Optical Coherence Tomography Advanced Parameters in Patients With Multiple Sclerosis: Ophthalmological and Neurological Assessments

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• PURPOSE: To evaluate ophthalmological, neurological, radiological, and laboratory data in patients with multiple sclerosis (MS) and to identify new ophthalmological factors that could be helpful as biomarkers of the disease, potentially leading to an earlier prediction of disease course and disability progression.

• DESIGN: Retrospective, cross-sectional-study.

• METHODS: Best-corrected visual acuity (BCVA), ophthalmological biomicroscopy of the anterior segment and fundus, structural optical coherence tomography (OCT) with retinal nerve fiber layer (RNFL) and ganglion cell complex (GCC), and OCT angiography (OCTA) with vascular density (VD) were performed. The following clinical and neuro-radiological features were assessed: MS phenotype, disease duration, clinical severity, type of treatment, and T2-weighted lesion and T1-weighted Gd+ enhancing lesion number on the brain and spinal cord MRI.

• RESULTS: One hundred and six patients (212 eyes) were analyzed. Sixty-six of them (62.2%) had MS and 40 (37.8%) were matched healthy controls (HCs). patients with MS showed lower RNFL, GCC, and VD in the radial peripapillary capillary plexus than controls in both eyes ($P < .05$). By Performing a logistic regression with a distinct MS outcome for both eyes, we were able to demonstrate that the value that was most predictive of MS was the average GCC thickness ($P = .009$). Regression analysis demonstrated that patients with a higher T2-weighted lesions showed a lower RNFL thickness value and reduced GCC and VD values than those with a low lesion load ($P < .01$ and $P < .05$, respectively). Similarly, relapsing MS patients showed lower RNFL values ($P < .05$).

• CONCLUSIONS: Several OCT and OCTA-optic nerve parameters could be useful prognostic biomarkers for the MS disease course in clinical practice. However, it is necessary to do additional research with larger sample sizes in order to validate these findings. (Am J Ophthalmol 2024;267: 41–49. © 2024 The Author(s). Published by Elsevier Inc. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/))

INTRODUCTION

Multiple sclerosis (MS) is a chronic condition characterized by inflammation, degeneration, and demyelination within the central nervous system (CNS). The cause of MS remains unknown, and it typically manifests in individuals during early adulthood.[1] The condition is characterized by broad inter- and intra-individual heterogeneity with diverse clinical manifestations, subgroups, and prognostic outcomes.[1]

The precise aetiology of multiple sclerosis (MS) remains unclear; however, it is widely believed to result from a complex combination between genetic susceptibility and environmental factors.[1]

Multiple sclerosis (MS) can give rise to a variety of neurological manifestations due to its capacity to impact various areas of the brain, spinal cord, and optic nerve.[2] Approximately 85% of patients initially exhibit a disease form known as relapsing-remitting multiple sclerosis (RRMS),

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characterized by the occurrence of acute exacerbations followed by periods of remission. Based on the inherent progression of the disease, a majority of individuals diagnosed with relapsing-remitting multiple sclerosis (RRMS) eventually transition to a secondary progressive form of the disease (SPMS). This particular manifestation is distinguished by a slow decline in neurological capabilities, either with or without additional episodes of relapse. In a small percentage of cases, approximately 5%-10% of patients have a primarily progressive course, known as primary progressive multiple sclerosis (PPMS), which is characterized by an ongoing progression of impairment from the onset of the disease.[2]

The pathogenesis of multiple sclerosis (MS) is distinguished by the presence of modified multidirectional interactions between several types of immune cells in both the peripheral and resident central nervous system (CNS) cells. The precise aetiology of the immunological response that is responsible for this abnormality has yet to be fully understood[3-5]

Multiple localized areas of demyelination, accompanied by varying degrees of inflammation and neurodegeneration, represent the pathophysiological hallmark of MS.[6,7]

In the context of relapsing-remitting multiple sclerosis (RRMS), the presence of classical active white matter lesions accompanied by significant lymphocytic inflammation is a distinguishing feature. Conversely, in progressive forms of the disease, lesions typically exhibit an inactive lesion core surrounded by a periphery consisting of activated macrophages and microglia.[6] In the initial stages of the disease, there is a certain degree of preservation observed in axons. However, as the disease progresses, permanent degeneration of axons occurs.[8] Neuronal loss and axonal injury are the principal aetiologies of progressive irreversible disability.[9]

To date, despite technological advancements, there are no prognostic biomarkers for the MS disease course and disability progression that are easily available in clinical practice.[10]

Optical coherence tomography (OCT) is an important diagnostic tool for MS. It can detect thinning of the retinal nerve fiber layer (RNFL) and retinal ganglion cell layer (GCL).[11] Recently, OCT angiography (OCTA) has been developed as a non-invasive method for measuring vessel density and flow velocity in the optic disc and macula.[11,12] Systematic reviews and meta-analyses of studies reporting OCTA measurements of individuals with MS have revealed decreased vessel density in the macular and optic disc regions, specifically in the superficial capillary plexus (SCP), deep capillary plexus (DCP), and radial peripapillary capillary (RPC) vessel densities.[13]

The objective of our study was to compare ophthalmological, neurological, radiological, and laboratory data to evaluate the anatomical and functional characteristics of patients with MS and to identify new ophthalmological factors that could be helpful as prognostic biomarkers of the disease course in clinical practice.

METHODS

This retrospective, cross-sectional observational study was conducted as a collaboration between the Department of Ophthalmology and Neurology at the Fondazione Policlinico Universitario A. Gemelli, IRCCS, between September 2021 and December 2022. We consecutively enrolled 106 (212 eyes) patients, 66 patients diagnosed with MS according to McDonald 2017[14] criteria, thus performing blood samples, MRI and CSF examinations, and 40 age-matched healthy controls. The enrollment exclusion criteria for patients and controls were the presence of vascular comorbidities and overall hypertension, diabetes, heart disease, ocular pathologies, ongoing chemotherapy, and drug abuse. The study adhered to the Declaration of Helsinki and was approved by the Ethical Committee "Università Cattolica Sacro Cuore" in Rome, Italy (protocol ID number: 3860, NCT05747144). Signed informed consent was obtained from each enrolled patient.

• **OPHTHALMOLOGICAL EXAMINATIONS:** A detailed ophthalmological examination was conducted, including best-corrected visual acuity (BCVA), biomicroscopy of the anterior segment and fundus, SD-OCT, OCT, and OCTA, and fundus photography using the Solix device (Visionix International SAS, Pont-de-l'Arche, France). Only OCT and OCTA data with quality indexes (QI) $\geq$ 8/10 were included in the analysis. With the Solix device, study participants underwent scanning protocols consisting of 6.4 $\times$ 6.4 mm field of view centered on the fovea, evaluating the ganglion cell complex (GCC), i.e., the sum total of retinal fiber layer (RNFL), ganglion cell layer (GCL) and inner-plexiform layer (IPL). Likewise, we performed an optic disc cube scan of 6 $\times$ 6 mm to evaluate the RNFL and mean peripapillary vessel density (VD). Each participant was instructed to fixate on an internal predefined target to ensure that comparable anatomical locations were recorded for each participant. The correct fixation point was verified by the individual who acquired the images. Retinal layer segmentation was performed autonomously by using the built-in algorithm of the OCT device. The accuracy of the segmentation was reviewed and manually corrected where necessary. Ophthalmological examination results are shown in Figures 1 and 2. To assess significant differences in OCT and OCTA parameters between the MS and healthy groups, we decided to split the data of the two eyes of each participant, comparing all right eyes (RE) of one group with all RE of the other, and likewise for the left eye (LE).

• **NEUROLOGICAL EXAMINATION:** During ophthalmological evaluation, the following neurological clinical features were evaluated: MS phenotype (relapsing-remitting, secondary-progressive, and primary-progressive forms), disease duration neurological examination with evaluation of

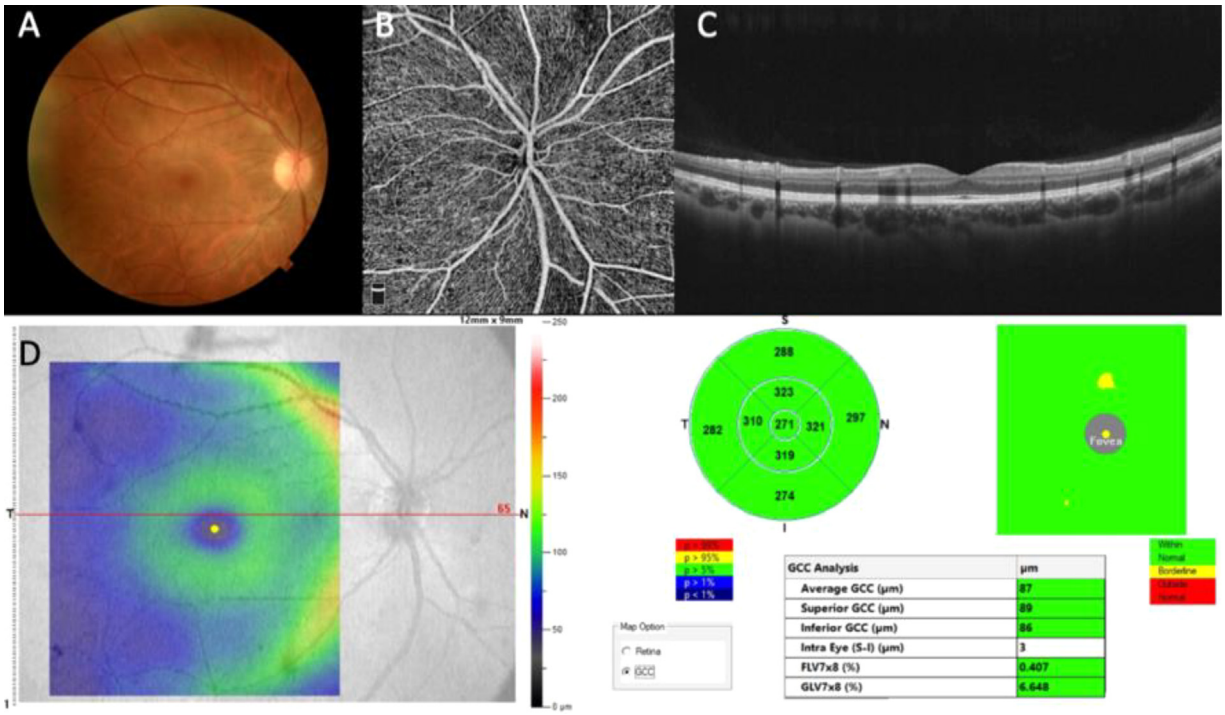

FIGURE 1. Ophthalmological imaging of healthy control eye: (A) Color fundus imaging shows a normal optic nerve. The posterior pole and midperiphery shows no severe morphologic changes. (B) Optic disc OCT angiography cube scan of 6 mm × 6 mm has a normal vascular pattern; (C) OCT B-scans of the retina (12 mm length) reveals a normal profile. (D) Ganglion cell complex map option in 12.0 × 9.0 mm OCT-scan shows normal values in the ganglion cell layer at the posterior pole.

the Expanded Disability Status Scale (EDSS score), and ongoing treatment. We also recorded the following neuroradiological features at the last brain and spinal cord MRI performed within the last two months: T2 weighted-demyelinating lesions number (considering >9 lesions as a cut-off to identify MS patients with a high T2 weighted-lesion load and ≤9 lesions in MS patients with a low T2 weighted-lesion load) and T1-weighted Gd+ enhancing lesions. We assessed the EDSS scores at diagnosis, ophthalmological examination, and the disease progression index (EDSS score/disease duration).

Neurological examinations were performed by a consultant neurologist qualified to perform the EDSS assessments according to the standardized scoring system in neurostatus (http://www.neurostatus.net/scoring/index.php).

In addition, we evaluated the patient's anti-aquaporin4 (anti-AQP4) and anti-myelin oligodendrocyte glycoprotein (anti-MOG) antibody status and the frequency of retrobulbar optic neuritis.

• **STATISTICAL ANALYSIS:** The researchers conducted descriptive analyses, utilizing frequencies and percentages to examine categorical data, and mean and standard deviation (SD) to evaluate continuous data. Statistical measures of skewness and kurtosis were employed to examine the distribution of the quantitative data that was gathered. Additionally, the Shapiro-Wilk test was utilized to assess the normality of the distribution. Inferential statistics were performed using the chi-square test or Fisher's exact test for qualitative variables and the t-test or Wilcoxon rank-sum test (Mann-Whitney) for quantitative data, according to the normality of the distribution by applying Fisher correction. Statistical significance was set at $P < .05$. Statistical analysis was performed using the STATA software (version 17.0; Stata Corporation, College Station, TX, USA).

RESULTS

A total of 106 patients (212 eyes) were included in this analysis. Sixty-six of them (62.2 %) had MS and 40 (37.8%) were matched healthy controls (HCs). The mean age at evaluation was 41.5 ± 13.3 (range 19-75), that is, 42.2 ± 11.7 (range 19-65) in the MS group and 40.4 ± 15.6 (range 21-75) in the control group). The female to male distribution on the other hand, was 60.6% females/39.4% male in the former and 72.5%/27.5% in the latter.

In the MS group, 27 (40.9%) patients had retrobulbar optic neuritis at disease onset of their disease. Fifty-nine (89.4%) patients showed an RR clinical course, seven (10.6%) had SP, no patient had a PPMS. The mean dis-

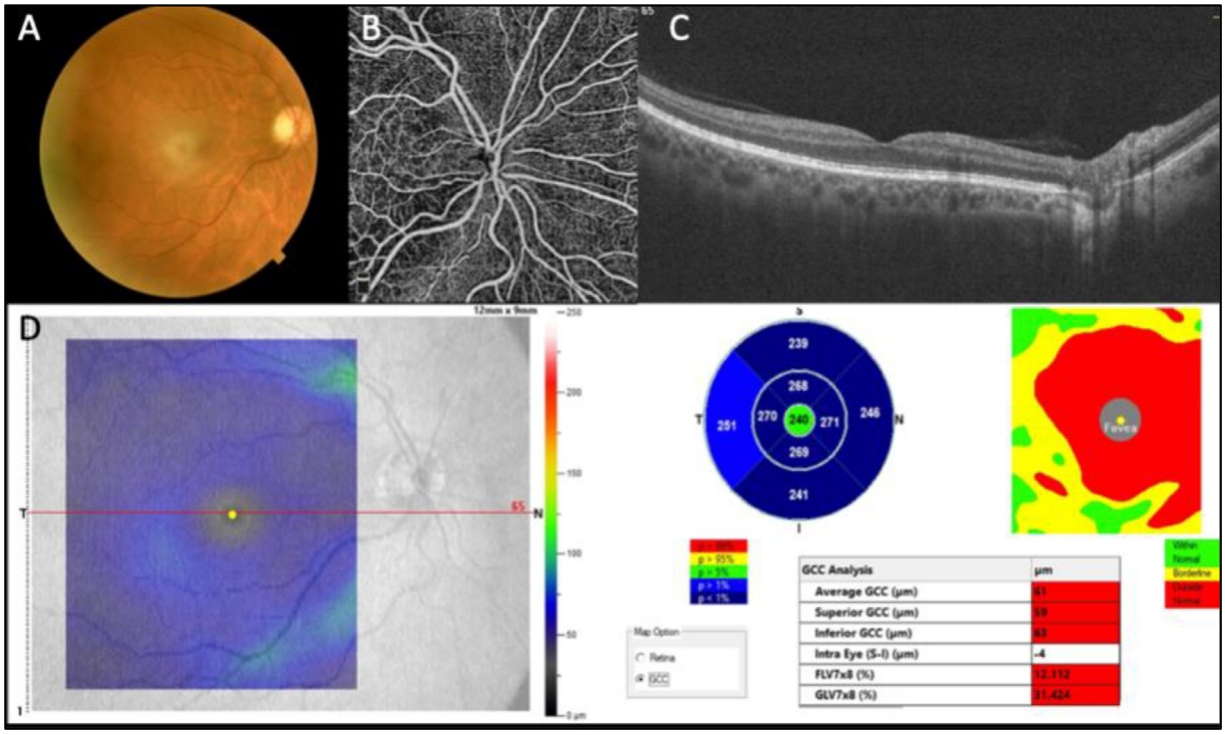

FIGURE 2. Ophthalmological imaging of SM left eye: (A) color fundus imaging shows a normal optic nerve. The posterior pole and midperiphery shows no severe morphologic changes. (B) Optic disc OCT angiography cube scan of 6 mm × 6 mm has a reduction in the deep vascular pattern most evident at the posterior pole and particularly at the interpapillary-macular bundle; (C) OCT B-scans of the retina (12 mm length) reveals a reduction in the inner retinal layers where nerve fiber layers are located. (D) Ganglion cell complex map option in 12.0 × 9.0 mm OCT-scan shows an average reduction in the ganglion cell layer at the posterior pole compared with normative data (blue color map).

TABLE 1. Baseline Demographic and Clinical Characteristics of the MS Population

	MS (n = 66)
Mean age, yrs	42.2 ± 11.7
Gender, female, no (%)	40 (61%)
Retrobulbar ON onset, no (%)	27 (41%)
Disease duration, months	126.7 ± 114.1
Clinical course	
• RR, no (%)	59 (89%)
• SP, no (%)	7 (11%)
• PP, no (%)	0 (0%)

MS, multiple sclerosis; RR, relapsing refitting; SP, secondary progressive; PP, primary progressive.

ease duration was 126.7 ± 114.1 (range 1-444) months. The baseline demographic and clinical characteristics of patients with MS are shown in Table 1. Mean BCVA in the MS-group was 0.95 ± 0.16 (range 0.2-1) in the RE and 0.92 ± 0.17 (range 0.1-1) in the LE. In the control-group on the other hand, BCVAs were 1.0 ± 0.3 (range 0.4-1) in RE and 1.0 ± 0.0 in LE (MS vs. HCs P = .03).

• **OCT ANALYSIS:** Mean average RNFL thickness values were 87.50 ± 12.10 µm in right eye (Res) and 86.80 ± 13.90 µm in left eye (Les), i.e., 84.95 ± 13.64 µm and 83.78 ± 16.03 µm in the MS group, and 91.80 ± 7.40 µm and 91.80 ± 7.10 µm, in the control group, respectively.

Mean average GCC thickness was 98.32 ± 10.08 µm in REs and 98.22 ± 11.68 µm in LEs. In the MS group and the control group, these values were, respectively, 94.92 ± 11.00 µm – 94.84 ± 13.28 µm, and 103.92 ± 4.46 µm – 103.80 ± 4.71 µm.

The differences in RNFL and GCC thicknesses in the REs and LEs between the two groups were statistically significant (P = .009 and P = .0017 for the RNFL and P < .001 and P < .001 for the GCC, respectively) (Figures 3 and 4).

Furthermore, we evaluated eyes that were previously diagnosed with retrobulbar (RB) optic neuritis. In particular, we found that when assessing the eyes independently for RE and LE, there is a statistically significant difference for RNFL in the RE (P < .02), but not for LE (P < .09). Regarding the GCC, there is a notable distinction for both RE and LE, with statistical significance at P = .005 and P < .03, respectively. In addition, we conducted an assessment to compare the two groups by analyzing the average values

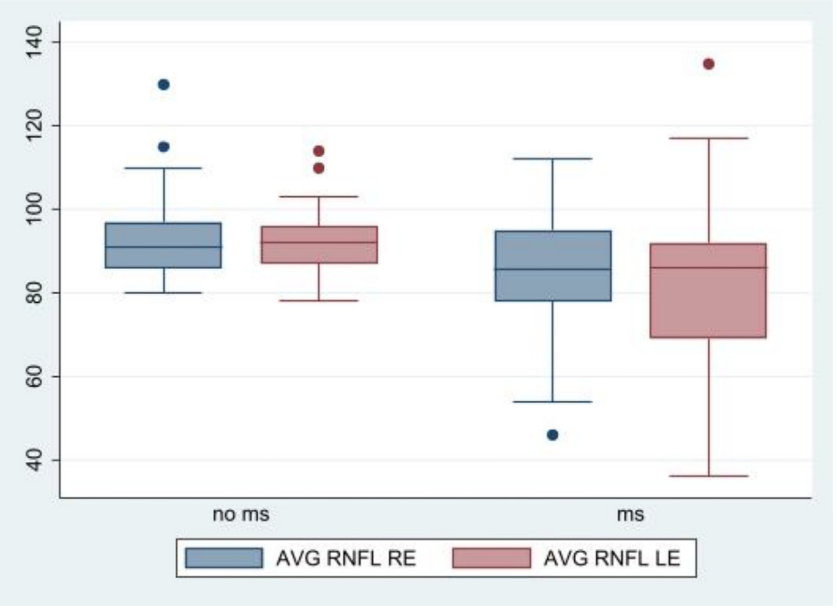

FIGURE 3. Distribution graphs of the mean value of retinal nerve fiber layer (RNFL) in heathy group (on the left) and with multiple sclerosis (on the right) divided in right eye (RE) and left eye (LE).

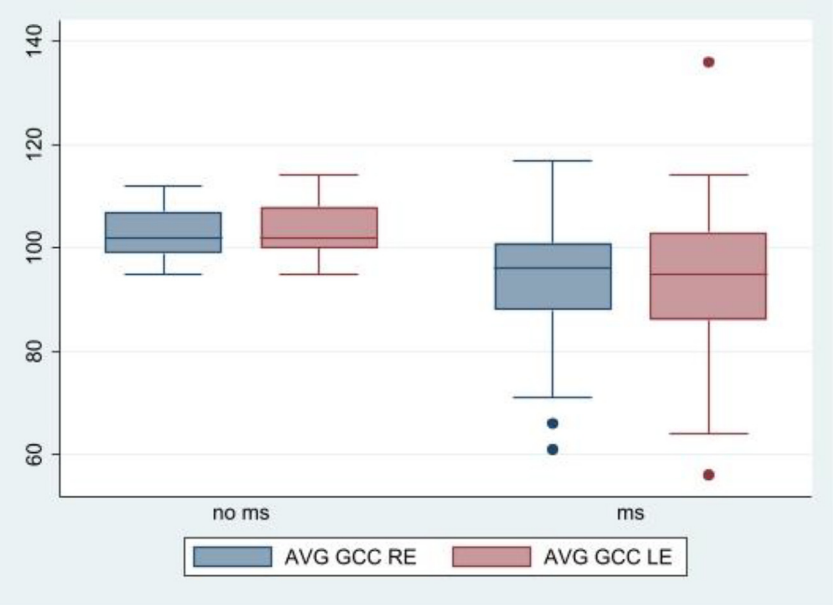

FIGURE 4. Distribution graphs of the mean value of ganglion cell complex (GCC) in heathy group (on the left) and with multiple sclerosis (on the right) divided in right eye (RE) and left eye (LE).

for both eyes. The results showed a significant difference in both the RNFL and in the GCC, respectively $P < .04$ and $P = .008$. Figure 5 shows a chart that highlights the differences between these two groups.

• **OCTA ANALYSIS:** Mean peripapillary vessel density (VD) in right and LE was 48.63 ± 3.38% and 47.77 ± 5.86%, respectively. In the MS group, mean average peripapillary VD in RE and LE was 48.06 ± 0.28% and 47.3 ± 4.17% respectively, whereas in the control group, mean average peripapillary VD in RE and LE was 49.88 ± 2.30% and 48.84 ± 8.55% respectively. The difference between the two groups was statistically significant for both RE ($P < .02$) and LE ($P < .001$) (Figure 6).

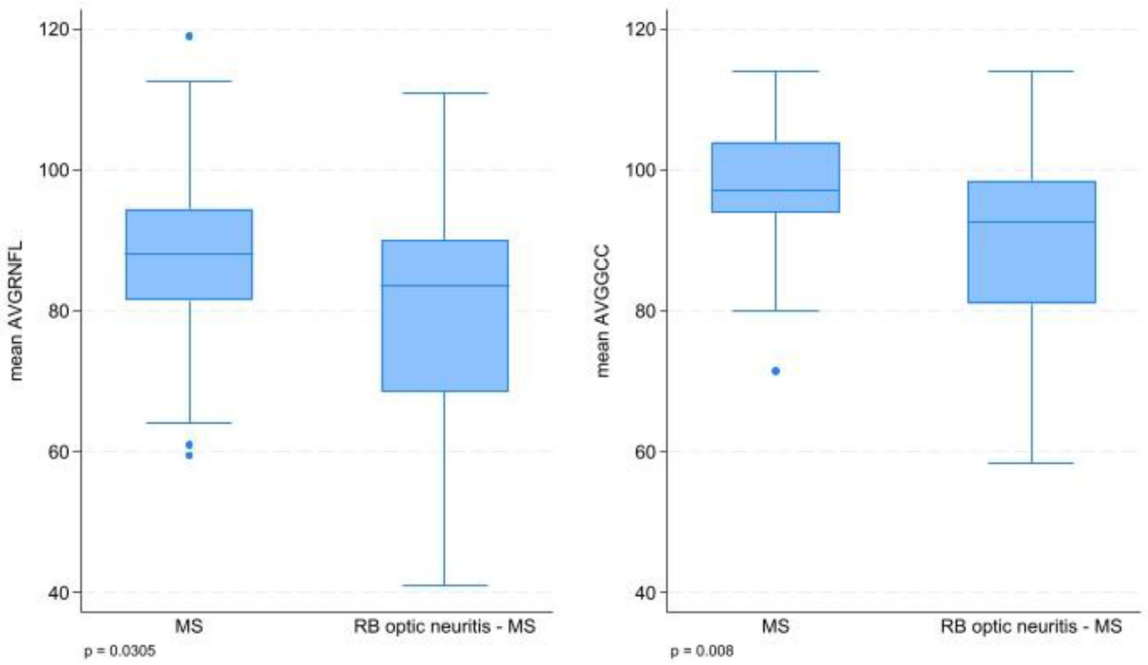

FIGURE 5. The chart shows the statistically significant difference between the two groups (with a history of retrobulbar optic neuritis versus without) by assessing the average values of RNFL (on the left) and GCC (on the right) between right and left eye.

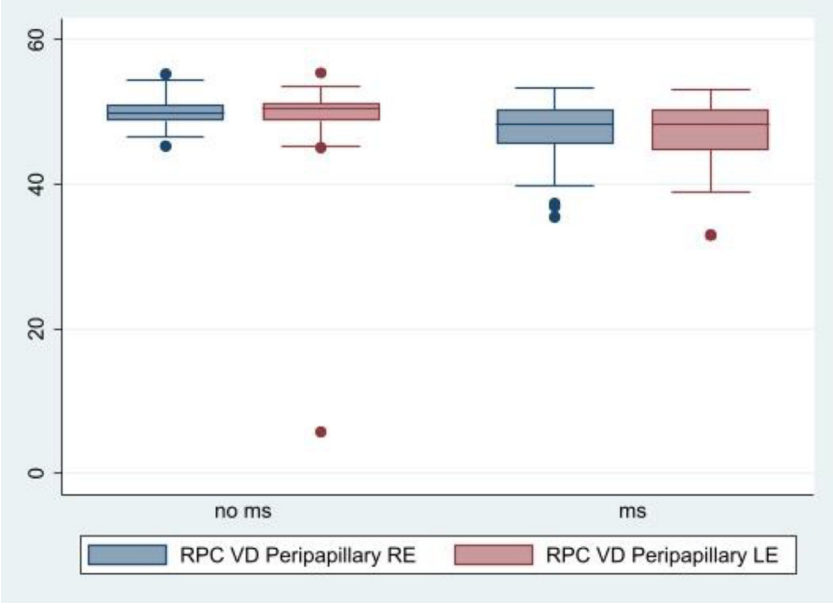

FIGURE 6. Distribution graphs of the mean value of retinal capillary plexus peripapillary (RPC) vessel density in heathy group (on the left) and with multiple sclerosis (on the right) divided in right eye (RE) and left eye (LE).

• **NEUROLOGICAL FEATURES:** At the time of diagnosis, 40% (27 out of 66) of the patients exhibited NORB. Among these cases, 60% (16 patients) involved the left eye, 33% (9 patients) involved the right eye, and 7% (2 patients) were bilateral.

The mean EDSS score for ophthalmological management was 2.51 (±1.89). The average progression index was 0.38 (±1.15).

A history of disease reactivation was also evaluated in patients with MS. Fifty-nine (89.39%) had at least one reac-

tivation, compared to 7 (10.61%) who experienced a single episode. Overall, we identified active retrobulbar ON in only three (4.54%) of 66 cases. On brain and spinal cord MRI, 49 MS patients (74.24%) had a high T2-weighted lesion load and 55 patients (83.33%) had no T1-weighted Gd+ enhancing lesions.

All patients were negative for anti-AQP4 and anti-MOG antibodies.

Regression analysis demonstrated that patients with a higher T2-weighted lesion load had a lower RNFL thickness and reduced GCC and VD values than those with a low lesion load ($P < .01$ and $P < .05$, respectively). Similarly, relapsing MS patients showed lower RNFL values ($P < .05$).

• **POST-HOC ANALYSIS:** Successively, performing a logistic regression with a distinct MS outcome for the RE and LE, we were able to demonstrate that the value that is most predictive of MS is the average GCC thickness ($P = .009$).

Moreover, we investigated which of these variables was most dependent on MS, and using linear regression analysis, we demonstrated that MS had a statistically significant effect on each of these variables. Indeed, MS had a statistically significant effect on mean RNFL and GCC thickness ($P = .004$ and $P = .001$, respectively, for both eyes).

Furthermore, linear logistic regression analysis demonstrated a robust association between the peripapillary VD values in the left eye and the EDSS score at diagnosis and ophthalmological examination ($P = .004$ and $P = .048$, respectively). No statistical significance was found in the OCTA parameters or progression index.

In addition, the Mann-Whitney test revealed a statistically significant difference ($P = .004$) between the RR and non-RR groups in terms of disease duration.

DISCUSSIONS

To date, despite technological advancements, there are few prognostic biomarkers of the MS disease course and disability that are not easily applicable in clinical practice.[10]

The primary objective of our study was to identify new ophthalmological factors to help clinicians identify MS patients with a worse disease course.

OCT is a non-invasive imaging technique that permits high-resolution quantification of retinal structures, including the ganglion cell complex (GCC) and their axons (RNFL). In contrast to most axons in the central nervous system, those from the eye are unmyelinated until they exit the globe, making them ideal for studying neurodegeneration and neuroprotection.[15,16] Indeed, the earliest applications of OCT technology in the study of MS were reported by Parisi et al. in 1999 and Trip in 2005, when they found a 26% and 27% reduction in RNFL thickness, respectively, when compared to controls.[17,18]

In our study, we analyzed the RNFL, GCC, and VD in the peripapillary area of 66 patients with and without a history of optic neuritis due to MS. Similar to previous studies, we found a statistically significant reduction in these values compared with the control group.[13,19]

Optic nerve perfusion has been investigated using OCTA. Compared with the control group, Cennamo et al.[19] showed that the VD of the superficial and deep capillary plexuses and average vascular density were substantially lower in patients with MS. These findings are consistent with those of Lanzillo et al.,[20] Wang et al.,[21] Spain et al.,[22] and Ulusoy et al.,[23] who found a marked decrease in ONH circulation in patients with MS without a history of ON. In accordance with these findings, our team found a statistically significant decrease in peripapillary VD in eyes with (8 eyes) and without (124 eyes) a history of optic neuritis compared with the control group.

Moreover, it is likely that the significant association between EDSS values at both diagnosis and at ophthalmological control and peripapillary VD values, specifically in the left eye, might be attributed to the fact that 40% of the evaluated patients experienced NORB at the outset, and among these cases, 66% showed involvement of the left eye.

In addition to analyzing a control population, the objective of our study was to assess the potential presence of a parameter that could serve as a disease predictor. Consequently, using linear regression analysis, we demonstrated that the average GCC thickness was a predictor of MS. Furthermore, we observed that structural OCT and OCTA parameters correlated significantly with neurological parameters of a worse MS disease course. Specifically, patients with a higher T2-lesion load on MRI had significantly reduced average RNFL, GCC, and peripapillary VD values, and average RNFL thickness values were correlated with disease reactivation. Consequently, our data support the importance of OCT and OCTA as minimally invasive, rapid, and non-operator-dependent examinations to provide crucial data regarding the potential clinical course of MS.

The lack of follow-up to assess changes over time, the presence of a small number of active optic neurites, and the possibility of assessing isolated values of the GCL due to instrumentation are limitations of our research.

CONCLUSIONS

Several OCT and OCTA-optic nerve parameters could be useful prognostic biomarkers for the MS disease course, which are easily accessible in clinical practice. Nonetheless, further studies with larger sample sizes are required to confirm these findings.

CREDIT AUTHORSHIP CONTRIBUTION STATEMENT

MARIA CRISTINA SAVASTANO: Validation, Conceptualization. **VIVIANA NOCITI:** Validation, Methodology, Investigation, Conceptualization. **FEDERICO GIANNUZZI:** Supervision, Methodology, Investigation, Conceptualization. **VALENTINA CESTRONE:** Visualization, Software, Resources, Data curation. **MATTEO MARIO CARLÀ:** Resources, Project administration, Formal analysis, Data curation. **CLAUDIA FOSSATARO:** Software, Resources, Formal analysis, Data curation. **ILARIA BIAGINI:** Funding acquisition, Formal analysis, Data curation, Conceptualization. **CLARA RIZZO:** Visualization, Validation, Supervision, Software. **RAPHAEL KILIAN:** Writing – review & editing, Writing – original draft, Visualization, Validation. **MARCO BISURGI:** Writing – review & editing, Writing – original draft, Visualization, Validation. **PAOLO CALABRESI:** Resources, Project administration, Methodology, Investigation. **MASSIMILIANO MIRABELLA:** Resources, Project administration, Methodology, Investigation, Conceptualization. **STANISLAO RIZZO:** Project administration, Methodology, Investigation, Funding acquisition.

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