Axons of retinal ganglion cells on the optic nerve disc following vessel density correction at different IOP values

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Abstract. The present study aimed to determine how the vascular density (VD) in each segment peripapillary influences the retinal nerve fiber layer (RNFL) and to eliminate its contribution to RNFL in pathological intraocular pressure (IOP). In a cohort of 69 subjects (mean age, 45±6 years old) with untreated ocular hypertension (122 eyes in total) enrolled in this study, Ocular Response Analyser IOP was measured during routine outpatient care. Its value was >21 (range, 21-36) mmHg in all eyes. Furthermore, peripapillary VD and RNFL were measured using optical coherence tomography in the following eight segments: Inferior temporal (segment 1); temporal inferior (segment 2); temporal superior (segment 3); superior temporal (segment 4); superior nasal (segment 5); nasal superior (segment 6); nasal inferior (segment 7); and inferior nasal (segment 8). The visual field examination was performed with the fast threshold glaucoma program using the Medmont M 700. The overall defect was evaluated. Person's correlation coefficient was used to assess the correlation between VD and IOP. The largest changes were observed in peripapillary segments 1, 4, 5, 6, 7 and 8. The second part of the work was to eliminate the contribution of VD to RNFL. The partial correlation coefficient r was used to adjust RNFL from VD to assess the dependence between the selected parameters. The largest changes in RNFL were in segments 5 and 8 after they had been 'cleaned' of peripapillary VD. In conclusion, the present study revealed that the largest changes in RNFL after VD adjustment were observed for the incipient hypertensive glaucoma in segments 5 and 8.

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Introduction

Hypertensive glaucoma (HTG) is a progressive disease, therefore early diagnosis and thus initiation of treatment is very important for preserving visual function (1). At present, HTG can be detected by measuring changes in the nerve fiber layer (RNFL), ganglion cell complex and visual field, in addition to the observation of high intraocular pressure (IOP) (1). The introduction of optical coherence tomography angiography (OCTA) has brought new possibilities to the field of glaucoma over the past decade, by allowing the examination of peripapillary vessel density (VD) (1).

Our previous study investigating the relationship between IOP, RNFL and VD revealed no correlations between the variables assessed in eyes with normal IOP (≤20 mmHg). In healthy eyes, a moderate correlation was observed between VD and RNFL (-0.43<r<-0.73). In pathological IOP, there was a moderate correlation between VD and IOP (-0.34<r<0.59) and a moderate correlation between IOP and RNFL (-0.42<r<0.59). The correlation between VD and RNFL in eyes with IOP >20 mmHg showed a moderate to strong correlation (0.59<r<0.87) (2).

In another previous study that examined the relationship between pathological IOP and RNFL in individual peripapillary segments, the highest correlation was found in segments 1, 4, 5 and 8. This is where the axons of the predominantly damaged retinal ganglion cells (magnocellular) enter (3).

As not only the nerve fiber layer itself but also the vascular component contributes to the overall RNFL, the aim of the present study was to determine how VD in each segment peripapillary correlated with pathological IOP. Another aim was to exclude the contribution of the vascular component to RNFL at high IOP values.

Patients and methods

The study consisted of 69 individuals (122 eyes) with untreated ocular hypertension that were recruited between January to May 2022 at Ophthalmology Clinic JL (Prague, Czech Republic), including 32 males (6 with one eye examined and 26 with both eyes examined; age range, 21-76 years; mean age, 55±13 years) and 37 females (4 with one eye examined and 30 with both eyes examined; age range, 22-75 years; mean age, 52±14 years). Their IOP was measured to be >21 (21-36) mmHg

during routine ambulatory care and were not diagnosed with pseudoexfoliative or pigmentary glaucoma and had the same stage of POAG. The inclusion criteria were set as follows: Visual acuity of 1.0 with a possible correction of ≤3 dioptres; approximately equal changes in the visual fields [in the overall defect (OD) parameter] in all patients compared with the physiological range and no other ocular or neurological diseases and no prior treatment for hypertensive glaucoma.

IOP was measured using a non-contact Ocular Response Analyser II device (Reichert, Inc.), which was averaged from three measurements in the same eye. VD in the radial peripapillary capillaries region was measured using the in-built software of the Avanti RTVue XR instrument (version 2018.0.018; Optovue, Inc.) in eight peripapillary segments. As presented in Fig. 1, the first image was divided and marked to show the inferior temporal (IT; segment 1) followed by the temporal inferior (TI; segment 2), temporal superior (TS; segment 3), superior temporal (ST; segment 4), superior nasal (SN; segment 5), nasal superior (NS; segment 6), nasal inferior (NI; segment 7) and inferior nasal (IN; segment 8) segments. Examination of all segments was performed simultaneously in a single measurement. As presented in Fig. 2, similar peripapillary RNFL measurements in identical segments were performed with the same instrument (OCT Avanti RTVue XR instrument).

The visual field was examined using a fast threshold glaucoma strategy that measured the visual field in range 50˚ in nasal direction and 22˚ in temporal direction (Medmont M 700 automated perimeter; Medmont International Pty Ltd.). Therefore, visual field OD was assessed, which also served as an identifier for the study of unsuitable patients with visual field abnormalities potentially affecting outcomes.

The statistics were calculated using the software STATISTICA 13 (version 13.3.721.1; StatSoft). All quantitative data were expressed as mean ± SD. Box-plots were used for visual comparison of the samples. Relationships between IOP and RNFL and IOP and VD were measured using Pearson's coefficient of correlation. The partial correlation coefficient was used to 'adjust' RNFL from VD to assess the dependence between the selected parameters and tells how the IOP value would correlate with the thickness of nerve fibers in a given segment if all patients in that segment had the same VD. $P<0.05$ was considered to indicate a statistically significant difference.

The present study was performed according to the Declaration of Helsinki and was approved by the internal ethics committee of the Ophthalmology Clinic JL (approval no. OKJL/220606/13; Prague, Czech Republic). Written informed consent for participation was obtained from all patients.

Results

The mean values of age, IOP, OD, VD and RNFL of the whole population used the in present study are presented in Table I. The correlation results between IOP and VD of all 122 eyes are included in Table II. No correlation was revealed between IOP and OD (r=-0.08, data not shown) (3). Furthermore, the correlation matrix showed that IOP was significantly but weakly correlated with VD in segments 1

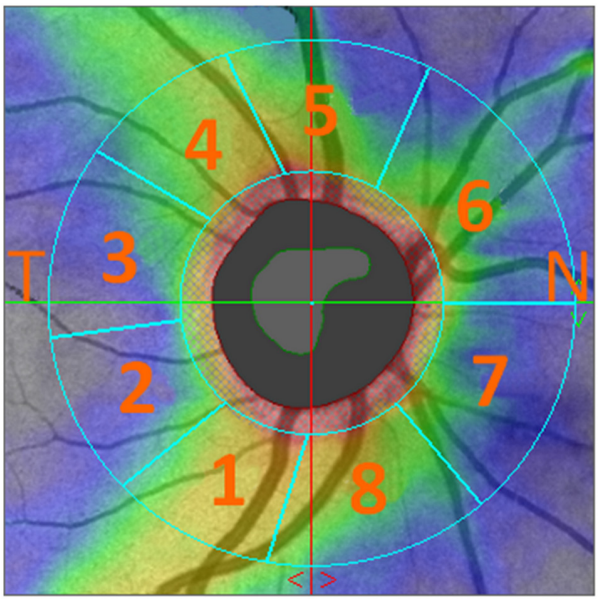

Figure 1. Designation of each peripapillary segment in which RNFL and VD were evaluated. 1=Inferior temporal; 2=temporal inferior; 3=temporal superior; 4=superior temporal; 5=superior nasal; 6=nasal superior; 7=nasal inferior; 8=inferior nasal. T-temporal, N, nasal area.

(r=-0.29), 4 (r=-0.33), 5 (r=-0.29), 6 (r=-0.37), 7 (r=-0.32) and 8 (r=-0.23). The strongest correlation was revealed in segment 6, although the strength of the correlation was medium (r=-0.37). However, the differences of correlation coefficients among these segments were relatively small. For all segments, the correlation with IOP was negative. Therefore, it appeared that the higher the IOP value, the lower the VD value in each segment.

Values of correlation coefficients of the RNFL and VD parameters in each evaluated segment are presented in Table II. The values for the first were obtained from the same set of a previous study (3). The relationship between IOP and the observed nerve fiber segments after VD 'cleaning' were assessed using partial correlation coefficients. The partial correlation coefficient suggests how the IOP value would correlate with the thickness of nerve fibers in a given segment if all patients had the same VD in that segment. The partial correlation coefficient always takes values between -1 and 1. If r comes out close to -1, it indicates an inverse linear dependence between the variables (the larger one variable is, the smaller the other is); if r comes out close to +1, it indicates a direct linear dependence (the larger one variable is, the larger the other is). If *r* comes out close to 0, it indicates that the variables are linearly independent. One is unrelated to the other and vice versa. According to the value of the partial correlation coefficient it is possible to distinguish: Weak ($|r|<0.3$), medium ($0.3<|r|<0.8$) and strong ($|r|>0.8$) linear dependence (correlation).

Table II reveals that significant correlations between IOP and RNFL were found in segments 1 (r=-0.23), 4 (r=-0.24), 5 (r=-0.31) and 8 (-0.28). As aforementioned, the highest correlations between VD and IOP were revealed in segments 1, 4, 5, 6 ,7 and 8. After VD-adjustment, significant correlations of medium strength between IOP vs. RNFL-VD were revealed in segments 5 (r=-0.32) and 8 (r=-0.39).

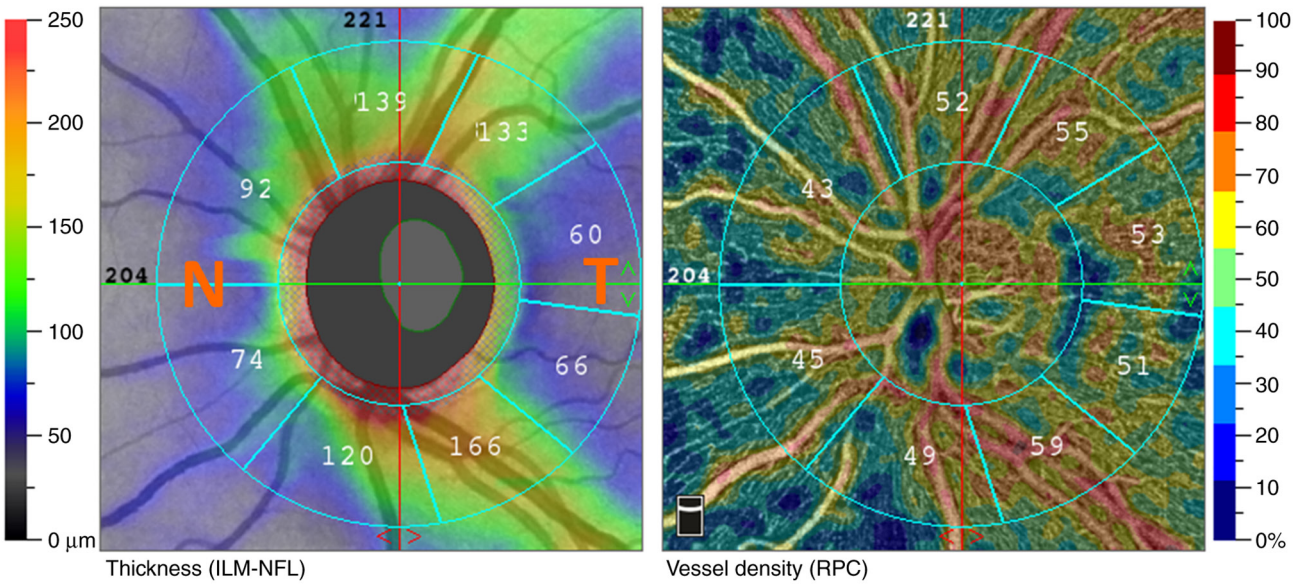

Figure 2. Optical coherence tomography angiography report: Retinal nerve fiber layer thickness values (left image) and vessel density in each segment (right image). ILM, internal limiting membrane; NFL, neuro fibre layer; RPC, radial peripapillary capillaries.

Discussion

For the early diagnosis of changes in the nerve fiber layer, which segment in the peripapillary region of the retina to focus on is important. Since the vascular component contributes non-negligibly to its total thickness, the present study sought to determine its 'true' value after elimination of the VD. (2) Our previous work has demonstrated that as IOP increases, VD decreases and the relationship between VD and RNFL increases (2).

Accumulating evidence suggests that abnormalities in the retinal microcirculation and ocular blood flow disturbances contribute to the development of primary open angle glaucoma (4-6). However, the precise role of vascular abnormalities in the pathogenesis of glaucoma remain poorly understood (7). The significant effect of VD on visual field changes in HTG was also demonstrated in a previous study (8).

Chen *et al* (9) previously investigated the effect of latanoprost (a drug that lowers IOP) on VD. This study found statistically significant increases of VD in the IT, TS, ST, SN, NI and IN segments. This corresponds to segments 1, 3, 4, 5, 7 and 8 in the current study. Moreover, the present study similarly revealed the greatest effect of pathological IOP on VD in segments 1, 4, 5, 6, 7 and 8. Similar findings were also observed by Shin *et al* (10), where peripapillary microvascular improvement was observed in 61.3% eyes at 3 months after anti-glaucomatous surgery. Improvements in VD after anti-glaucomatous surgery have also been observed by Park *et al* (11). By contrast, Zeboulon *et al* (12) revealed no significant changes in the peripapillary VD of their cases, with a mean baseline IOP of 23.7±9.5 mmHg and a mean IOP of 12.2±3.5 mmHg (≥40% reduction) at 1 month after the operation. Diaz *et al* (13) previously reported that high IOP significantly reduces the VD per unit area in the laminar and retrolaminar regions of the optic nerve. It should be noted that after the application of latanoprost and timolol for glaucoma, neither the capillary density nor the capillary volume fraction in the cribriform disc region returned to their original values. Wang *et al* (14) previously found an immediate change in VD values when IOP was experimentally increased using OCTA. Therefore, this may be one of the reasons why vascular alterations may occur before the degeneration of RNFL, even in patients with normal IOP. Conversely, following RNFL atrophy, changes in VD also occur (15). Glutamate, as the main neurotransmitter in the visual pathway, may play a major role, by not only acting excitotoxically on the visual analyzer cells (16,17) but by also affecting the vascular system (15).

In the present study, a weak correlation was found between IOP and VD, specifically in segments 1, 4, 5, 6, 7 and 8, where the highest degree of correlation was found in segment 6 (r=-0.37).

This is probably due to the larger number of retinal vessels that nourish the inner retinal layers, as the upper nasal quadrant of the retina contains the largest number of ganglion cells (18). These results only partially correlate with the results of our previous study (3), where pathological IOP values were shown to be inversely proportional to RNFL in segments 1, 4, 5, and 8. Therefore, the role of VD in RNFL loss is probably not direct, yet the highest correlation between these parameters has been previously observed in segment 5 (r=-0.31) according to a review study (3).

IOP is a major risk factor for the development of glaucoma and its progression (9,10). After an increase in IOP, the ganglion cells of the retina are altered, which is demonstrated by studies of different animal models of glaucoma revealing a higher sensitivity (compared with other parvocellular ganglion cells) to IOP in magnocellular ganglion cells (19-21). As described by Weber *et al* (22) and Naskar *et al* (23), the first changes after IOP elevation begin in the ganglion cells themselves, and their axons change later. The notion that these are predominantly magnocellular fibers has been confirmed by Quigley *et al* (24), where larger diameter fibers died faster compared with smaller fibers, although no fibre size was completely spared at any stage of atrophy. Retinal magnocellular cells die before their

Table I. Values of dataset age, RNFL, VD and OD and their standard deviations.

Parameter	Value ± standard deviation
No. of eyes (male/female)	122.00 (32.00/37.00)
Mean age, years	45.00±6.00
Mean intraocular pressure, mmhg	23.65±2.70
Mean OD (-)	1.93±1.19
VD, %	
1-IT	56.44±6.33
2-TI	52.85±4.02
3-TS	55.80±3.58
4-ST	54.44±6.00
5-SN	49.36±8.38
6-NS	48.30±5.13
7-NI	46.81±5.34
8-IN	50.66±13.43
RNFL thickness, μm	
1-IT	139.64±24.85
2-TI	69.49±12.02
3-TS	72.93±12.15
4-ST	123.07±22.02
5-SN	127.33±23.33
6-NS	103.07±16.72
7-NI	85.69±15.74
8-IN	129.94±21.99

IT, inferior temporal segment; TI, temporal-inferior; TS, temporal-superior; ST, superior temporal; SN, superior nasal; NS, nasal-superior; NI, nasal-inferior; IN, inferior-nasal; RNFL, retinal nerve fiber layer; OD, overall defect of visual field; VD, vessel density.

Table II. Pearson correlation coefficients between IOP and RNFL, IOP and VD in each segment and partial correlation coefficient between IOP and RNFL adjusted by VD.

	Pearson's correlation coefficient (n=122)		
Segments	IOP vs. RNFL	IOP vs. VD	IOP vs. RNFL-VD
1-IT	-0.23[a] (P=0.010)	-0.29[a] (P=0.001)	-0.13 (P=0.192)
2-TI	-0.02 (P=0.790)	-0.17 (P=0.060)	-0.03 (P=0.876)
3-TS	-0.04 (P=0.670)	-0.10 (P=0.259)	-0.05 (P=0.613)
4-ST	-0.24[a] (P=0.007)	-0.33[a] (P=0.000)	-0.13 (P=0.189)
5-SN	-0.31[a] (P=0.001)	-0.29[a] (P=0.006)	-0.32[a] (P=0.002)
6-NS	-0.14 (P=0.117)	-0.37[a] (P=0.000)	-0.11 (P=0.252)
7-NI	-0.06 (P=0.532)	-0.32[a] (P=0.000)	-0.01 (P=0.971)
8-IN	-0.28[a] (P=0.002)	-0.23[a] (P=0.010)	-0.39[a] (P=0.001)

[a]P<0.05. IT, inferior temporal segment; TI, temporal-inferior; TS, temporal-superior; ST, superior temporal; SN, superior nasal; NS, nasal-superior; NI, nasal-inferior; IN, inferior-nasal; RNFL, retinal nerve fiber layer; VD, vessel density; IOP, intraocular pressure.

axons in hypertensive glaucoma, therefore, our previous study investigated RNFLs in different peripapillary segments, which revealed that their greatest atrophy is where magnocellular fibers enter the optic disc (3). To refine their reduction, the present study decided to 'clean' them from VD. Hood *et al* (25) demonstrated that VDs play a significant role in RNFL thickness, and that ~13% of the total peripapillary RNFL thickness in healthy subjects is attributable to blood vessels. Patel *et al* (26) similarly revealed that blood vessels account for 9.3% of the total RNFL thickness or area, but vary by retinal location. On average, 17.6% of the upper and 14.2% of the lower RNFL are vascular, whereas blood vessels comprise only 2.3%

of the areas of the temporal and nasal RNFL. Pereira *et al* (27) reported that, according to their model, the circumpapillary distribution of retinal vessels is affected by VD in up to 70% of RNFL thickness. In addition, Allegrini *et al* (28) revealed a vascular contribution to RNFL thickness of 29.07±3.945%.

The present study also demonstrated that the greatest effect of pathological IOP is on VD in segments 1, 4, 5, 6, 7 and 8, and for RNFL in segments 1, 4, 5 and 8. After 'cleaning' VD for RNFL thickness, the current study observed the highest IOP correlations in segments 5 and 8; that is, at the points where the strongest axons of ganglion cells, which correspond to magnocellular cells, enter the optic disc. Therefore, in early stages of hypertensive glaucoma, the present study recommends focusing on RNFL assessment in the upper and lower segments of the optic nerve disc peripapillary region. In conclusion, the present study revealed the most significant RNFL damage in the lower and upper segments after 'cleaning' from VD, which is the location where the magnocellular fibers are found.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

JL conceptualized and designed the study. MF and JK designed and implemented the clinical investigations and outcome assessment. JK provided the data analyses. MF wrote the manuscript. MF, JK and JL confirm the authenticity of all the raw data. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

The present study was performed according to the Declaration of Helsinki and was approved by the internal ethics committee of the Ophthalmology Clinic JL (approval no. OKJL/220606/13; Prague, Czech Republic). Written informed consent for participation was obtained from all patients.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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