Title	Microvascular Abnormalities on Optical Coherence Tomography Angiography in Macular Edema Associated With Branch Retinal Vein Occlusion
Author(s)	Norihiro Suzuki, Yoshio Hirano, Munenori Yoshida, Taneto Tomiyasu, Akiyoshi Uemura,Tsutomu Yasukawa, Yuichiro Ogura
Citation	American journal of ophthalmology 161: 126-132
Issue Date	2016-01
Right	
Type	article (author version)

Elsevier Editorial System(tm) for American Journal of Ophthalmology

Manuscript Draft

Manuscript Number: AJO-15-973R2

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Article Type: Original Article

Keywords: optical coherence tomography angiography; fluorescein angiography; branch retinal vein occlusion; multimodal imaging

Corresponding Author: Dr. Yoshio Hirano, M.D., Ph.D.

Corresponding Author's Institution: Nagoya City University Graduate School of Medical sciences

First Author: Norihiro Suzuki

Order of Authors: Norihiro Suzuki; Yoshio Hirano, M.D., Ph.D.; Munenori Yoshida; Taneto Tomiyasu; Akiyoshi Uemura; Tsutomu Yasukawa; Yuichiro Ogura

ABSTRACT

PURPOSE: To determine the ability of optical coherence tomography (OCT) angiography to image the microvascular structures compared with fluorescein angiography (FA) in patients with macular edema associated with branch retinal vein occlusion (BRVO).

DESIGN: Retrospective, observational, consecutive case series.

METHODS: Twenty-eight eyes of 27 patients (14 men, 13 women; mean age, 68.4 years) with macular edema associated with BRVO were enrolled. Simultaneous OCT angiography and FA were performed in all patients to evaluate the microvascular abnormalities and non-perfused areas.

RESULTS: OCT angiography detected non-perfused areas in 28 eyes and FA in 18 eyes. The respective findings of superficial capillary telangiectasias by OCT angiography and FA were 13 and 11 eyes, for deep capillary telangiectasias 28 eyes and 11 eyes, for collateral vessels 18 eyes and 16 eyes, and for microaneurysms 13 eyes and 14 eyes. OCT angiography facilitated differential layer analysis of microaneurysms and collaterals in the retina.

CONCLUSIONS: OCT angiography can visualize microvascular abnormalities equally well or better than FA in eyes with BRVO. Multimodal imaging using OCT angiography and FA can be a powerful tool to evaluate the pathology in BRVO.

Dear Editors and Reviewers,

We would like to submit our manuscript entitled,

“**Microvascular Abnormalities on Optical Coherence Tomography Angiography in Macular Edema Associated with Branch Retinal Vein Occlusion**”.

Optical coherence tomography angiography (OCTA) is an evolutional tool for clinicians to evaluate the microvascular abnormalities in BRVO. We compared the findings of OCTA with conventional fluorescein angiography (FA) in BRVO. As a result, we found not only the advantage of OCTA but also the effectiveness of multimodal imaging using OCTA and FA in order to evaluate the pathology in BRVO. Moreover, in the current study we could identify the location of microaneurysms and speculate the cause of the formation, causing persistent macular edema. This manuscript has never been submitted to any other journals. Each of co-authors (N.S., Y.H., M.Y., T.T., A.U., T.Y., Y.O.) has seen and agrees with each of the changes made to this manuscript in the revision and to the way his name is listed. We would appreciate it if you could consider our manuscript for the publication in your journal.

Sincerely yours,

Yoshio Hirano, M.D, PhD.
Department of Ophthalmology and Visual Science
Nagoya City University Graduate School of Medical Sciences
1-Kawasumi, Mizuho-cho, Mizuho-ku, Nagoya 467-8601
JAPAN

Microvascular Abnormalities on Optical Coherence Tomography Angiography in Macular Edema Associated with Branch Retinal Vein Occlusion

NORIHIRO SUZUKI, YOSHIO HIRANO, MUNENORI YOSHIDA, TANETO TOMIYASU, AKIYOSHI UEMURA, TSUTOMU YASUKAWA, AND YUICHIRO OGURA

From the Department of Ophthalmology & Visual Science, Nagoya City University Graduate School of Medical Sciences, Nagoya, Japan.

Short title: OCT ANGIOGRAPHY IN BRANCH RETINAL VEIN OCCLUSION

Inquiries to Yoshio Hirano, MD, PhD, Department of Ophthalmology & Visual Science, Nagoya City University Graduate School of Medical Sciences, 1-Kawasumi, Mizuho-cho, Mizuho-ku, Nagoya 467-0001, Japan; phone: +81-52-8538251; fax: +81-52-8419490, e-mail: yoshio.hirano@gmail.com

Key words; optical coherence tomography angiography, fluorescein angiography, branch retinal vein occlusion, multimodal imaging

Branch retinal vein occlusion (BRVO) usually results from a thrombus at the arteriovenous crossings where a thickened artery compresses the underlying venous wall, resulting in elevated venous pressure and consequent macular edema, retinal ischemia, and rupture of the retinal wall with intraretinal hemorrhage.[1] In the chronic phase, after absorption of the intraretinal hemorrhage, several retinal vascular abnormalities, such as capillary nonperfusion, capillary dilation, microaneurysms, telangiectatic vessels, and collateral vessels.[2]

Fluorescein angiography (FA) is useful to confirm the diagnosis of BRVO and evaluate complications. The characteristic findings on FA are delayed filling of the occluded retinal vein, capillary nonperfusion, microaneurysms, and dye extravasation from macular edema or retinal neovascularization. Ultra-wide-field FA using the Optos 200Tx (Optos PLC, Dunfermline, Scotland, UK) shows non-perfused areas in the peripheral retina. However, dense retinal hemorrhages and/or macular edema make interpretation of FA images difficult because of blocked or pooled fluorescein. In addition, FA does not visualize the deeper capillary network in the retina well, possibly because of light scattering in the retina.[3]

Optical coherence tomography (OCT) is a rapid, non-invasive technique that provides high-resolution retinal images. Recently, three-dimensional and non-invasive vascular mapping without dye was accomplished using OCT angiography.[4-6] OCT angiography has the advantage of three-dimensional noninvasive vascular mapping in the retina and choroid in just a few seconds, thus displaying several vascular layers, i.e., the superficial and deep capillaries, outer retina, and choriocapillaris. Recently, de Carlo reported the microvascular changes in patients with BRVO.[7] We evaluated the ability of OCT angiography to visualize several vascular abnormalities and compared the findings with conventional FA.

METHODS

STUDY DESIGN AND SETTING: This was a retrospective, observational, consecutive case series conducted in an institutional setting. The study design was approved by the Institutional Review Board of Nagoya City University Graduate School of Medical Sciences. All patients provided written informed consent for participation in the study. The described research methods and analysis adhered to the tenets of the Declaration of Helsinki.

This study was conducted at Nagoya City University Hospital from November 2014 through May 2015. Twenty-eight eyes of 27 patients (14 men, 13 women; mean age, 68.4 years; range, 41-86 years) with macular edema associated with BRVO were enrolled. The mean follow-up period was 28.8 months (range, 4-122 months). All patients underwent a complete ophthalmic examination including measurement of the best-corrected visual acuity, indirect ophthalmoscopy, fundus photography, OCT (Cirrus HD-OCT, Carl Zeiss Meditec, Jena, Germany), OCT angiography (RTVue XR Avanti, AngioVue, Optovue Inc., Fremont, CA), and/or FA. FA was performed using the wide-field laser ophthalmoscope Optos 200Tx and/or confocal scanning laser ophthalmoscopy (Heidelberg Retina Angiograph 2 [HRA2], Heidelberg Engineering, Heidelberg, Germany). OCT angiography and

FA also were performed on the same day. The mean measurement time of OCT angiography and FA was 25.1 months (range, 1-120) after disease onset. The incidence rates of non-perfused areas, superficial and deep capillary telangiectasias, collateral vessels, and microaneurysms were determined using OCT angiography and FA. Patients whose OCT angiography images were of inadequate quality for evaluation because of eye movement or cataract or who had not undergone FA because of renal and/or liver dysfunction or allergy to fluorescein were excluded.

OPTICAL COHERENCE TOMOGRAPHY ANGIOGRAPHY: OCT angiography images were obtained using the RTVue XR Avanti AngioVue with a split-spectrum amplitude decorrelation angiography algorithm as previously described.[5-9] The instrument obtains 70,000 A-scans/second, using a light source centered on 840 nm; the tissue resolution is 5 µm in depth and 15 µm in width. Two consecutive B-scans (M-B frames) were captured at a fixed position before proceeding to the next sampling position, split into 11 decorrelation sets, compared to detect motion, and merged. The merging of the sets increased the signal-to-noise ratio and provided the high-detail, motion contrast “angioflow” image.

RESULTS

NON-PERFUSED AREAS: OCT angiography detected non-perfused areas in all 28 eyes compared with FA that detected them in 18 eyes. In some FA images, the non-perfused areas were masked by hyperfluorescence from the leaking vessels or hypofluorescence due to retinal hemorrhage (Figure 1), whereas OCT angiography detected them clearly because of the absence of fluorescein dye. OCT angiography also visualized the non-perfused areas clearer than FA (Figure 1) and was superior for differentiating foveal avascular zone from non-perfused areas because the OCT angiography system provided higher resolution images than FA. OCT angiography did not detect non-perfused areas in the peripheral retina. However, the Optos 200Tx visualized the non-perfused areas in the peripheral retina (Figure 1).

SUPERFICIAL CAPILLARY TELANGIECTASIA: OCT angiography detected superficial capillary telangiectasia in 13 of 28 eyes and FA in 11 eyes; even with high-resolution confocal scanning laser ophthalmoscopy HRA2 imaged the superficial capillary telangiectasia in 11 eyes maximally, but it was difficult to observe the normal capillary network in the superficial capillary layer (Figure 2). OCT angiography detected even the very thin capillary networks in the superficial layer (Figure 2).

DEEP CAPILLARY TELANGIECTASIA: OCT angiography detected deep capillary telangiectasia in all 28 eyes, and FA in 11 eyes. On FA, the deep capillary network was barely visible because of the two-dimensional image photography and blockage by choroidal vessels (Figure 2). OCT angiography easily detected the capillary telangiectasia and the normal capillary networks in the deep layer (Figure 2).

COLLATERAL VESSELS (VENO-VENOUS SHUNT): OCT angiography detected collateral vessels in 18 eyes and FA in 16 eyes. Pre-existing collateral vessels that connected adjacent venous beds were distended and carried more blood, which tended to decrease the retinal venous pressure in the occluded segment. The collateral vessels were present where the occlusive vein was connected to another vein (Figure 3) or a normal (circulated) vein that traversed the non-perfused retina (Figure 3). OCT angiography facilitated differential layers analysis of the collateral vessels (Figure 3) and showed that the collateral vessels were in the superficial and deep layers in all 18 eyes. FA did not clarify the location of the collateral vessels with differential layer analysis in the retina (Figure 3).

MICROANEURYSMS: OCT angiography detected microaneurysms in 13 of 28 eyes and FA in 14 eyes. OCT angiography identified microaneurysms in the superficial capillary layer in six eyes, the deep capillary layer in 13 eyes, and both layers in six eyes (Figure 4). The microaneurysms were at the edge of the non-perfused areas in 10 eyes, the collateral vessels in seven eyes, and both in five eyes. The microaneurysms in the collateral vessels caused macular edema despite collateral vessel formation (Figure 4). FA did not clarify the location of the collateral vessels in the superficial or deep layer (Figure 4).

DISCUSSION

In the current study, OCT angiography using AngioVue visualized the microvascular abnormalities the same as or better than FA in macular edema associated with BRVO. OCT angiography with its higher resolution provided clearer images of the non-perfused areas and foveal avascular zone than FA. The OCT angiography images were not obstructed by hyperpermeable vessels because of no use of fluorescein dye, whereas the FA images became obscure by leaking dye. Also retinal hemorrhage resulted in hypofluorescence because of the blockages of both excitation and emission lights but minimally impaired the OCT angiography images from longer wavelength light (840 nm). Moreover, OCT angiography facilitated differential layer analysis of the microaneurysms and the collateral vessels in the retina.

The major advantage of OCT angiography is the ability to perform differential layer analysis. OCT angiography can visualize multiple layers of the retinal and choroidal blood vessels, i.e., the superficial and deep capillaries, outer retina, and choriocapillaris.[8] OCT angiography detects the retinal artery, vein, and superficial capillaries in the superficial capillary mode and the deep capillary network running from the inner plexiform layer to the outer plexiform layer through the inner nuclear layer in the deep capillary mode, respectively. The absence of a need for dye facilitates more frequent and repeated imaging than FA and enables evaluations of children, pregnant women, and patients with renal or cardiac disorders. Moreover, lack of dye leakage allows observation of capillary abnormalities and choroidal neovascularization better than with FA.

However, a disadvantage of OCT angiography was that the captured area was limited and that in some cases the microaneurysms without flow could not be visualized. This is because OCT angiography visualizes ocular blood flow in the vessels. Moreover, the changes in vessels visualized on OCT angiograms did not directly indicate the structural regression and regeneration of capillary dropout.[9] Therefore, the retinal non-perfused area, capillary occlusion, and capillary dropout cannot be differentiated.[9] In addition, OCT angiography did not provide flow information, i.e., filling speed, leakage, dye pooling, and tissue staining, all of which are important findings that facilitate evaluation of the activity in BRVO. Therefore, FA is an essential diagnostic tool for BRVO. OCT angiography requires that patients fixate steadily for about 4 seconds, whereas useful FA images can be obtained in a fraction of a second.

FA requires intravitreous injection of a dye, which rarely causes serious adverse effects such as allergy, cutaneous rashes, Quincke's edema, infarctions, pulmonary edema, or anaphylactic shock.[10] Mild adverse effects such as nausea and hives are common.[10] FA using even high-resolution confocal scanning laser ophthalmoscopy (HRA2) barely can visualize the capillary network but cannot distinguish between the superficial and deep capillary plexuses because of the two-dimensional analysis. Fluorescein dye, which should provide important information, can obstruct visualization of the capillary abnormalities and non-perfused areas.

In eyes with BRVO, venous obstruction leads to turbulent blood flow, elevated venous pressure, and overload of drainage capacity that might cause telangiectatic vessels, dilated capillaries, and collateral vessel formation.[11] In the current study, deep capillary telangiectasia was more observable than that in the superficial capillary network. A previous analysis of human retinas showed that the capillary densities were much greater in the deep capillary layer than in the superficial capillary layer.[12] Moreover, no retinal veins drain the overloaded blood flow in the deep capillary layer. Therefore, the capillaries in the deep capillary layer may dilate to compensate for the retinal veins.

Most microaneurysms, which seem to be a microvascular response to vascular endothelial growth factor (VEGF) generated from hypoxic retinal tissue, originate in the inner nuclear layer and the border zones.[13] In the current study, microaneurysms were more frequently in the deep capillary layer than in the superficial capillary layer. Microaneurysms developed with particular frequency on the edge of the non-perfused retina in BRVO,[14] consistent with the hypothesis that they are a secondary reaction to hypoxia and increased local VEGF concentration. Interestingly, in the current study microaneurysms were in the collateral vessels and at the edge of the non-perfused areas. The wall of a retinal capillary is comprised of endothelial cells, pericytes, and a basement membrane. Capillary pericytes are within the endothelial basement membrane. Loss of the pericytes, which occurs in diabetic retinopathies, weakens the capillary walls and leads to development of microaneurysms and macroaneurysms.[15] In BRVO eyes, the same phenomenon might cause the formation of microaneurysms. Compression of the vein causes increased retinal venous blood flow velocity, shear stress of blood on the venous endothelium, and endothelial injury.[16-18] High shear stress

gradients and turbulent blood flow can cause reactive proliferative changes in the endothelial cells and endothelial damage.[19] We speculated that these factors also may cause formation of microaneurysms in the collateral vessels. Once collateral vessels mature and retinal venous pressure decreases, vascular permeability decreases, leading to spontaneous resolution of macular edema.[17] However, microaneurysms sometimes formed in the collateral vessels, resulting in recurrent macular edema around the microaneurysms (Figure 4).

OCT angiography is superior to FA for visualizing microvascular abnormalities in the presence of retinal hemorrhages and retinal edema. However, it is sometimes difficult to evaluate the precise microvascular abnormalities despite using OCT angiography (Figure 5). Therefore, analyzing OCT angiography images obtained without macular edema is preferable.

The current study had several limitations, the first being the limited sample size. OCT angiography and FA both have advantages and disadvantages. OCT angiography using AngioVue is beneficial for visualizing the flow in various retinal layers without dye injection, but the ability to image a larger retinal area and a shorter acquisition time are required in the future. Multimodal imaging with OCT angiography, FA, and OCT can be a powerful tool for clinicians to evaluate the microvascular abnormalities in eyes with BRVO.

ACKNOWLEDGEMENTS/DISCLOSURES

a. Funding/Support: This study was supported by a Grant-in-Aid for Scientific Research (C) 15K10875 (Y.H.), 60273447 (M.Y), 25462758 (T.Y.), and Scientific Research (B) 25293078 (A.U.), 15H04997 (Y.O.) from the Japan Society for the Promotion of Science, Tokyo, Japan.

b. Financial Disclosures: Munenori Yoshida received lecture fee from CHUO SANGIO CO (Nishinomiya, Japan). The other authors have no proprietary/ financial interest.

c. Other Acknowledgements: We thank Fumie Shibuya, Yasuyo Matsuda, Sayaka Oshio, and Ayano Maruyama (Nagoya City University Graduate School of Medical Sciences) for contribution to data collection.

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LEGENDS

FIGURE 1. Non-perfused areas in macular edema associated with branch retinal vein occlusion. (Top left and Top, second from left) Fluorescein angiography (FA) images obtained by Optos 200Tx and (Bottom left and Bottom, second from left) optical coherence tomography (OCT) angiography images obtained by AngioVue (scan size, 3 x 3 mm). (Top, second from left) Magnified image of the yellow square in scan top left. (Bottom left) Superficial layer. (Bottom, second from left) Deep layer. (Top, second from left) Hyperfluorescence from leaking vessels masks the non-perfused areas. (Bottom left and Bottom, second from left) OCT angiography clearly visualizes the non-perfused areas and microaneurysms (arrowhead). (Top, third from left and Top, fourth from left) The images were obtained by Heidelberg Retina Angiograph 2. (Top, fourth from left) Magnified image of the yellow square in scan top, third from left. (Bottom, third from left and Bottom, fourth from left) The images were obtained by AngioVue (scan size, 3 x 3 mm). (Bottom, third from left) Superficial layer. (Bottom, fourth from left) Deep layer. The border between the non-perfused areas and foveal avascular zone is obscured on FA (Top, third from left and Top, fourth from left), whereas OCT angiography clearly visualizes the non-perfused areas and foveal avascular zone (Bottom, third from left and Bottom fourth from left). (Right) A FA image obtained by Optos 200Tx. The non-perfused areas in the peripheral retina are imaged well.

FIGURE 2. Telangiectasia in macular edema associated with branch retinal vein occlusion. (Top row) Fluorescein angiography (FA) images (Top left and Top middle) obtained by Heidelberg Retina Angiograph 2 and optical coherence tomography (OCT) angiography image (Top right) obtained by AngioVue (scan size 3 mm x 3 mm, superficial layer). (Top middle) Magnified image of the yellow square in scan top left. Collateral vessels (arrows) are seen on FA and OCT angiography, but a normal capillary network in the superficial layer is hardly visible on FA (Top middle). OCT angiography clearly visualizes the microvascular network (Top right). (Bottom row) FA (Bottom left and Bottom middle) and OCT angiography (Bottom right). (Bottom middle) Magnified image of the yellow square in scan bottom left. Deep capillary telangiectasia and normal deep capillary network are not visible on FA (Bottom left and Bottom middle). OCT angiography clearly visualizes the deep capillary, deep telangiectasia, and microaneurysms (arrowheads) (Bottom right).

FIGURE 3. Collateral vessels in macular edema associated with branch retinal vein occlusion. Fluorescein angiography (FA) images (Top row) obtained by Optos 200Tx and optical coherence tomography (OCT) angiography images (Bottom row) obtained by AngioVue (scan size, 3 x 3 mm). (Top, second from left and Top right) Magnified images of the yellow squares in scans top left and top, second from right, respectively. (Bottom left) Superficial layer. (Bottom, second from left) Deep layer. The collateral vessel network is obscured on FA (Top left and Top, second from left), but OCT angiography visualizes the collateral vessels formation in the superficial layer (arrows) (Bottom left and Bottom, second from right).

(Top right and Bottom, second from right) Bridging collateral vessels (arrows) between occlusive vein and normal circulated vein are observed. Microaneurysms (arrowheads) are detected in the collateral vessels as well.

FIGURE 4. Microaneurysms in macular edema associated with branch retinal vein occlusion. Fluorescein angiography (FA) images (Top left 4 images) obtained by Heidelberg Retina Angiograph 2 and optical coherence tomography (OCT) angiography images (Bottom left 4 images) obtained by AngioVue (scan size, 3 x 3 mm). (Top, second from left and Top, fourth from left) Magnified images of the yellow square in scans top left and top, third from left, respectively. (Bottom left and Bottom, third from left) Superficial layer. (Bottom, second from left and Bottom, fourth from left) Deep layer. (Top right and Bottom right) Images by Cirrus high-definition OCT. Microaneurysms (arrowheads) are detected in the superficial layer in scan bottom left and the deep layer in scan bottom, fourth from left. The microaneurysms (Bottom, fourth from left) are in the collateral vessels and cause macular edema (Right 2 images).

FIGURE 5. Artifacts on optical coherence tomography angiograms in macular edema associated with branch retinal vein occlusion. Optical coherence tomography (OCT) angiography images (Top row) and OCT images (Bottom row) obtained by AngioVue in the same patient. (Top left, Top, second from left, and Bottom left) Images without macular edema after injection of an anti-vascular endothelial growth factor agent. (Top right, Top second from right, and Bottom right) Images with recurrent macular edema in the same patient. The microvascular network is clearly visualized by OCT angiography (Top left and Top, second from left) without macular edema, whereas some artifacts at the foveal avascular zone are observed (Top right and Top, second from right) due to cystoid macular edema (Bottom right).

Figure 1

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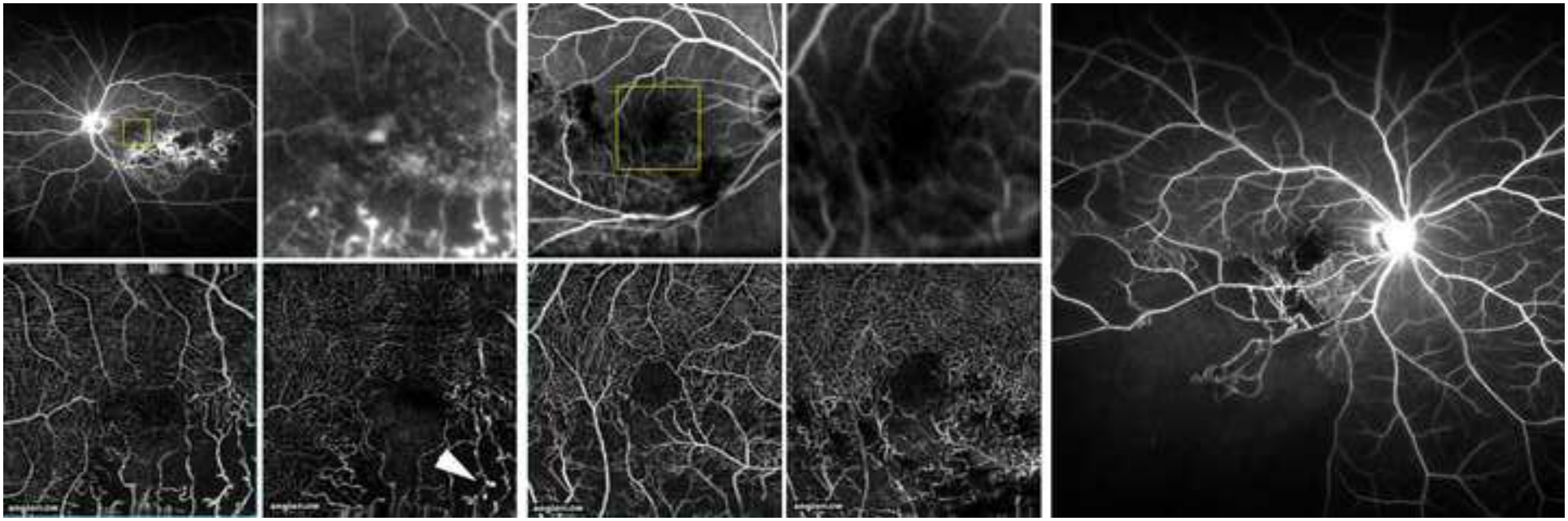

Figure 2

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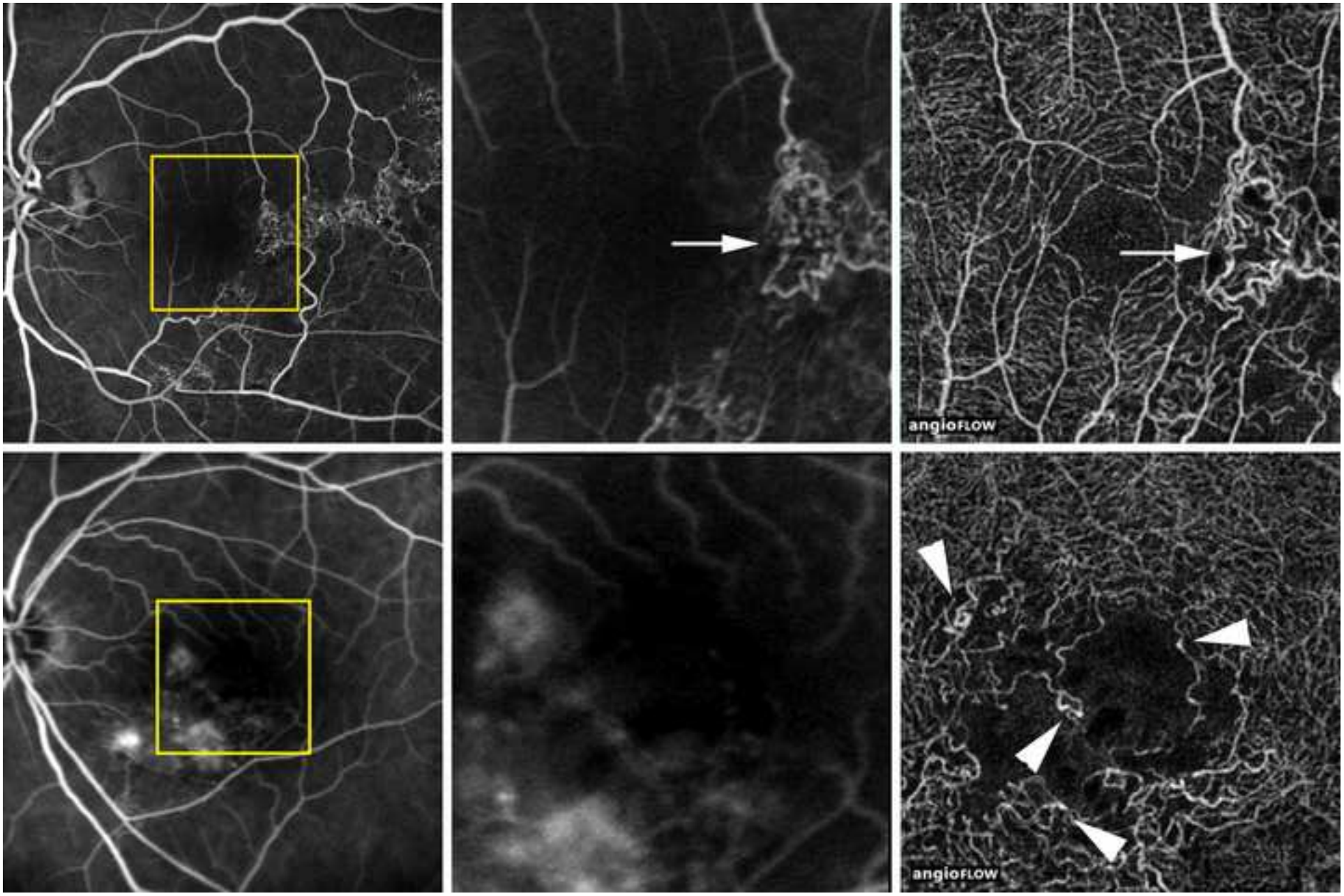

Figure 3

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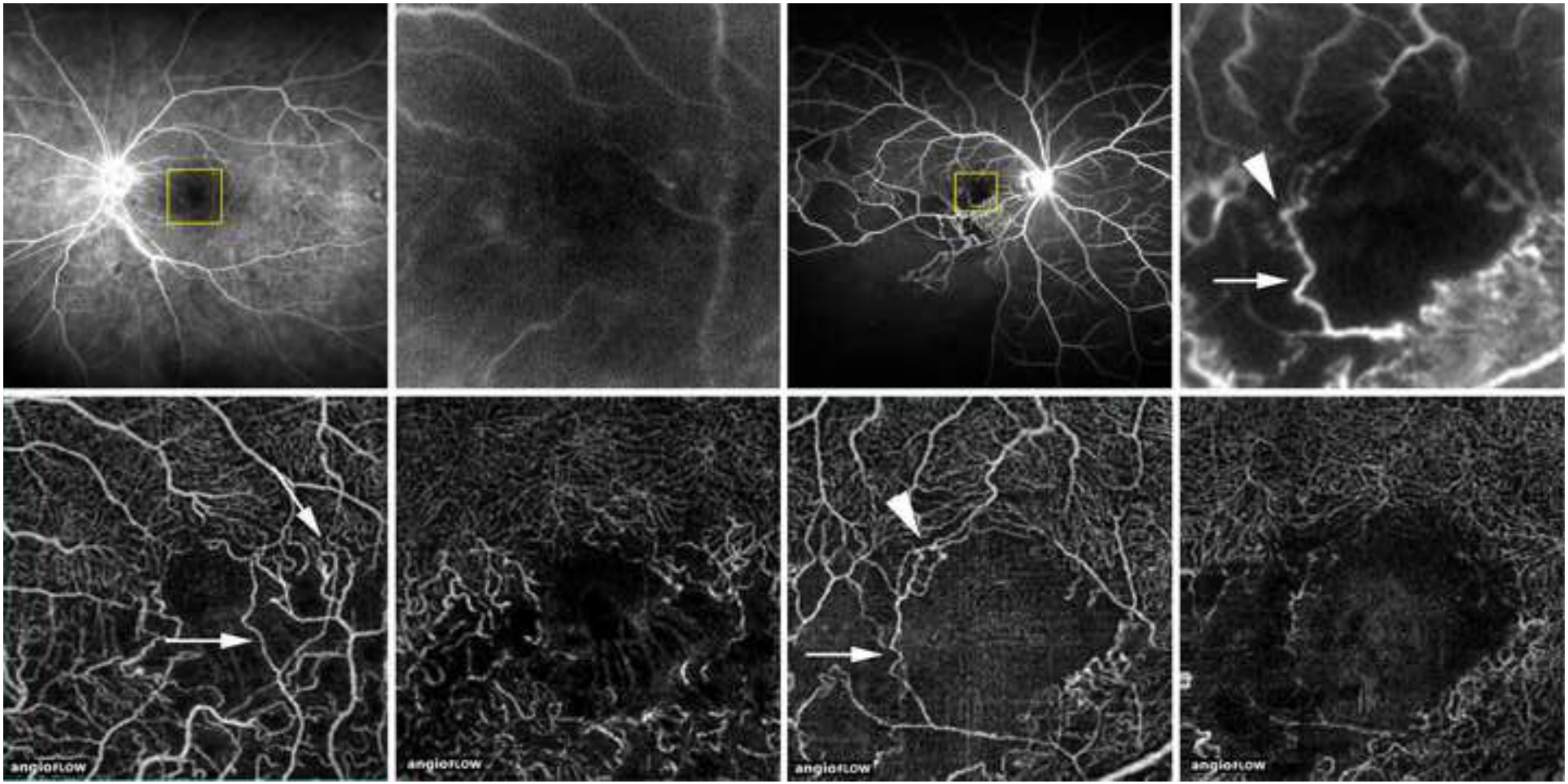

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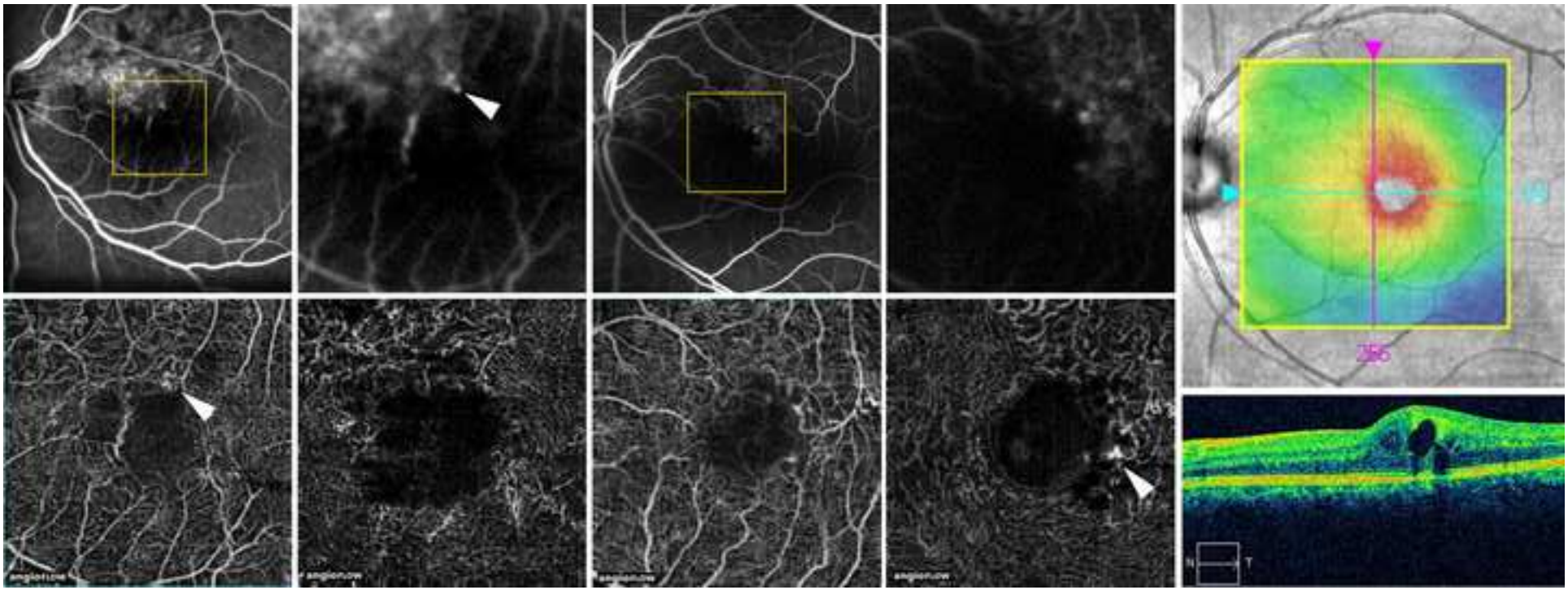

Figure 5

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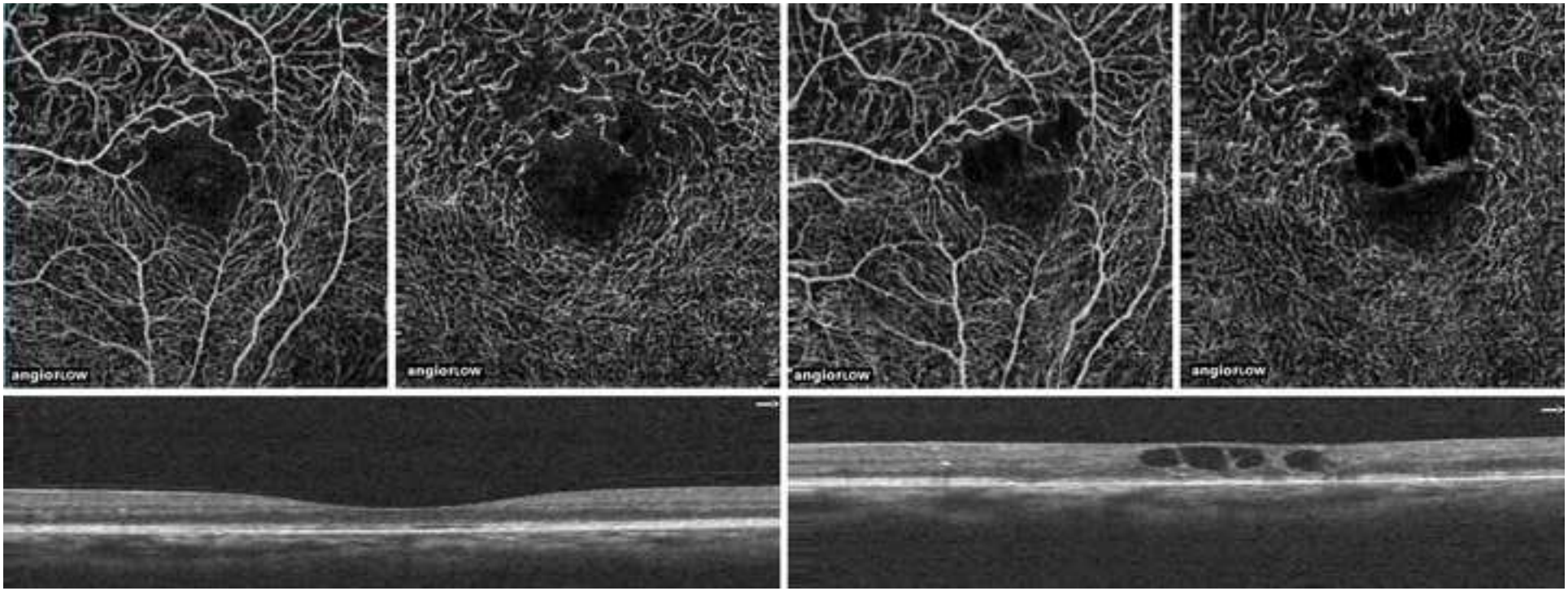

***Table of Contents Statement (Original Articles and Perspectives only)**

Manuscript No. AJO-15-973, Title “Microvascular Abnormalities on Optical Coherence Tomography Angiography in Macular Edema Associated with Branch Retinal Vein Occlusion”

A retrospective, observational study was conducted to determine the ability of optical coherence tomography angiography to image the microvascular structures compared with fluorescein angiography in patients with branch retinal vein occlusion. Optical coherence tomography angiography could visualize microvascular abnormalities equally well or better than fluorescein angiography in branch retinal vein occlusion. Multimodal imaging using optical coherence tomography angiography and fluorescein angiography can be a powerful tool to evaluate the pathology in branch retinal vein occlusion.

Biosketch

Norihiro Suzuki, MD, completed a medical residency program and is a PhD student at Nagoya City University Graduate School of Medical Sciences. Dr. Suzuki is interested in vitreoretinal diseases involving diabetic retinopathy, age-related macular degeneration, and retinal vein occlusion. The main subject of his research is to elucidate the mechanisms of vascular development and angiogenesis in retinal vascular diseases.

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