Macular vessel density differs in multiple sclerosis and neuromyelitis optica spectrum disorder: an optical coherence tomography angiography study.

Małgorzata Rogaczewska
Department of Ophthalmology. Chair of Ophthalmology and Optometry. Poznan University of Medical Sciences. ul. Grunwaldzka 16/18. 60-780, Poznan

Sławomir Michalak
Department of Neurochemistry and Neuropathology. Department of Neurology. Poznan University of Medical Sciences. ul. Przybyszewskiego 49, 60-355, Poznan

Marcin Stopa (✉ stopa@ump.edu.pl)
Department of Ophthalmology. Chair of Ophthalmology and Optometry. Poznan University of Medical Sciences. ul. Grunwaldzka 16/18. 60-780, Poznan

Research Article

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Abstract

Multiple sclerosis (MS) and neuromyelitis optica spectrum disorder (NMOSD) are inflammatory and demyelinating diseases that commonly manifest with optic neuritis (ON) but differ in the pathogenic mechanism. Although it was shown that retinal vessels might alter in MS and NMOSD, a comparative study has not been reported. This study evaluated the macular vessel density in 40 MS patients, 13 NMOSD patients, and 20 controls by using optical coherence tomography angiography. The vessel density of superficial capillary plexus (SCP) was significantly lower in ON eyes (MS + ON, NMOSD + ON) than in non-ON eyes (MS-ON, NMOSD-ON) and controls. The density of deep capillary plexus (DCP) was significantly increased in MS + ON, and MS-ON eyes compared to healthy eyes and decreased in NMOSD + ON compared to MS + ON. A significant positive correlation was noted between SCP and ganglion cell complex (GCC) thickness in MS + ON, MS-ON, and NMOSD + ON. The DCP did not significantly correlate with GCC thickness, but it increased or decreased with ganglion cell loss in MS and NMOSD, respectively. In conclusion, our findings suggest that the capillary changes in MS patients are secondary to the reduced metabolic demand of the atrophied ganglion cell layer, while the vasculopathy seems to be a primary process in NMOSD patients.

Introduction

Multiple sclerosis (MS) and neuromyelitis optica spectrum disorder (NMOSD) are inflammatory and demyelinating diseases of the central nervous system (CNS)\(^1,2\). Although optic neuritis (ON) is a common manifestation in both diseases, in NMOSD patients, optic nerve involvement is often bilateral with poorer visual outcomes\(^3\).

In contrast to MS, the disease-specific serum immunoglobulin G exists and can be detected in up to 80% of NMOSD patients\(^4\). These autoantibodies target the protein aquaporin-4 (AQP4), a water channel presented in the membrane of astrocytes in the CNS. Within the retina, the AQP4 is found in astrocytes and Müller glial cells and strongly expressed in their perivascular and end-foot processes\(^5,6\). Retinal capillaries, ensheathed by the membranes of astrocytes and Müller cells, form the inner blood-retinal barrier (iBRB). The disruption of the iBRB allows the anti-aquaporin-4 antibodies (AQP4-IgG) to bind to the water channels and dysregulates retinal water homeostasis\(^6\).

The retinal capillaries can be visualized in vivo using non-invasive optical coherence tomography angiography (OCTA) and expressed quantitatively with a vessel density (VD) parameter. Moreover, OCTA software enables us to divide macular vasculature into superficial capillary plexus (SCP) and deep capillary plexus (DCP)\(^7\).

Several studies evaluated the retinal vessel density in MS\(^8–12\) and NMOSD\(^13–16\) patients, but the SCP and DCP were distinguished only in three studies concerning multiple sclerosis\(^8–10\), and in two NMOSD studies\(^14,15\). However, the results of the studies were not consistent. The common observation was that the SCP is reduced in MS patients when compared with healthy controls\(^9,10\). The study of Feucht et al. revealed that lower vessel density of SCP and DCP was associated only with prior ON, but Farci et al. did not found the differences between eyes with or without former optic neuritis\(^8,10\). According to Cennamo et al., the ganglion cell complex (GCC) thickness was associated only with SCP\(^9\). In NMOSD, the vessel density of SCP and DCP was evaluated in two studies by Kwapong et al\(^14,15\). They reported that the capillary reduction of both plexuses was noted in ON and non-ON eyes when compared with controls. Additionally, the density of SCP was significantly lower in ON than non-ON eyes\(^14,15\).

It is still a matter of debate whether the reduction of retinal vessels is primary or secondary to ganglion cells loss\(^8–10,13–16\). Anatomically, the DCP is supplied by vertical anastomoses from the SCP; thus, the reduction of the SCP should influence the DCP\(^7\). In the case of primary retinal vasculopathy, the vessel loss of both plexuses should be observed. Regarding the ganglion cells damage as a causative mechanism, the plexus supplying this retinal layer, i.e., SCP, due to the decreased cell metabolic demand, should be more affected than DCP. In view of the distinct pathogenic mechanism
of MS and NMOSD, the comparative characteristics of superficial and deep vascular plexuses may clarify this issue. Such an analysis was not previously reported.

In this study, we aimed to identify macular perfusion abnormalities in patients with MS and NMOSD by using OCTA. Furthermore, we evaluated the SCP and DCP in relation to GCC thickness and clinical parameters.

**Methods**

**Study participants**

In this observational study, patients with multiple sclerosis and patients with neuromyelitis optica spectrum disorder were recruited from the Department of Ophthalmology and the Department of Neurology of the Poznan University of Medical Sciences between June 2018 and September 2020. All patients with MS fulfilled the revised 2017 McDonald criteria\(^\text{17}\). Clinical data, including disease duration, age at disease onset (defined as years since first symptoms), number of ON attacks, and ongoing therapy, were recorded. The enrolled eyes of MS and NMOSD were divided into subgroups: eyes with a history of optic neuritis (MS + ON, NMOSD + ON), and eyes with no history of ON (MS-ON, NMOSD-ON). Age- and sex-matched healthy volunteers served as controls. Anti-aquaporin-4 antibodies were detected by means of indirect fluorescence using a commercial cell-based assay with aquaporin 4 transfected cells (EUROIMMUN AG, Lübeck, Germany). Analyzes were performed in the Department of Neurochemistry and Neuropathology at Poznan University of Medical Sciences, which participates in an international external quality control system and receives regular certification for the detection of AQP4-IgG (Institut für Qualitätssicherung, Lübeck, Germany).

The examination protocol included best-corrected visual acuity (BCVA) measurement, Goldmann applanation tonometry (adjusted for central corneal thickness), slit-lamp biomicroscopy, indirect ophthalmoscopy, spectral-domain OCT (SD-OCT), and OCT angiography. BCVA was assessed with the Early Treatment of Diabetic Retinopathy Study chart and expressed as logMAR.

Eligibility criteria were age \(\geq 18\) years, no ON attack within 6 months prior to enrollment, and at least 2 years of disease duration for MS patients. We excluded participants with myopia > 6 diopters, macular disease, hypertensive or diabetic retinopathy, glaucoma, history of uveitis or eye surgery, and low OCT images quality.

The research was performed in accordance with the Declaration of Helsinki and was approved by the medical ethics committee of Poznan University of Medical Sciences. Written informed consent was obtained from each participant after an explanation of the nature of this study.

**SD-OCT**

The ganglion cell complex (GCC) thickness was obtained with RTVue XR Avanti with AngioVue (Optovue Inc., Fremont, CA, USA; software version 2017.1.0.151). The GCC scan, which covers a 7 × 7 mm area of the macula, was centered 1 mm temporal to the fovea. The device automatically measured GCC thickness from the internal limiting membrane (ILM) to the outer boundary of the inner plexiform layer (IPL).

**OCT angiography**

OCTA is based on a split-spectrum amplitude-decorrelation angiography algorithm, which detects the motion of erythrocytes in the vessels through sequentially obtained OCT cross-sectional scans. The generated blood flow map presented the vessel density (VD), i.e., the percentage area occupied by the perfused retinal blood vessels in the analyzed region\(^\text{18,19}\).
The OCTA en face image acquisition was performed with RTVue XR Avanti with AngioVue (Optovue Inc., Fremont, CA, USA; software version 2017.1.0.151). Macular vessel density was visualized using a 3 × 3 mm scan centered on the foveola. The AngioVue software automatically segmented the 3-dimensional image of the inner retinal capillaries into two plexuses: the superficial capillary plexus comprising the vessels of the nerve fiber layer (NFL), the ganglion cell layer (GCL), and the inner plexiform layer (IPL); and the deep capillary plexus consisting of the inner nuclear layer (INL) and the outer plexiform layer (OPL) vasculature. The whole vessel density of SCP and DCP were taken into analysis.

The low-quality images with the signal strength index < 50 or significant motion artifacts were not analyzed.

**Statistical analysis**

Statistical analysis was performed using Statistica v13.1 (StatSoft, Inc., Tulsa, USA). The distribution of continuous variables was evaluated using the Shapiro–Wilk test. A Chi-square test was used to determine sex differences among groups. A Student’s *t*-test and a Mann-Whitney *U*-test were used to evaluate differences in GCC thickness and OCTA parameters between two unpaired groups, as appropriate. To compare patients with controls in age, BCVA, GCC thickness, and OCTA parameters, we performed ANOVA with Dunnett’s post hoc analysis or Mann-Whitney *U*-test with Bonferroni correction. Pearson’s or Spearman’s correlations were calculated between OCTA parameters and GCC thickness, as appropriate. Statistical significance was set at *p* < 0.05.

**Results**

**Study population**

In total, 40 patients with MS, 13 patients with NMOSD, and 20 healthy controls were enrolled into this study. Due to the low-quality OCTA images, we excluded 3 eyes of MS patients and 9 eyes of NMOSD patients from analysis. The number of eyes with a history of optic neuritis in MS and NMOSD patients was 31 and 8, respectively. At baseline, there were no significant differences between patients and controls on age, sex, and BCVA of enrolled eyes. Demographic and clinical features are summarized in Table 1.

| Table 1 | Demographic and clinical characteristics of MS, NMOSD patients, and controls. |
|---------|-----------------------------|
|         | MS      | NMOSD   | Controls |
| Number of subjects | 40      | 13      | 20       |
| Number of eyes enrolled | 77      | 17      | 40       |
| ON+     | 31      | 8       | -        |
| ON-     | 46      | 9       | 40       |
| Age, mean ± SD (years) | 35.15 ± 7.38 | 42.1 ± 9.83 | 37.9 ± 11.18 |
| Sex (female/male) | 32/8    | 11/2    | 17/3     |
| Age at disease onset, mean ± SD (years) | 24.3 ± 6.53 | 30.85 ± 7.22 | - |
| Disease duration, mean ± SD (years) | 10.85 ± 6.06 | 11.23 ± 7.76 | - |
| BCVA of enrolled eyes (logMAR), median (min-max) | 0.00 (0.00-0.20) | 0.00 (0.00-0.20) | 0.00 (0.00–0.00) |

**BCVA**, best-corrected visual acuity; *logMAR*, the logarithm of the minimum angle of resolution; *max*, maximum; *min*, minimum; **MS**, multiple sclerosis; **NMOSD**, neuromyelitis optica spectrum disorder; **ON**, optic neuritis; **SD**, standard deviation.
SD-OCT

The average ganglion cell complex thickness was significantly lower in the MS + ON, MS-ON, and NMOSD + ON groups than in the controls ($p < 0.001$; Table 2; Fig. 1). Among patients with the same diagnosis, a significant difference in GCC thickness was seen between eyes with or without ON (Table 3). However, regarding the same eye status (ON + or ON-) between MS and NMOSD patients, the thickness was comparable in these groups ($p > 0.05$; Table 3).

Table 2
Differences in spectral-domain OCT and OCT angiography parameters between patients and controls.

| Groups                  | No. of eyes analyzed | Vessel density (%) | GCC (µm) |
|------------------------|----------------------|--------------------|----------|
|                        |                      | SCP                | DCP      | DCP-SCP | Average |
|                        |                      | Mean ± SD p-value vs controls | Mean ± SD p-value vs controls | Mean ± SD p-value vs controls | Mean ± SD p-value vs controls |
| MS + ON                | 31                   | 41.03 ± 4.02 < 0.001 | 54.91 ± 2.29 < 0.001 | 13.88 ± 4.67 < 0.001 | 83.61 ± 8.66 < 0.001 |
| MS-ON                  | 46                   | 44.0 ± 2.7 < 0.001 | 53.97 ± 2.22 0.004 | 9.97 ± 3.2 < 0.001 | 90.74 ± 7.72 < 0.001 |
| NMOSD + ON             | 8                    | 39.61 ± 6.02 < 0.001 | 53.39 ± 2.18 0.507 | 13.78 ± 6.14 < 0.001 | 77.75 ± 10.01 < 0.001 |
| NMOSD-ON               | 9                    | 45.08 ± 2.96 0.385 | 53.01 ± 1.32 0.366 | 7.96 ± 2.93 0.168 | 94.33 ± 8.58 0.291 |
| Controls               | 40                   | 46.96 ± 2.31 - | 52.19 ± 2.7 - | 5.22 ± 2.58 - | 99.15 ± 5.05 - |

DCP, deep capillary plexus; GCC, ganglion cell complex; MS + ON, multiple sclerosis with optic neuritis; MS-ON, multiple sclerosis without optic neuritis; NMOSD + ON, neuromyelitis optica spectrum disorder with optic neuritis; NMOSD-ON, neuromyelitis optica spectrum disorder without optic neuritis; OCT, optical coherence tomography; SCP, superficial capillary plexus; SD, standard deviation.

Table 3
Comparison of vessel density and ganglion cell complex thickness between selected groups.

| Groups                  | Vessel density (%) | GCC (µm) |
|------------------------|--------------------|----------|
|                        | SCP    | DCP    | DCP-SCP | Average |
|                        | p-value | p-value | p-value | p-value |
| MS + ON vs MS-ON       | < 0.001 | 0.081  | < 0.001 | < 0.001 |
| NMOSD + ON vs NMOSD-ON | 0.038   | 0.736  | 0.031   | 0.004   |
| MS + ON vs NMOSD + ON  | 0.446   | 0.035  | 0.958   | 0.116   |
| MS-ON vs NMOSD-ON      | 0.296   | 0.235  | 0.092   | 0.224   |

DCP, deep capillary plexus; MS + ON, multiple sclerosis with optic neuritis; MS-ON, multiple sclerosis without optic neuritis; NMOSD + ON, neuromyelitis optica spectrum disorder with optic neuritis; NMOSD-ON, neuromyelitis optica spectrum disorder without optic neuritis; SCP, superficial capillary plexus.
**OCTA**

The vessel density of SCP was significantly lower in MS + ON, MS-ON, and NMOSD + ON when compared with controls \((p < 0.001)\) and in ON eyes of MS and NMOSD groups when compared with non-ON eyes (Tables 2 and 3; Fig. 1). On the contrary, the density of DCP was significantly increased in MS + ON and MS-ON eyes compared to control eyes \((p < 0.001 \text{ and } p = 0.004, \text{ respectively})\). The NMOSD patients, with or without ON, have similar DCP parameters to controls (Table 2). Additionally, the vessel density of DCP was significantly lower in NMOSD + ON patients than in MS + ON group \((p = 0.035; \text{ Table 3; Fig. 1})\).

**The discrepancy between DCP and SCP**

The difference between the deep and superficial capillary plexuses (DCP-SCP) was significantly higher in MS + ON, MS-ON, and NMOSD + ON than in controls \((p < 0.001; \text{ Table 2; Fig. 2f})\) and in ON eyes of MS and NMOSD patients as compared to the non-ON eyes \((p < 0.001 \text{ and } p = 0.031, \text{ respectively; Table 3})\).

**Association of OCTA and SD-OCT**

A significant positive correlation was observed between SCP and GCC thickness in MS + ON, MS-ON, and NMOSD + ON groups \((p < 0.001; \text{ Fig. 2a,b,d})\). Although the vessel density of DCP was not significantly correlated with GCC thickness, the DCP of MS and NMOSD patients tended to increase or decrease with ganglion cell loss, respectively (Fig. 2a,b,d,e). The DCP-SCP strongly negatively correlated with GCC thickness in MS + ON, MS-ON and NMOSD + ON patients \((r = -0.81, p < 0.001; r = -0.699, p < 0.001; r = -0.863, p = 0.006, \text{ respectively})\).

**Disease duration**

The disease duration did not significantly correlate with SCP, DCP, DCP-SCP, and GCC thickness in all groups.

**Discussion**

In this study, we used OCTA to evaluate the macular vessel density in superficial and deep capillary plexuses in patients with MS and NMOSD. We found that SCP and GCC thickness were significantly lower in ON eyes of MS and NMOSD patients than in the controls, and the parameters strongly correlated to each other. Notably, such association was also observed in MS-ON eyes, indicating that former ON is not obligatory to cause ganglion cell loss and SCP reduction in this group.

In MS patients, the SCP’s blood perfusion was more decreased in eyes with previous ON than in non-ON eyes. On the contrary, the DCP did not differ between ON+ and ON- eyes but had significantly higher vessel density than controls. Although the DCP did not correlate with GCC thickness, the negative trend could be easily observed (Fig. 2a,b). Moreover, the discrepancy between SCP and DCP was strongly negatively correlated with GGC thickness and was significantly higher in ON eyes.

Nesper et al. reported that retinal vessels could actively adapt to the metabolic demand of retinal cells. The coupling mechanism allows for regulation of the retinal blood flow between plexuses, and, e.g., the higher DCP perfusion may result from vessel dilation or increased velocity of flow. According to these observations, the relationship between SCP and DCP in MS eyes can be explained. SCP and DCP, respectively, supply the ganglion cell layer and inner nuclear layer. It was shown that after optic neuritis, the GCL becomes atrophic, whereas the INL remains unchanged, or its volume increases. It was consistent with our findings that the reduced vessel density was observed only in SCP because of the lower metabolic demand of injured ganglion cells. Additionally, the redistribution of blood between plexuses through
vertical anastomoses resulted in an increased density of DCP. The results of our study nicely demonstrate that the reduction of vessel density in SCP is secondary to ganglion cell loss in MS + ON and MS-ON eyes.

In contrary to MS-ON group, the NMOSD-ON eyes had comparable SCP, DCP, and GCC parameters to controls. In the studies of Huang et al. and Chen et al., the vessel density was measured only in SCP, and in NMOSD + ON and NMOSD-ON patients, they found it lower than in healthy eyes. Kwapong et al. presented that, besides the reduced density of SCP, the density of DCP was also reduced in ON and non-ON eyes. Moreover, the authors suggested that it might be evidence for subclinical primary retinal vasculopathy. In our study, the only significant difference in vessel density was noted in SCP between NMOSD + ON eyes and controls. In accordance with other studies, the GCC thickness was significantly lower in ON eyes than in healthy eyes, and in non-ON eyes – was similar to controls.

Comparing the features of MS + ON and NMOSD + ON patients, we made an interesting observation. Although the SCP and GCC thickness parameters are comparable (Table 3), the groups significantly differ in DCP, which is also well seen on the plots (Fig. 2a,d). While the DCP of MS + ON eyes tends to increase with the ganglion cell loss, in NMOSD + ON eyes, it tends to decrease. It shows that the blood flow distribution pattern between plexuses in NMOSD + ON is different from this previously described in MS. The increasing trend of DCP with GCC thickness loss is also observed in controls (Fig. 2c), and we may assume that the typical network of healthy vessels can adapt and act this way. Thus, the reduction of vessel density in DCP indicates the capillary loss in NMOSD + ON eyes. Although statistically insignificant, a weak tendency of DCP reduction is observed in NMOSD-ON eyes, suggesting that vascular loss may appear prior to optic neuritis, as Huang et al. and Kwapong et al. reported. Inconsistent results were published on hemodynamics in the optic neuritis eye vasculature using ultrasound examination. There are reports available on the effects of the upregulation of Th17 cells, that transforming growth factor-beta, which can cause myointimal fibrosis in NMO patients. Moreover, the vascular reactivity, e.g., effects of nitric oxide, should be considered during the interpretation of results, however, no disturbances in cerebrovascular reactivity in MS patients were recently reported.

The retinal capillary network evaluation showed the difference between superficial and deep capillary plexuses in MS and NMOSD patients. The compensatory vascular mechanism of DCP was only seen in MS eyes, indicating that vessel density of DCP in NMOSD patients was reduced. We think that the explanation may be found in the distinct pathogenic mechanism of NMOSD. Retinal capillaries of SCP and DCP are ensheathed by macroglial cells, i.e., astrocytes and Müller cells, contributing to the formation and maintenance of the inner blood-retinal barrier. While the astrocytes’ bodies and processes are found exclusively in the nerve fiber layer, Müller cells’ bodies are located in the inner nuclear layer, and they project processes through entire retinal thickness. The high density of aquaporin-4 expressed on these cells is targeted by disease-specific IgG under inflammatory conditions. The T cells get access to the retina from SCP and DCP and open the BRB for the AQP4-IgG and complement. It was experimentally shown on an animal model that retinal damage may appear independently of optic neuritis.

The limitation of our study is a small group of NMOSD patients. However, at the beginning of study enrollment, we had to exclude some patients who could not undergo ophthalmic examination because of visual and physical disability. Therefore, further studies with larger cohorts are necessary to confirm our observations.

In conclusion, we demonstrated for the first time that in the eyes of MS patients, the vascular changes are secondary to the decreased metabolic demand of atrophied ganglion cell layer, while in the eyes of NMOSD patients, the vasculopathy seems to be a primary process.

**Declarations**
Competing Interests

The authors declare no competing interests.

Author Contributions

M.R. – conception and design of work; acquisition, analysis, and interpretation of data; review of the literature; drafting the manuscript. S.M. – conception of work; analysis and interpretation of data; review of the literature; critical revision of the manuscript. M.S. – conception of work; interpretation of data; critical revision of the manuscript. All authors read and approved the final manuscript and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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