MULTIPLE SCLEROSIS JOURNAL | MSJ

Original Research Paper

Optical coherence tomography angiography indicates associations of the retinal vascular network and disease activity in multiple sclerosis

Multiple Sclerosis Journal

1–11

DOI: 10.1177/1352458518750009

Nikolaus Feucht, Mathias Maier, Gildas Lepennetier, Moritz Pettenkofer, Carmen Wetzlmair, Tanja Daltrozzo, Pauline Scherm, Claus Zimmer, Muna-Miriam Hoshi, Bernhard Hemmer, Thomas Korn and Benjamin Knier

Abstract

Background: Patients with multiple sclerosis (MS) and clinically isolated syndrome (CIS) may show alterations of retinal layer architecture as measured by optical coherence tomography. Little is known about changes in the retinal vascular network during MS.

Objective: To characterize retinal vessel structures in patients with MS and CIS and to test for associations with MS disease activity.

Method: In all, 42 patients with MS or CIS and 50 healthy controls underwent retinal optical coherence tomography angiography (OCT-A) with analysis of the superficial and deep vascular plexuses and the choriocapillaries. We tested OCT-A parameters for associations with retinal layer volumes, history of optic neuritis (ON), and the retrospective disease activity.

Results: Inner retinal layer volumes correlated positively with the density of both the superficial and deep vascular plexuses. Eyes of MS/CIS patients with a history of ON revealed reduced vessel densities of the superficial and deep vascular plexuses as compared to healthy controls. Higher choriocapillary vessel densities were associated with ongoing inflammatory disease activity during 24 months prior to OCT-A examination in MS and CIS patients.

Conclusion: Optic neuritis is associated with rarefaction of the superficial and deep retinal vessels. Alterations of the choriocapillaries might be linked to disease activity in MS.

Keywords: Multiple sclerosis, optical coherence tomography, optical coherence tomography angiography, optic neuritis, disease activity, biomarker

Date received: 27 July 2017; revised: 24 November 2017; accepted: 1 December 2017

Correspondence to:
B Knier
Department of Neurology, Klinikum rechts der Isar, Technische Universität München, Ismaninger Street 22, Munich 81675, Germany.
benjamin.knier@tum.de

T Korn
Department of Neurology, Klinikum rechts der Isar, Technische Universität München, Ismaninger Street 22, Munich, 81675, Germany.
thomas.korn@tum.de

Nikolaus Feucht
Mathias Maier
Moritz Pettenkofer
Pauline Scherm
Department of Ophthalmology, Klinikum rechts der Isar, Technische Universität München, Munich, Germany

Gildas Lepennetier
Benjamin Knier
Department of Neurology, Klinikum rechts der Isar, Technische Universität München, Munich, Germany/ Department of Experimental Neuroimmunology, Klinikum rechts der Isar, Technische Universität München, Munich, Germany

Carmen Wetzlmair
Tanja Daltrozzo
Muna-Miriam Hoshi
Department of Neurology, Klinikum rechts der Isar, Technische Universität München, Munich, Germany

Claus Zimmer
Department of Neuroradiology, Klinikum rechts der Isar, Technische Universität München, Munich, Germany

Bernhard Hemmer
Department of Neurology, Klinikum rechts der Isar, Technische Universität München, Munich, Germany/

Introduction

Retinal optical coherence tomography (OCT) is a non-invasive and well-tolerated imaging technique which allows reproducible high-resolution analysis of different retinal structures.[1] Since the retina reflects central nervous system (CNS) tissue directly accessible to optical imaging, the OCT technique has already been introduced into clinical neuroimmunology about 20 years ago and has developed into a sensitive method for the evaluation of optic nerve and retinal pathology in multiple sclerosis (MS).[2] MS-associated optic neuritis (ON) leads to axonal loss within the optic nerve and results in thinning of the retinal nerve fiber layer (RNFL) and the common ganglion cell and inner plexiform layer (GCIPL).[3–5] OCT-derived retinal layer volumes correlate with both brain[6] and spinal cord volumes[7] suggesting that OCT might serve as a potential tool for the evaluation of neurodegeneration in the course of MS. In addition, retinal OCT might be helpful as a prognostic marker for disease outcomes in MS patients with both short[8] and longer[9] disease durations. Although MS-associated changes in retinal layer architecture have been described in

Munich Cluster for Systems Neurology (SyNergy), Munich, Germany

Thomas Korn
Department of Neurology, Klinikum rechts der Isar, Technische Universität München, Munich, Germany/ Department of Experimental Neuroimmunology, Klinikum rechts der Isar, Technische Universität München, Munich, Germany/ Munich Cluster for Systems Neurology (SyNergy), Munich, Germany

detail, only little is known about alterations in retinal vessel integrity during MS.

OCT-angiography (OCT-A) is a novel and non-invasive imaging technique that generates high-resolution information of retinal blood vessels. In principle, OCT-A obtains consecutive scans at one location of the retina. After removal of stationary tissue signals, the remaining signal reflects the complete scan area-intrinsic motion of corpuscular blood constituents in vessels including both venous and arterial blood vessels.[10] Thus, OCT-A allows assessment of retinal vessel structures within different vessel plexuses of the retina and the choriocapillary layer. In this study, we aimed to provide a descriptive and detailed overview of alterations in retinal vessel densities linked to the disease course of MS during clinical practice. We furthermore searched for associations of the retinal vessel plexuses and disease activity patterns in patients with CIS or MS.

Materials and methods

Subjects and study design

In this retrospective study, individuals with relapsing-remitting MS (RRMS), clinically isolated syndrome (CIS), and healthy controls (HCs) were recruited from the Department of Neurology and the Department of Ophthalmology at the Klinikum rechts der Isar, Technical University of Munich, between 2016 and 2017. RRMS and CIS patients were included from an ongoing observational cohort study of patients with CIS or MS (TUM-MS). RRMS and CIS were defined using the 2010 McDonald criteria.[11] Age- and sex-matched HCs served as controls. We included patients from 18 to 60 years of age and a disease duration of at least 12 months. We excluded patients with substantial eye disease, a refractive error ⩾6 diopter, occurrence of ON or use of corticosteroids within 30 days prior to enrollment, or poor OCT-A quality on both eyes. After enrollment into the study, both RRMS/CIS patients and HCs underwent an OCT-A examination. Only RRMS/CIS patients received a clinical neurological examination with evaluation of the Expanded Disability Status Scale (EDSS) and were studied by OCT within 1 week after OCT-A examination. The patients' disease history during the last 24 months prior to study enrollment or since first clinical episode (if disease duration was shorter than 24 months) was studied. Here, annualized relapse rates (ARRs), annualized increases in T2 lesion counts, or annualized numbers of gadolinium-enhancing (Gd+) lesions as measured by cerebral magnetic resonance imaging (MRI) were calculated. Patients who were free of relapses and free of Gd+ lesions and who showed stable T2 lesion counts and EDSS values within this period of time were defined to have no evidence of disease activity (NEDA-3). The patients' disease-modifying therapies (DMTs) during the last 24 months prior to enrollment were categorized into first-line DMT including interferon beta-1a/b, glatiramer acetate, dimethyl fumarate, or teriflunomide and second-line DMT including natalizumab, fingolimod, alemtuzumab, or rituximab. The study was approved by the ethics commission of the Technical University of Munich and followed the Declaration of Helsinki. All patients gave written informed consent. We followed Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statements for reporting cohort studies.

OCT-A

OCT-A image acquisition was performed using a RTVue XR Avanti spectral-domain OCT device (Optovue Inc., Fremont, CA, USA). This instrument has an A-scan rate of 70,000/s using a light source of 840 nm. En face images were acquired focusing the fovea centralis. Each scan consisted of 304 × 304 A-scans with two consecutive B-scans at each fixed position. To reduce motion artifacts, each scan consisted of one orthogonal horizontal and vertical scan.[12,13] Pictures with visible motion artifacts or projection artifacts were excluded. To extract information on vessel structures, the device uses the split-spectrum amplitude-decorrelation angiography (SSADA) algorithm, which is a mathematical algorithm fully implemented in the used OCT-A device. In principle, SSADA detects variations in reflected OCT signal amplitudes between two consecutive scans.[13] Decorrelation is a mathematical function that quantifies this variation. SSADA splits the OCT signal into different spectral bands, thus increasing the number of usable image frames, in which each undergoes a decorrelation analysis.[13] Static tissues reveal low decorrelation, whereas blood vessels show high decorrelation values.[14] Thus, OCT-A provides information on perfused retinal blood vessel structures at the scanning timepoint, but not on blood flow velocities. The exact mathematical algorithm is described elsewhere.[13,14] In this study, we investigated the superficial retinal capillary plexus, deep capillary plexus, and the choriocapillary layer. For the analysis of the superficial and deep retinal capillary plexuses, we assessed decorrelation signals representing vessel densities of a 6 mm × 6 mm scanning area focused on the fovea centralis (Figure 1(a) and (b)). Further segmentation was done automatically by the implied AngioVue software (beta version 1206.1.0.26) without manual correction.

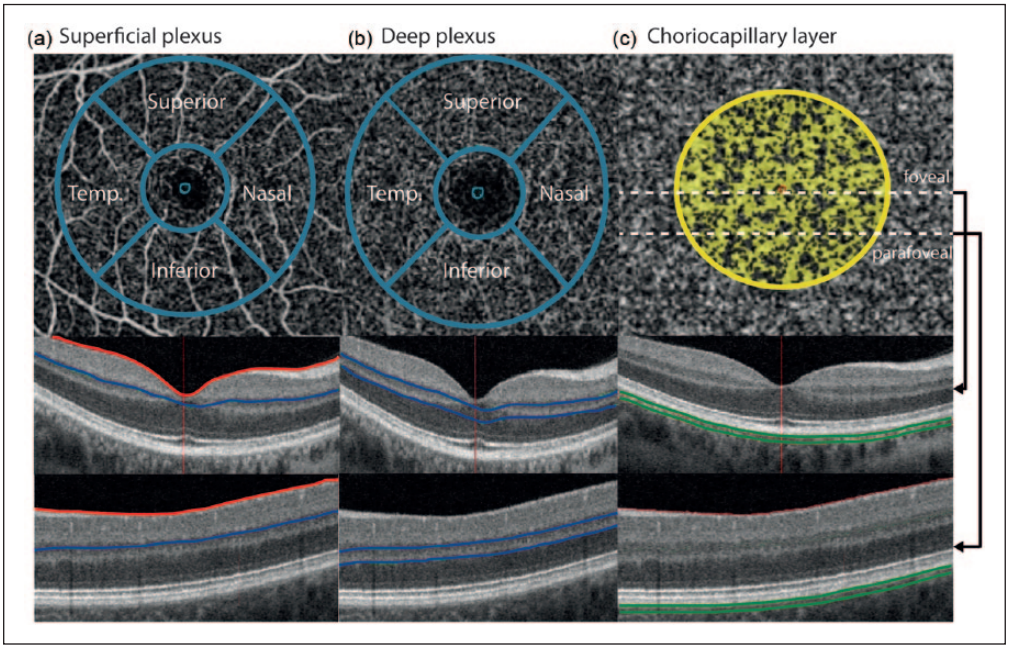

Figure 1. Image analysis of optical coherence tomography angiography (OCT-A) of the parafoveolar macular area. (a) The superficial vascular plexus was analyzed within a scanning circle (diameter = 3 mm) automatically centered at the fovea between 1 and 3 mm eccentricity excluding the foveal avascular zone. Decorrelation signal was detected within an inner offset at −3 to −15 µm from the inner limiting membrane (ILM; between red and blue lines) within the superior, nasal, inferior, and temporal quadrants. Exemplary segmentation of the foveal (second row) and parafoveal (third row) areas of the macula. (b) The deep plexus was analyzed within the same scanning circle as in (a) between 1 and 3 mm eccentricity within four quadrants. Decorrelation signal was detected between an inner offset of −15 to −70 µm from the ILM (between blue lines). Exemplary segmentation of the foveal (second row) and parafoveal (third row) areas of the macula. (c) The choriocapillary layer was analyzed using a scanning circle (radius = 1 mm) manually centered on the fovea centralis within an inner offset at −31 to −60 µm from the retinal pigment epithelium (between green lines). Exemplary segmentation of the foveal (second row) and parafoveal (third row) areas of the macula.

The segmentation algorithm has been used within different studies.[15–17] In principle and according to the manufacturer, segmentation process relies on anatomical retinal structures such as the inner limiting membrane (ILM) and the retinal pigment epithelium (RPE). For evaluation of the superficial retinal vascular plexus, the software detected perfused vessel structures within an inner offset at −3 to −15 µm from the ILM (Figure 1(a)). The deep retinal plexus (Figure 1(b)) was analyzed within an inner offset at −15 to −70 µm from the ILM. Vessel densities (% blood flow signal within the defined area) of the superficial and deep plexuses were measured automatically in four quadrants (superior, nasal, temporal, and inferior) between 1 and 3 mm eccentricity using the implemented software tools. We excluded the central area around the fovea (diameter = 1 mm) from the analysis to avoid artifacts from the foveal avascular zone (Figure 1(a) and (b)). For the analysis of the choriocapillary layer (Figure 1(c)), the flow signal within −31 to −60 µm from the RPE was acquired. Here, vessel densities were analyzed within a circle (radius = 1 mm) manually centered on the fovea. The device provides a "flow index" for the choriocapillaries. The term "flow index" describes the average area (in mm^2) of detected blood flow signal reflecting vessel structures within a selected region of the retina.[18] Flow index values were converted into vessel density (in %) values by flow index (in mm^2) × 100/(3.14 mm^2).

OCT

RRMS and CIS patients, but not HC, underwent OCT analysis. We used a Spectralis SD-OCT (Heidelberg Engineering, Heidelberg, Germany) as previously described[8] using room light without pupil dilatation. Briefly, evaluation of the peripapillary RNFL (pRNFL) was performed by a 3.4-mm ring scan centered on the optic nerve head (1536 A-scans, automatic real time ART 100). The macular area was scanned with 61 vertical B-scans (512 A-scans, scanning angle 30° × 25°) focusing the fovea centralis (ART 13). Two experienced operators performed OCT analysis. We checked all examinations for sufficient quality using

OSCAR-IB criteria.[19] Every B-scan was segmented automatically into different layers using Eye Explorer software (version 6.0.9.0; Heidelberg Engineering). One rater checked segmentations manually and corrected in a blinded manner if necessary. Layer volumes were calculated by the software's segmentation algorithm (6 mm diameter circle around the fovea).

MRI

Brain images were acquired on a 3-T scanner (Achieva; Philips, Amsterdam, the Netherlands) using a 16-channel head coil. The scanning protocol included a three-dimensional (3D) fluid-attenuated inversion-recovery (FLAIR) sequence (TE: 140 ms, TR: 10,000 ms, TI: 2750 ms, 144 contiguous axial 1.5-mm slices; field of view: 230 × 185 mm^2; voxel size: 1.0 × 1.0 × 1.5 mm^3). Contrast-enhanced images were acquired using a volumetric 3D gradient-echo (GRE) T1-weighted sequence (TE: 4 ms, TR: 9 ms, 170 contiguous sagittal 1-mm slices; field of view: 240 × 240 mm^2; voxel size: 1.0 × 1.0 × 1.0 mm^3) 6 minutes after injection of the contrast agent (gadoterate meglumine, Dotarem; Guerbet, Aulnay-sous-Bois, France; dose 0.1 mmol/kg).

Statistics

For statistical analyses, R version 3.2.3 (R Core Team 2015) with packages geepack (version 1.2–1) and ppcor (version 1.1), and GraphPad Prism 6 (GraphPad Software, Inc., La Jolla, CA, USA) were used. For demographic differences, we performed bivariate analyses with Fisher's exact test for categorical and Mann–Whitney *U*-test for quantitative variables. As previously described,[20] OCT or OCT-A parameters were analyzed using generalized estimating equations (GEEs). GEE model relying on the geeglm function was used to measure associations of OCT-A parameters on different patterns of disease activity. Here, dummy variables were calculated whether MRI progression (increase in T2 lesion load and/or occurrence of Gd+ lesions), relapses, or NEDA-3 occurred or not. We used an exchangeable correlation structure with jackknife variance estimation. We adjusted for inter-eye correlations if both eyes of one individual were taken into analysis. Furthermore, the covariates right/left eye, age, sex, disease duration, EDSS, and therapy group were included in the additive model. Selected tests underwent bootstrap analysis (10,000 tests) with calculation of *p*-value standard deviations (SDs) using the sample function as implemented in R. Values are given as median value with 25%–75% interquartile range (IQR) if not stated otherwise. Statistical significance was established at $p < 0.05$.

Results

Study population

In total, 37 patients with RRMS, 7 patients with CIS, and 50 HCs were enrolled into the study. Two RRMS patients and one eye of one RRMS patient were excluded from the analysis due to motion artifacts during OCT-A analysis, and patients with CIS and RRMS were merged for further analysis. Thus, 42 RRMS/CIS patients (83 eyes) and 50 HCs (100 eyes) were included into the analysis. Individuals with RRMS/CIS and HCs showed similar distributions of age and sex (Table 1). A total of 21 eyes of 17 patients in the RRMS/CIS group had suffered from former ON at variable intervals before OCT-A analysis (28.0 (16.0–35.5) months). These 17 patients comprised 2 of 7 CIS and 11 of 35 RRMS patients who suffered from unilateral ON, as well as 4 RRMS patients who suffered from ON on both sides. No ON took place within the last 3 months before enrollment into the study. All patients received corticosteroid treatment in the past, whereas last treatments were administered 29.0 (13.3–41.8) months prior to OCT-A analysis. Patients with RRMS and CIS had disease durations of 49.0 (25.8–76.0) months and EDSS values of 1.0 (0–1.0). Most of the patients were on first- or second-line DMT (Table 1). Patients in the RRMS/CIS group exhibited rather low disease activity during the last 2 years prior to enrollment (Table 1). Totally, 38.1% fulfilled NEDA-3 criteria during that period.

Retinal architecture and retinal perfusion

In a first step, eyes of patients with CIS/MS were analyzed by conventional SD-OCT. As compared to eyes without former ON, ON eyes revealed reduced thicknesses of the pRNFL (ON 87.0 (64.5–95.5) µm, no ON 95.0 (88.0–107.0) µm, $p < 0.001$), lower total macular volume (ON 8.3 (8.0–8.5) mm^3, no ON 8.9 (8.6–9.0) mm^3, $p < 0.001$), and lower GCIPL volume (ON 1.7 (1.5–1.9) mm^3, no ON 2.0 (1.9–2.1) mm^3, $p < 0.001$). No differences were seen in the inner nuclear layer (INL) or the outer retinal layers (data not shown). In a second step, CIS/MS and HC eyes were analyzed separately depending on the former history of ON by OCT-A. Here, eyes of RRMS/CIS patients with former ON revealed lower vessel densities of the superficial and deep plexuses as compared to eyes without former ON or eyes of HC (Figure 2(a) and (b)). All macular sectors were affected (Figure 2(a) and (b)). In contrast, no ON-related alterations in vessel densities were seen for the choriocapillary layer (Figure 2(c)). Eyes of RRMS/CIS patients unaffected by ON revealed similar vessel densities of both the superficial and deep retinal vascular plexuses as HC.

Table 1. Study population.

	HC (n = 50, 100 eyes)	RRMS/CIS (n = 42, 83 eyes)	p-Value
Female, no. (%)	34 (68.0)	29 (69.0)	>0.99
Age, years	32.0 (26.8–38.3)	30.0 (28.5–26.0)	0.42
Disease duration, months	n.a.	49.0 (25.8–76.0)	n.a.
EDSS score	n.a.	1.0 (0–2.0)	n.a.
History of optic neuritis, eyes (%)	0 (0)	21 (25.3)	<0.0001
Disease activity 2 years before enrollment			
No. of relapses/year	n.a.	0 (0–1.0)	n.a.
No. of new T2 lesions/year	n.a.	0.5 (0–2.1)	n.a.
No. of Gd+ lesions/year	n.a.	0 (0–0.4)	n.a.
Disease-modifying therapies, no. (%)			
None	n.a.	7 (16.7)	
First line	n.a.	22 (52.4)	
Second line	n.a.	13 (30.9)	

HC: healthy control; RRMS: relapsing-remitting multiple sclerosis; CIS: clinically isolated syndrome; n.a.: not applicable; EDSS: Expanded Disability Status Scale.
Demographic parameters in HCs and patients with RRMS and CIS.

We found no association between inter-eye differences of vessel densities within the superficial or deep retinal vascular plexus and the time interval elapsed since the last ON episode (data not shown). In RRMS/CIS patients, both the superficial and deep retinal vessel plexus densities (ON and non-ON eyes included) correlated with the total macular volume, GCIPL, and INL volumes (Figure 3(a) and (b)). No associations were seen for the remaining retinal layers (data not shown). In summary, the densities of both retinal plexuses were associated with prior ON but were not altered in MS/CIS patients independently of ON.

Association of retinal vessel density and retrospective disease activity

Next, we tested for associations between retinal vessel density measures and retrospective disease activity patterns during the last 24 months prior to OCT-A analysis. When correcting for sex, age, disease duration, EDSS at time of OCT-A analysis, and DMT group during the last 24 months, we found a positive association between the choriocapillary vessel densities and the retrospective ARR (Figure 4(a)) before OCT-A analysis. This finding also remained statistically robust after performing a bootstrapping analysis (p-value SD 0.0774). This association was found in both eyes with or without former ON (Figure 4(a)). Higher vessel densities of the choriocapillaries were associated with the occurrence of relapse, MRI progression as defined by an increase in T2 lesion load or the occurrence of Gd+ lesions (Figure 4(b)), and violation of NEDA-3 criteria during 2 years prior to OCT-A analysis (Figure 4(c)). RRMS/CIS patients with ongoing disease activity had higher vessel densities of the choriocapillaries as compared to patients fulfilling NEDA-3 criteria (no NEDA: 63.2 ± 1.2; NEDA-3: 62.4 ± 1.2; p = 0.01) and by trend higher vessel densities than HC (HC: 62.8 ± 1.2; p = 0.07). Choriocapillary vessel densities correlated negatively with RRMS/CIS disease duration (r = −0.22; p = 0.04). Thus, while the choriocapillary layer is not affected by ON, the vascularization of the choroid might indicate ongoing disease activity in patients with MS independently of ON.

Discussion

In this study, we show that MS-associated ON causes alterations in the macular retinal vascular network. Furthermore, increased capillary densities of the choriocapillary layer might indicate increased recent inflammatory disease activity in MS.

In the current understanding, perfusion of the human macula is organized by three different vascular plexuses.[21] The superficial vascular plexus is supplied by the central retinal artery and extends with larger arteries and arterioles through the whole RNFL, ganglion cell layer, and the inner plexiform layer.[21] The deep plexus, which is supplied by anastomoses of the superficial plexus, feeds the INL and outer plexiform

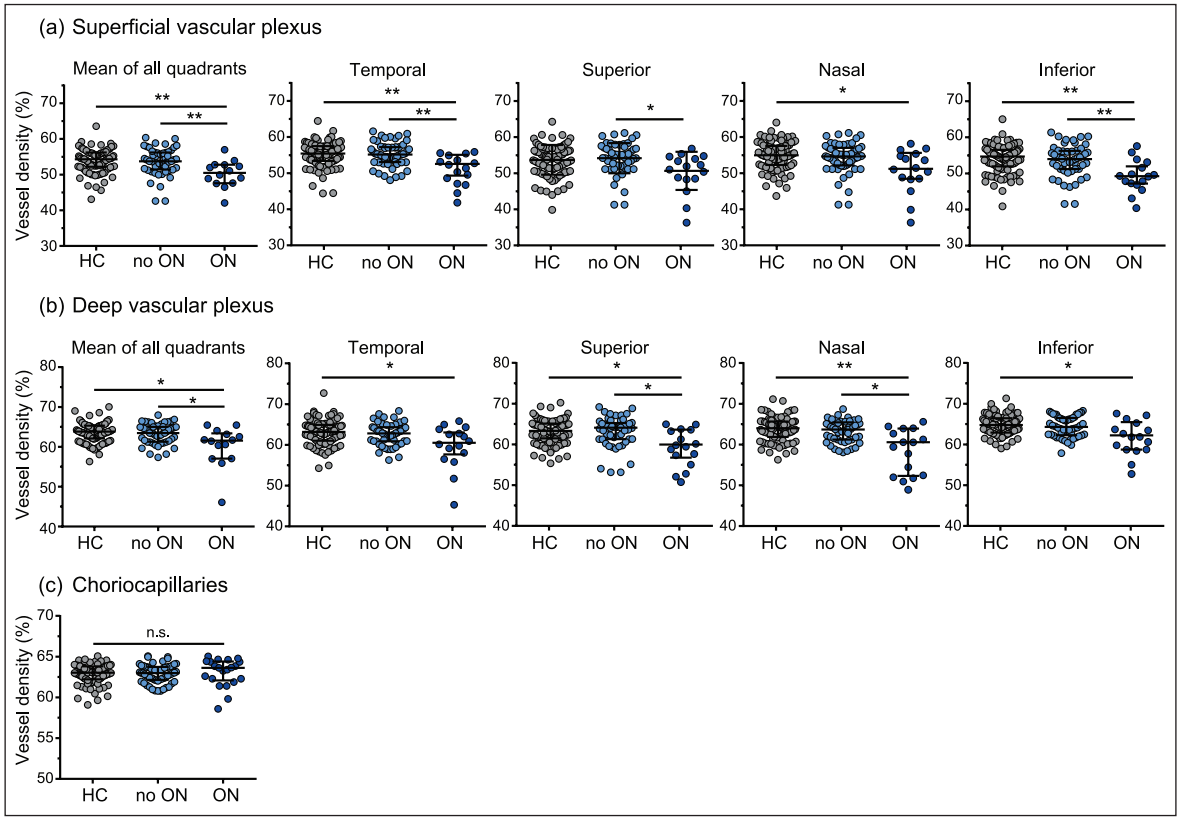

Figure 2. Retinal vessel densities in patient groups. Retinal vessel densities in eyes from healthy controls (HCs), eyes of patients with clinically isolated syndrome or multiple sclerosis without a history of former optic neuritis (no ON), and eyes with a positive history of optic neuritis (ON). (a) Vessel densities (%) in different quadrants of the superficial retinal vascular network in HCs (n = 100 eyes), no ON (n = 56), and ON (n = 16 eyes). (b) Vessel densities (%) in different quadrants of the deep retinal vascular network in HC (n = 100 eyes), no ON (n = 56), and ON (n = 16 eyes). (c) Vessel densities (%) of the choriocapillary layer in HCs (n = 100 eyes), no ON (n = 62), and ON (n = 21 eyes). (a, b) Six eyes from no ON group and five eyes from ON group were excluded due to motion artifacts during analysis. Median values with 25%–75% interquartile range; generalized estimating equations (GEEs); n.s.: not significant; *$p < 0.05$, **$p < 0.01$.

layer. The outer nuclear and photoreceptor layer are largely devoid of any blood vessels.[21] The choriocapillaries, which are originating from posterior ciliary arteries of the ophthalmic artery, supply the RPE and thus indirectly through the outer blood–retina barrier also the photoreceptors.[21]

MS-associated ON causes atrophy of the inner retinal layers RNFL and GCIPL, but not of the INL and outer retinal layers,[22] most likely via retrograde axonal degeneration due to an inflammatory optic nerve damage.[3] Beside atrophy of the inner retinal layers, we found a rarefaction of retinal vessel density within both the superficial and deep vascular plexuses of the central macula. Consistent with this, reduced retinal macular vessel densities in seven patients after isolated, MS-associated, or neuromyelitis optica–related ON were recently reported.[23] Also, using a prototype swept source OCT system, decreased retinal vessel densities within the optic nerve,[24] but not within the macular area were observed in MS patients with former ON.[25] Differentiation into different retinal vascular plexuses, however, was not performed.

The reason for ON-associated macular vessel rarefaction is unclear. One possible explanation is a reduced need of blood supply due to an ON-related retinal damage. Different groups have shown that ON leads to atrophy of the retinal ganglion cells and the RNFL.[3–5,22] Thus, it is possible that neuronal and axonal decline results in reduced metabolic activity within the inner retinal layers with consecutive lower oxygen and blood demand and regression of vessels of the superficial vascular plexus. Since the deep vascular plexus is supplied by anastomoses from superficial vessels, this could secondarily also affect deeper retinal vessel structures although deeper retinal layers remain largely unaffected after ON.[22] Consistent with this idea, MS patients reveal reduced brain perfusion patterns in areas of normal appearing white matter as

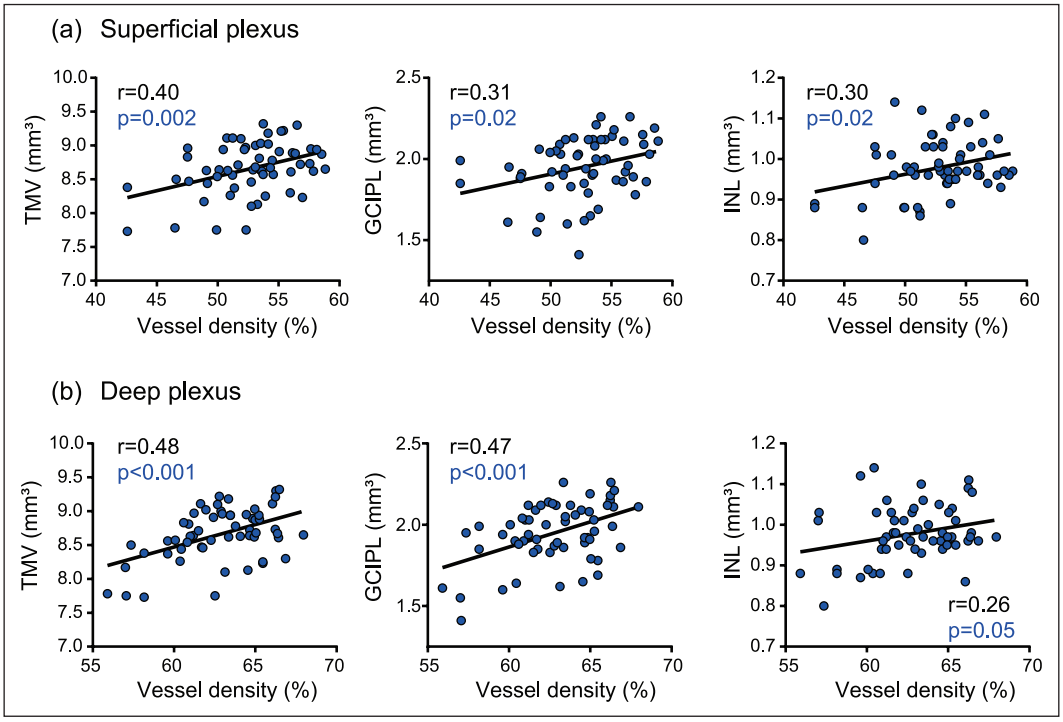

Figure 3. Association of OCT and OCT-A measures. Association of optical coherence tomography (OCT) and OCT angiography (OCT-A) measures in patients with clinically isolated syndrome or multiple sclerosis (n = 72 eyes). (a) Correlation of vessel densities of the superficial vascular plexus of the retina (%) and the total macular volumes (TMV) as well as volumes of the common ganglion cell and inner plexiform layer (GCIPL) and the inner nuclear layer (INL). (b) Association of vessel densities of the deep vascular plexus of the retina (%) and TMV, GCIPL, and INL volumes; generalized estimating equations (GEEs).

compared to healthy individuals.[26] MS-associated cerebral hypoperfusion might be due to an altered energy metabolism of astrocytes with impaired astrocytic K^+ uptake and subsequent impaired arteriolar vasodilatation.[27] Also within the retina, astrocytic Müller cells are essential for synaptic transmission and for maintaining the blood–retina barrier.[28] It is still unclear, however, whether retinal astrocytes are also affected in MS.

Alternatively, rarefaction of retinal vessel structures could also be the consequence of a direct inflammatory process affecting retinal vessels during ON. While this idea would be in line with the profound changes in vessel density, the relative protection of outer retinal layers from atrophy appears very discordant with the significant rarefaction of the deep retinal plexus. About 11% of all MS patients show signs of retinal periphlebitis during autopsy[29] and MS patients may show lower numbers of epiretinal blood vessels and reduced vessel diameters irrespective of whether they experienced former ON or not.[30] In this study, we did not see any alterations of vessel densities within the retinal vascular network in eyes of MS patients without former ON. Alterations of the superficial and deep vascular plexuses were exclusively linked to a history of ON and eyes without former ON showed comparable vessel densities as HCs. Thus, we could not detect any clear signs of a subclinical, ON-independent alteration of retinal vessel structures during MS.

The occurrence of MS-associated ON did not affect vessel densities within the choriocapillary layer. However, we found a surprising association of the vessel density within the choriocapillary layer with the retrospective disease activity prior to analysis. Although vessel network architecture of the choriocapillary layer in MS patients was not different as compared to healthy individuals, higher vessel densities were associated with ongoing disease activity before OCT-A analysis. The reason for this observation is at present unclear. It is still a matter of debate, whether choroid structures are affected in the disease course of MS. Using enhancing depth imaging OCT analysis, MS patients revealed decreased choroid thickness values as compared to HC, and choroidal thinning was linked to MS disease duration.[31]

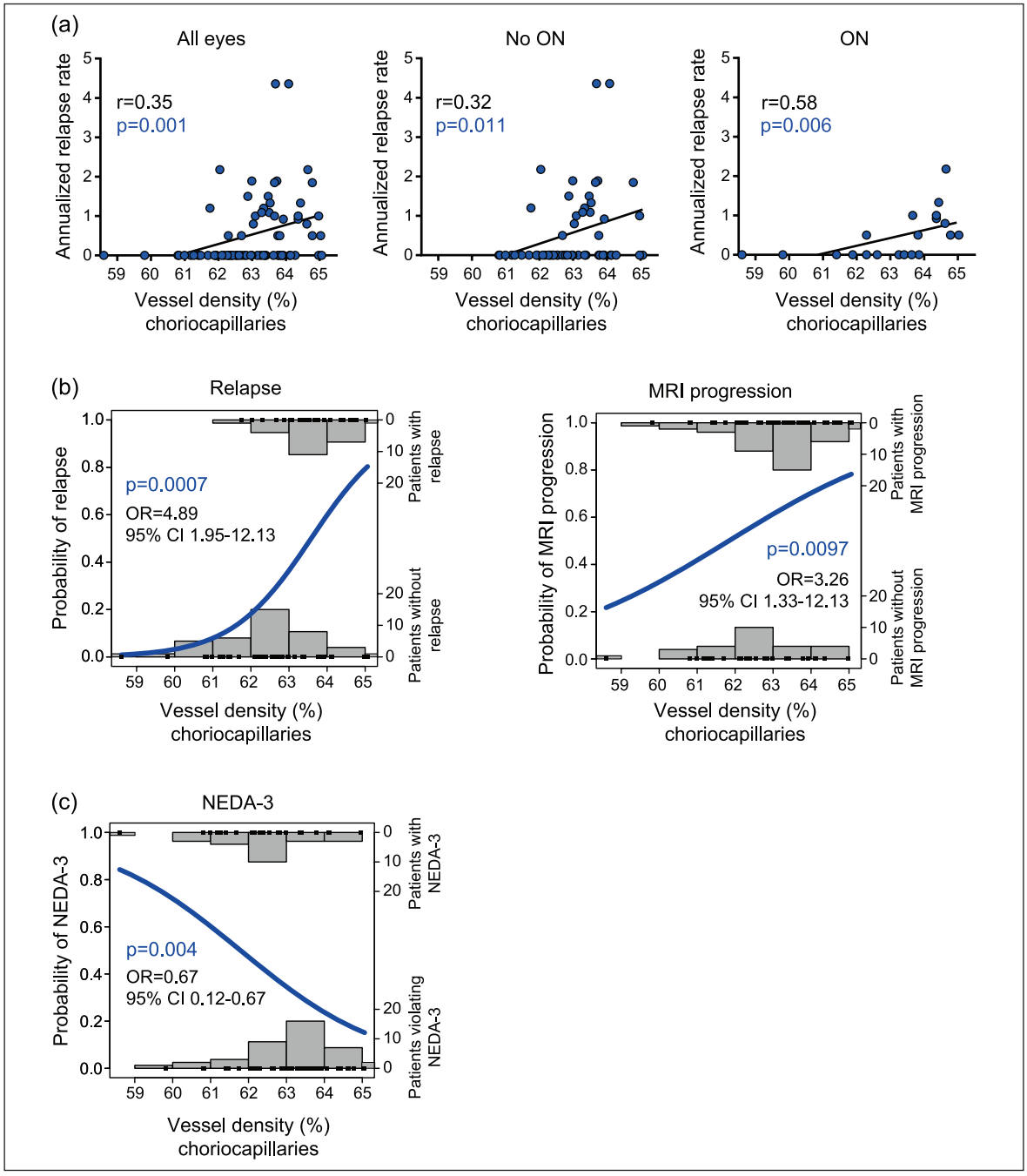

Figure 4. Association of the retrospective disease activity and the choriocapillary vessel densities. (a) Association of the annualized relapse rates (ARRs) during 24 months before analysis and the choriocapillary vessel densities in patients with clinically isolated syndrome or multiple sclerosis (all eyes; $n = 81$ eyes), seperated in eyes without history of optic neuritis (no ON, $n = 60$) and with positive history of optic neuritis (ON, $n = 21$); generalized estimating equations (GEEs) corrected for age, sex, disease duration, disease-modifying therapy, and the Expanded Disability Status Scale; one patient (no ON) was excluded due to short disease duration of 2 months. (b, c) Effect of choriocapillary vessel densities (%) on the retrospective occurrence of relapse, MRI progression (defined by an increase in T2 lesion load or the occurrence of gadolinium-enhancing lesions), or successful achievement of NEDA-3 status during 24 months before analysis. Left y-axis indicates probability of relapse, MRI progression, or NEDA-3 status; gray bars and right y-axis describe the distribution of choriocapillary vessel density measures (%) of patients with (top distribution) and without (bottom distribution) occurrence of relapse, MRI progression, or NEDA-3; odds ratio (OR) for a 1% increase in choriocapillary vessel density on the probability of relapse, MRI progression, or achievement of NEDA-3 status with 95% confidence intervals; generalized estimating equations (GEEs) corrected for age, sex, disease duration, disease-modifying therapy, former optic neuritis, and the Expanded Disability Status Scale.

Furthermore, between 0.4% and 2.0% of all MS patients suffer from additional uveitis[32] and MS patients with uveitis might exhibit a more benign disease course.[33] Our current data add further data to the idea of a possible subclinical involvement of choroid structures during MS. It has already been shown that inflammation within the CNS might alter perfusion patterns. Patients with acute MS-associated inflammation and enhanced inflammatory disease activity might show increased brain perfusion with enhanced blood volumes and blood flow.[34,35] Of course, further studies focusing on choroidal changes in MS patients are needed to corroborate this hypothesis.

This study has several limitations. First, our sample size is rather small, and we have not yet used a second cohort to validate our findings. Our study population showed relatively low EDSS values at baseline as would be expected for study populations with early disease.[36] Thus, it remains to be determined whether similar findings also apply for MS patients with longer standing disease and more severe disability. Second, we only analyzed associations of OCT-A measures with retrospective, but not prospective patterns of disease activity. Consequently, the diagnostic value of our findings is unclear. OCT-A, however, is a very novel imaging technique, which was introduced into ophthalmologic diagnostic work-up only 3 years ago. Third, the OCT-A technique has methodological limitations. The biological meaning of several measures is unclear. The influence of different disturbances and environment factors on OCT-A measures is unclear although the OCT-A technique per se seems to reveal good repeatability and reproducibility.[37] Thus, we cannot completely exclude false-positive findings due to technical and methodological issues. Furthermore, OCT-A only allows for the assessment of vessel density but fails to provide quantitative information on blood flow velocity, vessel morphology, or alterations of the vessel barrier.

In conclusion, our study shows that MS-associated ON leads to a vessel rarefaction within both the superficial and deep macular vascular plexuses, but not within the choriocapillary layer. Higher vessel densities within the choriocapillary layer, however, might be linked to an increased recent clinical inflammatory disease activity during MS.

Acknowledgements

The authors thank Janice Schneider and Göran Joost-Zentgraf (both from the Department of Neurology, Technical University of Munich) and Serife Aslantas (Department of Ophthalmology, Technical University of Munich) for expert assistance during OCT and OCT-A analysis. They are grateful to Andrea Hilgers, Sanja Kramberger, Kim Obergfell, and Lisa-Ann Voithenleitner (all from the Department of Neurology, Technical University of Munich) for database maintenance. They thank Jan Dechent and Kai Zeitzmann (both Heidelberg Engineering, Germany) for technical support. N.F. and M.M. are equal contributing first authors. T.K. and B.K. are equal contributing senior authors.

Declaration of Conflicting Interests

The author(s) declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article: C.Z. has served on scientific advisory boards for Philips and Bayer Schering; serves as co-editor on the Advisory Board of Clinical Neuroradiology; has received speaker honoraria from Bayer Schering and Philips; and has received research support and investigator fees for clinical studies from Biogen Idec, Quintiles, MSD Sharp & Dome, Boehringer Ingelheim, Inventive Health Clinical UK Ltd, Advance Cor, Brainsgate, Pfizer, Bayer Schering, Novartis, Roche, Servier, Penumbra, WCT GmbH, Syngis, SSS International Clinical Research, PPD Germany GmbH, Worldwide Clinical Trials Ltd, Phenox, Covidien, Actelion, Medivation, Medtronic, Harrison Clinical Research, Concentric, Penumbra, Pharmtrace, Reverse Medical Corp., Premier Research Germany Ltd, Surpass Medical Ltd, and GlaxoSmithKline. M.-M.H. reports grants from Bayer Healthcare, personal fees from Novartis, personal fees from Biogen Idec, outside the submitted work. B.H. has served on scientific advisory boards for F. Hoffmann-La Roche Ltd, Novartis, Bayer Schering, AllergyCare, and Genentech; has received speaker honoraria from Biogen Idec, Teva Neuroscience, Merck Serono, Medimmune, and F. Hoffmann-La Roche Ltd; has received research support from Chugai Pharmaceuticals; holds part of two patents: one for the detection of antibodies and T cells against KIR4.1 in a subpopulation of MS patients and one for genetic determinants of neutralizing antibodies to interferon-beta. N.F., M.M., G.L., M.P., C.W., T.D., P.S., T.K., and B.K. have nothing to disclose.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: B.K., B.H., and T.K. are supported by the Bundesminsterium für Bildung und Forschung (Kompetenznetz Multiple Sklerose KKNMS). B.K. receives intramural funding from the Technical University of Munich. B.H. receives funding from the Deutsche Forschungsgemeinschaft DFG (TR 128) and the EU Horizon 2020 project

MultipleMS. T.K. is supported by the Deutsche Forschungsgemeinschaft DFG (SFB 1054 and TR 128) and the European Research Council ERC (CoG 647215). No sponsor had any influence on design and conduct of the study, collection, management, analysis, and interpretation of the data, preparation, review, or approval of the manuscript and decision to submit the manuscript for publication.

ORCID iD

Gildas Lepennetier http://orcid.org/0000-0002-1899-3149

References

1. Oberwahrenbrock T, Weinhold M, Mikolajczak J, et al. Reliability of intra-retinal layer thickness estimates. *PLoS ONE* 2015; 10: e0137316.
2. Petzold A, de Boer JF, Schippling S, et al. Optical coherence tomography in multiple sclerosis: A systematic review and meta-analysis. *Lancet Neurol* 2010; 9: 921–932.
3. Petzold A, Wattjes MP, Costello F, et al. The investigation of acute optic neuritis: A review and proposed protocol. *Nat Rev Neurol* 2014; 10: 447–458.
4. Trip SA, Schlottmann PG, Jones SJ, et al. Retinal nerve fiber layer axonal loss and visual dysfunction in optic neuritis. *Ann Neurol* 2005; 58: 383–391.
5. Costello F, Coupland S, Hodge W, et al. Quantifying axonal loss after optic neuritis with optical coherence tomography. *Ann Neurol* 2006; 59: 963–969.
6. Saidha S, Sotirchos ES, Oh J, et al. Relationships between retinal axonal and neuronal measures and global central nervous system pathology in multiple sclerosis. *JAMA Neurol* 2013; 70: 34–43.
7. Oh J, Sotirchos ES, Saidha S, et al. Relationships between quantitative spinal cord MRI and retinal layers in multiple sclerosis. *Neurology* 2015; 84: 720–728.
8. Knier B, Leppenetier G, Wetzlmair C, et al. Association of retinal architecture, intrathecal immunity, and clinical disease course in multiple sclerosis. *JAMA Neurol* 2017; 74: 847–856.
9. Martinez-Lapiscina EH, Arnow S, Wilson JA, et al. Retinal thickness measured with optical coherence tomography and risk of disability worsening in multiple sclerosis: A cohort study. *Lancet Neurol* 2016; 15: 574–584.
10. Chalam KV and Sambhav K. Optical coherence tomography angiography in retinal diseases. *J Ophthalmic Vis Res* 2016; 11: 84–92.
11. Polman CH, Reingold SC, Banwell B, et al. Diagnostic criteria for multiple sclerosis: 2010 revisions to the McDonald criteria. *Ann Neurol* 2011; 69: 292–302.
12. Kraus MF, Liu JJ, Schottenhamml J, et al. Quantitative 3D-OCT motion correction with tilt and illumination correction, robust similarity measure and regularization. *Biomed Opt Express* 2014; 5: 2591–2613.
13. Huang D, Jia Y, Gao SS, et al. Optical coherence tomography angiography using the optovue device. *Dev Ophthalmol* 2016; 56: 6–12.
14. Koustenis A, Harris A, Gross J, et al. Optical coherence tomography angiography: An overview of the technology and an assessment of applications for clinical research. *Brit J Ophthalmol* 2017; 101: 16–20.
15. Zhang Z, Huang X, Meng X, et al. In vivo assessment of macula in eyes of healthy children 8 to 16 years old using optical coherence tomography angiography. *Sci Rep* 2017; 7: 8936.
16. Nesper PL, Scarinci F and Fawzi AA. Adaptive optics reveals photoreceptor abnormalities in diabetic macular ischemia. *PLoS ONE* 2017; 12: e0169926.
17. Al-Sheikh M, Phasukkijwatana N, Dolz-Marco R, et al. Quantitative OCT angiography of the retinal microvasculature and the choriocapillaris in myopic eyes. *Invest Ophthalmol Vis Sci* 2017; 58: 2063–2069.
18. Gao SS, Jia Y, Zhang M, et al. Optical coherence tomography angiography. *Invest Ophthalmol Vis Sci* 2016; 57: 27–36.
19. Tewarie P, Balk L, Costello F, et al. The OSCAR-IB consensus criteria for retinal OCT quality assessment. *PLoS ONE* 2012; 7: e34823.
20. Cruz-Herranz A, Balk LJ, Oberwahrenbrock T, et al. The APOSTEL recommendations for reporting quantitative optical coherence tomography studies. *Neurology* 2016; 86: 2303–2309.
21. Campbell JP, Zhang M, Hwang TS, et al. Detailed vascular anatomy of the human retina by projection-resolved optical coherence tomography angiography. *Sci Rep* 2017; 7: 42201.
22. Gabilondo I, Martinez-Lapiscina EH, Fraga-Pumar E, et al. Dynamics of retinal injury after acute optic neuritis. *Ann Neurol* 2015; 77: 517–528.
23. Higashiyama T, Nishida Y and Ohji M. Optical coherence tomography angiography in eyes with good visual acuity recovery after treatment for optic neuritis. *PLoS ONE* 2017; 12: e0172168.
24. Spain RI, Liu L, Zhang X, et al. Optical coherence tomography angiography enhances the detection of optic nerve damage in multiple sclerosis. *Brit J*

Ophthalmol 2017. Epub ahead of print. DOI:10.1136/bjophthalmol-2017-310477

25. Wang X, Jia Y, Spain R, et al. Optical coherence tomography angiography of optic nerve head and parafovea in multiple sclerosis. *Brit J Ophthalmol* 2014; 98: 1368–1373.
26. Adhya S, Johnson G, Herbert J, et al. Pattern of hemodynamic impairment in multiple sclerosis: Dynamic susceptibility contrast perfusion MR imaging at 3.0 T. *Neuroimage* 2006; 33: 1029–1035.
27. D'Haeseleer M, Cambron M, Vanopdenbosch L, et al. Vascular aspects of multiple sclerosis. *Lancet Neurology* 2011; 10: 657–666.
28. Newman EA. Glial modulation of synaptic transmission in the retina. *Glia* 2004; 47: 268–274.
29. Green AJ, McQuaid S, Hauser SL, et al. Ocular pathology in multiple sclerosis: Retinal atrophy and inflammation irrespective of disease duration. *Brain* 2010; 133: 1591–1601.
30. Bhaduri B, Nolan RM, Shelton RL, et al. Detection of retinal blood vessel changes in multiple sclerosis with optical coherence tomography. *Biomed Opt Express* 2016; 7: 2321–2330.
31. Esen E, Sizmaz S, Demir T, et al. Evaluation of choroidal vascular changes in patients with multiple sclerosis using enhanced depth imaging optical coherence tomography. *Ophthalmologica* 2016; 235: 65–71.
32. Marrie RA, Reider N, Cohen J, et al. A systematic review of the incidence and prevalence of autoimmune disease in multiple sclerosis. *Mult Scler* 2015; 21: 282–293.
33. Shugaiv E, Tuzun E, Kurtuncu M, et al. Uveitis as a prognostic factor in multiple sclerosis. *Mult Scler* 2015; 21: 105–107.
34. Bester M, Forkert ND, Stellmann JP, et al. Correction: Increased perfusion in normal appearing white matter in high inflammatory multiple sclerosis patients. *PLoS ONE* 2015; 10: e0142464.
35. Wuerfel J, Bellmann-Strobl J, Brunecker P, et al. Changes in cerebral perfusion precede plaque formation in multiple sclerosis: A longitudinal perfusion MRI study. *Brain* 2004; 127: 111–119.
36. Miller AE, Wolinsky JS, Kappos L, et al. Oral teriflunomide for patients with a first clinical episode suggestive of multiple sclerosis (TOPIC): A randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet Neurol* 2014; 13: 977–986.
37. Lei J, Durbin MK, Shi Y, et al. Repeatability and reproducibility of superficial macular retinal vessel density measurements using optical coherence tomography angiography en face images. *JAMA Ophthalmol* 2017; 135: 1092–1098.