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Measurement of Blood Flow Velocity in Feeder Vessels of Choroidal Neovascularization by a Scanning Laser Ophthalmoscope and Image Analysis System

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Purpose: To measure the blood flow velocities in feeder vessels of patients with choroidal neovascularization (CNV) secondary to age-related macular degeneration.

Methods: We examined the early images of indocyanine green videoangiography (v-ICGA) in 29 patients (29 eyes) with CNV, in whom feeder vessels could be identified by v-ICGA. The v-ICGA images were installed in a personal computer. With original software, optical density measurements were performed for the determination of dye-dilution curves. The flow velocity in a CNV feeder vessel was obtained by analyzing the dye-dilution curves.

Results: The mean flow velocity in feeder vessels was 33.8 ± 32.5 mm/s. The flow velocity in feeder vessels of CNV with a diameter of 1.5 mm or larger was significantly higher than that in feeder vessels of CNV with a diameter smaller than 1.5 mm ($P < .05$, unpaired t-test).

Conclusions: The flow velocity in CNV feeder vessels can be measured with v-ICGA and a computer-based image analysis system. This system would be useful in the evaluation of choroidal circulation. **Jpn J Ophthalmol 2003;47:53–58** © 2003 Japanese Ophthalmological Society.

Key Words: Age-related macular degeneration, choroidal neovascularization, dye dilution curves, feeder vessel, image analysis system.

Introduction

Recent studies have analyzed choroidal hemodynamics by the laser speckle method,[1–3] the laser Doppler method,[4] and fluorescein angiography (FA).[5,6] Each method has limitations in its range of flow velocity or in its area of measurement, and none of them allows measurement in one selected vessel such as the feeder vessels of choroidal neovascularization (CNV). We have developed a system for measuring the blood flow velocity in the retinal artery using fluorescein videoangiography (v-FA) obtained by the use of a scanning laser ophthalmoscope (SLO) and dye dilution techniques.[7–9] In the present study, we simultaneously obtained measurements by v-FA and indocyanine green videoangiography (v-ICGA) using the double detector of SLO, and evaluated the applicability of making simultaneous measurements with these two systems. In addition, blood flow velocities in feeder vessels of CNV secondary to age-related macular degeneration (AMD) were measured simultaneously by v-ICGA and v-FA. The association between the flow velocity in feeder vessels and the size of CNV was evaluated.

Materials and Methods

Materials

Between January 1993 and December 1998, in our department, we examined the early images of v-ICGA in 29 eyes of 29 patients with CNV secondary to AMD, in which feeder vessels could be identified by using SLO. Informed consent was orally obtained from these 29 patients. The subjects consisted of 22 men and 7 women with a mean age of 69.7 years. In addition, v-FA and v-ICGA images were simultaneously obtained using a double detector and SLO

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(Rodenstock Instrument, Munchen, Germany), and the influence of the different dyes, fluorescein sodium and indocyanine green, on measurement values was evaluated in 10 eyes of 10 patients in whom the temporosuperior retinal artery could be clearly visualized. The exclusion criteria were subretinal hemorrhage, vitreous hemorrhage, a feeder vessel 2-disc diameter (DD) or further apart from the fovea, and refractive error outside the range of −1.0 D to +1.0 D. In addition, the subjects were limited to patients in whom only one vessel was identified as a feeder vessel.

Simultaneous v-FA and v-ICGA Using Double Detector of SLO

To perform v-FA and v-ICGA simultaneously, we used the double detector of SLO, an argon laser (wavelength, 480 nm) for v-FA and a diode laser (wavelength, 810 nm) for v-ICGA. V-FA and v-ICGA images were simultaneously recorded as serial images (30 frames per second) on s-VHS videotapes using two video recorders. The intensity of the argon laser was B5 (240 μW), and that of diode laser was 12 (2000 μW). The Autogain system was not used, and the gain was set at 7. A 20-degree-field size was used for identification of feeder vessels. Indocyanine green (25 mg; Dai-ichi Pharmaceuticals, Tokyo) was dissolved in 5 mL of 10% fluorescein sodium solution. To more sharply obtain the ascending portion of the dye dilution curve, this solution was pushed into a cubital vein by a flash of 10 mL of saline so that a bolus of ICG dye could reach the ocular fundus.[7]

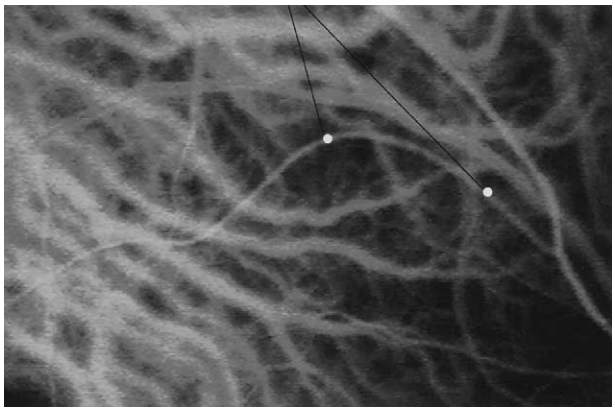

Figure 1. Measurement points in indocyanine green angiographic (ICGA) images. Two measurement points (white circles) were chosen in ICGA images for the measurement of fluorescence intensity at sites on a temporosuperior retinal artery, where the retinal vessel and large choroidal vessels did not overlap.

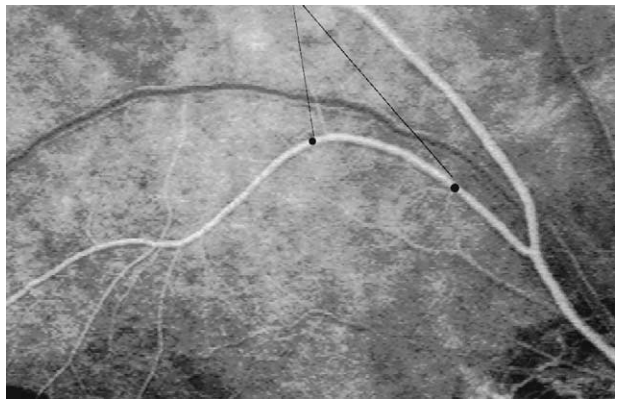

Figure 2. Measurement points in fluorescein angiographic (FA) images. Two measurement points (black circles) were chosen in FA images for the measurement of fluorescence intensity at the same sites as those on the ICGA images shown in Figure 1.

Image Analysis and Calculation of Blood Flow Velocity

Image Analysis

The v-FA and v-ICGA images obtained were captured with an analogue-digital converter board (Dig98; Ditect, Tokyo) loaded into a computer (PC9821Xa; NEC, Tokyo). Two measurement points were chosen at an interval of about 1/2 DD (Figures 1, 2, and 4), and the fluorescence intensity at each point was measured in each frame (30 frames per second). The two points did not involve areas showing overlapping of

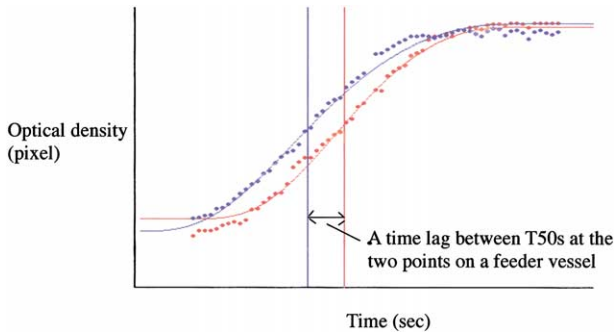

Figure 3. Serial changes in fluorescence intensity at two locations measured in choroidal neovascularization (CNV), and dye dilution curves. Serial changes in fluorescence intensity at two points were plotted on a graph, and the data were regressed to theoretical dye-dilution curves by the least squares fitting methods. The difference in the time until A50 between the two dye dilution curves (ΔT50) was obtained from this graph. (See text for more details.)

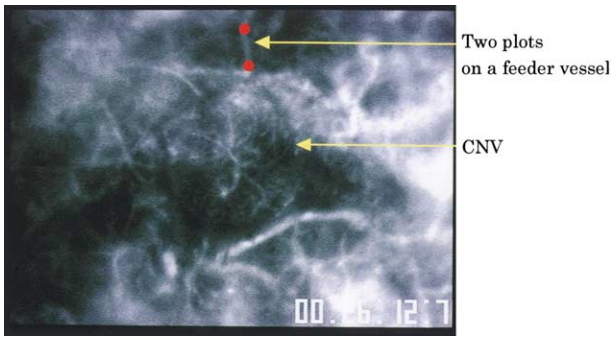

Figure 4. Two locations on a choroidal neovascularization (CNV) feeder vessel. Two sites (circles) on a CNV feeder vessel were chosen for the measurement of fluorescence intensity identified on indocyanine green angiographic images.

the choroidal and retinal vessels so that the measurements were not affected by background fluorescence. For shifts of the two points due to ocular movements, their sites were manually corrected for each frame.

Regression of the Dye Dilution Curve to Theoretical Formula

Fluorescent intensity at each point in each frame (I) was plotted on the ordinate, and time (t) on the abscissa. With a program of least-squares method running on the computer, the data was regressed to the following theoretical function:

$$I = K + Ip\mathrm{EXP}[-\alpha\{ \log (t - to/tp - to)^2]$$

where K = background intensities before appearance of the dye in blood vessels; Ip = peak fluorescence intensities; EXP = exponent with e as the base; α = coefficients; to = times at the beginning of the regression curves; tp = times at which peak intensities were reached.

To determine the differences in fluorescence intensity between the systolic and diastolic phase, the robust estimation method was applied to measurement values.[9]

Measurement of the Time Difference in Dye Filling Between Two Points

In the measurement of retinal circulation time in the dye dilution technique, the highest reproducibility has been reported to be obtained at 50% of the peak intensity on the ascending part of the dye dilution curve.[8] Therefore, we calculated the time difference [ΔT (50)] between the ascending parts of the dye dilution curves obtained at two points at 50% of the peak intensity (Figure 3).

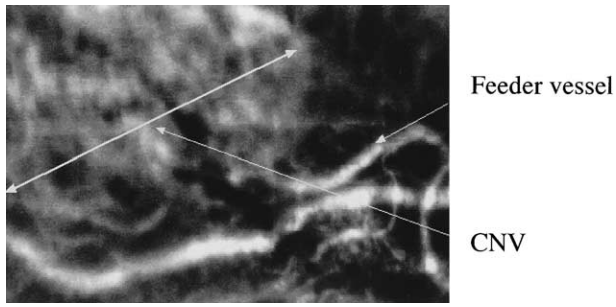

Figure 5. Choroidal neovascularization (CNV) with a diameter of 1.5 mm or larger and the feeder vessel.

Calculation of Blood Flow Velocity

The distance between two points about 1/2 DD apart on the blood vessel was obtained in terms of dot units by the third spline function and divided by ΔT (50) to obtain the blood flow velocity (dot/s). The dot unit was converted to the mm unit based on a previous study using a similar method.[9]

Influence of the difference in dyes on measurement values. Many fluorescein sodium molecules and ICG are bound with plasma albumin in the blood vessels. Under appropriate conditions, fluorescence intensity is proportional to the dye concentration for both dyes.[5,10] Therefore, it may be possible to obtain blood flow velocity from the plasma velocity in blood vessels by the analysis of v-ICGA images as well as v-FA images by the dye dilution technique. However, the two dyes differ in molecular weight, composition, and proportion of molecules bound with albumin. There may be a difference in movement in blood vessels between protein-binding molecules and free molecules. It was necessary to elucidate whether or not the dye-dilution technique could be used to obtain v-ICGA images and v-FA images simultaneously for the measurement of blood velocities. Therefore,

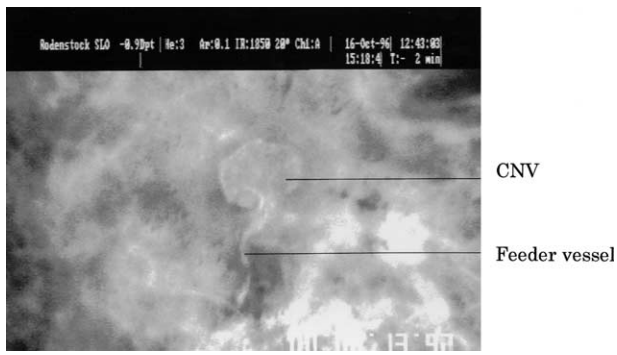

Figure 6. Choroidal neovascularization (CNV) with a diameter smaller than 1.5 mm and the feeder vessel.

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on the v-FA and v-ICGA images simultaneously obtained using the double detector of SLO, serial changes in fluorescence intensity were analyzed at the same site (Figures 1 and 2) on the temporosuperior retinal artery. The blood flow velocity in the retinal artery that was obtained from v-ICGA images was compared with that obtained from v-FA images, and their correlation was evaluated by linear regression analysis.

Measurement of blood flow velocity in feeder vessel and evaluation of measurement values. In early image of v-ICGA, video recording confirmed serial visualization of the vascular connection from the choroid to neovascular loops. This vascular connection was regarded as a feeder vessel,[11] and two points were chosen on it (Figure 4). Blood flow velocity in the feeder vessel was measured by the above-mentioned method. The velocity in a large CNV with a diameter of 1.5 mm or larger (17 eyes) (Figure 5) was compared with that in small CNV with a diameter smaller than 1.5 mm (12 eyes) (Figure 6), and the difference between them was analyzed by unpaired *t*-test.

Results

Influence of the Difference in Dyes on Measurement Values

Table 1 shows the blood flow velocity in a retinal artery obtained by v-FA image analysis and that obtained in the same artery by v-ICGA image analysis. There was a close correlation between the two measurement values ($y = 1.087x$, regression coefficient = 0.98) (Figure 7).

Table 1. Blood Flow Velocities Obtained Simultaneously in Retinal Arteries

From FA Images (dot/s)*	From ICGA Images (dot/s)†
2,670	2,700
546	841
1,553	1,462
1,134	1,525
1,185	1,312
3,000	3,400
1,700	1,439
1,254	1,425
1,516	1,446
1,695	2,376
1,625 ± 726‡	1,793 ± 778‡

*FA: Fluorescein angiography.
†ICGA: indocyanine green angiography.
‡Mean ± SD.

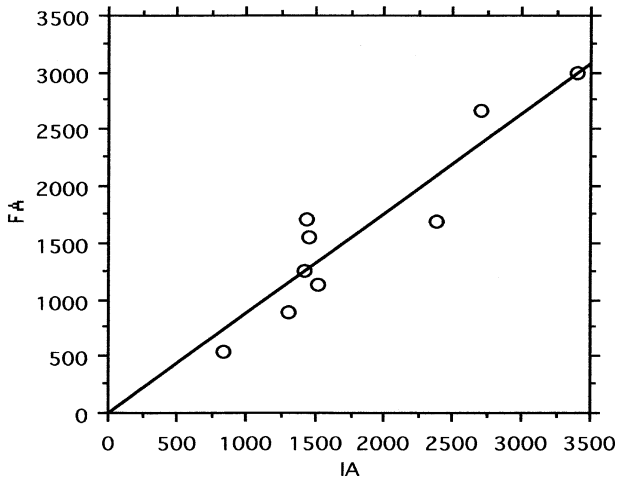

Figure 7. Correlation between the blood flow velocity in retinal arteries obtained from indocyanine green angiographic (IA) images (abscissa) and those obtained from fluorescein angiography (FA) images (ordinate). A regression line was obtained ($y = 1.087x$, regression coefficient = 0.98).

Blood Flow Velocity in Feeder Vessels

Blood flow velocity in feeder vessels ranged from 1.9 to 108.0 mm/sec (mean ± SD, 33.8 ± 32.5 mm/s). In 17 eyes with large CNV with a diameter of 1.5 mm or larger, the blood flow velocity was 43.4 ± 30.6 mm/s. In contrast, in 12 eyes with small CNV with a diameter smaller than 1.5 mm (Table 2), the flow velocity was 20.3 ± 20.2 mm/s. There was a significant difference between the two measurements ($P < .05$, unpaired *t*-test).

Discussion

Recent advances in the SLO imaging system have enabled us observe the choroidal circulation and to determine the direction of blood flow in choroidal vessels. Although various methods have been used for the quantitative analysis of choroidal circulation in vivo, there have been no studies using v-ICGA with SLO. Mihara,[9] of our department, reported the measurement of blood flow velocity in retinal arteries by the analysis of v-FA images obtained using SLO and

Table 2. Blood Flow Velocities in Feeder vessels (mm/s)

Mean Diameter of CNV*	n	Mean ± SD
≥1.5 mm	17	43.4 ± 30.6
≤1.5 mm	12	20.3 ± 20.2
	29	33.8 ± 32.5

*CNV: choroidal neovascularization.

the dye dilution technique. If feeder vessels can be identified on v-ICGA images obtained using a SLO, we thought it may be possible to measure blood flow velocity by analyzing the blood flow in feeder vessels. Therefore, we analyzed v-ICGA images obtained using SLO and the dye dilution technique, and measured the blood flow velocity in feeder vessels.

When this system using the dye dilution technique is applied to v-ICGA images, we should study the influence of the difference in dyes on measurement values. In the present study, there was a close correlation between flow velocity in the retinal artery measured using v-FA images and the same artery measured using v-ICGA images (correlation coefficient >0.9; slope of regression equation = 1.087). This suggests that the influence of the difference in dyes on measurement values obtained by using this system is negligible, and that analysis of v-ICGA images by the dye dilution technique is also possible. In this system, the influence of pulse waves on blood flow velocity was compensated for by the robust estimation method. However, when blood flow velocity is high, and the time to reach peak fluorescence intensity is shorter than the heartbeat cycle (about one second), the influence of pulse waves may be strong. In such cases, measurement of blood flow velocity in the choroidal artery is not appropriate. Because flow velocities in feeder vessels identified on v-ICGA images were lower than those in the choroidal artery, however, their measurement was possible by the method used in the present study.

On v-ICGA images, the effect of fluorescence in the choroidal vascular system and in the retinal vascular system should be considered. Therefore, the measurement of fluorescence in our study was performed at sites on the feeder vessel where retinal vessels were not seen on angiographic images. In the present study, the measurement was performed only in the eyes in which one single feeder vessel running straight about 1/2 disc diameter could be identified. The measurement was performed in about 22% of the feeder vessels identified on v-ICGA images. Although this method involves the intravenous injection of ICG dye, it allows measurement of blood flow velocity in a feeder vessel that cannot be detected by the laser speckle method or the laser Doppler method.

The blood flow velocity in feeder vessels markedly varied from 1.9 mm/s to 108.0 mm/s compared with the blood flow velocity in the retinal artery.[9,12] Feeder vessels clinically identified by v-ICGA are not always choroidal arterioles but can be a part of CNV. Moreover, the blood flow velocity might be affected by measurement sites and by CNV size, vascular diameter, and the maturity of CNV that varies widely from case to case. As mentioned above, the mean blood flow velocity in feeder vessels was 33.8 ± 32.5 mm/s, which was lower than that in the choroidal artery[13,14] and slightly higher than that previously reported in the retinal artery.[12] The feeder vessels defined in the present study consist of a newly arising afferent vessel and a choroidal vessel. When matured, CNV is considered to have a vascular structure similar to the normal circulation system.[15] The morphology and hemodynamics of feeder vessels change with maturity, which may be reflected by the blood flow velocity.

In our study, serial changes in dye concentration in the feeder vessel were evaluated, and the blood flow velocity was obtained from the plasma transfer velocity. Blood flow velocity was higher in feeder vessels feeding large CNV than in those feeding small CNV. With a decrease in the vascular diameter, blood flow resistance increases and blood flow velocity decreases (the Poiseuille law) in microcirculation. Blood flow velocity could be strongly affected by the activity of leukocytes that produce the highest flow resistance among blood components.[16]

Thus, there may be many factors involved in hemodynamics in addition to plasma transfer velocity. Further analysis of vascular diameter, blood flow volume, and the velocity of blood cells in feeder vessels is needed to more precisely clarify the hemodynamics of the feeder vessels of CNV. Concerning feeder vessel treatment, studies on an association between its outcome and the vascular diameter or blood flow velocity are also needed. Indeed, Staurenghi et al reported that occlusion can be easily achieved in feeder vessels with small diameters.[17] There might be a possibility that the blood flow velocity in feeder vessels is associated with the anatomical success of feeder vessel treatment.

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