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Alterations in the retinal vasculature occur in multiple sclerosis and exhibit novel correlations with disability and visual function measures

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Abstract

Background: The retinal vasculature may be altered in multiple sclerosis (MS), potentially acting as a biomarker of disease processes.

Objective: To compare retinal vascular plexus densities in people with MS (PwMS) and healthy controls (HCs), and examine correlations with visual function and global disability.

Methods: In this cross-sectional study, 111 PwMS (201 eyes) and 50 HCs (97 eyes) underwent optical coherence tomography angiography (OCTA). Macular superficial vascular plexus (SVP) and deep vascular plexus (DVP) densities were quantified, and poor quality images were excluded according to an artifact-rating protocol.

Results: Mean SVP density was 24.1% (SD = 5.5) in MS eyes (26.0% (SD = 4.7) in non-optic neuritis (ON) eyes vs. 21.7% (SD = 5.5) in ON eyes, $p < 0.001$), as compared to 29.2% (SD = 3.3) in HC eyes ($p < 0.001$ for all MS eyes and multiple sclerosis optic neuritis (MSON) eyes vs. HC eyes, $p = 0.03$ for MS non-ON eyes vs. HC eyes). DVP density did not differ between groups. In PwMS, lower SVP density was associated with higher levels of disability (expanded disability status scale (EDSS): $R^2 = 0.26$, $p = 0.004$; multiple sclerosis functional composite (MSFC): $R^2 = 0.27$, $p = 0.03$) and lower letter acuity scores (100% contrast: $R^2 = 0.29$; 2.5% contrast: $R^2 = 0.40$; 1.25% contrast: $R^2 = 0.31$; $p < 0.001$ for all).

Conclusions: Retinal SVP density measured by OCTA is reduced across MS eyes, and correlates with visual function, EDSS, and MSFC scores.

Keywords: Multiple sclerosis, optical coherence tomography, angiography, retinal vasculature

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Introduction

Multiple sclerosis (MS) is thought to be an autoimmune demyelinating disorder in which neuroaxonal degeneration is the principal driver of disability. Microvascular abnormalities in the central nervous system (CNS) may contribute to or be a biomarker of the disease process. Global reductions in cerebral perfusion have been repeatedly illustrated in imaging studies of MS,[1–4] which could simply be a sequelae of neurodegeneration or could be mediated by concomitant inflammatory vasculopathy. Endothelial cell dysfunction,[5] excessive platelet activation,[6] evidence of oxidative stress,[7] altered blood-brain barrier permeability,[8] vascular occlusion within demyelinating lesions,[9] and hypoxia-like tissue injury[10] have all been reported in people with MS (PwMS).

The brain and retina share common embryological origins and vascular supply, and the idea of using the retinal vasculature to examine cerebral disease processes has been applied to other CNS disorders.[11] Optical coherence tomography angiography (OCTA) is a new technology allowing rapid, non-invasive imaging of the retinal vasculature. Initial studies of OCTA in MS have identified reduced retinal vascular measures in relatively small cohorts of PwMS as compared to healthy controls (HCs).[12–14]

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The goal of our study was to compare retinal vascular densities in a large cohort of PwMS to HCs, and to explore whether the retinal vasculature may be a biomarker of disability. Furthermore, we aimed to employ a pragmatic method for quality control of artifact-degraded images—an important but often overlooked limitation of OCTA.

Methods

Study design and participants

One hundred and eleven patients with relapsing-remitting MS (RRMS) or high-risk clinically isolated syndrome (CIS) were recruited for this cross-sectional study by convenience sampling from the Johns Hopkins MS center between 2017 and 2018. Diagnosis was made by the treating neurologist according to the 2010 McDonald criteria.[15] HCs ($n=50$) were healthy volunteers, primarily recruited from among Johns Hopkins University (JHU) staff. Exclusion criteria included potentially confounding neurological or ophthalmological disorders, eyes with prior ocular surgery or trauma or acute optic neuritis (ON) within the preceding 6 months, refractive errors of ±6 diopters, moderate to poorly controlled hypertension, or moderate to poorly controlled diabetes mellitus. Expanded disability status scale (EDSS) assessments were performed in all PwMS by neurostatus qualified raters. A subset of PwMS additionally underwent processing speed test ($n=49$) or multiple sclerosis functional composite (MSFC, $n=61$) assessment by qualified assessors. MSFC evaluation included timed 25-foot walk, 9-hole peg test, and paced auditory serial addition test. *Z* scores for each component and composite *Z* score were calculated according to the MSFC taskforce database.[16] Visual function was evaluated monocularly (with the participant's usual glasses or contact lenses when applicable) using retroilluminated 100% high-contrast Early Treatment of Diabetic Retinopathy Study charts at 4 m, and 2.5% and 1.25% low-contrast Sloan letter charts at 2 m.

OCTA and OCT image acquisition

OCTA scans were acquired for both eyes of each participant by experienced technicians under low-lighting conditions using Spectralis OCTA (model Spec-CAM S2610 with OCT2 and OCTA modules, software version 6.9a-US-IRB, Heidelberg Engineering, Germany). This device uses a wavelength of 870 nm to acquire 85,000 A-scans per second, with an axial tissue resolution of 7 µm. Full-spectrum amplitude decorrelation algorithm (FS-ADA) is employed for motion detection and image creation, and active eye tracking is facilitated by TruTrack. Average scan time was 40–60 seconds. Images with a sustained signal strength of <25 dB were excluded. Scans measuring 3 × 3 mm were automatically segmented by the device into images of the superficial vascular plexus (SVP) and deep vascular plexus (DVP).

OCT scans were acquired using spectral domain OCT by experienced technicians without pupillary dilation, as described in detail in prior publications.[17,18] At the time this study originally commenced, we routinely tracked all participants with Cirrus HD-OCT (model 5000, software version 8.1, Carl Zeiss Meditec, CA, USA). Spectralis SD-OCT was not routinely performed on all participants (in order to minimize participant burden and fatigue), although, regardless, 93 of 111 PwMS and 25 of 50 HCs were agreeable to also additionally complete OCT scans on this device. All acquired OCT scans were reviewed for quality in accordance with the OSCAR-IB criteria.[19] Peri-papillary retinal nerve fiber layer (pRNFL) thickness was quantified by the conventionally incorporated Cirrus HD-OCT and Spectralis SD-OCT automated algorithms. Macular retinal layer thicknesses (ganglion cell + inner plexiform layer (GCIPL), inner nuclear layer (INL), and outer nuclear layer (ONL)) were quantified from both devices using a validated segmentation algorithm developed at JHU that has been previously shown to consistently and reliably segment macular scans from both of these devices cross-sectionally and longitudinally.[20,21]

OCTA image quality control

A standardized process was applied for image acquisition, image processing, and identification of artifact (Supplemental Table 1). All OCTA images were qualitatively reviewed in a masked fashion by a single rater (O.C.M.) for the presence of artifact (Figure 1). The proportion of the capillary architecture affected by artifacts was classified as follows (Figure 2): (1) major artifact: causing substantial disruption of >25% of the capillary architecture; (2) intermediate artifact: causing substantial disruption of <25% of the capillary architecture; and (3) minimal artifact: minor artifact may be present but it does not cause disruption of the capillary architecture. The earliest imaging set to meet quality control criteria was used for each participant (Figure 3).

Vessel density calculation

Images of the SVP and DVP for each eye were manually cropped to 955 × 955 pixels (total 912,025 pixel area), centered around the fovea. Quantitative analysis of OCTA images was performed using open source

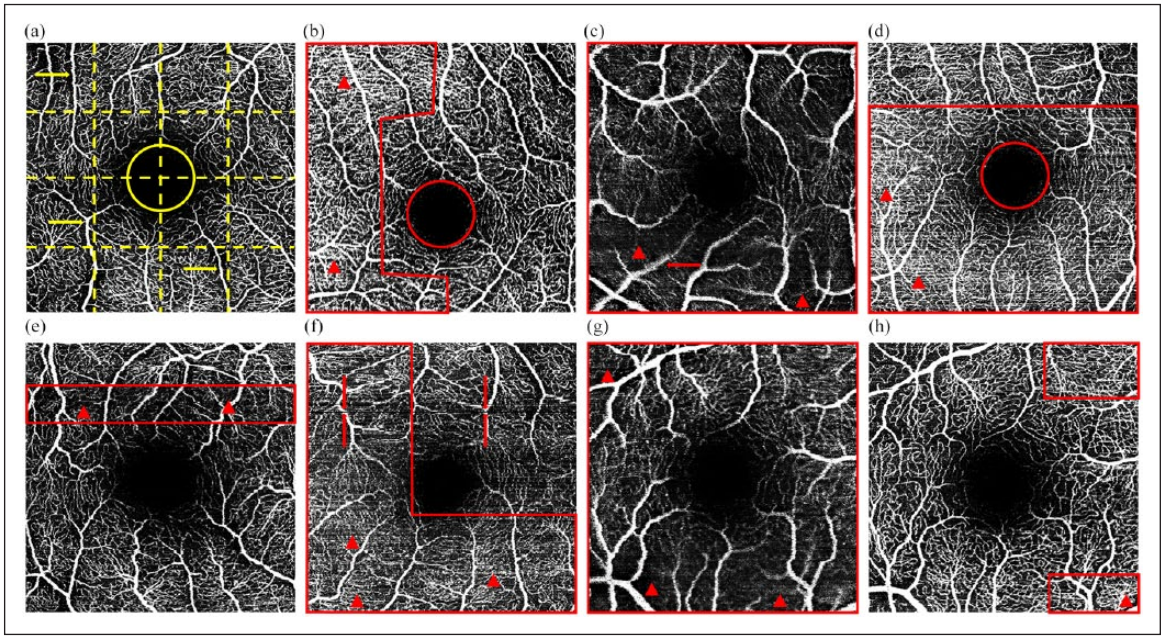

Figure 1. Assessment of artifact using images of the superficial vascular plexus. (a) A high quality image is demonstrated here. The fovea (yellow circle) is well-centered and the major arterioles have clear definition (yellow arrows). The contrast between the vasculature (white) and the background image (black) is crisp. Visualizing the image as divided in four vertical and four horizontal sections, the capillary architecture is not substantially disrupted by artifact in any segment. Artifact in this image was rated "minimal." (b) In this image, the fovea is off-center (circle). In addition, motion artifact is present in more than 25% of the image (outlined in red), which can be recognized by the "streaking" effect it creates. Areas where blurring/duplication of the capillary architecture is most marked are indicated by triangles. Artifact in this image was rated "major." (c) Loss of focus affects this entire image. This can be appreciated by loss of definition of the major arterioles (arrow), reduced contrast between the vasculature and the background, and complete dropout of the capillary architecture in large sections of the image (examples indicated by triangles). Artifact in this image was rated "major." (d) The fovea is off-center (circle) and motion artifact results in "streaking" of the majority of the image (outlined area). This creates blurring/duplication of the capillary architecture (triangles). Artifact in this image was rated "major." (e) A banding effect is demonstrated in this image, with some loss of definition of the capillary architecture (triangles) in the affected area (outlined in red), which represents $<25\%$ of the image. Artifact was therefore rated as "intermediate." (f) A blink line can be seen in this image, recognized as a horizontal black line (indicated by arrows). Blink lines do not result in a substantial quantitative alteration in vascular plexus densities. However, a large portion of this image is also affected by motion artifact resulting in poor contrast between the vasculature and the background (outlined in red), with loss of definition of the capillary architecture. Artifact in this image was rated as "major." (g) Loss of focus results in poor contrast between the vasculature and the background (outlined area) with consequent poorly defined arterioles and capillary dropout (triangles). Artifact was rated as "major." (h) This image is acquired from the same eye as (g) and is now affected by minimal artifact. The small outlined areas with some minor motion artifact represent $<25\%$ of the image. The difference between (g) and (h) demonstrates how artifact can substantially impair interpretation of OCTA images. Thus, quality control measures should be considered an essential element of any research study in OCTA.

ImageJ software (https://imagej.nih.gov/ij/). This software utilizes Otsu's thresholding method to convert each image into binary form (Figure 2) and quantify the number of black pixels in the image, which we used to calculate percentage vessel density.

Statistical analysis

Statistical analyses were performed using Stata version 13 (StataCorp, College Station, TX, USA). Patients with RRMS and high-risk CIS were grouped together for analysis, with this group referred to herein as "MS." Demographic details of PwMS and HCs were compared using chi-square test or Wilcoxon rank-sum test (for non-normally distributed data according to the Shapiro–Wilk test), as appropriate. Wilcoxon rank-sum test was used to compare vascular plexus densities according to artifact rating, and based on these results (presented later), images with major artifact were excluded from all further analyses. Mixed-effects linear regression analyses accounting for within-participant inter-eye correlations were used for all further analyses.

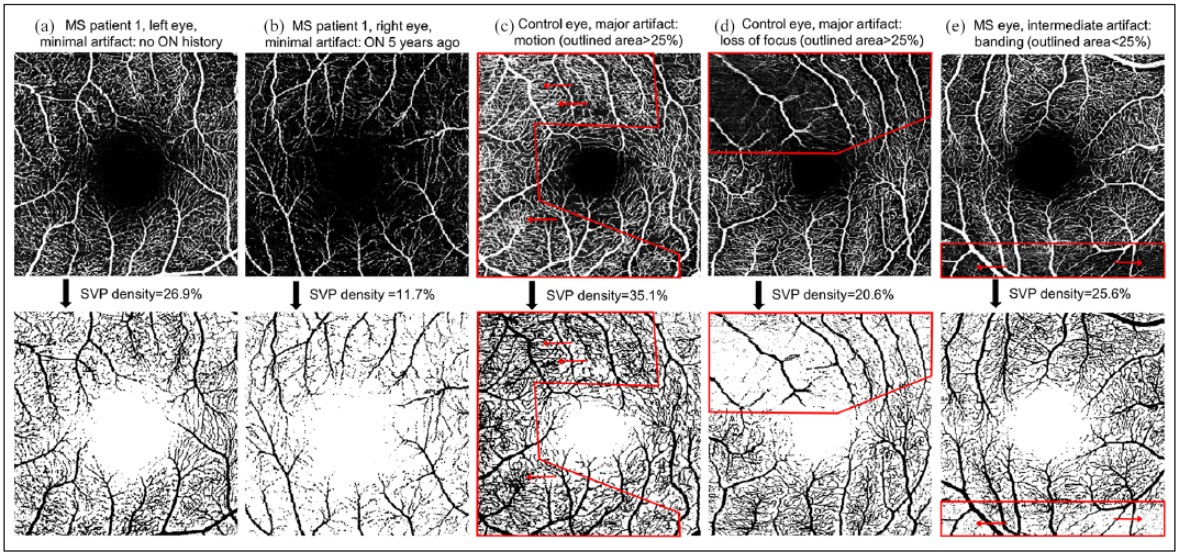

Figure 2. Examples of OCTA images of the superficial vascular plexus (SVP). Images of the SVP acquired using Heidelberg Spectralis OCTA device are presented here, in the raw form (upper row) and after processing into binary form using ImageJ software (lower row). (a, b) In an MS patient with a history of prior unilateral optic neuritis (ON), reduced SVP density can be appreciated qualitatively in the right eye (ON eye) as compared to the left eye. The images demonstrate minimal artifact with no substantial disruption of the capillary architecture. (c) In a control eye, the SVP image demonstrates how motion artifact duplicating the capillary vascular plexus margins (arrows indicate particularly affected areas) can result in the translation of artifact "noise" into additional dark pixels during binarization of the image, and therefore overestimation of the SVP density. The artifact rating is major as the artifact results in disruption of >25% of the capillary architecture. (d) In a control eye, loss of focus artifact affecting the SVP image can result in reduced definition of the capillary vascular plexus, with "dropout" of the plexus during binarization, and therefore underestimation of the SVP density. The artifact rating is major as the artifact results in disruption of >25% of the capillary architecture. (e) In an MS eye, banding artifact is present (arrows indicate areas with marked loss of capillary definition) but affects <25% of the capillary architecture, resulting in an intermediate artifact rating.
MS: multiple sclerosis.

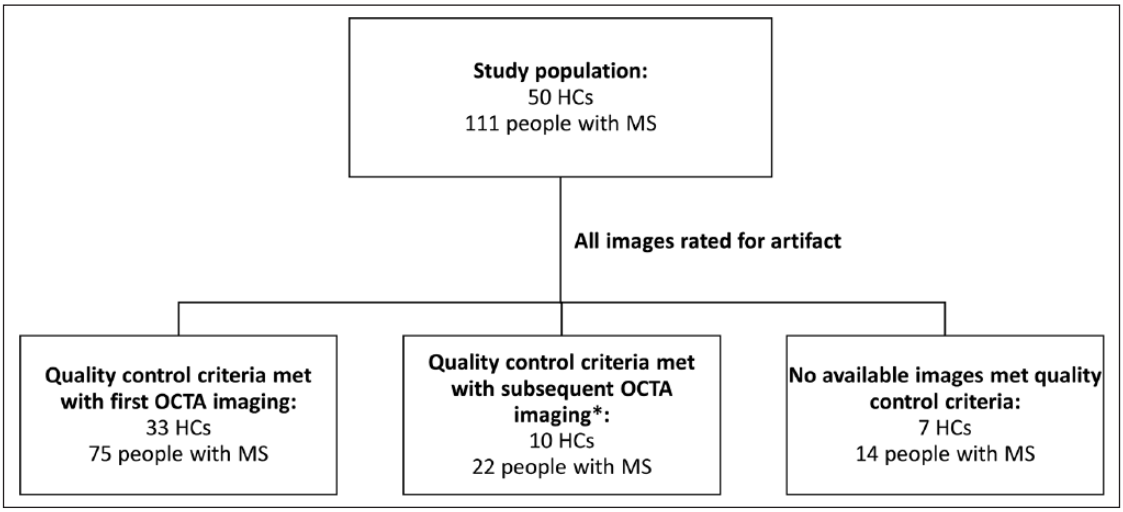

Figure 3. Impact of quality control criteria for OCTA images on the study population. All OCTA images were rated for artifact according to outlined criteria (see "methods" section). Where possible, images without major artifact were identified for each participant. In 7 HCs and 14 people with MS, all available OCTA images were affected by major artifact.
HCs: healthy controls; MS: multiple sclerosis; quality control criteria: OCTA images without major artifact obtained from at least 1 eye.
*Subsequent imaging occurred as part of a test–retest substudy or at a later study visit.

Table 1. Demographic characteristics of the population.

	HC	MS	*p* value
Participants, *n*	50	111	–
Eyes			
Total	97	201	–
Optic neuritis eyes (%)	–	81 (40%)	–
Age, mean (SD), years	34.5 (12.1)	40.0 (11.3)	0.002[a]
Female, sex (%)	29 (58%)	89 (80%)	0.003[b]
Ethnicity			
Caucasian (%)	32 (64%)	83 (75%)	0.009[b]
African American (%)	6 (12%)	22 (20%)	–
Other (%)	12 (24%)	6 (5%)	–
Disease duration, median, (IQR), years	–	10 (3–15)	–
EDSS score, median (IQR)	–	1.5 (1.5–2.5)	–
MSFC score, mean (SD) [$n=63$]	–	0.50 (0.50)	–
Disease-modifying therapy			
None (%)	–	23 (21%)	–
Dimethyl fumarate (%)	–	20 (18%)	–
Interferons (%)	–	19 (17%)	–
Glatiramer (%)	–	16 (14%)	–
Natalizumab (%)	–	14 (13%)	–
Rituximab/Ocrelizumab (%)	–	8 (7%)	–
Other (%)	–	11 (10%)	–

HC: healthy control; MS: multiple sclerosis; IQR: inter-quartile range; EDSS: expanded disability status scale; MSFC: multiple sclerosis functional composite; SD: standard deviation.
[a]Wilcoxon rank-sum test.
[b]Chi-square test.

Models were adjusted for age and sex (and in PwMS were additionally adjusted for disease duration and ON history). R^2 values were estimated at a participant level from mixed-effects models, according to methodology described by Snijders and Bosker.[22] Sensitivity analyses excluding all eyes with a history of ON were also performed in all regression models, and yielded similar results to the primary analyses.

Standard protocol approvals, registrations, and patient consents

JHU Institutional Review Board approval was obtained for study protocols, and all participants provided written informed consent.

Results

Demographic and clinical characteristics

One hundred and eleven PwMS (201 eyes) and 50 HCs (97 eyes) were included. Comparing demographic characteristics of PwMS to HCs (Table 1), there were significant differences in age ($p=0.003$) and sex ($p=0.003$). Ethnicity breakdown differed between PwMS and HCs ($p=0.009$), which was driven by higher numbers of Hispanic and Asian HCs. In PwMS, 40% of eyes had a history of ON, median disease duration was 10 years (interquartile range (IQR) = 3–15), and median EDSS score was 1.5 (IQR = 1.5–2.5). In accordance with the large body of evidence on retinal layer thicknesses in MS,[23] pRNFL and GCIPL thicknesses were reduced in PwMS versus HCs ($p \leqslant 0.001$ for both using mixed-effects linear regression, data not presented).

Artifact rating and quality control

After quality control criteria were applied (Figures 1 and 2, Supplemental Table 1), retinal vascular plexus densities were compared according to artifact rating. In MS eyes, lower retinal vascular plexus densities were found in OCTA images with major artifact, as compared to images with intermediate artifact ($p=0.001$ for SVP density and $p<0.001$ for DVP density, Table 2). Similar differences were identified in HC images affected by major or intermediate artifact. There was no significant difference in SVP

Table 2. Retinal vascular plexus densities stratified by OCTA image artifact rating.

	Major artifact		Intermediate artifact		Minimal artifact		*p* value (major vs. intermediate artifact)	*p* value (intermediate vs. minimal artifact)	*p* value (major vs. intermediate/ minimal artifact)
	n	Mean (*SD*)	*n*	Mean (*SD*)	n	Mean (*SD*)			
Superficial vascular plexus density (%)									
HC eyes	26	27.4 (4.9)	36	29.6 (3.5)	35	28.9 (3.1)	0.07	0.31	0.07
MS eyes	42	20.8 (11.1)	78	23.7 (5.7)	81	24.5 (5.3)	**0.002**	0.37	**<0.001**
MSON eyes	10	18.2 (6.3)	35	21.4 (6.1)	36	22.1 (5.0)	0.14	0.66	0.07
MSNON eyes	32	21.7 (12.0)	43	25.5 (4.6)	45	26.5 (4.7)	**0.003**	0.28	**<0.001**
Deep vascular plexus density (%)									
HC eyes	21	26.3 (3.4)	31	30.2 (3.9)	45	31.5 (2.8)	**<0.001**	0.13	**<0.001**
MS eyes	39	23.8 (5.7)	75	29.7 (3.7)	87	30.7 (2.8)	**<0.001**	0.08	**<0.001**
MSON eyes	10	25.2 (3.9)	33	30.3 (3.9)	38	30.8 (2.4)	**0.002**	0.56	**<0.001**
MSNON eyes	29	23.3 (6.1)	42	29.4 (3.6)	49	30.8 (2.8)	**<0.001**	0.06	**<0.001**

HC: healthy control; MS: multiple sclerosis; MSON: multiple sclerosis optic neuritis; MSNON: multiple sclerosis non-optic neuritis; OCTA: optical coherence tomography angiography; SD: standard deviation.
The *p* values are calculated using Wilcoxon rank-sum test. *p* value for significance (i.e. bold type) was taken as <0.05.

density between images with intermediate versus minimal artifact. Using mixed-effects linear regression adjusted for within-participant inter-eye correlations, there were no significant associations between the artifact rating and participant characteristics including age, MS or HC status, sex, disease duration, ON status, or visual acuity; however, there was a weak association in PwMS between a greater degree of artifact and higher level of disability as measured by EDSS score ($R2 = 0.03$ and $p = 0.04$ for both SVP and DVP images).

Retinal vascular plexus densities in MS versus HCs

After exclusion of SVP images with major artifact (42 MS eyes and 26 HC eyes, with Table 3 showing updated demographic characteristics of the cohort), mean SVP density was lower in MS eyes with and without a history of ON (21.7% (SD = 5.5) for multiple sclerosis optic neuritis (MSON) eyes and 26.0% (SD = 4.7) for MSNON eyes), as compared to HC eyes (29.2% (SD = 3.3), $p < 0.001$ for MSON eyes and $p = 0.03$ for MSNON eyes), using mixed-effects linear regression adjusted for age, sex, and within-participant inter-eye correlations. Furthermore, reductions in SVP density in MSON eyes were significantly greater than in MSNON eyes ($p < 0.001$). Mean DVP density did not differ in MS eyes as compared to HC eyes ($p = 0.92$), or in MSON eyes as compared to MSNON eyes ($p = 0.18$). Given the differences in demographic characteristics identified between PwMS and HCs (Tables 1 and 3), we also performed sensitivity analyses: (1) limiting analysis to participants between the age of 30 and 50, which removed any age difference between PwMS and HCs, (2) limiting analysis to either male or female participants, and (3) limiting analysis to Caucasian and African American participants and excluding participants identifying as "other" ethnicity. In all of these sensitivity analyses, robust reductions in SVP density were identified between PwMS as compared to HCs (similar or greater to differences found in the primary model), and no differences in DVP density were identified.

Relationships between retinal vascular plexus densities and retinal layer thicknesses

In both HC and MS eyes, lower SVP density was associated with lower pRNFL and GCIPL thicknesses ($p < 0.001$ for all) while lower DVP density was associated with lower GCIPL and INL thicknesses ($p = 0.003$ for GCIPL thickness and $p = 0.005$ for INL thickness in HC eyes; $p = 0.03$ for GCIPL thickness and $p = 0.01$ for INL thickness in MS eyes, Figure 4). ONL thickness correlated with DVP density in MS eyes ($p = 0.02$) but not in HC eyes ($p = 0.68$).

Relationships between retinal vascular plexus densities or retinal layer thicknesses and disability (EDSS, MSFC, visual function)

Lower SVP density was associated with longer disease duration (every 1-year increase in disease

Table 3. Demographic characteristics of the population used in analyses of superficial vascular plexus densities, after exclusion of images with major artifact.

	HC	MS	*p* value
Participants, *n*	39	94	–
Eyes			
Total	71	159	–
Optic neuritis eyes (%)	–	71 (45%)	–
Age, mean (*SD*), years	32.4 (11.2)	39.9 (10.6)	0.04[a]
Female, sex (%)	22 (56%)	75 (80%)	0.006[b]
Race			
Caucasian (%)	24 (62%)	74 (79%)	0.03[b]
African American (%)	6 (15%)	16 (17%)	–
Other (%)	9 (23%)	4 (4%)	–
Disease duration, median, (IQR), years	–	10 (3–16)	–
EDSS score, median (IQR)	–	1.5 (1.0–2.0)	–
MSFC score, mean (*SD*) (*n*=57)	–	0.56 (0.45)	–
Disease-modifying therapy			
None (%)	–	20 (21%)	–
Dimethyl fumarate (%)	–	16 (17%)	–
Interferons (%)	–	17 (18%)	–
Glatiramer (%)	–	13 (14%)	–
Natalizumab (%)	–	10 (11%)	–
Rituximab/Ocrelizumab (%)	–	7 (7%)	—
Other (%)	–	11 (12%)	–

HC: healthy control; MS: multiple sclerosis; IQR: inter-quartile range; EDSS: expanded disability status scale; MSFC: multiple sclerosis functional composite; SD: standard deviation.
[a]Wilcoxon rank-sum test.
[b]Chi-square test.

duration was associated with a 0.2% (95% confidence interval (CI)=0.05–0.3) decrease in mean SVP density, $R^2=0.20$, $p=0.02$, Table 4). After additional adjustment for disease duration, lower SVP density was associated with higher EDSS scores (every 1.0-point increase in EDSS score was associated with a 1.1% (95% CI=0.4–1.9) decrease in mean SVP density, $R^2=0.20$, $p=0.004$, Table 4 and Figure 5). Furthermore, lower SVP density was also associated with increasing disability as measured by the MSFC score (every 1-point decrease in MSFC was associated with a 2.8% (95% CI=0.4–5.2) decrease in mean SVP density, $R^2=0.27$, $p=0.03$). DVP density did not demonstrate associations with disease duration or disability measures.

To explore how the relationships between retinal vascular densities and disability compare with those between retinal layer thicknesses and disability, we performed similar analyses using GCIPL thickness—the retinal layer most strongly linked to disability outcomes in previous studies of MS.[24] GCIPL thickness did demonstrate significant associations with EDSS score (every 1.0-point increase in EDSS score was associated with a 2.2 µm (95% CI=1.1–3.3) decrease in GCIPL thickness, $R^2=0.26$, $p<0.001$, Table 4 and Figure 6). On the contrary, and in keeping with previously published work,[25] there were no relationships between GCIPL thickness and MSFC score.

Lower SVP density and lower GCIPL thickness were both associated with lower monocular letter acuity scores at 100% high contrast, 2.5% low contrast, and 1.25% low contrast in PwMS ($p<0.001$ for all, Table 4).

Discussion

Results of our study provide compelling evidence that OCTA-derived retinal macular SVP densities are reduced in PwMS as compared to HCs, supporting and expanding on previous work in smaller cohorts.[12,13] Interestingly, our findings within the retina mirror the body of evidence from imaging research that diffuse cerebral hypoperfusion which might occur in PwMS.[1–4] This is perhaps unsurprising given that the retina can be considered a "window into the brain" and retinal

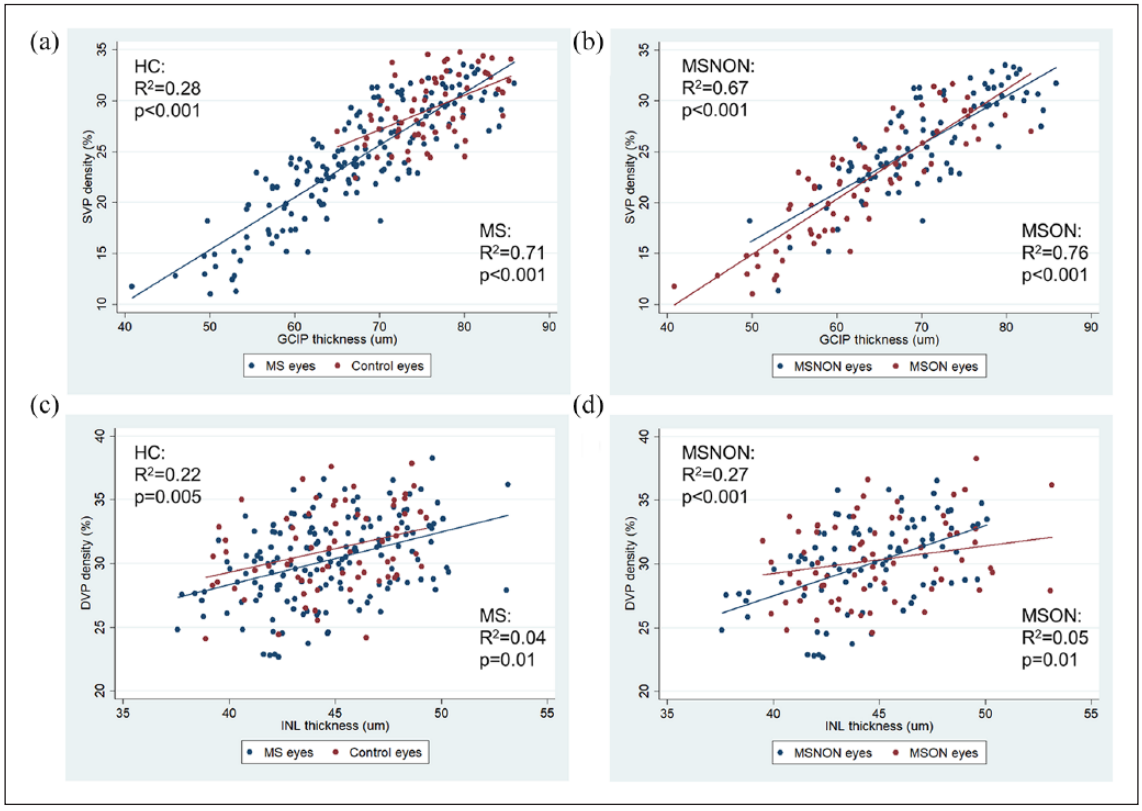

Figure 4. Relationships between retinal vascular plexuses and retinal layer thicknesses. *P* values were derived from mixed-effects linear regression adjusted for age, sex, and within-participant inter-eye correlations (and additionally adjusted for disease duration in people with MS, and ON history in (a) and (c)). R^2 values were estimated at a participant level from mixed-effects regression models using the Snijders and Bosker method. Results are presented from analysis using Cirrus HD-OCT. Sensitivity analysis using retinal layer thicknesses derived from Spectralis SD-OCT demonstrated similar associations. Anatomically, the SVP is located primarily in the GCIPL, and strong relationships between SVP density and GCIPL thickness are identified in (a) MS eyes and HC eyes, and (b) MSON eyes and MSNON eyes. The DVP is located primarily in the INL, and moderate relationships are demonstrated between DVP density and INL thickness in (c) MS eyes and HC eyes, and (d) MSON eyes and MSNON eyes.
HC: healthy control eyes; MS: multiple sclerosis eyes; MSNON: multiple sclerosis non-optic neuritis eyes; MSON: multiple sclerosis optic neuritis eyes; SVP: superficial vascular plexus; DVP: deep vascular plexus; GCIPL: ganglion cell + inner plexiform layer; INL: inner nuclear layer.

measures show relationships with gray matter volumes[26] and brain atrophy rates.[27] Indeed, abnormal cerebral and retinal vascular measures in MS may simply reflect neurodegeneration. Importantly, and similar to one smaller study,[13] we have shown that SVP densities are reduced in MS eyes both with and without a history of ON. Furthermore, results from our study show that lower SVP densities in MS patients are associated with poorer visual function, longer disease duration, and higher levels of global disability (as estimated by both EDSS and MSFC scores), suggesting that OCTA may be a useful biomarker of neurodegeneration in PwMS, particularly when interpreted in conjunction with retinal layer thickness measurements.

A primary question that arises from our study and others is the mechanism underlying reductions in retinal vascular densities in MS eyes. It is now well-established that MS eyes exhibit reduced thicknesses of certain retinal layers (primarily the pRNFL and GCIPL) as compared to HCs, and that reductions are most marked in eyes with a history of ON.[23] Furthermore, pathological studies have confirmed that retinal inflammation and ganglion cell loss are frequent, and optic nerve pathology is almost ubiquitous in MS.[28] In the retina, the vascular plexuses are supplied by the central retinal artery (a branch of the ophthalmic artery). The SVP supplies the RNFL and GCIPL layers, and provides diving arterioles to the DVP which supplies the INL and outer plexiform layer. Given that ON and optic neuropathy cause atrophy of the pRNFL and GCIPL, a rational explanation for reductions in SVP density observed in MS eyes may simply relate to a reduction in metabolic demand

Table 4. Correlations between retinal vascular plexus densities and disease variables in MS patients.

	SVP density[a] (%)			DVP density[a] (%)			GCIPL thickness (μm)		
	N eyes, subjects	R^2[b]	*p* value[c]	*N* eyes, subjects	R^2[b]	*p* value[c]	*N* eyes, subjects	R^2[b]	*p* value[c]
Disease duration, years	159, 94	0.20	**0.02**	162, 96	0.04	0.24	199, 111	0.16	**0.005**
EDSS score	159, 94	0.26	**0.004**	162, 96	0.04	0.43	199, 111	0.26	**<0.001**
MSFC score	101, 57	0.27	**0.03**	105, 59	0.01	0.32	118, 63	0.22	0.21
Average T25W, seconds	118, 67	0.19	**0.02**	122, 69	0	0.69	139, 75	0.09	0.12
Leg *Z* score[d]	118, 67	0.19	**0.02**	122, 69	0	0.69	139, 75	0.09	0.12
Average 9HPT, seconds	115, 65	0.22	**0.006**	119, 67	0	0.14	134, 72	0.14	0.05
Arm *Z* score[d]	115, 65	0.25	**0.001**	119, 67	0	0.19	134, 72	0.14	0.05
PASAT	104, 59	0.20	0.43	108, 61	0.02	0.27	123, 66	0.16	0.79
Cognitive *Z* score[d]	104, 59	0.20	0.43	108, 61	0.02	0.27	123, 66	0.16	0.79
Processing speed test, seconds	68, 40	0.42	0.30	69, 40	0.16	0.19	89, 49	0.24	0.25
Letter acuity, monocular									
100% contrast	154, 92	0.29	**<0.001**	157, 94	0.04	0.22	194, 109	0.29	**<0.001**
2.5% contrast	154, 92	0.40	**<0.001**	157, 94	0.06	0.27	194, 109	0.44	**<0.001**
1.25% contrast	154, 92	0.31	**<0.001**	157, 94	0.05	0.25	194, 109	0.35	**<0.001**

SVP: superficial vascular plexus; DVP: deep vascular plexus; GCIPL: ganglion cell + inner plexiform layer; EDSS: expanded disability status scale; MSFC: multiple sclerosis functional composite; T25W: timed 25-foot walk; 9HPT: nine hole peg test; PASAT: paced auditory serial addition test; MS: multiple sclerosis. *p* value for significance (i.e. bold type) was taken as <0.05.
[a]Images with major artifact excluded (42 SVP images and 39 DVP images).
[b]Estimated R^2 values derived from mixed-effects regression models using the Snijders and Bosker method.
[c]Mixed-effects regression adjusted for age, sex, disease duration, optic neuritis history, and inter-eye correlations.
[d]Calculated according to the MSFC taskforce database.

within these layers, that is, a secondary effect of neurodegeneration or marked mitochondrial dysfunction in damaged neurons. This hypothesis is supported by the strong correlation between SVP density and GCIPL thickness identified, with this correlation being stronger in MS eyes than HC eyes, and even stronger in MS eyes with a history of ON. In other words, these measures are closely linked and with greater neuroaxonal loss the degree of variability in GCIPL thickness is increasingly better explained by SVP density. It is also possible that reduced SVP densities in MS eyes reflect endothelial injury as a direct effect of prior inflammation. However, these hypotheses assume inflammation or neurodegeneration precede or occur in tandem with reductions in vascular supply—an assumption that cannot be proven without detailed longitudinal analysis. Another potential explanation for our findings is that vascular aberrations in the retina are not just a "bystander" effect, but a possible contributory process in the pathophysiology of MS. Indeed, support for a possible uncoupling between neurodegeneration and hypoperfusion in MS includes brain imaging studies suggesting that gray matter perfusion is reduced even in regions without evidence of atrophy.[29] Finally, regardless of whether vascular abnormalities in PwMS are a primary contributor to the pathophysiology of the disease, there is accumulating evidence that co-morbidities that are traditionally considered to be cardiovascular risk factors may play a role in modifying the clinical course of PwMS.[30–34] Thus, the potential to provide information on the combined effects of neurodegeneration and vasculopathy makes OCTA an attractive avenue of investigation in MS.

The histopathological correlate of reduced retinal SVP density in MS eyes likely holds important clues to pathobiology, and yet, this correlate is essentially unknown, as there is a paucity of histopathological studies quantifying the retinal vasculature in MS eyes. It is possible that reduced SVP densities measured by OCTA represent a reduction in capillary vessel number, capillary vessel volume, or simply low capillary flow undetectable by motion-based OCTA technology. Retinal periphlebitis could also potentially affect flow rates, and has been identified in MS eyes hispathologically,[28,35] and in studies using fundus photography and/or fluorescein angiography.[36–38] Indeed, using another retinal imaging device, retinal blood flow velocities have been shown to be reduced in MS eyes.[39,40] Future angiographic and histopathologic studies may provide further useful information regarding the mechanisms underlying OCTA measurement aberrations in MS.

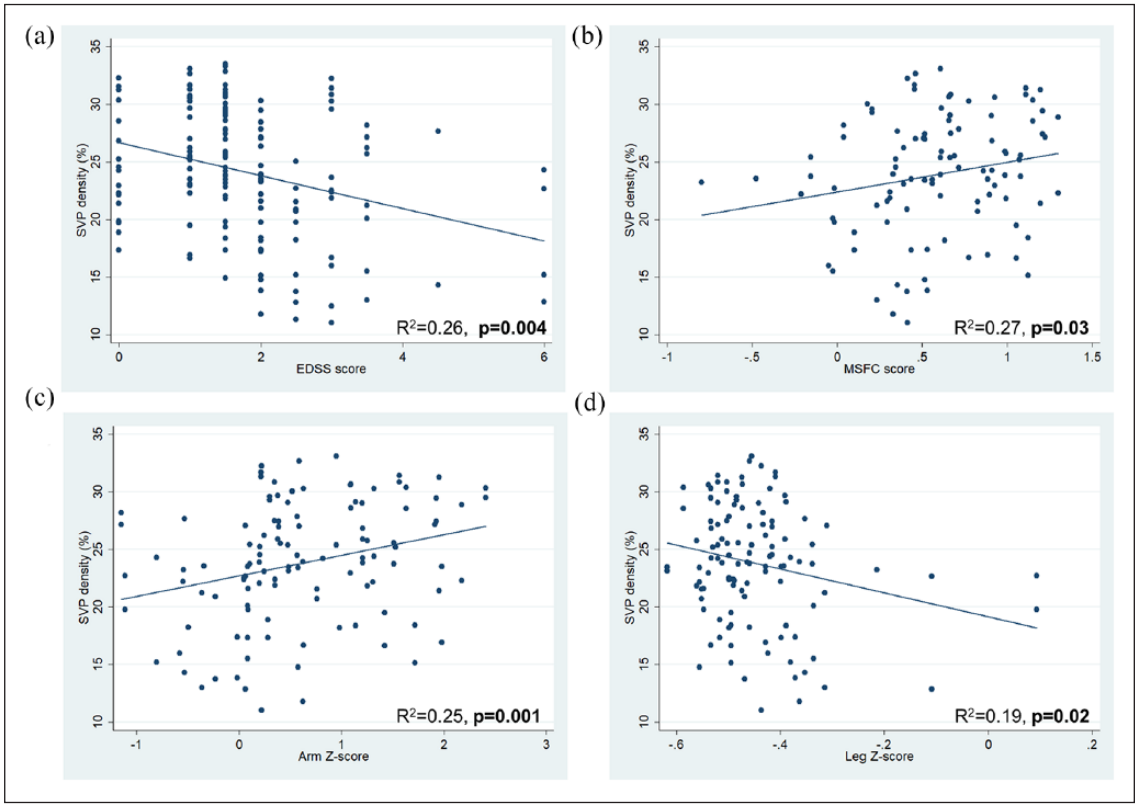

Figure 5. Relationships between SVP density and disability measures in people with MS. *P* values were derived from mixed-effects linear regression adjusted for age, sex, disease duration, optic neuritis history, and inter-eye correlations. R^2 values were estimated at a participant level from mixed-effects regression models using the Snijders and Bosker method. Relationships are demonstrated between lower SVP density and higher levels of disability measured with (a) EDSS, (b) overall MSFC score, (c) arm *Z* score (calculated using nine hole peg test), and (d) leg *Z* score (calculated using timed 25-foot walk).
SVP: superficial vascular plexus; EDSS: expanded disability status scale; MSFC: multiple sclerosis functional composite. *Z* scores (arm and leg) derived using the MSFC taskforce database.

Relationships between lower SVP densities and both longer disease duration and higher levels of global disability were an important finding in our cohort. One study reported similar associations between retinal vessel densities and disability measured by EDSS score and multiple sclerosis severity score (MSSS) in a cohort of 50 PwMS.[13] Our findings of relationships between lower SVP densities and higher levels of disability measured by MSFC scores, component leg, and arm *Z* scores are novel and of particular interest. Furthermore, previous studies of standard OCT measures in MS have failed to identify relationships between retinal layer thicknesses and MSFC scores.[25] We suggest that lower SVP densities could represent a marker of more global disease processes in MS, particularly neurodegeneration and otherwise challenging to estimate vasculopathy contribution to the MS disease process. Another novel and, furthermore, important observation in our study was that lower SVP densities in PwMS correlate with decreased high and low contrast visual function, findings similar to those described using OCT[24] (and indeed also identified in this study). Collectively, studies support strong structure-function relationships within the retina in MS.

Our cross-sectional study examining retinal vascular densities in MS eyes represents one of the largest cohorts examined to date. Furthermore, we have noted the importance of image artifact as a potential confounding variable in analyses and employed a pragmatic quality-control protocol to exclude artifact-degraded images. This is important because ocular dysmetria in MS patients may result in loss of focus artifact and falsely reduced vascular densities. Our exclusion of images degraded by loss of focus artifact means that the differences we identified between MS eyes and HC eyes are not due to ocular dysmetria alone. However, our study has a number of limitations. First, the quantification of retinal vessel densities from OCTA images is a relatively new technique with some technical limitations including frequent imaging artifact. Indeed, implementation of

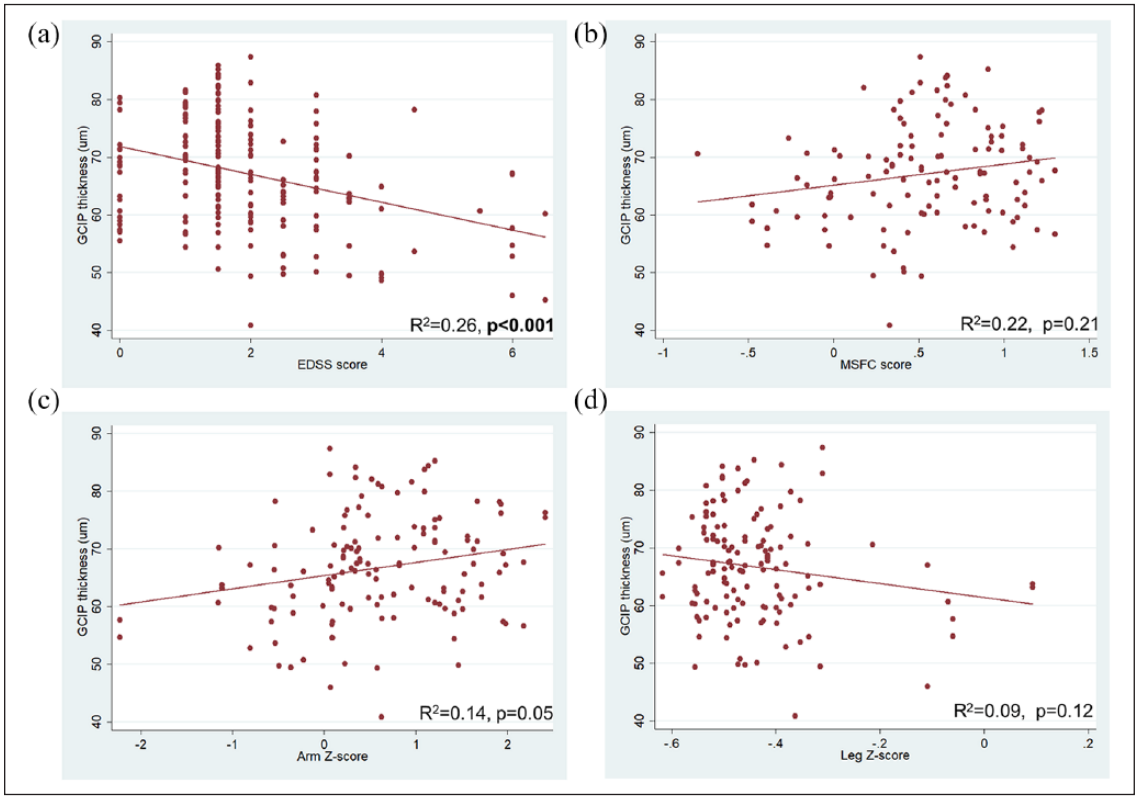

Figure 6. Relationships between GCIP thickness and disability measures in people with MS. *P* values were derived from mixed-effects linear regression adjusted for age, sex, disease duration, optic neuritis history, and inter-eye correlations. R^2 values were estimated at a subject level from mixed-effects regression models using the Snijders and Bosker method. Lower GCIP thickness demonstrated significant relationships with (a) higher EDSS score, (b) but not with overall MSFC score, (c) arm *Z* score (calculated using nine hole peg test), or (d) leg *Z* score (calculated using timed 25-foot walk).
GCIP: ganglion cell + inner plexiform layer; EDSS: expanded disability status scale; MSFC: multiple sclerosis functional composite. *Z* scores (arm and leg) derived using the MSFC taskforce database.

our quality control protocol led to exclusion of a substantial proportion of images (approximately 20%). In addition, while our quality control criteria performed very well with SVP images, the imaging artifact seen in DVP images tends to be more subtle and difficult to rate. Flow-projection artifact from the SVP has been previously noted as a significant confounder in evaluating the DVP, leading to the development of algorithms for "projection-resolved OCTA."[41] Indeed, Heidelberg Spectralis OCTA software was updated in 2018 to include post-acquisition measures reducing flow-projection artifact, which we applied to all images in this study. The image processing and vessel density method we have utilized is not specific and applies a thresholding method for binarization of images (i.e. transforming a grayscale image to a binary black and white image), with potential underestimation or overestimation of vascular plexus densities when contrast between the vasculature and background is poor. In addition, while we examined macular retinal vessel densities, we included peripapillary RNFL measurements in our analysis of correlations—a region which does not receive arterial supply from the vascular plexuses analyzed. We opted for this approach because the RNFL in the peripapillary region is a widely used and reproducible[17] measurement in OCT analysis of PwMS, whereas in the macular region, the RNFL is thin and more difficult to reliably segment. Our study is cross-sectional in nature and we did not include patients with progressive MS. Finally, patients were recruited using convenience sampling from a clinic setting, which may introduce some selection bias, for example, less disabled patients may be more likely to attend clinic and patients with visual symptoms may be more interested in research participation.

Conclusion

Retinal SVP densities measured by OCTA are reduced in MS eyes (including MSNON eyes) and are closely related to GCIPL thickness. Furthermore, reduced SVP densities correlate with reduced visual function, longer disease duration, and higher levels of global disability,

suggesting that OCTA may have additive value as a biomarker in MS, in conjunction with routine OCT measures. Our findings add to the growing body of evidence that both cerebral and retinal vascular abnormalities are widespread in PwMS. As the retina is an ideal location for rapid non-invasive imaging of the microvasculature, further work may elucidate important clues to the pathophysiology of MS. Based on our preliminary findings which suggest SVP density may be a marker of neurodegeneration in MS with strong structure–function relationships, SVP density and other OCTA derived measures may have a role to play as outcomes in MS clinical trials in the future.

Declaration of Conflicting Interests

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Supplemental Material

Supplemental material for this article is available online.

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