• Clinical Research •

Quantitative analysis of retinal intermediate and deep capillary plexus in patients with retinal deep vascular complex ischemia

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Abstract

● **AIM:** To quantitatively analyze the retinal intermediate and deep capillary plexus (ICP and DCP) in patients with retinal deep vascular complex ischemia (RDVCI), using 3D projection artifacts removal (3D PAR) optical coherence tomography angiography (OCTA).

● **METHODS:** RDVCI patients and gender- and age-matched healthy controls were assessed and underwent OCTA examinations. The parafoveal vessel density (PFVD) of retinal deep vascular complex (DVC), ICP, and DCP were analyzed, and the percentage of reduction (PR) of PFVD was calculated.

● **RESULTS:** Twenty-four eyes in 22 RDVCI patients (20 in acute phase and 4 in chronic phase) and 24 eyes of 22 healthy subjects were enrolled as the control group. Significant reduction of PFVD in DVC, ICP, and DCP was observed in comparison with the controls (DVC: acute: 43.59%±6.58% *vs* 49.92%±5.49%, PR=12.69%; chronic: 43.50%±3.33% *vs* 51.20%±3.80%, PR=15.04%. ICP: acute: 40.28%±7.91% *vs* 46.97%±7.14%, PR=14.23%; chronic: 41.48%±2.87% *vs* 46.43%±3.29%, PR=10.66%. DCP: acute: 45.44%±8.27% *vs* 51.51%±9.97%, PR=11.79%; chronic: 37.78%±3.48% *vs* 51.73%±5.17%, PR=26.97%; all $P<0.05$). No significant PR difference was found among DVC, ICP, and DCP of RDVCI in acute phase ($P=0.812$), but significant difference in chronic phase ($P=0.006$, DVC *vs* DCP, ICP *vs* DCP). No significant difference in PR between acute and chronic phases in the DVC ($P=0.735$) or ICP ($P=0.681$) was found, but significant difference in the DCP ($P=0.041$).

● **CONCLUSION:** The PFVD of DVC, ICP, and DCP in RDVCI is significantly decreased in both acute and chronic phases. ICP impairment is stabilized from acute to chronic phase in RDVCI, whereas subsequent DCP impairment is uncovered and can be explained by ischemia-reperfusion damage.

● **KEYWORDS:** intermediate and deep capillary plexus; 3D projection artifacts removal; optical coherence tomography angiography; retinal deep vascular complex ischemia

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INTRODUCTION

Optical coherence tomography (OCT) angiography (OCTA) permits noninvasive and rapid three-dimensional (3D) visualization of retinal and choroidal vasculature with high resolution and innovates the understanding of enormous pathologies in ophthalmology[1-2]. However, image defects called artifacts often occur and can lead to data misinterpretation. Projection artifacts, appearing as a shadow of superficial blood perfusion in a deep layer, is one of the non-negligible types[3]. Previously, slab subtraction (SS) algorithms were often applied to remove projection artifacts, but an SS algorithm also leads to false-negative voxels and underestimation of blood perfusion on OCTA images[4-6]. Moreover, it does not attenuate flow projection within the slab, so we cannot use it to delineate separate vascular plexuses without pre-defining their slab boundaries[7-8]. Recently, 3D projection artifact removal (3D PAR) OCTA, with a refined projection-resolved algorithm, has been proposed. This novel algorithm filters projection artifacts but keeps flow signals, on the basis that *in situ* flow demonstrates higher intensity-

decorrelation value than shallower voxels do[7-8]. With 3D PAR OCTA, projection artifacts from the superficial vascular plexus (SVP) are largely reduced; two distinct sublayers of the DVC, in accordance with the retinal anatomy in human eyes, the intermediate capillary plexus (ICP), and the deep capillary plexus (DCP), can be observed more authentically, and quantitative analysis of retinal vascular perfusion is obviously more convincing[8-10].

Retinal deep vascular complex ischemia (RDVCI), previously mentioned as deep capillary ischemia (DCI), also called paracentral acute middle maculopathy (PAMM), is associated with various retinal vascular diseases such as retinal artery occlusion (RAO), retinal vein occlusion (RVO), diabetic retinopathy (DR), Purtscher retinopathy, and other ischemic retinopathies[11-13]. Our previous study indicated thickening and hyper-reflectivity of the inner nuclear layer (INL; where located ICP and DCP) in acute phase, while thinning and normal/hypo-reflectivity of INL in chronic phase, using spectral domain optical coherence tomography (SD-OCT)[14]. Although RDVCI has a strong pathophysiological link to DVC ischemia, and previous study conducted in a small population using OCTA with SS algorithms showed some ischemic changes, direct evidence with more convincing and advanced methodology still remains to be seen, especially in the acute phase[15]. Moreover, the DVC can be subdivided into ICP and DCP, according to human anatomy. Previous study focused only on the DVC; the ICP and DCP impairment pattern remains unknown[16]. Traditional fluorescein angiography (FA) is often unable to identify retinal ischemia in deep layers in RDVCI patients, as ICP and DCP are largely covered by SVP[14]. Previous OCTA also had great difficulties in revealing deep ischemia, as the hyper-reflectivity of inner plexiform layer (IPL) results in enormous projection artifacts in RDVCI patients, leading to a false-positive bias in measuring the actual deep vascular perfusion[16]. The development of 3D PAR OCTA brings novel insight to DVC evaluation[10].

The purpose of this study is to characterize the spectrum of retinal deep capillary vascular perfusion changes in RDVCI. In this study, we observed and evaluated the parafoveal vessel density (PFVD) of the DVC and its two sublayers, using 3D PAR OCTA, identifying the pattern of ICP and DCP involvements in patients with acute and chronic RDVCI.

SUBJECTS AND METHODS

Ethical Approval This case-control study was approved by the Institutional Review Board and Ethics Committee of Shanghai General Hospital and adhered to the tenets of the Declaration of Helsinki. Informed consent was waived because of the retrospective nature of the study.

Subjects This was a retrospective, nonconsecutive, observational study, which included 24 eyes of 22 RDVCI patients and 1:1 gender- and age-matched (range: 12mo) healthy controls in the Department of Ophthalmology of Shanghai General Hospital between December 1, 2014, and May 31, 2018. Clinical data and multimodal imaging data, including high-resolution SD-OCT, OCTA, and color and red-free fundus photography for each patient and control were obtained at the time of presentation and reviewed. The diagnostic criteria of RDVCI were based on the original description of DCI as a history of acute-onset scotoma, adapted from previous reports[11,14]. All patients displayed characteristic abnormalities in their imaging, including plaque-like, hyper-reflective bands on an edematous INL during the acute phase or thinning and atrophy of the INL during the chronic phase on SD-OCT, with or without retinal whitening on color fundus photographs and hyper-reflectivity on red-free fundus photographs.

Instruments and Scan Patterns The OCTA images were obtained as 3×3 mm^2 scans centered on the macula, using the AngioVue OCT angiography system (Optovue, Inc.; Fremont, CA, USA). AngioVue applied a light source centered on 840 nm and with a bandwidth of 45 nm, operates 304×304 A-scans at 70 000 A-scans per second. It uses a split-spectrum amplitude decorrelation angiography algorithm (SSADA) and motion correction technique that merges two consecutive orthogonal scans to calculate and generate angiograms[17]. All scans were performed by well-trained operators (Li XX, Qian TQ, and Lyu YN), following manufacturers' guidelines.

The OCT angiography software (Angio Analytics, Optovue, Inc.; Fremont, CA, USA) was applied to segment the DVC, ICP, and DCP on 3×3 mm^2 scans. In each patient, the layer segmentation settings stay consistent as default. The DVC was taken from IPL (offset -10 μm) to outer plexiform layer (OPL; offset 10 μm). The DVC can be further divided into ICP and DCP. The ICP was taken from the IPL (offset -10 μm) to the IPL (offset 30 μm), and the DCP was taken from the IPL (offset 30 μm) to the OPL (offset 10 μm)[18]. In cases of automatic segmentation failure resulting from INL thinning, two thinner 20 μm bands dividing the INL were manually adjusted by a well-trained operator (Li XX) to include ICP and DCP. The parafoveal area was defined according to the default EDTRS grid setting, as a concentric ring with an inner diameter of 1 mm and an outer diameter of 3 mm. The area center was automatically provided by the software and examined by a well-trained operator (Li XX) to confirm. The PFVD was then calculated automatically by the software. To compare the angiograms generated with and without 3D PAR technology, all OCTA images underwent two analyses successively (version 2017.02 without 3D PAR and then version 2018.01 with 3D PAR). The percentage of reduction (PR) was defined as the reduction proportion of PFVD in the RDVCI patients compared to the mean PFVD of the control group.

Table 1 Mean PFVD of the DVC, ICP, and DCP in eyes affected by retinal deep capillary complex ischemia compared with the healthy control eyes mean±SD

Parameters	PFVD (median, 25% percentile, 75% percentile)			P^a
	Total (n=24)	Acute lesions (n=20)	Chronic lesions (n=4)	
DVC				
RDVCI eyes (%)	43.57±6.10 (43.15, 40.55, 46.68)	43.59±6.58 (43.15, 39.90, 46.68)	43.50±3.33 (42.35, 35.65, 47.03)	
Control eyes (%)	50.13±5.20 (50.25, 45.10, 53.33)	49.92±5.49 (50.25, 44.68, 53.33)	51.20±3.80 (50.95, 47.68, 54.98)	
PR (%)	13.08±12.17 (13.92, 6.89, 19.11)	12.69±13.18 (13.56, 6.50, 20.07)	15.04±6.51 (17.29, 8.15, 30.37)	0.438
P^b	<0.001	0.002	0.047	
ICP				
RDVCI eyes (%)	40.48±7.21 (40.75, 34.73, 44.35)	40.28±7.91 (38.20, 33.68, 46.58)	41.48±2.87 (40.75, 35.13, 42.10)	
Control eyes (%)	46.88±6.60 (45.60, 42.33, 53.65)	46.97±7.14 (45.20, 40.43, 54.15)	46.43±3.29 (45.90, 43.68, 49.70)	
PR (%)	13.65±15.38 (13.08, 5.40, 25.93)	14.23±16.84 (18.98, 1.22, 28.58)	10.66±9.99 (10.39, 8.89, 12.71)	0.794
P^b	0.003	0.008	0.029	
DCP				
RDVCI eyes (%)	44.16±8.16 (45.30, 35.25, 51.00)	45.44±8.27 (46.10, 41.80, 51.85)	37.78±3.48 (38.80, 34.10, 40.43)	
Control eyes (%)	51.55±9.25 (55.55, 43.13, 59.00)	51.51±9.97 (56.35, 42.15, 59.65)	51.73±5.17 (53.15, 46.28, 55.75)	
PR (%)	14.34±15.83 (12.12, 1.07, 25.80)	11.79±16.05 (10.50, 0.66, 18.85)	26.97±6.72 (24.99, 21.85, 34.07)	0.036
P^b	0.005	0.043	0.029	

PFVD: Parafoveal vessel density; DCP: Deep capillary plexus; DVC: Deep vascular complex; ICP: Intermediate capillary plexus; PR: Percentage of reduction; RDVCI: Retinal deep capillary complex ischemia; [a]P values of comparison between acute and chronic lesions using Mann-Whitney test; [b]P values of comparison between total and acute RDVCI and control eyes using independent t-test, chronic RDVCI and control eyes using Mann-Whitney test.

Statistical Analysis Data analysis was performed using Prism version 5.0 (GraphPad Software, Inc., La Jolla, CA, USA) and SPSS software version 18 (SPSS Inc., Chicago, IL, USA). The distributions of the data sets were checked for normality by using Kolmogorov-Smirnov tests, and P>0.10 indicated that the data was normally distributed. The repeated measures one-way analysis of variance (ANOVA), with Bonferroni's post-test, independent t-test, and Mann-Whitney test were used to compare the PFVD and PR. P-value <0.05 was considered to be statistically significant.

RESULTS

Demographics This study included 24 eyes of 22 RDVCI patients (15 males, 68.18% and 7 females, 31.82%) with a mean age of 59.31±12.80y; 20 patients were unilaterally affected and two were bilaterally affected. Of the 24 affected eyes, 20 in 18 patients demonstrated hyper-reflectivity and edema of the INL consistent with acute RDVCI lesions, and 4 of 4 patients demonstrated thinning and atrophy of the INL consistent with chronic RDVCI lesions according to SD-OCT. Etiologies leading to RDVCI included RAO (n=15), artery perfusion deficiency secondary to RVO (n=7), and RVO (n=2). Twenty-four eyes of 22 healthy subjects (15 males, 68.18% and 7 females, 31.82%) with a mean age of 59.36±12.75y were enrolled as the control group (age and gender matched with each patient separately). The age displayed no significant difference between RDVCI patients and healthy controls (P=0.622).

Optical Coherence Tomography Angiography The deep vascular angiograms in OCTA demonstrated extensive and profound projection artifacts from the superficial capillary plexus inconsistent with actual vascular perfusion without the application of 3D PAR on both B-scan and C-scan images. After 3D PAR, the projection artifacts were greatly attenuated. On account of the vivid demonstration of the DVC without disturbance of projection artifacts, DVC can be subdivided into ICP and DCP in accordance with human anatomy, and convincing analysis of the ICP and DCP can be done. Manual segmentation was conducted in 7 of 24 OCTA images.

Quantitative analysis was carried out in all cases and the measurements are summarized in Tables 1 and 2. The PFVD of the DVC, ICP, and DCP in RDVCI patients was significantly decreased in both the acute and chronic phases.

Between the acute and chronic RDVCI phases, the PR revealed no significant difference in the DVC (P=0.735) and ICP (P=0.681), whereas significant reduction was observed in the DCP (P=0.041).

The PR displayed no significant difference among the DVC, ICP, and DCP in the acute phase (P=0.812), whereas significant difference was found in the chronic phase (P=0.006). The mean difference and 95%CI of Bonferroni's post-test are listed in Table 1. Bonferroni' post-test demonstrated no significant difference in any of the three pairs in the acute phase, whereas in the chronic phase, no significant difference was found in

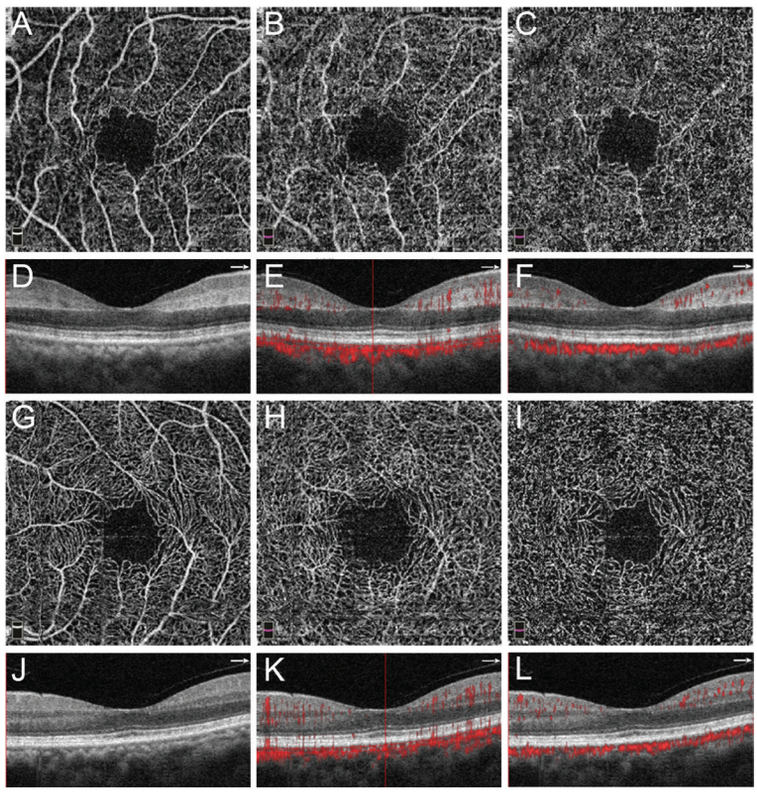

Figure 1 OCTA images of a 63-year-old woman with CRVO in her right eye (OD) A: En-face OCTA images of SVC OD shows blood flow perfusion of the retinal SCP; B: En-face OCTA images of DVC OD shows tremendous projection artifacts resembling SVC without 3D projection artifacts removal (PAR), overlapping perfusion of retinal ICP and DCP; C: With 3D PAR, OD DVC OCTA demonstrates reduced blood flow perfusion; D: SD-OCT shows hyper-reflectivity and thickening of INL OD; E: OD OCTA B-scan demonstrates projection artifacts on deeper retina (especially on INL); F: Projection artifacts are enormously attenuated after 3D PAR; G: En-face SVC OCTA in the left eye (OS) shows SCP perfusion; H: En-face OS DVC OCTA shows projection artifacts from SVC, though less perceptible than in the OD; I: After 3D PAR, OS DVC OCTA demonstrates more authentic blood flow perfusion; J: OS SD-OCT shows normal macular morphous; K: OS OCTA B-scan shows projection artifacts, less than in OD comparatively, corresponding C-scan respectively; L: Projection artifacts are attenuated after 3D PAR. SVC: Superficial vascular complex; SCP: Superficial capillary plexus; DVC: Deep vascular complex; ICP and DCP: Intermediate and deep capillary plexus; INL: Inner nuclear layer.

Table 2 Post-hoc comparison of PFVD PR concerning DVC, ICP, and DCP in acute and chronic lesions

Parameters	Acute lesions (*n*=20)		Chronic lesions (*n*=4)	
	Mean difference (%)	95%CI	Mean difference (%)	95%CI
DVC-ICP	1.54	-10.50 to 13.59	-4.38	-15.83 to 7.08
DVC-DCP	-0.90	-12.94 to 11.15	11.93	0.463 to 23.39
ICP-DCP	-2.44	-14.48 to 9.603	16.31	4.851 to 27.76

PFVD: Parafoveal vessel density; DCP: Deep capillary plexus; DVC: Deep vascular complex; PR: Percentage of reduction; ICP: Intermediate capillary plexus.

one pair out of three (DVC *vs* ICP) and significant difference appeared in the other two pairs (DVC *vs* DCP and ICP *vs* DCP).

Representative Cases Representative cases are described in detail, with corresponding figures.

Case 1 (Figure 1): A 63-year-old woman with systemic hypertension presented with sudden painless vision loss in her right eye. Best-corrected visual acuity (BCVA) was 20/400 in the right eye and 20/25 in the left eye. Clinical examination revealed a central retinal artery occlusion (CRAO) of the right eye, but the left eye was unremarkable. In the right, affected eye, SD-OCT demonstrated thickening and hyper-reflectivity of the INL consistent with RDVCI. En-face OCTA of the DVC without 3D PAR showed obvious projection artifacts from SVP (resembling large retinal vessels that do not exist), and the PFVD was 60.7%. With 3D PAR, projection artifacts were attenuated and the PFVD was 45.9%. B-scans also revealed removal of projection artifacts on the level of the INL, using 3D PAR OCTA. In the normal left eye, SD-OCT

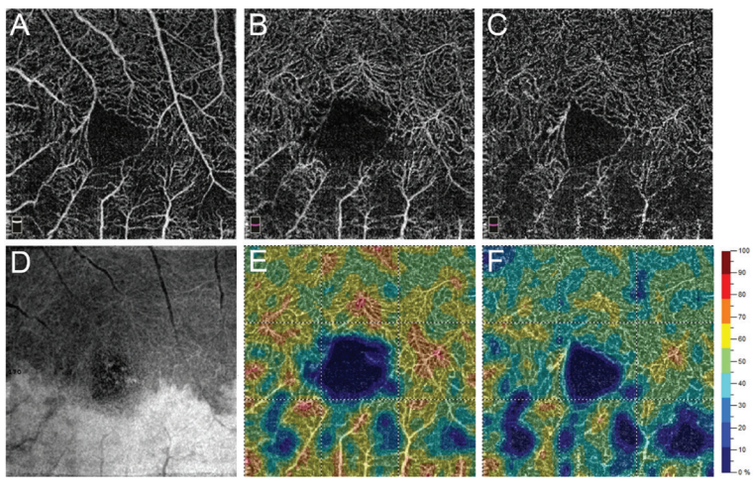

Figure 2 OCTA images of a 71-year-old woman with retinal artery perfusion deficiency secondary to BRVO in her left eye A: En-face OCTA images of SVC shows normal blood flow perfusion in area superior to macula and reduced perfusion in area inferior to macula; B: En-face OCTA images of DVC shows enhanced projection artifacts in affected inferior area corresponding to SVC OCTA; C: DVC OCTA with 3D PAR demonstrates reduced perfusion in affected area; D: DVC Infrared image shows retinal whitening of inferior area consistent with OCTA; E-F: Corresponding DVC vessel density images with and without 3D PAR generated by Angio Analytics. SVC: Superficial vascular complex; DVC: Deep vascular complex.

was unremarkable, slight projection artifacts from SVP were removed after using 3D PAR, and the PFVD of the DVC was 61.5% without 3D PAR and 51.1%, using 3D PAR. This case demonstrated the importance of 3D PAR technology in evaluating deep retinal vasculature of the RDVCI patient.

Case 2 (Figure 2): A 71-year-old woman with systemic hypertension and aortic calcification presented with a new paracentral scotoma three months after sudden painless superior visual field loss in the left eye. BCVA was 20/30 and clinical examination demonstrated an inferotemporal artery perfusion deficiency secondary to branch retinal vein occlusion (BRVO) in the affected eye. The 3×3 mm^2 OCTA scan of SVP was unremarkable, and the infrared (IR) image of the INL revealed retinal hyper-reflectivity (white) involving the inferior retina just adjacent to the macula. Without 3D PAR, the en-face OCTA of the DVC displayed remarkable projection artifacts in the affected area. However, projection artifacts were largely removed with 3D PAR technology. The vessel density image generated by Angio Analytics revealed vessel density reduction of the affected area and the removal of false-positive large retinal vessels on the DVC attenuated vessel density of the entire scan area, though in the inferior parafoveal area (from 52.1% to 38.7%) more than in the superior parafoveal area (from 58.1% to 49.1%). This case shows that the projection artifact removal facilitates vessel density evaluation within the same OCTA scan.

Case 3 (Figure 3): A 63-year-old man with no systemic diseases presented with sudden painless loss in vision and BCVA of 20/100 in the left eye. Clinical examination revealed a branch retinal artery occlusion (BRAO). SD-OCT revealed characteristic thickening and hyper-reflectivity on the INL level. IR showed diffuse INL hyper-reflectivity of the superior retina and scattered INL hyper-reflectivity of the inferior retina (avoidant artery-adjacent area). 3D PAR attenuated projection artifacts on the DVC, which could be observed on both B-scan and C-scan, facilitating ICP and DCP observation. The IR and 3D PAR OCTA of the DVC, ICP, and DCP demonstrated a similar affecting pattern. This case illustrates the pattern of ICP and DCP involvement in the acute lesion of RDVCI.

Case 4 (Figure 4): A 30-year-old woman with no systemic diseases presented with sudden painless vision loss in the right eye. Her BCVA was 20/80 and clinical examination revealed a BRAO in the affected right eye. SD-OCT demonstrated thinning and atrophy of the INL. The IR and 3D PAR OCTA of the DVC, ICP, and DCP demonstrated an unbalanced affecting pattern, and the DCP demonstrated subsequent impairment and more attenuation in vessel density. The PFVD of the DVC, ICP, and DCP were 43.8%, 41.2%, and 37.7%, respectively. This case illustrates the pattern of ICP and DCP involvement in a chronic lesion of RDVCI.

DISCUSSION

Previously, retinal vasculature was divided into SVC and DVC in OCTA due to projection artifact removal limits, but in the new OCTA segmentation nomenclature proposed by Zhang *et al*[7], retinal vasculature is divided into four layers: radial peripapillary capillary plexus (RPCP), SVP, ICP, and DCP corresponding to human anatomy, with the latter three layers in the macula.

In this study, we performed quantitative OCTA analysis of the DVC, ICP, and DCP on eyes with RDVCI and on normal eyes.

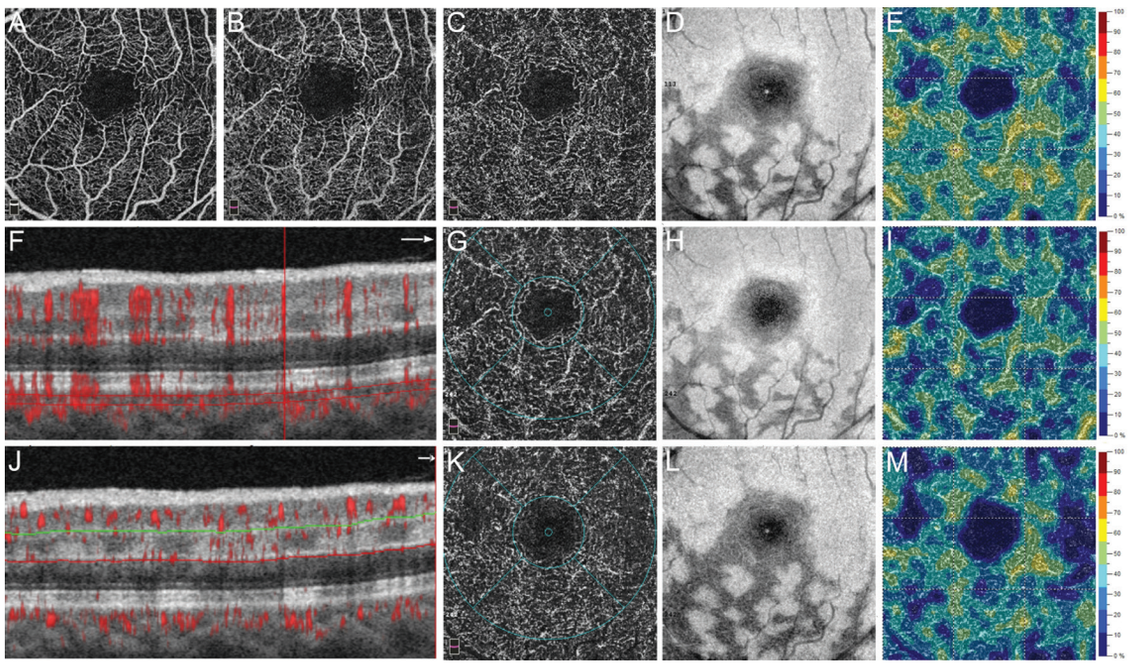

Figure 3 OCTA images of a 63-year-old man with BRVO in his left eye A: En-face OCTA images of SVC shows blood flow perfusion of retinal SCP; B: En-face OCTA images of DVC without 3D PAR shows projection artifacts resembling SVC; C: With 3D PAR, DVC OCTA demonstrates reduced blood flow perfusion; D, H, and L: Infrared image of DVC, ICP, and DCP shows similar entire retinal whitening of superior area and scattered inferior area in accordance with OCTA; E, I, and M: Corresponding vessel density images with 3D PAR generated by Angio Analytics reveal similar perfusion attenuation pattern; F: OCTA B-scan demonstrates artifacts projecting on deeper retina, especially on INL with band-like, hyper-reflective lesions; G and K: En-face 3D PAR OCTA images of ICP and DCP shows similar lesion pattern; J: Projection artifacts are attenuated after 3D PAR. SVC: Superficial vascular complex; SCP: Superficial capillary plexus; DVC: Deep vascular complex; PAR: Projection artifact removal; ICP and DCP: Intermediate and deep capillary plexus; INL: Inner nuclear layer.

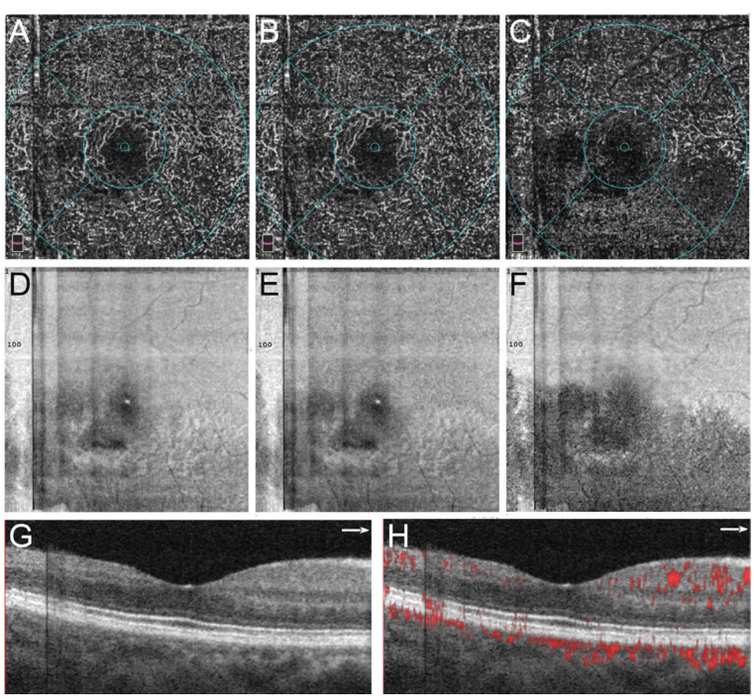

Figure 4 OCTA images of a 30-year-old woman with BRVO in her right eye A-C: En-face OCTA images with 3D PAR of DVC, ICP and DCP shows blood flow perfusion reduction, whereas DCP OCTA demonstrates more attenuation; D-F: Infrared image of DVC, ICP, and DCP shows retinal lesions in inferior area adjacent to macula, corresponding to OCTA; G: SD-OCT across macula shows thinning and atrophy of INL temporal to macula and normal INL nasal to macula; H: 3D PAR OCTA B-scan across macula reveals perfusion attenuation in lesion area. DVC: Deep vascular complex; PAR: Projection artifact removal; ICP and DCP: Intermediate and deep capillary plexus; INL: Inner nuclear layer.

As described previously, we elaborated the involvement pattern of vessel perfusion on the DVC (ICP and DCP) in RDVCI eyes by using 3D PAR OCTA. In the acute phase, reduction of DVC perfusion was observed, and ICP and DCP perfusion was impaired simultaneously and equivalently. As RDVCI evolved, the DVC perfusion stabilized with unequal evolution of the ICP and DCP. ICP perfusion was stable, whereas DCP perfusion attenuation was continuous.

RDVCI, previously mentioned as DCI, also called PAMM in its acute phase, is a recently reported SD-OCT lesion defined as middle retinal layer involvement at the level of the INL flanked by the ICP and DCP. It was reported in association with various vascular ischemic diseases such as RAO, RVO, and DR, with (in most cases) or without retinal superficial capillary ischemia (RSCI)[19]. RSCI has been well-defined in our previous study as the involvement of the capillaries of SVP which resides in the ganglion cell layer, appearing in acute or chronic lesion patterns as RDVCI but on superficial retinal layers[12]. RSCI can also exist alone without affecting RDVCI and thus diagnosed as cotton-wool spot in the acute phase and retinal depression sign in the chronic phase in clinical practice[20]. The ischemia of SVP in RAO, RVO, and various diseases has been convincingly proven as vascular perfusion defect by FA, and en-face OCTA of the SVP is not overlaid by other vasculature in physiological status[12,21]. However, the "ischemia" of the DVC, so far, lacks direct evidence because of the limitation in imaging technology concerning the DVC with SVP overlays on it. Moreover, although the ICP and DCP always demonstrate their edema/atrophy simultaneously on SD-OCT, the quantitative analysis of their separate impairment remains undiscovered. Our report uses the advantage of 3D PAR OCTA to analyze and compare the ICP and DCP vascular perfusion separately and provides novel insight into RDVCI.

The significant reduction of the DVC in both acute and chronic phases is not surprising and agrees with the report by Nemiroff *et al*[15]. The insignificant difference of the DVC between acute and chronic phases demonstrates no evolution of DVC vessel density attenuation along the course of RDVCI as a first impression. However, when the ICP and DCP were subdivided and evaluated separately, the results were detailed and the stereotype was altered. The ICP, which is located on the upper part of the INL, showed significant flow reduction compared to healthy controls but no evolution in vascular perfusion from acute lesion to old lesion. The DCP, which is located in the deeper part of the INL, revealed significant flow reduction and even more vasculature loss from the edema phase to the atrophy phase. These analyses tell us that the ICP impairment is completed at the acute phase, or at least no later than the acute phase. On the other hand, the DCP was affected in another pattern. The DCP's vascular loss was not one-off, but progressive. The comparisons of the PR among layers in the same disease period also confirmed the subsequent DCP impairment. The PR of the three layers in the acute phase showed no significant difference in the acute phase; this result corresponded with the SD-OCT findings that the ICP and DCP were affected simultaneously. Although the ICP and DCP can be segmented separately anatomically, for ischemic pathology, their acute impairment involvement is similar, showing that there is no obvious functional segmentation of the ICP and DCP in the acute phase of ischemia. In the chronic phase, however, the significant difference of PR among the layers was found. This difference was only discovered when comparing the ICP and DCP, but not when comparing the DVC and the ICP/DCP, indicating that the difference exists but is subtle and can be easily ignored when the ICP and DCP are robustly combined in analysis.

Visual acuity impairment can be made a few hours after artery perfusion rupture and is sometimes irreversible[22]. However, both favorable and unfavorable factors contribute to the ischemia-reperfusion progress after ischemia and vascular perfusion finally stabilizes. The reperfusion progress begins two days after the ischemia; blood vessels are partially revascularized and the blood flow is partially preserved[23]. The stress and hypoxia also result in multiple pathogenic factors and aggravate secondary capillary dropout[24]. In our study, the PFVD of the ICP remained stable as RDVCI progressed, suggesting that the balance of perfusion reconstruction was reached (or almost reached, more specifically) in the acute phase, because no significant ICP perfusion change was observed in the chronic phase in comparison with the acute phase. Despite some severe visual function loss in RDVCI, the average PR of the three layers ranged from 10% to 20%, indicating that the vessel perfusion impairment was less severe than the vision loss. The ischemia-reperfusion theory may account for this phenomenon[25-26].

The subsequent DCP impairment in the chronic phase is discovered for the first time in RDVCI lesions, and the anatomical characteristics of the DCP may contribute to this pathology. The DCP resides in the middle retina where the INL lies and is the deepest retinal capillary plexus[9,27]. When ICP and DCP ischemia happens, the oxygen from the retinal artery/arterioles and choroidal capillaries diffuse and compensate for their oxygen deficiency. The deep retina has no capillary plexus inside it, so it has the lowest oxygen saturation in the retina, and its tissue function relies on oxygen diffused mainly by the DCP and choroidal capillaries. The DCP functions as a vascular terminal, and the deep retina is therefore more sensitive to perfusion loss[28-29]. Similar to the ICP as described earlier, oxidative stress, inflammation, and various pathophysiologies resulting from oxygen deficiency can contribute to secondary

damage and may lead to subsequent DCP impairment[30-31]. Meanwhile, the reconstruction of capillary vasculature in RDVCI is inevitable, and multiple local microenvironmental factors contribute to the DCP's atrophy compared to the ICP due to their depth difference, as described earlier. The ICP and DCP appear as parallel connections in the anatomy; the slightly greater atrophy in the vascular lumen may also result in higher resistant circulation and therefore less perfusion, called "subsequent DCP impairment"[32].

The advantages of our methodology included projection-resolved OCTA analysis, parafoveal flow perfusion detection, and a gender- and age-matched (range: 12mo) healthy control group selection. OCTA generates high-resolution angiograms rapidly and noninvasively by detecting blood flow signals from sequentially scanned cross-sectional OCT images, using special algorithms such as SSADA[1-2,33-34]. OCTA can display angiograms as en-face frontal sections (C-scans), offering the possibility to observe and analyze vascular layers separately[2-3,9]. However, projection artifacts, appearing as the shadow of superficial blood vessels, easily occur on deep vascular angiograms, impairing image depth resolution[3,7,10,35]. SS algorithms can remove projection artifacts by subtracting superficial signals from deep slabs but can also lead to vessel integrity disruption and false-negative voxels on angiograms of the deep layer[4-6], whereas the 3D PAR algorithm filters projection artifacts but keeps flow signals, on the basis that in situ flow demonstrates higher intensity decorrelation value than shallower voxels do[7-8]. With 3D PAR OCTA, not only projection artifacts from superficial to deep vascular plexus are largely reduced, but projection artifacts from upper to deeper layers within the same plexus are as well. This permits convincing quantitative vessel density evaluation of the ICP and DCP, exempt from the influence of projection artifacts[8-10]. In this study, the parafoveal area of a 3×3 mm^2 scan focused on the macula, chosen as the study area. The macula is normally free from retinal vessels and nourished by choroidal vessels[36]. The removal of the macular area therefore strengthens the sensitivity of quantitative analysis. Moreover, this study is a retrospective one, and the earliest examination dated back to December 2014, when the 6×6 mm^2 scan included the low-resolution mode of 304×304 A-scans only, which is not supported by 3D PAR OCTA analysis. The 3×3 mm^2 scan, on the contrary, is high-resolution with an optical transverse resolution of 15 μm. The PFVD was hence decided as the measurement, based on these reasons. The control group was selected with gender- and age-matched healthy subjects, rather than with the fellow eye of RDVCI patients. This selection was made on the basis of two concerns. Two patients were affected bilaterally, and there was no control fellow eye to compare in their cases. The systemic diseases in some patients may result in capillary dropout imperceptibly and unilaterally, leading to analysis bias. It should be mentioned that the control group was selected with a one-to-one correspondent, so it could be divided into two subgroups, corresponding to the controls for the acute and chronic patient groups separately, and the PFVD between these two sub-control groups revealed no significant difference[37].

Our study also had several limitations. Although the stable ICP impairment and subsequent DCP impairment were observed and explained by the ischemia-reperfusion theory, the definitive mechanisms still need to be explored and confirmed on fundamental models. As a cross-sectional study, the comparison of acute and chronic cases was conducted in groups, but not by individuals, because of the lack of follow-up data. In addition, the PR of PFVD in the ICP and the DCP displayed no difference; whether the ICP and the DCP are involved in combination or randomly remains unsolved. Further investigation by linear regression analysis needs to be conducted. Our study only included RDVCI patients related to RAO and RVO, not other diseases such as DR. More patients need to be included in future investigations.

The high-resolution angiograms of the DVC and its subdivision of the ICP and DCP can be visualized without disturbance of projection artifacts, using 3D PAR OCTA in RDVCI eyes and healthy eyes. The PFVD of the ICP remained unchanged from the acute to the chronic phase, whereas the PFVD of the DCP reduced in the chronic phase, showing subsequent DCP impairment. The ischemia-reperfusion theory and anatomical characteristics may be responsible for the perfusion involvement pattern in RDVCI lesions.

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