported that vitreous retinal traction can cause reversible changes in retinal vascular perfusion, indicating that vitreous traction has direct force on retinal vessels [20]. Therefore, we believe that in the process of MH, the number and total area of cysts in the OPL+HFL complex continue to increase, which produces certain tension to the SCP of the retina and changes the structure of the capillaries located in the layer of ganglia cells and nerve fibers, which may cause the congestion of blood vessels, and then cause the change of vascular density. However, this theory is not perfect, and we need to carry out more relevant research.

The number and the total area of cysts in the INL and OPL+HFL complex were not correlated with the postoperative retinal VD of SCP and DCP. At present, it is very rare to compare the retinal capillary vascular density of IMH patients before and after surgery. However, in our study, there was no significant difference in the VD of retinal SCP and DCP after surgery compared with those before surgery. It is assumed that the effect of cysts on retinal capillaries is mainly based on the "vascular sliding" theory [11], so the VD of retinal capillaries should be improved after surgery compared with before surgery. It is assumed that the effect of cysts on retinal capillaries is mainly "mechanical damage" [15], so the total area of cysts before surgery should be correlated with the VD of retinal capillaries after surgery. Therefore, we believe that the effect of the cysts in retinas on the capillaries during the formation of IMH should be complex, including the preservation of the vasculature between the cysts, and mechanical damage. After surgery, the capillaries of the retina also have a certain capacity to repair after the absorption of the cysts. These combined factors resulted in no significant difference in retinal capillary VD before and after surgery. However, due to the small sample size, short follow-up time and different follow-up times, the

results may be affected to some extent. Therefore, studies with larger sample size and longer follow-up time are needed to explain this phenomenon.

The diameter of IMH and the VD of retinal capillaries in the SCP and DCP were significantly correlated with the number and total area of cysts in the OPL+HFL complex, but not significantly correlated with the number and total area of cysts in the INL. There was no significant correlation between the diameter of IMH and the VD of retinal SCP and DCP. Therefore, we speculate that the diameter of IMH does not directly affect the retinal VD, but indirectly affects the retinal VD through the accumulation of intraretinal fluid and the appearance of cystic space around the macular hole. For the structure of the cystic cavity in the INL of the retina, both the total area of the cystic cavity and the average area of the cystic cavity in the INL were significantly reduced when compared with that in the OPL+HFL complex (Table 2). Therefore, the effect of the cystic cavity in the INL on the VD of retinal capillaries was relatively small, and the correlation coefficient was reduced in the comprehensive analysis.

This study has several limitations as follows. 1. The sample size was small, especially in the postoperative follow-up. 2. Most of the images were selected manually and measured by software, with certain errors. 3. Injected vitreous cavity filling was different in surgery. 4. Postoperative follow-up time was different, and the follow-up time was short.

## Conclusions

1. The diameter of the idiopathic macular hole was related to the formation of cysts around the hole.
2. Multiple mechanisms are involved in the interaction between the cystic space around the idiopathic macular hole and the retinal capillary plexuses.

## Abbreviations

<b>IMH</b>	<b>idiopathic macular hole</b>
OCT	Optical coherence tomography
OCTA	Optical coherence tomography angiography
SD-OCT	Spectral-domain optical coherence tomography
INL	Inner nuclear layer
ILM	Internal limiting membrane
OPL	Outer plexiform layer
HFL	Henle fiber layers
BCVA	Best correct visual acuity
VD	Vascular density
SCP	Superficial capillary plexuses
DCP	Deep capillary plexuses
PVD	Posterior vitreous detachment
PPV	Par plana vitrectomy
ICG	Indocyanine green
FFA	Fundus fluoresceine angiography

## Declarations

Ethics approval and consent to participate: The informed consent was in writing and I can confirm that informed consent was obtained from all participants. This research was performed in accordance with the declaration of Helsinki and was approved by the Second hospital of Hebei medical university ethics committee. The committee's reference number is 2018-P052.

Consent for publication: No applicable.

Availability of data and materials: The data is presented in the additional supporting files.

Competing interests: The authors declare that they have no competing interests.

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Authors' contributions: YY was the major contributor in writing the manuscript. YH was the corresponding author. NL and DL helped getting data. RJ had made contributions to the design of the work and interpretation of data. All authors read and approved the final manuscript.

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## Additional Files

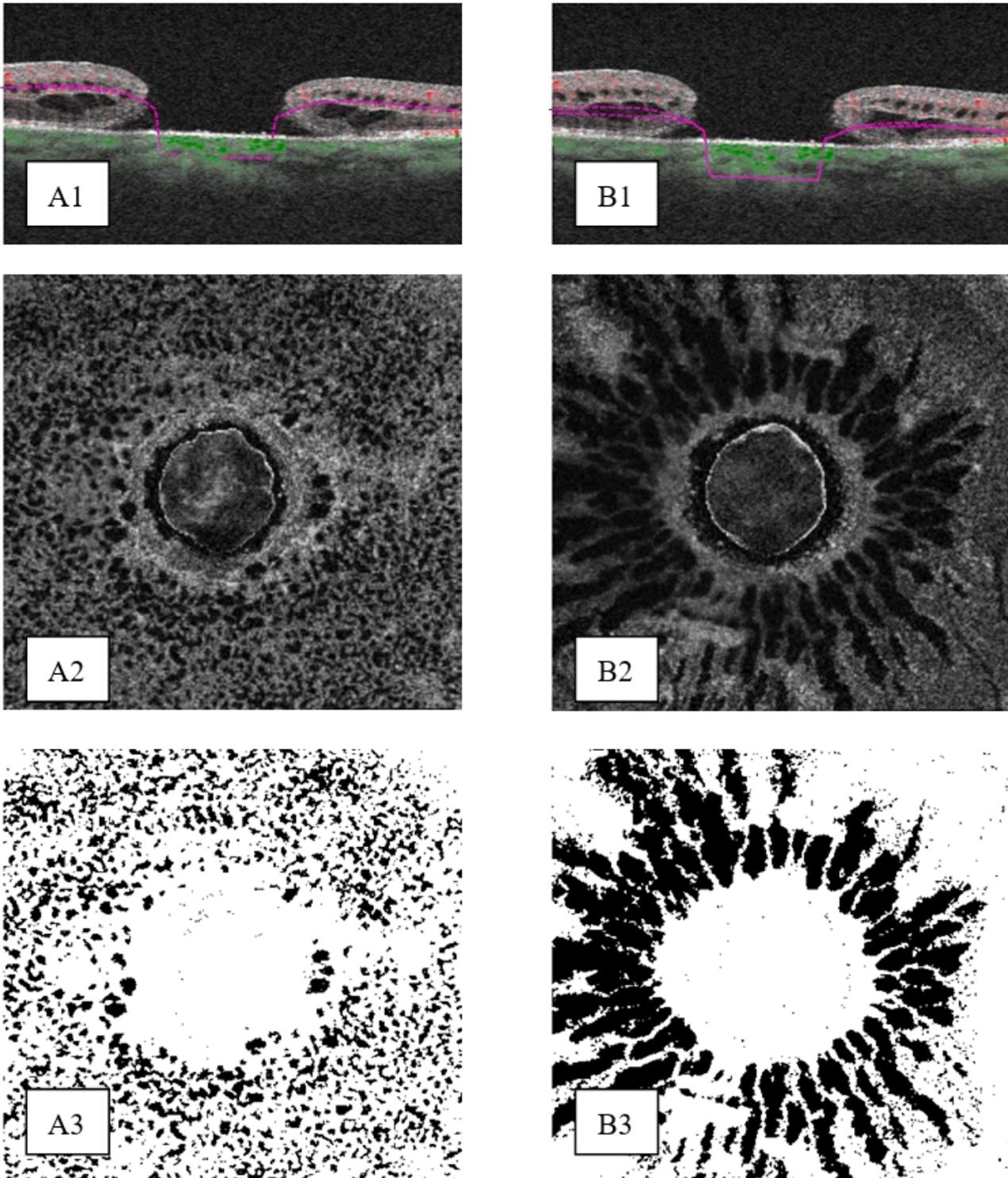
Additional. file 1. The data of my manuscript. This additional file contains all the original data for the research.

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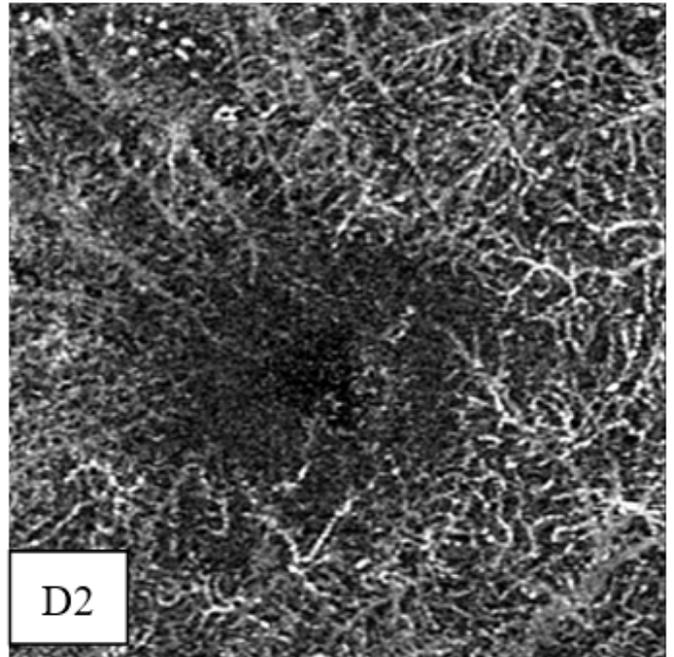
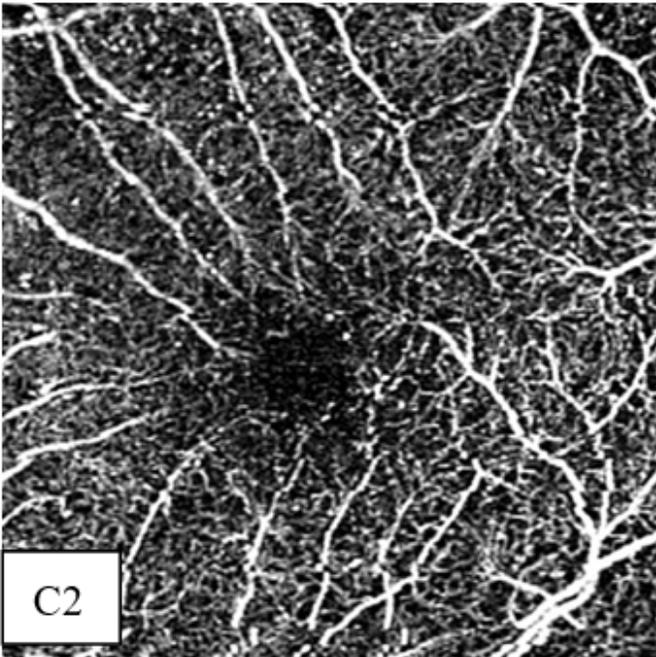
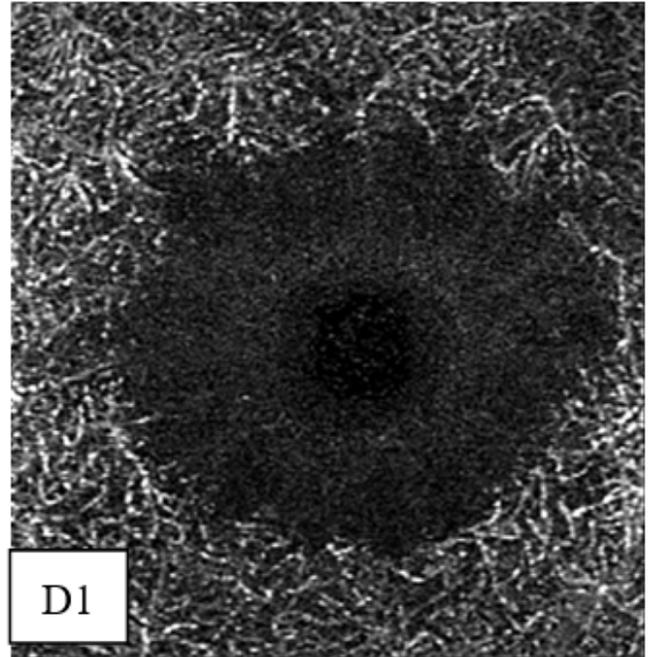
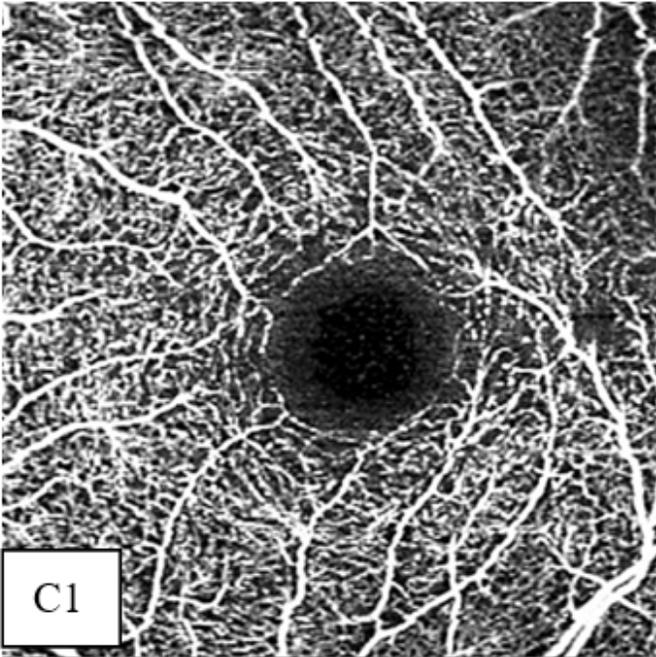
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## Figures



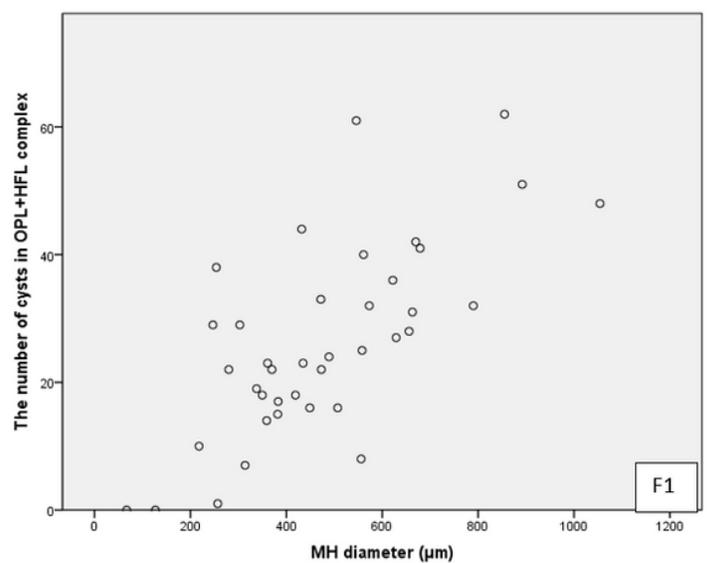
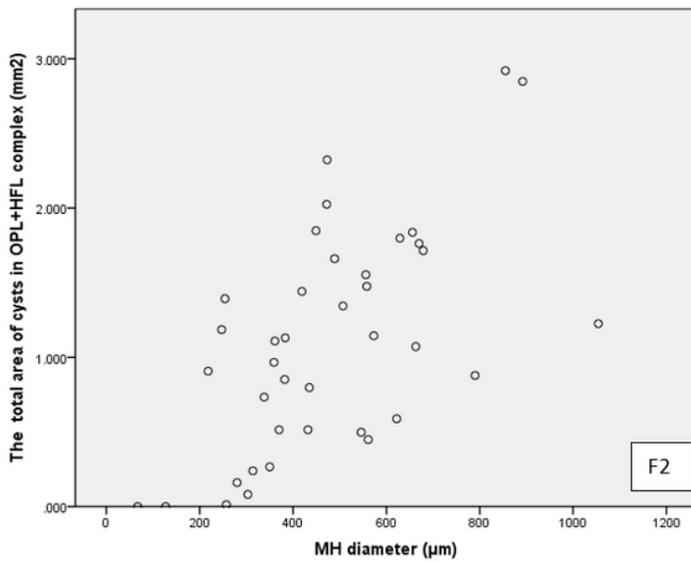
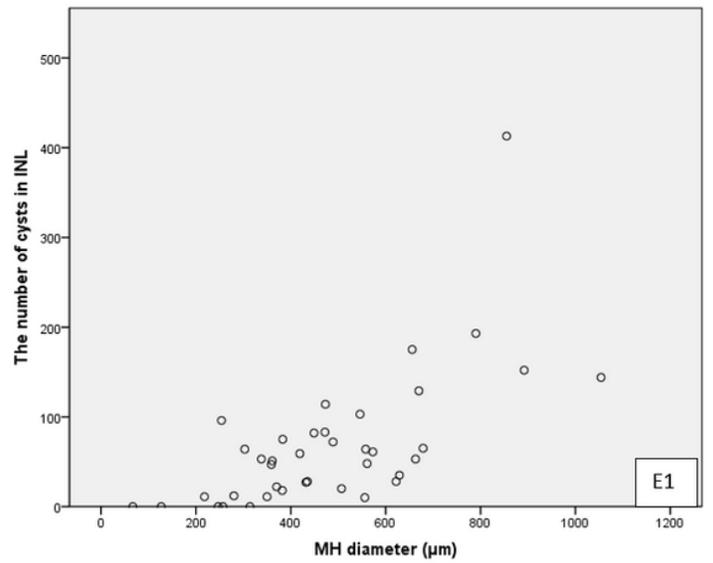
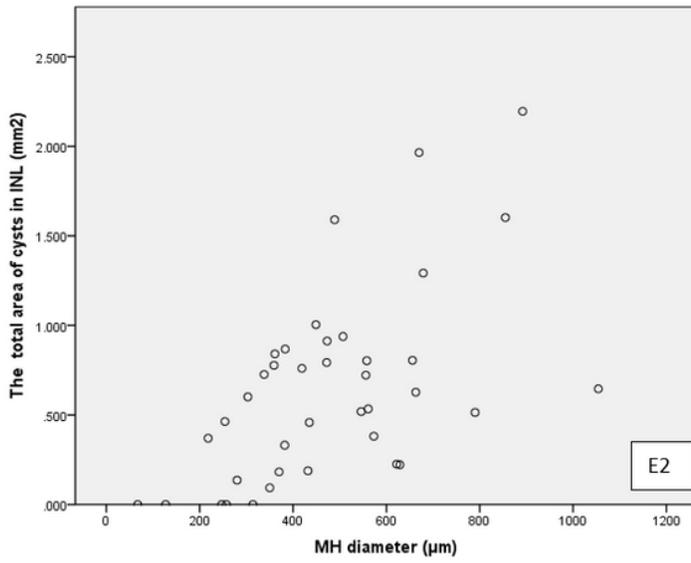
**Figure 1**

The form of cystic cavities in INL and OPL+HFL complex. A1-A3 respectively show the B-scan image of IMH, the en face image of INL (corresponding to the red line position in A1), and the marked cystic cavities in INL (the black part in the figure). B1-B3 respectively show the B-scan image of IMH, the en face image of the OPL+HFL complex (corresponding to the red line position in B1), and the marked cystic cavities in the OPL+HFL complex (the black part in the figure).



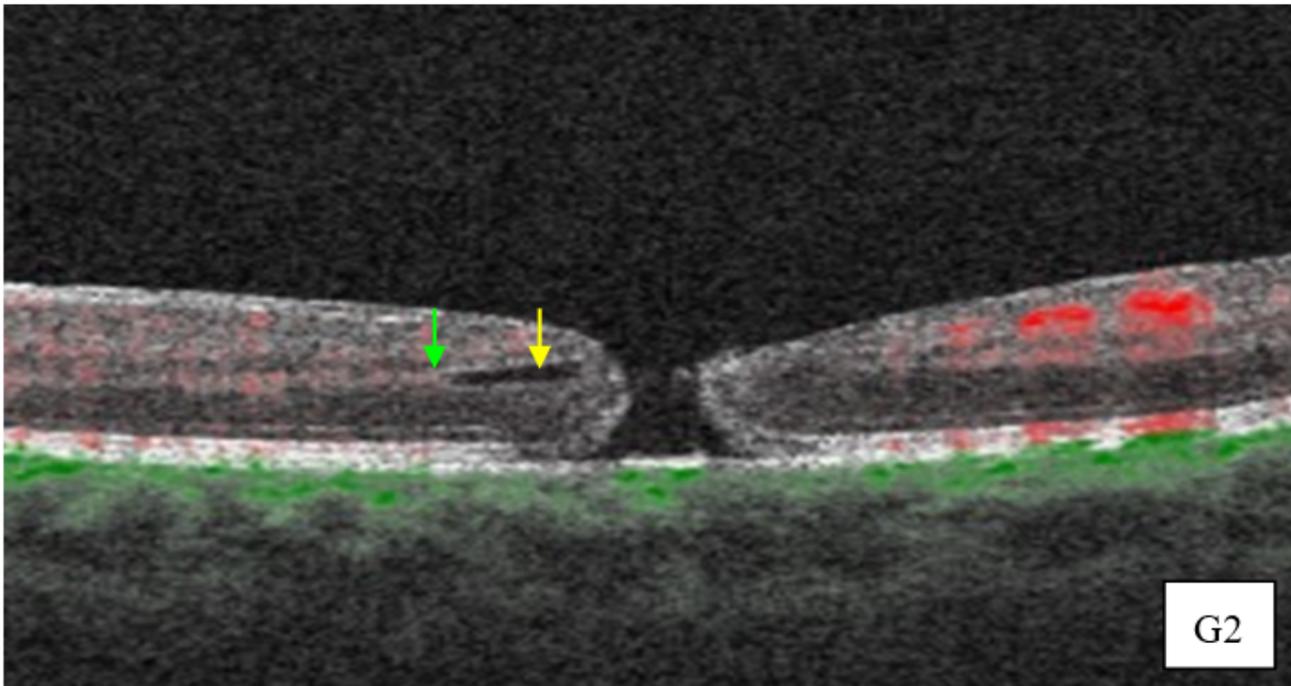
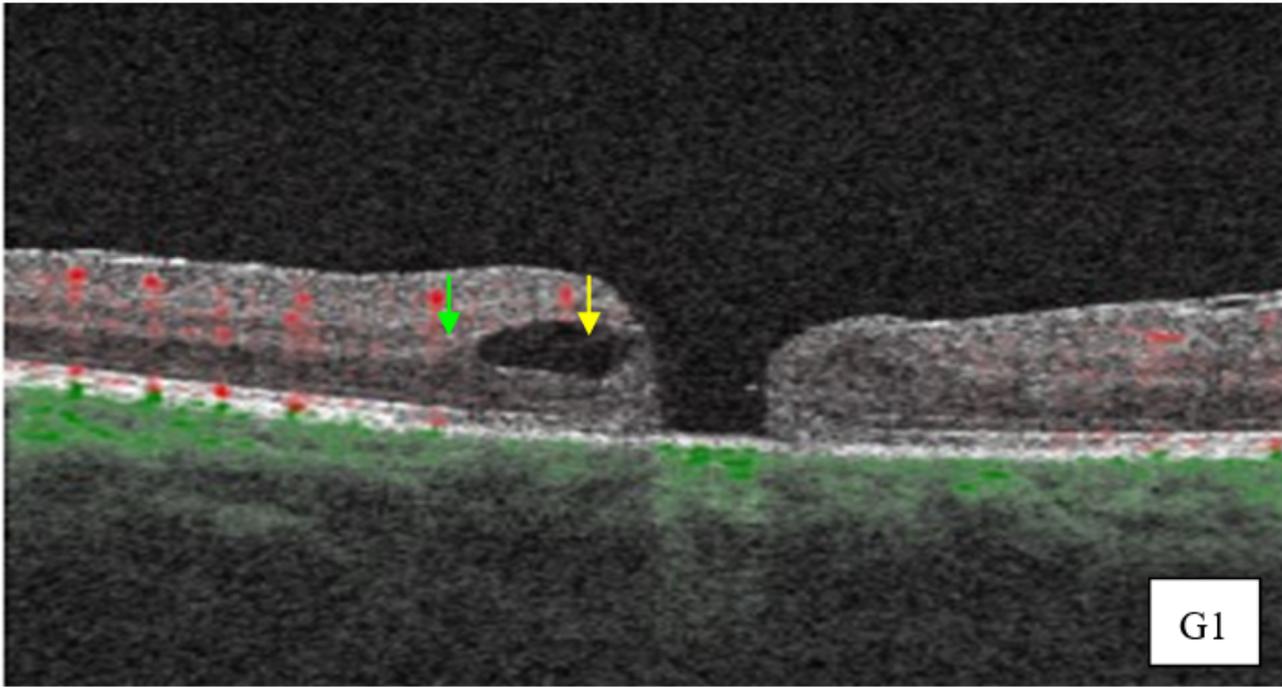
**Figure 2**

The morphology of retinal capillary. C1 shows the preoperative retinal SCP, C2 shows the postoperative retinal SCP, D1 shows the preoperative retinal DCP, and D2 shows the postoperative retinal DCP.



**Figure 3**

The scatter plots of MH diameter with the parameters of cysts in different retinal layers.



**Figure 4**

The formation of cysts in two eyes. In the two eyes, the formation of cysts can be seen in the retinal OPL+HFL complex around the hole (yellow arrow in the figure), but not in the INL (green arrow in the figure).

## Supplementary Files

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