Nonhomogenous Hyperreflectivity in the Choriocapillaris Layer on Optical Coherence Tomography Angiography Implies Early Treatment with Anti-VEGF for Central Serous Chorioretinopathy

Zhenzhen Zhao\textsuperscript{a,b} Jingfa Zhang\textsuperscript{a,b}

\textsuperscript{a}Department of Ophthalmology, Shanghai General Hospital (Shanghai First People’s Hospital), Shanghai Jiao Tong University, School of Medicine, Shanghai, China; \textsuperscript{b}National Clinical Research Center for Eye Diseases, Shanghai Key Laboratory of Ocular Fundus Diseases, Shanghai Engineering Center for Visual Science and Photomedicine, Shanghai Engineering Center for Precise Diagnosis and Treatment of Eye Diseases, Shanghai, China

Keywords
Central serous chorioretinopathy · Nonhomogenous hyperreflectivity · Anti-vascular endothelial growth factor · Optical coherence tomography angiography

Abstract

Introduction: Optical coherence tomography angiography (OCTA) facilitates the detection of choroidal neovascularization (CNV). This study explored the role of nonhomogenous hyperreflectivity implying putative CNV in the choriocapillaris layer on OCTA in central serous chorioretinopathy (CSCR). Methods: Thirteen eyes out of 12 patients with CSCR were examined with OCTA. The nonhomogenous hyperreflectivity was compared with the histological morphology of experimental CNV. The effect of intravitreal anti-vascular endothelial growth factor (VEGF) was evaluated by analyzing the changes in central macular thickness (CMT) and the height of subretinal fluid (SRF). Results: Comparison of the nonhomogenous hyperreflectivity on OCTA with the established CNV in two animal models strongly indicated these signals are putative CNV. During following-up, these nonhomogenous hyperreflectivity in CSCR developed into visible CNV on OCTA. Moreover, anti-VEGF treatment was effective to reduce both the SRF and CMT in CSCR with nonhomogenous hyperreflectivity or secondary CNV within 2 months. Conclusion: This study suggested that the nonhomogenous hyperreflectivity on OCTA could be served as a diagnostic biomarker for putative CNV in CSCR, implying early treatment with anti-VEGF.

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Introduction

Central serous chorioretinopathy (CSCR) is a common chorioretinal disease which is mainly characterized by serous detachment of the neurosensory retina due to fluid leakage through retinal pigment epithelium (RPE) [1]. CSCR is usually self-limiting with good visual recovery, whereas in some cases, CSCR may be complicated by persistent serous subretinal fluid (SRF) accumulation or
multiple recurrences with increased risk of irreversible photoreceptor damage and poor visual outcomes, thus complete elimination of SRF is the goal of CSCR treatment [2, 3]. Moreover, choroidal neovascularization (CNV), which has been reported with the incidence ranging from 2% to 15.6% in CSCR, is the most serious complication that causes vision loss [4, 5]. Although the etiology of CSCR remains poorly understood, choroidal changes are believed to play an important primary pathogenic role [6] and intravitreal anti-vascular endothelial growth factor (anti-VEGF) agents have been tried as a therapeutic strategy for CSCR [7, 8].

Optical coherence tomography angiography (OCTA) is a new noninvasive imaging technique which uses the motion contrast of moving blood flow to create images of the retinal and choroidal vasculature and shows significant textural changes in the choriocapillaris flow pattern [9], allowing diagnosis of CNV in CSCR with a greater sensitivity and specificity than traditional angiographic modalities [10]. Using OCTA, vascular abnormalities, such as neovascularization, can be detected at the choriocapillaris level with enhanced variable morphologies of CNV on OCTA, while vascular signals at the choriocapillaris level in normal eyes were found to be relatively consistent, demonstrating homogenous reflectivity on OCTA [8, 9]. In this study, “visible CNV” implies the tubular, cord-like, or plexiform hyperreflectivity in the choriocapillaris layer on en face of OCTA, which is created out of abnormal moving blood flow. Although the resolution of OCTA images is approaching histology level, there is still a certain gap between the two [11], implying that OCTA may not be sensitive enough to detect the fine fibrovascular changes of incipient CNV.

There are currently a variety of in vivo models used for the study of CNV which mimic the histological changes as shown in patients like neovascular age-related degeneration [12]. The laser-induced CNV model uses a laser to break Bruch’s membrane, which enables the growth of

Fig. 1. OCTA examinations of both eyes in a 50-year-old male, who was diagnosed as CSCR in the left eye. The images were obtained as the area of 3 × 3 mm², and both en face and b-scan of OCTA images were shown. OCTA showed the relatively normal appearance on en face (a) and b-scan (c) in the right eye. OCTA showed the representative nonhomogeneous hyperreflectivity in the choriocapillaris layer on en face (b) and the characteristic appearance of subretinal fluid (SRF) on b-scan (d) in the left eye. The nonhomogeneous hyperreflectivity (marked with * and outlined with yellow dotted line) were putative CNV.
abnormal neovessels originating from the choroid to the subretinal space [13]; in addition, we found traumatic injury of RPE-Bruch’s membrane-choriocapillaris complex (RBCC) by the needle tip during subretinal injection can also induce the formation of CNV.

During the clinical practice, we found some patients with CSCR, when detected with OCTA, demonstrated both SRF on b-scan and nonhomogenous hyperreflectivity in the choriocapillaris layer on en face of OCTA. We hypothesized that the nonhomogenous hyperreflectivity might indicate the incipient CNV (or putative CNV) even when no obvious vascular structure was detected on OCTA. To prove this hypothesis, we compared the microstructure of nonhomogenous hyperreflectivity in CSCR patients with the established CNV lesions derived from two animal models. The CNV morphologies in animal models detected with the fluorescence microscopy with a higher resolution and on the same scale strongly

Fig. 2. Development of nonhomogenous hyperreflectivity to visible CNV in the right eye of a 49-year-old male patient with CSCR. The images were obtained as the area of 3 × 3 mm², and the en face images of the choriocapillaris layer on OCTA were shown. a Nonhomogenous hyperreflectivity was marked with red arrowhead and outlined with yellow dotted line at the first presentation. The follow-ups were performed at 2 weeks (b), 5 weeks (c), and 2 months (d) after the first presentation. The development and progression to CNV with cord-like morphologies was outlined with yellow dotted line.
Fig. 3. OCTA showed that a 49-year-old female patient with chronic CSCR in the left eye, who developed the visible CNV from nonhomogenous hyperreflectivity within 2 years. The images were obtained as the area of $3 \times 3\, \text{mm}^2$, and both b-scan and the en face images of the choriocapillaris layer on OCTA were shown. a The en face of the choriocapillaris layer on OCTA showed several non-homogenous hyperreflectivity, which were magnified separately as I-IV and marked with yellow dotted lines in I-IV. Although no visible vessels were observed, the patients demonstrated obvious SRF with degenerating photoreceptor outer segments. b After 2-year follow-up, the visible CNV was developed on en face of OCTA, the SRF on b-scan was present with the deteriorated degeneration of photoreceptor outer segments. c After 2 intravitreal injections of aflibercept, the SRF was diminished on b-scan and no significant enlargement of the CNV.
persuade us that the nonhomogenous hyperreflectivity in CSCR patients are putative or incipient CNV. We retrospectively reviewed 13 eyes from 12 treatment-naïve patients, who were diagnosed as CSCR with nonhomogenous hyperreflectivity in the choriocapillaris layer on en face of OCTA and treated with anti-VEGF reagents. These patients benefited from the absorption of SRF and decreased nonhomogenous hyperreflectivity after anti-VEGF therapy, further demonstrating these nonhomogenous hyperreflectivity are putative CNV.

The present study provided a new opinion for the treatment of CSCR with nonhomogenous hyperreflectivity. We proposed that the nonhomogenous hyperreflectivity may serve as a diagnostic biomarker of putative CNV in patients with CSCR, who should initiate early anti-VEGF treatment.

**Methods**

**Study Design and Patients**

This study was performed at the Department of Ophthalmology, Shanghai General Hospital affiliated to Shanghai Jiao Tong University, Shanghai, China. A total of 13 eyes of 12 patients with CSCR were enrolled from April 2018 to February 2022. All patients underwent a comprehensive ophthalmological examination, including best-corrected visual acuity (BCVA), intraocular pressure, slit-lamp biomicroscopy, fundus photography, and OCTA (Optovue, Inc., Fremont, CA, USA). In this study, CSCR were diagnosed mainly according to SRF on b-scan of OCTA and the clinical features. One very important inclusion criterion is that OCTA images show obvious nonhomogeneous hyperreflectivity (Fig. 1b, Fig. 2a, Fig. 3a, Fig. 4a) or visible CNV (Fig. 2b–d, Fig. 3b, c). Patients with other ocular conditions commonly associated with serous SRF, such as CNV, polypoidal choroidal vasculopathy (PCV), diabetic macular edema, retinal vascular occlusion, and myopia < −6 diopters were excluded as well as patients with any active ocular inflammation. Other exclusion criteria include low image quality and any previous intraocular surgery history.

**OCTA Evaluation**

RTVue XR Avanti OCT system (Optovue, Inc., Fremont, CA, USA) was employed to obtain the images. The scan covered an area of 3 × 3 mm² volume scan (304 × 304 A-scans) or a 6 × 6 mm² volume scan (400 × 400 A-scans) sections centered on the fovea. Using the manufacturer’s software, we collected and analyzed the changes of the choriocapillaris layer in patients with CSCR, which is between the depths of 30 and 60 μm below the RPE- Bruch’s membrane complex. We also measured the height of SRF and central macular thickness (CMT) with the manufacturer’s software.

**Intravitreal Injection of Anti-VEGF Agents**

Patients diagnosed with CSCR with the nonhomogenous hyperreflectivity or visible CNV on OCTA images received the intra-
vitreal injections of aflibercept (2 mg/0.05 mL, 8 eyes), ranibizumab (0.5 mg/0.05 mL, 4 eyes), or conbercept (0.5 mg/0.05 mL, 1 eye). The intravitreal injection was performed at the infratemporal limbus through the eyeball’s pars plana under aseptic conditions using a 30-gauge needle. One week after intravitreal injection, the efficacy of anti-VEGF was evaluated, including the changes of SRF and CMT before and after anti-VEGF treatments. The number of intravitreal injections was determined according to the curative effect and the patient wishes.

**Histology of CNV in Animal Models**

The laser-induced CNV mice model was generated as described previously [14]. Traumatic CNV in rats was accidentally found when performing subretinal injections [15] in which the needle destroys the integrity of RBCC and then induces the ingrowth of neovessels into subretinal space.

For CNV immunofluorescence, the animals were killed and perfused intracardially with phosphate-buffered saline (PBS) buffered with 4% paraformaldehyde. Eyeballs were enucleated and RBCCs were isolated for immunofluorescence with isolectin B4 (1:1,000).

**Statistical Analyses**

GraphPad Prism Version 8.0 was used for statistical analysis. All values were presented as the number or mean ± standard deviation (mean ± SD). A p value <0.05 was considered as statistical significance.

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**Results**

**Baseline Characteristics of the Patients**

In this retrospective cohort study, as shown in Table 1, 13 eyes out of 12 patients were diagnosed as CSCR, including 9 males and 3 females, with the mean age 49.4 ± 7.7 years old. Among 13 eyes diagnosed as CSCR, 9 eyes demonstrated nonhomogenous hyperreflectivity in the choriocapillaris layer on OCTA, 3 eyes showed visible CNV, and 1 eye, initially displaying nonhomogenous hyperreflectivity, developed visible CNV during the follow-up within 2 months.

**Development and Progression of CNV from Nonhomogenous Hyperreflectivity**

Figure 1 shows the difference between both eyes detected with OCTA in a 50-year-old male. In his right eye, there were homogenous reflective signals in the choriocapillaris layer on en face of OCTA, and the sensory neuroretina was well attached to the RPE on b-scan of OCT. While in his left eye diagnosed as CSCR, there was nonhomogenous hyperreflectivity in the choriocapillaris layer on en face of OCTA, and the sensory neuroretina was detached from the RPE with obvious SRF on b-scan of OCTA. It was not surprising that the nonhomogenous

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**Table 1**. Characteristics of the patients with CSCR

| Criteria                                      | Distribution                                      |
|-----------------------------------------------|---------------------------------------------------|
| Patients, n                                   | 12                                                |
| Eyes, n                                       | 13                                                |
| Ages, years old                               | 49.4±7.7                                         |
| Gender, n                                     | Male (9) Female (3)                               |
| OCTA features in the choriocapillaris layer   | Only nonhomogenous hyperreflective signals (9 eyes)|
|                                               | Visible CNV (3 eyes)                              |
|                                               | Developed from nonhomogenous hyperreflective signals to visible CNV (1 eye) |
| The average injection number                  | 1.31±0.48                                        |
| The interval of follow-up, months             | 1.11±0.46                                        |
| Anti-VEGF reagents                            | Aflibercept (8 eyes)                              |
|                                               | Ranibizumab (4 eyes)                              |
|                                               | Conbercept (1 eye)                                |

The data are represented as the number or mean ± standard deviation.
Hyperreflectivity on OCTA could develop into CNV. Figure 2 shows the rapid development of CNV from the nonhomogenous hyperreflectivity on OCTA in a 49-year-old male patient, who was diagnosed as CSCR in the right eye (Fig. 2a). Two weeks after the first presentation, the CNV displayed as the cord-like morphology on OCTA, with increased size and changeable morphology 2 months later (Fig. 2b–d). Thus, we proposed that the nonhomogeneous hyperreflectivity could be served as a new biomarker for putative CNV in CSCR.

Moreover, we retrospectively reviewed a 49-year-old female patient who was diagnosed as CNV secondary to chronic CSCR in the left eye. When checking her medical record, we found that she was diagnosed as CSCR in the left eye 2 years ago and the OCTA showed several nonhomogenous hyperreflectivity in the choriocapillaris layer on en face, with SRF and degeneration of photoreceptor outer segment on b-scan (Fig. 3a). But at the visit 2 years later, she was demonstrated with visible CNV and persistent SRF with degeneration of photoreceptor outer segment on OCTA (Fig. 3b). After aflibercept treatment, the SRF was significantly decreased, but no obvious change for CNV with large caliber (Fig. 3c). These results implied that initial nonhomogenous hyperreflectivity may be served as a predictable biomarker for putative CNV in CSCR.

Relatively Low Resolution May Serve as a Possible Reason that Incipient CNV Appears as Nonhomogenous Hyperreflectivity in OCTA

To verify that these nonhomogenous hyperreflectivity are putative CNV, we compared the nonhomogenous hyperreflectivity in the right eye of a 49-year-old male patient with two CNV animal models, i.e., laser-induced mouse model and traumatic rat model (Fig. 4). When magnifying the area with nonhomogenous hyperreflectivity, the image was blurry and the detailed morphology cannot be clarified (Fig. 4a, b). However, in two CNV animal models, the CNV lesions are well identified and clearly observed under fluorescence microscopy with higher magnification and resolution, showing the ring-like structure in laser-induced mouse or flower-like structure of CNV in traumatic rat (Fig. 4c, d). Thus, at the same scale (200 μm), the nonhomogenous hyperreflectivity in the choriocapillaris detected with OCTA are comparable to the established CNV in two animal models detected under the fluorescence microscopy with higher magnification and resolution, further indicating the nonhomogenous hyperreflectivity in the choriocapillaris detected with OCTA are putative CNV and also suggesting if detected in higher resolution these signals may show more distinguished vascular structure. Overall, in this part, we demonstrated that OCTA images were with relatively low resolution and insufficient to reveal the fine microvascular structures in the regions of nonhomogenous hyperreflectivity compared with the examination under microscopy with high magnification and high resolution, which partially explained the reason that the incipient CNV in OCTA appeared as nonhomogenous hyperreflectivity rather than ring-like or strip-like structures.

Early Treatment with Anti-VEGF Is Effective for CSCR with Nonhomogeneous Hyperreflectivity

To figure out whether early anti-VEGF therapy can promote the absorption of SRF, we compared the changes of SRF and CMT in CSCR patients presented with nonhomogeneous hyperreflectivity on OCTA en face before and after anti-VEGF treatments within 2 months of observation. The interval between initial and final OCTA examinations within 2 months was 1.11 ± 0.46 months, and the number of anti-VEGF injections was 1.31 ± 0.48 (Table 1). As shown in Table 2 and Figure 5, the height of SRF was reduced from 209.4 ± 129.0 μm to 55.2 ± 38.3 μm (n = 13, p < 0.005), and CMT was reduced from 420.6 ± 128.6 μm to 237.2 ± 42.2 μm (n = 13, p < 0.0001), demonstrating anti-VEGF is effective to decrease SRF and CMT in CSCR patients with nonhomogeneous hyperreflectivity or secondary CNV. Besides, no side effects related to either ocular or systemic events were observed after anti-VEGF treatments.

Discussion

In this study, we found that the nonhomogenous hyperreflectivity in the choriocapillaris layer on en face of OCTA are putative CNV in CSCR patients, and these sig-

| Baseline | Treatment | p value |
|----------|-----------|---------|
| SRF, μm  | 209.4±129.0 | 55.2±38.3 | 0.005* |
| CMT, μm  | 420.6±128.6 | 237.2±42.2 | <0.0001* |

SRF, subretinal fluid; CMT, central macular thickness. n = 13 eyes. * Paired t test.
Nonhomogenous Hyperreflectivity on OCTA Is Putative CNV

Nonhomogenous Hyperreflectivity on OCTA was comparable with CNV in both the laser-induced mouse model and traumatic rat model when evaluated at the same scale. Under fluorescence microscopy, the morphology of CNV was well evidenced and characterized with high magnification and high resolution. The appearance of SRF and CMT might be secondary to the leakage of these nonhomogenous hyperreflectivity, which is responsive to anti-VEGF treatment as a proof of concept, further confirming that these nonhomogenous hyperreflectivity might be presented as the incipient CNV. Thus, we proposed that the nonhomogenous hyperreflectivity in the choriocapillaris layer on en face of OCTA might be served as a sensitive biomarker for putative CNV.

To our knowledge, this is the first study to track the nonhomogenous hyperreflectivity of OCTA in CSCR and propose its role to develop into CNV in patients with CSCR. Nowadays, OCTA is widely used in routine clinic examinations, as a promising, noninvasive imaging technique that enables us to view different layers of blood flow and perfusion in both the retina and the choroid [16]. Owing to the short acquisition time, OCTA is beneficial to patients and can also be repeated at any time during follow-up. Recent studies have demonstrated the value of OCTA in patients with CSCR, and the choriocapillaris images in OCTA may facilitate the elucidation of the pathogenesis of CSCR [17, 18]. In a direct comparison of fluorescein angiography (FA), indocyanine green angiography (ICGA), OCT, and OCTA, it has been reported that CNV secondary to CSCR was not visible with other imaging techniques except OCTA, which suggests the high-sensitivity of OCTA in the detection of neovascularization [19]. Our study demonstrates that the normal eye exhibits a relatively homogeneous choriocapillaris vascular network as described before, while patients with CSCR exhibit areas of increased and decreased OCTA signals at the level of the choriocapillaris in line with previous study [20]. By collecting the OCTA images of different patients with CSCR, we found some patients had the similar feature of nonhomogenous hyperreflectivity in the choriocapillaris layer on en face of OCTA with persistent SRF as reported before [18], and anti-VEGF therapy had a good short-term effect in the absorption of SRF in these patients, thus we suggest that the nonhomogenous hyperreflectivity could be served as a sensitive biomarker for putative or incipient CNV, implying the early initiation of anti-VEGF treatment.

VEGF is an important factor leading to the development of a neovascular network and exudation. Anti-VEGF therapy has been reported effective to treat neovascularization-associated ocular diseases [21]. Intravitreal anti-VEGF agents have been reported to treat CSCR [2] with controversial results due to the large variation in therapeutic efficacy among previous studies [22, 23]. However, these previous studies did not analyze the patients according to the presence or absence of CNV on OCTA. Recently, Song et al. [24] reported that anti-VEGF therapy showed more effective short-term outcomes in chronic CSCR patients with CNV than those without CNV classified by OCTA, and what they called CNV was similar to the visible CNV in our study (Fig. 3). In accordance with this report, we also found that anti-VEGF therapy could effectively reduce both SRF and CMT.
CMT in CSCR patients (Fig. 5) with nonhomogenous hyperreflectivity in the choriocapillaris layer on en face in OCTA, further validating the active leakage of fluid from putative CNV into subretinal space or neural retina. Besides, we observed 2 patients initially displayed nonhomogenous hyperreflectivity in the choriocapillaris layer developed into CNV (Fig. 2, 3), which were also responsive to anti-VEGF treatment. Moreover, we also examined the change in visual acuity in 8 patients before and after anti-VEGF treatment and found no significant difference.

Although the pathogenesis of CSCR remains poorly understood, choroidal hyperpermeability is presumably related to abnormal choroidal circulation especially the choriocapillaris attenuation and the breakdown of the RPE monolayer is also thought to result in SRF accumulation [2, 25]. CSCR is now considered as one of the pachychoroid spectrum diseases, characterized as thick choroid and choroidal pachyvessels [26]. Due to the not-high-enough resolution, OCTA failed to detect fine CNV at every early stage, in which the vascular endothelial cells proliferate and start to form incipient CNV with active leakage, which could be well detected and characterized under the microscope. In addition, by 2-year follow-up of a patient with chorionic CSCR, we found that the nonhomogenous hyperreflectivity detected initially did not share the same location with the final visible CNV, indicating the putative CNV, presented as nonhomogenous hyperreflectivity in OCTA, might undergo a dynamic remodeling during the process of growth, development, and maturation.

This study has several limitations. First, the sample size is relatively small. Second, the number of long-term follow-ups needs to be increased for statistical analysis. Also, our study did not compare efficacy among different anti-VEGF drugs. Thus, a multicenter study with a large number of patients is needed to specialize in the feature of nonhomogenous hyperreflectivity in OCTA of patients with CSCR and confirm these signals as a potential biomarker of putative CNV in patients with CSCR. Also, the long-term effect of various anti-VEGF drugs needs to be further evaluated in patients with CSCR.

**Conclusion**

In conclusion, we proposed the nonhomogenous hyperreflectivity in the choriocapillaris layer on en face of OCTA as a potential biomarker for putative or incipient CNV in patients with CSCR. By comparing these putative CNV signals on OCTA with the histological morphology of CNV in animal models, this nonhomogenous hyperreflectivity on OCTA was supposed to be putative CNV due to the relatively low resolution of OCTA. In addition, early initiation of anti-VEGF treatment was beneficial to CSCR patients with nonhomogenous hyperreflectivity in OCTA in terms of decreased SRF and CMT, which might be due to the diminishment of putative CNV by anti-VEGF.

**Statement of Ethics**

All procedures performed in studies involving human participants were in accordance with the ethical standards of the Institutional Research Committee and with the Declaration of Helsinki. Written informed consent was obtained from all individual participants included in the study. The study was approved by the Ethics Committee of the Shanghai General Hospital, Shanghai Jiao Tong University, Shanghai, China (Approval No. 2020KY205-2).

**Conflict of Interest Statement**

The authors have no conflicts of interest to declare.

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**Author Contributions**

Z.Z. and J.Z. drafted the article, contributed to the conception and design of the experiments, and were responsible for data collection, analysis, and interpretation. Z.Z. contributed to methodology, investigation, and data curation. J.Z. contributed to the article revision and funding acquisition and is the guarantor of this work, who has full access to all the data in this study and takes responsibility for the integrity and accuracy of the data.

**Data Availability Statement**

The data that support the findings of this study are available from the corresponding author upon reasonable request.
