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Biological and imaging changes in patients recently diagnosed with multiple sclerosis

PHD THESIS SUMMARY

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INTRODUCTION

Multiple sclerosis (MS) is a chronic, demyelinating, inflammatory, autoimmune, degenerative condition that mainly affects young adults.

MS usually begins in an extremely productive stage of life, when people plan to start a family and build a career and has a significant impact on the affected individuals, their families and society. The ever-growing arsenal of therapies that modify the course of the disease offers opportunities to reduce disability and extend the survival of patients with multiple sclerosis. However, a curative treatment is still missing and the etiology of the disease remains incompletely understood [1].

The research topic chosen in this doctoral thesis brings to the attention an extremely current topic in the studies from the medical field that are carried out worldwide, namely MS.

This paper includes the general part, divided into several chapters dedicated to multiple sclerosis. Current information on the national and international situation of MS, etiology, pathophysiology and immunology, clinical manifestations, MS investigations, diagnostic criteria, clinical course of the disease, prognosis and treatment are presented. In the chapter dedicated to the etiology are provided informations on vitamin D and its role in immunity, the risk of developing MS associated with serum vitamin D levels, and the association between serum vitamin D levels and disease activity. In the chapter dedicated to investigations in MS are provided informations regarding optical coherence tomography and its utility in MS.

The second part of this research is the personal contribution in which are presented the results of 3 studies.

In the first study are presented the results of the analysis of 25-hydroxy-vitamin D (25 (OH)D) level determined in patients recently diagnosed with relapsing remitting MS and in healthy subjects. In this study there were also investigated the possible correlations between the level of 25(OH)D determined in MS patients and disease duration, the number of relapses from diagnosis, degree of disability and immunomodulatory treatment.

In the second study are presented the results obtained by measuring the thickness of the total retinal nerve fiber layer (RNFL) and in four quadrants using optical coherence tomography (OCT) in patients with recently diagnosed relapsing remitting MS compared to healthy subjects. For this study, the patients eyes were included into two groups as follows: the group of eyes with history of optic neuritis, that included the eyes of the patients with history of optic neuritis (more than 6 months prior to the inclusion in the study) and the group of eyes without history of optic neuritis. The control group was formed from the eyes of healthy subjects.

In the third study are presented the results obtained by measuring the total RNFL thickness and in four quadrants in the eyes of MS patients and those of healthy subjects. In contrast to the second study, in this study, the eyes of patients with MS were divided into three groups as follows: eyes with history of optic neuritis (more than 6 months prior to enrollment in the study), eyes without history of optic neuritis and fellow eyes group. The fellow eye is the eye unaffected by optic neuritis, contralateral to the one in which the patient had a history of optic neuritis. The control group was represented by the eyes of healthy subjects. In this study, are also investigated the correlations between the results obtained by measuring optic parameters in MS patients and data like degree of disability, number of relapses from diagnosis and disease duration. Also, are investigated the correlations between the results obtained by quantification of ocular parameters using OCT in MS patiens and healthy subjects and 25-hydroxy-vitamin D level.

1. Working hypothesis / objectives

1.1. The purpose of the doctoral thesis

The aim of this doctoral thesis is to analyze the results obtained by measuring certain ocular parameters using optical coherence tomography (OCT) and the level of 25-hydroxy-vitamin D (25(OH)D) in patients with relapsing remitting multiple sclerosis (MS) compared to the results obtained in healthy people and to determine how these results influence the factors related to the disease activity.

1.2. The objectives of the doctoral thesis

In order to achieve the purpose of this doctoral thesis, the following objectives were established:

- Quantification of certain ocular parameters using OCT in patients with relapsing remitting MS and healthy subjects.
- Measurement of 25(OH)D level in patients with relapsing remitting MS and in healthy individuals.
- Analysis of the results obtained by measuring ocular parameters and 25(OH)D level in patients with relapsing remitting MS compared to those of healthy subjects.
- Quantification of the degree of disability in the patients from the study using the EDSS Scale (Expanded Disability Status Scale).
- Identifying the associations between the results obtained from the measurement of ocular parameters by OCT and the level of 25(OH)D in patients suffering from relapsing remitting MS, with disease specific elements.

2. General methodology

2.1. Patient selection

We performed a study in which we analyzed the results obtained from imaging and biological explorations in patients diagnosed with relapsing remitting MS and in healthy subjects. The study took place between 2015-2021. The patients and healthy subjects were enrolled after the **informed consent was signed.**

For the enrollment of the patients in the study, the approval of the Ethics Commission for the approval of the clinical studies and of the research works constituted within the County Emergency Clinical Hospital "St. Apostol Andrei" Constanța, the annex being attached at the end of the thesis.

The specific criteria on which **patient** enrollment in the study depended were:

- ✓ **Inclusion criteria**: age over 18 years, diagnosis of relapsing remitting MS established at most 7 years prior to the enrollment in the current study
- ✓ **Exclusion criteria**: history of documented optic neuritis in the last 6 months, history of neurological diseases, history of ophthalmic diseases, diabetes, poor image quality obtained after performing optical coherence tomography, patient refusal.

The specific criteria on which the enrollment of **healthy subjects** in the study depended were:

- ✓ **Inclusion criteria**: age over 18 years
- ✓ **Exclusion criteria**: history of neurological diseases, history of ophthalmic diseases, diabetes mellitus, poor image quality obtained after performing optical coherence tomography, refusal of the subject.

2.2. Study and evaluation procedure

In the the study were included 44 patients diagnosed with relapsing remitting MS (based on McDonald criteria in effect at the time of diagnosis) and 28 healthy subjects.

The data used for documentation in the case of **healthy subjects** were: sex, age.

The following **patient** data were used for documentation: gender and age.

From the medical history of the patients we extracted data on: disease duration (the number of years that have passed since the confirmation of the diagnosis), the existence or absence of the diagnosis of optic neuritis documented in the medical history (we reiterate that the existence of optic neuritis in the last 6 months was an exclusion criterion from this research), the number of documented relapses that patients presented since the definite diagnosis of MS, the existence or absence of immunomodulatory treatment, the duration of immunomodulatory treatment (for patients undergoing such treatment). A complete neurological examination was performed to the enrolled patients in the study in order to assess the disability within the disease course.

To quantify the degree of disability we used the **Expanded Disability Status Scale (EDSS)**, attached to the doctoral thesis. The EDSS Disability Scale is the standard tool used to quantify neurological impairment and disability in clinical practice and research studies [2].

Biological evaluation of both patients and healthy subjects consisted of the determination of serum levels of 25-hydroxy-vitamin D (25(OH)D).

The determination was performed at an analysis laboratory in the city of Constanța using the Immunology Analyzer Cobas e601, by immunochemical method, with detection by electrochemiluminescence (ECLIA). The specimen used was blood serum obtained by centrifugation of venous blood collected on vacutainer without anticoagulant, with a separating gel.

Imaging evaluation of patients and healthy subjects consisted in performing **Optical Coherence Tomography** (OCT). The machine used was TOPCON DRI OCT Triton (plus), software version 10.0x. The OCT examination was performed at an ophthalmology clinic in Constanța city. From the result of each OCT examination, we used the following ocular parameters for the present research: total peripapillary retinal nerve fiber layer (RNFL) –represents the average thickness of the peripapillary retinal nerve fiber layer measured in the four quadrants, RNFL measured in the superior, inferior, temporal and nasal peripapillary quadrants.

Figure 1- TOPCON DRI OCT Triton (plus) machine used in the study

(Personal archive)

2.3. Statistical analysis

The data were processed using the *IBM SPSS Statistics 23* statistical processing software.

The procedures used were: *Descriptive statistics* (for characterizing categorical and continuous variables defined at the database level), *Graphs* (Bar, Bar + Error Bar), *Parametric statistical tests* (t-Test for comparing the average of two independent samples, One- Way ANOVA Test, Two-Way ANOVA Test), *Nonparametric statistical tests* for categorical variables (association test χ^2, link between two categorical variables, test χ^2 for comparing two proportions), *Nonparametric statistical tests* for ordinal data or for numerical variables when the normal condition is not satisfied (Mann-Whitney U test, Median test), *Correlation analysis* (Pearson, Sperman) [3], [4], [5], [6].

3. Study I- Personal contribution regarding evaluation of biological exploration results in patients with multiple sclerosis compared to healthy subjects

3.1. Results

3.1.1. Analysis of study groups according to age and sex

Analyzing the distribution of the 72 people according to sex, we notice that in the Study group out of a total of 44 patients, 11 (25%) were men and 33 (75%) women, and in the Control group out of a total of 28 subjects, 7 (25%) were men and 21 (75%) were women. In the Study group, the proportion of men 61.1% (11/18) is found not to differ significantly from the proportion of women 61.1% (33/54) and in the Control group, the proportion of men 38.9% (7/18) is found not to differ significantly from the proportion of women 38.9% (21/54), $p > \alpha = 0.05$.

Regarding the age, the mean age value for the patients in the Study group was 38.48 years and for the Control group, the mean age value was 35.00 years. There were no significant differences between the mean age values of the Study and Control groups.

3.1.2. Analysis of specific data for patients with multiple sclerosis

The mean value of disease duration for the patients in the study is 3.66 years.

After applying the EDSS scale for the patients in the study, we obtained a mean value for the EDSS score of 2.17.

Regarding the number of relapses that patients have presented since the diagnosis of MS, their mean value is 2.14.

In the Study Group, 34 patients (77.27%) are receiving immunomodulatory treatment and 10 (22.73%) recive no treatment. For patients undergoing immunomodulatory treatment, the mean value for treatment duration is 2.91 years.

For the group of patients receiving immunomodulatory treatment, the mean value of the EDSS score is 1.99 and for those not receiving treatment, the mean value of the EDSS score is 2.80, the differences not being statistically significant.

By analyzing the data by sex and disease duration, we noticed that for male patients, the mean value for the disease duration is 3.90 years and for females, the mean value for the disease duration is 3.57 years, the differences were not statistically significant. We wanted to analyze the EDSS score calculated in patients in the study depending on sex. In males the mean value of the EDSS score is 1.95, and in females the mean value was 2.24, the differences were not statistically significant.

In our study, the EDSS score is correlated with age and with the number of relapses from diagnosis.

We noticed that the number of relapses from the diagnosis is correlated also with age. Also, the duration of the disease is correlated with the number of relapses from the diagnosis.

3.1.3. Analysis according to the level of 25-hydroxy-vitamin D

The mean value of the 25-hydroxy-vitamin D level (25(OH)D) in the Study group (21.91µg/L) was significantly lower compared to that quantified in the Control group (27.93 µg/L).

The mean value of 25(OH) D in women in the Study group (22.32 µg/L) is lower compared to the one of women in the Control group (28.23 µg/L).

We wanted to analyze the vitamin D level according to the immunomodulatory treatment, so, we divided the Study group into Immunomodulatory Treatment Yes and No. In the group Immunomodulatory treatment Yes (N= 34), the mean value of 25(OH)D level was 22.80 µg/L. In the group Immunomodulatory Treatment No (N= 10) the mean value was 18.88 µg/L. The differences were not statistically significant.

3.2. Discussions

3.2.1. Discussions regarding analysis of the study groups according to age and sex

In this study, patients (44) were included in the **Study** group and healthy subjects (28) were included in the **Control** group. In the Control group were 25% men and 75% women. In the Study group, the one of the relapsing remitting multiple sclerosis (MS) patients, there were also 25% men and 75% women, with a predominance of females, which is consistent with the data in the literature that showed that multiple sclerosis affects more females [7], [8].

Regarding the age, for the patients in our study, the mean age was 38.48 years and in the healthy individuals was 35 years. There were no statistically significant differences between the mean age values of the two groups of patients and healthy individuals.

3.2.2. Discussions regarding specific data for patients with multiple sclerosis

Regarding the number of years that have passed since the time of diagnosis, in the patients in our study, the mean value for disease duration is 3.66 years. Given that MS is a chronic inflammatory disease of the central nervous system [9] and that the longevity of patients is comparable to that of the general population [10], we consider that the mean value for disease duration for the patients in our study places them among those recently diagnosed.

Regarding the EDSS (Expanded Disability Status Scale) score of the patients in this study, the mean value was 2.17. Given that the disease progresses unpredictably, with a broad clinical spectrum and progresses over time [11] and that EDSS scores in the range 0-3.5 indicate a low level of disability [12], we consider that the mean value of the EDSS score of the patients in our study is also suggestive of a low degree of disability.

Regarding the EDSS score, in the patients in our study taking immunomodulatory treatment it had a value of 1.99 and in those who didn't take any, the value was 2.80, the differences between the values not being significant, which allows us to state that the existence immunomodulatory treatment does not influence the present study.

3.2.3. Discussions regarding the level of 25-hydroxy-vitamin D

In our study, patients with multiple sclerosis had a lower 25(OH)D value compared to the healthy subjects enrolled in the study.

Numerous studies have reported that hypovitaminosis D is widespread among patients with MS, but is also common in healthy subjects. The possible cause of hypovitaminosis D among the general population is most likely caused by a combination of low sun exposure and low dietary intake [13].

By analyzing the level of 25(OH)D in people of the same sex in each group, we noticed that in women with MS the mean 25(OH)D value was significantly lower compared to that of healthy women in the study. This may be due to the low sun exposure of MS patients, who are known to have a high temperature sensitivity manifested by the transient worsening of neurological deficits within the disease in this context (Uhthoff phenomenon) [13].

3.3. Conclusions of study I

• In our research, in the group of patients with relapsing remitting multiple sclerosis, the female sex was predominant, as well as in the group of healthy people.
• The mean age of the patients in our study was 38.48 years, and that of healthy subjects was 35 years.
• The mean disease duration for the patients in our study was 3.66 years.
• The degree of disability of the patients in this study was low.
• The mean value of the number of relapses from diagnoses for patients in the study was 2.14.
• In this research, the 25-hydroxy-vitamin D level is lower in patients with relapsing remitting multiple sclerosis compared to the healthy subjects enrolled.
• Women with relapsing remitting multiple sclerosis have a lower level of serum 25-hydroxy-vitamin D compared to healthy women in the present study.
• In our study, the degree of disability quantified by the EDSS scale, the number of relapses from the time of diagnosis and the disease duration are correlated with age.
• The degree of disability quantified by the EDSS scale correlates with the number of relapses from the time of diagnosis, in the patients in this study.

4. Study II- Analysis of the ocular parameters measured by Optical Coherence Tomography in patients with multiple sclerosis compared to healthy subjects

The eyes of patients and healthy subjects enrolled in the first study according to the specific inclusion criteria were included in this study.

Patients were included in 2 groups as follows:

• The **NO-Yes** group- includes 19 eyes, consists of the eyes of patients who had a history of optic neuritis (more than 6 months prior to inclusion in the study)
• The **NO-No** group- includes 69 eyes, consists of the eyes of patients who have relapsing remitting MS, but in whom there has been no history of optic neuritis
• **Control group** - consisting of 56 eyes of healthy subjects

Imaging evaluation of patients and healthy subjects was performed using optical coherence tomography (OCT).

4.1. Results

Figure 2 shows the mean values of the total RNFL thickness for the analyzed groups

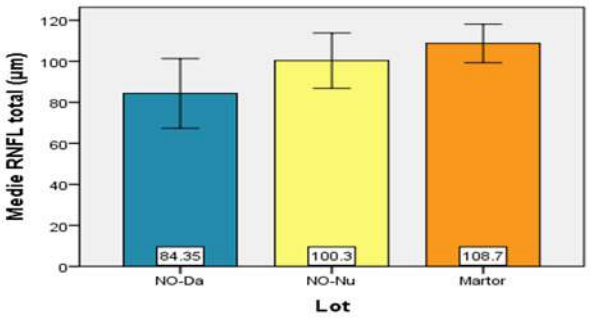

Figure 2- Bar + Error bar chart for mean values of total RNFL for the analyzed groups

Tamhane's PostHoc Multiple Comparisons analysis shows that there are statistically significant differences between the mean **total RNFL thickness values** in the groups:

- ✓ NO-Yes versus NO-No ($p = 0.003 < \alpha = 0.05$), with a lower mean total RNFL thickness in the group of the eyes of patients with a history of optic neuritis (NO-Yes)
- ✓ NO-Yes versus Control ($p < 0.001 < \alpha = 0.05$), with lower mean value for total RNFL thickness in the group of the eyes of patients with history of optic neuritis (No-Yes)
- ✓ NO-No versus Control ($p < 0.001 < \alpha = 0.05$), the lower mean value for total RNFL thickness being in the eyes of MS patients with no history of optic neuritis (NO-No group)

For the **RNFL thickness in the superior quadrant**, Bonferroni's PostHoc Multiple Comparisons analysis shows that there are significant differences between the mean values in the following groups:

- ✓ NO-Yes versus NO-No ($p = 0.002 < \alpha = 0.05$), with lower mean RNFL thickness in the superior quadrant for the eyes of patients with a history of optic neuritis (NO-Yes group)
- ✓ NO-Yes versus Control ($p < 0.001 < \alpha = 0.05$), with lower mean value for superior RNFL thickness in eyes with history of optic neuritis (NO-Yes group)
- ✓ NO-No versus Control ($p < 0.001 < \alpha = 0.05$), with lower mean value for RNFL in the superior quadrant in the eyes of patients without history of optic neuritis (NO-No group)

Regarding the **thickness of the RNFL in the inferior quadrant**, the PostHoc Multiple Comparisons-Bonferroni analysis shows that there are significant differences between the average values in the groups:

- ✓ NO-Yes and NO-No ($p < 0.001 < \alpha = 0.05$), with lower mean value for RNFL thickness in the inferior quadrant in the eyes of MS patients and history of optic neuritis (NO-Yes group)
- ✓ NO-Yes and Control ($p < 0.001 < \alpha = 0.05$), the lower mean value of the inferior RNFL being in the NO-Yes group

Regarding the **RNFL thickness in the temporal quadrant,** the PostHoc Multiple Comparisons-Tamhane analysis shows that there are significant differences between the mean values of the groups:

- ✓ NO-Yes versus NO-No ($p = 0.001 < \alpha = 0.05$), with lower mean value in eyes with history of optic neuritis (NO-Yes group)
- ✓ NO-Yes versus Control ($p < 0.001 < \alpha = 0.05$), with lower mean value for temporal RNFL in the NO-Yes group
- ✓ NO-No versus Control ($p < 0.001 < \alpha = 0.05$), with lower mean value for temporal RNFL in the eyes of MS patients with no history of optic neuritis (NO-No group)

In the case of **RNFL thickness measured in the nasal quadrant**, we did not observe statistically significant differences of the mean values in the analyzed groups.

4.2. Discussions

In our study we noticed that the total RNFL thickness in the eyes of the patients with history of optic neuritis (NO-Yes group) and in those without history of optic neuritis (NO- No group) is significantly lower compared to that of the healthy eyes in the control group.

Practically in this study we show that the total RNFL is thinner in the eyes of patients with multiple sclerosis (MS) compared to the eyes of healthy subjects, regardless of the history of optic neuritis, which is consistent with the literature [14]. A 2021 study also found that the retinal nerve fiber layer (RNFL) was significantly thinner in patients diagnosed with MS who had optic neuritis, and also in the ones that did not have optic neuritis, compared to healthy subjects [15].

Also, in our study we found that the total RNFL thickness in the eyes of patients with history of optic neuritis (NO-Yes group) is significantly lower ($p < \alpha = 0.05$) compared to the one of the eyes of those with no history of optic neuritis (group NO- No). This result is consistent with studies conducted by Jankowska-Lech [16], Loughran-Fjeldstad [17] and the one conducted by Oberwahrenbrock [18].

Regarding RNFL in the superior quadrant, in our study its thickness is significantly reduced ($p < \alpha = 0.05$) in the eyes with history of optic neuritis (NO-Yes group) but also in those without such a history (NO- No group) compared to that measured in the eyes of healthy subjects.

We also found that the thickness of the RNFL in the superior quadrant is significantly ($p < \alpha = 0.05$) lower in the eyes of patients with history of optic neuritis (NO-Yes group) compared to those without history of optic neuritis (NO- No group).

Regarding the thickness of the RNFL in the inferior quadrant, it is significantly reduced ($p < \alpha = 0.05$) in the eyes of patients with history of optic neuritis (NO-Yes group) compared to the eyes of healthy individuals in the study. Also, the RNFL measured in the inferior quadrant is significantly thinner in the eyes of our patients with history of optic neuritis compared to those without history of optic neuritis.

The RNFL thickness in the temporal quadrant is also significantly ($p < \alpha = 0.05$) lower in the eyes of patients with a history of optic neuritis (group NO-Yes) and in those without history of optic neuritis (group NO- No) compared to the one in the eyes of healthy subjects in the control group. Also, in the case of RNFL in the temporal quadrant we found that it has a significantly ($p < \alpha = 0.05$) smaller thickness in the eyes of patients in the study with history of optic neuritis compared to those without such a history.

The results we obtained are similar to those of a study conducted by Jiang in 2018, which also found that RNFL in the temporal and inferior quadrants was thinner in patients with multiple sclerosis without history of optic neuritis compared to healthy people [19].

Regarding the retinal nerve fiber layer (RNFL) thickness measured in the nasal quadrant, in our study, there were no significant differences of the mean values in the eyes of patients with history of neuritis (NO-Yes group) or with no history of optic neuritis (NO- No) compared to the eyes of healthy subjects in the Control group.

Our results are consistent with those of a study from, which also found no significant differences in mean RNFL values in the nasal quadrant between the groups of MS patients and healthy subjects [20].

4.3. Conclusions of study II

• In our study, the total peripapillary retinal nerve fiber layer thickness is lower in the eyes of patients with relapsing remitting multiple sclerosis and history of optic neuritis but also in those without a history of optic neuritis, compared to the one of the healthy subjects enrolled in the study.

• The total peripapillary retinal nerve fiber layer had a reduced thickness in the eyes of the patients with multiple sclerosis and history of optic neuritis compared to the eyes of the patients without optic neuritis history.

• In the superior, inferior and temporal quadrants, the retinal nerve fiber layer thickness was reduced in the eyes of patients with multiple sclerosis and history of optic neuritis compared to the thickness of the retinal nerve fiber layer measured in the same quadrants in the eyes of healthy people in the study.

• The thickness of the retinal nerve fiber layer in the superior and temporal quadrants was reduced in the eyes of patients with multiple sclerosis with no history of optic neuritis compared to the thickness of the retinal nerve fiber layer in the upper and lower quadrants measured in the eyes of healthy people in the study.

• The retinal nerve fiber layer in the superior, inferior, and temporal quadrants had a lower thickness in the eyes of patients with history of optic neuritis compared to the eyes of patients without history of optic neuritis.

5. Study III- Personal contribution regarding the study of changes in ocular parameters measured by OCT in multiple sclerosis, with focus on the fellow eye

We conducted a study on 44 (88 eyes) patients diagnosed with relapsing remitting MS and 28 (56 eyes) healthy subjects. We mention that this study also includes the eyes of the patients and of the healthy subjects enrolled in the first study, according to the specific inclusion criteria previously described.

Patients were included in 3 groups as follows:

- ✓ **Fellow Eye Group (OC)** - the fellow eye represents the unaffected eye, contralateral to the one in which the patient had optic neuritis (but not in the last 6 months). This group consists of 19 eyes.
- ✓ The **NO-Yes group** - includes 19 eyes, consists of the eyes of patients who had documented optical neuritis in the medical history (more than 6 months before inclusion in the study)
- ✓ The **NO- No group** - consisting of 50 eyes, includes the eyes of patients that didn't have history of optic neuritis.
- ✓ The eyes of the healthy subjects were included in the **Control** group which consists of 56 eyes.

The degree of disability was quantified by applying the Expanded Disability Status Scale (EDSS) as detailed above.

Biological evaluation was represented by the determination of the 25-hydroxy-vitamin D (25(OH)D) serum level according to the method previously described.

Imaging evaluation consisted of performing optical coherence tomography (OCT), according to the method previously described. From the OCT result we used the following eye parameters for the study:

- Total peripapillary retinal nerve fiber layer (RNFL);
- RNFL measured in the peripapillary quadrants Superior, Temporal, Inferior and Nasal.

5.1. Results

5.1.1. Presentation of the results obtained following the analysis of the ocular parameters depending on the group

In figure 3 are presented the mean values of total RNFL thickness of the analyzed groups.

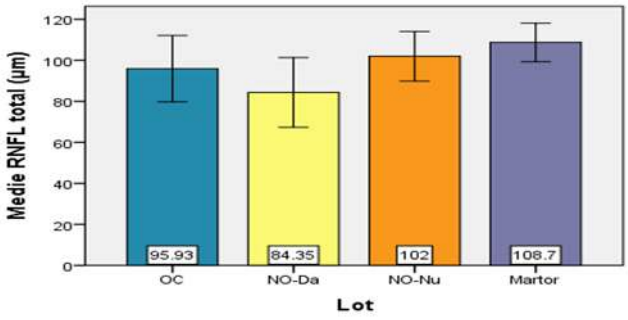

Figure 3- Barr+Error chart for mean values of total RNFL thickness for the analyzed groups.

The PostHoc Multiple Comparisons-Tamhane analysis shows that there are significant differences between the mean values of **total RNFL thickness** for the following groups:

- ✓ Control and OC ($p = 0.021 < \alpha = 0.05$), with a lower mean value of total RNFL thickness in the fellow eye group (OC group)
- ✓ Control and NO-Yes ($p = 0.001 < \alpha = 0.05$), with a lower mean value for total RNFL thickness in the eyes of patients with history of optic neuritis (NO-Yes group)
- ✓ Control and NO-No ($p = 0.012 < \alpha = 0.05$), with a lower mean value for total RNFL thickness in the eyes of MS patients with no history of optic neuritis (NO-No group)
- ✓ NO-Yes and NO-No, the lower mean value for the total RNFL thickness being in the NO-Yes group: $p = 0.002 < \alpha = 0.05$

Regarding the **RNFL thickness** in **the superior quadrant**, the PostHoc Multiple Comparisons-Bonferroni analysis shows that there are significant differences between the mean values for the following groups:

- ✓ OC versus Control ($p < 0.001 < \alpha = 0.05$), the lower mean value of RNFL in the superior quadrant being in the fellow eye group (OC group)
- ✓ NO-Yes versus Control ($p < 0.001 < \alpha = 0.05$), the lower mean value being in the NO-Yes group
- ✓ NO-No versus Control ($p < 0.001 < \alpha = 0.05$), the lower mean value being in the eyes of patients without a history of optic neuritis (NO- No group)
- ✓ NO-Yes versus No-No ($p = 0.001 < \alpha = 0.05$), with a lower mean value in the eyes with a history of optic neuritis (NO-Yes group)

For the **RNFL thickness in the inferior quadrant**, Bonferroni's PostHoc Multiple Comparisons analysis shows that there are significant differences between the mean values of the following groups:

- ✓ NO-Yes versus Control ($p = 0.001 < \alpha = 0.05$), with a lower mean value of RNFL in the inferior quadrant in the eyes of MS patients with optic neuritis history (NO-Yes group)
- ✓ NO-Yes versus NO- No ($p < 0.001 < \alpha = 0.05$), with a lower mean RNFL value in the inferior quadrant for the NO-Yes group

By using the PostHoc Multiple Comparisons-Tamhane analysis we noticed that there are significant differences between the mean values of **RNFL thickness measured in the temporal quadrant** for the following groups:

- ✓ OC and NO-Yes ($p = 0.038 < \alpha = 0.05$), with a lower mean value of temporal RNFL thickness for the eyes of patients with a history of optic neuritis (NO-Yes group)
- ✓ OC and Control ($p = 0.006 < \alpha = 0.05$), with a lower average value of RNFL thickness in the temporal quadrant in fellow eye group (OC group)
- ✓ NO-Yes and NO-No ($p = 0.02 < \alpha = 0.05$), with a lower mean temporal RNFL value for the eyes of patients with a history of optic neuritis (NO-Yes group)
- ✓ NO-Yes and Control ($p < 0.001 < \alpha = 0.05$), the lower mean value of RNFL thickness measured in the temporal quadrant being in the NO-Yes group
- ✓ NO- No and Control ($p < 0.001 < \alpha = 0.05$), the lower average value being in the eyes of patients without optic neuritis history (NO-No group)

There were no significant differences between the mean RNFL thickness values in the **nasal quadrant** in our groups.

5.1.2. Presentation of the results obtained after the analysis of the ocular parameters in each group

Regarding the **RNFL thickness in the OC group**, the PostHoc Multipe Comparisons-Tamhane analysis shows that there are significant differences between the mean values in the following quadrants:

- ✓ Superior and Temporal ($p < 0.001 < \alpha = 0.05$) with lower mean value for RNFL thickness in the temporal quadrant.
- ✓ Inferior and Temporal ($p < 0.001 < \alpha = 0.05$), the average value of RNFL thickness being lower in the temporal quadrant.
- ✓ Inferior and Nasal ($p = 0.010 < \alpha = 0.05$), with a lower mean value of RNFL thickness in the nasal quadrant.

For the **NO-Yes group**, by using the PostHoc Multipe Comparisons-Tamhane analysis we identified significant differences between the mean values of RNFL thickness for following quadrants:

- ✓ Superior versus Temporal ($p < 0.001 < \alpha = 0.05$), with lower mean value for RNFL thickness in the temporal quadrant.
- ✓ Superior versus Nasal ($p = 0.030 < \alpha = 0.05$), with lower mean value for RNFL thickness in the nasal quadrant.
- ✓ Inferior versus Temporal ($p < 0.001 < \alpha = 0.05$) with lower mean value for RNFL thickness in the temporal quadrant.
- ✓ Inferior versus Nasal ($p = 0.002 < \alpha = 0.05$), with lower mean value for RNFL thickness in the nasal quadrant
- ✓ Temporal versus Nasal ($p = 0.021 < \alpha = 0.05$), with a lower mean value for RNFL thickness in the temporal quadrant.

For the **NO-No group**, by using the PostHoc Multiple Comparisons-Tamhane analysis we noticed that there are significant differences between the mean **RNFL thickness** values for the following quadrants:

- ✓ Superior and Inferior ($p < 0.001 < \alpha = 0.05$), with lower average value for RNFL thickness in the superior quadrant

- ✓ Superior and Temporal ($p < 0.001 < \alpha = 0.05$), RNFL thickness having lower mean value in the temporal quadrant
- ✓ Superior and Nasal ($p < 0.001 < \alpha = 0.05$), with lower mean RNFL thickness value in the nasal quadrant
- ✓ Inferior and Temporal ($p < 0.001 < \alpha = 0.05$), RNFL thickness has lower mean value in the temporal quadrant
- ✓ Inferior and Nasal ($p < 0.001 < \alpha = 0.05$), with lower mean value for RNFL thickness in the Nasal quadrant.
- ✓ Temporal and Nasal ($p < 0.001 < \alpha = 0.05$), the mean value of RNFL thickness being lower in the temporal quadrant

In the **Control group**, by using the PostHoc Multiple Comparisons-Tamhane analysis we noticed that there are significant differences between the mean RNFL thickness values for the quadrants:

- ✓ Superior versus Temporal ($p < 0.001 < \alpha = 0.05$), with lower mean RNFL thickness value in the temporal quadrant
- ✓ Superior versus Nasal ($p < 0.001 < \alpha = 0.05$), with lower mean value for RNFL thickness in nasal quadrant
- ✓ Inferior versus Temporal ($p < 0.001 < \alpha = 0.05$), with lower mean value for RNFL thickness in the temporal quadrant
- ✓ Inferior versus Nasal ($p < 0.001 < \alpha = 0.05$), with lower RNFL thickness value in the nasal quadrant
- ✓ Temporal versus Nasal ($p < 0.001 < \alpha = 0.05$), with lower mean value for RNFL thickness in temporal quadrant

We wanted to investigate how the thickness of the retinal nerve fiber layer (RNFL) is influenced by Location (Quadrant) and Group.

The TwoWay ANOVA analysis shows that the RNFL dependent variable is influenced separately from the quadrant to a greater extent and from the group to a moderate extent.

5.1.3. Analysis of ocular parameters according to age, sex and level 25 of hydroxy-vitamin D

The t test for independent samples showed significant differences for the mean values of the total RNFL thickness of the Male and Female groups in fellow eyes group (OC group): $p < \alpha = 0.05$, with a lower mean value of the total RNFL thickness for the Male group.

The t-test for independent samples showed significant differences between the mean values of RNFL thickness in the superior quadrant of the Male and Female groups for the fellow eyes group (OC group): $t = -2.282$, $df = 1$, $p = 0.036 > \alpha = 0.05$, with a lower RNFL thickness mean value for the Male group.

By applying the t test for independent samples, we observed significant differences for the mean values of RNFL thickness in the inferior quadrant for the males and females in fellow eyes group (OC group), with a lower mean value for RNFL thickness in the inferior quadrant in the male group: $t = -0.204$, $df = 17$, $p = 0.028 < \alpha = 0.05$.

The t test for independent samples shows that there are statistically significant differences for the mean values of RNFL thickness in the nasal quadrant in the Male and Female groups in the NO- No group ($p = 0.029 < \alpha = 0.05$), with a lower mean value of RNFL thickness in the nasal quadrant in the Male group.

Next, we present the results obtained by applying of correlation tests between the results obtained by measuring ocular parameters and age. We noticed that there is no correlation between total RNFL thickness, RNFL measured in superior, inferior, temporal and nasal quadrants and age for the studied groups: $p > \alpha = 0.05$.

In the following we present the data obtained as a result of the analysis of the correlations between ocular parameters measured in groups OC, NO-Yes, NO- No and Control by OCT and the level of 25-hydroxyvitamin D (25(OH)D) determined in the same groups. The total RNFL value correlates with the level of 25(OH)D in fellow eyes (OC group). There is also a significant correlation between total RNFL value and the level of 25(OH)D in the eyes of patients with MS and history of optic neuritis (NO-Yes group). We found that the value corresponding to the thickness of the RNFL in the inferior quadrant of the OC group correlates with the level of 25(OH)D. The RNFL value in the inferior quadrant correlates with the level of 25(OH)D also in the eyes of patients with optic neuritis history (NO-Yes group). There is a statistically significant correlation between the RNFL value in the nasal quadrant and the 25(OH)D level in the OC group.

5.1.4. Correlations between the results obtained from the measurement of ocular parameters and specific data of multiple sclerosis patients

We wanted to investigate if there are correlations between the EDSS score and the results obtained by measuring the ocular parameters in the NO-Yes, NO- No and OC groups.

The results we obtained were the following:

- ✓ The total RNFL value correlates with the EDSS score in the OC group
- ✓ The RNFL value measured in the superior quadrant correlates with the EDSS score in the OC group

We wanted to investigate if there are correlations between the results we obtained by measuring the ocular parameters in the OC, NO-Yes and NO- No groups and the number of relapses from diagnosis in the same groups and we obtained the following results:

- ✓ There is a statistically significant correlation between RNFL measured in the superior quadrant and the number of relapses from the time of diagnosis in the eyes of patients with optic neuritis history (NO-Yes group)
- ✓ There is a statistically significant correlation between the RNFL thickness measured in the inferior quadrant and the number of relapses from the time of diagnosis in the NO-Yes group.
- ✓ There is a statistically significant correlation between the value of RNFL in the temporal quadrant and the number of relapses from the time of diagnosis in the OC group.

5.2. Discussions

5.2.1. Discussions regarding the results obtained after the analysis of the ocular parameters according to the group

In our study, the thickness of the total retinal nerve fiber layer (RNFL) was significantly lower ($p < \alpha = 0.05$) in the eyes of patients with history of optic neuritis (NO-Yes), no history of optic neuritis (NO- No) and in the fellow eyes (OC) compared to the eyes of healthy subjects from the Control group. We note, therefore, that the total thickness of RNFL is reduced in the eyes of patients with multiple sclerosis (MS) regardless of the history of optic neuritis compared to the eyes of healthy individuals.

This aspect is also indicated in the literature, where it is specified that the axonal loss was objectified by measuring the thickness of RNFL in recent stages of demyelinating diseases such

as MS, even in eyes without a history of optic neuritis, retinal nerve fiber layer (RNFL) being thinner in MS eyes compared to healthy ones [21].

Also, in our study, the total RNFL thickness was significantly ($p < \alpha = 0.05$) lower in the eyes of patients who had history of optic neuritis (NO-Yes group), than those who did not (NO-No group).

A possible explanation for the thinning of the retinal nerve fiber layer in the absence of optic neuritis could be the retrograde trans-synaptic degeneration of the retinal ganglion cells due to demyelinating lesions in the posterior optic pathways. Progressive axonal loss could also explain the reduction in the thickness of the retinal nerve fiber layer in MS patients in the absence of optic neuritis [22].

Regarding the measurement of the retinal nerve fiber layer (RNFL) in the superior quadrant, we found that its thickness is significantly lower in the eyes of patients with history of optic neuritis (group NO-Yes), without history of optic neuritis (group NO- No) and fellow eyes (OC group), compared to the healthy eyes in the study.

Also, the RNFL in the superior quadrant has a significantly lower thickness ($p < \alpha = 0.05$) in the eyes of patients with history of optic neuritis (group NO-Yes) than in those without history of optic neuritis (group NO- No).

By measuring RNFL in the inferior quadrant, we found that in the eyes of patients with history of optic neuritis (NO-Yes group) the thickness of RNFL is significantly ($p < \alpha = 0.05$) reduced compared to the eyes of healthy individuals but also to those of patients without a history of optic neuritis (group NO- No).

Regarding the temporal quadrant, here also the thickness of the retinal nerve fiber layer (RNFL) was significantly ($p < \alpha = 0.05$) lower in the eyes of patients with history of optic neuritis (NO-Yes group), those without history of optic neuritis (group NO- No) but also in the fellow eyes (group OC), compared to the eyes of healthy people in the study. Also, the thickness of the RNFL in the temporal quadrant was significantly lower in the eyes with history of optic neuritis (NO-Yes group) compared to fellow eyes (OC group) and with eyes without history of optic neuritis (NO-No group).

Regarding the thickness of the RNFL in the nasal quadrant, there were no significant differences between the mean values in the eyes we studied.

In patients with MS, thinning of the RNFL occurs in the eyes with optic neuritis history, but can also be found in the fellow eye without history of optic neuritis [23], which we also found by highlighting the reduced thickness of the total RNFL in fellow eyes compared with the eyes of the healthy people in the study.

5.2.2. Discussions regarding the results obtained after the analysis of the ocular parameters in each group

We measured the thickness of the retinal nerve fiber layer (RNFL) in the fellow eye group (OC) and found that in the temporal quadrant it is significantly ($p < \alpha = 0.05$) smaller than in the superior and inferior quadrants. Also the RNFL in the nasal quadrant is significantly thinner than in the lower quadrant.

Regarding the eyes of our patients with history of optic neuritis (NO-Yes group), the thickness of the RNFL in the temporal quadrant is significantly ($p < \alpha = 0.05$) lower than in the superior, inferior and nasal quadrants. Also, in these eyes of our patients with optic neuritis history, the thickness of the RNFL in the nasal quadrant was significantly smaller than in the superior and inferior quadrants.

In the patients from our study who didn't have a history of optic neuritis (NO- No group) we found that RNFL is significantly ($p < \alpha = 0.05$) lower in the temporal quadrant compared to the superior, inferior and nasal quadrants. Also, in these eyes without optic neuritis history, the RNFL in the nasal quadrant was significantly lower than in the superior and inferior quadrants. At the same time, the thickness of the RNFL in the superior quadrant was significantly smaller compared to that in the inferior quadrant.

We observe, therefore, that in eyes with history of optic neuritis, those without optic neuritis history and in the fellow eyes, there is a particular impairment of the retinal nerve fiber layer in the temporal quadrant in the sense of reducing its thickness.

Meta-analysis of the data provided by OCT devices regarding the thickness of the retinal nerve fiber layer in patients with optic neuritis show that the degree of reduction in RNFL thickness in eyes without history of optic neuritis is lower compared to those with history of optic neuritis and that it is more pronounced in the temporal quadrant [24].

The results obtained in our study are similar to these data.

The temporal quadrant of the retina, containing the papillomacular bundle, which serves the central vision, is the most affected by optic neuritis and MS. The papillomacular bundle is composed mainly of parvocellular axons. A plausible hypothesis suggests that selective size loss is a consequence of the failure of small axons to remyelinate [25].

Regarding the eyes of healthy people in our study (Control group), the thickness of the RNFL in the temporal quadrant is significantly reduced ($p < \alpha = 0.05$) compared to that in the superior, inferior and nasal quadrants. Also, the thickness of the RNFL in the superior and inferior quadrants is significantly higher than in the nasal quadrant.

The data we obtained are similar to the results of other studies that were performed in previous years.

In a study that analyzed the RNFL thickness measured by OCT in healthy individuals, in some of the subjects in the study, the maximum thickness was in the inferior quadrant, followed by the superior quadrant, nasal quadrant, and then the temporal quadrant, where RNFL was the thinnest. In this case, the ISNT rule was applied for the thickness of the retinal nerve fiber layer. In the same study, in some of the normal enrolled individuals, the ISNT rule could not be applied to the thickness of the RNFL. The authors say that normal eyes probably show some variations in the number and distribution of retinal ganglion cell axons, which is why in some normal eyes ISNT rule cannot be applied for RNFL thickness [26].

The ISNT rule refers to the normal retina where the thickness of the neuroretinal rim is greater in the inferior quadrant, followed by the superior quadrant, then the nasal quadrant and finally the temporal quadrant where the thickness is the smallest [27]. We mention that the neuroretinal rim is that part of the optic disc that contains the fibers of the optic nerve [28].

Another study conducted in 2016 in which RNFL was measured in the four quadrants in healthy individuals showed that it has a greater thickness in the inferior quadrant, followed by the superior quadrant, then the nasal and temporal [29].

In our study we showed that both the group and the quadrant influence the thickness of the retinal nerve fiber layer (RNFL). Therefore, the thickness of the RNFL is influenced by the quadrant, the largest thickness being in the inferior quadrant, followed by the superior, nasal and temporal quadrant where the RNFL is the thinnest.

Regarding the group, in our study, the greater thickness of RNFL was in the group of healthy eyes, followed by the group of eyes of patients without optic neuritis history, then the

group of fellow eyes and finally the group of eyes with history of optic neuritis where the RNFL had the most reduced thickness.

5.2.3. Discussions regarding the analysis of ocular parameters according to age, sex and the level of 25-hydroxy-vitamin D

Regarding the analysis of the total retinal nerve fiber layer (RNFL) thickness according to sex, we found that in the group of fellow eyes (OC), in males, the total RNFL thickness is significantly ($p < \alpha = 0.05$) lower compared to that of the female sex. Also, the thickness of the RNFL in the superior and inferior quadrants is lower in males compared to females in the fellow eye group (OC).

Regarding the thickness of the RNFL in the nasal quadrant, in the group of eyes without history of optic neuritis (NO- No), males had significantly lower RNFL compared to females. These changes may be due to the fact that men often have an unfavorable clinical course and faster progression of the disease compared to women [30] possibly due to sex hormones, especially estrogen which is thought to have a protective role [31].

Following the analysis of the ocular parameters according to the level of 25-hydroxy-vitamin D (25(OH)D) we found that the total RNFL thickness and in the inferior quadrant correlates with the level of 25(OH)D in fellow eyes and in the eyes of patients with history of optic neuritis. In fellow eyes, there is also a correlation between RNFL thickness in the nasal quadrant and the level of 25(OH)D.

It is known that vitamin D has an anti-inflammatory action in MS, proving its impact over the inflammatory clinical aspects of the disease, its neuroprotective role is being proven by more and more evidence [32], but its value in clinical practice is still a highly debated topic [33].

5.2.4. Discussions regarding the correlations between the results obtained from the measurement of ocular parameters and specific data of patients with multiple sclerosis

Regarding the correlation between the degree of disability quantified by applying the EDSS scale (Expanded Disability Status Scale) and ocular parameters we found that in the fellow eyes group of MS patients in our study, the EDSS score correlates with the thickness of the total retinal nerve fiber layer (RNFL) and in the superior quadrant.

Also, the thickness of the RNFL in the superior quadrant correlates with the EDSS score in the group of eyes with history of optic neuritis.

In a 2015 study, the authors identified significant correlations between the EDSS score and total RNFL thickness and RNFL in the inferior and superior quadrants in the eyes of MS patients with no history of optic neuritis. They also identified correlations between the EDSS score and RNFL in the temporal quadrant in those with MS and optic neuritis in their history [17].

In another study, the authors identified significant correlations between the EDSS score and the total retinal nerve fiber layer (RNFL) and RNFL in the superior, inferior, and temporal quadrants of the eyes with no history of optic neuritis. In the eyes with history of optic neuritis, this correlation was not significant in their study [34].

Regarding the number of relapses from the time of diagnosis and ocular parameters, we found in our study that, in the eyes of patients with history of optic neuritis, the thickness of the RNFL in the superior and inferior quadrants correlates with the number of relapses since the diagnosis. Also, the thickness of the RNFL in the temporal quadrant is correlated with the number of relapses from the time of diagnosis to fellow eyes in our study.

5.3. Conclusions of study III

• In this study we showed that the thickness of total retinal nerve fiber layer is smaller in the eyes of patients with multiple sclerosis and history of optic neuritis, in the eyes without history of optic neuritis but also in fellow eyes compared to that measured in the eyes of healthy individuals from the study.
• The thickness of the retinal nerve fiber layer in the superior and temporal quadrants is reduced in the eyes of patients with history of optic neuritis, with no history of optic neuritis but also in fellow eyes, compared to the thickness of the retinal nerve fiber layer measured in the same quadrants in the eyes of healthy people from our study.
• The retinal nerve fiber layer measured in the temporal quadrant in the eyes with history of optic neuritis of the multiple sclerosis patients was less thick compared to the one measured in the temporal quadrant in the fellow eyes of the study patients.
• In healthy individuals in our study, the ISNT rule applies to the thickness of the RNFL in the four quadrants, in the inferior quadrant being the thickest and in the temporal being the thinner.
• The thickness of the total retinal nerve fiber layer and in the four quadrants does not correlate with age in patients with multiple sclerosis from our study or in the healthy subjects.
• In this research, the 25-hydroxy-vitamin D serum level correlates with the thickness of the total retinal nerve fiber layer and in the inferior quadrant in the eyes of patients who had a medical history of optic neuritis but also in fellow eyes.
• The EDSS score correlates with the thickness of the total peripapillary retinal nerve fiber layer and in the superior quadrant in the fellow eyes.
• The disease duration does not correlate with the thickness of the retinal nerve fiber layer in the patients in our study, regardless of the history of optic neuritis.

6. General conclusions

• Recently diagnosed patients with multiple sclerosis have a decreased level of 25-hydroxy-vitamin D compared to healthy individuals.
• Women with multiple sclerosis have lower 25-hydroxy-vitamin D levels compared to healthy women.
• The total peripapillary retinal nerve fiber layer measured using optical coherence tomography is thinner in the eyes of recently diagnosed patients with relapsing remitting multiple sclerosis compared to that of the healthy individuals, regardless of the history of optic neuritis of the patients.
• The total peripapillary retinal nerve fiber layer is thinner in the eyes of patients with multiple sclerosis who have had a history of optic neuritis compared to those who have not.
• In this study we found that the fellow eyes, unaffected by optic neuritis, of patients with multiple sclerosis who had optic neuritis in the opposite eye, show a reduction in the thickness of the peripapillary nerve fiber layer compared to the eyes of healthy people.
• The temporal quadrant is most likely to lose retinal nerve fiber thickness in the context of multiple sclerosis, regardless of the history of optic neuritis.
• The presence of the medical history of optic neuritis influences the thickness of the retinal nerve fiber layer, which has the largest thickness in the eyes of patients without a history of optic neuritis, followed by fellow eyes, being thinner in patients who have had a medical history of optic neuritis.
• The thickness of the retinal nerve fiber layer does not correlate with age regardless of the history of optic neuritis in the present research.

• We appreciate that optical coherence tomography is a useful imaging examination for investigating patients with recently diagnosed multiple sclerosis, as it helps to highlight the retinal nerve fiber layer thickness reduction in the absence of clinically manifested optic neuritis.
• We consider that optical coherence tomography can be used as a tool to dynamically monitor changes in the papilla of the optic nerve in recently diagnosed patients with multiple sclerosis in whom the clinical manifestations did not make their presence felt yet.

7. Originality of the thesis

The original element of this research is represented by the quantification through optical coherence tomography of the total peripapillary retinal nerve fiber layer and in the four quadrants in patients recently diagnosed with relapsing remitting multiple sclerosis and their comparison with the results of healthy subjects.

A particularity of this research is the study of 25-hydroxy-vitamin D level in patients with multiple sclerosis and healthy individuals and the study of possible correlations between ocular parameters measured by optical coherence tomography and serum levels of 25-hiroxy-vitamin D.

Up to now nationally no such research was carried out.

We consider that this research has a strong impact on the medical practice in the field of multiple sclerosis because it provides evidence on the role of optical coherence tomography, a non-invasive accessible imaging investigation, in highlighting the subclinical changes within this condition.

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