Progression of Macular Vessel Density in Primary Open-Angle Glaucoma: A Longitudinal Study

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• PURPOSE: To evaluate the rate of progression of macular vessel density (mVD) in primary open-angle glaucoma (POAG) and explore the relationship between the progression of mVD and macular ganglion cell–inner plexiform layer (mGCIPL) thickness and parapapillary retinal nerve fiber layer (pRNFL) thickness.
• DESIGN: Prospective cohort study.
• METHODS: In this study, 102 eyes with POAG were followed for 36.6 ± 6.4 months. The rates of progression were estimated by linear models. The agreement of progression detection among the 3 parameters was evaluated with Kappa statistics. The influence of baseline measurements on the rates of progression of mGCIPL thickness, pRNFL thickness, and mVD was investigated by linear mixed modeling. Kaplan-Meier survival analysis was adopted to calculate the survival probabilities.
• RESULTS: The respective rate of progression by linear regression was −0.102 ± 0.054 μm/month, −0.160 ± 0.086 μm/month, and −0.199 ± 0.073 %/month for mGCIPL thickness, pRNFL thickness, and mVD. The agreement in detection of progression among them was poor with the Conger's Kappa coefficient of 0.098 (95% confidence interval: −0.025~0.220, $P = .116$). The significant factors influencing the rate of progression of mVD were baseline mGCIPL thickness, baseline pRNFL thickness, and baseline mVD ($P \leq .001$), while baseline mVD was not a significant factor influencing the rates of progression of mGCIPL thickness and pRNFL thickness ($P \geq .659$). Also, pRNFL thickness had a better survival probability compared with the other 2 parameters ($P = .025$).
• CONCLUSIONS: The mGCIPL thickness, pRNFL thickness, and mVD decreased over time in POAG eyes. The rate of reduction of mVD was significantly influenced by the baseline measurements of mGCIPL thickness, pRNFL thickness, and mVD. (Am J Ophthalmol 2021;223:259–266. © 2020 Elsevier Inc. All rights reserved.)

AS THE LEADING CAUSE OF IRREVERSIBLE BLINDNESS, glaucoma is expected to affect about 76.0 million and 111.8 million people aged between 40 and 80 years in 2020 and 2040, respectively.[1] To halt or retard the deterioration of visual function in glaucoma, prompt initiation or adjustment of intraocular pressure (IOP)-lowering therapy is of importance; achieving this objective often relies on the early diagnosis of glaucoma and strict monitoring of its progression. The advent of optical coherence tomography (OCT) has greatly enhanced the ability of early detection and reliable quantification of glaucomatous damage to the optic nerve, which demonstrates the invaluable role of OCT in the management of glaucoma.[2]

In recent years, dye-free angiography based on OCT technology, named optical coherence tomography angiography (OCT-A), enables the noninvasive visualization of the vasculature of the retina and optic disc.[3,4] Since vascular insufficiency is one of the classic pathophysiologic theories of glaucoma, OCT-A has also been studied in glaucoma to explore whether this new imaging modality can provide additional information for the detection and monitoring of glaucoma. On the one hand, although there are discrepancies in existing literature regarding the diagnostic performance of OCT-A measurements in glaucoma detection, OCT-A measurements seemed to be similarly or slightly less accurate in differentiation of glaucoma or its suspect from normal, when compared to OCT parameters.[5–8] On the other hand, longitudinal study investigating the progression of OCT-A measurements in glaucoma is still scarce. Shoji and associates[9] characterized the rate of macula vessel density (mVD) loss in primary open-angle glaucoma (POAG), glaucoma suspect, and healthy eyes and found that eyes with POAG had significantly faster loss of macula vessel density than either glaucoma suspect or healthy eyes, with a mean follow-up duration less than 14 months. More recently, Hou and associates,[10] from the same group, reported that in POAG eyes, mVD decrease was faster than ganglion cell complex thinning and was associated with severity of disease, with

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larger sample size and longer follow-up duration compared to the first study. However, the relationship between the decrease of mVD and thinning of ganglion cell complex still remains unclear. In this regard, the longitudinal change of OCT-A measurements in glaucoma remains to be further elucidated.

In this longitudinal study, we evaluated the rate of progression of mVD measured by OCT-A in subjects with POAG and explored the relationship between the progression of mVD and the baseline macular and optic nerve head OCT measurements.

METHODS

THIS IS A PROSPECTIVE COHORT STUDY. APPROVAL FROM the Institutional Review Board of the Eye Hospital of Wenzhou Medical University was obtained for this study, and written informed consent was acquired from all study participants. This study was conducted in accordance with the tenets of the Declaration of Helsinki.

• **STUDY PARTICIPANTS:** A total of 74 patients (102 eyes) with POAG were consecutively recruited at the glaucoma clinic of the Eye Hospital of Wenzhou Medical University and followed at the Clinical Research Center of the same hospital from March 2015 to October 2019. These subjects were enrolled for a prospective, longitudinal study aiming at investigating the change of retinal microvasculature in patients with POAG. The inclusion criteria were as follows: (1) age ≥18 years, (2) confirmation of POAG diagnosis by a glaucoma specialist (Y.L.), and (3) best-corrected visual acuity better than 20/40. The exclusion criteria were as follows: (1) visual field mean deviation (MD) <-20 dB, (2) history of macular disease and neurologic disease at the time of recruitment, and (3) history of refractive or retinal surgery at the time of recruitment. All subjects were followed with a frequency of every 3 or 6 months for a comprehensive ophthalmic examination, including visual acuity test, IOP measurement (Goldmann applanation tonometer), axial length measurement (Lenstar; Haag-Streit, Köniz, Switzerland), central corneal thickness measurement (Lenstar), slit-lamp examination, gonioscopy, visual field test (Humphrey Field Analyzer; Carl Zeiss Meditec, Dublin, California, USA), anterior segment OCT imaging (Casia OCT; Tomey, Nagoya, Japan), OCT imaging (Cirrus HD-OCT; Carl Zeiss Meditec), OCT-A imaging (AngioVue OCT; Optovue, Fremont, California, USA), fundus photography (Visucam; Carl Zeiss Meditec), and questionnaires, which are performed by a designated team of certified technicians and clinicians. In this cohort, patients with IOP less than 24 mm Hg and visual field MD more than -6 dB were not treated after recruitment. During follow-up period, subjects were treated with IOP-lowering eye drops if the IOP raised to 24 mm Hg and above or progression of visual field damages was identified. None of the subjects included in this study received ocular surgeries during the follow-up period. All 102 eyes included in this study had more than 3 reliable OCTA scans, 3 OCT macular scans, and 3 OCT parapapillary scans, which spanned at least 2 years.

• **DEFINITION OF PRIMARY OPEN-ANGLE GLAUCOMA:** Subjects with (1) the presence of narrowed neuroretinal rim and retinal nerve fiber layer defects with corresponding visual field defects in standard automated perimetry in at least 1 eye, (2) open anterior chamber angles on gonioscopy, and (3) no signs of secondary open-angle glaucoma were diagnosed as POAG. The level of IOP was not considered when making the diagnosis.

• **OPTICAL COHERENCE TOMOGRAPHY IMAGING:** Measurements of macular ganglion cell–inner plexiform layer (mGCIPL) thickness and parapapillary retinal nerve fiber layer (pRNFL) thickness were obtained with the Cirrus HD-OCT. The 6×6 mm^2 area centered at the optic nerve head or fovea was imaged by a cube scan (200×200 A-scans). A measurement circle 3.46 mm in diameter (256 A-scans) was then placed around the optic disc automatically to calculate the pRNFL thickness. For the mGCIPL thickness, it was derived as the average thickness of the elliptical annulus with a vertical radius of 2.0 mm and a horizontal radius of 2.4 mm, excluding the central elliptical foveal region (1×1.2 mm^2). Follow-up scans were automatically aligned to the baseline scan based on the retinal blood vessel trajectories to ensure the measurements were derived from the same location. Images with signal strength less than 6, artifacts, missing data, poor centration, and segmentation errors were not included in this study.

• **OPTICAL COHERENCE TOMOGRAPHY ANGIOGRAPHY IMAGING:** The mVD was measured with AngioVue OCT. The 3×3 mm^2 area centered at the fovea was imaged by 2 repeated volumetric scans (304×304 A-scans) with vertical and horizontal priorities, respectively, which were merged into a 3-dimensional OCT angiogram by an orthogonal registration algorithm to reduce motion artifacts and increase image quality.[11] The split-spectrum amplitude-decorrelation angiography algorithm was used to detect the motion contrast generated by the moving of red blood cells inside the vessels, which was then translated into flow signals for angiography.[12] Vessel density was calculated as the proportion of the flow signals within the range of predefined area and depth. In this study, the term mVD only referred to the superficial macular vessel density within the depth from the inner limiting membrane to the posterior boundary of the inner plexiform layer of the whole 3×3 mm^2 macular area centered at the fovea. Similarly, images with signal strength index less than 48 or quality index less than 6, artifacts, missing data, poor centration, and segmentation errors were not included in

TABLE 1. Demographics and Characteristics of Study Subjects at Baseline

Parameters	Summaries (102 Eyes From 74 Subjects)
Sex (male/female)	36/38
Age (years)	60.78 ± 12.19
Follow-up duration (months)	36.55 ± 6.40
Axial length (mm)	23.71 ± 0.96
Central corneal thickness (μm)	534.51 ± 30.74
IOP at baseline (mm Hg)	15.43 ± 3.57
IOP at the latest visit (mm Hg)	13.60 ± 2.61
Number of IOP-lowering eye drops used at baseline	0.18 ± 0.45
Number of IOP-lowering eye drops used at the latest visit	0.47 ± 0.66
Visual field mean deviation (dB)	−5.56 ± 4.95
Visual field index (%)	85.79 ± 13.96
mGCIPL thickness (μm)	72.12 ± 10.44
pRNFL thickness (μm)	74.46 ± 13.47
mVD (%)	44.63 ± 4.18

IOP = intraocular pressure; mGCIPL = macular ganglion cell–inner plexiform layer; mVD = macular vessel density; pRNFL = parapapillary retinal nerve fiber layer.
Data are mean ± standard deviation unless indicated.

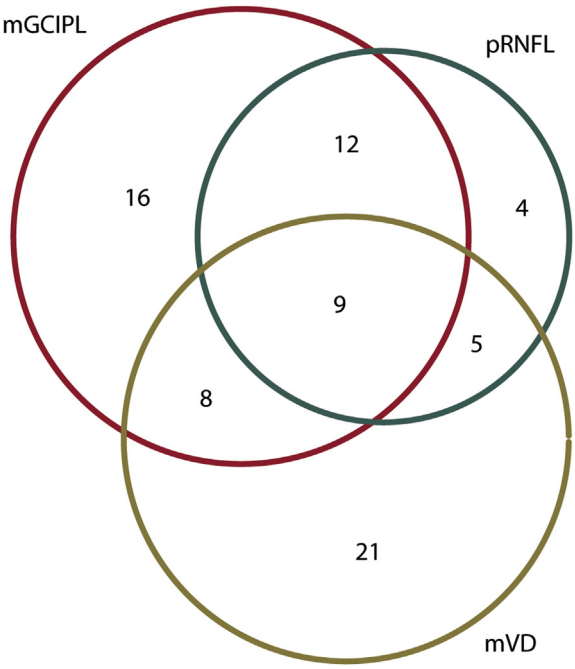

FIGURE 1. Proportional Venn diagram of the eyes with progression detected by the parapapillary retinal nerve fiber layer (pRNFL) thickness, macular ganglion cell–inner plexiform layer (mGCIPL) thickness, and macular vessel density (mVD).

this study. Data were extracted from the commercially available AngioAnalytics software (version 2017.1.0.155; Optovue).

• **STATISTICAL ANALYSIS:** Statistical analyses were performed using Stata Statistical Software (version 14.2; StataCorp, College Station, Texas, USA). Progression of individual eye was defined by linear regression of mGCIPL thickness, pRNFL thickness, and mVD to follow-up duration, when the slope was <0 and $P < .05$. Kappa coefficient and Venn diagram were used to evaluate the agreement in detection of progression among mGCIPL thickness, pRNFL thickness, and mVD. Cohen's Kappa and Conger's Kappa coefficients were used to evaluate the agreement in detection of progression between 2 and all measurements, respectively. Kappa coefficients of less than 0.2, 0.21-0.40, 0.41-0.60, 0.61-0.80, and 0.81-1.0 indicates poor, fair, moderate, good, and very good agreement, respectively.[13] The overall rate of progression of mGCIPL thickness, pRNFL thickness, and mVD was estimated using linear mixed modeling with adjustment of confounding factors and correlation between fellow eyes. Measurements of mGCIPL, pRNFL, and mVD were fitted with linear mixed models with follow-up duration, baseline age, baseline axial length, image signal strength index, and baseline measurement as fixed effects. Random intercepts and coefficients for the effect of follow-up duration were included at both the subject and eye levels as random effects. The influence of baseline measurement on the rate of progression was investigated by incorporating the interaction effect between baseline measurement and follow-up duration ("baseline measurement × duration") into the linear mixed models. Kaplan-Meier survival analysis was adopted to study the survival probability of mGCIPL thickness, pRNFL thickness, and mVD, by defining the endpoint as progression in individual eye recorded in 2 consecutive follow-up visits using linear regression. The time of the prior visit of the 2 consecutive visits was documented as the time of reaching endpoint. $P < .05$ was considered as statistical significance.

RESULTS

TABLE 1 SHOWS THE DEMOGRAPHICS AND CHARACTERISTICS of study subjects at baseline. The mean follow-up duration was 36.6 ± 6.4 months and there were 981 OCT macular scans (average number of scans per eye: 9.6), 909 OCT optic nerve head scans (8.9), and 743 OCT-A macular scans (7.3) analyzed in this study. The average mGCIPL thickness, pRNFL thickness, and mVD at baseline were 72.12 ± 10.44 μm, 74.46 ± 13.47 μm, and 44.63 ± 4.18 %, respectively.

TABLE 2. Coefficient Estimates of Linear Mixed Modeling to Evaluate the Progression of Macular Ganglion Cell–Inner Plexiform Layer Thickness, Parapapillary Retinal Nerve Fiber Layer Thickness, and Macular Vessel Density

	Parameter	Coefficient	95% CI	*P* Value
mGCIPL thickness	Duration	−0.053	−0.060 ~ −0.046	<.001
	Baseline age	0.021	−0.027 ~ 0.07	.39
	Axial length	−0.221	−0.861 ~ 0.420	.499
	Signal strength	0.001	−0.100 ~ 0.101	.99
	Baseline mGCIPL Thickness	0.946	0.890 ~ 1.002	<.001
	Intercept	7.628	−10.279 ~ 25.534	.404
pRNFL thickness	Duration	-0.064	−0.079 ~ −0.048	<.001
	Baseline age	0.011	−0.069 ~ 0.092	.780
	Axial length	1.090	0.035 ~ 2.146	.043
	Signal strength	0.758	0.494 ~ 1.022	<.001
	Baseline pRNFL Thickness	0.910	0.839 ~ 0.981	<.001
	Intercept	−25.076	−54.075 ~ 3.923	.090
mVD	Duration	−0.134	−0.146 ~ -0.122	<.001
	Baseline age	0.054	0.017 ~ 0.092	.005
	Axial length	−0.213	−0.680 ~ 0.255	.373
	Signal strength index	0.228	0.196 ~ 0.259	<.001
	Baseline mVD	0.872	0.769 ~ 0.975	<.001
	Intercept	−7.866	−22.338 ~ 6.607	.287

CI = confidence interval; mGCIPL = macular ganglion cell–inner plexiform layer; mVD = macular vessel density; pRNFL = parapapillary retinal nerve fiber layer.

• **PROGRESSION ANALYSIS:** By simple linear regression, 45 (44.1%), 30 (29.4%), and 43 (42.2%) eyes showed significant negative trends for mGCIPL thickness, pRNFL thickness, and mVD, respectively, during the follow-up period. The mean ± standard deviation of the rate of progression was -0.102 ± 0.054 (95% confidence interval: −0.118 ~ −0.086) μm/month for mGCIPL thickness, −0.160 ± 0.086 (−0.192 ~ −0.128) μm/month for pRNFL thickness, and −0.199 ± 0.073 (−0.221 ~ −0.176) %/month for mVD.

The agreement in detection of progression among mGCIPL thickness, pRNFL thickness, and mVD was poor, with Conger's Kappa coefficient 0.098 (−0.025 ~ 0.220, $P = .116$). The respective Cohen's Kappa coefficients were 0.320 (0.138 ~ 0.502, $P = .001$), −0.079 (−0.275 ~ 0.117, $P = .427$), and 0.057 (−0.134 ~ 0.248, $P = .557$) between mGCIPL thickness and pRNFL thickness, mGCIPL thickness and mVD, and pRNFL thickness and mVD. Figure 1 illustrates the proportional Venn diagram of the eyes with progression detected by the 3 parameters.

The overall rates of progression estimated by linear mixed modeling were −0.053 (−0.060 ~ −0.046, $P < .001$) μm/month, −0.064 (−0.079 ~ −0.048, $P < .001$) μm/month, and −0.134 (−0.146 ~ −0.122, $P < .001$) %/month, respectively, for mGCIPL thickness, pRNFL thickness, and mVD, after adjusting for baseline age, baseline axial length, image signal strength index, and baseline measurement (Table 2). When incorporating the interaction effect between baseline measurement and follow-up duration ("baseline measurement × duration") into the linear mixed models, the significant factors influencing the rate of progression of mVD were baseline mGCIPL thickness (coefficient of "baseline mGCIPL thickness × duration" = 0.0026, $P < .001$), baseline pRNFL thickness (coefficient of "baseline pRNFL thickness × duration" = 0.0016, $P = .001$), and baseline mVD (coefficient of "baseline mVD × duration" = 0.0169, $P < .001$) (Table 3). The greater the baseline measurements, the slower was the rate of mVD reduction. However, baseline mVD was not a significant factor influencing the rates of progression of both mGCIPL thickness and pRNFL thickness, with *P* values ≥ .659 (Table 3).

• **SURVIVAL ANALYSIS:** The numbers of eyes reaching the predefined endpoint during the follow-up period were 43 (42.2%) for mGCIPL thickness, 27 (26.5%) for pRNFL thickness, and 34 (33.3%) for mVD. Figure 2 shows the Kaplan-Meier survival curves of the above 3 parameters. There was a significant difference of survival probability among mGCIPL thickness, pRNFL thickness, and mVD ($P = .025$, log-rank test), and the pRNFL thickness had a better survival probability compared with the other 2 parameters. Pairwise comparison of survival probabilities detected a significant difference between mGCIPL and pRNFL thicknesses ($P = .013$), but not between mGCIPL thickness and mVD or pRNFL and mVD ($P \geq .074$).

TABLE 3. Coefficient Estimates of Linear Mixed Modeling to Evaluate the Influence of Baseline Measurements on the Progression of Macular Ganglion Cell–Inner Plexiform Layer Thickness, Parapapillary Retinal Nerve Fiber Layer Thickness, and Macular Vessel Density

	Parameter	Coefficient	95% CI	*P* Value
mVD	Baseline mGCIPL thickness × duration	0.0026	0.0015 ~ 0.0037	<.001
	Duration	−0.318	−0.395 ~ −0.241	<.001
	Baseline age	0.040	0.007 ~ 0.073	.017
	Axial length	−0.010	−0.419 ~ 0.398	.961
	Signal strength index	0.224	0.194 ~ 0.255	<.001
	Baseline mVD	0.622	0.501 ~ 0.744	<.001
	Baseline mGCIPL thickness	0.096	0.045 ~ 0.148	<.001
	Intercept	−7.366	−20.117 ~ 5.385	.258
mVD	Baseline pRNFL thickness × duration	0.0016	0.0007 ~ 0.0026	.001
	Duration	−0.253	−0.321 ~ −0.184	<.001
	Baseline age	0.050	0.014 ~ 0.087	.007
	Axial length	−0.121	−0.582 ~ 0.341	.608
	Signal strength index	0.224	0.192 ~ 0.256	<.001
	Baseline mVD	0.759	0.647 ~ 0.871	<.001
	Baseline pRNFL thickness	0.038	0.000 ~ 0.075	.050
	Intercept	−7.267	−21.663 ~ 7.129	.322
mVD	Baseline mVD × duration	0.0169	0.0155 ~ 0.0183	<.001
	Duration	−0.803	−0.860 ~ −0.745	<.001
	Baseline age	0.026	0.002 ~ 0.051	.037
	Axial length	−0.084	−0.386 ~ 0.218	.587
	Signal strength index	0.130	0.105 ~ 0.155	<.001
	Baseline mVD	0.580	0.509 ~ 0.651	<.001
	Intercept	9.975	0.387 ~ 19.563	.041
mGCIPL thickness	Baseline mVD × duration	0.0002	−0.0011 ~ 0.0014	.774
	Duration	−0.061	−0.111 ~ −0.011	.016
	Baseline age	0.031	−0.019 ~ 0.080	.226
	Axial length	−0.220	−0.853 ~ 0.414	.496
	Signal strength	0.000	−0.122 ~ 0.122	.996
	Baseline mGCIPL thickness	0.892	0.816 ~ 0.967	<.001
	Baseline mVD	0.182	−0.007 ~ 0.371	.059
	Intercept	2.859	−16.006 ~ 21.724	.766
pRNFL thickness	Baseline mVD × duration	−0.0006	−0.0030 ~ 0.0019	.659
	Duration	−0.045	−0.143 ~ 0.053	.37
	Baseline age	0.028	−0.055 ~ 0.110	.51
	Axial length	1.239	0.172 ~ 2.306	.023
	Signal strength	0.731	0.429 ~ 1.033	<.001
	Baseline pRNFL thickness	0.864	0.784 ~ 0.945	<.001
	Baseline mVD	0.302	0.041 ~ 0.562	.023
	Intercept	−39.438	−71.382 ~ −7.494	.016

CI = confidence interval; mGCIPL = macular ganglion cell–inner plexiform layer; mVD = macular vessel density; pRNFL = parapapillary retinal nerve fiber layer.

DISCUSSION

IN THIS STUDY, WITH A MEAN FOLLOW-UP DURATION OF 36.6 ± 6.4 months, all 3 parameters—including mGCIPL thickness, pRNFL thickness, and mVD—decreased over time in POAG eyes, although the agreement in detection of progression among them was poor. Linear mixed modeling revealed that the rate of progression of mVD was significantly influenced by the baseline measurements of mGCIPL thickness, pRNFL thickness, and mVD, separately. In contrast, the rates of progression of mGCIPL thickness and pRNFL thickness were not influenced by the baseline mVD measurement.

To date, there have been only 2 studies on the longitudinal change of mVD in eyes with glaucoma.[9,10] By recruiting 32 POAG, 30 glaucoma suspect, and 38 healthy eyes followed for at least 1 year with at least 2 OCT-A scans, Shoji and associates found that the rate of decrease of

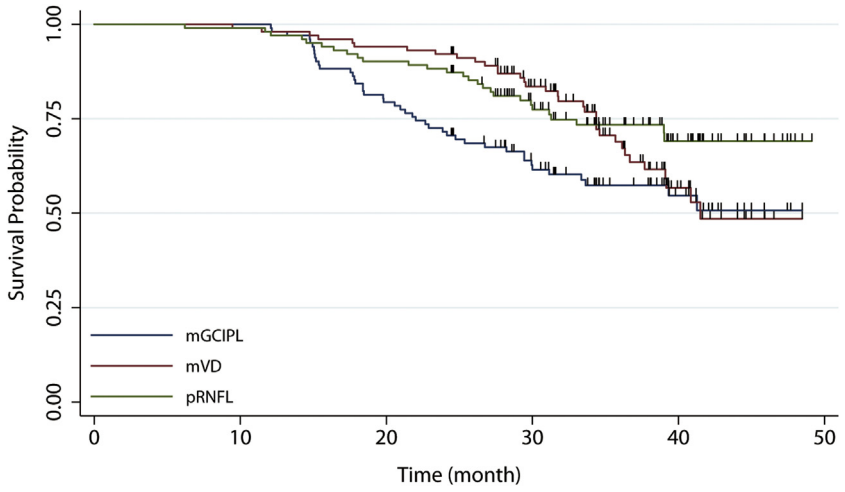

FIGURE 2. Kaplan-Meier survival curves of the parapapillary retinal nerve fiber layer (pRNFL) thickness, macular ganglion cell–inner plexiform layer (mGCIPL) thickness, and macular vessel density (mVD).

mVD over time was significantly faster in the glaucoma group than in the other 2 groups.[9] However, with the mean follow-up duration less than 14 months and with a minimum of 2 visits for study subjects, this study may not clearly reflect the pattern of the progression of mVD in glaucoma eyes. More recently, Hou and associates, from the same group, published another study with the purpose to characterize the change rate of ganglion cell complex thickness and mVD in healthy, preperimetric glaucoma, and POAG eyes.[10] In the second study, the mean follow-up time and number of visits were increased to 2.0 years and 3.4 visits for healthy eyes (n = 23), 2.6 years and 3.8 visits for preperimetric glaucoma eyes (n = 36), and 2.6 years and 3.8 visits for POAG eyes (n = 80). Conclusions were made that thinning of ganglion cell complex thickness and reduction of mVD were found for all diagnostic groups, and in the POAG group the rate of progression of mVD was faster than that of ganglion cell complex thickness and was associated with the severity of disease. Findings of our study are in agreement with the 2 previous studies. With a mean follow-up duration of 36.6 ± 6.4 months, progression of mGCIPL thickness, pRNFL thickness, and mVD was detected in eyes with POAG using both linear regression and linear mixed modeling for adjustment of confounders. The rate of progression of mVD (normalized rate of progression: −5.35% per year) seemed to be faster than that of mGCIPL thickness (−1.70% per year) and pRNFL thickness (−2.58% per year) by roughly comparing the proportions of the mean rates of progression calculated by linear regression to the respective mean baseline measurement, which is also similar to Hou's paper.

The relationship between OCT-A measurements at baseline and future glaucoma progression was also studied previously. Park and associates reported an association between lower parapapillary choroidal vessel density within the β-zone parapapillary atrophy at baseline and higher risk of glaucoma progression defined by visual field tests.[14] Moghimi and associates also found that lower macular and optic nerve head vessel densities are associated with a faster rate of retinal nerve fiber layer progression in mild-to-moderate glaucoma.[15] However, results from the current study do not support the previous findings. The rates of progression of mGCIPL and pRNFL thicknesses were not significantly influenced by baseline mVD. Instead, baseline mGCIPL, mRNFL, and mVD measurements were significantly influencing the rate of progression of mVD. The greater the baseline measurements, the slower was the rate of mVD reduction. Lower baseline measurements of mGCIPL and pRNFL indicated a more severe stage of disease; therefore, the rate of mVD progression would be higher in eyes with more severe glaucoma, which is in concordance with Hou's finding.[10] Furthermore, as the mean visual field MD of the study cohort was −5.56 ± 4.95 dB, it is plausible that this cohort had developed certain levels of optic nerve damage related to glaucoma at recruitment. Thus, our finding might also suggest that in POAG eyes with existing optic nerve damage, further decreasing of mVD over time might be partially attributable to a reduced demand of blood supply resulting from existing optic nerve damage, demonstrated as thinning of mGCIPL and pRNFL thicknesses at baseline. Yet, the complex relationship between reduction of blood flow and retinal ganglion cell (RGC) damage during the development of glaucoma remains to be studied. And it is worth mentioning that OCT-A quantifies the microvasculature of the retina, which is not a complete picture of the blood supply to the retina, and only the vessels with signals of the

motion contrast generated by the moving of red blood cells above the threshold of the device could be visualized. Caution should be taken when interpreting the results related to OCT-A measurements. Nevertheless, OCT-A provides an opportunity to explore the role that microvasculature plays in the pathophysiology of glaucoma.

In this study, poor agreement among mGCIPL thickness, pRNFL thickness, and mVD was found when identifying the progression of glaucoma. This might be because progressions of the 3 metrics may not happen concurrently. A similar phenomenon was reported by Hou and associates[16] that among 88 glaucoma eyes with progressive pRNFL or mGCIPL thinning, only 35 (39.8%) eyes demonstrated progressive thinning in both pRNFL and mGCIPL. This could be explained by the findings of the animal study from Leung and associates,[17] which found that RGC damage in a glaucoma model was observed prospectively to begin with progressive dendritic shrinkage, followed by loss of the axon and the cell body, using an in vivo RGC imaging technique. Furthermore, progression of visual field, pRNFL, mGCIPL, mVD, and other parameters may not happen in a linear manner, and the rate of progression of a certain parameter varies at different stages of the disease. There are pitfalls when using linear regression to model glaucoma progression, but it does provide a simple and straightforward way for patients to understand the idea of glaucoma progression in many clinical scenarios; and therefore, trend analysis using linear regression remains one of the most popular methods to identify glaucoma progression.

Performing of Kaplan-Meier survival analysis in this study was to mimic the typical clinical scenario of defining glaucoma progression. The pRNFL thickness was significantly higher in survival probability among the 3 parameters under study. It has been reported that age-related change affected the analysis of glaucoma progression and the impact was more substantial in mGCIPL progression than in pRNFL progression.[18] In Leung's paper, survival probability of mGCIPL thickness became significantly higher than that of pRNFL thickness, after accounting for age-related change. Progression of mVD in healthy eyes was documented by previous studies,[9,10,19] and the rate ranged from 0.29% to 1.30% per year. However, with a mean rate of progression of 2.388% per year, it is not implausible that the progressive decrease of mVD in the current cohort was more disease-related rather than age-related. Nevertheless, the impact of age-related change on mVD progression in glaucoma is of interest for future studies and needs to be considered in clinical practice. Meanwhile, floor effect also needs to be considered when using OCT to monitor glaucomatous progression. Moghimi and associates[20] found that in late-stage glaucoma with visual field MD worse than −14 dB, OCT-A-derived vessel density measurements did not have a detectable measurement floor and thickness parameters reached the floor earlier than OCTA measurements. With the fact that the subjects in the current study have mean visual field MD of −5.56 ± 4.95 dB, indicating that most of the subjects were at the early stage of glaucoma, it would be reasonable to believe that the impact of floor effect was not significant for this study. Progression of mVD in glaucoma patients reminds the attending ophthalmologist that IOP alone may not be satisfactory in explanation of glaucoma progression. Systemic factors like vascular dysregulation should draw adequate attention when the disease continues to deteriorate under the condition of reaching targeted IOP after treatment.[21]

There are limitations to this study. The sample size is not large enough for subgroup analysis based on disease severities. As most of the study subjects were referred from community screening programs, this cohort had early-stage glaucoma. The demographic makeup and clinical feature of this cohort limited the generalization of findings of this study to other populations. In addition, investigation of the influence of mVD on the visual field progression might need longer follow-up duration. Furthermore, mVD measurements derived from a small portion of the macular area were used in this study. With the development of wide-field OCT-A technology, additional information may further increase the value of OCT-A measurements in the management of glaucoma.

In conclusion, the mGCIPL thickness, pRNFL thickness, and mVD decreased over time in POAG eyes, although the agreement in the progression analyses was poor. The rate of reduction of mVD was significantly influenced by the baseline measurements of mGCIPL thickness, pRNFL thickness, and mVD.

CRediT AUTHORSHIP CONTRIBUTION STATEMENT

CONG YE: CONCEPTUALIZATION, METHODOLOGY, WRITING - original draft, Funding acquisition. **Xiaoyan Wang:** Investigation, Data curation, Project administration. **Marco Chak-yan Yu:** Formal analysis. **Xiao Shang:** Investigation. **Kun Zhou:** Investigation. **Yan Tao:** Investigation. **Fan Lu:** Writing - review & editing, Supervision. **Yuanbo Liang:** Writing - review & editing, Resources.

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