



Evaluation of Factors Predicting Glaucoma Damage in Patients with Primary Open-angle Glaucoma

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Abstract

Purpose: To evaluate factors predicting glaucoma damage in primary open-angle glaucoma (POAG) eyes.

Methods: 20 POAG patients were examined in this retrospective study. The most affected eye was analyzed. Peripapillary (P) choroidal thickness (CT) and macular (M) CT were measured by Swept Source Optical Coherence Tomography. Correlation between CT retinal nerve fiber layer thickness (RNFLT) and ganglion cell-inner plexiform layer thickness (GCIPLT) and various other factors including mean deviation (MD), pattern standard deviation (PSD), mean superior and inferior hemifield sensitivity was also assessed by Pearson's r correlation coefficients and linear regression model.

Results: Superior and inferior nasal MCT and PCT were significantly correlated with MD ($r = 0.509$, $r = 0.507$, $r = 0.577$, respectively). MD was positively associated with mean GCIPLT ($r = 0.517$, $p = 0.02$) and each GCIPLT sector ($p < 0.05$), except for the nasal sector. A positive relationship was found between RNFL thickness (mean and each sector) and MD ($p < 0.05$). PSD was negatively correlated to both GCIPLT ($r = -0.695$, $p = 0.001$) and RNFL thickness ($p < 0.05$). Among mean PCT, mean MCT, RNFL thickness and mean GCIPLT, only RNFL thickness ($\beta = 0.85$, $p = 0.011$) and mean PCT ($\beta = 0.521$, $p = 0.021$) were predictive factors of MD in the linear regression model.

Conclusions: RNFL thickness was the most predictive factor of MD, followed by mean PCT. RNFL thickness seems to be the most useful parameter to predict glaucoma damage in clinical practice.

Keywords: Glaucoma; Swept Source Optical Coherence Tomography; Choroidal Thickness; Retinal Nerve Fiber Layer

Introduction

Primary open-angle glaucoma (POAG) is a chronic, progressive, optic neuropathy with characteristic morphological changes at the optic nerve head (ONH) and retinal nerve fiber layer in the absence of other ocular disease or congenital anomalies. Progressive reti-

nal ganglion cell loss is associated with macular, papillary and peripapillary changes [1]. The reason of damage is not yet understood, but it could be multifactorial [1]. Vascular pathogenetic theory is still under investigation and since the introduction of new technologies able to make visible the choroid, choroidal thickness (CT) is

aimed of new researches into normal and pathological processes, because choroidal vessels perfuse the prelaminar part of the optic disc [2]. Post-mortem histological studies have reported the choroid to be thinner in glaucoma, but it is unclear whether this finding represents a risk factor or a consequence of the disease. Furthermore, histology is unlikely to represent the thickness of the living choroid, which consists of prominently of blood vessels [3-5]. With the introduction of enhanced depth imaging optical coherence tomography (EDI-OCT), it has been possible to obtain *in vivo* images of the choroid [5]. However, EDI-OCT has some limitations because it is difficult to distinguish the choroidal-scleral boundary and no choroidal segmentation software is not available yet, so users must identify choroidal-scleral boundary manually [6]. Recently, because of the deeper penetration of the Swept Source OCT (SS-OCT), the ability to assess the choroid has improved [7].

In most of the OCT studies between open-angle glaucoma (OAG) and healthy eyes no difference was found for peripapillary or macular CT (PCT and MCT, respectively), and no relationship was shown between CT and different glaucoma stages [8-16]. However, in some other studies a thinning of PCT or MCT has been found in OAG patients [17-20]. In particular, Hirooka, *et al.* [21] found a relationship between glaucoma stages and CT especially in the nasal region 3 mm from the fovea toward to the ONH. This area is close to the peripapillary choroid which could be connected to the ONH blood supply.

It's has been shown that the CT depends on several factors [5] such as older age, higher intraocular pressure (IOP), higher myopia, thicker central corneal thickness (CCT) and longer axial length (AXL), which could be associated with a thinner choroid [5,22]. While higher diastolic perfusion pressure, a lower IOP, male gender and water drinking test in POAG could be associated with a thicker choroid [5,22]. Changes in IOP, alterations in blood pressure (BP) or ocular perfusion pressure (OPP) could change CT [5] suggesting that choroid also varies on a diurnal basis [22]. All these data suggested that choroid is a dynamic structure.

The purpose of this study is to evaluate and compare factors predicting glaucoma damage in POAG eyes, such as CT as a predictive factor of this damage using the following parameters visual field indices, retinal nerve fiber layer (RNFL), ganglion cell layer-inner plexiform layer (GCIPL) and ganglion cell complex (GCC) [23].

Methods

This study was a clinical retrospective one, and it was approved by the ethics committee (CER Liguria 138/2021). Furthermore, all

patients attending the University Eye Clinic of Genoa give their consent to future use of data in retrospective studies.

This study included 20 POAG patients treated at the University Eye Clinic of Genoa, San Martino Hospital, Genoa, Italy that were assessed in June 2019. The most affected eye was analyzed according to Mean Deviation (MD) and Pattern Standard Deviation (PSD) values.

All subjects were required to have a refractive error less than -6.0 diopters of sphere or 3 diopters of cylinder, no history of retinal diseases (i.e. diabetic retinopathy, macular degeneration, optic neuritis), a normal anterior chamber, a clinical diagnosis of POAG at a previous visit (at least one year before the study) and a last follow-up visit within one month before the SS-OCT scan. Exclusion criteria included those who were under 18 years old, a history of ophthalmic diseases that could affect the interpretation of the visual field, a history of ocular trauma or glaucoma surgery, poor quality SS-OCT images (defined as those with signal strength ≤ 40 and with motion artifacts, involuntary saccades, or overt misalignment of decentration), visual fields with more than 33% fixation losses or false-negative errors, or more than 15% false-positive errors.

POAG patients were defined when optic nerve head had typical glaucomatous damage and/or retinal nerve fiber layer changes, glaucomatous visual field defects and an open anterior angle on gonioscopy [1].

Data of the last follow-up visit were recorded and included: review of medical history, body mass index (BMI), water intake in the previous two hours, systolic blood pressure (SBP), diastolic blood pressure (DBP), number of glaucoma medications being used, best-corrected visual acuity (BCVA) (using a Snellen chart at 4 m), spherical equivalent (SE), slit-lamp biomicroscopy, IOP measurement (using a calibrated Goldmann applanation tonometer), gonioscopy, dilated funduscopy examination, cup-to-disc ratio (using a 90D lens), CCT, axial length (AXL), CT, retinal nerve fiber layer thickness (RNFLT) and ganglion cell-inner plexiform layer thickness (GCIPLT).

Mean blood pressure (BP) was calculated using the following formula: Mean BP = DBP + (1/3*[SBP-DBP]). Diastolic and systolic ocular perfusion pressure was calculated as diastolic or systolic BP minus IOP respectively. Mean ocular perfusion pressure was calculated as the difference between mean BP and IOP.

CCT was measured using RTVue-100 Fourier-domain optical coherence tomography (OCT) device (Optovue, Inc., Fremont, CA),

while AXL was obtained through Optical Biometer AL- scan (Nidek Co, Ltd., Gamagori, Japan).

CT, RNFLT and GCIPLT were assessed using SS-OCT (DRI OCT Triton, Topcon, Oakland, NJ). All eyes were imaged using the 3D Wide Scan (12 mm x 9 mm) with the scan centered on the posterior pole through dilated pupils. All SS-OCT images were obtained by a single and well- trained technician. Using the SS-OCT segmentation software (version 9.11, Topcon, Inc., Tokyo, Japan) the limits of the choroid were identified and to assess CT. The data were exported using the manufacturer’s OCT-Batch (version 9.1.10) utility.

After segmentation, 6 and 12 sectors of peripapillary RNFL thickness (pRNFLT) and 6 and 12 sectors of PCT were calculated automatically by a 3,4 mm diameter peripapillary circle centered on the optic disc (Figure 1A, 1B). The localized and global pRNFLT and PCT were calculated as the thicknesses in each sector or as the mean thicknesses of the localized pRNFLT and PCT, respectively.

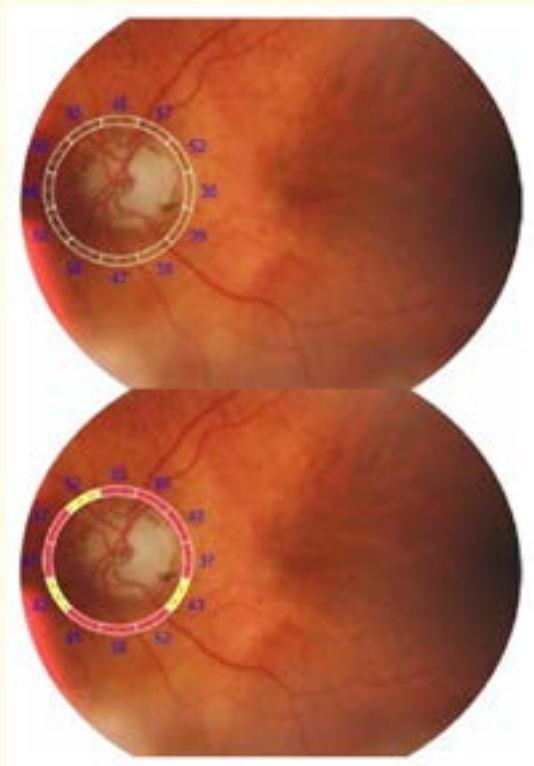


Figure 1: Measurement of 12 clock-hour peripapillary choroidal thickness (PCT) (A) and retinal nerve fiber layer thickness (RNFLT) (B) using 3D Wide Scan (12 mm x 9 mm).

By way of a 6x6 mm diameter parafoveal circle centered on the fovea GCIPLT was calculated automatically in the six (6) sectors (superotemporal (ST), superior (S), superonasal (SN), inferotemporal (IT), inferior (I), inferotemporal (IT)) of the MCT (Figure 2A, 2B).

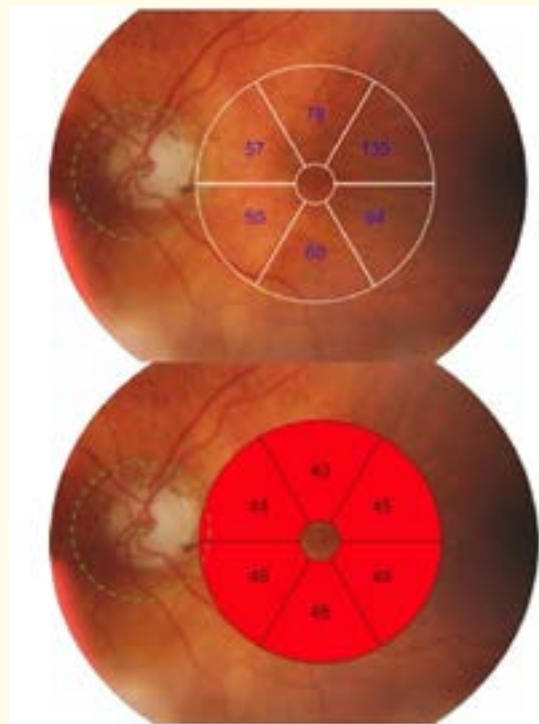


Figure 2: Measurement of macular choroidal thickness (MCT) (A) and ganglion cell- inner plexiform layer thickness (GCIPLT) (B) within 6x6 mm diameter parafoveal circle using 12x9 mm Wide scan.

Each patient performed standard automated perimetry (SAP) using the Humphrey Field Analyzer II (Carl Zeiss Meditec, Dublin, CA, USA) within 6 months of the OCT study and the 24-2 Swedish interactive threshold algorithm (SITA Standard 24-2, Carl Zeiss Meditec, Inc., Dublin, CA, USA) program was used. We additional calculated the mean sensitivity of superior and inferior hemifield from each patient’s visual field report.

Statistical analysis

The statistical analyses were performed using SPSS 21.0 (SPSS, Inc., Chicago, IL, USA).

The relationships between CT and various factors including age, gender, race, diabetes mellitus, systemic hypertension, BMI, water intake in the previous two hours, SBP, DBP, OPP (systolic, diastolic and mean)multi, SE, IOP, AXL, CCT, visual field MD, visual field PSD, mean superior and inferior hemifield sensitivity were analyzed by Pearson’s r correlation coefficient and linear regression analysis. The relationships between any OCT parameter (GCIPLT, RNFLT, PCT, MCT) and the visual field indices (MD, PSD) and mean superior and inferior hemifield sensitivity were analyzed by using Pearson’s correlation coefficient.

When variables had a p value < 0.05 in the univariate regression, those were included in the subsequent linear regression model. A p value < 0.05 were considered statistically significant in all of the analyses.

Results

Twenty eyes of 20 PAOG patients were included in this study. The mean age was 74.15 ± 8.39 (range 56-87 years) and 8/20 (40%) were females. 18/20 (90%) patients were Caucasian, while 2/20 (10%) were Latin American. The descriptive parameters are list in table 1 and 2.

Number of eyes	20
Eye laterality, right eye, % (n)	40% (8)
Pseudophakia , % (n)	55% (11)
Median Spherical equivalent, Diopters	-0,25
Mean IOP at imaging, mmHg	15.35 (2.80)
Systolic OPP, mmHg	118.90 (13.32)
Diastolic OPP, mmHg	64.15 (9.48)
Mean OPP, mmHg	80.15 (10.06)
Cup-to-disc ratio	0.80 (0.12)
Axial length, mm	24.06 (1.20)
Mean CCT, µm	530.20 (40.10)
MD, dB	-6.52 (6.39)
PSD, dB	6.23 (3.95)
Mean superior hemifield sensitivity, dB	21.51 (5.18)
Mean inferior hemifield sensitivity, dB	21.05 (7.26)

Table 2: Ocular characteristics of subjects.

Mean and standard deviation (SD) unless specified otherwise. IOP = Intraocular Pressure; OPP = Ocular Perfusion Pressure, CCT = Central Corneal Thickness; MD = Mean Deviation; PSD = Pattern Standard Deviation.

	POAG patients
Number	20
Age, years (SD)	74.15 (8.39)
Female Gender, % (n)	40% (8)
Race, % (n) Caucasian	90% (18)
Latin American	10% (2)
BMI, kg/m ² (SD)	27.82 (5.01)
Diabetes mellitus, % (n)	15% (3)
Systemic hypertension, % (n)	65% (13)
N° glaucoma meds, (SD)	2.15 (1.04)
SBP, mmHg (SD)	134.25 (14.07)
DBP, mmHg (SD)	78.50 (10.01)
MAP, mmHg (SD)	95.50 (11.11)
Water intake in the previous two hours, ml (SD)	142.50 (185.16)

Table 1: Demographic characteristics of subjects.

Mean and standard deviation (SD) unless specified otherwise. POAG = Primary Open Angle Glaucoma; BMI = Body Mass Index; SBP = Systolic Blood Pressure; DBP = Diastolic Blood Pressure; MAP = Mean Arterial Pressure.

Superior and inferior nasal MCT were positively correlated with MD (r = 0.509 and r = 0.507, p < 0.05, respectively) (Table 3). Mean PCT and each of the 6 sectors of PCT showed significant correlation with MD (p < 0.05), above all the inferior nasal and the inferior temporal sectors (r = 0.594 and r = 0.628, p < 0.01, respectively) (Table 4). Similar correlation was obtained between the 12 sectors of PCT and MD. There was no association between CT and PSD, mean superior and inferior hemifield sensitivity (p > 0.05) (Table 3 and 4).

MD was positively associated with mean GCIPLT (r = 0.517, p = 0.02) and each GCIPLT sector (p < 0.05), except with the superior and inferior nasal sectors. PSD was negatively correlated with GCIPLT(p < 0.05). Mean GCIPLT and some of the six sectors of GCIPLT showed a positive correlation with mean superior and inferior hemifield sensitivity (p < 0.05) (Table 5).

A positive relationship was found between RNFLT (mean, each 6 sector and some of the 12 sectors) and MD (p < 0.05), in particular the nasal and the inferior temporal sector showed the most positive correlation (r = 0.579, r = 0.624, p < 0.01, respectively). Each 6

		Mean MCT	MCT_TS	MCT_S	MCT_NS	MCT_NI	MCT_I	MCT_TI
MD	r	0.40	0.14	0.39	0.51*	0.51*	0.37	0.20
	P value	0.083	0.553	0.086	0.022	0.023	0.109	0.402
PSD	r	0.15	0.14	0.002	0.06	0.07	0.20	0.30
	P value	0.533	0.548	0.993	0.799	0.758	0.386	0.197
Mean superior hemifield sensitivity	r	-0.05	-0.15	0.07	0.12	0.09	-0.10	-0.28
	P value	0.849	0.539	0.779	0.607	0.693	0.684	0.235
Mean inferior hemifield sensitivity	r	0.19	0.25	0.40	0.18	0.11	0.07	-0.01
	P value	0.434	0.291	0.079	0.447	0.644	0.780	0.977

** . Correlation is significant at the 0.01 level
 * . Correlation is significant at the 0.05 level

Table 3: Pearson correlations between visual field indices and choroidal thickness.
 MD = mean deviation; PSD = pattern standard deviation; MCT = choroidal macular thickness.

		Mean PCT	PCT6T	PCT6TS	PCT6NS	PCT6N	PCT6NI	PCT6TI
MD	r	.58**	.49*	.53*	.52*	.49*	.59**	.63**
	P value	0.008	0.030	0.016	0.019	0.030	0.006	0.003
PSD	r	.06	.16	.13	.14	.07	-.16	-.017
	P value	0.807	0.495	0.596	0.556	0.761	0.512	0.475
Mean SHS	r	.14	.04	.09	.08	.15	.23	.26
	P value	0.547	0.876	0.704	0.740	0.533	0.325	0.269
Mean IHS	r	.11	.05	.11	.08	.02	.24	.25
	P value	0.652	0.838	0.649	0.737	0.925	0.301	0.280

** . Correlation is significant at the 0.01 level.
 * . Correlation is significant at the 0.05 level.

Table 4: Pearson correlations between visual field indices and peripapillary choroidal thickness.
 MD = Mean Deviation; PSD = Pattern Standard Deviation; PCT = Peripapillary Choroidal Thickness.
 SHS: Superior Hemifield Sensitivity; IHS: Inferior Hemifield Sensitivity

		Mean GCIPL	GCIPL_TS	GCIPL_S	GCIPL_NS	GCIPL_NI	GCIPL_I	GCIPL_TI
MD	r	.52*	.49*	.59**	.25	.3	.47*	.53*
	P value	0.020	0.030	0.007	0.289	0.206	0.036	0.016
PSD	r	-.70**	-.60**	-.67**	-.45*	-.53*	-.61**	-.60**
	P value	0.001	0.006	0.001	0.047	0.016	0.004	0.005
Mean superior hemifield sensitivity	r	.52*	0.41	.48*	0.33	.47*	.48*	.44*
	P value	0.018	0.075	0.034	0.152	0.039	0.033	0.050
Mean inferior hemifield sensitivity	r	.56**	.56*	.67**	.367	.33	.42	.43
	P value	0.010	0.011	0.001	0.110	0.155	0.064	0.059

** . Correlation is significant at the 0.01 level.
 * . Correlation is significant at the 0.05 level.

Table 5: Pearson correlations between visual field indices and GCIPL thickness.
 MD = Mean Deviation; PSD = Pattern Standard Deviation; GCIPL = Ganglion Cell-Inner Plexiform Layer.

and 12 sector of RNFLT was negatively correlated with PSD ($p < 0.05$). Most of the 6 e 12 sectors of RNFLT showed a positive correlation with both mean superior and inferior hemifield sensitivity ($p < 0.05$) (Table 6). No significant correlation was shown between

CT and age, gender, race, diabetes mellitus, systemic hypertension, BMI, water intake in the previous two hours, SE, IOP, CCT, AXL, BP (systolic, diastolic and mean) and OPP ($p > 0.05$).

		Mean RNFL	RNFL 6T	RNFL 6TS	RNFL 6NS	RNFL 6N	RNFL 6NI	RNFL 6TI	RNFL 12T	RNFL 12 TS	RNFL 12ST	RNFL 12S	RNFL 12SN	RNFL 12NS	RNFL 12N	RNFL 12NI	RNFL 12IN	RNFL 12I	RNFL 12IT	RNFL 12TI
MD	r	.64**	.49*	.45*	.49*	.58**	.55*	.62**	.4	.49*	.43	.30	.55*	.51*	.39	.58**	.4	.66**	.58**	.44
	P value	0.002	0.029	0.047	0.027	0.007	0.011	0.003	0.080	0.027	0.057	0.089	0.012	0.021	0.088	0.007	0.078	0.001	0.008	0.055
PSD	r	-.82**	-.75**	-.73**	-.52*	-.64**	-.66**	-.82**	-.60**	-.76**	-.74**	-.5*	-.51*	-.5*	-.47*	-.7**	-.5*	-.8**	-.78**	-.66**
	P value	0.001	0.001	0.001	0.018	0.002	0.002	0.001	0.005	0.001	0.001	0.026	0.021	0.026	0.038	0.001	0.025	0.001	0.001	0.001
Mean SHS	r	.7**	.65**	.42	.37	.67**	.69**	.65**	.58**	.57**	.43	.29	.38	.47*	.55*	.78**	.53*	.77**	.6**	.67**
	P value	0.001	0.002	0.069	0.112	0.001	0.001	0.002	0.008	0.009	0.057	0.215	0.098	0.035	0.012	0.001	0.017	0.001	0.006	0.001
Mean IHS	r	.65**	.54*	.7**	.61**	.46*	.46*	.58**	.45*	.61**	.66**	.57**	.63**	.43	.16	.45*	.33	.6**	.536*	.39
	P value	0.002	0.014	0.001	0.004	0.044	0.040	0.007	0.047	0.004	0.002	0.009	0.003	0.062	0.503	0.047	0.160	0.005	0.015	0.088

** Correlation is significant at the 0.01 level.

* Correlation is significant at the 0.05 level.

Table 6: Pearson correlations between visual field indices and RNFL thickness

MD = Mean Deviation; PSD = Pattern Standard Deviation; RNFL = Retinal Nerve Fiber Layer.

SHS: Superior Hemifield Sensitivity; IHS: Inferior Hemifield Sensitivity

Among mean PCT, mean MCT, mean RNFLT and mean GCIPLT, only mean RNFLT ($\beta = 0.85$, $p = 0.011$) and mean PCT ($\beta = 0.521$, $p = 0.021$) were predictive factors of MD in the linear regression model (Table 7) (Figure 3). Between mean RNFLT and mean PCT, mean RNFLT was more predictive of both MD and PSD.

Variable	Beta	t	P*
Mean RNFLT	0.85	2.878	0.011
Mean PCT	0.521	2.58	0.021
Mean MCT	0.084	0.401	0.694
Mean GCIPLT	-0.22	-0.74	0.47
Dependent variable: MD.			
*P < 0.05 significant			

Table 7: Univariate regression analysis of OCT parameters with MD.

RNFLT = Retinal Nerve Fiber Layer Thickness; PCT = Peripapillary Choroidal Thickness; MCT: Macular Choroidal Thickness; GCIPLT = Ganglion Cell-Inner Plexiform Layer Thickness; MD = Mean Deviation.

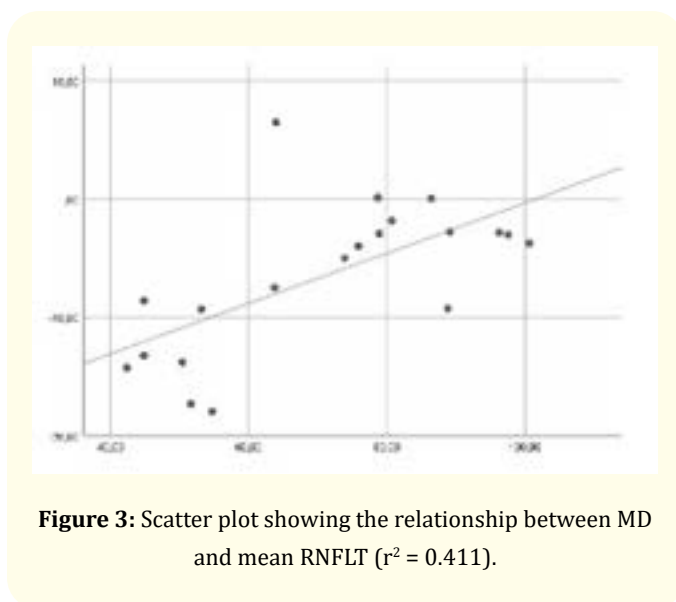


Figure 3: Scatter plot showing the relationship between MD and mean RNFLT ($r^2 = 0.411$).

Discussion and Conclusion

Glaucoma is a multi-factorial optic neuropathy, often asymptomatic disease, characterized by structural damage of retinal ganglion cells (RGC) followed by vision loss [24]. Different are the pathophysiological theories of glaucoma, but there are still under investigation. As the prelaminar area of the ONH is perfused by choroidal derived vessels, there is interest in understanding the role of the choroid in the pathogenesis [2]. Because glaucomatous damage is a chronic, progressive and irreversible neurodegenerative disease and it is possible to reduce the loss of ganglion cells with a treatment, it's mandatory to try to improve the detection of glaucoma progression [25-27].

The introduction of OCT has enabled the evaluation of optic nerve head and retina [28,29] and in particular the RNFL which is well detectable in the peripapillary area [25]. First peripapillary RNFL measurement was used to detect glaucoma and its progression, then in longitudinal studies other parameters such as GCC, GCIPL and ONH parameters (rim area, cup area and cup-to-disc ratio) have been shown to be useful for evaluating glaucoma progression [30-32].

In the present study we evaluated factors predicting glaucoma damage in POAG patients using a SS-OCT which is a newer generation of OCT with a better accuracy of the measurements. Among mean PCT, mean MCT, mean RNFLT and mean GCIPLT, only mean RNFLT and mean PCT were predictive factors of MD in the linear regression model. Between mean RNFLT and mean PCT, mean RNFLT was more predictive of both MD and PSD.

Lin., *et al.* [23] reported significant correlations between the blue-on-yellow (B/Y) MD and PCT, while for the white-to-white (W/W) MD [9,19]. Hirooka, *et al.* [21] showed a relationship between CT and glaucoma damage in particular in the nasal region 3 mm from the fovea, which is close to the ONH and it could affect its ONH blood supply.

Several previous studies explored the relationship between OAG and PCT, reporting conflicting results. A meta-analysis conducted by Lin., *et al.* [33] showed that the average PCT in OAG was significantly reduced compared to healthy individuals, while previous meta-analyses conducted by Wang and Zhang [34] and Zhang, *et al.* [8] demonstrated no correlation between PCT and OAG.

Recent studies also evaluated possible differences in macular CT between glaucoma and healthy patients. In the study of Lin., *et al.* [23], no significant difference was found for macular CT. Mwanza, *et al.* [11] also compared the macular CT among 56 POAG, 20 NTG

and 38 healthy subjects and no significant difference was found. Also Nakakura, *et al.* [35] using swept-source OCT found similar results between 40 POAG with 48 healthy subjects. Furthermore, recent meta-analyses [8,34] found that the macular CT did not change significantly in open-angle glaucoma, discarding the possibility to use it as a parameter to detect glaucoma.

In our study, mean MCT and PCT (152.27 μm and 98.46 μm , respectively) appeared thinnest when compared with results of others studies among healthy individuals, in particular Ruiz-Medrano, *et al.* showed that mean macular CT was $229.7 \pm 66.1 \mu\text{m}$ in subjects older than 60 years [36], while Yang, *et al.* reported a PCT ranged from 108.81 μm to 172.47 μm [37]. Furthermore, we did not find any associations between CT and age, gender, race, diabetes mellitus, hypertension, BMI, water intake in the previous two hours, spherical equivalent, IOP, CCT, AXL, BP and OPP (systolic, diastolic and mean) ($p > 0.05$).

The present study has some limitations such as the lack of the control group was lacking; the small number of eyes included in the study; and a potential confounding effect of anti-glaucoma drugs on the hemodynamics of peripapillary vessels exists. It is unknown if anti-glaucoma eyedrops could affect peripapillary perfusion and this aspect was not investigated in this study. Furthermore, there is a possible confounding effect of several systemic conditions that could influence the vascular physiology and cause choroidal changes.

In conclusion, RNFL thickness was the most predictive factor of MD, followed by mean PCT. Therefore RNFL thickness seems to be the most useful parameter to predict glaucoma damage in clinical practice.

Synopsis

Structure-function analysis is fundamental to detect glaucomatous changes, the visual field correlation with retinal nerve fibers has been already shown by several authors, interesting is the possible relationship with the choroidal thickness.

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MM and MI conceived the study; MM, AP, CAC and GA collected the data and patients; MI analyzed the data; MM, AF, CET and MI wrote the manuscript, all the authors revised the manuscript.

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