学位論文

Glaucoma Diagnostic Performance of Retinal Blood Flow Measurement With Doppler Optical Coherence Tomography
（ドップラーOCT を用いた網膜血流測定による緑内障の診断能）

旭川医科大学大学院医学系研究科博士課程医学専攻

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Glaucoma

Glaucoma Diagnostic Performance of Retinal Blood Flow Measurement With Doppler Optical Coherence Tomography

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Purpose: The purpose of this study was to evaluate the diagnostic performance of retinal blood flow (RBF) measured with the Doppler optical coherence tomography (OCT) segmental scanning method to distinguish between healthy and glaucoma eyes.

Methods: Fifty-eight patients with normal tension glaucoma (NTG) who had a single-hemifield visual field defect and 44 age-matched healthy subjects were enrolled. Retinal nerve fiber layer thickness (RNFLT) was measured with swept-source OCT. Superior and inferior temporal arteries (TAs) and temporal veins (TVs) RBF were measured with Doppler OCT. The area under the curve (AUC) of the receiver operating characteristic (ROC) was used to compare the diagnostic performances in the damaged and normal hemispheres.

Results: Multivariate regression analysis showed TA RBF and TV RBF were significantly reduced in the damaged and normal hemispheres. The ROC analysis showed that the AUC for quadrant RNFLT, TA RBF, and TV RBF were 0.973, 0.909, and 0.872 in the damaged hemisphere, respectively. The AUC values in the normal hemisphere were 0.783, 0.744, and 0.697, respectively. The combination of quadrant RNFLT and TA/TV RBF had a greater AUC than quadrant RNFLT alone in both damaged (AUC = 0.987) and normal (AUC = 0.825) hemispheres.

Conclusions: In NTG eyes with single-hemifield damage, the RBF was found to be significantly reduced in the damaged and normal hemispheres independent from structural changes. The combination of RNFLT and RBF could improve diagnostic performances for glaucoma.

Translational Relevance: Combining morphological and blood flow measurements with Doppler OCT may be useful in glaucoma diagnosis.

Introduction

Glaucoma is a neurodegenerative disease characterized by progressive loss of the retinal ganglion cells (RGCs) and associated with characteristic structural damage to the optic nerve and visual field (VF).[1] Previous studies have examined the relationship between structure and function in glaucoma eyes. Medeiros et al.[2] reported that, with the decrease in RGCs, the retinal nerve fiber layer thickness (RNFLT) began to decrease in the initial stages, but the standard automated perimetry (SAP) mean deviation (MD) changes were small. Thus, they found

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a nonlinear relationship between MD and RGC counts. Gadi et al.[3] have identified the "tipping point," where the VF threshold values are associated with the structural measurements. Because VF loss becomes evident at a later stage of glaucomatous damage, the ability to diagnose glaucoma before the tipping point is an important aspect of disease management.

Recently, vascular factors have been suggested to be significant contributors to the development and progression of glaucoma. In recent studies, use of the Doppler optical coherence tomography (OCT) double circular scan method has shown there is decreased retinal blood flow (RBF) in glaucoma eyes.[4–6] Studies on the associations between RBF and the pathophysiology of glaucoma have been more prevalent recently. Some studies have reported that RNFLT is a useful parameter for glaucoma diagnosis and is considered as the gold standard,[7–12] because thinning of the RNFL is directly correlated with loss of ganglion cells.[13] However, it remains unclear how useful RBF is in diagnosing glaucoma.

A newly developed Doppler OCT uses OCT technology and it provides absolute velocity and flow data of RBF.[14–16] We have developed a Doppler OCT instrument with novel software and a segmental scanning method[17–19] that enables continuous measurement of the RBF in the retinal arterioles and venules during one cardiac cycle. Additionally, we reported, in normal tension glaucoma (NTG) eyes with single-hemifield damage, the RBF was decreased in damaged and normal hemispheres when compared to the healthy hemisphere independent from RNFLT.[20] With Doppler OCT, RBF could be a useful parameter for glaucoma diagnosis, different from a structural change perspective.

The purpose of this study was to evaluate the diagnostic performance of RBF measured with the Doppler OCT's segmental scanning method to distinguish between normal and glaucoma eyes.

Methods

Study Population

A total of 90 consecutive Japanese patients with NTG who had a single-hemifield VF defect and 54 age-matched healthy subjects without retinal diseases were enrolled from Asahikawa Medical University Hospital (from January 5, 2018, to July 15, 2021). The initial NTG diagnosis was made by glaucoma specialists (authors T.Y., Y.S., and M.K.). Informed consent was obtained from all subjects. This cross-sectional prospective study was approved by the Institutional Review Board of Asahikawa Medical University (approval number: 17114-2). The present study adhered to the tenets of the Declaration of Helsinki.

All subjects underwent a comprehensive ophthalmologic examination, including a review of their medical history, best corrected visual acuity, slit-lamp biomicroscopy, intraocular pressure (IOP) measurement with Goldman applanation tonometry, gonioscopy, dilated fundoscopy examination using a 90-diopter (D) lens, fundus and optic disk photography, and axial length with an A-mode ultrasound system (IOL Master 500; Carl Zeiss Meditec, Jena, Germany). SAP was performed using the Swedish Interactive Threshold Algorithm standard 24-2 threshold test (Humphrey Field Analyzer 850-III, 750-II; Carl Zeiss Meditec, Tokyo, Japan). Swept-source OCT (DRI OCT-1 triton; Topcon Corp., Tokyo, Japan) was used to measure the circumpapillary RNFLT. Only good quality circumpapillary RNFL images, defined by scans with a manufacture signal index ≥45, without segmentation failure or motion artifacts (i.e. missing or blank areas), were included in the analysis. Mean arterial blood pressure (MABP) and heart rate (HR) were measured using an electronic sphygmomanometer (EP-88Si; Colin, Tokyo, Japan). MABP was calculated as 1/3 systolic blood pressure (SBP) +2/3 diastolic blood pressure (DBP). Mean ocular perfusion pressure (MOPP) was calculated as 2/3 MABP − IOP. After pupil dilation with 0.5% tropicamide and 0.5% phenylephrine eye drops (Mydrin P; Santen Pharmaceuticals, Osaka, Japan), the participants sat in a quiet dimly lit room for 5 minutes, in which the temperature was maintained at 25°C. Subsequently, their blood pressure and HR were measured and RBF was measured with Doppler OCT by experienced observers (authors T.A. and Y.T.). The temporal arteries and veins with relatively straight segments that were sufficiently far from the bifurcations were selected for the measurement of RBF.

Inclusion criteria common to both the healthy and NTG groups were aged ≥40 and ≤80 years, best corrected visual acuity of 20/40 or better, spherical equivalent refractive error of −6.00 D or higher and less than +3.00 D, and reliable SAP results (fixation losses <20%; false-positive and false-negative errors <33%). Further inclusion criteria for the NTG group were: (1) glaucomatous optic neuropathy; (2) corresponding glaucomatous VF damage defined as an abnormal Glaucoma Hemifield Test and pattern standard deviation (PSD) outside 95% of the normal limits; (3) glaucomatous VF damage, matching the Anderson–Patella criteria had to be confined to a single hemifield. All patients were familiar with SAP testing from earlier exposure to at least two VF examinations. The sensitivity in decibels (dBs) at each test location was converted to a linear scale of 1/Lambert (1/Lambert = $10^{0.1 \times \text{dB value}}$).[21] The retinal sensitivity (RS) values in 1/Lambert were calculated in each hemifield using the average of 26/52 VF locations. Finally, the average of the 1/Lambert scale was converted to the dB scale. Individuals in the healthy group were required to have: (1) an IOP ≤21 mm Hg; (2) normal appearing optic disks and an intact neuroretinal rims and RNFL; (3) normal SAP defined as a Glaucoma Hemifield Test result within normal limits, and PSD within the 95% confidence limits.

Exclusion criteria common to both groups were history of intraocular/laser surgery (except for uncomplicated cataract surgery), existing retinal pathologies, uveitis, ocular trauma, or diabetes. Participants with systemic hypertension were included unless they were diagnosed with hypertensive retinopathy. Participants with unreliable VF results and poor quality swept-source OCT scans were excluded from this study. Supplementary Figure S1 shows a flow diagram of the selection process in this study.

RBF Measurement with Doppler OCT

Our prototype Doppler OCT flowmeter system is based on a version of a commercially available spectral-domain OCT device (3D OCT-1 Maestro; Topcon Corp., Tokyo, Japan). In Doppler OCT, the flow velocity $v(z)$ can be derived from the Doppler shift incurred by the moving blood:

$$v(z) = \frac{\lambda \Delta \Phi (z, t)}{4\pi nt} \cdot \frac{1}{\cos\theta}$$

where z is the depth location, $\Delta\Phi$ is the phase difference at the same depth location between adjacent profiles after Fourier transform, λ is the center wavelength, n is the refractive index of blood, t is the time interval between the adjacent profiles, and θ is the Doppler angle between the flow vector and the incident probe beam. The detailed description of blood flow measurement has been published previously.[17–19] We first obtained color fundus images and selected a region of a retinal vessel. When the Doppler angle θ was close to 90 degrees, the measured velocity became very sensitive to the accuracy of θ. To minimize this potential measurement error, our Doppler blood flow measurements were performed where the θ was considerably less than 90 degrees (i.e. at approximately 80 degrees). Automated alignment software was integrated to seek the proper Doppler angle. The Doppler angle estimation was performed in locations where a pair of B-scan images were captured 200 µm apart across the blood vessels. The median value for nine repeated measurements was used to define the Doppler angle. The blood vessels were detected automatically and identified from the OCT structured and phase images. The vessel diameter was measured from OCT phase images. The blood flow rate can be calculated by integrating the velocity in the blood vessel. Example images of RBF measurements are shown in Figure 1.

Data and Statistical Analysis

The data were analyzed using Stata (StataBE 17; Stata Corp, College Station, TX, USA). All data are shown as the median value (interquartile range [IQR]). Demographic characteristics of the healthy and NTG groups were compared using the Wilcoxon rank sum test for continuous variables and chi-squared tests for categorical variables. Kruskal Wallis test and Steel Dwass test (using EZR; Saitama Medical Center, Jichi Medical University, Saitama, Japan) were used to compare the averages of variables between superior and inferior in healthy hemispheres and damaged and normal hemispheres of NTG. Univariate linear regression models were built using temporal artery (TA) RBF or temporal vein (TV) RBF as the dependent variable, and RNFLT, RS, and demographic ocular and systemic characteristic variables were the independent variables. Axial length and factors that were significant in the univariate analyses ($P < 0.05$) were then further included in the multivariable linear regression model. The areas under the curve (AUC) of the receiver operating characteristic (ROC) were calculated by logistic regression model adjusting for age, gender, MOPP, and axial length. The AUCs for quadrant RNFLT and the combination of quadrant RNFLT and TA/TV RBF were compared using the Delong test. Logistic regression analysis was conducted to examine the impact of using antiglaucoma drops on the TA and TV RBF in patients with NTG.

Sample Size

In our pilot study, TA RBF and TV RBF (mean [standard deviation]) in the normal hemisphere in NTG eyes and the healthy hemisphere in healthy eyes were 6.62 (2.35) vs. 8.47 (3.57) µL/min and 6.97 (2.5) vs. 8.33 (2.29) µL/min. The calculated sample sizes (50 NTG eyes and 50 healthy eyes) allowed for the detection of significant difference in RBF between the normal hemisphere and the healthy hemisphere, with a 2-sided type I error probability of 0.05 and the statistical power of 80%.

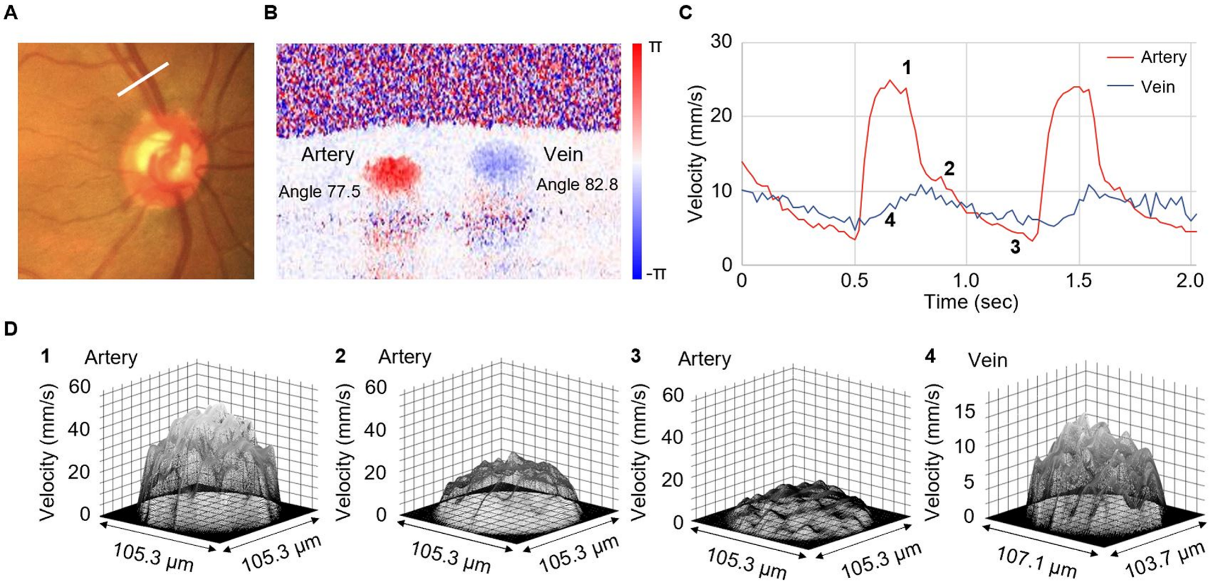

Figure 1. Example images of retinal blood flow measured by Doppler optical coherence tomography by segmental scan method. (**A**) Fundus image and white line shows the measurement location. (**B**) Phase image with color coding. The artery is coded by *red* and the vein is coded by *blue*. The angle is the Doppler angle between the flow vector and the incident probe beam. (**C**) Acquired blood velocity waveform of the average velocity. The *red line* shows the artery and the *blue line* shows the vein. (**D**) The velocity profiles. (1), (2), and (3) show the arterial velocity profiles at time (1), (2), and (3) of **C**, respectively. (4) shows the venous velocity profile at time (4) of **C**.

Results

The analysis included 58 eyes of 58 patients with NTG and 44 healthy subjects. Table 1 shows the demographics of the study population. There were no significant differences in age, gender, spherical equivalent, axial length, IOP, SBP, DBP, MOPP, HR, and proportion of hypertension. The median (IQR) of the VF MD was −4.29 dB (IQR = −6.81 to −1.21) and

Table 1. Demographics of the Study Population

Variable	Healthy Eyes ($n = 44$)	NTG Eyes ($n = 58$)	P Value
Age, median (IQR), years	63 (55 to 70)	66 (56 to 72)	0.295*
No. of men/women	19/25	26/32	0.868**
VF defect location, no. of superior/inferior	–	38/20	
Spherical equivalent, median (IQR), D	−0.78 (−2.73 to 0.40)	−0.78 (−2.93 to 0.26)	0.670*
Axial length, median (IQR), mm	24.0 (23.3 to 24.7)	24.0 (23.1 to 24.4)	0.511*
IOP, median (IQR), mm Hg	15 (13 to 17)	14 (12 to 15)	0.149*
SBP, median (IQR), mm Hg	129 (121 to 136)	130 (124 to 135)	0.748*
DBP, median (IQR), mm Hg	77 (71 to 85)	79 (73 to 85)	0.494*
MOPP, median (IQR), mm Hg	49 (43 to 52)	50 (47 to 53)	0.215*
HR, median (IQR), bpm	67 (61 to 71)	66 (60 to 71)	0.590*
Hypertension, no. (%)	8 (18)	14 (24)	0.469**
SAP MD, median (IQR), dB	0.14 (−0.51 to 1.37)	−4.29 (−6.81 to −1.21)	**<0.001***
SAP PSD, median (IQR), dB	1.76 (1.52 to 2.31)	8.71 (5.21 to 12.61)	**<0.001***

Abbreviations: IQR, interquartile range; NTG, normal tension glaucoma; VF, visual field; IOP, intraocular pressure; SBP, systolic blood pressure; DBP, diastolic blood pressure; MOPP, mean ocular perfusion pressure; HR, heart rate; SAP, standard automated perimetry; MD, mean deviation; dB, decibel; PSD, pattern standard deviation.

*Calculated as Wilcoxon rank sum test, with $P < 0.05$ considered to be statistically significant.

**Calculated as chi–squared test, with $P < 0.05$ considered to be statistically significant.

Boldface indicates statistically significant difference.

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Table 2. The Comparisons Between Damaged and Normal Hemispheres of Normal Tension Glaucoma (NTG) Eyes; and Normal Hemispheres of NTG Eyes and Healthy Eyes

	NTG Eyes, Median (IQR)			Healthy Eyes Median (IQR)[b]	
Variable	Damaged Hemisphere $n = 58$	Normal Hemisphere $n = 58$	P Value[*]	Mean Hemispheric $n = 44$	P Value[*]
RNFLT, µm[a]	71.5 (59.0 to 83.0)	96.5 (83.0 to 125.0)	**<0.001**	125.3 (114.5 to 137.8)	**<0.001**
RS, dB	26.9 (24.3 to 28.8)	30.1 (29.5 to 31.0)	**<0.001**	30.0 (29.3 to 31.6)	0.899
TA RBF, µL/min	3.82 (2.76 to 4.91)	6.51 (4.21 to 8.25)	**<0.001**	7.99 (6.20 to 9.92)	**0.002**
TA diameter, µm	78.7 (70.3 to 89.6)	87.0 (80.9 to 96.7)	**0.007**	93.6 (85.3 to 101.9)	**0.018**
TA average velocity, mm/s	13.1 (10.9 to 17.1)	15.9 (12.7 to 18.6)	**0.046**	16.9 (14.0 to 21.7)	0.327
TV RBF, µL/min	5.03 (3.86 to 7.18)	7.51 (5.55 to 9.50)	**<0.001**	8.75 (6.70 to 12.0)	**0.013**
TV diameter, µm	109.0 (100.2 to 117.8)	112.1 (102.8 to 125.7)	0.621	120.7 (109.9 to 131.0)	**0.048**
TV average velocity, mm/s	9.1 (6.6 to 11.2)	10.8 (8.3 to 13.9)	0.060	12.5 (9.6 to 15.2)	0.053

Abbreviations: IQR, interquartile range; NTG, normal tension glaucoma; RNFLT, retinal never fiber layer thickness; RS, retinal sensitivity; TA, temporal artery; RBF, retinal blood flow; TV, temporal vein.

[*]Kruskal–Wallis test and Steel Dwass test were used to compare the variables between healthy hemispheres and damaged and normal hemispheres of normal tension glaucoma eyes, with $P < 0.05$ considered to be statistically significant.

[a]Measured in superior (46 degrees to 135 degrees) and inferior (226 degrees to 315 degrees) quadrants.

[b]Calculated from the average of superior and inferior hemispheres.

Boldface indicates statistically significant difference.

the median (IQR) VF PSD was 8.71 dB (IQR = 5.21 to 12.61). In the NTG group, the glaucomatous VF defect was localized to the superior hemifield in 38 of 58 eyes (65.5%) and to the inferior hemifield in 20 eyes (34.5%).

Table 2 shows comparisons of the RNFLT, RS, and RBF parameters between the damaged and normal hemispheres, and normal hemispheres of NTG eyes and healthy hemispheres. TA and TV RBF and RNFLT were reduced in the damaged hemisphere compared with the normal hemisphere. Those values in the normal hemisphere of NTG eyes also decreased compared with the healthy hemisphere of the healthy eyes.

Table 3 shows the univariate and multivariate regression analysis for TA RBF. The reduced TA RBF was significantly associated with the axial length ($\beta = -0.56$, $P = 0.004$), RNFLT ($\beta = 0.39$, $P = 0.001$), damaged hemisphere ($\beta = -3.00$, $P < 0.001$), and normal hemisphere ($\beta = -1.42$, $P = 0.016$). Similar results were observed for TV RBF in the damaged hemisphere ($\beta = -3.27$, $P = 0.001$) and normal hemisphere ($\beta = -1.57$, $P = 0.023$; Table 4). Scatter plots illustrating the relationships between TA RBF and RNFLT ($r = 0.572$, $P < 0.001$) and TV RBF and RNFLT ($r = 0.454$, $P < 0.001$) are shown in Figure 2.

Figure 3 shows ROC curves for the damaged and normal hemispheres in NTG eyes and Table 5 shows the AUC values for RNFLT and RBF parameters. In the damaged hemisphere in NTG eyes, the AUC (95% confidence interval [CI]) for quadrant RNFLT, TA RBF, and TV RBF were 0.973 (95% CI = 0.948–0.997), 0.909 (95% CI = 0.855–0.964), and 0.872 (95% CI = 0.806–0.938), respectively. The AUC values in the normal hemisphere in NTG eyes were 0.783 (95% CI = 0.693–0.873), 0.744 (95% CI = 0.649–0.839), and 0.697 (95% CI = 0.594–0.800), respectively. We also compared the AUC values for quadrant RNFLT with the combination of quadrant RNFLT and RBF. We found that the combination of quadrant RNFLT and TA/TV RBF had a greater AUC than quadrant RNFLT alone in both damaged (AUC = 0.987 [95% CI = 0.971–1.000]) and normal (AUC = 0.825 [95% CI = 0.747–0.904]) hemispheres of NTG eyes, however, there was no statistical significance of AUC in both damaged ($P = 0.075$) and normal ($P = 0.147$) hemispheres.

Discussion

The purpose of this study was to evaluate the diagnostic performance of RBF, measured with the Doppler OCT segmental scanning method, to distinguish between normal and glaucoma eyes. We observed that the TA RBF and TV RBF were significantly

Table 3. Univariate and Multivariate Linear Regression Analysis for Temporal Artery Retinal Blood Flow

	Univariate Analysis			Multivariable Analysis		
Factors	Coefficient	95% CI	*P* Value	Coefficient	95% CI	*P* Value
Age, per 10 years	−0.14	−0.64 to 0.36	0.583	–	–	–
Gender, female	0.64	−0.03 to 1.31	0.061	–	–	–
Axial length, mm	−0.75	−1.16 to −0.32	**0.001**	−0.56	−0.94 to −0.18	**0.004**
IOP, mm Hg	−0.01	−0.20 to 0.19	0.971	–	–	–
SBP, mm Hg	0.01	−0.04 to 0.06	0.714	–	–	–
DBP, mm Hg	0.01	−0.05 to 0.06	0.876	–	–	–
MOPP, mm Hg	0.01	−0.07 to 0.09	0.791	–	–	–
HR, bpm	−0.01	−0.07 to 0.05	0.859	–	–	–
Hypertension	−0.85	−2.13 to 0.44	0.195	–	–	–
RS, dB	0.27	0.12 to 0.42	**0.001**	−0.13	−0.30 to 0.04	0.142
RNFLT,[a] per 10 µm	0.65	0.50 to 0.80	**<0.001**	0.39	0.16 to 0.63	**0.001**
Group:						
Healthy hemisphere		Reference			Reference	
Normal hemisphere	−2.21	−3.43 to −0.98	**<.001**	−1.42	−2.58 to −0.26	**0.016**
Damaged hemisphere	−4.38	−5.56 to −3.20	**<.001**	−3.00	−4.56 to −1.43	**<0.001**

Abbreviations: IOP, intraocular pressure; SBP, systolic blood pressure; DBP, diastolic blood pressure; MOPP, mean ocular perfusion pressure; HR, heart rate; RS, retinal sensitivity; RNFLT, retinal never fiber layer thickness.

[a]Measured in superior (46 degrees to 135 degrees) and inferior (226 degrees to 315 degrees) quadrants.

Boldface indicates statistically significant difference.

Table 4. Univariate and Multivariate Linear Regression Analysis for Temporal Vein Retinal Blood Flow

	Univariate Analysis			Multivariable Analysis		
Factors	Coefficient	95% CI	*P* Value	Coefficient	95% CI	*P* Value
Age, per 10 years	0.33	−0.21 to 0.87	0.228	–	–	–
Gender, female	0.26	−0.47 to 0.99	0.481	–	–	–
Axial length, mm	−0.56	−1.01 to −0.10	**0.018**	−0.45	−0.89 to −0.01	**0.047**
IOP, mm Hg	−0.01	−0.21 to 0.20	0.972	–	–	–
SBP, mm Hg	0.01	−0.05 to 0.05	0.973	–	–	–
DBP, mm Hg	−0.01	−0.06 to 0.05	0.942	–	–	–
MOPP, mm Hg	−0.01	−0.09 to 0.09	0.982	–	–	–
HR, bpm	0.02	−0.05 to 0.08	0.632	–	–	–
Hypertension	−1.12	−2.51 to 0.26	0.111	–	–	–
RS, dB	0.26	0.10 to 0.42	**0.002**	−0.08	−0.28 to 0.12	0.416
RNFLT,[a] per 10 µm	0.56	0.39 to 0.74	**<0.001**	0.26	−0.01 to 0.53	0.067
Group:						
Healthy hemisphere		Reference			Reference	
Normal hemisphere	−2.08	−3.45 to −0.71	**0.003**	−1.57	−2.92 to −0.22	**0.023**
Damaged hemisphere	−4.17	−5.50 to −2.84	**<0.001**	−3.27	−5.10 to −1.44	**0.001**

Abbreviations: IOP, intraocular pressure; SBP, systolic blood pressure; DBP, diastolic blood pressure; MOPP, mean ocular perfusion pressure; HR, heart rate; RS, retinal sensitivity; RNFLT, retinal never fiber layer thickness.

[a]Measured in superior (46 degrees to 135 degrees) and inferior (226 degrees to 315 degrees) quadrants.

Boldface indicates statistically significant difference.

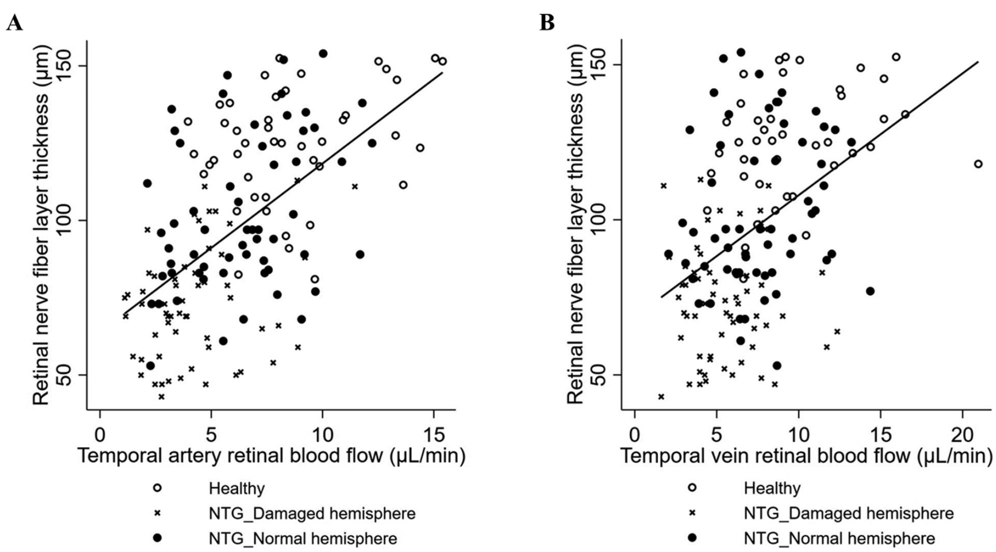

Figure 2. (**A**) Correlation between retinal nerve fiber layer thickness and temporal artery retinal blood flow. (**B**) Correlation between retinal nerve fiber layer thickness and temporal vein retinal blood flow.

reduced in the damaged and normal hemispheres of the NTG eyes with single-hemifield VF damage. In addition, the combination of quadrant RNFLT and RBF had a greater AUC than quadrant RNFLT alone in both damaged and normal hemispheres. These results suggested that RBF measurement can be useful in assessing glaucoma.

The multivariate regression analysis revealed that the hemisphere in NTG was associated with RBF reduction independent from RNFLT. These results were similar to our previous report,[20] notably with a larger study population. Findings of decreased RBF reduction in glaucoma eyes are in agreement with previous reports. Consistent with our results,

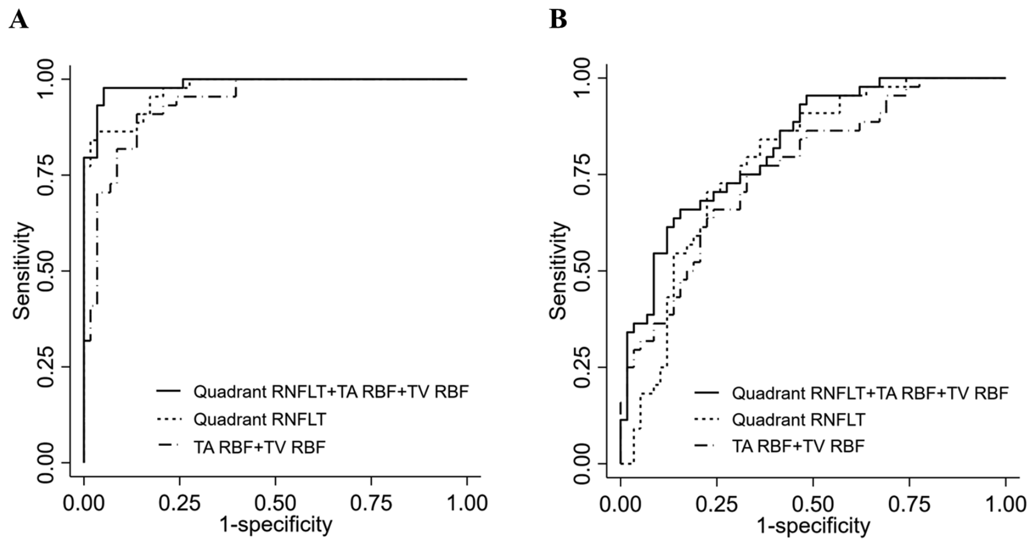

Figure 3. Receiver operating characteristic curves (ROCs) of quadrant retinal nerve fiber layer thickness (RNFLT), the combination of temporal artery (TA), retinal blood flow (RBF), and temporal vein (TV) RBF, and the combination of quadrant RNFLT and TA/TV RBF in damaged hemisphere (**A**) and normal hemisphere (**B**) in normal tension glaucoma with single-hemifield damage. The ROCs were adjusted for age, gender, mean ocular perfusion pressure, and axial length.

Table 5. Area Under the Receiver Operating Characteristics Curve (AUC) Values Between Normal Tension Glaucoma Eyes and Healthy Eyes

	Damaged Hemisphere in NTG Eyes Versus Healthy Eyes		Normal Hemisphere in NTG Eyes Versus Healthy Eyes	
Parameter	AUC	95% CI	AUC	95% CI
TA RBF	0.909	0.855 to 0.964	0.744	0.649 to 0.839
TV RBF	0.872	0.806 to 0.938	0.697	0.594 to 0.800
Quadrant RNFLT[a]	0.973	0.948 to 0.997	0.783	0.693 to 0.873
TA RBF + TV RBF	0.938	0.894 to 0.982	0.764	0.672 to 0.856
Quadrant RNFLT[a] + TA RBF + TV RBF	0.987	0.971 to 1.000	0.825	0.747 to 0.904

Abbreviations: NTG, normal tension glaucoma; TA, temporal artery; RBF, retinal blood flow; TV, temporal vein; RNFLT, retinal never fiber layer thickness.

[a]Measured in superior (46 degrees to 135 degrees) and inferior (226 degrees to 315 degrees) quadrants.

The AUC values were adjusted for age, gender, mean ocular perfusion pressure, and axial length.

Hwang et al.[5] reported an independent relationship between RBF reduction and structural loss. Additionally, Yarmohammadi et al.[22] reported a statistically significant association between the decreased vessel density measured with OCT angiography and the severity of VF damage, independent from structural loss. These results may indicate the independence of a blood flow factor and structural changes. In addition, a multivariate regression analysis demonstrated an association with longer axial length and RBF reduction. This might be due to effects of ocular magnification of the scan area.[23–25] The scan could change the measurement of retinal blood vessel diameters, measuring lower RBF. Although high myopic eyes (spherical equivalent, <-6 D) were excluded in this study, results of longer axial length eyes should be interpreted carefully.

The ROC analysis showed that there was good diagnostic performance for quadrant RNFLT, TA RBF, and TV RBF (AUC = 0.973, 0.909, and 0.872, respectively) in the damaged hemisphere of NTG eyes, and the combination of these parameters had the best diagnostic performance (AUC = 0.987). Although diagnostic performance is influenced by the population, the severity of the disease, and the measurement methods, previous studies have reported AUCs for an average RNFLT ranged from 0.892 to 0.962 in mild and moderate glaucoma.[7–12] These AUC values were similar to our results. Because the RNFLT and TA/TV RBF were significantly reduced in the damaged hemisphere compared with the healthy hemisphere in healthy eyes and the visual field defects were biased toward the single hemifield, the above large AUCs were confirmed. On the other hand, in the normal hemisphere, we found lower AUCs for quadrant RNFLT, TA RBF, and TV RBF (0.783, 0.744, and 0.697, respectively) compared to the damaged hemisphere. The combination of these parameters had a greater AUC (0.825) than the quadrant RNFLT alone. A previous study reported the AUC for an average RNFLT was 0.799 in early glaucoma (VF MD = −2.6dB).[26] The earlier the severity of glaucoma was, the lower the AUC. However, the normal hemisphere in this study differed from early glaucoma eyes in that the eye was glaucomatous but did not have a VF defect. As Medeiros et al.[2] reported, in glaucomatous eyes, there was a nonlinear relationship between MD and RGC counts. Gadi et al.[3] reported that it was not until the mean RNFLT reached 75.3 µm that the first VF defect was detected. In this study, the median quadrant RNFLT in a normal hemisphere was 96.5 µm (see Table 2). Therefore, the normal hemisphere of glaucoma eyes with single hemifield damage may be a suitable model for the stage before a VF defect becomes clinically evident.

Several studies have reported relationships between RBF and glaucoma using the Doppler OCT by the double circle scan method.[4–6] This method can measure total RBF by summing flow from all detectable veins. Because RNFLT thinning in early to moderate glaucoma is more localized, especially for parts of the temporal quadrant, measuring total RBF includes parts of the nasal quadrant flow that are less likely to be associated with glaucoma. On the other hand, our study evaluated only the RBF of the temporal arteries and veins, which may be more reflective of the blood flow changes in glaucoma. Another advantage of this study was the ability to obtain the waveforms by continuously measuring one heartbeat, so the RBF may be measured more accurately. Other studies have evaluated ocular blood flow using laser speckle flowgraphy (LSFG) whose main parameter is the mean blur rate, which represents the ocular blood cell velocity.[27–29] However, the values of mean blur rate are not absolute values of direct blood flow velocity or flow. It is therefore impossible to compare the blood vessels in different parts or different individuals. Thus, measuring RBF with Doppler OCT may be useful for assessing glaucoma diagnostic performance.

There are several limitations in this study. First, we could not measure all retinal arteries and veins because the reproducibility is poor for vessels less than 50 µm. Therefore, we selected four temporal vessels (TA and TV) per eye. To calculate all arterial blood flow, technical problems must be solved. Second, this study was a cross-sectional study and it did not determine the causal effect of RBF reduction. More recently, a longitudinal study reported that, when an open angle glaucoma patient was older and had a higher pulse rate, the optic nerve head tissue blood flow measured by LSFG decreased, preceding glaucomatous neurodegeneration.[30] In the future, a longitudinal study should be conducted to evaluate progressive RBF changes in a time series using Doppler OCT. Third, because this study was a case-control design, selection bias might have influenced the results. In order to overcome this bias as much as possible, the observers were masked to the diagnosis. Fourth, the sample size was smaller than that of previous studies that reported diagnostic performances for glaucoma. Our results should therefore be interpreted with caution. A fifth limitation is the potential effects of medication on RBF.[31] Our logistic regression analysis showed that the use of antiglaucoma drops was not significantly associated with average TA RBF and TV RBF in NTG eyes (Table 6). Furthermore, multivariate regression analysis showed no association among BP, IOP, and MOPP with RBF reduction. Because these association

Table 6. List of Antiglaucoma Drops Used in the Present Study

	NTG Eyes ($n = 58$)		
Medications	No. (%)	OR per µL/Min Average TA RBF (*P* Value)	OR per µL/Min Average TV RBF (*P* Value)
Prostaglandin analogs	50 (86)	0.86 (0.43)	0.69 (0.06)
Topical beta blockers	29 (50)	1.23 (0.14)	1.08 (0.52)
Topical carbonic anhydrase inhibitors	21 (36)	0.94 (0.68)	0.99 (0.94)
α2-adrenergic agonists	13 (22)	1.20 (0.26)	1.05 (0.75)
Rho-associated protein kinase inhibitor	3 (5)	0.58 (0.25)	1.29 (0.37)

Abbreviations: NTG, normal tension glaucoma; OR, odds ratio; TA, temporal artery; RBF, retinal blood flow; TV, temporal vein. Calculated as logistic regression analysis, with $P < 0.05$ considered to be statistically significant.

results might be due to the small sample size, we cannot exclude the potential effects of using medication on various ocular vascular beds. A future study with a larger sample size is needed.

In conclusion, the current study suggested that RBF reduction may be independent from RNFLT thinning in NTG. Thus, RBF measurement has the potential to be a useful tool for assessment of glaucoma. Although the diagnostic performance of RBF measurement may be inferior to that of RNFLT, the combination of the two measurements could help improve the diagnostic performance for glaucoma, even before VF damage appears clinically.

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