

**”CAROL DAVILA UNIVERSITY OF MEDICINE AND PHARMACY,
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**EVALUATION OF MICROVASCULAR CHANGES IN
HYPERTENSIVE RETINOPATHY IN PATIENTS WITH
DYSLIPIDEMIA**

- ABSTRACT -

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“Evaluation of microvascular changes in hypertensive retinopathy in patients with dyslipidemia”

The structure of this thesis comprises two sections:

- the general section, which presents data on primary arterial hypertension, notions related to the anatomy and histology of the retina and choroid, Optical Coherence Tomography Angiography (OCTA), and hypertensive retinopathy; and
- the special section, in which the studies underlying our research are presented: monitoring the progression of hypertensive retinopathy in patients with dyslipidemia using OCTA, improving artificial intelligence (AI) systems, the relevance of OCTA in the screening and monitoring of patients with arterial hypertension (AH) and carotid artery stenosis, and the evaluation of the potential of OCTA to demonstrate retinal dysfunction in hypertensive patients with carotid atherosclerosis.

General Part

Chapter 1. Primary arterial hypertension

Arterial hypertension is classified as primary or essential hypertension when no identifiable cause is found, and as secondary hypertension when it is caused by nephropathies, congenital heart anomalies, endocrinopathies, or certain classes of medications.

Primary arterial hypertension accounts for approximately 90% of hypertension cases. The staging of blood pressure (BP) levels used in Romania, according to the European Society of Hypertension (ESH) and the European Society of Cardiology (ESC), is [1]:

- Optimal BP: <120 mmHg systolic BP (SBP) and <80 mmHg diastolic BP (DBP)
- Normal BP: 120–129 mmHg SBP and/or 80–84 mmHg DBP
- High-normal BP: 130–139 mmHg SBP and/or 85–89 mmHg DBP
- Grade 1 hypertension: 140–159 mmHg SBP and/or 90–99 mmHg DBP
- Grade 2 hypertension: 160–179 mmHg SBP and/or 100–109 mmHg DBP
- Grade 3 hypertension: ≥ 180 mmHg SBP and/or ≥ 110 mmHg DBP
- Isolated systolic hypertension: ≥ 140 mmHg SBP and <90 mmHg DBP

- Isolated diastolic hypertension: <140 mmHg SBP and $\geq$ 90 mmHg DBP

Hypertension is asymptomatic until it becomes associated with the development of cardiac and cardiovascular conditions such as acute myocardial infarction, congestive heart failure, stroke, nephropathy, hypertensive retinopathy, choroidopathy, and optic neuropathy with a risk of blindness.

Arterial hypertension remains the leading preventable and treatable cause of death in the European Union and worldwide [2-4].

Chapter 2. Concepts related to the anatomy and histology of the retina and choroid

The eyeball is located within the orbit. Structurally, the human eye is composed of three layers, which from the inside outward are represented by the retina, uvea, sclera, and cornea.

The retina extends anteriorly up to the ora serrata and its primary function is the absorption and processing of photons from the visible light spectrum. Histologically, the retina consists of multiple layers, and Optical Coherence Tomography (OCT) enables their visualization. Thus, on OCT, the histological layers and visible zones, arranged from the outermost to the innermost, are: the retinal pigment epithelium (RPE), the interdigitation zone, the outer segment of photoreceptor cells, the inner segment of photoreceptor cells (including the myoid and ellipsoid zones), the external limiting membrane, the outer nuclear layer, the outer plexiform layer, the inner nuclear layer, the inner plexiform layer, the ganglion cell layer, the retinal nerve fiber layer, and the internal limiting membrane.

The choroid, or the middle tunic of the eyeball, maintains optimal retinal function. It is involved in light absorption, thermoregulation, and the regulation of intraocular pressure through aqueous humor drainage from the anterior chamber via the uveoscleral pathway.

Retinal and choroidal circulation ensures the nutrition of the posterior pole of the eye. Retinal blood vessels supply the inner two-thirds of the retina, while the choroid provides vascularization to the outer retina. Retinal vessels are located at the level of the retinal nerve fiber layer.

Chapter 3. Optical Coherence Tomography Angiography (OCTA)

OCTA allows visualization of the microcirculation and blood flow within retinal capillaries and the choroid. It provides in vivo imaging of the choriocapillaris as well as the superficial and deep retinal vascular plexuses. This is a rapid, reproducible, and non-invasive method for assessing retinal and choroidal vascularization in depth, without requiring the administration of contrast agents [5-7].

The obtained scans classify the results into: the superficial inner retinal vascular plexus (which includes the vasculature of the ganglion cell layer and the retinal nerve fiber layer), the deep retinal vascular plexus (including the vasculature at the junctions of the inner nuclear–outer plexiform layers and the inner nuclear–inner plexiform layers), the outer retina, and the choriocapillaris.

OCTA is based on variations in the OCT signal generated by moving red blood cells, which contrast with the surrounding static neurosensory tissue, making it similar to the motion of particles assessed in Doppler vascular imaging. This method allows early identification of ischemia through impaired blood flow, thereby enabling the assessment of disease severity [8].

Angiographic changes in hypertensive subjects with poor blood pressure (BP) control include: reduction of superficial and deep vascular flow, decreased deep foveal and parafoveal vascular flow density, reduced thickness of the parafoveal inner retina relative to total retinal thickness, and an increase in both the area and perimeter of the avascular zone.

OCTA provides information on preclinical microvascular changes in hypertensive patients, such that alterations in retinal microcirculation can be considered biomarkers for assessing the risk of complications and ocular, cardiac, cerebral, and renal damage.

Potential biomarkers obtained through OCTA include the density of the superficial and deep capillary vascular plexuses, the foveal avascular zone, and the choriocapillaris. In patients with poorly controlled blood pressure, there is a progressive reduction in the number of choriocapillaris vessels, along with an increase in areas exhibiting choriocapillaris flow deficits [9].

Chapter 4. Hypertensive retinopathy

Hypertensive retinopathy is an ocular manifestation secondary to elevated systemic blood pressure. In this condition, both acute changes—caused by sudden increases in BP—and, more commonly, chronic changes due to long-standing hypertension can be observed.

Arterial hypertension is a risk factor for retinal vascular occlusions (both venous and arterial), for the development and progression of diabetic retinopathy, retinal arteriolar emboli, retinal arterial macroaneurysm, non-arteritic ischemic optic neuropathy, glaucoma, and macular degeneration.

Advanced retinopathy is independently associated with clinical and subclinical stroke, congestive heart failure, cardiovascular mortality, end-stage renal disease, dementia, and cognitive decline [10, 11].

Ocular manifestations of hypertension are classified into hypertensive retinopathy, hypertensive choroidopathy, and hypertensive optic disc edema. The diagnosis of hypertensive retinopathy is clinical, based on biomicroscopic visualization of characteristic ocular changes at the level of the retina, choroid, and optic disc, associated with elevated blood pressure [12].

Imaging investigations complement the clinical diagnosis. Fundus photography, fluorescein angiography (FA), and OCTA are among these evaluation methods.

The treatment of arterial hypertension follows two main approaches: pharmacological therapy and the adoption of a healthy lifestyle.

II. Personal contributions

Chapter 5. Working hypothesis and general objectives

Hypertension leads to a progressive reduction in the number of retinal choriocapillaris vessels, as well as an increase in choriocapillaris flow deficits.

Microcirculatory impairment in hypertension is associated with increased cardiovascular risk, caused by abnormal vascular tone, vascular wall thickening, and reduced lumen diameter [11, 13].

The main objective of this doctoral thesis was to use OCTA to achieve the following:

- early identification of the progression of ocular pathology from stage II to stage III, in the absence of visible fundus changes or ocular symptoms;
- early identification of cases at risk of progression and complications;
- updating the classification of hypertensive retinopathy based on instrumental criteria;
- establishing new correlations between retinal vascular markers and biochemical markers with an impact on reducing premature cardiovascular mortality (through myocardial infarction and stroke) and morbidity associated with hypertensive retinopathy;
- evaluating the usefulness of including OCTA in the assessment protocol of hypertensive patients, alongside fundus examination, cardiac ultrasound, and Doppler ultrasound of the carotid and vertebral arteries;
- establishing OCTA–Doppler correlations in subjects with internal carotid artery stenosis (ICAS);
- improving screening, monitoring, and therapeutic protocol adjustment in patients with hypertensive angiopathy and ICAS;
- reducing the rate of ocular, cardiovascular, cerebral, and renal complications of hypertension and dyslipidemia;
- accurately identifying microvascular changes in hypertensive patients with dyslipidemia and carotid atherosclerosis using OCTA;
- correlating OCTA findings with carotid Doppler ultrasound in antihypertensive and lipid-lowering therapies to reduce cardiovascular risk.

Chapter 6. General research methodology

This work includes three analytical, observational, prospective cohort studies.

In the first study, we analyzed whether BP values, total cholesterol, HDL, LDL, and triglycerides correlate with the progression of hypertensive retinopathy. Progression was evaluated using OCTA by calculating vascular parameters such as the foveal avascular zone (FAZ), non-perfusion area (NFA), vascular perfusion area (VFA), ETDRS grid metrics, vascular density, and capillary skeleton.

The second study aimed to establish new correlations between retinal vascular and biochemical markers, as well as OCTA–Doppler correlations in patients with carotid stenosis.

The third study investigated whether OCTA can demonstrate retinal vascular dysfunction in subjects with hypertension, dyslipidemia, and carotid atherosclerosis, with the goal of reducing complication rates and improving therapeutic strategies.

Chapter 7. Study number 1. Monitoring progression of hypertensive retinopathy in patients with dyslipidemia using OCTA. Can we improve the A.I. system?

7.1. Introduction

Hypertension predominantly affects adults aged 30–79 years. It is a major cause of premature mortality and a significant risk factor for vascular events. Dyslipidemia increases the risk of cardiovascular diseases [3, 4].

Using OCTA, the following study hypotheses were formulated:

- potential OCTA parameters can be identified for analysis using A.I.;
- OCTA can establish new correlations between retinal vascular markers and biochemical markers with an impact on reducing premature cardiovascular mortality (myocardial infarction and stroke) and morbidity associated with hypertensive retinopathy;
- early identification of the progression of ocular pathology from stage II to stage III in the absence of fundus changes or symptoms;
- subclinical identification of retinal microvascular changes in superficial and deep plexuses associated with hypertension and dyslipidemia;
- justification for including OCTA in the evaluation protocol of hypertensive patients;
- potential updating of hypertensive retinopathy classification using instrumental criteria.

7.2. Materials and Methods

A prospective observational cohort study was conducted on 77 subjects with hypertension and dyslipidemia, monitored over 18 months (154 eyes analyzed).

The protocol included: bi-monthly BP measurements; lipid profile, blood glucose, HbA1c, and BMI every 3 months; complete ophthalmologic examination (visual acuity, autorefractometry, slit-lamp exam, Goldmann tonometry, fundus exam, OCTA).

Subjects were divided into right eye (RE) and left eye (LE) groups, then further into hypercholesterolemia and hypertriglyceridemia subgroups. OCTA parameters analyzed included FAZ, NFA, VFA, vascular Density, and Skeleton metrics.

Statistical analysis included t-tests, Pearson correlations, and factorial analysis (Principal Component Analysis), identifying major components influencing vascular changes.

7.3. Results

Statistical analysis was performed using SPSS. Subjects were divided into two groups: the right eye and the left eye. Correlations were established with the hypercholesterolemia and hypertriglyceridemia groups.

In the group of patients with hypercholesterolemia, in the right eye, correlations were found between VFA Area and HDL levels ($r = 0.274$; $p = 0.016$), showing that higher HDL levels are associated with improved vascular flow. Elevated HDL is also a protective factor in dyslipidemia.

In the group of subjects with hypercholesterolemia, in the left eye, statistically significant results were observed for total cholesterol with FAZ Circularity ($r = 0.287$; $p = 0.011$), NFA Area ($r = 0.245$; $p = 0.032$), VFA Area ($r = 0.273$; $p = 0.016$), VFA Flow ($r = -0.257$; $p = 0.024$), Skeleton Total ($r = -0.249$; $p = 0.029$), and for total cholesterol with Skeleton ETDRS ($r = -0.254$; $p = 0.026$).

Correlations between FAZ Circularity and LDL ($r = 0.313$; $p = 0.006$), NFA Area and LDL ($r = 0.233$; $p = 0.042$), and VFA Area and LDL ($r = -0.354$; $p = 0.002$) were statistically significant. Elevated Skeleton Total values correlated with high HDL levels ($r = 0.230$; $p = 0.044$), indicating that improving HDL levels leads to better capillary circulation. Subjects with elevated LDL and total cholesterol lost more capillaries, presented larger non-perfusion areas, had reduced vascular flow, and consequently showed progression of hypertensive retinopathy in patients with poor dyslipidemia control.

Analyzing the RE, four OCTA parameters were positively correlated (values increased when systolic and diastolic blood pressure were elevated) and statistically significant with blood pressure values: FAZ Area ($r = 0.276$; $p = 0.015$), FAZ Perimeter ($r = 0.253$; $p = 0.026$), and FAZ Circularity ($r = 0.256$; $p = 0.025$). Parameters such as VFA Area ($r = -0.368$; $p = 0.001$), VFA Flow Area ($r = -0.364$; $p = 0.001$), Total Density ($r = -0.382$; $p = 0.001$), ETDRS Density ($r = -0.399$; $p < 0.01$), Skeleton Total ($r = -0.364$; $p = 0.001$), and Skeleton ETDRS ($r = -0.351$; $p = 0.002$) showed a negative correlation (values decreased when blood pressure was elevated) and were highly significant, with $p < 0.01$.

These results demonstrate that capillary loss, increased avascular zones, and reduced retinal vascular flow were observed in subjects with hypertension. Thus, the progression of hypertensive retinopathy can be demonstrated using OCTA.

Correlations between OCTA parameters were also calculated using the two-tailed significance test and Pearson correlation. In patients with increased NFA Area, FAZ Area and FAZ Perimeter were increased, while VFA Area, VFA Flow Area, Density (Total and ETDRS), and Skeleton Total were significantly reduced. In both eyes, elevated Density (Total and ETDRS) levels correlated with increased Skeleton parameters (Total and ETDRS). Differences between OCTA parameters of the RE and the LE were analyzed.

The t-test yielded p-values > 0.05 for all pairs of OCTA parameters. Therefore, no statistically significant differences were found between OCTA parameters of both eyes.

With the MIP algorithm implemented in the OCT software and our statistical results, the A.I. software of the OCT device can be improved by integrating new mathematical formulas that correlate blood pressure and lipid values with vascular parameters.

Our most relevant statistical correlations showed that variables such as VFA Area, VFA Flow Area, Total and ETDRS Density, and Skeleton Total were inversely proportional to blood pressure, total cholesterol, and LDL, whereas NFA Area, FAZ Area, Perimeter, and Circularity were directly proportional to the same clinical variables.

To determine how these factors interact collectively, a factor analysis was performed using Principal Component Analysis. This revealed five main components for the RE and four for the LE. Within each component, OCTA parameters are highlighted, with importance given by the highest positive values. For example, in the right eye, the order is: Total Density (0.928), ETDRS Density (0.925), Total Skeleton (0.847), and Skeleton ETDRS (0.699). In

the left eye, the same parameters appear in component 1, in the same order of importance but with different values.

Tests used for factor analysis included: Kaiser-Meyer-Olkin (KMO), Bartlett's test, Rotated Component Matrix, Total Variance Explained, and Scree Plot. The KMO value in the RE group was 0.619, higher than in the LE group, indicating that the RE patient group is more statistically relevant. Bartlett's test yielded a significance value < 0.01 for both eyes, demonstrating that factor analysis was relevant in both groups. The Rotated Component Matrix was calculated using Varimax rotation with Kaiser normalization.

7.4. Discussions

In this study conducted on subjects with hypertension and dyslipidemia, correlations between blood pressure and clinical variables showed that higher blood pressure is associated with higher BMI, total cholesterol, and LDL levels, while HDL levels are lower. Our subjects did not have diabetes, and glucose and HbA1c values were not statistically significant, being used only as confounding factors.

OCTA is a high-precision technique for evaluating retinal vascularization. The results can detect retinal pathology early, monitor its progression, and provide a more detailed staging of hypertensive retinopathy with more specific therapeutic targets [8, 14].

Our study analyzed the relationship between blood pressure, retinal vessels, choriocapillaries, and systemic risk factors in hypertension.

We identified reduced vascular flow and oxygen supply, as well as reduced HDL in these subjects. These microvascular alterations may represent an early step in organ damage and could serve as markers for monitoring anti-hypertensive treatments.

Other changes identified in our study included increased FAZ Area and FAZ Perimeter, reduced VFA Flow Area, and decreased vascular density and skeleton in hypertensive participants.

With ongoing development of A.I. systems and human research, more accurate and specific results can be achieved, enabling early detection, treatment, and detailed monitoring of at-risk patients.

7.5. Conclusions

- Strong correlation exists between hypertensive retinopathy, dyslipidemia, and OCTA parameters;
- Improving BP and microcirculation reduces cardiovascular risk and organ damage;
- OCTA is a valuable tool for early detection and monitoring;
- A.I. systems in OCTA can be further improved for better diagnosis and classification.

Chapter 8. Study number 2. Relevance of OCTA in screening and monitoring hypertensive patients with carotid artery stenosis

8.1. Introduction

Carotid artery stenosis is one of the most important causes of cerebrovascular ischemia and ocular impairment, representing a significant public health issue. It is estimated that approximately 5–10% of the population over 70 years old is affected by carotid artery stenosis. Microvascular pathological changes involved in the development of cardiovascular diseases occur long before they are clinically diagnosed, making early identification essential [15-18].

Carotid artery Doppler ultrasound is a non-invasive, simple, and highly accurate method (92%) for assessing ICAS. When applied as a screening tool, ultrasound is valuable in evaluating cardiovascular risk and developing preventive strategies. While patients with severe ICAS often present with visual symptoms, those with mild to moderate ICAS are typically asymptomatic. Therefore, identifying the latter group becomes a priority.

The retinal microvasculature has anatomical and physiological features almost identical to the cerebral and coronary microcirculation. It can be studied non-invasively using OCTA, which provides qualitative and quantitative information about the retina's small vessels before changes appear on fundus examination. This highlights the utility of studying microcirculation in patients with ICAS.

Our study aimed to quantify and compare retinal vascular changes detected by OCTA in subjects with different stages of carotid artery stenosis. We correlated OCTA parameters with data obtained from carotid and vertebral Doppler ultrasound. We hypothesized that retinal microvascular changes are correlated with mild and moderate stenosis, and that

OCTA-derived parameters could serve as biomarkers for identifying ICAS. Thus, OCTA screening can be appropriately performed during the same visit as carotid Doppler ultrasound.

8.2. Materials and Methods

We conducted a prospective cohort study involving 98 eyes. All participants underwent a complete ophthalmologic evaluation, cardiovascular risk assessment through blood pressure measurement and laboratory tests, and carotid and vertebral Doppler ultrasound evaluation. Subjects with chronic hypertension, secondary dyslipidemia, and chronic hypertensive retinopathy were selected. Retinopathy was classified according to the Keith–Wagener–Barker and Mitchell–Wong systems, based on the Radiological Society’s evaluation. Subjects were divided into four stenosis groups.

Assessment of subjects was performed using OCTA Angio-OCT Optopol Revo NX 130 with A.I. technology. The entire cohort was divided into RE and LE groups. Subgroups with different degrees of carotid artery stenosis were obtained: normal (no stenosis), mild, and moderate.

Descriptive analysis was used for age, sex, and smoking status. The Kruskal–Wallis test was applied to determine whether each OCTA parameter showed statistically significant differences among the three stenosis groups. Dunn’s post hoc test was used to identify pairs of groups with significant differences. Spearman and Pearson correlations were used to correlate OCTA parameters from both eyes with systolic (PSV) and diastolic (EDV) velocities. Both velocities were measured for the internal carotid artery (ICA), external carotid artery (ECA), common carotid artery (CCA), and vertebral artery (VA). Normality of distribution was assessed using the Shapiro–Wilk test. QQ plots were used to determine data distribution, regression line, type of correlation, and confidence intervals for both eyes and the ipsilateral carotid arteries.

8.3. Results

A total of 49 subjects were enrolled in our study. According to the Radiological Society classification, 21 patients were assigned to the normal group (no stenosis), 9 to the mild stenosis group, and 9 to the moderate stenosis group. No subjects with severe stenosis were identified.

The chi-square test (χ^2) was used to analyze whether statistically significant differences existed between the three stenosis groups in terms of sex, age, and smoking status. The mean age was 60.9 ± 8.2 years in the mild stenosis group, 54.4 ± 10.0 years in the normal group, and 58.2 ± 13.1 years in the moderate stenosis group. The proportion of male subjects was 63.2% in the mild stenosis group, 52.4% in the normal group, and 44.4% in the moderate group. Non-smokers were the majority, representing 80.9% of the normal group, 63.2% of the mild stenosis group, and 1.1% of the moderate stenosis group.

The Kruskal–Wallis test was used to determine whether there were statistically significant differences between stenosis groups for each OCTA parameter. Dunn’s post hoc test was subsequently applied to compare different stenosis groups in the RE.

Analysis of the RE revealed statistically significant results for FAZ Circularity when comparing normal vs. mild stenosis ($p = 0.025$) and mild vs. moderate stenosis ($p = 0.006$). Significant correlations were found for NFA when comparing normal vs. moderate stenosis ($p = 0.004$) and mild vs. moderate stenosis ($p = 0.011$). Comparing normal vs. moderate groups, statistically significant differences were observed for FAZ Area ($p = 0.016$) and VFA Area ($p = 0.037$).

In the left eye, comparing normal vs. moderate stenosis groups, statistically significant results were obtained for VFA Area ($p = 0.041$), NFA ($p = 0.045$), and VFA Flow Area ($p = 0.029$). Comparing mild vs. moderate stenosis groups, significant differences were identified for NFA ($p = 0.001$), FAZ Area ($p = 0.007$), and VFA Area ($p = 0.013$).

Spearman and Pearson correlations revealed relationships between OCTA parameters from RE and LE. PSV and EDV were measured for the ICA, ECA, CCA, and VA in both carotid and vertebral arteries. Statistically significant correlations ($p < 0.05$) in the RE group included: ICA PSV and VFA ($\rho = -0.286$), ICA EDV and NFA ($r = 0.365$), ECA PSV and VFA ($r = -0.288$; $\rho = -0.317$), CCA PSV and NFA ($\rho = -0.354$), CCA EDV and NFA ($\rho = -0.292$), VA PSV and VFA ($r = -0.327$; $\rho = -0.379$), and NFA and VFA ($r = -0.374$; $\rho = -0.288$).

For the LE group, statistically significant results were observed for: ICA PSV and NFA ($r = -0.351$; $\rho = -0.313$), ICA EDV and VFA ($r = -0.421$; $\rho = -0.314$), ECA PSV and NFA ($r = -0.412$; $\rho = -0.457$), CCA PSV and NFA ($p = -0.288$; $\rho = -0.339$), and CCA EDV and NFA Flow Area ($r = -0.404$; $\rho = -0.417$).

Normality of distribution was assessed using the multivariate Shapiro–Wilk test and QQ plots, which illustrate data distribution, regression line, confidence intervals, and type of correlation.

8.4. Discussion

The study of retinal vessels provides valuable insights in analyzing patients with ICAS. Retinal blood vessels can be directly visualized, offering rapid access for monitoring circulatory and vascular function [19, 20]. Microvascular abnormalities significantly contribute to the development of cardiovascular diseases. ICAS is a major risk factor for ischemic ocular complications, such as amaurosis fugax, ocular ischemic syndrome, and central retinal artery occlusion. Retinal vessel involvement is usually asymptomatic and occurs in 29% of ICAS patients, with 1.5% of cases progressing annually to ocular ischemic syndrome. OCTA can monitor cardiovascular risk by quantifying retinal microvascular impairment.

Our research aimed to determine the microvascular changes in subjects with different stages of ICAS, quantify them, and non-invasively evaluate vascular structural changes using OCTA. We measured and compared vascular parameters provided by OCTA and correlated them with ipsilateral parameters obtained from carotid and vertebral Doppler ultrasound, hypothesizing that these correlations exist.

Statistically significant results were obtained for three OCTA parameters: FAZ, NFA, and VFA, and correlations were demonstrated when comparing normal carotid stenosis groups with mild and moderate groups, as well as between the latter two. Consequently, we propose that carotid Doppler ultrasound screening should be complemented with OCTA in ICAS patients.

It is important for clinicians to be familiar with ocular signs caused by carotid atherosclerosis to prevent stroke. However, retinal microvascular changes precede both fundus exam signs and patient-reported symptoms. Early identification of vascular alterations can provide cardiologists with more precise information and help adjust the therapeutic protocol for hypertensive patients.

8.5. Conclusions

Using OCTA, we identified retinal and choroidal microcirculation changes correlated with carotid artery stenosis. Alterations in small retinal vessels contribute to carotid artery

disease, and even patients with mild stenosis exhibited detectable retinal circulation changes via OCTA. Since most subjects in our study had mild or moderate stenosis, we conclude that OCTA can detect arterial disease at these stages.

OCTA can serve as a monitoring tool for microvascular changes associated with carotid artery stenosis, providing quantitative data on vascular density and flow. Changes in small retinal vessels assessed by OCTA may serve as a novel biomarker reflecting ICAS severity and potentially identifying ICAS [17, 18, 21, 22].

Therefore, we propose that OCTA be used as a complementary screening method alongside fundus examination and carotid/vertebral Doppler ultrasound, ideally performed during the same clinical visit. Ophthalmic signs and symptoms may sometimes be the only manifestations in patients with atherosclerotic disease. Early identification allows timely referral of at-risk subjects for medical or surgical intervention, potentially preventing stroke or myocardial infarction.

Chapter 9. Study number 3. Retinal dysfunction in hypertensive patients with carotid atherosclerosis: an OCTA evaluation

9.1. Introduction

Hypertension is associated with multiple retinal microvascular changes, including generalized and focal arteriolar narrowing and arteriovenous crossing abnormalities [23-25]. Several studies have examined the impact of different stages of hypertensive retinopathy assessed by OCTA [26, 27]. Anti-hypertensive treatment has been shown to influence retinal microcirculation [25].

OCTA can detect abnormalities in small retinal vessels before they become symptomatic or clinically visible, providing a more precise evaluation than other diagnostic methods [28, 29]. Cardiovascular risk in patients with hypertension and dyslipidemia can also be assessed and monitored using OCTA [22, 29-31]. This method can quantify vessel caliber, vascular tortuosity, capillary perfusion, and foveal areas. These parameters can be associated with coronary artery disease, stroke, and myocardial infarction [32].

Imaging of carotid artery plaques and measuring the degree of internal carotid artery stenosis are simple, accessible, and highly accurate methods (92%) [33, 34]. A significant

proportion of hypertensive patients have clinically detectable atherosclerosis on carotid Doppler ultrasound [35, 36].

Coronary artery calcifications, assessed via intima-media thickness (IMT) measurements, as well as the identification of atheromatous plaques through Doppler ultrasound, can reveal subclinical atherosclerosis [37]. Both an $IMT \geq 1.0$ mm and the presence of atherosclerosis are predictors of cardiovascular disease and both can be detected via carotid Doppler ultrasound, defining subclinical atherosclerosis [38, 39].

Our study aimed to quantify and compare retinal microvascular changes obtained via OCTA in subjects with hypertension, dyslipidemia, and carotid artery plaques. All patients were receiving anti-hypertensive treatments, including ACE inhibitors (ACEI), calcium channel blockers (CCB), ACEI/ARB combinations, as well as statin therapy, either in combination or as monotherapy.

The goal of our study was to detect retinal dysfunction in patients with hypertension, dyslipidemia, and atherosclerosis, resulting in areas of reduced vascular flow, decreased density, and reduced vascular skeleton.

We aimed to investigate early detection of microvascular changes so that OCTA-derived information could be used to guide adjustments in therapeutic protocols.

9.2. Materials and Methods

We selected patients with chronic dyslipidemia, hypertension, and retinopathy. Statin and anti-hypertensive therapies administered to participants were evaluated to establish correlations between retinal microvascularization and atherosclerotic disease.

Based on carotid Doppler ultrasound, we determined the presence of atheromatous plaques and calculated IMT. Participants included those with secondary dyslipidemia and essential hypertension, with disease duration of 5–10 years (11 subjects), 10–15 years (34 patients), and over 15 years (53 participants). All included subjects therefore had an elevated risk of complications and mortality.

Ophthalmologic evaluation included: visual acuity testing, refraction assessment, intraocular pressure measurement, slit-lamp biomicroscopy, dilated fundus examination, and OCTA. OCTA was performed using Angio-OCT Optopol Revo NX 130 with A.I. technology, evaluating the macula with a 6.0×6.0 mm² HD scan. Carotid Doppler

ultrasound using the G.E. Vivid T8 Cardiovascular Ultrasound assessed the presence of atheromatous plaques and measured IMT.

Participants were divided into three groups according to cardiologist-prescribed medication: monotherapy (ACEI) + statins, monotherapy (CCB) + statins, and combination therapy (ACEI/ARB) + statins.

The study cohort included 196 eyes from 98 patients on statin therapy. They were divided into three groups according to medication: monotherapy anti-hypertensive (34 ACEI and 30 CCB subjects) and combination therapy anti-hypertensive (ACEI/ARB) with 34 participants.

ANOVA was used to compare means across the three groups to determine whether at least one group differed significantly from the others. Post hoc tests with Bonferroni corrections were then performed for multiple comparisons across the three study groups. The Brown-Forsythe test was used to verify homogeneity of variance across groups. Normality of data distribution was assessed using the Shapiro–Wilk test. For further analysis, the Kruskal–Wallis test was also applied, followed by Dunn’s post hoc test to compare group pairs.

Multivariate MANCOVA analysis was subsequently used to highlight the effect of cardiologist-prescribed medications across the three groups and it included multiple tests: Box's Test, Pillai's Trace and Wilks' Lambda, Between-Subjects Effects, Estimated Marginal Means, Post Hoc Pairwise Comparisons, and Spearman and Pearson correlations.

9.3. Results

A total of 98 subjects (196 eyes) with hypertensive retinopathy and dyslipidemia were enrolled in our study. Males (55.1%) and non-smokers (75.5%) comprised the majority across all three therapy groups in the demographic analysis.

Our study included patients receiving statins in combination with anti-hypertensive therapy, either as monotherapy (ACEI or CCB) or as combination therapy (ACEI/ARB). This resulted in three study groups:

- Monotherapy anti-HTA (ACEI) + statins: 34 patients,
- Monotherapy anti-HTA (CCB) + statins: 30 subjects,
- Combination therapy (ACEI/ARB) + statins: 34 participants.

With increasing age and BMI, the risk of developing atherosclerosis rises [40]. Pearson and Spearman correlations were calculated to assess relationships between demographic parameters and carotid Doppler measurements. BMI showed the most statistically significant correlation with ultrasound parameters. Increased age correlated with higher ICA EDV and CCA IR values, while smokers exhibited lower ECA PSV values.

IMT above the normal threshold (>1.0 mm) and the presence of atheromatous plaques in the carotid arteries were defined as subclinical atherosclerosis. Fifty-one subjects (52.04%) were identified with carotid plaques on Doppler ultrasound, and 5 participants had an IMT ≥ 1.0 mm (5.1%).

Multivariate Shapiro–Wilk tests for normality indicated deviation from multivariate normality in all groups: ACEI + statins (SW = 0.932, $p < 0.001$), CCB + statins (SW = 0.944, $p < 0.001$), and ACEI/ARB + statins (SW = 0.923, $p < 0.001$).

ANOVA was used to compare means across the three medication groups. Significant differences were observed for the following OCTA parameters: NFA Area, FAZ Area, FAZ Circularity, VFA Flow Area, and Density (Total and ETDRS) ($p < 0.05$). Post hoc tests revealed:

- NFA Area and FAZ Area: significant differences between ACEI + statins vs. ACEI/ARB + statins and CCB + statins vs. ACEI/ARB + statins ($p \leq 0.001$)
- FAZ Circularity: significant difference between CCB + statins vs. ACEI/ARB + statins ($p = 0.033$)
- VFA Flow Area: significant difference between ACEI + statins vs. ACEI/ARB + statins ($p = 0.040$)
- Density Total and ETDRS: significant differences between ACEI + statins vs. ACEI/ARB + statins ($p = 0.002$ and $p = 0.003$, respectively)

Kruskal–Wallis non-parametric testing confirmed the ANOVA results, with Dunn’s post hoc comparisons yielding consistent findings using Bonferroni correction ($p < 0.05$).

Box's Test of Equality of Covariance Matrices was significant (Box M = 455.876, $F = 3.517$, $p < 0.001$), indicating heterogeneity of the data.

All four multivariate tests indicated a significance threshold of $p < 0.001$, demonstrating significant differences in the effects of the three medication regimens on

OCTA parameters overall. Covariates generally did not show significant multivariate effects on OCTA parameters ($p > 0.05$), except for total cholesterol ($p = 0.017$).

Tests of Between-Subjects Effects identified the impact of independent variables and covariates on OCTA parameters. Medication group had a statistically significant effect ($p < 0.05$) on NFA Area, FAZ Area, FAZ Circularity, VFA Flow Area, and Skeleton ETDRS. Partial Eta squared indicated a significant effect on FAZ Area. Clinical parameters such as blood pressure and lipid levels also had significant effects on OCTA parameters ($p < 0.05$).

Estimated Marginal Means and Pairwise comparisons presented adjusted means in therapy groups for each OCTA parameter after controlling for covariates. Significant differences between the three medication groups were confirmed using ANOVA post hoc with Bonferroni correction.

Spearman and Pearson correlations were established between OCTA and clinical parameters.

In the ACEI + statins group, elevated SBP and DBP correlated with larger areas of low vascular flow or FAZ and reduced density and vascular skeleton. Total cholesterol correlated with NFA Area ($r = 0.477$, $p = 0.004$; $\rho = 0.541$, $p = 0.001$). Presence of carotid plaques significantly correlated with FAZ Area ($r = 0.540$, $p < 0.001$; $\rho = 0.526$, $p = 0.001$) and VFA Area ($r = -0.409$, $p = 0.016$; $\rho = -0.430$, $p = 0.011$).

In the CCB + statins group, higher blood pressure and LDL/HDL levels were associated with larger avascular areas, reduced vascular flow, and decreased capillary perfusion. Total cholesterol correlated with SBP ($r = 0.379$, $p = 0.039$). Carotid plaques correlated with FAZ Circularity ($r = -0.406$, $p = 0.026$; $\rho = -0.409$, $p = 0.025$) and Density Total ($r = 0.400$, $p = 0.028$; $\rho = 0.389$, $p = 0.034$).

In the ACEI/ARB + statins group, the strongest correlations were between clinical variables (blood pressure with LDL and HDL) and OCTA parameters: FAZ (Area and Perimeter) with Density and Skeleton. Carotid plaques correlated with three OCTA parameters: NFA ($r = -0.558$, $p < 0.001$; $\rho = -0.555$, $p < 0.001$), FAZ Area ($r = 0.405$, $p = 0.017$; $\rho = 0.394$, $p = 0.021$), and Density Total ($r = -0.527$, $p = 0.001$; $\rho = -0.509$, $p = 0.002$).

Overall, elevated SBP, DBP, total cholesterol, and LDL were associated with reduced VFA, increased avascular areas, and higher NFA. Subjects on combination therapy (anti-

HTA + statins) exhibited higher blood pressure, dyslipidemia, and progressive hypertensive retinopathy. Among the most relevant correlations, the presence of carotid plaques was associated with: VFA Area ($r = -0.409$, $p = 0.016$; $\rho = -0.430$, $p = 0.011$), NFA Area ($r = -0.242$, $p = 0.017$; $\rho = -0.237$, $p = 0.019$), FAZ Area ($r = 0.540$, $p < 0.001$; $\rho = 0.526$, $p < 0.001$; $r = 0.405$, $p = 0.017$; $\rho = 0.394$, $p = 0.021$), FAZ Circularity ($r = -0.406$, $p = 0.026$; $\rho = -0.409$, $p = 0.025$), Density Total ($r = -0.400$, $p = 0.028$; $\rho = -0.389$, $p = 0.034$; $r = -0.527$, $p = 0.001$; $\rho = -0.509$, $p = 0.002$).

Thus, reduced retinal perfusion—manifested as decreased capillary density, increased avascular areas, and non-perfusion zones—was associated with the presence of carotid atherosclerosis.

9.4. Discussion

OCTA has been widely used in studies evaluating systemic cardiovascular diseases. Numerous studies indicate that retinal microvascular abnormalities may serve as potential indicators of microvascular, cardiovascular, and neurodegenerative damage [29, 41, 42]. Detecting subclinical carotid artery changes in asymptomatic patients is a public health concern and global priority [19]. OCTA is a valuable tool for identifying retinal microvascular anomalies before they are clinically apparent. Retinal circulation can act as a surrogate marker for cardiovascular pathology [43]. OCTA may aid in early detection of vascular alterations, providing cardiologists with additional information for therapeutic decision-making in patients with hypertension, dyslipidemia, and carotid atherosclerosis.

Our study found that the presence of carotid plaques correlated with older age across all three anti-hypertensive therapy groups. It also correlated with decreased retinal vascular density in the CCB and ACEI/ARB groups, reduced VFA in the ACEI group, and elevated NFA in the combination therapy group. The most relevant OCTA parameters were FAZ Area and Density Total.

9.5. Conclusions

Significant correlations were identified between hypertension, carotid artery plaques, and reduced retinal microcirculation in patients receiving combination therapy (anti-HTA + statins).

Key findings included:

- ACEI group: increased FAZ Area and decreased VFA Area

- CCB group: reduced total density and increased FAZ Circularity
- ACEI/ARB group: increased NFA and FAZ areas, with decreased total density

Subjects on combination therapy exhibited elevated blood pressure, dyslipidemia, and progressive hypertensive retinopathy. The most relevant correlations involved carotid plaques with NFA Area, FAZ Area, and total density.

Reduced retinal vascular perfusion—manifested as lower capillary density, increased non-perfusion areas, and avascular zones—was observed in patients with hypertension and carotid atherosclerosis. Our findings indicate that patients on multiple therapies, with poor dyslipidemia control, high blood pressure, progressive hypertensive retinopathy, and carotid atherosclerosis, exhibit impaired retinal vascularization.

OCTA can detect microvascular changes early in hypertension, carotid artery stenosis, and dyslipidemia. By correlating OCTA data with carotid Doppler findings, treatment protocols for hypertensive patients can be adjusted. Additionally, early risk stratification and personalized interventions may reduce ocular and systemic complications of chronic disease.

Chapter 10. Final conclusions and personal contributions

- The obtained data confirmed the correlation between hypertensive retinopathy, dyslipidemia, and OCTA parameters.
- The improvement in retinal microcirculation, associated with the reduction of blood pressure values, contributed to decreasing cardiovascular mortality and morbidity, as well as to the prevention and reduction of target organ damage: eyes, heart, brain, and kidneys.
- The information obtained through OCTA provided additional details that enabled a more precise identification of retinal vascular alterations.
- The early identification of microvascular changes through OCTA, prior to their detection in fundoscopic examination, allowed for a more accurate staging of hypertensive retinopathy and a better understanding of the pathology involved in its progression.
- During my research, I identified limitations of the OCTA device's artificial intelligence system. This system can be improved to achieve a more accurate

diagnosis, facilitate the discovery of new therapies, enhance communication with patients, and possibly contribute to a new classification of hypertensive retinopathy.

- My research confirmed that OCTA is a highly accurate technique for evaluating retinal vascularization. The obtained results enabled early detection of retinal pathology, monitoring of disease progression, and a more detailed assessment of hypertensive retinopathy with more specific therapeutic targets.
- The doctoral thesis confirms the link between hypertensive retinopathy, dyslipidemia, and OCTA parameters. Based on the data collected during the doctoral research, the possibility emerges of developing an algorithm that includes the staging of hypertensive retinopathy, characteristics of dyslipidemia, and OCTA parameters. This algorithm would allow prediction of the evolution of ocular and systemic pathologies, early diagnosis, and early identification of at-risk patients.
- The use of OCTA allowed me to identify subtle changes in retinal and choroidal microcirculation, changes correlated with carotid artery stenosis that cannot be detected using other techniques.
- Using OCTA, I quantified retinal microvascular impairment in hypertensive patients with dyslipidemia.
- I found that alterations in small retinal vessels are directly proportional to carotid lesions.
- The results demonstrated that OCTA is a highly accurate tool for monitoring microvascular alterations associated with carotid artery stenosis, providing objective data on vascular density and flow.
- I propose that retinal microvascular changes assessed by OCTA should serve as a biomarker reflecting the severity of stenosis and may even raise suspicion of internal carotid artery stenosis.
- I propose that OCTA be used as a screening method, complementary to fundoscopic examination and carotid Doppler ultrasound, thus allowing OCTA evaluation to become a gold standard in assessing hypertensive patients with dyslipidemia.
- Carotid artery stenosis represents a significant public health issue, as carotid pathology leads to ocular and cerebral ischemic events that can cause permanent disability and even death. For this reason, I consider the results of this study highly valuable in establishing an early and accurate diagnosis in hypertensive patients.

- I obtained statistically significant correlations between elevated blood pressure, impaired retinal microvascularization, and the presence of atherosclerotic plaques in subjects receiving simultaneous antihypertensive and statin therapy.
- I found that subjects with hypertensive retinopathy and atherosclerosis, undergoing combined treatments, exhibited reduced retinal and choroidal vascular perfusion.
- By correlating OCTA results with carotid Doppler ultrasound, I obtained information that allowed me to provide recommendations to cardiologists regarding the adjustment of combined therapies, aiming to reduce patients' cardiovascular risk.
- By repeating OCTA during follow-up evaluations, I was able to confirm improvement in retinal microcirculation in patients whose therapeutic protocol had been adjusted by the cardiologist.
- There is a limited number of studies published in the international literature addressing the correlations between retinal vascular perfusion, hypertension, and atherosclerosis. For this reason, I believe my research provides valuable contributions to this field.
- The results of my research showed that subjects receiving combined treatments, with poor control of dyslipidemia, elevated blood pressure, progressive hypertensive retinopathy, and carotid atherosclerosis, exhibited a more severe deficit in retinal vascularization.

Practical implications

I addressed the issue of retinal changes in patients with hypertension, as arterial hypertension, dyslipidemia, and cardiovascular mortality represent major public health concerns worldwide and in Romania. Through the comprehensive study of clinical parameters, OCTA, carotid artery Doppler ultrasound, and with the support of AI tools, I developed a more complete profile of the hypertensive patient with dyslipidemia, correlating ocular and systemic pathology.

I correlated the treatments administered for hypertension and dyslipidemia with OCTA and ultrasound parameters, obtaining information that enabled optimization of the patient's therapeutic protocol, with positive outcomes objectively demonstrated by OCTA. These achievements represent the novelty of the doctoral thesis and contribute to the originality of the present research.

In current practice, OCTA represents an efficient, non-invasive, quantifiable, and cost-effective method for evaluating ocular microcirculation. OCTA, when correlated with another non-invasive and accessible investigation such as carotid artery Doppler ultrasound, provides a more precise, detailed, and comprehensive assessment of both retinal and systemic circulation.

During the doctoral research, I implemented a multidisciplinary approach involving ophthalmology, cardiology, internal medicine, laboratory medicine, neurology, pathophysiology, and imaging techniques. This approach led to increased diagnostic accuracy, early identification of hypertensive patients at risk of death and comorbidities, and allowed for treatment adjustment.

I propose that OCTA be used as a screening method, complementary to fundoscopic examination and carotid Doppler ultrasound, so that OCTA evaluation may become a gold standard in assessing hypertensive patients with dyslipidemia.

The doctoral thesis confirms the link between hypertensive retinopathy, dyslipidemia, and OCTA parameters. In future postdoctoral studies, I aim to develop an algorithm that incorporates the staging of hypertensive retinopathy, characteristics of dyslipidemia, and OCTA parameters, in order to predict the evolution of ocular and systemic pathologies, enable early diagnosis, and allow early identification of at-risk patients.

A comprehensive ophthalmological evaluation, including OCTA, combined with carotid artery Doppler examination and other paraclinical tests, will allow rapid identification of patients at risk of complications (myocardial infarction, stroke) and enable adjustment of therapeutic regimens aimed at reducing mortality and morbidity in these patients.

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List of published scientific papers

1. Journal of Clinical Medicine - section: AI in Medical Imaging and Image Processing; I.F. 3.0, PubMed; J. Clin. Med. 2024, 13(24), 7584; <https://doi.org/10.3390/jcm13247584>, "Monitoring Progression in Hypertensive Patients with Dyslipidemia Using Optical Coherence Tomography Angiography: Can A.I. Be Improved?".

<https://www.mdpi.com/2077-0383/13/24/7584>

Paper 1 – Related to Chapter 7 of the doctoral thesis can be found on page 47.

2. Diagnostics – section Special Issue Advances in Optical Coherence Tomography in 2025; I.F. 3.0, PubMed; Diagnostics 2025,15(11), 1393; <https://doi.org/10.3390/diagnostics15111393>, "The Relevance of Optical Coherence Tomography Angiography in Screening and Monitoring Hypertensive Patients with Carotid Artery Stenosis".

<https://www.mdpi.com/2075-4418/15/11/1393>

Paper 2 – Related to Chapter 8 of the doctoral thesis can be found on page 64.

3. Life – section Special issue: Research on Glaucoma Diseases; Life 2026, 16(3), 436; <https://doi.org/10.3390/life16030436> "Retinal dysfunction in hypertensive patients with atherosclerotic plaque detected by carotid Doppler ultrasound: an Optical Coherence Tomography Angiography assessment."

<https://www.mdpi.com/2075-1729/16/3/436>

Paper 3 – Related to Chapter 9 of the doctoral thesis can be found on page 90.