

# OCT Angiography and Color Doppler Imaging in Glaucoma Diagnostics

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## Abstract

The purpose of the study was to evaluate the perfusion of optic nerve head (ONH), peripapillary retina and inner macula in glaucoma, and determine the relation between retinal and ONH microcirculation, retrobulbar blood flow and structural parameters. The study involved patients suffering from primary open-angle glaucoma (POAG) (65 eyes) and healthy subjects matched with respect to age (22 eyes). The eyes were examined by means of Angiovue SD-OCT-A (RTVue-XR Avanti) and color Doppler imaging (CDI). Also, the study included assessment of retinal thickness and vessel density (VD). VD inside Disc and Peripapillary VD were evaluated in ONH and in 750  $\mu\text{m}$  wide elliptical annulus extending from the optic disc boundary, as Whole En Face VD (disc scan). Whole En Face VD (macula scan) was evaluated in macula (area bounded by a circle with a diameter of 3 mm) comprising fovea and parafovea in superficial and deep plexuses of the inner layers of retina. The authors used CDI to measure retrobulbar blood flow parameters, including ophthalmic artery (OA) and short posterior ciliary artery (SPCA). To perform a statistical analysis, SPSS Statistics 21 and R were used. To determine the diagnostic ability of each diagnostic parameter indicating retinal hemoperfusion, the authors employed the Wilcoxon-Mann-Whitney test and AUC. Indicator values for OCT-A and CDI appeared to be reduced in glaucomatous eyes against healthy ones. The largest AUC for differentiating the early glaucoma from the norm was characteristic of such indicators as: Whole En Face Superficial VD in macula (AUC 0.8 (0.69-0.90)), Peripapillary VD (AUC 0.75 (0.63-0.87)), end-diastolic flow velocity in OA (AUC 0.74 (0.61-0.86)) and temporal SPCA (AUC 0.72 (0.58-0.86)). In order to differentiate the early glaucoma from the moderate/severe stages of the disease, the most useful were Inferotemporal Peripapillary VD (AUC 0.94 (0.86-1.0)) and mean flow velocity in the CRA (AUC 0.81 (0.69-0.92)). In normal eyes, end-diastolic flow velocity in SPCA correlated with Inferotemporal RNFL ( $r = 0.747$ ,  $p < 0.001$ ) and the inferior macular thickness (0.634,  $p < 0.001$ ). In early glaucoma, the inferotemporal RNFL correlated with Whole En Face Superficial VD in macula ( $r = 0.590$ ,  $p < 0.001$ ), Peripapillary VD ( $r = 0.340$ ,  $p = 0.034$ ) and global loss volume of the ganglion cells complex ( $r = -0.5$ ,  $p = 0.001$ ). Inverse relation was observed between the blood flow parameters in retrobulbar veins and the inferotemporal RNFL and macular thickness in the inferior and nasal areas. There was no significant correlation between IOP and OCT-A parameters in glaucoma subjects. The relation between retinal and ONH microcirculation and retrobulbar blood flow and structural parameters were revealed in normal eyes and early glaucoma. Both methods are useful for diagnosis and monitoring of early glaucoma.

**Keywords:** optical coherence tomography, OCT angiography, primary open-angle glaucoma, ocular blood flow, early diagnosis of glaucoma.

## INTRODUCTION

There is a growing body of evidence suggesting that glaucoma pathogenesis is related to vascular dysfunction [4; 6; 8; 9; 23; 32; 33]. The consensus on this issue, however, still has not been found due to the lack of adequate techniques for the study of ocular blood flow despite of different measurement tools [2; 11; 12; 25]. This makes it reasonable to search for new methods of the vascular bed's visualization for the early diagnosis and monitoring of glaucoma. Optical coherence tomography (OCT) is commonly used in clinical settings for the diagnosis and management of glaucoma. Doppler OCT has been used to obtain precise measurements of total retinal blood flow [30]. Although Doppler OCT is appropriate for large vessels around the disc, it is not sensitive enough to measure accurately low velocities in small vessels that make up the disc microcirculation. The same refers to the most widely used method – color Doppler imaging (CDI). A new method – OCT angiography (OCT-A) – has been recently introduced. This method allows measuring vessel density in the retina and choroid in the peripapillary and

macular areas using high-speed OCT to perform quantitative angiography. OCT-A is based on the evaluation of decorrelation between consecutive B-scans (split-spectrum amplitude-decorrelation angiography, SSADA). This method was firstly defined in 2012 by Jia et al. [9]. The application of OCT using SSADA algorithm allows detecting changes in the microvascular in perifovea and peripapillary areas of glaucomatous eyes. According to previous studies, quantitative analysis of OCT-A allows distinguishing diseased eyes from the norm by means of analyzing the entire peripapillary vasculature and macula [10; 16; 19; 22; 28; 29; 31]. It was found that perimetric indices correlated better with peripapillary blood flow parameters than with morphometric parameters of ONH and peripapillary retina [10]. However, data on the OCT-A results in glaucoma are scarce, and up to date there have been no studies comparing the retinal microcirculation measured by OCT-A and retrobulbar blood flow measured by CDI.

The purpose of the study was to evaluate the perfusion of optic nerve head (ONH), peripapillary retina

and inner macula in glaucoma and to determine the relation between retinal and ONH microcirculation, retrobulbar blood flow and structural parameters.

#### SUBJECTS AND RESEARCH METHODS

The cross-sectional study was conducted at the eye clinic of the Institution of Federal Medical and Biological Agency of Russia in May-October 2015. The methodology fulfilled the conditions of the Helsinki Declaration. All participants gave written informed consent.

Among the participants of the study there were 95 patients with primary open-angle glaucoma (POAG) (65 eyes: 38 – with early glaucoma, 27 eyes – with moderate and severe glaucoma. The diagnosis of glaucoma was made based on specific changes in ONH, which were determined by means of ophthalmoscopy (pathological deviation from the normal neural rim, glaucomatous optic disc cupping, peripapillary atrophy, wedge-shaped defects of the retinal nerve fiber layer (RNFL) and hemorrhages contiguous to the optic disc border). The results of standard automated perimetry (SAP) were outside normal limits.

All participants were Caucasian. Patients taking anti-glaucoma drops were asked to stop the administration of drug for three weeks in order to wash out the drug.

The study enrolled only subjects with ametropia  $\leq 0.5$  D and open anterior chamber angle  $\geq 30^\circ$ , which was confirmed by OCT performed in the anterior segment (Zeiss Visante OCT), and having no any eye surgery in the past.

The subjects were excluded from the study in case of systemic administration of beta-blockers and calcium-channel blockers, symptoms of primary or secondary vascular dysregulation, any concomitant ophthalmic pathology (except initial cataract), chronic autoimmune diseases, diabetes mellitus, acute circulatory disorders, and any other concomitant diseases requiring administration of steroid drugs.

The controls had intraocular pressure (IOP)  $< 21$  mm Hg for both eyes, Humphrey Swedish Interactive Threshold Algorithm (SITA) 24-2 within the normal limits, standard visual field with mean deviation (MD) and pattern standard deviation (PSD) within 95% limits of the normal reference, glaucoma hemifield test within 97% limits, central corneal thickness  $\geq 500$   $\mu\text{m}$ , normal optic disc appearance (as assessed by two expert on disc photographs), RNFL within the norm, open anterior chamber angle (proved by gonioscopy), and no chronic ocular diseases and no history of systemic administration of corticosteroids. As to the age and race distribution, it matched that of the patients with glaucoma.

One eye per patient was enrolled in the study. In the glaucoma patients, it was the most damaged eye, and in the controls – a random eye.

#### Measuring devices

SAP was performed by Humphrey Field Analyzer (Carl Zeiss Meditec): 30-2 threshold test library, SITA Standard threshold test strategy, 176 points in the central  $30^\circ$ , Goldmann III white stimulus, stimulus duration 100 ms, background illumination 31.5 ASB. Also, we have determined MD and PSD. SAP results were considered

normal in case pattern deviation plot had less than 3 neighboring points significantly differing from the norm ( $p < 0.05$ ) and at least one point ipsilateral to horizontal meridian, with  $p < 0.01$ .

We have carefully collected anamnesis of the patients, and particular attention was paid to the symptoms of primary and secondary vascular dysregulation. To exclude pathology of the brachiocephalic vessels, the study involved Doppler ultrasound scanning for all study subjects.

For the purpose of IOP measuring, the Ocular Response Analyzer (ORA) (Reichert Ophthalmic Instruments) was utilized.

Based on IOP and arterial blood pressure (BP), the mean ocular perfusion pressure (MOPP) was calculated:  $\text{MOPP} = (2/3\text{diastolicBP} + 1/3\text{systolicBP}) \times 2/3 - \text{IOP}$ . MOPP was measured immediately before the OCT scanning and investigation of retrobulbar blood flow, after having a 10-minute rest in sitting position. Systemic BP was evaluated by the Riva Rocci technique.

OCT was performed by means of Optovue RTVue 100 OCT. OCT was used to study the ONH area (ONH and 3D Disc protocols) and macular area (GCC protocol) in tracking mode. Retinal nerve fiber layer thickness (RNFLT) was investigated in the ONH protocol; the average ganglion cell complex (GCC) thickness, focal loss volume (FLV), and global loss volume (GLV) were measured in the GCC protocol.

Choroidal thickness (CT) was investigated in tracking mode as well (Retina Cross Line protocol) by means of RTVue 100 OCT. CT was determined as a distance between the hyperreflective signal line from the retinal pigment epithelium (RPE) to the continuous hyperreflective line on the sclera/choroid border. At the foveal center, CT was found as an average of the vertical and horizontal scanning results. We have chosen 13 measuring points in the area of 6x6 mm for statistical processing of the CT at the foveal center. Also, in the analysis we used a point situated 3 mm nasal from the foveal center towards the disc center (a point closest to the peripapillary area) [17].

#### Color Doppler imaging

Blood flow velocity in retrobulbar vessels was investigated by grey scale ultrasound, color Doppler imaging (CDI), and pulsed-wave Doppler (PWD). The ultrasound investigations were performed by VOLUSON 730 Pro ultrasound system (GE Medical Systems Kretztechnik) with SP 10-16 transducer. The patient was in supine position. Grey scale ultrasound allowed obtaining images of the globe and orbit. CDI displayed the fine orbital vessels directly. The studied vessels included ophthalmic artery (OA) and its branches, central retinal artery (CRA), lateral and medial posterior ciliary arteries (PCAs), central retinal vein (CRV), vortex veins (VV) (Figure 2), and superior ophthalmic vein. The blood flow in OA was measured at a depth of 35 mm. The blood flow velocity in CRA was examined in the optic nerve's canal, 5-6 mm from the posterior wall of the globe. PCAs were identified on either side of the optic nerve, approximately at the same distance from the fundus as the CRA and CRV.

PWD allowed measuring the blood flow spectrum of vessels and its main indices: peak systolic velocity (PSV), end-diastolic velocity (EDV), mean velocity (Vmean), resistive index (RI), and pulsatility index (PI).

#### OCT angiography

The study was performed in the peripapillary and macular areas using spectral-domain optical coherence tomography (SD-OCT) by means of RTVue-XR Avanti (Optovue Inc., Fremont, CA, USA) with Angiovue function (OCT-A), software version: 2016.1.0.26. We evaluated thickness of the macular area and RNFL by sector (Figure 1).

Two vascular plexuses were studied during OCT-A: a *superficial plexus* located in the layer with the upper boundary 3  $\mu\text{m}$  below the surface of the internal limiting membrane (ILM) and the lower boundary 15  $\mu\text{m}$  below the inner plexiform layer (IPL); and a *deep plexus* located in the retina from 15  $\mu\text{m}$  to 70  $\mu\text{m}$  below the IPL.

Two parameters were measured in the macular area: retinal thickness and relative vessel density (VD) of the retinal microvasculature. VD (%) is the ratio of the area of the vessels in the test spot to the area of the test spot.

Measurements were performed in the foveal area (1 mm diameter circle) and parafovea (in the border area between the fovea and 3 mm diameter circle around the center). Also, the fovea VD, parafovea VD and averaged value – *Whole En Face VD* (macula scan) were measured (Figure 1).

We measured relative density of the ONH microvasculature – *Whole En Face VD (disc scan)* – and Peripapillary VD in ONH and 750- $\mu\text{m}$ -wide elliptical annulus extending from the optic disc boundary in the layer of 100  $\mu\text{m}$  thickness from ILM, and only in the peripapillary area (*Peripapillary VD*), as shown in Figure 1.

Signal strength index (SSI) was reliable enough in all studied groups:  $53.2 \pm 6.9$  for disc scans and  $61.2 \pm 7.9$  for macula scans.

#### Statistical analysis

For the statistical analysis, a two-tailed Wilcoxon rank sum test was applied. Parameters having p-value < 0.05 were considered statistically significant. As a measure of the parameter importance in distinguishing the groups, we used AUC (area under the ROC curve). Three groups were compared by means of Kruskal–Wallis rank test. We studied correlations between most important parameters and selected just one (the most important) parameter from each strongly interconnected group. Correlation between parameters was measured by the Spearman's rank correlation coefficient  $r$ .

A number of parameters – GCC, GLV, systolic and mean perfusion pressure, choroidal thickness – depend on the anterior-posterior axis and the age of the subjects, thus, we carried out an adjustment for these parameters on the base of the linear regression model. Statistical analysis was performed using R library and SPSS Statistics 21.

## RESULTS

Structural and functional parameters of all studied subjects are shown in Table 1.

OCT-A parameters were reduced in glaucoma patients as compared to healthy subjects and between the stages of glaucoma (Table 2).

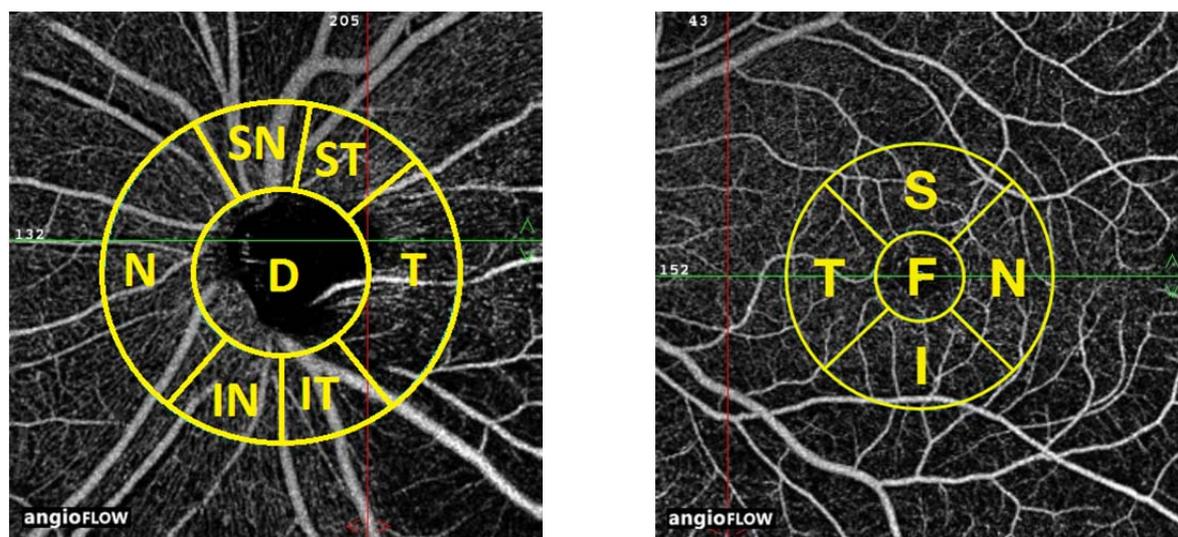
Blood flow parameters in OA, CRA, CRV and short PCA (SPCA) were significantly reduced in the early glaucoma as compared with the normal subjects (Table 3). This coincides with the results of our previous studies [15]. Significant difference in the CDI parameters between the stages of glaucoma was obtained only for CRA (Table 3).

We have revealed a link between VD in foveal-parafoveal (Whole En Face) superficial plexus and RI in OA in early glaucoma (Figure 2). No other significant correlations between the OCT-A and CDI parameters have been revealed in the studied groups.

**Table 1. Patients' characteristics**

Parameter	Controls (norm)	p-value*	Early POAG	p-value**	Moderate and severe POAG	total p-value***
Age, years	61.9(6.2)	0.622	62.5(7.6)	0.367	64.8(4.7)	0.071
BP systolic, mm Hg	126.6(4.6)	0.010	135.4(17.4)	0.472	130.9(13.7)	0.018
BP diastolic, mm Hg	81.4(6.9)	0.183	83.6(9.9)	0.726	83.15(8.2)	0.344
IOPcc, mm Hg	15.5(3.1)	0.001	19.4(4.6)	0.549	19.4(6.2)	<0.001
MOPP, mm Hg	50.4(2.4)	0.198	47.8(9.8)	0.594	46.6(7.7)	0.081
MD, dB	-0.03(0.84)	<0.001	-1.95(3.48)	<0.001	-12.13(6.2)	<0.001
PSD, dB	1.41(0.19)	0.006	2.20(1.62)	<0.001	9.79(3.59)	<0.001
RNFL, $\mu\text{m}$	101.9(6.1)	<0.001	91.8(10.3)	<0.001	69.3(14.3)	<0.001
GCC, $\mu\text{m}$	98.3(7.6)	0.001	89.2(10.4)	<0.001	70.5(10.4)	<0.001
FLV, %	0.21(0.23)	0.004	2.18(2.05)	<0.001	9.50(3.68)	<0.001
GLV, %	1.62(1.06)	0.001	7.60(6.10)	<0.001	25.91(9.44)	<0.001
Foveal CT, $\mu\text{m}$	312.3(88.5)	0.342	279.9(105.3)	0.906	276.6(82.6)	0.581
Peripapillary CT, $\mu\text{m}$	181.4(51.3)	0.348	179.3(93.3)	0.472	161.9(64.9)	0.346

Source: The table shows the median of data and standard deviation (in parentheses), the exact two-sided Wilcoxon rank sum test (p-value\*) between the control group and initial POAG, p-value\*\* between the POAG groups, total p-value\*\*\* obtained when comparing the three groups by means of Kruskal–Wallis test by ranks. Abbreviation expansions are given in the List of abbreviations.



A. Zones of ONH and peripapillary retina

B. Zones of the macular area (scan, 6x6 mm)

**Figure 1.** ONH, peripapillary and macular retina studied during OCT-A.

A: outer circumference located at a distance of 750 μm from the edge of ONH that is outlined by an inner ellipse. Peripapillary area: SN+ST+T+IT+IN+N, where SN – superonasal, ST – superotemporal, N – nasal, T – temporal, IN – inferonasal, IT – inferotemporal. Whole En Face VD (disc scan): D+peripapillary area, where D – ONH (Inside Disc). B: circumference with a diameter of 3 mm includes foveal zone (delimited by the inner circle of 1 mm in diameter) and parafovea. Parafovea: N+I+T+S, where N – nasal, I – inferior, T – temporal, S – superior. Whole En Face VD (macula scan): F + parafovea, where F – fovea.

**Table 2. Vessel density measured in study participants**

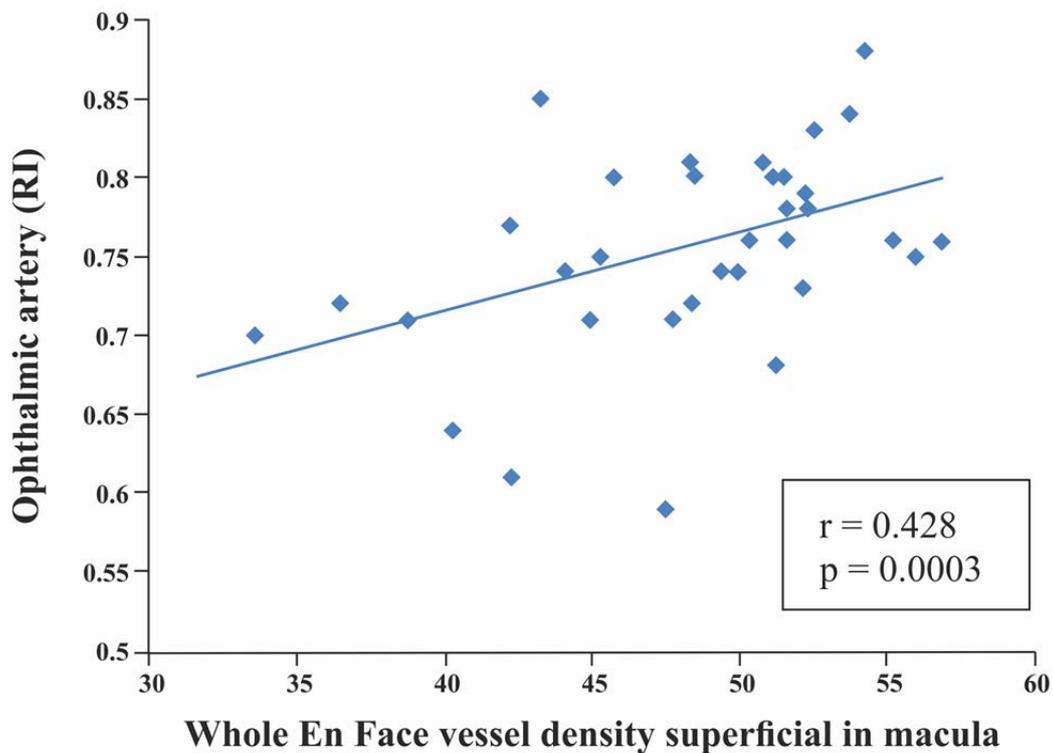
Parameter	Controls (norm)	p-value*	Early POAG	p-value**	Moderate and severe POAG
Whole En Face VD (disc scan)	55.8(2.2)	0.002	52.9(4.1)	<0.001	43.1(6.2)
Inside Disc VD	50.2(4.3)	0.001	45.3(6.5)	<0.001	37.7(5.6)
Average peripapillary VD	58.1(2.8)	0.396	56.9(4.9)	<0.001	46.3(7.5)
Nasal VD	56.9(4.1)	0.096	54.8(5.7)	<0.001	46.5(6.4)
Inferonasal VD	55.8(4.1)	0.02	57.6(7.4)	<0.001	45.0(8.2)
Inferotemporal VD	60.5(3.5)	0.730	59.8(4.7)	<0.001	44.4(8.9)
Superotemporal VD	60.4(3.6)	0.391	59.0(4.8)	<0.001	49.2(10.0)
Superonasal VD	55.6(4.3)	0.936	54.7(7.3)	<0.001	44.8(10.3)
Temporal VD	59.4(4.2)	0.619	58.0(7.0)	<0.001	47.0(11.8)
Whole En Face VD Superficial (macula scan)	50.7(3.0)	<0.001	45.9(5.0)	0.019	42.2(6.0)
Foveal VD superficial	36.4(5.7)	0.015	32.1(6.4)	0.444	30.8(6.5)
Parafoveal VD	52.0(5.2)	0.001	47.0(5.9)	0.765	46.3(5.2)
Temporal VD	52.4(5.2)	0.002	47.2(5.9)	0.585	46.1(6.0)
Superior VD	53.2(5.2)	<0.001	47.3(5.3)	0.799	47.4(5.4)
Nasal VD	51.2(5.9)	0.009	46.8(6.4)	0.743	46.3(6.2)
Inferior VD	50.8(6.1)	0.016	46.5(6.5)	0.390	45.3(5.8)
Whole En Face VD Deep	57.3(4.1)	0.001	52.2(6.9)	0.013	46.6(8.5)
Foveal VD Deep	35.4(7.6)	0.187	32.5(8.0)	0.005	26.3(8.6)
Parafoveal VD Deep	60.3(3.7)	0.003	56.3(5.4)	0.008	51.5(6.9)
Temporal VD	60.0(4.1)	0.013	55.8(6.7)	0.001	49.2(7.3)
Superior VD	62.1(4.0)	<0.001	57.0(5.1)	0.116	54.1(6.7)
Nasal VD	59.6(4.9)	0.021	56.3(6.0)	0.009	50.7(8.7)
Inferior VD	59.3(4.6)	0.049	56.0(6.6)	0.042	52.0(7.3)

Source: The table shows the median of data and standard deviation (in parentheses), the exact two-sided Wilcoxon rank sum test (p-value\*) between the control group and initial POAG, p-value\*\* between the POAG groups. Abbreviation expansions are given in the List of abbreviations.

**Table 3. Retrobulbar blood flow parameters in patients with different stages of glaucoma**

Parameters	Controls (norm)	p-value*	Early POAG	p-value**	Moderate and severe POAG
OA, PSV, cm/s	37.5(11.1)	0.011	31.7(7.7)	0.590	33.7(11.9)
OA, EDV, cm/s	11.3(5.6)	0.004	8.0(3.2)	0.957	7.8(3.7)
OA, Vmean, cm/s	19.2(7.4)	0.008	14.9(4.6)	0.536	15.6(6.1)
OA, RI	0.70(0.09)	0.054	0.74(0.09)	0.475	0.77(0.07)
OA, PI	1.49(0.39)	0.109	1.68(0.38)	0.831	1.69(0.46)
CRA, PSV, cm/s	12.4(1.8)	0.074	13.7(2.7)	<0.001	10.7(2.6)
CRA, EDV, cm/s	4.3(1.1)	0.277	4.2(0.9)	0.001	3.2(1.5)
CRA, Vmean, cm/s	6.8(1.4)	0.037	7.8(1.6)	<0.001	6.0(1.6)
CRA, RI	0.66(0.08)	0.037	0.70(0.06)	0.684	0.70(0.11)
CRA, PI	1.26(0.21)	0.591	1.22(0.18)	0.545	1.29(0.57)
TPCA, PSV, cm/s	14.3(2.8)	0.130	13.2(3.5)	0.669	12.9(4.6)
TPCA, EDV, cm/s	5.7(1.1)	0.005	4.7(1.3)	0.333	4.6(2.6)
TPCA, Vmean, cm/s	8.8(1.9)	0.231	8.1(2.7)	0.379	7.9(3.6)
TPCA,RI	0.63(0.09)	0.420	0.65(0.08)	0.553	0.68(0.12)
TPCA, PI	0.99(0.16)	0.390	1.31(1.14)	0.553	1.86(3.7)
CRV, PSV, cm/s	7.7(1.5)	0.031	6.8(1.4)	0.655	6.6(1.8)
CRV, EDV, cm/s	5.5(1.4)	0.073	4.7(1.4)	0.434	4.5(1.5)
CRV, Vmean, cm/s	7.9(2.5)	0.064	6.4(3.0)	0.294	6.0(3.4)
CRV, RI	0.37(0.11)	0.985	0.39(0.14)	0.327	0.35(0.15)
CRV, PI	0.53(0.15)	0.533	0.88(0.82)	0.657	0.98(0.92)
VV, PSV, cm/s	11.2(3.0)	0.205	9.8(4.1)	0.846	9.7(4.2)
VV, EDV, cm/s	6.9(1.6)	0.302	6.3(3.1)	0.878	6.3(2.4)
VV, Vmean, cm/s	9.5(1.9)	0.036	7.6(3.7)	0.734	7.2(3.7)
VV, RI	0.39(0.13)	0.607	0.37(0.15)	0.939	0.36(0.16)
VV, PI	0.54(0.19)	0.192	0.51(0.30)	0.917	0.48(0.23)

Source: The same as for Table 2.



**Figure 2.** Correlations between the perfusion of inner macula and ophthalmic artery blood flow (resistive index) in early glaucoma.

**Table 4. Correlation between RNFL thickness and blood flow parameters for early glaucoma and for the norm**

Blood flow parameters	RNFL Inferotemporal	
	r	p
TPCA, EDV	<b>0.747</b>	<b>&lt;0.001</b>
CRV, RI	0.516	<0.001
Whole En Face Superficial VD (macula scan)	0.590	<0.001
Whole En Face Deep VD (macula scan)	0.409	0.015
Peripapillary VD	0.340	0.034
VV, RI	0.52	0.001
VV, PI	0.53	0.001

Source: The Spearman's correlations and corresponding p-values for healthy subjects are given in bold italics. Abbreviation expansions are given in the List of abbreviations.

**Table 5. Correlation between the thickness of retina in the macula and parameters of blood flow for early glaucoma and for the norm**

Blood flow parameters	Mean parafoveal thickness from ILM to RPE				
	Average	Temporal	Superior	Nasal	Inferior
TPCA, EDV	<b>0.634</b>	<b>0.610</b>	<b>0.622</b>	<b>0.617</b>	<b>0.647</b>
	<b>0.002</b>	<b>0.003</b>	<b>0.002</b>	<b>0.002</b>	<b>0.001</b>
CRV, EDV	-0.254	-0.343	-0.284	-0.462	-0.191
	0.481	0.091	0.111	0.005	0.225
CRV, Vmean	-0.132	-0.213	-0.332	-0.261	-0.455
	0.335	0.124	0.181	0.534	0.005

Source: The same as for Table 4.

**Table 6. Correlation between VD in the inner layers of the macula and the GCC parameters in early glaucoma**

Parameters	Peripapillary VD		Superficial Parafoveal VD		Whole En Face Superficial VD	
	r	p	r	p	r	p
GCC	0.38	0.002	0.481	0.003	0.486	0.002
GLV	-0.42	<0.001	-0.505	0.001	-0.505	0.001
FLV	-0.36	0.003	-0.203	0.321	-0.105	0.451

**Table 7. Correlation between the capillary density in inner macula, optic nerve head, peripapillary retina and IOP in glaucoma subjects**

OCT-A parameters	IOP	
	r	p-value
Whole En Face VD (disc scan)	-0.162	0.353
Inside Disc VD	-0.191	0.264
Average peripapillary VD	-0.133	0.461
Whole En Face VD Superficial (macula scan)	-0.115	0.781
Foveal VD superficial	-0.142	0.371
Parafoveal VD	-0.374	0.291
Whole En Face VD Deep	-0.283	0.192

In healthy subjects, we have revealed a high correlation between diastolic blood flow velocity in PCA and the RNFL thickness in the inferotemporal sector (Table 4) and the mean macula thickness (Table 5). In early glaucoma there was link between RNFL thickness in the inferotemporal sector and VD in macula and peripapillary retina (Table 4). We have also found an inverse relation between the blood flow parameters in retrobulbar veins and the RNFL thickness in inferotemporal sector (Table 4) and macular thickness in the inferior and nasal areas (Table 5).

There was no relation between macular thickness and the OCT-A parameters in early glaucoma, and between blood flow parameters and peripapillary thickness of RNFL and macula in moderate/severe glaucoma.

A correlation was revealed between VD in the inner layers of macula and peripapillary retina and the GCC parameters in early glaucoma, but was not found in healthy subjects and in moderate/severe glaucoma (Table 6).

There was no significant correlation between IOP and OCT-A parameters in glaucoma subjects (Table 7).

On the basis of the obtained data, we selected parameters of hemoperfusion, which appeared most useful for differentiating all studied groups: Whole En Face VD in superficial plexus of the macula scan (AUC 0.8), average peripapillary VD (AUC 0.74), EDV in OA (AUC 0.74), and TPCA (AUC 0.72) (Table 8). Inferotemporal peripapillary VD (AUC 0.94) and Vmean in CRA (AUC 0.81) had the highest diagnostic value for distinguishing between the early POAG and moderate/severe glaucoma.

**Table 8. Diagnostic ability of hemoperfusion parameters studied by OCT-A and CDI in differentiating study groups**

Hemoperfusion parameters	p-value	AUC	AUC LCL*	AUC UCL**
Differentiating early POAG from control eyes				
Whole En Face VD Superficial (macula scan)	<0.0001	0.80	0.69	0.90
Peripapillary VD	0.001	0.75	0.63	0.87
OA, EDV	0.002	0.74	0.61	0.86
TPCA, EDV	0.005	0.72	0.58	0.86
Differentiating early POAG from moderate/severe glaucoma				
Inferotemporal peripapillary VD	<0.0001	0.94	0.86	1.0
CRA, Vmean	<0.0001	0.81	0.69	0.92

Source: AUC – area under the ROC curve; LCL – lower confidence limit of 95% confidence interval for AUC; UCL – upper confidence limit of 95% confidence interval for AUC.

## DISCUSSION

In this study we used two methods – CDI and OCT angiography – to evaluate the blood supply to the retina and optic nerve in glaucoma patients.

The literature suggests that CDI is a useful method for early diagnosis of glaucoma and makes it possible to determine various parameters of retrobulbar vessels [23; 25]. Unlike the CDI, OCT-A does not allow direct measurement of the blood flow volume passing through the vessel per time unit and does not give information about blood flow resistance (resistive index). However, OCT-A has significant advantages over CDI as it allows visualizing the microvasculature in different areas of the retina and at various depths. This is achieved due to the selection of blood vessels from the surrounding tissue at the entire scan depth without any contrast agents. OCT-A allows exploring not only the superficial plexus of the retina, but also the deep plexus [9; 28]. Thus, both methods – CDI and OCT-A – allow evaluation of the ocular hemoperfusion, but in different ways.

The present study showed that a reduction in the retrobulbar and retinal blood flow occurred already in early glaucoma. According to the literature, the reduction of hemoperfusion may be an important risk factor for glaucomatous optic neuropathy progression. For example, when studying patients with glaucoma in the dynamics, Martinez and Sanchez-Salorio [20] revealed that glaucomatous progression was associated primarily with low diastolic blood flow velocity in SPCA. SPCA are the main vessels involved in the blood supply to the optic nerve [6]. Our previous studies [14-17] demonstrated that there was a decrease in blood flow parameters in SPCA, as well as in OA, CRA and CRV already in pre-perimetric glaucoma. However, in spite of the CDI ability to measure blood flow in such small vessels as CRA and SPCA, the method does not allow visualization of the smallest capillaries of the peripapillary and macular areas.

According to histologic findings there are four capillary plexuses in the retina. Only three of them are located in the macula: superficial plexus, deep plexus, and intermediate plexus [27]. In 2015, Spaide et al. [24] suggested combining the intermediate plexus with the deep one.

In the present study, we revealed a decrease in the vessel density in the macular inner layers for both vascular plexuses in the early glaucoma. Moreover, this reduction reflected the loss of ganglion cells and their axons as it is shown in Table 6. Other authors also described the link

between structure and hemoperfusion in healthy subjects and in glaucoma [11; 26; 35]. This data is consistent with the understanding that the inner macular retina is supplied by the oxygen and nutrition from the small capillaries of the CRA system.

When comparing with the peripapillary blood flow, the diagnostic ability the macular microcirculation for early detection of the disease was greater (Table 8). In contrast to patients with early glaucoma, patients with moderate/severe glaucoma had a more pronounced deficit of capillaries in the peripapillary area, particularly in the inferior temporal sector.

The possible role of OCT-A in glaucoma diagnostics and monitoring has been demonstrated by Jia et al. [9; 10]. Some papers state the possibility of distinguishing between glaucomatous and normal eyes by means of OCT-A quantitative analysis of the entire peripapillary vasculature, from ILM to Bruch's membrane [18]. According to Yarmohammadi et al. [34], the decrease in the VD is strongly connected with the severity of visual field damage, and not the structural loss. Other authors found that the VD reduction inside optic disc was closely related to GCC thickness [29; 31].

To the best of our knowledge, we were the first who studied the microcirculation in the macular area in glaucoma with the use of OCT-A [16]. The present study supports the importance of evaluation vessel density in macula for early glaucoma detection: our results showed a significant decrease in the capillary density in early glaucoma as compared to healthy individuals of similar age. In contrast to our data, Rao and coauthors have recently reported that the parameters with the highest diagnostic abilities in differentiating glaucoma eyes from control eyes were the Whole En Face VD of the disc scan (AUC 0.90) and the inferotemporal sector peripapillary VD (AUC 0.89) [22]. This may be explained by the more severe glaucoma in their cohort (median MD -6.3 dB compared to -1.95 dB in our patients). As we have mentioned above, the peripapillary VD of the inferotemporal sector had the highest diagnostic ability in distinguishing between early and moderate/severe glaucoma. Hence, this data is consistent with the results obtained by Rao.

Besides, in contrast to the design of the present study, Rao and coauthors assessed only the pretreatment IOP. They revealed that the pretreatment IOP had a significant positive relation with the AUC of the inside disc VD, but did not influence the AUC of the peripapillary or

the parafoveal measurements. In our study, we failed to find a correlation between the IOP and OCT-A parameters in any of the studied regions (ONH, peripapillary retina, macula), though the patients had stopped using hypotensive eye drops three weeks before the examination. This suggests that the VD decrease in the macula and peripapillary retina in glaucoma might be independent on the IOP level.

According to the results of this study, one can assume that trophic deterioration in the capillaries of the inner retina layers explains the involvement of the macula in the pathological process at the very beginning of the disease. This was confirmed by the fact that in early glaucoma macular OCT-A parameters had a high correlation with the GCC characteristics (Table 5). In early glaucoma, the higher RI of OA was associated with less VD in foveal and parafoveal superficial plexus. We explain this by the autoregulation of ocular blood flow that can be still undamaged at the very beginning of the disease. According to Pechauer and his coauthors [21], the reduction of retinal microcirculation may occur as an autoregulatory response during hyperoxia. It can be assumed that significant blood supply to the retina due to the higher blood flow in OA causes the constriction of small retinal capillaries. This leads to the blood flow reduction in retinal vascular plexuses, and some of plexus' vessels can't be detected by OCT-A.

In the normal subjects, RNFLT in the peripapillary retina and macula correlated with the diastolic blood flow velocity in SPCA; this speaks to the fact that SPCA are the main vessels that supply ONH [6]. In early glaucoma, we also detected a link between the venous blood flow and macula thickness: disturbed blood outflow in CRV and VV was associated with the greater thickness of macula and RNFL. This fact is noteworthy since it can be associated with venous stasis at the very beginning of the glaucomatous process and it explains the increase in pressure in CRV during the disease [3]. The venous blood flow reduction was revealed in our previous studies [13-17]. Correlation between the morphometric parameters and venous blood flow, revealed only in early glaucoma and absent in healthy subjects or in advanced glaucoma, may suggest that disturbed blood outflow from the eye is one of the triggers of the glaucoma development. It also should be noted that a high correlation was revealed between VD in the inner layers of macula and peripapillary retina and the GCC parameters in early glaucoma, but not in healthy subjects and in moderate/severe glaucoma. In advanced glaucoma, we did not reveal any correlation between the retrobulbar blood flow parameters of structural changes in the retina. It is not still clear which correlation is stronger: between the microcirculation and structure or between the microcirculation and functional parameters as well as between the structural and functional change. According to Hollo [7], the relation between the inferotemporal sector peripapillary VD and superotemporal cluster of visual fields (MD) was stronger than that between inferotemporal sector RNFLT and superotemporal cluster MD. Akil et al. [1] found that that ONH VD in POAG group was more strongly linked to RNFL thickness than to any other

variables. Further studies are needed for better understanding the relations between structural, functional parameters and retinal microcirculation.

Our findings suggest that blood flow parameters studied by means of CDI and OCT-A give different diagnostic information. CDI method allows better evaluation of the pathogenic processes that occurs early in the disease. Reduced blood flow in the retrobulbar vessels may indicate failure of the ocular blood supply autoregulation, development of venous stasis and reduced blood supply to retina via OA. At the same time, OCT angiography allows obtaining information about the retinal blood flow deficiency in the conditions of incipient atrophy of the nerve tissue.

We carried out a regression analysis, which demonstrated that the diagnostic value of OCT-A in glaucoma exceeds that of the retrobulbar blood flow measured by CDI (Table 8). This conclusion is valid for both – the early detection of the disease, when the reduction in the capillary density in the macular inner layers is the most informative, and the monitoring of glaucoma, when the state of the peripapillary retina hemoperfusion becomes more essential.

There are limitations to be taken into account when considering the results of our study. After three weeks of washing out the eye drops, IOP was rather low (Table 1). However, according to anamnesis of patients, previously all of them had IOP values > 21 mm Hg.

CDI data should be carefully interpreted taking into account subjectivity of CDI measurements [5].

However, our study has an advantage as, in contrast to the studies mentioned above, we stopped the administration of anti-glaucoma medications for POAG patients because they could affect the vessel density.

The obtained results do not resolve the question of the cause-and-effect relations in POAG: whether the reduction in hemoperfusion is primary or secondary event in relation to the structural changes of the retina. It has been repeatedly shown that ocular blood flow reduction generally was characteristic of glaucoma [6]. However, it should be recognized that these data do not give evidence that the reduction in the ocular blood flow is the cause or even trigger of the POAG development. Indeed, the reduction of hemoperfusion may occur due to the degeneration of the retina and optic nerve structures; blood supply to them becomes unnecessary as the disease progresses. Another explanation for this phenomenon is the increased IOP in glaucoma as compared to healthy individuals. However, this explanation can be disposed by the fact that blood flow parameters did not correlate with the IOP data in any of the studied groups. We believe that, in the context of our study and in accordance with the study purpose, we ascertained that both methods – CDI and OCT-A – are useful for the diagnosis of glaucoma and contribute to the early detection and monitoring of the disease.

## CONCLUSIONS

According to our findings, there is a decrease in VD in glaucoma in optic disc, peripapillary area and macula, based on OCT-A measurements. CDI provided

extra data on ocular blood circulation in glaucoma. The relation between the retinal and ONH microcirculation and retrobulbar blood flow and structural parameters were revealed in normal eyes and early glaucoma. Both methods are useful for early glaucoma detection and monitoring.

#### LIST OF ABBREVIATIONS

AUC – area under the receiver operating characteristic curve  
 BP – arterial blood pressure  
 CDI – color Doppler imaging  
 CRA – central retinal artery  
 CRV – central retinal vein  
 CT – choroidal thickness  
 EDV – end-diastolic velocity  
 FLV – focal loss volume  
 GCC – ganglion cell complex  
 GLV – global loss volume  
 ILM – internal limiting membrane  
 IOP – intraocular pressure  
 IOPcc – corneal-compensated intraocular pressure  
 IPL – inner plexiform layer  
 MD – mean deviation  
 MOPP – mean ocular perfusion pressure  
 OA – ophthalmic artery  
 OCT – optical coherence tomography  
 OCT-A – optical coherence tomography angiography  
 ONH – optic nerve head  
 PCA – posterior ciliary artery  
 PI – pulsatility index  
 POAG – primary open-angle glaucoma  
 PSD – pattern standard deviation  
 PSV – peak systolic velocity  
 PWD – pulsed-wave Doppler  
 RI – resistive index  
 RNFL – retinal nerve fiber layer  
 RNFLT – retinal nerve fiber layer thickness  
 ROC – receiver operating characteristic  
 RPE – retinal pigment epithelium  
 SAP – standard automated perimetry  
 SD-OCT – spectral-domain optical coherence tomography  
 SITA – Swedish Interactive Threshold Algorithm  
 SSADA – split-spectrum amplitude-decorrelation angiography  
 SPCA – short posterior ciliary artery  
 TPCA – temporal short posterior ciliary artery  
 VD – vessel density  
 Vmean – mean velocity  
 VV – vortex vein

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