

Retinal blood flow correlates to aqueous vascular endothelial growth factor in
central retinal vein occlusion

Yoshihisa Yamada MD,PhD¹⁾, Kiyoshi Suzuma MD,PhD¹⁾, Makiko Matsumoto
MD,PhD¹⁾, Eiko Tsuiki MD,PhD¹⁾, Azusa Fujikawa MD,PhD¹⁾, Takafumi Harada
MD²⁾, Takashi Kitaoka MD,PhD¹⁾

¹⁾Department of Ophthalmology and Visual Sciences, Graduate School of
Biomedical Sciences, Nagasaki University, 1-7-1 Sakamoto, Nagasaki 852-8501,
Japan

²⁾Yaizukogawa Eye Clinic, 2-15-4 Higashikogawa, Yaizu, Shizuoka 425-0035,
Japan

Correspondence to: Kiyoshi Suzuma

Department of Ophthalmology and Visual Sciences

Graduate School of Biomedical Sciences, Nagasaki University

1-7-1 Sakamoto, Nagasaki 852-8501, Japan

Tel: +81-95-819-7345; Fax: +81-95-819-7347

E-mail: suzuma@nagasaki-u.ac.jp

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Key Words: arteriovenous passage time; central retinal vein occlusion; laser speckle flowgraphy; retinal blood flow; vascular endothelial growth factor

Summary Statement: This study used laser speckle flowgraphy to analyze the relationship between retinal blood flow and aqueous vascular endothelial growth factor (VEGF) concentration in central retinal vein occlusion. A correlation was observed between lower blood flows in large vessels at the optic disc and higher aqueous VEGF concentrations.

Abstract

Purpose: Since laser speckle flowgraphy (LSFG) can measure blood flow distribution in the ocular fundus, we analyzed the relationship between retinal blood flow and aqueous vascular endothelial growth factor (VEGF) concentration in central retinal vein occlusion (CRVO).

Methods: This prospective observational study examined 45 eyes of 45 CRVO patients prior to treatment. Blood flow in large vessels around and at the optic disc, aqueous VEGF concentration, and arteriovenous passage time were examined. Blood flow was evaluated as mean blur rate (MBR) by LSFG.

Results: Fluorescein angiography found 20 ischemic and 25 non-ischemic type eyes. Aqueous VEGF concentration in the ischemic type was significantly higher than that in the non-ischemic type ($P = 0.01$). Arteriovenous passage time was significantly correlated to the logarithm of the aqueous VEGF concentration ($P = 0.0001$). MBR of the affected eye / MBR of the unaffected eye of the ischemic type was significantly lower than the non-ischemic type ($P = 0.039$). Additionally, MBR was significantly correlated both to the logarithm of the aqueous VEGF concentration ($P < 0.0001$) and to the arteriovenous passage time ($P = 0.0001$).

Conclusion: LSFG may be useful for predicting aqueous VEGF concentration and severity of CRVO.

INTRODUCTION

Central retinal vein occlusion (CRVO) is an ophthalmologic vascular disorder that is the leading cause of visual loss due to macular edema or neovascular glaucoma. Of the two CRVO types, visual outcomes have historically been reported to be good in non-ischemic CRVO, while they are poor in ischemic CRVO.¹ Vascular endothelial growth factor (VEGF) is related to the CRVO pathology. Induction of VEGF and pro-inflammatory cytokines by vascular occlusion has been shown to be associated with a breakdown of the blood-retinal barrier.² VEGF also causes macular edema, which results in a poor visual acuity. It has been reported that the anti-VEGF agent, ranibizumab and aflibercept, significantly improved the visual acuity in eyes with macular edema after branch retinal vein occlusion (BRVO) or CRVO.³⁻⁵

Ocular blood flow measurements are a commonly used parameter in a variety of ophthalmic diseases. The methodologies used to obtain ocular blood flow measurements can involve both invasive and noninvasive techniques. For example, the dye dilution method⁶ is an invasive technique, while color Doppler imaging,⁷ laser Doppler velocimetry,⁸ laser speckle flowgraphy (LSFG) (LSFG-Navi, Softcare, Fukuoka, Japan),⁹⁻¹⁶ and the retinal functional imager methodologies¹⁷ are considered to be noninvasive techniques. The LSFG device has been used to visualize blood flow distributions in the ocular fundus.¹² LSFG is convenient for measuring blood flows and thus, can be utilized for clinical applications. Therefore, we used this instrument to analyze the relationship between the retinal blood flow and the aqueous VEGF concentration in CRVO.

METHODS

This prospective study was conducted on 45 eyes of 45 CRVO patients (18 females, 27 males) with no history of previous treatment. Exclusion criteria consisted of cases of poor measurement (cataract with severe opacity, vitreous hemorrhage, poor mydriasis, corneal opacity), and previous vitreoretinal surgery. After providing informed consent, all patients were evaluated for blood flow in the large vessels at the optic disc, aqueous VEGF concentration, and arteriovenous passage time. The blood flow was evaluated as the mean blur rate (MBR) by LSF. Aqueous humor of the VEGF concentration was evaluated by an enzyme-linked immunosorbent assay. Arteriovenous passage time was evaluated by fluorescein angiography using a Heidelberg Retina Angiograph II (Heidelberg Engineering GmbH, Heidelberg, Germany). A previous study that used fluorescein angiography to investigate CRVO showed that the ischemic type of CRVO was observed in more than 10 disc areas of the nonperfusion areas.¹⁸

Our current research followed the tenets of the Declaration of Helsinki, with approval for this study obtained from the Ethics Committee of the Nagasaki University School of Medicine.

Instrument

The methodology used by the LSF instrument is based on the laser speckle phenomenon.¹² The most current instrument available now uses a fundus camera equipped with a diode laser (wavelength, 830 nm) and a highly sensitive charge-coupled device (CCD) camera (750 × 360 pixels), which has a scanning

speed of 30 frames per second. To observe the blood flow in the fundus, the area of interest is first illuminated by a wide laser spot, which causes the back-scattered laser from the spot to form a speckled pattern in the image plane of the fundus. The intensity variation of the pattern is then detected by the CCD camera, with the viewable area corresponding to a field of 8.6×4.6 mm (21° visual angle of the fundus camera) in the human fundus.¹²

Mean Blur Rate Measurement

In the present study, 30 min prior to taking the measurements, the patient's pupils were dilated using one drop of Mydrin P[®] (1% tropicamide and 2.5% phenylephrine hydrochloride; Santen Pharmaceutical, Osaka, Japan). During the measurement collection period, patients were asked to focus on a target light in order to fix the line of sight, thereby ensuring capture of the image speckles from the optic disc. The relative blood flow velocity (AU) was evaluated by the MBR and displayed as a 2-dimensional color-coded map. After circling the area around the optic disc, we then investigated the MBR of the major vessel (artery and vein) within this circle. Figure 1 shows a composite map (Figure 1A) within the optic disc and its histogram (Figure 1B). The vertical axis corresponds to the number of pixels, while the horizontal axis corresponds to the MBR in the histogram. The appropriate threshold between the tissue and vessel areas was automatically determined by the software. The histogram seen in Figure 1D shows the division between the tissue and vessel areas that was determined from the image shown in Figure 1C. The area to the left of the threshold line corresponds to the tissue area of the optic disc (black area in Figure 1C), while

the area to the right corresponds to the vessel area of the optic disc (white area in Figure 1C). Since the MBR in the vessel area includes the choroidal blood flow, we subtracted the mean MBR in the tissue area (MT) from the mean MBR in the vessel area (MV). Thus, the MBR used to evaluate the blood flow in the retinal vessel excluded the choroidal blood flow.

Measurements were performed three times, with the average used as the MBR value. Since the eye position was recorded using LSFG measurements, this made it possible to capture the same area during each of the subsequent examinations.

To examine the correlation between the preoperative MBR and the postoperative MBR in this study, we calculated the percentage of the preoperative MBR of the same eye. For comparisons with the MBR of different eyes, cases of comparison with other patients, or with an unaffected eye, we used the percent of the MBR of the unaffected eye.

Statistical analysis

All results are expressed as the means \pm standard deviation. Statistical analysis was performed using Statflex ver. 5.0 software (Artech Corporation, Osaka, Japan). Statistical significance was considered at $P < 0.05$.

RESULTS

A total of 45 unilateral CRVO patients were enrolled in this study. Mean age was 69 ± 10 years old. The average CRVO duration was 1.2 ± 1.6 months (range, 0.1 to 6 months) (Table).

MBR of the affected eye / MBR of the unaffected eye ($81.2 \pm 23.0\%$) was significantly lower than the MBR of the fellow eyes (100%) ($P < 0.0001$, paired t -test) (Figure 2). After first classifying the 45 cases of CRVO as either non-ischemic type (25 cases) or ischemic type (20 cases), we evaluated the relationship between ischemia, blood velocity, and aqueous VEGF concentration.

The relationship between ischemia and blood velocity is shown in Figure 3A. MBR of the affected eye / MBR of the unaffected eye in the ischemic type ($73.3 \pm 20.9\%$) was significantly lower than that in the non-ischemic type ($87.6 \pm 24.0\%$) ($P = 0.039$, unpaired t -test) (Figure 3A). VEGF is an important cytokine that causes macular edema. VEGF is produced in response to the ischemia that results from the vascular occlusion; therefore, we decided to evaluate the relationship between ischemia and the aqueous VEGF concentration. Aqueous VEGF concentration in the ischemic type (1945 ± 2216 pg/ml) was significantly higher than that found in the non-ischemic type (515 ± 495 pg/ml) ($P = 0.01$, unpaired t -test) (Figure 3B).

Since the arteriovenous passage time is correlated to the degree of ischemia, we subsequently evaluated the relationship between the arteriovenous passage time and the aqueous VEGF concentration. Results indicated a significant correlation between the arteriovenous passage time and the logarithm of the aqueous VEGF concentration ($R^2 = 0.28$, $P = 0.0001$) (Figure 4). To examine whether the blood velocity is also correlated with ischemia, we evaluated the relationship between the arteriovenous passage time and the blood velocity. Our analysis indicated that the MBR was significantly correlated to the arteriovenous

passage time ($R^2 = 0.30$, $P = 0.0001$) (Figure 5). Our final comparison of the blood velocity and aqueous VEGF concentration showed that the MBR was significantly correlated to the logarithm of the aqueous VEGF concentration ($R^2 = 0.32$, $P = 0.0001$) (Figure 6).

DISCUSSION

LSFG measurements and analysis can be easily performed, have good reproducibility, and are noninvasive; therefore, it was used in the current study to evaluate blood velocity. Since we investigated the MBR of the major vessel (artery and vein) located within a circle on the optic disc, our study was also able to measure and analyze the mean blood velocity of the artery and vein, which reflected the total retinal circulation. While investigations of the blood velocity of the major vessels on the disc may reflect the entire retinal circulation, we believe it is better to separately measure the blood velocity of the artery and vein, as it can be difficult to automatically separate and measure the blood velocity.

MBR of the affected eye / MBR of the unaffected eye was significantly lower than the MBR of the fellow eyes. Many previous studies have reported finding that the blood velocity of CRVO was lower than that observed in the normal eye. When using color Doppler imaging, Michelson et al. reported the mean blood velocity of the central retinal vein (CRV) in CRVO (2.1 cm/sec) was significantly slower than that found in the controls (3.3 cm/sec).¹⁹ In addition, they also reported finding no significant difference between the blood velocity of the central retinal artery in CRVO (8.2 cm/sec) and that in the controls (9.3 cm/sec). Crama et al. have also reported that color Doppler imaging showed a decrease

in the mean blood velocity of the CRV in CRVO (4.9 cm/sec) as compared to the controls (8 cm/sec).²⁰ Horio et al. found that blood velocity in the vein in CRVO (28.5 pixel²/sec) was slower than that of the fellow eyes (39.9 pixel²/sec) when using the dye dilution technique.²¹ In the current study, the MBR of the affected eye / MBR of the unaffected eye was 81.2%. Our results indicate that there was a slightly faster rate than that observed in the other studies. For example, the Michelson study determined the percentage to be 63%,¹⁹ while the Crama study found 61%,²⁰ and the Horio study found 71%.²¹ The reason why our blood velocity results were faster than those previously reported was most likely that we measured the blood velocity in both the vein and artery. It might also be possible that our study contained higher numbers of the non-ischemic type of CRVO as compared to the other reports.

We also examined the number of ischemic and non-ischemic types for CRVO, and then compared the blood velocity between these two types. Our results showed that the MBR of the affected eye / MBR of the unaffected eye in the ischemic type was significantly lower than that found for the non-ischemic type. Williamson et al. reported that the values of the maximum and minimum blood velocities of the CRV were reduced in accordance with the degree of the increase in the ischemia, with significantly lower values found in ischemic versus non-ischemic eyes.²² Arsène et al. have also reported that the minimum blood velocity of CRV in the ischemic type of CRVO (1.83 cm/sec) was lower than that of the non-ischemic type (2.28 cm/sec).²³

Our study also indicated that the aqueous VEGF concentration for the ischemic type was significantly higher than that for the non-ischemic type. Noma

et al. found that the vitreous levels of VEGF were significantly higher in ischemic versus non-ischemic CRVO patients.²⁴ Moreover, after demonstrating that the aqueous humor levels of the cytokines were correlated with the vitreous levels, they further proposed that the aqueous levels might be indicative of the severity of the macular edema in BRVO.²⁵ Our present results are in agreement with the findings of Noma's previous studies.

We additionally evaluated the relationship between the arteriovenous passage time and the aqueous VEGF concentration. Our results showed there was a significant correlation between the longer arteriovenous passage times and the higher aqueous VEGF concentrations. To the best of our knowledge, no previous studies in human subjects have evaluated the relationship between the arteriovenous passage time and the aqueous VEGF concentration.

In the final step of our study, we examined the relationship between the blood velocity and the aqueous VEGF concentration. Our results indicated there was a significant correlation between lower blood velocities and higher aqueous VEGF concentrations. Williamson et al. demonstrated that a minimum blood velocity of less than 3.0 cm/sec in CRVO patients was highly predictive of the development of iris neovascularization, with a sensitivity rate of 75% and a specificity rate of 86%.²² When all of these results are taken together, our findings suggest that it might be possible to determine the presumed aqueous VEGF concentration from the blood velocity. Thus, CRVO prognosis could be inferred from this presumed aqueous VEGF concentration.

There were several limitations for the present study, including having only a small number of cases, and not confirming whether ischemic CRVO met

Hayreh's high risk criteria.^{26, 27} In addition, we did not estimate nonperfusion areas quantitatively. When using the LSFG methodology, it is important to remember that the MBR is only able to provide relative and not absolute values for the blood velocity. Therefore, when comparing different eyes in this study, we only evaluated the differences using percentages derived from the following formula: MBR of the affected eye / MBR of the unaffected eye.

In conclusion, lower blood flows in large vessels in the optic disc were correlated to the higher aqueous VEGF concentrations in CRVO. Thus, LSFG may be a useful device for determining presumed aqueous VEGF concentrations. This is a very important finding, as VEGF is used in the visual acuity prognosis in CRVO.

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FIGURE CAPTIONS

Figure 1. A composite map and a histogram within the optic disc was created using LSFG. A) A false-color composite map within the optic disc is shown. Red indicates a faster blood flow, while blue indicates a slower blood flow. B) A histogram within the optic disc is shown. The vertical axis corresponds to the number of pixels, while the horizontal axis corresponds to the MBR. C) Representative binary format images for segmentation between the vessel (white area) and tissue (black area) are shown. D) The histogram was analyzed using image viewer software that utilizes an automated definitive threshold. The area to the left of the threshold line corresponds to the tissue of the optic disc (black area in Fig. 1C), while the area to the right of the line corresponds to the vessel of the optic disc (white area in Fig. 1C).

Figure 2

MBR of the affected eye / MBR of the unaffected eye ($81.2 \pm 23.0\%$) was significantly lower than the MBR of the fellow eyes (100%).

Figure 3

A) The relationship between ischemia and blood velocity. The vertical axis represents the ratio of the MBR of the affected eye to the MBR of the unaffected eye. Blood velocity in the ischemic type CRVO was significantly lower than that in the non-ischemic type CRVO. B) The relationship between the ischemia and the aqueous VEGF concentration. Aqueous VEGF concentration in ischemic type eyes (1945 ± 2216 pg/ml) was significantly higher than that in the

non-ischemic type eyes (515 ± 495 pg/ml).

Figure 4

This figure shows the relationship between the arteriovenous passage time and the logarithm of the aqueous VEGF concentration. Arteriovenous passage time was significantly correlated to the logarithm of the aqueous VEGF concentration ($R^2 = 0.28$, $P = 0.0001$).

Figure 5

This figure shows the relationship between the arteriovenous passage time and the blood velocity. MBR was significantly correlated to the arteriovenous passage time ($R^2 = 0.30$, $P = 0.0001$).

Figure 6

This figure shows the blood velocity and the logarithm of the aqueous VEGF concentration. MBR was significantly correlated to the logarithm of the aqueous VEGF concentration ($R^2 = 0.32$, $P = 0.0001$).

Fig. 1

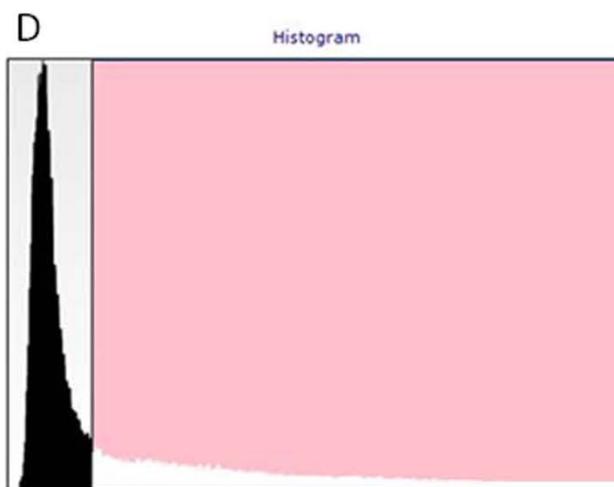
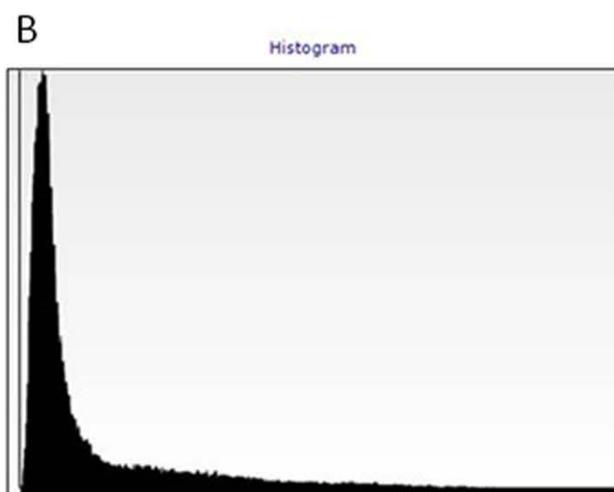
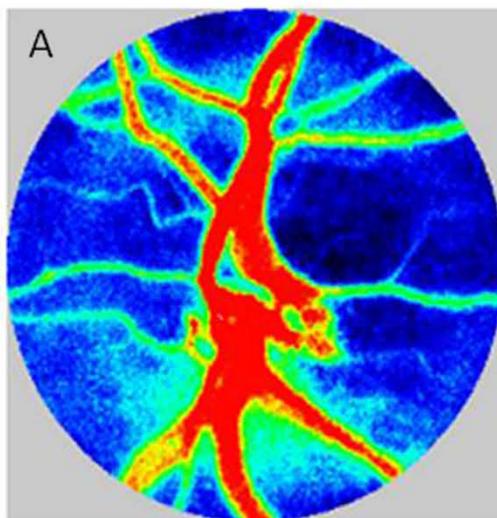


Fig. 2

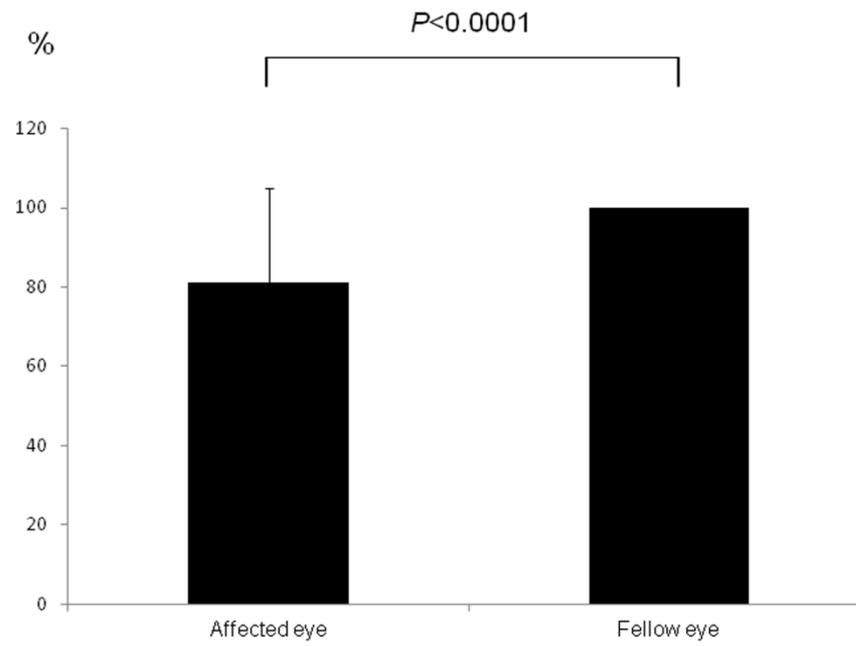


Fig. 3

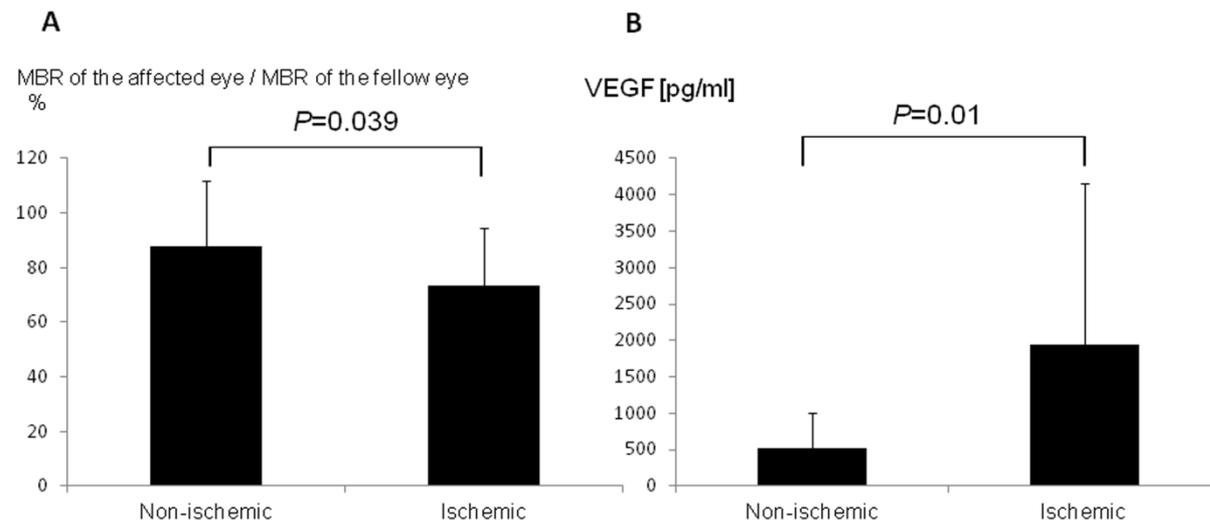


Fig. 4

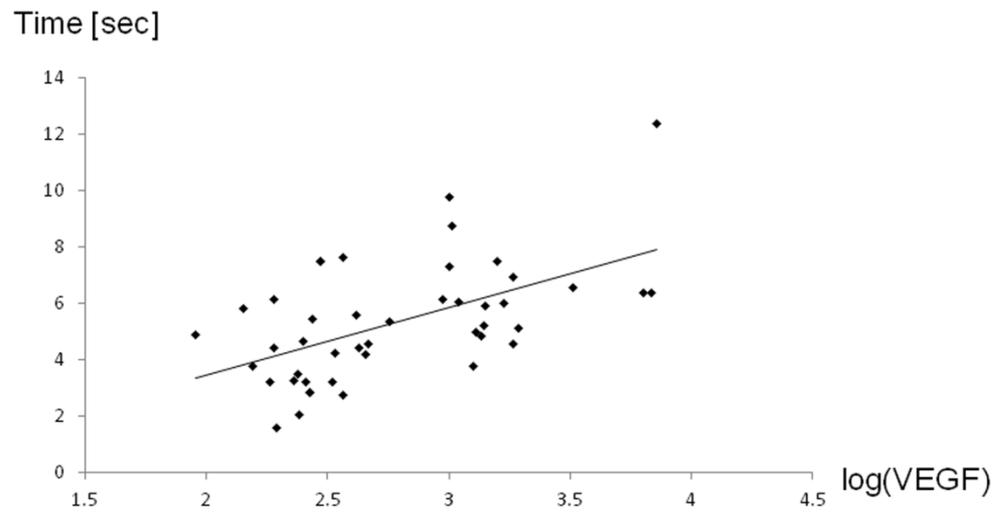


Fig. 5

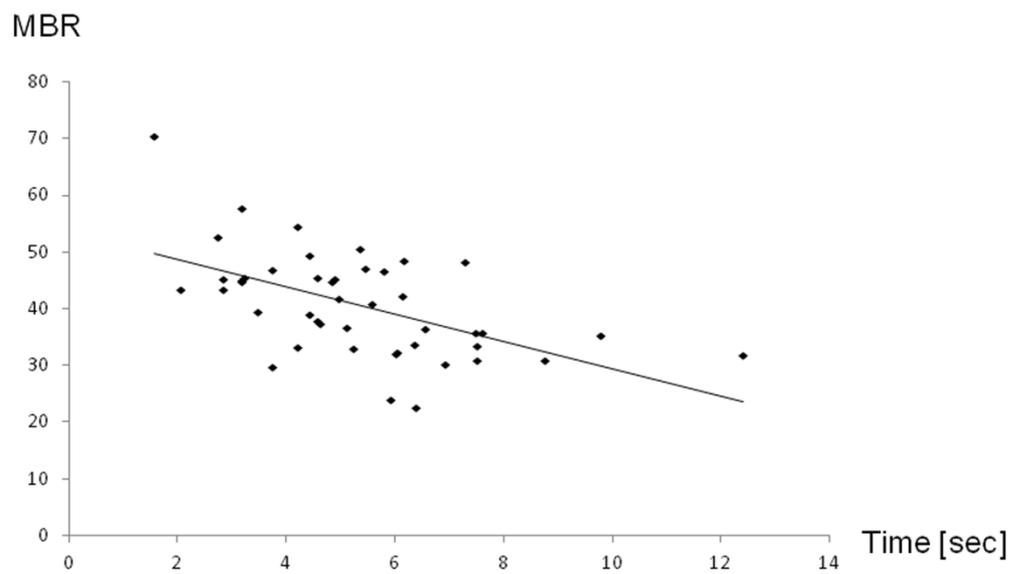


Fig. 6

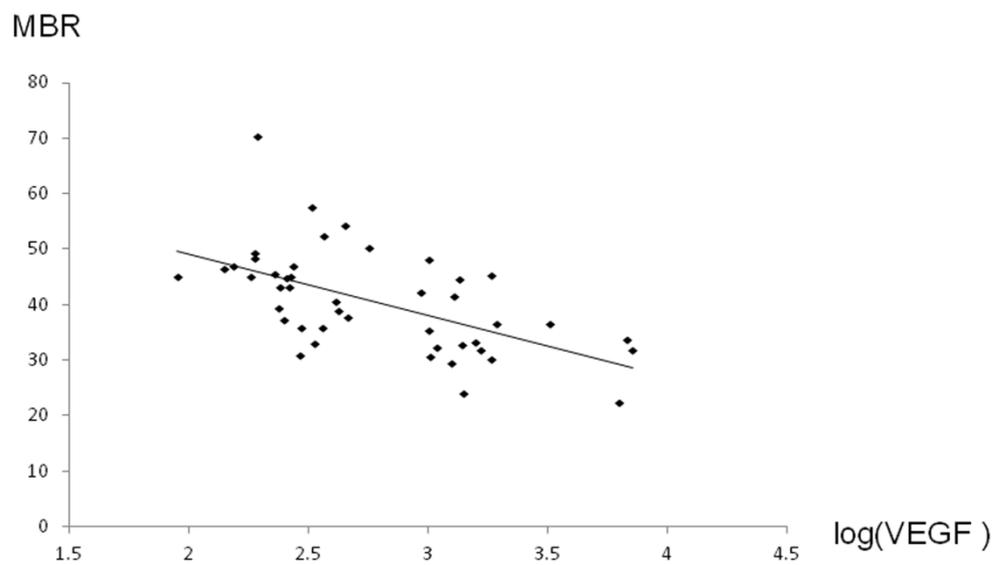


TABLE. Characteristics of patients with central retinal vein occlusion

Demographics	
Age (years) (mean \pm SD)	69 \pm 10
Sex (female:male)	18:27
Clinical	
Hypertension	13
Diabetes	7
Systolic blood pressure (mm Hg) (mean \pm SD)	133 \pm 15
Diastolic blood pressure (mm Hg) (mean \pm SD)	74 \pm 11
Ischemic type of CRVO (eye)	20
Duration of CRVO (months) (mean \pm SD)	1.2 \pm 1.6

SD = standard deviation; CRVO = central retinal vein occlusion