„CAROL DAVILA" UNIVERSITY OF MEDICINE
AND PHARMACY, BUCHAREST
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Retinal changes in patients with relapsing-remitting multiple sclerosis

Ph.D. THESIS SUMMARY

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THE CONTENT OF THE PhD THESIS

Introduction

The present paper-work is entitled "Retinal changes in patients with relapsing-remitting multiple sclerosis" and consists of a general part, in which I presented the current state of knowledge regarding multiple sclerosis (MS), and an experimental part, in which I investigated the ability of retinal structural and vascular parameters to be used in the complementary diagnosis of MS.

MS is a pathology that can be regarded from several points of view.

For the patient, this pathology is the everyday chameleon, having a huge variety of symptoms, with certain recurring manifestations and an unpredictable evolution.[1]

For the pathologist, MS is an inflammatory disease of the central nervous system (CNS), characterized by acute focal demyelination, axonal loss and partial remyelination, resulting in the formation of chronic multifocal sclerotic plaques.[1]

For the neurologist, MS is a condition of young adults, diagnosed on the basis of clinical and paraclinical evidence, in which demyelinating lesions in the brain or spinal cord are evident, with evolution in time and space.[1]

For the clinician, MS is the prototype autoimmune inflammatory disease of the CNS, for which there are rational treatment strategies.[1]

For all these groups, MS remains a difficult disease.[1]

MS is a topic of great current interest, especially as new questions about definition, nosology, cause, mechanisms and management challenge many existing concepts.[2] Meanwhile, the affected population is waiting for a curative solution for this unpredictable CNS pathology.[2, 3] Patients' personal experiences, hopes and fears of an uncertain neurological future are expressed with emotion, at times, through writing, music, drama and visual arts.[1–3]

Currently, the diagnosis of MS remains a challenge, with substantial interest in using Optical Coherence Tomography (OCT) and Optical Coherence Tomography Angiography (OCT-A) as complementary diagnostic tools.[4, 5] Early detection of MS allows initiation of Disease Modifying Therapies (DMT), increasing the quality of life.[4]

I. GENERAL PART

Chapter 1. Current state of knowledge regarding multiple sclerosis

1.1. Definition

MS is a chronic CNS disease,[2] characterized by autoimmune-mediated inflammation, demyelination and axonal loss, resulting in loss of motor and sensory function.[6]

1.2. Epidemiology

MS typically affects young patients aged between 20 and 50 years.[4] The female gender is predominant, with a ratio of 2 to 1 compared to the male gender.[7] In general, life expectancy is reduced by 7-10 years, but it is at least 25 years after the onset of the disease.[1, 8]

1.3. Ethiopathogenesis

The etiology of MS is unknown,[8] but it is assumed to be multifactorial and there is a complex interaction between genetic factors (although it is not considered a hereditary disease) and environmental factors, that is not fully understood.[9]

1.4. Diagnosis

1.4.1. Systemic clinical manifestations

Clinical signs and symptoms in MS can result from involvement of the brainstem, cerebellum, cerebrum and spinal cord, by affecting sensory and motor pathways, and are therefore highly variable between patients.[10, 11]

1.4.2. Ocular clinical manifestations

Involvement of the visual system is the second cause of decrease in the quality of life of patients with MS, after the involvement of the locomotor system. Ocular clinical manifestations include optic neuritis (ON), chronic optic neuropathy, uveitis, retrochiasmatic visual defects, Pulfrich phenomenon, Uhthoff phenomenon, motility dysfunctions, internuclear ophthalmoplegia, nystagmus, other ocular pathologies that should not be associated with the underlying disease and ocular side effects caused by drugs used in MS. [12, 13]

1.4.3. Diagnostic criteria

The diagnosis of MS is based on the 2017 McDonald criteria.[4] MS typically occurs in young patients, with a first attack equivalent to clinically isolated syndrome (CIS).[4] Subsequently, three clinical forms are described: relapsing-remitting multiple sclerosis (RRMS), secondary progressive multiple sclerosis (SPMS) and primary progressive multiple sclerosis (PPMS).[11] To establish the diagnosis of MS, patients must meet the criteria for temporal and spatial dissemination of CNS lesions and there must be no better explanation for the clinical manifestations.[4]

1.5. Evaluation of disability status

The Expanded Disability Status Scale (EDSS) was developed in 1983 by Kurtzke and is currently a frequently used method in clinical trials and in the long-term evaluation of MS patients. EDSS reflects the clinical status through a number that extends from 0 (normal neurological status) to 10 (death due to MS) and is calculated based on two components: the functional systems (FS) that may be affected in MS and the limitations related to ambulation.[14]

1.6. Evolution and prognosis

Disability in MS occurs through two distinct mechanisms: residual disability (resulting from incomplete recovery after attacks) and disability due to disease progression. The disability in RRMS accumulates much more slower from disease onset than in PPMS.[1]

Without treatment, natural progression causes significant worsening.[10] DMT improve disease progression by significantly decreasing the rate of disability progression.[10]

1.7. Treatment strategies

Current treatment strategies are focused on treating acute attacks, symptomatic therapies and DMT.[10]

DMT reduce the number and intensity of seizures, reduce the occurrence of MRI lesions, and stabilize, delay, or even improve neurological disability.[10] DMT treatments have become much more varied, which allows for a personalized treatment for each patient depending on the clinical form of the disease, the mechanism of action, the administration route, the effectiveness and the tolerability of the drugs.[15]

Chapter 2. Current state of knowledge regarding OCT and OCT-A retinal changes in patients with multiple sclerosis

The visual apparatus is easily accessible optically and is considered a window to the brain,[16] so that structural and vascular changes in the retina may be associated with changes in the brain.[17, 18]

OCT studies in MS are numerous and relatively homogeneous. Thus, it was demonstrated that the decrease in the thickness of the peripapillary retinal nerve fiber layer (pRNFL) and the decrease in the thickness of the macular ganglion cell layer (mGCL) is correlated with clinical and paraclinical variables - more precisely, it is directly proportional to the decrease in visual acuity and MRI changes,[19] and inversely proportional to the disease duration [20] and disability status.[21] In addition, retinal and cerebral atrophic changes were correlated using OCT and MRI scans,.[18, 21]

OCT-A studies investigating the superficial capillary plexus (SCP), deep capillary plexus (DCP) and choriocapillaris (CC) in MS are less numerous and have conflicting results.[18, 22] Studies have reported that vessel density (VD) in SCP can be decreased,[23] increased[24] or unaffected.[25] Similarly, VD in DCP can be decreased,[26] increased[24] or unaffected[25] in MS patients compared to the control group. The choroidal vasculature has rarely been investigated, and studies report that the VD in CC can be unaffected[27] or increased in MS patients without a history of ON.[18, 28]

The practical utility of OCT and OCT-A in detecting MS is not yet known, but it is promising.[20] The diagnosis of MS is difficult, expensive and time-consuming, therefore there is a substantial interest in using OCT and OCT-A parameters as biomarkers for screening, complementary diagnosis and monitoring of MS.[4, 18]

Previous studies have focused either on OCT structural changes,[29] either on OCT-A vascular changes.[23] Therefore, in the current literature, the associated features of OCT + OCT-A and their impact as potential biomarkers in MS have not been evaluated, and there are no longitudinal studies on this topic.[18]

II. EXPERIMENTAL PART

Chapter 3. Working hypothesis and general objectives

The working hypothesis that was the basis of this PhD thesis was the possibility of OCT and OCT-A parameters to be used in the complementary diagnosis of MS.

The main objective was to investigate the diagnostic accuracy of MS patients without a history of ON by using the combined model between OCT structural parameters (compensated pRNFL + macular ganglion cell complex – mGCC) and OCT-A vascular parameters (VD in SCP + VD in DCP).

The secondary objectives are as follows:

1. investigation of measured pRNFL;

2. application of multiregression analysis to compensate for pRNFL thickness according to individual characteristics;

3. segmentation of individual macular layers;

4. integration of the optic disc informations (compensated pRNFL) with the macular informations (macular ganglion cell complex, mGCC);

5. investigation of the ability of OCT structural parameters to detect MS;

6. comparison of vascular perfusion density (PD) in SCP and DCP between the two groups;

7. investigation of the ability of OCT-A vascular parameters to detect MS;

8. association between OCT structural parameters and OCT-A vascular parameters to investigate the effect on MS diagnosis.

Chapter 4. General research methodology

Ethical approval and consent to participate

The studies are approved by the Emergency University Hospital Bucharest Institutional Review Board (ID 11285) and followed the principles of the Declaration of Helsinki. After providing a detailed explanation of the study, written informed consent was obtained from all participants.

Study design

The current studies have a cross-sectional, case-control design and include MS patients and age- and sex-matched healthy participants.

Study participants

MS diagnosis was confirmed by the treating neurologist based on 2017 McDonald criteria.[4] Only patients with RRMS who were under DMT treatment were included in the study, and patients with SPMS and PPMS were excluded. The medical records corresponding to the MS group were checked to determine the clinical form of the disease, the duration of the disease, the number of attacks, the history of ON and the type of DMT used by each patient.[5, 27, 30]

The control group consists of healthy subjects who presented to the same clinic, matched for age and sex with the group of MS patients, but who did not have neurological and/or other relevant medical conditions.[5, 27, 30]

Participants in both groups were excluded from the study if they had eye surgery within the last 3 months from the start of the study, ocular diseases that could interfere with the aims of the study, such as glaucoma, diabetic retinopathy, retinal vein occlusion, age-related macular degeneration, choroidal neovascular membranes and/or other sight-threatening ocular disease. Glaucomatous optic neuropathy was defined by decreased neuroretinal RIM, vertical cup-to-disc ratio > 0.7 or inter-eye asymmetry > 0.2, and/or notching attributed to glaucoma. All participants with a history of ON and with poor quality OCT / OCT-A scans were also excluded from the study.[5, 27, 30]

All participants received a complete ophthalmological examination, which included measurements of visual acuity, intraocular pressure (IOP), refractive error, axial length, slit lamp biomicroscopy, OCT +/- OCT-A scans and visual field test.

OCT and OCT-A

All scans were performed by the same trained technician, at the same location and on the same day as the other measurements, using the Cirrus AngioPlex HD-5000 Spectral-Domain OCT (Carl Zeiss Meditec, Inc, Dublin, CA, USA).

Depending on the objectives of each study, they were achieved macular OCT scans (6 x 6 mm^2), peripapillary OCT scans (6 x 6 mm^2) and/or macular OCT-A scans (3 x 3 mm^2).

A single trained grader, who was masked to participant's characteristics according to APOSTEL recommendations and the OSCAR-IB protocol, reviewed the quality of OCT and OCT-A scans.[31, 32]

Macular retinal layer thickness analysis

The segmentation of the macular layers (RNFL; GCL; inner plexiform layer - IPL) was performed using the customized program Iowa Reference Algorithm (Retinal Image Analysis Lab, Iowa Institute for Biomedical Imaging, Iowa City, IA, USA, **Figure 4.1.A**). All analyzes were corrected for the magnification effect. The average retinal thickness was determinated within a ring centered on the fovea, with an internal diameter of 1 mm and an external diameter of 2.5 mm. We computed mGCC (RNFL + GCL + IPL) and macular ganglion cell and inner plexiform layer (mGCIPL; GCL + IPL).[5, 27, 30]

Peripapillary retinal nerve fiber layer thickness compensation analysis

We extracted the pRNFL thickness from the peripapillary OCT scans using Cirrus Review Software (Carl Zeiss Meditec, software version 11.0.0.29946). The OCT scans were then imported into a customized MATLAB algorithm (MathWorks Inc., R2018b, Natick, MA) to extract the relevant factors (**Figure 4.1.B**). We then used a multi-regression analysis to adjust pRNFL thickness for several factors: optic disc (area, ratio, orientation), fovea (distance and angle), retinal vascular density, refractive errors and age , using a multivariate linear regression-based model.[33] Optic disc ratio is the quotient between major axis and minor axis, and orientation refers to the angle between the horizontal axis and the major axis of the optic disc. We generated the compensated pRNFL thickness and obtained the averaged measurements within the 3.4 mm annulus around the optic disc center.[5]

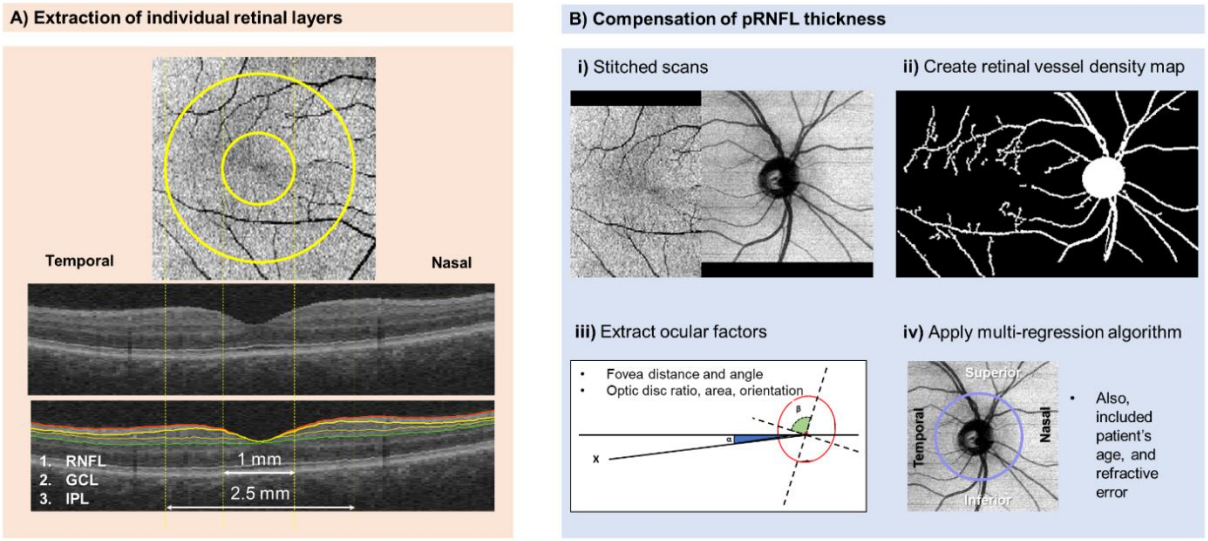

Figure 4.1. Technical processing of OCT scans.
A) extraction of individual macular retinal layers (RNFL, GCL and IPL). **B)** compensation of peripapillary RNFL.
(Adapted - **Bostan M**, Li C, Sim YC, et al. Combining retinal structural and vascular measurements improves discriminative power for multiple sclerosis patients. Ann N Y Acad Sci. Epub ahead of print 2023. DOI: 10.1111/nyas.15060).[5]

Retinal vasculature analysis

Each OCT-A scan was automatically segmented into two plexuses, SCP and DCP, by Cirrus Review Software (Carl Zeiss Meditec, software version 11.0.0.29946). We checked the scans to ensure that the automatic segmentation performed by the software was correct and no manual adjustment was required. Projection artefacts from the overlying retinal circulation were removed from the DCP using the built-in Cirrus software.

OCT-A scans of the SCP and DCP were processed with a custom MATLAB algorithm (The MathWorks Inc., Natick, MA) to assess the capillary densities of these plexuses (**Figure 4.2.**).[5]

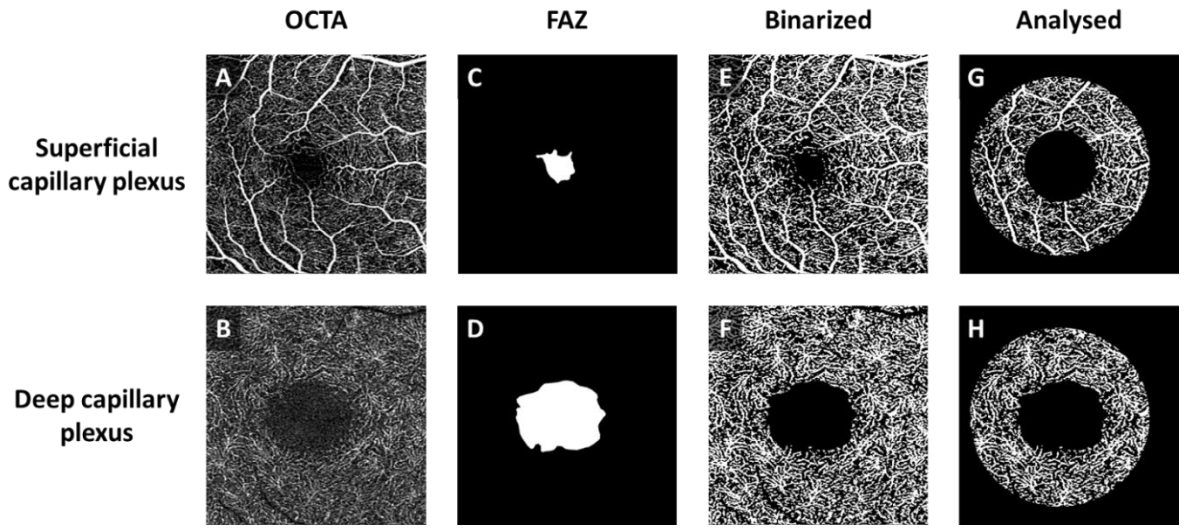

Figure 4.2. Technical processing of OCT-A scans.
(A, B) Raw OCT-A images extracted from the OCT-A device. **(C, D)** Foveal avascular zone (FAZ) manually delineated from SCP and DCP. **(E, F)** Vessels binarized from SCP and DCP. FAZ regions were masked from the binarized images. **(G, H)** For analysis, a magnification-corrected fovea-centered annulus mask (internal diameter = 1.0 mm and external diameter = 2.5 mm) overlaid on the binarized images was used. Capillary density in SCP and DCP was computed as the percentage of vessel area per annulus area.
(Adapted - **Bostan M**, Li C, Sim YC, et al. Combining retinal structural and vascular measurements improves discriminative power for multiple sclerosis patients. Ann N Y Acad Sci. Epub ahead of print 2023. DOI: 10.1111/nyas.15060).[5]

Statistical analysis

The main outcome variables depended on each study. Overall, these are represented by the abilities of retinal structural and vascular parameters to differentiate MS patients without a history of ON from healthy participants.

We compared the mean thickness and vasculature of different retinal layers between the two groups using a multivariate linear regression model with generalized estimating equations (GEE), adjusted for potential confounders such as age, sex, systemic hypertension, IOP, signal strength scans and inter-eye correlation. We generated the receiver operating characteristic (ROC) curves and compared the OCT and OCT-A parameters according to the Area Under the Curve (AUC). Data analysis was performed with Stata version 16.1 (StataCorp LLC, College Station, TX).[5]

Chapter 5. Study 1 - A multi-regression approach to improve optical coherence tomography diagnostic accuracy in multiple sclerosis patients without previous optic neuritis

5.1. Background

The objective of this study was to determine if the OCT discriminative power of MS patients without a history of ON from healthy subjects can be improved by compensating pRNFL thickness according to individual characteristics and by associating the optic disc informations with the macular informations.[30]

5.2. Materials and methods – these were presented in chapter 4.

5.3. Results

The analysis included 74 MS patients (n = 129 eyes) and 84 healthy participants (n = 149 eyes).[30]

After adjusting for age, sex, systemic hypertension, IOP, signal strength of OCT scans and correlation between eyes, pRNFL thickness was statistically significantly decreased in the MS group compared to the control group (87 ± 10 μm vs 95 ± 8 μm; $p < 0.001$; **Figure 5.1.**). After compensation for anatomical factors, compensated pRNFL thickness remained statistically significantly decreased in MS group compared to control group (86 ± 9 μm vs 98 ± 7 μm; $p < 0.001$). Macular layers are statistically significantly thinner in MS patients: mRNFL (MS group: 22 ± 3 μm vs control group: 23 ± 3 μm; $p < 0.001$) and mGCL (MS group: 46 ± 9 μm vs control group: 54 ± 5 μm; $p = 0.026$).

Furthermore, we investigated if the diagnostic performance of MS can be improved by combining the optic disc informations with the macular informations. The combined mGCC + compensated pRNFL model achieved the best diagnostic performance compared to the measured pRNFL (AUC = 0.85 vs 0.75; $p < 0.001$), with an average sensitivity improvement of 24% for detecting MS patients (71.8% vs 47.7%; **Table V.1.**).[30]

Table V.1. Diagnostic performance for discriminating multiple sclerosis from healthy controls

No	Parameters	AUC (95% CI)	Sensitivity at 80% Specificity	Best Cutoff (µm)	p
1	Measured pRNFL thickness	0.75 (0.69-0.81)	47.7	97.0	Ref
2	Compensated pRNFL thickness	0.80 (0.75-0.85)	62.4	98.4	**0.020**
3	mGCIPL	0.74 (0.68-0.80)	46.3	92.1	0.612
4	mGCC	0.76 (0.70-0.81)	52.4	115.7	0.952
5	Combined (#2 and #4)	0.85 (0.80-0.89)	71.8	-	**<0.001**
6	Combined (#1 and #4)	0.82 (0.78-0.87)	67.1	-	**0.001**

Results for sensitivity are expressed as percentages.
p-value indicates the paired comparisons with the best parameter (reference).
Statistically significant p-values are highlighted with **bold**.
CI = confidence interval
Ref = reference
(Adapted - Chua J, **Bostan M**, Li C, et al. A multi-regression approach to improve optical coherence tomography diagnostic accuracy in multiple sclerosis patients without previous optic neuritis. NeuroImage Clin; 34. Epub ahead of print 2022. DOI: 10.1016/j.nicl.2022.103010).[30]

5.4. Discussion

The current study demonstrates that the OCT diagnostic performance of RRMS patients without a history of ON is more robust if pRNFL thickness compensation is performed according to each participant's individual characteristics. Furthermore, by observing the combined models 5 and 6 in the **Table V.1.**, we can state that the detection of MS is improved by using combined optic disc and macular scans compared to using individual optic disc scans.[30]

Chapter 6. Study 2 - Microvascular changes in the macular and parafoveal areas of multiple sclerosis patients without optic neuritis

6.1. Background

The retinal vasculature can be viewed as a surrogate for the cerebral vasculature, and the retina is a neural tissue easily accessible for OCT-A scanning.[16] The aim of the current study was to analyse the microvascular changes occurring in RRMS patients without a history of ON compared to age- and sex-matched healthy participants.[27]

6. 2. Materials and methods – these were presented in chapter 4.

6.3. Results

The analysis included 58 MS patients (n = 100 eyes) and 78 age- and sex-matched healthy participants (n = 136 eyes) with good quality OCT and OCT-A scans.

After adjusting for age, sex, hypertension, IOP and signal strength of OCT-A scans, a statistically significant difference was obtained regarding PD in SCP (MS group: 43.1 ± 0.3%; control group: 41.9 ± 0.3 %;p = 0.003; **Figure 6.1.**) and regarding PD in DCP between the two groups (MS group: 39.2 ± 0.6%; control group: 41.5 ± 0.3%; p < 0.001; **Figure 6.1.**). When large vessels (LV) in the SCP were removed from the PD calculation, a statistically significant difference was still present (p = 0.004). Regarding FAZ characteristics in SCP, there were no statistically significant differences between the two groups in terms of area and circularity (**Figure 6.1.**). However, the FAZ region in DCP was statistically significantly larger (p = 0.005; **Figure 6.1.**) and less circular (p < 0.001; **Figure 6.1.**) in MS group compared to control group. There was no statistically significant difference in LV PD and CC flow deficit between the two groups ($p \geq 0.186$). [27]

6.4. Discussion

In this study, we accounted for various potential confounders, such as potential bias generated by FAZ measurements, OCT magnification correction with axial length measurements and projection artifacts in DCP. The MS group showed a significant increase of PD in SCP and a significant decrease of PD in DCP compared to the control group. Our results support the concept of the existence of retinal microvascular changes detectable by OCT-A in MS patients and the concept of using OCT-A parameters as imaging biomarkers for MS detection and screening.[27]

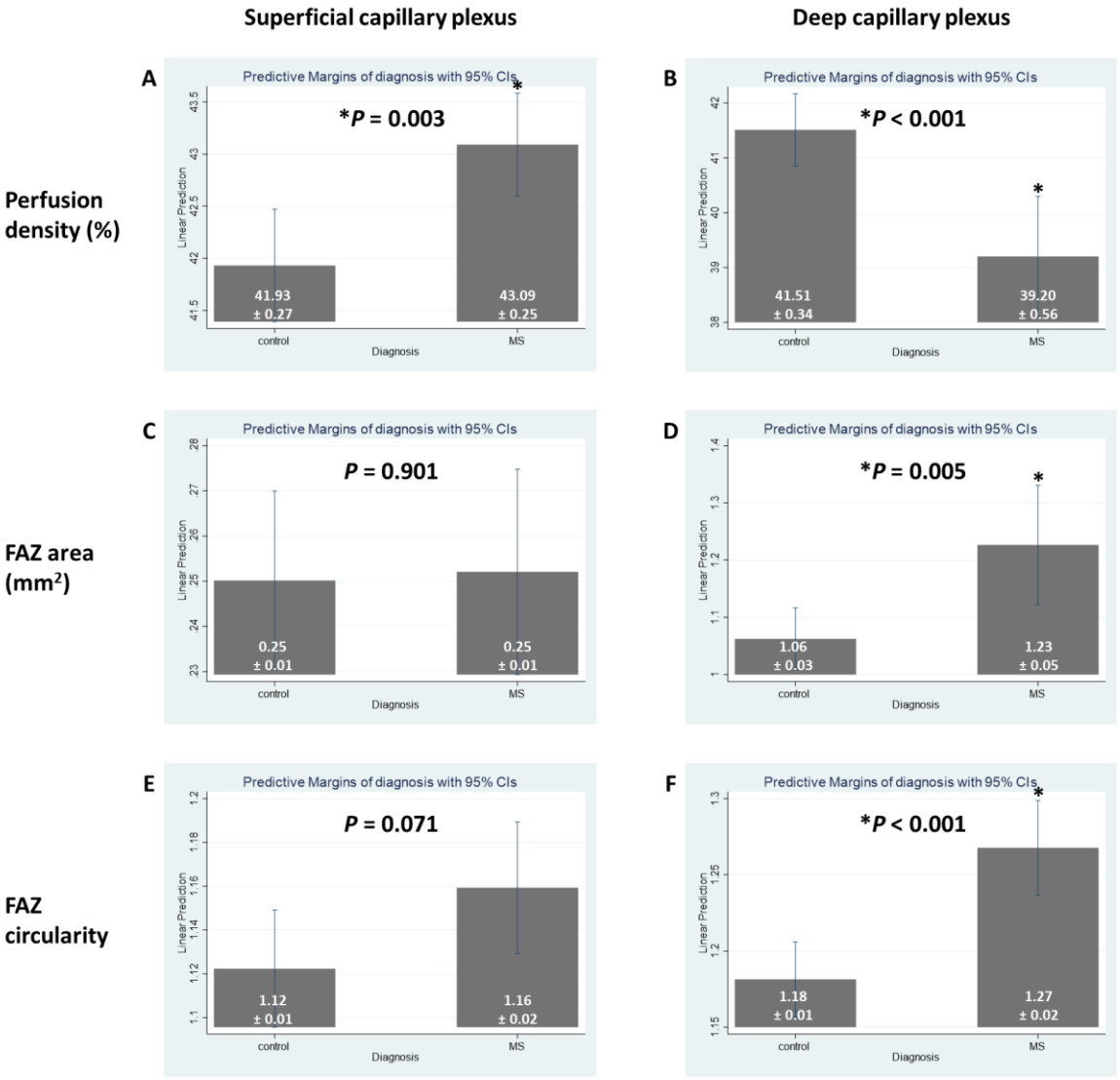

Figure 6.1. Distribution of OCT-A parameters in control group and MS group.
(**A**) PD in SCP. (**B**) PD in DCP. (**C**) superficial FAZ area. (**D**) deep FAZ area. (**E**) superficial FAZ circularity. (**F**) deep FAZ circularity.
The asterisk symbol (*) indicates a statistical significance of $p < 0.05$ when MS group was compared to the control group.
(Adapted - **Bostan M**, Chua J, Sim YC, et al. Microvascular changes in the macular and parafoveal areas of multiple sclerosis patients without optic neuritis. Sci Rep 2022; 12: 13366).[27]

Chapter 7. Study 3 - Combining retinal structural and vascular measurements improves discriminative power for multiple sclerosis patients

7.1. Background

Taking into account the two studies previously presented, where we demonstrated the high detection accuracy of MS using individual OCT [30] or OCT-A[27] parameters, the aim of the current study was to investigate the diagnostic performance of combined OCT and OCT-A parameters to discriminate MS patients without a history of ON from healthy participants.[5]

7.2. Materials and methods – these were presented in chapter 4.

7.3. Results

The analysis included 51 MS patients (n = 76 eyes) and 71 age- and sex-matched healthy participants (n = 117 eyes).[5]

We adjusted pRNFL thickness for age, sex, IOP, signal strength of OCT scans and correlation between eyes. After adjustment, pRNFL thickness was significantly lower in MS group compared with control group (87.4 ± 1.5 μm vs. 95.4 ± 1.0 μm; difference, 8.0 ± 1.3 μm; F (5, 187) = 6.79; $p < 0.001$). After compensation, pRNFL thickness remained statistically significantly decreased in MS group compared to control group (87.3 ± 1.4 μm vs. 96.3 ± 0.9 μm; difference, 9.0 ± 1.1 μm; F (5, 187) = 12.06; $p < 0.001$). Also, macular structural parameters, mGCIPL (83.9 ± 1.5 μm vs. 92.4 ± 0.7 μm; difference, 8.5 ± 1.1 μm; F (5, 187) = 15.15; $p < 0.001$) and mGCC (106.7 ± 1.6 μm vs. 115.8 ± 0.9; difference, 9.1 ± 1.2 μm; F (5, 187) = 14.13; $p < 0.001$), were statistically significantly thinner in MS group compared to control group. Regarding the vascular parameters, VD in SCP was statistically significantly increased in MS group compared to control group (43.4 ± 0.3% vs. 41.7 ± 0.3%; F (5, 187) = 16.59; $p < 0.001$), but VD in DCP was statistically significantly decreased in MS group compared to control group (39.7 ± 0.5% vs. 41.4 ± 0.4%; F (5, 187) = 6.35; $p = 0.012$).[5]

Subsequently, we examined the ability of neuronal and vascular parameters to discriminate between MS group and control group (**Table VII.1.**). The best structural

parameter for MS detection was compensated pRNFL (AUC = 0.85), followed by mGCC (AUC = 0.79), and the best vascular parameter was SCP (AUC = 0.66).[5]

Finally, we analysed whether the diagnostic performance of MS can be improved by combining neural and vascular parameters. The discrimination power of the combination of OCT-A parameters (SCP and DCP) with mGCC and with either measured pRNFL (AUC = 0.88; p = 0.002) or compensated pRNFL (AUC = 0.90; p < 0.001) exceeded the discriminative power of measured pRNFL (AUC = 0.79; **Table VII.1.**). Of note, the combined model with compensated pRNFL (AUC = 0.90) significantly improved the discriminative power of MS compared to the individual models (p = 0.027). With a specificity of 80% (or 20% false positive rate), 91.5% of MS participants had abnormal results when the combined model was used for analysis (98.4 µm for compensated pRNFL; 116.5 µm for mGCC; 45.6 % for VD in SCP; 42.8% for VD in DCP; **Table VII.1.**).[5]

7.4. Discussion

In the current study, MS patients without a history of ON showed decreased thickness of RNFL, GCIPL, and GCC, increased VD in SCP and decreased VD in DCP compared with healthy subjects. In general, structural OCT parameters are better than vascular OCT-A parameters for detecting MS, but combined structural OCT and vascular OCT-A parameters have a net superior diagnostic capacity for MS compared to individual parameters (**Table VII.2.**). Thus, the results of the study suggest that the integration of structural and vascular parameters could be advantageous for identifying subtle ocular changes in MS patients without a history of ON. The current study is the first cross-sectional study to combine structural OCT parameters and vascular OCT-A parameters with the aim of increasing the diagnostic power capacity of MS patients without a history of ON. Previous studies have mainly focused on the detection of MS based on OCT parameters,[29] and sometimes on MS detection based on OCT-A parameters.[5, 23]

Table VII.1. Diagnostic performance for discriminating MS patients without optic neuritis from healthy controls.

No	Parameters	AUC (95% CI)	Sensitivity at 80% Specificity	Specificity at 80% Sensitivity	Best Cutoff (µm or %)	p
Structural						
1	Measured RNFL thickness	0.79 (0.72-0.85)	58.1	63.2	97.1	Ref
2	Compensated RNFL thickness	0.85 (0.79-0.91)	73.5	73.7	98.4	**0.008**
3	mGCIPL	0.77 (0.70-0.84)	54.7	61.8	92.3	0.754
4	mGCC	0.79 (0.72-0.85)	60.7	65.8	116.5	0.968
Vascular						
5	SCP, %	0.66 (0.58-0.74)	36.8	30.3	45.6	**0.020**
6	DCP, %	0.65 (0.57-0.73)	47.9	44.7	42.8	**0.007**
Combined						
7	Combined (#2, #4, #5, and #6)	0.90 (0.84-0.95)	91.5	86.8	-	**<0.001**
8	Combined (#1, #4, #5, and #6)	0.88 (0.82-0.93)	87.2	85.5	-	**0.002**

Results for sensitivity and specificity are expressed as percentages.
Retinal parameters were compared with measured pRNFL thickness.
p-value indicates the paired comparisons with the best parameter (reference).
Statistically significant p-values are highlighted with **bold**.
(Adapted - **Bostan M**, Li C, Sim YC, et al. Combining retinal structural and vascular measurements improves discriminative power for multiple sclerosis patients. Ann N Y Acad Sci. Epub ahead of print 2023. DOI: 10.1111/nyas.15060).[5]

Chapter 8. Conclusions and personal contributions

The current PhD thesis is an exploratory research that presents a methodological approach designed to investigate topics that have not been addressed in depth in the specialized literature.

In study 1 (chapter 5), we demonstrated that the OCT differentiation accuracy of RRMS patients without a history of ON can be improved if pRNFL thickness compensation is performed according to individual ocular characteristics. The compensated pRNFL pattern reduces non-disease-dependent pRNFL variability, resulting in increased specificity by increasing the ability to recognize subtle MS-related pRNFL thickness changes. The study clearly demonstrates that axonal atrophy is a feature independent of ON history. Furthermore, the diagnostic performance becomes much more robust if peripapillary informations (compensated pRNFL) are integrated with macular informations (mGCC). The proposed model formed by the association of compensated pRNFL with mGCC showed a 24% increase in sensitivity over measured pRNFL in differentiating MS group from control group.

The significant improvements in diagnostic capability resulting from these strategies are particularly important to increase the potential applicability of OCT in the complementary diagnosis of MS. These observations imply monitoring of axonal degeneration and monitoring of neuronal loss, with the aim of being used as biomarkers in MS.

Although study 1 (chapter 5) and previous literature [34] demonstrated clear differences between MS group and control group in terms of retinal neuronal parameters, OCT results do not always allow a clear distinction between MS patients and healthy participants. This aspect is particularly relevant in patients with short disease duration (1–8 years), as the use of (traditionally) measured pRNFL in this phase of the disease may result in a greater overlap with the general population, especially in cases with non-visual onset, when a significant proportion of MS pacients may present with normal OCT examination. In contrast, the use of multiregression analysis to generate compensated pRNFL and the integration of mGCC offers much greater advantages in the early phase of MS, as increased performance of detecting MS is achieved.

Thus, the obtained results could promote in the near future the inclusion of OCT in the routine practice of MS diagnosis, together with other paraclinical techniques.

Study 2 (chapter 6) shows increased PD in SCP and decreased PD in DCP, confirming that there are microvascular changes in the parafoveal retina of MS patients without a history of ON. Thus, OCT-A parameters can be used as imaging biomarkers for the screening and diagnosis of MS, because they detect even the subclinical changes present in this pathology.

In study 3 (chapter 7), we demonstrated that structural parameters are better than vascular parameters for detecting MS, but the association between OCT structural parameters (compensated pRNFL + mGCC) and OCT-A vascular parameters (VD in SCP + VD in DCP) demonstrated clearly superior diagnostic performance over individual parameters. So, the combined model between OCT parameters and OCT-A parameters significantly increased the sensitivity and specificity of differentiating RRMS patients without a history of ON from healthy participants. Thus, we demonstrated that these strategies improve the identification capacity of ocular changes present in MS and support that the presented parameters can be used as imaging biomarkers for screening, complementary diagnosis and monitoring of MS.

These conclusions add to an area of great current interest, as they provide valuable information that improves the diagnosis and evaluation of MS patients without a history of ON. Several steps are required to validate the clinical application of these findings.

First, larger and more diverse study cohorts are needed to solidify the reliability and generalizability of diagnostic performance. Studies with international multicenter design would ensure the robustness of the results to the entire MS population.

Second, longitudinal studies are needed, as they are valuable to extrapolate the potential of combining structural and vascular parameters in monitoring retinal changes that occur over time. These could provide insights into the evolution and prognosis of MS without a history of ON. In addition, longitudinal approach would allow correlation of retinal findings with clinical changes occurring over time, such as ON or other neurological symptoms, which would improve the understanding of the disease evolution and would bring benefits in the development of new treatment directions targeting mechanisms other than the known ones.

Third, comparative studies are needed to evaluate the diagnostic performance of combined OCT and OCT-A parameters compared to existing biomarkers and diagnostic tools in the current clinical practice. Depending on sensitivity, specificity and positive predictive value, these parameters could differentiate between MS without a history of ON and other

pathologies, which could contribute to redefining diagnostic criteria and could increase the accuracy of the differential diagnosis.

The routine use of OCT and OCT-A parameters could be facilitated by the development of practice guidelines, which would allow the interpretation of measurements and the establishment of specific normative values for demographic and age characteristics. Standardized protocols, guidelines and reference databases are needed to incorporate structural and vascular parameters derived from OCT and OCT-A into clinical practice.

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List of published scientific papers

1. OCT and OCT-A biomarkers in multiple sclerosis - review.
Bostan M, Pîrvulescu R, Tiu C, Bujor I, Popa-Cherecheanu A.
Romanian Journal of Ophthalmology; year 2023; vol 67; no 2; p 107–110.
Index BDI.
Link: https://www.rjo.ro/oct-and-oct-a-biomarkers-in-multiple-sclerosis-review/
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2. A multi-regression approach to improve optical coherence tomography diagnostic accuracy in multiple sclerosis patients without previous optic neuritis.
Chua J, **Bostan M**, Li C, Sim YC, Bujor I, Wong D, Tan B, Yao X, Schwarzhans F, Garhöfer G, Fischer G, Vass C, Tiu C, Pîrvulescu R, Popa-Cherecheanu A, Schmetterer L.
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ISI - Impact Factor 4.891 (2022-2023).
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The article was written from the information presented in the chapter 5 (p 37-62).

3. Microvascular changes in the macular and parafoveal areas of multiple sclerosis patients without optic neuritis.
Bostan M, Chua J, Sim YC, Tan B, Bujor I, Wong D, Garhöfer G, Tiu C, Schmetterer L, Popa-Cherecheanu A.
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4. Combining retinal structural and vascular measurements improves discriminative power for multiple sclerosis patients.
Bostan M, Li C, Sim YC, Bujor I, Wong D, Tan B, Ismail MB, Garhöfer G, Tiu C, Pîrvulescu R, Schmetterer L, Popa-Cherecheanu A, Chua J.
Annals of the New York Academy of Sciences; year 2023; vol 1529; no 1; p 72-83.
ISI - Impact Factor 6.499 (2022-2023).
Link: https://nyaspubs.onlinelibrary.wiley.com/doi/10.1111/nyas.15060
The article was written from the information presented in the chapter 7 (p 85-113).