

Association of Vascular Versus Avascular Subretinal Hyperreflective Material With Aflibercept Response in Age-related Macular Degeneration



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- **PURPOSE:** To investigate flow signal within subretinal hyperreflective material (SHRM) using optical coherence tomography angiography (OCTA) and its association with aflibercept treatment responses in treatment-naïve neovascular age-related macular degeneration (nAMD).
- **DESIGN:** Prospective consecutive interventional case series.
- **METHODS:** Forty-four eyes of 44 patients with treatment-naïve nAMD manifesting SHRM on OCT were studied. All patients underwent OCTA and received 3 monthly aflibercept injections. The intrinsic flow signals within SHRM were quantitatively analyzed using OCTA, and eyes were classified into the vascular and avascular SHRM groups.
- **RESULTS:** Of 44 eyes, 21 (47.7%) and 23 (52.3%) showed vascular SHRM and avascular SHRM, respectively. Compared with eyes with avascular SHRM, eyes with vascular SHRM showed higher rates of external limiting membrane (ELM) disruption owing to SHRM ($P = .015$), classic choroidal neovascularization (CNV) (85.7% vs 26.1%, $P = .87 \times 10^{-4}$), and intraretinal fluid ($P = .008$) at baseline. After 3 aflibercept injections, 38 eyes (86.4%) showed dry macula despite persistent SHRM in 24 eyes (54.5%). Compared with the eyes with resolved SHRM, those with persistent SHRM showed higher rate of vascular SHRM (75.0% vs 15.0%, $P = .86 \times 10^{-4}$), classic CNV ($P = .032$), absence of polypoidal lesion ($P = .020$), ELM disruption owing to SHRM ($P = .042$), and intraretinal fluid ($P = .008$). Dry macula after loading injections was significantly associated with SHRM resolution ($P = .025$).
- **CONCLUSIONS:** In nAMD, SHRM can be categorized as vascular and avascular by quantitative OCTA analysis. Vascular SHRM persisted after treatment and was

associated with failure to achieve dry macula, suggesting that vascular SHRM is predictive of lower response to anti-VEGF therapy. (Am J Ophthalmol 2017;181:61–70. © 2017 Elsevier Inc. All rights reserved.)

AGE-RELATED MACULAR DEGENERATION (AMD) IS a leading cause of legal blindness among the aging populations of developed countries.^{1,2} The treatment of neovascular AMD was dramatically changed by the anti-vascular endothelial growth factor (VEGF) drug ranibizumab.^{3,4} More recently, aflibercept injections have shown similar or even better functional and anatomic outcomes than ranibizumab.^{5–7} Using optical coherence tomography (OCT), several studies have investigated the predictive factors of visual outcomes after anti-VEGF therapy.^{8–12} In this regard, poor visual acuity at baseline, the type 2 subtype of choroidal neovascularization (CNV),¹³ and subretinal hyperreflective material (SHRM) have been associated with poor visual outcomes after anti-VEGF therapy.⁸

SHRM is a morphologic finding of hyperreflective materials located in the subretinal space, often observed on OCT images of patients with macular diseases. In the Comparison of Age-related Macular Degeneration Treatments Trial (CATT) study, SHRM existed in 77% of eyes with neovascular AMD before anti-VEGF therapy.^{8,9} The components of SHRM in neovascular AMD may include exudates, hemorrhage, fibrosis, and neovascular tissue.⁶ The responses of SHRM to anti-VEGF therapy are variable and may depend on the components; some SHRMs show resolution after anti-VEGF therapy, but others do not. For instance, subretinal hyperreflective exudation, which is composed primarily of fibrin originating from the associated neovascular process, can be resolved by anti-VEGF therapy, resulting in better visual outcomes.¹⁴ In contrast, some cases of SHRM are associated with fibrotic scar formation and result in poor visual prognosis, possibly owing to the presence of neovascular tissue within SHRM.⁸ Because spectral-domain OCT (SD-OCT) cannot provide information about blood flow to visualize pathologic vascularization, we cannot distinguish neovascular tissue from other components of SHRM. Although fluorescein angiography



Supplemental Material available at AJO.com.

Accepted for publication Jun 21, 2017.

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(FA) and indocyanine green angiography are gold standards for detecting neovascular tissue,¹⁵ determining whether SHRM includes neovascular tissue is sometimes difficult because of the limited depth resolution and disturbances caused by dye leakage and staining effects.

OCT angiography (OCTA) is a noninvasive imaging tool that visualizes the CNV by detecting intravascular blood flow without the use of fluorescein.^{16,17} OCTA has some advantages over fluorescein/indocyanine green angiography, such as better depth resolution, no dye leakage, and no staining effects. Furthermore, structural OCTA B-scans allow for simultaneous visualization of structure and blood flow. Using OCTA, a previous study reported that SHRM could be categorized as vascular SHRM and avascular SHRM.¹⁸ However, the association of anti-VEGF treatment efficacy with vascular SHRM or avascular SHRM remains unknown. Although SHRM has been reported to be the marker of poor response to anti-VEGF therapy, we hypothesized that the distinction between vascular SHRM and avascular SHRM could improve the ability of SHRM as a predictive factor for anti-VEGF therapeutic efficacy. In this study, we investigated the various associations between vascular/avascular SHRM and wet/dry macula after anti-VEGF therapy.

METHODS

• **STUDY DESIGN:** This was a prospective, nonrandomized, interventional study conducted in an institutional setting. The study design was approved by the Institutional Review Board of Kyoto University Graduate School of Medicine, and all study conduct adhered to the tenets of the Declaration of Helsinki. Each patient provided written informed consent for participation in the study.

• **PATIENTS AND STUDY POPULATION:** Participants were recruited at the Macular Service, Department of Ophthalmology, Kyoto University Hospital between January 1, 2015 and March 31, 2016. We included the following participants: (1) patients aged more than 50 years, (2) those with an axial length less than 26.5 mm, (3) those with neovascular AMD, (4) those with SHRM in the OCTA scan area (3×3 mm), and (5) individuals willing to participate in the study. Only 1 eye from each patient was included; when a patient's second eye developed AMD, the second eye was not included in the study. The exclusion criteria were as follows: (1) any previous treatment for CNV; (2) the presence of retinal angiomatous proliferation (RAP) lesion; (3) the presence of other retinal diseases, such as angioid streaks, vitelliform macular dystrophy, retinal vein, or artery occlusion; (4) the presence of massive fibrotic lesions indicating a prolonged disease course; and (5) SHRM mainly consisting of subretinal hemorrhage (SRH) judged by fundus photography.

• **INTERVENTION AND OBSERVATION PROCEDURES:** Participants underwent 3 monthly injections of aflibercept (2.0 mg). Patients underwent comprehensive examinations, including the measurement of best-corrected visual acuity (BCVA), axial length (IOLMaster; Carl Zeiss Meditec, Dublin, California, USA), fundus photography, SD-OCT (Spectralis; Heidelberg Engineering, Heidelberg, Germany), swept-source OCT (SS-OCT; DRI OCT-1; Topcon, Tokyo, Japan), FA, indocyanine green angiography, fundus autofluorescence imaging (HRA2; Heidelberg Engineering), and OCTA (RTVue XR Avanti with AngioVue; Optovue, Inc, Fremont, California, USA). Fundus photography, SD-OCT, SS-OCT, FA, indocyanine green angiography, fundus autofluorescence, and OCTA imaging were performed under sufficient pupillary dilation. Although OCTA images can be obtained without pupil dilation depending on the patient's condition, we induced mydriasis before the procedure to obtain high-quality images. SD-OCT was performed at each visit, and the results before treatments and at 1 month after the loading treatments were analyzed. SD-OCT images included 30-degree horizontal and vertical, regular- and enhanced-depth scans through the fovea with 100 automatic retinal tracking (ART; using eye tracking, 100 B-scan images were acquired at each location of interest on the retina; these were then averaged), and 13 raster scans covering a 20×30 -degree oblong rectangle with 50 ART. The central retinal thickness (CRT) was measured in horizontal and vertical scans and averaged. Disruption of the external limiting membrane (ELM) owing to SHRM in the outer retina was determined on the OCT images, and the patients were classified into 2 groups on the basis of the presence or absence of ELM disruption ([Supplemental Figure](#), available at [AJO.com](#)). SS-OCT was performed to obtain horizontal and vertical line scans through the fovea (12 mm), 5-line cross scans, and 3-dimensional volumetric scans. Five-line cross scans (12 mm) were performed with 0.25-mm spacing. Three-dimensional volumetric scans were also performed to cover areas of 3×3 mm² and 6×6 mm² centered on the fovea. On angiography, we judged the presence of polypoidal lesions by using indocyanine green angiography at baseline in each case. The diagnosis of polypoidal choroidal vasculopathy (PCV) was made on the basis of the finding of characteristic polypoidal lesions at the border of the branching choroidal vascular networks. Those with the signs of retinochoroidal anastomosis were diagnosed with RAP, and the others were diagnosed as having typical AMD (tAMD). We also determined whether the subtype of the CNV adjacent to the SHRM was occult (occult with no classic) or classic type (predominantly classic and minimally classic). This classification was performed by 2 graders masked to OCTA information (Y.K. and M.H.). When the graders disagreed, they discussed the results until they had reached a consensus. The levels of intraretinal fluid and ELM disruption were judged by a single grader (Y.K.).

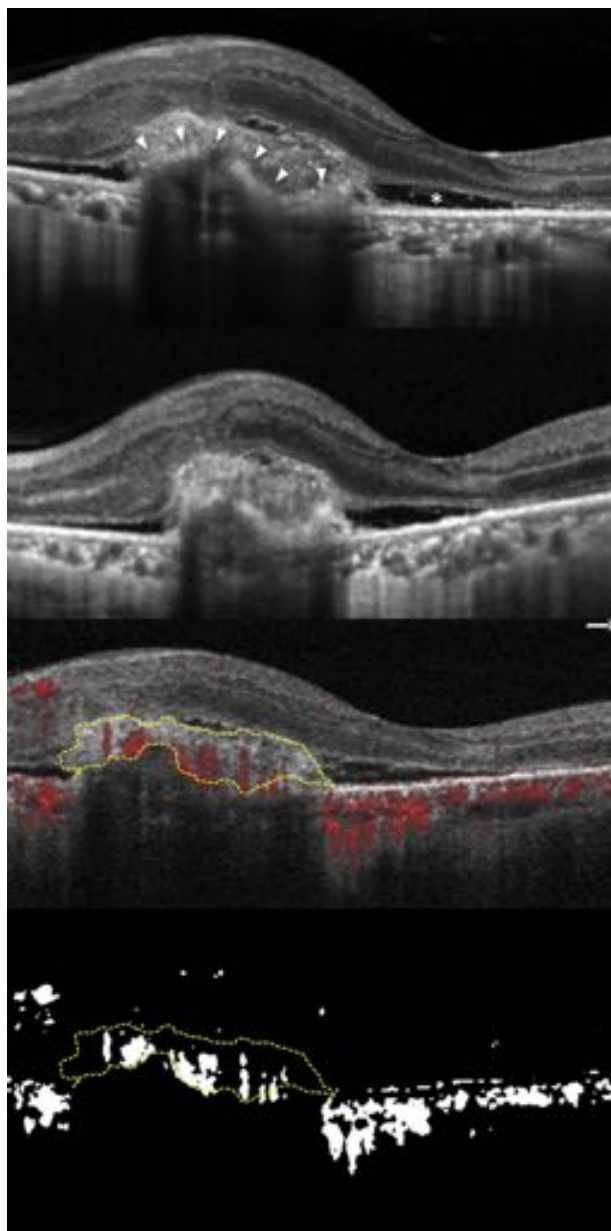


FIGURE 1. Detection of flow signal within subretinal hyperreflective material (SHRM) using optical coherence tomography angiography (OCTA). (Top row) Cross-sectional spectral-domain optical coherence tomography (SD-OCT) shows shallow pigment epithelial detachment and SHRM with adjacent subretinal fluid. The arrowheads show pigment epithelial detachment (PED), and the asterisk shows subretinal fluid. (Second row) Swept-source OCT, which can visualize the PED border line more clearly, showing the same section as the SD-OCT. (Third row) Cross-sectional OCT with angiographic overlay (red pixels) shows a flow signal within the SHRM (area within the yellow dotted line). (Bottom row) Only flow signal is extracted from the OCTA image using ImageJ. Red signal abundance ratio is 15.6% as calculated using the ImageJ plugin program.

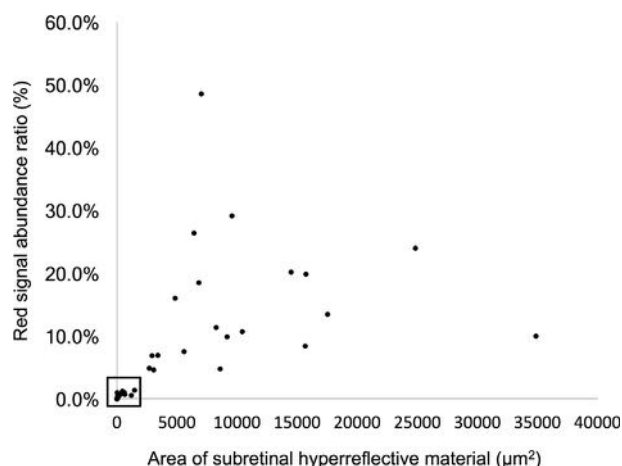


FIGURE 2. Relationship between the abundance ratio and the area of the subretinal hyperreflective material (SHRM). Red signal abundance ratio and the area of SHRM are plotted against each other as a scatter diagram. They are divided into 2 groups on the basis of a threshold of 2%. The box comprises the eyes defined as having avascular SHRM.

After 3 consecutive monthly injections, the presence of fluid accumulation was determined by SD-OCT. Dry macula was defined as the complete resolution of intraretinal and/or subretinal fluid detected on SD-OCT raster scan at 1 month after the loading treatment. Wet macula was defined as the existence of any retinal fluid detected on OCT at 1 month after the loading treatment. The persistence of only pigment epithelial detachment was not considered to indicate wet macula.

- **ANALYSIS OF SUBRETINAL HYPERREFLECTIVE MATERIAL WITH OPTICAL COHERENCE TOMOGRAPHY:** For analysis, the SD-OCT image on which the area of the SHRM was the largest was chosen for each patient from the records of raster scans or multiple single horizontal line scans over the SHRM. In cases in which the boundary between the retinal pigment epithelium (RPE) and SHRM was difficult to determine using SD-OCT images, we used SS-OCT images.

- **ANALYSIS OF SUBRETINAL HYPERREFLECTIVE MATERIAL WITH OPTICAL COHERENCE TOMOGRAPHY ANGIOGRAPHY:** All OCTA images were obtained using RTVue XR Avanti with the AngioVue macular cube (3×3 mm) protocol. Each imaging cube comprised 304 clusters of repeated B-scans that contained 304 A-scans each. Retinal layer segmentation was automatically performed by the built-in software. All the OCTA B-scan images were resegmented manually.

We quantitatively evaluated the flow signal within the SHRM by using OCTA B-scans. First, we chose the

OCTA B-scan image, which was at the same position as the SD-OCT image on which the area of the SHRM was the largest. Second, we demarcated the SHRM on the OCTA image. SD-OCT and SS-OCT images were used as reference to detect the boundary between the SHRM and RPE. Third, by using the public-domain software ImageJ (Wayne Rasband, National Institute of Mental Health, Bethesda, Maryland, USA; available at <http://rsb.info.nih.gov/ij/index.html>) and its plugin software “Measure RGB” (available at <http://rsb.info.nih.gov/ij/plugins/rgb-measure.html>), we extracted red flow signal from the OCTA B-scan images and calculated the abundance of red signals within the SHRM (Figure 1). Simultaneously, we calculated the SHRM area.

To evaluate the flow signal within the SHRM, we used red signal abundance ratio. Red signal abundance ratio was calculated as follows: Red signal abundance ratio (%) = (red signals within the SHRM/SHRM area) × 100.

We defined vascular SHRM as SHRM with a red signal abundance ratio equal to or more than 2% and avascular SHRM as SHRM with a red signal abundance ratio less than 2% (Figure 2). As a slight signal can exist in most cases owing to a projection artifact, we classified cases with a weak red signal abundance ratio as having avascular SHRM. As shown in Figure 2, a threshold of 2% is a valid point to dichotomize the range and to classify the avascular SHRM group.

When the SHRM was located within the foveal avascular zone (FAZ), retinal vessel projections were not observed. In contrast, when the SHRM was located outside the FAZ, retinal vessel projection artifacts were observed within the SHRM. We excluded patients with strong projection artifacts by comparing en face images of the retinal superficial vessel and deep capillary vessels with en face images of the outer retina.¹⁸

• **MAIN OUTCOME MEASURE:** The main outcome measure was the achievement of dry macula and resolved SHRM (complete disappearance of SHRM—judged using SD-OCT) after aflibercept injections.

• **STATISTICAL ANALYSIS:** All values are presented as median and interquartile range. The BCVA, measured using a Landolt chart, was converted to the logarithm of the minimal angle of resolution (logMAR) for statistical analyses. Mann-Whitney *U* tests were used to compare patient age, BCVA, and the CRT at baseline between eyes with vascular SHRM and avascular SHRM, and between eyes with resolved SHRM and with persistent SHRM after the loading dose. The χ^2 test was used to compare the sex ratio, presence of polypoidal lesion, presence of intraretinal fluid, ELM disruption owing to SHRM, and position of SHRM between eyes with vascular SHRM and avascular SHRM and between eyes with resolved SHRM and with persistent SHRM after the loading dose. For the subtype of the CNV, the κ statistic

TABLE 1. Characteristics of the Included Age-related Macular Degeneration Patients With Subretinal Hyperreflective Material

Characteristics	Values
Patient age (y) (median, IQR)	77.0 (69.0, 81.0)
Sex, n (%)	
Male	32 (73%)
Female	12 (27%)
Subtype, n (%)	
tAMD	26 (59%)
PCV	18 (41%)
Baseline visual acuity (median, IQR) (Snellen equivalents)	0.22 (0.046, 0.52) (20/33)
Position of SHRM, n (%)	
Subfovea	27 (61%)
Juxtafovea	17 (39%)
ELM disruption owing to SHRM in outer retina, n (%)	
ELM disruption (+)	17 (39%)
ELM disruption (−)	27 (61%)
Signal of cross-sectional OCT angiography	
Flow (+)	21 (48%)
Flow (−)	23 (52%)
Intraretinal fluid, n (%)	
Presence	20 (45%)
Absence	24 (55%)
Central macular thickness (μm) (median, IQR)	265 (193, 362)

ELM = external limiting membrane; IQR = interquartile range; logMAR = logarithm of minimal angle of resolution; OCT = optical coherence tomography; PCV = polypoidal choroidal vasculopathy; SHRM = subretinal hyperreflective material; tAMD = typical age-related macular degeneration.

was calculated. All statistical comparisons were performed using IBM SPSS Statistics for Windows/Macintosh, Version 19.0 (IBM Corp, Armonk, New York, USA). A *P* value < .05 was considered statistically significant.

RESULTS

FIFTY-TWO EYES OF 52 PATIENTS (38 MEN AND 14 WOMEN) with neovascular AMD met the inclusion and exclusion criteria. Among 52 eyes, 8 eyes were further excluded from this analysis because of the following reasons: 7 eyes showed SHRM with strong projection artifacts within the SHRM on OCTA images, and 1 eye had indistinct OCTA images. Eventually, 44 eyes of 44 patients (26 eyes with tAMD and 18 with PCV) were included in this study. The characteristics of the patients are summarized in Table 1. The reproducibility for the subtype of CNV gave a κ statistic of 0.82.

Of the 44 eyes, 21 (47.7%) showed OCTA intrinsic flow signal within the SHRM (vascular SHRM,

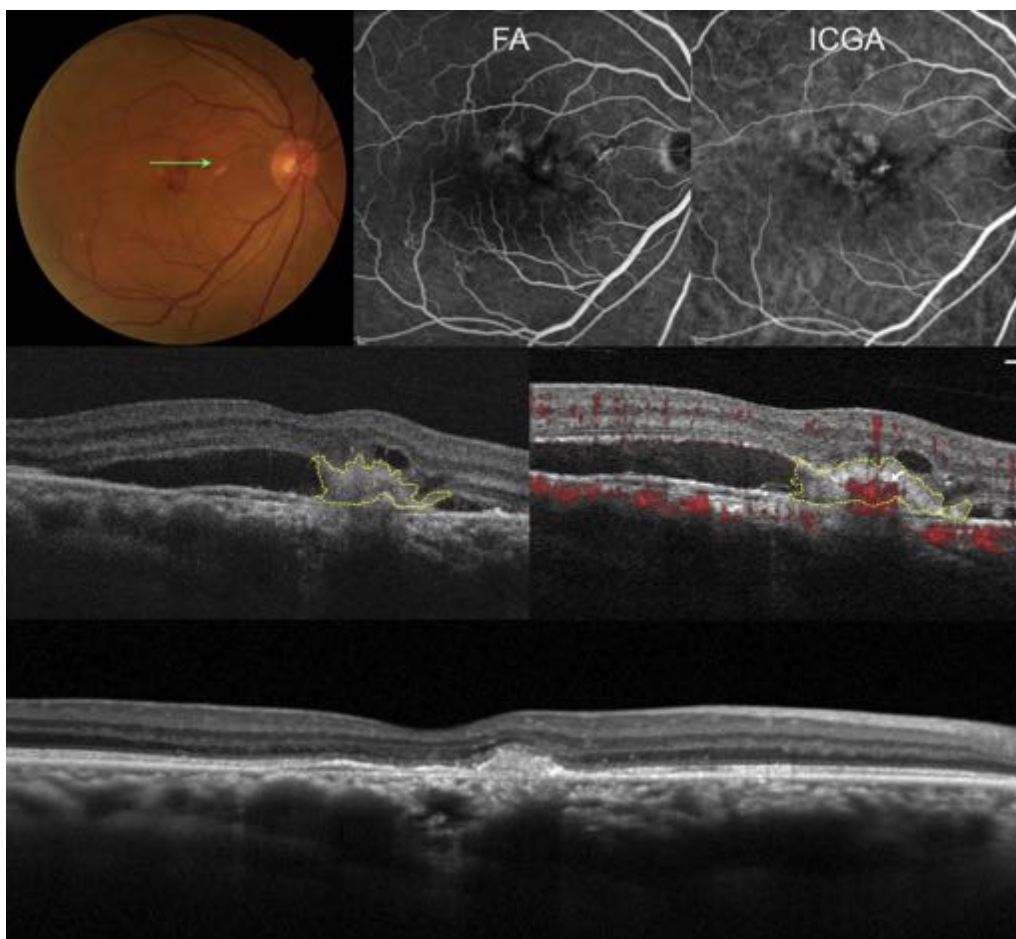


FIGURE 3. The right eye of a 70-year-old patient with vascular subretinal hyperreflective material (SHRM). (Top, left) Color fundus photograph of the patient shows serous retinal detachment and pigment epithelial detachment surrounded by subretinal hemorrhage. (Top, second and third from left) Fluorescein angiography (FA) and indocyanine green angiography (ICGA) reveal occult choroidal neovascularization (CNV). (Middle, left) Swept-source optical coherence tomography (OCT) shows SHRM, subretinal fluid, and intraretinal fluid. The SHRM is demarcated by a yellow dotted line. (Middle, right) Cross-sectional OCT with angiographic overlay (red pixels) shows strong flow signal within the SHRM. The SHRM is demarcated by a yellow dotted line. The arrow shows where the CNV has infiltrated the SHRM. (Bottom) After 3 dosing injections of aflibercept, the subretinal fluid is absorbed, but the SHRM persists.

Figure 3) and 23 (52.3%) showed no intrinsic flow signal within the SHRM (avascular SHRM, Figure 4). The SHRM was located subfoveally in 27 eyes (61.4%), and juxtafoveally in 17 (38.6%). Disruption of the ELM owing to SHRM in the outer retina was seen in 17 eyes (38.6%), while there was no ELM disruption in 27 eyes (61.4%). Twenty eyes (45.5%) showed intraretinal fluid at baseline (Table 1).

• **ASSOCIATION OF BASELINE FACTORS WITH OPTICAL COHERENCE TOMOGRAPHY ANGIOGRAPHY FLOW SIGNAL WITHIN THE SUBRETINAL HYPERREFLECTIVE MATERIAL:** Of the 21 eyes with vascular SHRM, 18 (85.7%) presented classic CNV and 3 (14.3%) showed occult CNV on FA. In contrast, of the 23 eyes with avascular SHRM, 17 (73.9%) showed occult CNV and 6 (26.1%) showed classic CNV (Table 2).

Compared with the eyes with avascular SHRM, the eyes with vascular SHRM showed higher rates of ELM disruption (81.0% vs 43.5%, $P = .015$), classic CNV on FA ($P = .87 \times 10^{-4}$), and intraretinal fluid (66.7% vs 26.1%, $P = .008$) at baseline. Furthermore, eyes with vascular SHRM tended to show the absence of polypoidal lesions with borderline significance (52.2% vs 28.6%, $P = .099$). Eyes with vascular SHRM showed significantly worse VA at baseline and after treatments than those with avascular SHRM ($P = .015$ and $P = .004$, respectively) (Table 2). However, VA change did not differ significantly between eyes with vascular SHRM and those with avascular SHRM ($P = .458$).

• **FACTORS ASSOCIATED WITH PERSISTENT/RESOLVED SUBRETINAL HYPERREFLECTIVE MATERIAL AFTER AFLIBERCEPT TREATMENTS:** Twenty-four of the 44 eyes (54.5%) showed persistent SHRM after 3 aflibercept

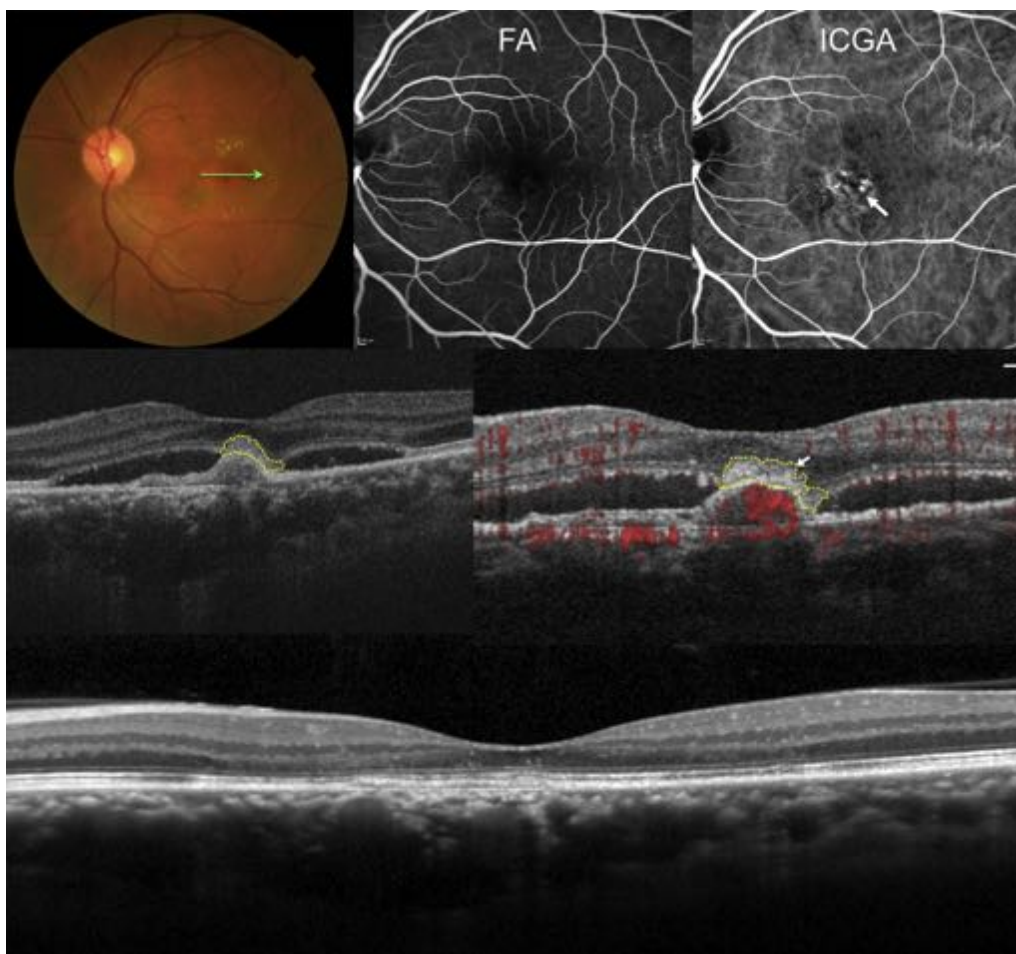


FIGURE 4. The left eye of a 62-year-old patient with avascular subretinal hyperreflective material (SHRM). (Top, left) Color fundus photograph shows serous retinal detachment and pigment epithelial detachment (PED). (Top, second and third from left) Fluorescein angiography (FA) reveals occult choroidal neovascularization (CNV). Indocyanine green angiography (ICGA) shows polypoidal lesions (arrow). (Middle, left) Swept-source optical coherence tomography (OCT) shows subfoveal SHRM. The SHRM is demarcated by a yellow dotted line. (Middle, right) Cross-sectional OCT with angiographic overlay (red pixels) shows strong flow signals within the pigment epithelium detachment but not in the SHRM. The SHRM is demarcated by a yellow dotted line. The arrow shows where the CNV has not infiltrated the SHRM. (Bottom) After 3 dosing injections of aflibercept, serous retinal detachment and SHRM have been absorbed.

injections. Compared with the eyes with resolved SHRM, the eyes with persistent SHRM showed a higher rate of vascular SHRM (75.0% vs 15.0%, $P = .86 \times 10^{-4}$), classic CNV on FA (70.8% vs 35.0%, $P = .032$), absence of polypoidal lesions (75.0% vs 40.0%, $P = .020$), subfoveal location of the SHRM (75.0% vs 45.0%, $P = .042$), ELM disruption owing to SHRM (75.0% vs 45.0%, $P = .042$), and intraretinal fluid (66.7% vs 20.0%, $P = .008$) at baseline (Table 3). In addition, eyes with vascular SHRM tended to show more persistent SHRM than those with classic CNV (85.7% vs 58.8%, $P = .078$). As for the association of SHRM resolution with the level of ELM disruption owing to SHRM, 64.7% (11/17) of the eyes with ELM disruption showed SHRM resolution, but only 25.0% (1/4) showed SHRM resolution if limited to cases with vascular

SHRM. In contrast, only 33.3% (9/27) of the eyes with ELM disruption owing to SHRM showed SHRM resolution, but 70.0% (7/10) showed SHRM resolution if limited to cases with avascular SHRM. Furthermore, 38 of the 44 eyes (86.4%) showed dry macula after 3 dosing injections. Dry macula after dosing injections was significantly associated with SHRM resolution after treatments (SHRM resolution rate in dry macula vs that in wet macula: 20 of 38 eyes (52.6%) vs 0 of 6 eyes (0%), respectively; $P = .025$). Dry macula was marginally associated with vascular SHRM (vascular SHRM rate in dry macula vs that in wet macula: 16 of 38 eyes (42.1%) vs 5 of 6 eyes (83.3%), respectively; $P = .088$). Macula status (dry/wet) did not differ between eyes with tAMD and those with PCV ($P = 1.000$; Supplemental Table, available at AJO.com).

TABLE 2. Associated Baseline Factors With Optical Coherence Tomography Angiography Flow Signal Within Subretinal Hyperreflective Material

	OCTA Image Flow (+) Vascular SHRM (N = 21)	OCTA Image Flow (–) Avascular SHRM (N = 23)	P Value
Patient age (y) (median, IQR)	78.0 (70.5, 84.0)	74.0 (67.0, 79.0)	.108
Sex, n (%)			.560
Male	15 of 32 (47%)	17 of 32 (53%)	
Female	6 of 12 (50%)	6 of 12 (50%)	
Subtype, n (%)			.099
tAMD	15 of 26 (58%)	11 of 26 (42%)	
PCV	6 of 18 (33%)	12 of 8 (67%)	
Subtype of FA pattern, n (%)			<.0001
Classic	18 of 24 (75%)	6 of 24 (25%)	
Occult	3 of 20 (15%)	17 of 20 (85%)	
Baseline visual acuity (median, IQR) (Snellen equivalents)	0.40 (0.10, 0.82) (20/50)	0.10 (0.00, 0.30) (20/25)	.015
Central macular thickness (μm) (median, IQR)	274 (222, 456)	246 (166, 310)	.127
Position of SHRM, n (%)			.159
Subfovea	15 of 27 (56%)	12 of 27 (44%)	
Juxtafovea	6 of 17 (35%)	11 of 17 (65%)	
ELM disruption owing to SHRM in outer retina, n (%)			.015
ELM disruption (+)	17 of 27 (63%)	10 of 27 (37%)	
ELM disruption (–)	4 of 17 (24%)	13 of 17 (76%)	
Intraretinal fluid, n (%)			.008
Presence	14 of 20 (70%)	6 of 20 (30%)	
Absence	7 of 24 (29%)	17 of 24 (71%)	

ELM = external limiting membrane; FA = fluorescein angiography; IQR = interquartile range; logMAR = logarithm of minimal angle of resolution; OCTA = optical coherence tomography angiography; PCV = polypoidal choroidal vasculopathy; SHRM = subretinal hyperreflective material; tAMD = typical age-related macular degeneration.

DISCUSSION

IN THE PRESENT STUDY, WE CATEGORIZED SHRM AS vascular and avascular by quantitative OCTA analysis. We found that at least 48% of SHRM is composed of vascular components, and vascular SHRM is significantly associated with ELM disruption owing to SHRM in the outer retina and the presence of intraretinal fluid at baseline. We also found that vascular SHRM is significantly associated with persistent SHRM after treatments, which was also associated with wet macula, suggesting that vascular SHRM could be a better predictive factor for less response to anti-VEGF therapy than is avascular SHRM.

SHRM seems to contain various components, such as fluid, fibrin, blood, fibrosis tissue, and CNV.⁸ In fact, a previous study qualitatively evaluated the intrinsic flow within the SHRM by using OCTA, and showed that 7 of 17 eyes had intrinsic flow within the SHRM in patients with CNV.¹⁸ In our study, by performing quantitative evaluation of intrinsic flow within the SHRM, we identified a flow signal in almost half (48%) of the eyes with SHRM.

We are unable to compare the results of the 2 studies because we did not include SHRM owing to apparent SRH and fibrosis to exclude the projection artifacts and signal blocking. Nevertheless, quantitative and objective methods may not be inferior to qualitative methods in view of detecting the rate of flow signal within the SHRM. Therefore, flow signal analysis using OCTA may provide additional information on the components of SHRM.

SHRM is an important biomarker in the treatment of AMD. The CATT study showed that SHRM persisted mostly after anti-VEGF therapy and was related with worse visual acuity.⁸ However, SHRM contains various components and the response of SHRM to treatments may differ according to these components. Considering the treatment for type 2 CNV often yields poor results because it sometimes causes fibrosis and photoreceptor damage,^{19,20} whether the SHRM included neovascular tissues or not would be important information in the treatment of AMD. In fact, the present study showed that 24 eyes (54.5%) had persistent SHRM after 3 injections, and that the persistence of SHRM was associated with classic

TABLE 3. Associated Baseline Factors With Status of Subretinal Hyperreflective Material After Aflibercept Treatment

	Resolution of SHRM (N = 20)	Persistence of SHRM (N = 24)	P Value
Patient age (y) (median, IQR)	78.0 (67.5, 80.5)	73.5 (69.3, 84.0)	.981
Sex, n (%)			.514
Male	15 of 32 (47%)	17 of 32 (53%)	
Female	5 of 12 (42%)	7 of 12 (58%)	
Subtype, n (%)			.020
tAMD	8 of 26 (31%)	18 of 26 (69%)	
PCV	12 of 18 (67%)	6 of 18 (33%)	
Central macular thickness (μm) (median, IQR)	239 (167, 308)	276 (222, 458)	.053
FA pattern, n (%)			.032
Classic	7 of 24 (29%)	17 of 24 (71%)	
Occult	13 of 20 (65%)	7 of 20 (35%)	
Status of OCTA, n (%)			<.0001
Vascular SHRM	3 of 21 (14%)	18 of 21 (86%)	
Avascular SHRM	17 of 23 (74%)	6 of 23 (26%)	
Position of SHRM, n (%)			.042
Subfovea	9 of 27 (33%)	18 of 27 (67%)	
Juxtafovea	11 of 17 (65%)	6 of 17 (35%)	
ELM disruption owing to SHRM in outer retina, n (%)			.042
ELM disruption (+)	9 of 27 (33%)	18 of 27 (37%)	
ELM disruption (–)	11 of 17 (65%)	6 of 17 (35%)	
Intraretinal fluid, n (%)			.008
Presence	4 of 20 (20%)	16 of 20 (80%)	
Absence	16 of 24 (67%)	8 of 24 (33%)	

ELM = external limiting membrane; FA = fluorescein angiography; IQR = interquartile range; OCTA = optical coherence tomography angiography; PCV = polypoidal choroidal vasculopathy; SHRM = subretinal hyperreflective material; tAMD = typical age-related macular degeneration.

CNV and vascular SHRM. Among the various types of SHRMs, vascular SHRM determined by OCTA in particular may be a predictive factor for the resistance to anti-VEGF therapy. Further investigation should be performed to reveal the long-term association between the responses to anti-VEGF therapy and the vascular/avascular status of SHRM.

Disruption of the ELM owing to SHRM in the outer retina is also an important predictive factor for treatment responses. Previous studies have showed that the prognosis of AMD differed according to the levels of ELM disruption owing to SHRM²¹ and that ELM discontinuity was a predictive factor for poor response to AMD treatments.²² The present study confirmed that ELM disruption owing to SHRM is associated with a poor response to anti-VEGF therapy. In addition to the ELM disruption level, our data suggested that the presence or absence of intrinsic flow on OCTA may predict the responses to anti-VEGF therapy.

In the current study, vascular SHRM was significantly associated with the presence of intraretinal fluid at baseline. Furthermore, 85.7% (12/14) of the eyes with vascular SHRM and intraretinal fluid showed ELM disruption, while only 29.4% (5/17) of the eyes with avascular

SHRM and no intraretinal fluid showed ELM disruption. ELM was a hypothetical line on OCT, but ELM was also considered a border line blocking macromolecules^{23,24} because it corresponds to Müller cells and photoreceptor junctional structures on electron microscopy.²⁵ A previous report showed that intraretinal fluid was detected frequently when ELM structures were damaged in diabetic retinopathy.²⁶ In addition, intraretinal fluid at baseline is reported to be a marker for poorer visual outcomes in AMD because of the underlying baseline retinal dysfunction and subsequent scarring.²⁷ Our result may suggest that vascular SHRM frequently causes ELM damage, resulting in fluid barrier impairment and photoreceptor damage.

The strengths of the current study were recruitment and treatment of prospective patients using an identical protocol and performance of systematic examinations. However, the study has several limitations, including a relatively small study population and a short observation period. Furthermore, the sensitivity of OCTA vascularity detection within the SHRM may be limited in the present study. Specifically, with quantitative OCTA, 18 of the 24 eyes with classic CNV on FA presented vascular SHRM. Therefore, these findings may indicate that our OCTA technique leads to false-negatives, especially in eyes with low-flow

neovascularization. Conversely, using the qualitative OCTA technique, 20 of the 24 eyes with classic CNV were judged to have vascular SHRM in the appropriate retinal plane (data not shown), suggesting that the sensitivity of our quantitative method is similar to that of the qualitative method, and that a combined strategy of quantitative and qualitative OCTA image analysis may improve the sensitivity of the detection of vascularity within SHRM using OCTA. Additionally, because of the lack of criteria for vascular SHRM and avascular SHRM, we defined them on the basis of the ratio of flow signal within the SHRM area by using quantitative OCTA. In fact, although our study population could apparently be divided into 2 groups, the classification of vascular SHRM and avascular SHRM may show variability. As for OCTA images, projection artifacts exist invariably. We excluded cases with strong projection artifacts by comparing en face images of the retinal superficial vessel and deep capillary vessels

with en face images of the outer retina. In particular, we excluded patients with massive hemorrhage, as well as those with fibrotic scar tissue, because these findings cause strong projection artifacts. Although some cases with weak projection artifacts could have remained, weak projection artifacts were canceled by setting a threshold of 2% for the ratio of flow signal within the SHRM area. Furthermore, posttreatment OCTA may be informative in cases of persistent SHRM; however, we did not include this in the study protocol. With regard to statistical analysis, our data should be interpreted carefully on account of the multiplicity of our statistical methods.

In conclusion, SHRM could be categorized as vascular and avascular by quantitative OCTA analysis. Vascular SHRM is associated with persistent SHRM and wet macula after anti-VEGF therapy, suggesting that vascular SHRM could be a predictive factor for less response to anti-VEGF therapy than is avascular SHRM.

FUNDING/SUPPORT: THIS RESEARCH WAS SUPPORTED IN PART BY A GRANT-IN-AID FOR SCIENTIFIC RESEARCH (NO. 24791847) from the Japan Society for the Promotion of Science, Tokyo, Japan, and the Innovative Techno-Hub for Integrated Medical Bio-Imaging of the Project for Developing Innovation Systems, from the Ministry of Education, Culture, Sports, Science, and Technology (MEXT), Tokyo, Japan. Financial Disclosures: Sotaro Ooto: grant: Alcon Japan (Tokyo, Japan); Akio Oishi: grant: Alcon Japan (Tokyo, Japan), Bayer (Leverkusen, Germany); Akitaka Tsujikawa: grant: Pfizer (New York, NY). The following authors have no financial disclosures: Yu Kawashima, Masayuki Hata, Kenji Yamashiro, Hiroshi Tamura, Manabu Miyata, Akihito Uji, and Naoko Ueda-Arakawa. All authors attest that they meet the current ICMJE criteria for authorship.

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