

Effects of Trabeculectomy on Waveform Changes of Laser Speckle Flowgraphy in Open Angle Glaucoma

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PURPOSE. To investigate the effect of trabeculectomy on the waveform changes of laser speckle flowgraphy (LSFG) in the optic nerve head (ONH) in patients with glaucoma.

METHODS. Forty-eight eyes of 48 patients with open angle glaucoma were included in this prospective study. LSFG was performed before and 1, 3, and 6 months after trabeculectomy. Longitudinal changes in average mean blur rate (MBR), blow out score (BOS), resistivity index (RI), falling rate, skew, acceleration time index, and blow out time in the tissue area of the ONH were analyzed by using mixed-effects models.

RESULTS. Intraocular pressure (IOP) decreased and ocular perfusion pressure increased significantly at each postoperative time point ($P < 0.001$, each). BOS increased ($P < 0.001$, each) and RI decreased ($P < 0.001$, each) significantly at each postoperative time point, although average MBR and other waveform parameters did not change significantly. Multivariate analyses revealed that younger age (coefficients = -0.13 and 0.0014 , $P = 0.006$ and 0.03 for BOS change and RI change, respectively), worse baseline mean deviation of visual fields (coefficients = -0.18 and 0.0026 , $P = 0.009$ and 0.005), larger IOP reduction (coefficients = -0.29 and 0.0037 , $P < 0.001$, each), and larger pulse rate increase (coefficients = 0.17 and -0.0024 , $P < 0.001$, each) are significantly associated with postoperative BOS increase and RI decrease.

CONCLUSIONS. Given that postoperative BOS increased and RI decreased with the average MBR remaining unchanged, IOP reduction by trabeculectomy may contribute to stable blood flow throughout the duration of the heartbeat in the tissue area of the ONH.

Keywords: trabeculectomy, laser speckle flowgraphy, glaucoma, waveform, optic nerve head

Although intraocular pressure (IOP) reduction is the only evidence-based therapy of glaucoma, multicenter randomized trials reveal that visual field (VF) defects still progress in a considerable proportion of patients despite achieving the target IOP reduction.¹⁻³ Besides IOP, several prognostic factors associated with VF progression have been identified in a systematic review.⁴ Among them, disc hemorrhage and stroke were identified as a definite risk and a probable risk in normal tension glaucoma (NTG), respectively, and higher ocular blood flow resistivity index (RI) was a probable risk factor for VF progression in open angle glaucoma. These findings indicate that the local or systemic pathologic vascular status may underlie disease progression in patients with glaucoma.

Various devices including scanning laser Doppler flowmetry (SLDF), color Doppler imaging (CDI), laser speckle flowgraphy (LSFG), and optical coherence tomography angiography have been used to study blood flow impairment in the optic nerve head (ONH) of glaucomatous eyes.⁵ Of these, LSFG measures the relative value of blood flow velocity, the mean blur rate (MBR), by using the laser speckle phenomenon.⁶ The MBR of the speckle images is highly correlated with actual tissue blood flow in the ONH of monkeys and rabbits, measured by microsphere and hydrogen gas clearance methods, respectively.⁷⁻⁹ Moreover, MBR in the capillary region of the ONH appears

to reflect the blood flow in the retrolaminar region, which is served primarily by the short posterior ciliary circulation.⁹ Several studies⁹⁻¹² consistently have shown lower MBR in the ONH capillary region in glaucomatous eyes than in control eyes. Furthermore, LSFG provides MBR waveform parameters, which characterize the hemodynamics within a single heartbeat in the measurement area quantitatively. Recently, differences in LSFG waveform parameters for glaucomatous eyes have been reported. Skew and acceleration time index (ATT) have the greatest ability to differentiate eyes with mild NTG from normal eyes.¹³ NTG patients have a flatter MBR curve as indicated by a higher blow out time (BOT) and lower flow acceleration index than normal subjects.¹²

Several recent studies¹⁴⁻¹⁶ have reported that surgical IOP reduction may improve VF sensitivity. Although better postoperative IOP control¹⁴ or a larger IOP reduction¹⁵ are predictive factors for long-term VF improvement during the 5-year study periods, a larger postoperative mean ocular perfusion pressure (OPP) increase, but not larger IOP decrease, is associated with short-term enhancement of VF sensitivity at 3 months postoperatively.¹⁶ The authors¹⁶ suggest that improved OPP through IOP reduction may help with the recovery from ganglion cell dysfunction and contribute to restoring visual function.



There are several reports¹⁷⁻²² on changes of ocular blood flow after trabeculectomy. However, the results are controversial because of differences in methods, including devices used for measuring blood flow, target area of blood flow measurements, and patient backgrounds. A previous study using a similar laser speckle method to the current LSFSG shows no significant changes in ONH blood flow until 8 weeks after trabeculectomy.²⁰ However, waveform changes of ONH blood flow within a single heartbeat after trabeculectomy are currently unknown.

The purpose of this prospective study was to investigate the MBR waveform changes in the capillary area of the ONH after trabeculectomy and to identify factors associated with the waveform changes.

MATERIALS AND METHODS

Study Subjects and Protocol

The current prospective study included 48 eyes of 48 patients with open angle glaucoma who underwent trabeculectomy without any combined surgeries at Kanazawa University Hospital from September 2012 to August 2015. The protocol of the study adhered to the tenets of the Declaration of Helsinki and was approved by the Ethical Committee of Kanazawa University Hospital, Japan. Written informed consent was obtained from each patient.

The inclusion criteria were primary open angle glaucoma (POAG) and exfoliation glaucoma. Cases in which the EX-PRESS glaucoma filtration device (Alcon Laboratories, Inc., Fort Worth, TX, USA) was used were included. Eyes with previous trabeculectomy, laser trabeculoplasty, and cataract surgery were also included if they were performed more than 6 months before trabeculectomy. Patients with previous intraocular surgery, except glaucoma and cataract surgery as listed above, were excluded. Patients who required postoperative topical ocular hypotensive drugs and intraocular surgeries, including bleb revision or cataract surgery, were also excluded. Moreover, patients with retinal diseases including any degree of diabetic retinopathy and chorioretinal atrophy, with past retinal photocoagulation, with a long axial length (AL) (≥ 27.00 mm), with inadequate LSFSG data having band-shaped artifacts, failure in heartbeat analysis, MBR image defects in the periphery of the measurement area, and marked decentration of the ONH, and with a short follow-up period (< 6 months) were excluded.

Trabeculectomy was performed with a fornix-based conjunctival flap and a half-thickness scleral flap. Mitomycin C (0.04%) was applied for 5 minutes, and the scleral flap was closed with 10-0 nylon sutures. EX-PRESS shunts were implanted at the surgeon's discretion. Postoperatively, topical levofloxacin and betamethasone were tapered as needed. Blebs were managed by argon laser suture lysis to enhance filtration.

Patients underwent a battery of preoperative ophthalmologic tests including best corrected visual acuity, refractive status by autorefractometry, slit-lamp examination, IOP measurement using Goldmann applanation tonometry, gonioscopy, dilated fundus examination, AL measurement (OA-1000; Tomey Corporation, Nagoya, Japan), VF testing using a Humphrey field analyzer (HFA; Carl Zeiss Meditec, Dublin, CA, USA) with the 24-2 Swedish Interactive Threshold Algorithm, and LSFSG. In addition, systemic blood pressure was measured by using an automated sphygmomanometer. OPP was calculated by using the following formula: $OPP = 2/3$ mean arterial pressure (MAP) – IOP, where $MAP = \text{diastolic blood pressure} + 1/3$ (systolic blood pressure – diastolic blood pressure). Measurements of

IOP, blood pressure, and LSFSG were repeated at 1, 3, and 6 months after surgery.

Measurement of LSFSG and MBR Waveform Parameters

The methods and principles of LSFSG (LSFG-NAVI; Softcare Ltd., Kyushu, Japan) have been described in detail previously.^{23,24} Briefly, this instrument is composed of an ordinary charge-coupled device (CCD) camera (750×360 pixels) and fundus camera equipped with a diode laser (wavelength, 830 nm). MBR is determined by blurring of the speckle pattern generated by the interference of reflected lights from the movement of blood cells in the ocular fundus. MBR represents the relative velocity of blood flow and is expressed in arbitrary units (AUs). The continuous MBR images are acquired at a rate of 30 frames per second over a 4-second period and automatically detected the beginning and the end of the heartbeat. Images corresponding to the identical phases within one heartbeat duration are synthesized to one image sequence. An MBR waveform is delineated by plotting MBRs derived from each frame.

Before performing LSFSG, the pupils of each patient were dilated with Mydrin M (0.4% tropicamide; Santen Pharmaceutical Co., Ltd., Osaka, Japan). Then, images of ONH blood flow were captured by using LSFSG, and an elliptical band was placed to identify the optic disc margin to measure MBR in the ONH.

The LSFSG analyzer software (version 3.1.68.2; Softcare Ltd.) separates large vessels from the tissue area according to the automated definitive threshold, and the demarcated ONH area was divided into the vessel area and the tissue area. Then, MBRs were calculated from each area: that is, MBR from the entire area, MBR from the vessel area, and MBR from the tissue area. In this study, we analyzed only the tissue area of the ONH.

LSFG images were consecutively captured three times for each measurement time, and averaged values were used for statistical analysis. We applied an identical elliptical band to the ONH in all measurements throughout a 6-month follow-up period in the same eye, and thus we could evaluate longitudinal changes after trabeculectomy.

The LSFSG-analyzer software enabled us to calculate pulse waveform parameters as follows.

$$\text{Average MBR} = \sum_{k=1}^N \{MBR_k \cdot P(k)\}$$

$$\text{BOS} = C \cdot \{[2 - (MBR_{max} - MBR_{min}) / \text{Average MBR}] / 2\}$$

$$\text{RI} = (MBR_{max} - MBR_{min}) / MBR_{max}$$

$$\text{FR} = C \cdot \frac{\{MBR_{max} \cdot (N - f_{max} + 1) - \sum_{k=f_{max}}^N MBR_k\}}{\{(MBR_{max} - MBR_{min}) \cdot (N - f_{max} + 1)\}}$$

$$\text{Skew} = C \cdot \int_{k=1}^N \{(MBR_k - \text{Average MBR}) / SD\}^3 \cdot P(k) dx$$

$$\text{ATI} = 100 \cdot f_{max} / N$$

$$\text{BOT} = C \cdot \frac{\sum_{k=1}^N b(k)}{N}$$

$$b(k) = \begin{cases} 1 & MBR_k > (MBR_{max} + MBR_{min}) / 2 \\ 0 & \text{otherwise} \end{cases}$$

Given that LSFG obtains 30 frames of MBR images per second, the MBR waveform during a single heartbeat consists of variable total number of frames (N), which depends on pulse rate (PR) (i.e., lower PR leads to higher N frames). The k indicates the number of frames. C is the constant of proportion. SD represents the standard deviation of MBR. $P(k)$ indicates the probability density function in the k -th frame. f_{max} represents the number of frames until the peak of MBR. Average MBR represents the average value of MBR in all frames during a single heartbeat. BOS (blow out score) represents the blood flow volume that is maintained in the vessels between each heartbeat. RI (resistivity index) is calculated by dividing the difference of the maximum and minimum MBR by the maximum MBR. Both BOS and RI are supposed to be associated with vascular resistance. FR (falling rate) is defined as the ratio of falling area to total area after the peak. A higher FR value indicates a more sudden MBR decrease. Skew is an indicator for asymmetry of the MBR waveform. A negative value indicates a rightward shift of MBR waveform and/or a gentler slope after the peak. ATI is derived from a ratio dividing the total time of a heartbeat by the time to the peak. A smaller ATI indicates a more rapid increase in MBR to the peak. BOT is the ratio of the duration of MBR being maintained more than half of the mean of the maximum and minimum MBR to the duration of one heartbeat. A higher BOT indicates that a high level of MBR is maintained for a larger proportion of a single heartbeat.

The average waveform of MBR in the tissue area of the ONH was determined by averaging MBR from all patients after normalizing the heartbeat duration to the range of 0% to 100% in each patient, and was compared between four measurement time points.

Statistical Analysis

Mixed-effects models with eye-specific random effects accounting for the repeated measurements of LSFG were used to evaluate postoperative changes of IOP, OPP, average MBR, BOS, RI, FR, skew, ATI, and BOT. Bonferroni tests were used for post hoc analyses. Correlations between baseline values of MBR waveform parameters, age, and baseline mean deviation (MD) of VFs were examined by using Spearman's rank correlation coefficients. The factors associated with the postoperative changes of waveform parameters with significant postoperative changes were analyzed by using mixed-effects models with eye-specific random effects accounting for the repeated measurements of LSFG. The following variables were examined: measurement time, sex, glaucoma type, presence of systemic hypertension, number of IOP-lowering medications, age, baseline AL, baseline MD, baseline value of the waveform parameter, baseline average MBR, average MBR change, IOP change, OPP change, and PR change. The final multivariate model was created by using backward elimination by successively removing variables with the least significant effects until only those variables with significant effects remained.²⁵ Statistical analyses were performed by using STATA 14.0 software for Windows (StataCorp, College Station, TX, USA). For all analyses, a P value of <0.05 was considered statistically significant.

RESULTS

Baseline patient characteristics are shown in Table 1. Of these patients, 52.1% (25/48) were female, and 64.6% (31/48) had a diagnosis of POAG. The age of the study population was 69.1 ± 8.9 years (mean \pm standard deviation), and the MD value was -19.7 ± 6.4 dB. Preoperative IOP and OPP were $19.6 \pm$

TABLE 1. Baseline Patient Characteristics Before Trabeculectomy

Age, y	69.1 ± 8.9 (62 to 76)
Sex, male/female, cases	23/25
Diagnosis, POAG/XFG, eyes	31/17
Systemic hypertension, cases (%)	25 (52.1)
Oral antihypertensive agents, cases (%)	16 (33.3)
Oral calcium channel blockers, cases (%)	14 (29.2)
Oral angiotensin II receptor blockers, cases (%)	11 (22.9)
Oral β blockers, cases (%)	2 (4.2)
Diabetes mellitus, cases (%)	2 (4.2)
Axial length, mm	24.2 ± 1.3 (23.3 to 25.3)
Spherical equivalent refractive error, diopters	-2.1 ± 2.3 (-3.0 to -0.5)
Intraocular pressure, mm Hg	19.6 ± 6.0 (16 to 21)
Mean deviation, dB	-19.7 ± 6.4 (-24.6 to -14.5)
Blood pressure, mm Hg	
Systolic blood pressure	130.3 ± 22.6 (118 to 145)
Diastolic blood pressure	71.8 ± 13.1 (63 to 80)
Mean arterial pressure	91.3 ± 15.4 (81.3 to 102.0)
Ocular perfusion pressure, mm Hg	41.4 ± 10.7 (34.0 to 47.0)
Pulse rate, beats/min	70.5 ± 12.4 (61.0 to 81.3)
Preoperative use of oral acetazolamide, eyes (%)	8 (16.7)
No. of preoperative antiglaucoma medications (range)	4.2 ± 0.8 (2 to 6)
Prostaglandin analogues (%)	48 (100)
β antagonists (%)	43 (89.6)
Carbonic anhydrase inhibitors (%)	44 (91.7)
α -1 antagonist (%)	16 (33.3)
α -2 agonist (%)	38 (79.2)
Rho kinase inhibitor (%)	3 (6.3)
Previous intraocular surgery, eyes (%)	
Cataract surgery	17 (35)
Trabeculectomy	8 (17)
Laser trabeculoplasty	3 (6)

Unless otherwise indicated, data are expressed as the mean \pm standard deviation (interquartile range). XFG, exfoliation glaucoma.

6.0 mm Hg and 41.4 ± 10.7 mm Hg, respectively. Systemic hypertension was present in 52.1% (25/48) of cases. Preoperatively, all eyes were treated with a topical prostaglandin analogue, and 85.4% (41/48) of eyes received four or more antiglaucoma medications.

Correlations between baseline values of MBR waveform parameters were examined by using Spearman's rank correlation coefficients (r_s ; Table 2). More than half of the correlations were statistically significant. Of note, baseline BOS had an almost perfect negative correlation with RI ($r_s = -0.996$, $P < 0.001$).

Baseline values of MBR waveform parameters and their relationship with age and baseline MD are shown in Table 3. Age had a significant negative correlation with baseline BOS ($r_s = -0.29$, $P = 0.046$) and a positive correlation with baseline RI ($r_s = 0.28$, $P = 0.0499$). The baseline MD value was not significantly correlated with any baseline values of MBR waveform parameters.

Following trabeculectomy, IOP decreased and OPP increased significantly. At 1, 3, and 6 months after trabeculectomy, the marginal mean of IOP decrease and OPP increase were 10.0 mm Hg (50.8%) and 12.4 mm Hg (29.8%), 10.8 mm Hg (55.2%) and 11.3 mm Hg (27.3%), and 10.0 mm Hg (51.1%) and 11.8 mm Hg (28.3%), respectively (Fig. 1).

TABLE 2. Spearman's Rank Correlation Coefficients Between Baseline Values of MBR Waveform Parameters

	rs, P Value					
	Average MBR	BOS	RI	FR	Skew	ATI
BOS	-0.28, 0.051					
RI	0.28, 0.055	-0.996, <0.001				
FR	0.45, 0.001	-0.47, 0.001	0.48, 0.001			
Skew	0.25, 0.086	-0.61, <0.001	0.63, <0.001	0.74, <0.001		
ATI	0.15, 0.317	0.21, 0.151	-0.23, 0.117	-0.061, 0.682	-0.54, <0.001	
BOT	-0.33, 0.021	0.31, 0.035	-0.33, 0.024	-0.87, <0.001	-0.70, <0.001	0.15, 0.299

Representative color-coded maps of the MBR from baseline to 6 months after trabeculectomy in the same eye are shown in Figure 2. There were no significant changes in the average MBR throughout the study period (Fig. 3). To visualize the average waveform of the MBR, the duration of the heartbeat in each patient was normalized from 0% to 100%, and the waveforms of all patients were averaged in each time point. The average waveform of the MBR flattened after trabeculectomy (Fig. 4).

In terms of the longitudinal changes of waveform parameters, BOS significantly increased and RI significantly decreased at all postoperative time points ($P < 0.001$, each), while there were no significant changes in FR, skew, ATI, and BOT throughout the study period (Fig. 5).

A mixed-effects model analysis was performed to determine the factors influencing changes of BOS and RI (Table 4). In the multivariate models, age (BOS change: coefficient = -0.13, $P = 0.006$; RI change: coefficient = 0.0014, $P = 0.03$), baseline MD (BOS change: coefficient = -0.18, $P = 0.009$; RI change: coefficient = 0.0026, $P = 0.005$), IOP change (BOS change: coefficient = -0.29, $P < 0.001$; RI change: coefficient = 0.0037, $P < 0.001$), and PR change (BOS change: coefficient = 0.17, $P < 0.001$; RI change: coefficient = -0.0024, $P < 0.001$) had significant inverse associations with BOS change and RI change. Baseline BOS (coefficient = -0.50; $P < 0.001$) and baseline RI (coefficient = -0.43; $P < 0.001$) were significantly associated with BOS change and RI change, respectively.

DISCUSSION

Several CDI studies^{17,18,22} have reported increased blood flow velocity and decreased RI in retrobulbar arteries after trabeculectomy. In terms of microvascular blood flow in the ONH, Tamaki et al.²⁰ have evaluated changes of ONH blood flow after trabeculectomy, using the laser speckle method similar to the current LSF. They have found that the normalized blur, which is analogous to MBR, in the tissue region of the ONH does not change significantly until 8 weeks after trabeculectomy. Our average MBR observations corroborate their results. In contrast, Berisha et al.¹⁹ have evaluated the effect of

trabeculectomy on blood flow in the neuroretinal rim area of the ONH, using SLDF in POAG patients. They have found a significant increase in ONH blood flow 10 weeks after trabeculectomy, which is associated with an increase in OPP. According to the experiments with monkeys, SLDF mainly measures the microcirculation on the anterior portion of the ONH supplied by the central retinal artery,²⁶ while LSF reflects ONH blood flow in the retrolaminar region supplied primarily by the short posterior ciliary arteries.⁹ The difference in the measurement principle between SLDF and LSF may account for the discrepancy in the results.

The present study is the first to evaluate waveform changes of ONH blood flow by using LSF in glaucoma patients after trabeculectomy. We observed a significant BOS increase and a significant RI decrease. The strong inverse relationship of BOS and RI to each other at baseline and in the postoperative changes was an expected result given the formula for BOS and RI. RI is a representative parameter of CDI and indicates the resistance to blood flow in the retrobulbar arterial vessels. A higher RI has been identified as a probable risk factor for VF progression in glaucoma.⁴ Although MBR in the tissue area of the ONH does not target arterial vessels, RI and BOS should reflect the resistance to blood flow there. Lower RI and higher BOS in MBR waveform indicate a flatter waveform, which is in agreement with the significantly positive correlation of RI with FR and Skew at baseline in our study. Mursch-Edlmayr et al.¹² have reported that the average MBR in the tissue area of the ONH in NTG eyes is significantly lower than that in control eyes. Therefore, lower RI in NTG eyes in their study does not necessarily mean maintenance of better perfusion.¹² Given that the average MBR remained unchanged after trabeculectomy, postoperative BOS increase and RI decrease should indicate more stable perfusion during a single heartbeat in the tissue region of the ONH than in the preoperative status.

We also found that younger age, larger IOP reduction, worse baseline MD value, and larger PR increase were associated with BOS increase and RI decrease (i.e., more stable blood flow) postoperatively. Tsuda et al.²⁷ have reported that age has a significant positive association with FR in normal subjects, which suggests that blood flow reduces steeply after the peak

TABLE 3. Baseline Values of MBR Waveform Parameters and Their Relationships With Age and Baseline MD

Waveform Parameters	Baseline Value, Mean \pm SD, AU	Correlation With Age, rs, P Value	Correlation With Baseline MD, rs, P value
Average MBR	8.8 \pm 2.5	0.095, 0.519	0.26, 0.073
BOS	75.5 \pm 8.0	-0.29, 0.046	-0.28, 0.054
RI	0.37 \pm 0.09	0.28, 0.0499	0.27, 0.065
FR	13.1 \pm 1.0	0.25, 0.084	0.27, 0.066
Skew	11.0 \pm 2.6	0.16, 0.279	0.16, 0.288
ATI	32.9 \pm 5.1	0.12, 0.415	0.11, 0.458
BOT	49.5 \pm 4.7	-0.23, 0.114	-0.23, 0.115

MD, mean deviation of visual fields; SD, standard deviation.

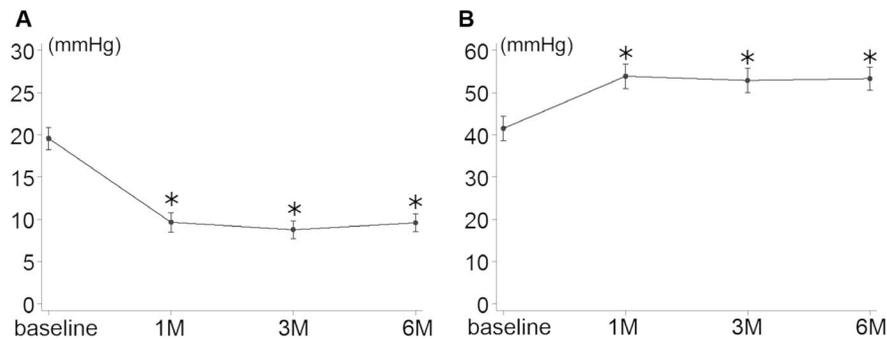


FIGURE 1. Longitudinal changes of intraocular pressure and ocular perfusion pressure. (A) Intraocular pressure. (B) Ocular perfusion pressure. Estimated marginal means from the linear mixed-effects models are plotted for each postoperative time point. Error bars denote 95% confidence interval. Significant changes compared to initial values are indicated with an asterisk (* $P < 0.05$ after Bonferroni correction for multiple comparisons).

in eyes of elderly subjects, possibly as a result of less elastic vessel walls owing to atherosclerosis. In our results, older age was associated with postoperative BOS decrease and RI increase, indicating an increase in resistance to blood flow. Thus, the beneficial effects of trabeculectomy on ONH hemodynamics may be less likely to be applicable to elderly patients.

In the univariate analysis, IOP change was significantly associated with BOS and RI changes, while OPP change had association with both of them at less significant levels than IOP changes. In the multivariate mixed-effects models with backward elimination, larger IOP reduction, but not larger OPP increase, was selected as a significantly associated factor with BOS increase and RI decrease. Reversal of structural changes in the ONH, such as posterior lamellar displacement and prelaminar tissue thinning, has been reported.²⁸⁻³⁰ Larger IOP reduction is associated with larger reversal of posterior lamellar displacement.^{28,29} Our results indicate that IOP reduction, which mitigates pressure-induced structural changes in the ONH, was more relevant to flattening of the MBR waveform (i.e., BOS increase and RI decrease) after trabeculectomy than OPP increase. The relationship between MBR waveform changes and structural changes in the ONH needs to be addressed in a future study.

There is an issue of multicollinearity in the multivariate analysis when using both IOP and OPP as independent variables, given that these two variables are not independent from each other, with a supposed considerably high correlation between them.^{31,32} In this study, the correlation between IOP change and OPP change at all postoperative time points was not so strong ($r = -0.52$, $P < 0.001$) as to be expected to induce serious multicollinearity. Furthermore, although the initial multivariate models had both IOP change and OPP change as independent variables, only IOP change remained after backward elimination in both final models showing the associated factors with BOS or RI changes. Therefore, interpretation of the final models is not relevant to the interaction between IOP and OPP. In addition, we performed a supplemental analysis for Table 4, where MAP was used as an independent variable instead of OPP. As shown in Supplementary Table S1, MAP was not significantly associated with either BOS or RI changes in the univariate analysis, and final multivariate models were the same as those using OPP as the independent variable, as shown in Table 4. Accordingly, IOP change, but not MAP change, had significant association with BOS and RI changes, and OPP change may be associated with them secondarily to the IOP change.

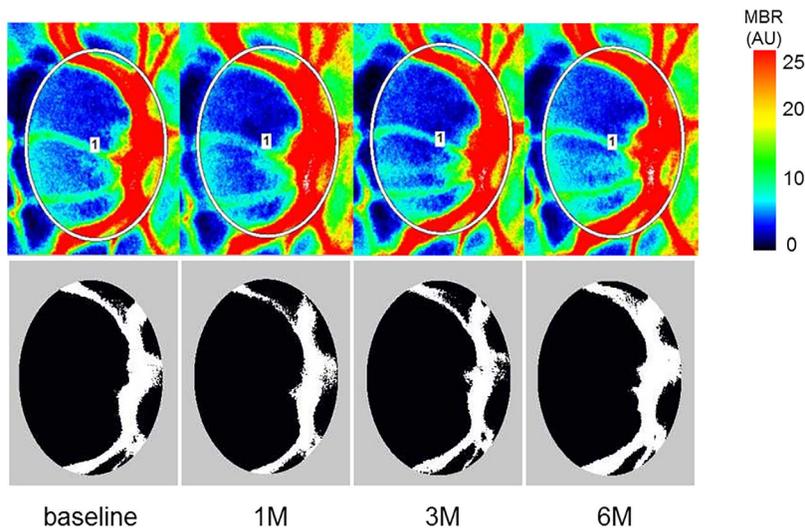


FIGURE 2. Representative MBR images at different time points in the optic nerve head of the same patient. Upper Images: Color-coded maps of MBR. The red color indicates a high average MBR and the blue color indicates a low average MBR. White elliptical bands represent the entire optic nerve head area. Lower Images: Binary images. White area represents large blood vessels, while black area represents the tissue area.

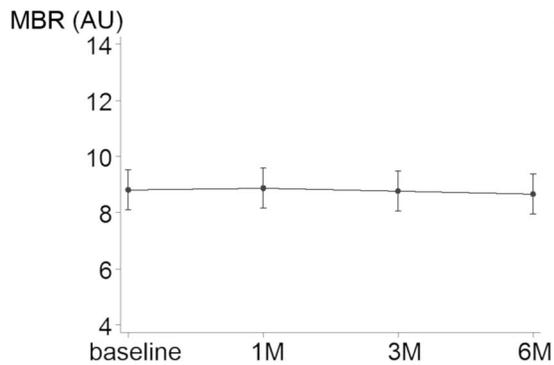


FIGURE 3. Longitudinal change of average mean blur rate. The estimated marginal means from the linear mixed-effects models are plotted for each time point. Error bars denote 95% confidence interval.

The study participants had relatively advanced glaucoma with a mean baseline MD value of -19.7 dB. Worse baseline MD values were associated with postoperative BOS increase and RI decrease. Previous studies have not addressed the association of blood flow changes and the severity of baseline VF defects. However, worse baseline MD is a prognostic factor for VF improvement after trabeculectomy in the Collaborative Initial Glaucoma Treatment Study (CIGTS).¹⁴ Further research is necessary to determine whether improved hemodynamics in the ONH after trabeculectomy may contribute to better VF prognosis in glaucoma.

Yanagida et al.³³ have reported that PR in healthy eyes has a significant positive association with BOS in their multiple regression analysis. Postoperative PR increase was associated with BOS increase and RI decrease in our study. However, a change in PR is not necessarily due to trabeculectomy. Care should be taken to consider the influence of PR on postoperative BOS and RI changes when PR at postoperative visits is markedly different from baseline.

Among 25 patients with systemic hypertension, 16 (33.3%) patients were taking oral antihypertensive agents including calcium channel blockers, angiotensin II receptor blockers, and β blockers, which may affect postoperative MBR changes.

However, none of these medications had any significant influences on postoperative changes in BOS and RI in the mixed-effects models accounting for the preoperative values.

Multiple glaucoma medications were used preoperatively in the study participants. A number of studies³⁴ have reported the alteration of ONH blood flow by IOP-lowering medications. Accordingly, we examined the effects of each IOP-lowering drug (prostaglandin analogues, β antagonists, carbonic anhydrase inhibitors, α -1 antagonist, α -2 agonist, and Rho kinase inhibitor) on postoperative changes in BOS and RI in the mixed-effects models accounting for the preoperative values. No drugs showed significant association with BOS or RI changes.

Our study has several limitations. We did not observe significant effects of the type of glaucoma on postoperative MBR changes, which may be due to a small sample size in this study. Preoperative usage of IOP-lowering medication might have had some effect on MBR changes over time. Tamaki et al.²⁰ have suggested the possibility that the effect of IOP reduction by trabeculectomy on ONH blood flow measured by a laser speckle method is partly masked by discontinuation of topical ocular hypotensive drugs because these drugs might have some effects on ONH blood flow. They have also analyzed another group undergoing needling revision of the bleb,²⁰ where no change in medication occurred before and after the IOP reduction. The results show that the ONH blood flow does not significantly change after the procedure. Therefore, the preoperative use of topical medication appears not to have a significant influence on ONH blood flow changes after surgery.²⁰

In summary, we evaluated the waveform change of ONH blood flow after trabeculectomy, using LSFG. BOS increased and RI decreased after trabeculectomy, although the average MBR remained unchanged. The postoperative waveform changes indicated more stable perfusion during a single heartbeat in the tissue region of the ONH than before trabeculectomy. Further studies are needed to elucidate the relationship between postoperative MBR waveform changes and structural changes in the ONH. The significance of MBR waveform changes to the prognosis of glaucomatous VF damage should also be assessed.

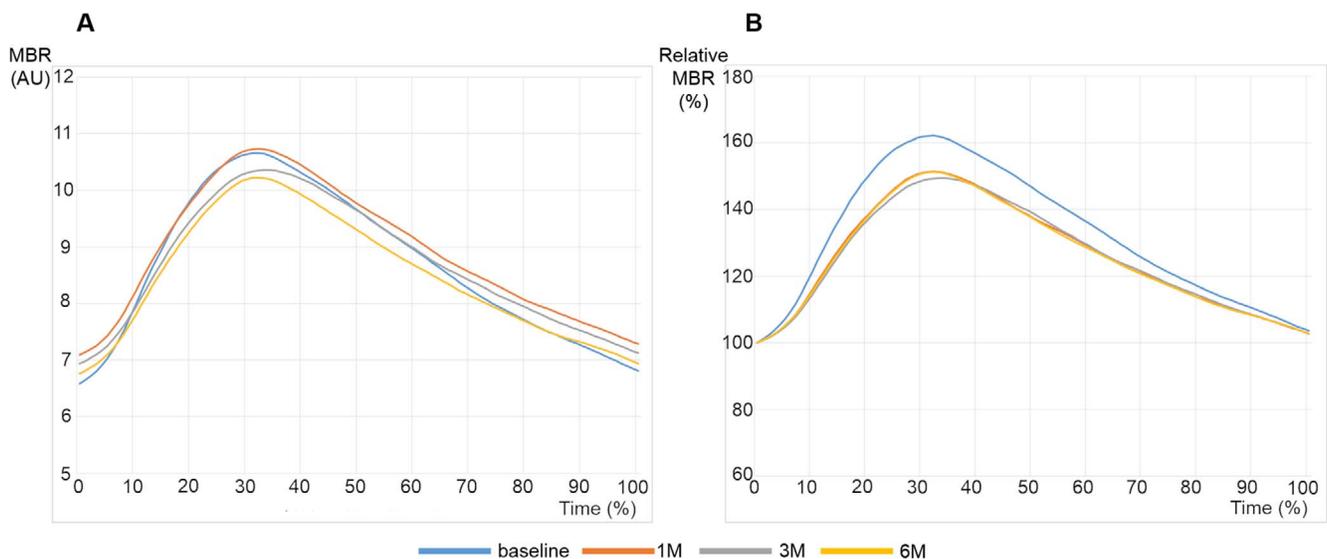


FIGURE 4. Changes of the average waveform of MBR in the tissue area of the optic nerve head after trabeculectomy. The average waveform of MBR in the tissue area of the optic nerve head at each time point was determined by normalizing the heartbeat duration of each patient. The horizontal axes represent time normalized with one heartbeat duration being 100%. The vertical axes in (A) and (B) represent MBR (AU) and the relative MBR (%) to MBR at the time of 0%, respectively. AU, arbitrary unit.

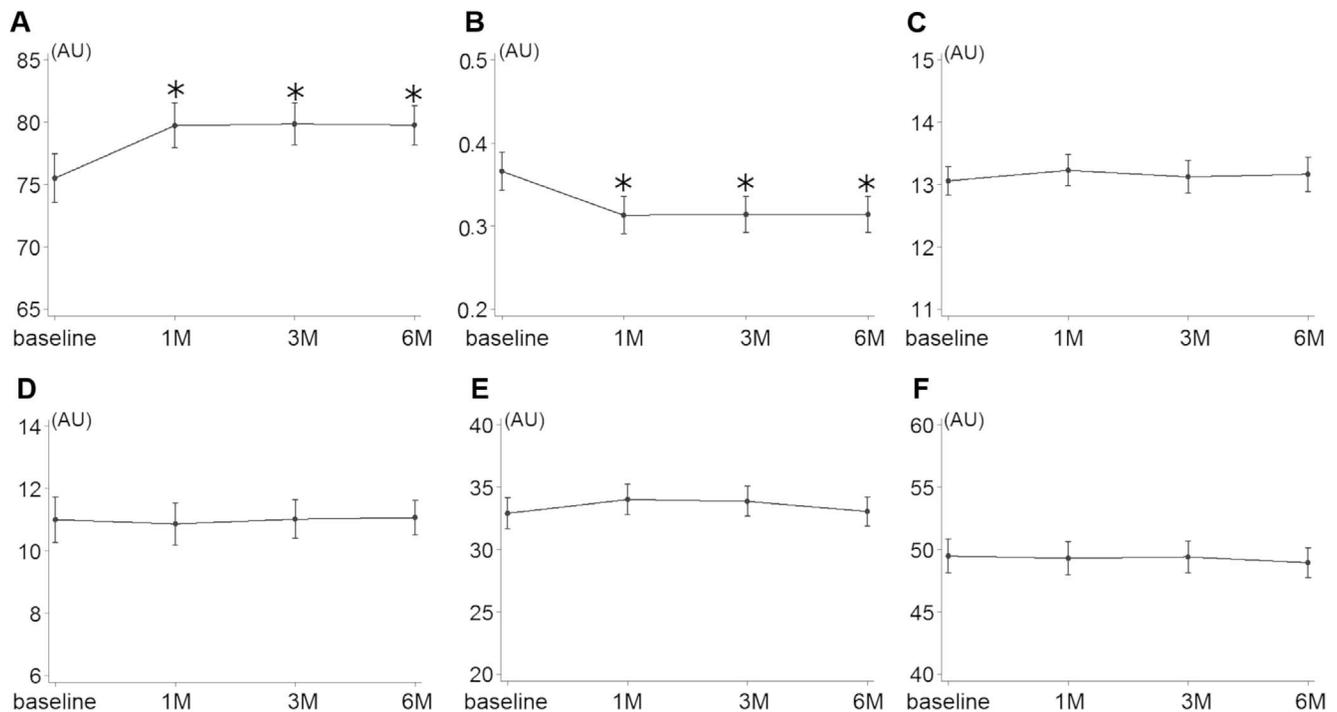


FIGURE 5. Longitudinal changes of MBR waveform parameters. (A) Blow out score. (B) Resistivity index. (C) Falling rate. (D) Skew. (E) Acceleration time index. (F) Blow out time. The estimated marginal means from the linear mixed-effects models are plotted for each time point. Error bars denote 95% confidence interval. Significant changes compared to initial values are indicated with an asterisk (* $P < 0.05$ after Bonferroni correction for multiple comparisons).

TABLE 4. Univariate and Multivariate Mixed-Effects Models Showing the Factors Associated With Postoperative Changes of BOS and RI

Independent Variables	BOS, Univariate Coefficient (SE), P Value	BOS, Multivariate Coefficient (SE), P Value	RI, Univariate Coefficient (SE), P Value	RI, Multivariate Coefficient (SE), P Value
Time, mo	0.040 (0.22), 0.85		-0.0001 (0.0029), 0.97	
Sex, male versus female	2.9 (1.6), 0.07		-0.030 (0.018), 0.10	
Glaucoma type, POAG versus XFG	-3.3 (1.7), 0.048		0.029 (0.019), 0.13	
Hypertension	-0.43 (1.6), 0.79		0.0085 (0.019), 0.65	
No. of preoperative antiglaucoma medications	-1.5 (1.0), 0.16		0.014 (0.012), 0.25	
Age, y	-0.074 (0.093), 0.43	-0.13 (0.05), 0.006	0.00075 (0.0011), 0.48	0.0014 (0.0006), 0.03
Baseline axial length, mm	0.067 (0.66), 0.92		-0.00075 (0.0075), 0.92	
Baseline mean deviation, dB	0.19 (0.13), 0.15	-0.18 (0.07), 0.009	-0.0012 (0.0015), 0.43	0.0026 (0.0009), 0.005
Baseline values of dependent variables, AU	-0.55 (0.070), <0.001	-0.50 (0.06), <0.001	-0.47 (0.079), <0.001	-0.43 (0.07), <0.001
Baseline average MBR, AU	0.39 (0.32), 0.23		-0.0040 (0.0037), 0.29	
Average MBR change, AU	0.20 (0.25), 0.43		-0.0012 (0.0033), 0.72	
IOP change, mm Hg	-0.44 (0.074), <0.001	-0.29 (0.06), <0.001	0.0055 (0.0009), <0.001	0.0037 (0.0007), <0.001
OPP change, mm Hg	0.063 (0.036), 0.08		-0.0010 (0.0005), 0.03	
PR change, beats/min	0.18 (0.024), <0.001	0.17 (0.02), <0.001	-0.0025 (0.0003), <0.001	-0.0024 (0.0003), <0.001

Blank cells indicate the variables not retained in the final models. SE, standard error.

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