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Segmental reproducibility of retinal blood flow velocity measurements using retinal function imager

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Abstract

Background—To evaluate the reproducibility of blood flow velocity measurements of individual retinal blood vessel segments using retinal function imager (RFI).

Methods—Eighteen eyes of 15 healthy subjects were enrolled prospectively at three centers. All subjects underwent RFI imaging in two separate sessions 15 min apart by a single experienced photographer at each center. An average of five to seven serial RFI images were obtained. All images were transferred electronically to one center, and were analyzed by a single observer. Multiple blood vessel segments (each shorter than 100 μ m) were co-localized on first and second session images taken at different times of the same fundus using built-in software. Velocities of corresponding segments were determined, and then the inter-session reproducibility of flow velocity was assessed by the concordance correlation co-efficient (CCC), coefficient of reproducibility (CR), and coefficient of variance (CV).

Results—Inter-session CCC for flow velocity was 0.97 (95% confidence interval (CI), 0.966 to 0.9797). The CR was 1.49 mm/sec (95% CI, 1.39 to 1.59 mm/sec), and CV was 10.9%. The average arterial blood flow velocity was 3.16 mm/sec, and average venous blood flow velocity was 3.15 mm/sec. The CR for arterial and venous blood flow velocity was 1.61 mm/sec and 1.27 mm/sec respectively.

Conclusion—RFI provides reproducible measurements for retinal blood flow velocity for individual blood vessel segments, with 10.9% variability.

Keywords

Retinal blood flow; retinal blood flow velocity; retinal function imager; retinal blood flow

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Introduction

Abnormalities in retinal or optic nerve blood flow are associated with many ocular diseases including diabetic retinopathy [1], vascular occlusion [2 , 3], age-related macular degeneration [4], and glaucoma [5 , 6]; as well as systemic diseases such as systemic vasculitis [7]. Further information about blood flow velocity would help us to understand the pathophysiology and help to plan treatment strategies for these diseases. Although the retina is one of the few human tissues where blood circulation can be observed directly and noninvasively, knowledge about retinal blood flow velocity remains limited due to the limitations of available blood flow measurement devices. Both invasive and non-invasive techniques have been reported for measuring retinal blood flow [8]. Invasive techniques using either radioactive or non-radioactive microspheres in experimental animals have been performed to measure retinal blood flow [9 - 11], but these technologies are not practical for human applications. Video fluorescein and indocyanine angiography have been used to measure blood flow velocity, but there is limited resolution with these methods [12]. Fluorescein-stained leukocytes have been used to calculate retinal blood flow velocity in experimental animals and humans; however, this method is not used in clinical practice [13 - 15]. Ultrasound color Doppler imaging cannot provide individual retinal blood flow measurements due to its poor spatial resolution [16]. For in-vivo measurements, several instruments, including the Heidelberg retina flowmeter (HRF, Heidelberg GmbH, Heidelberg Germany), bi-directional laser Doppler velocimetry (LDV), Canon laser blood flowmeter (CLBF 100, Canon, USA), color Doppler optical coherence tomography (CD-OCT), and Doppler Fourier-domain optical coherence tomography (FD-OCT) are currently available [17 - 20]. All these devices are based on the same principle of using the Doppler shift of reflected light from the moving blood cells to calculate blood flow velocity. The laser speckle method of using a laser Doppler flow meter measures blood flow velocity by calculating the change in speckle pattern caused by moving blood cells [21]. Laser Doppler velocimetry (LDV) uses light that is primarily directed perpendicular to the direction of blood flow. The ability to achieve Doppler-shifted signals depends on the sampling light being scattered into the plane of blood flow. While small angles from blood cell shifts allow detection of Doppler frequency shifts, greater turbulence can cause artifacts. Bi-directional LDV is independent of the angle between the incident beam and the direction of flow. LDV needs a relatively long time to make measurements; therefore, it requires precise eye fixation and eye alignment. The HRF uses a scanning laser ophthalmoscope, which helps to visualize small vessels; however, the measurements are done in small areas of 10×10 pixels, and the measurements are in arbitrary units. LDV is limited by the lack of direct information on the flow-velocity profile and blood vessel dimensions, and it is laborious to use because each blood vessel is measured individually. LDV is capable of measuring blood velocity only in retinal vessels of a large diameter. Thus, capillary circulation cannot be studied. CD-OCT has limited spatial and velocity resolution; only large retinal vessels can be analyzed [18]. Doppler FDOCT is limited by phase washout to a maximum speed that is dependent on the sampling frequency of the instrument [22]. In practice, due to the relatively slow sampling frequency of commercial instruments, peak arterial blood velocities cannot be accurately measured at this time. The Retinal Function Imager (RFI) is a novel, noninvasive device to measure blood flow velocity by identification of actual erythrocyte motion in retinal blood vessel in serial images, taken at short intervals (17.5 msec) without any dye. Relevant blood vessel segments on an acquired image can be drawn manually on a computer monitor with the aid of an automatic correction. The built-in software simultaneously measures the average blood flow velocity of manually drawn segments on multiple vessels after image acquisition. Recently, changes in retinal blood flow velocity, using RFI in various ocular and systemic diseases, have been reported [23 - 29]. However, there is no study available which reports inter-session reproducibility of RFI. In the present study, our goal was to find the inter-session reproducibility of the blood flow velocity

measurements of retinal blood vessel segments using the RFI in healthy subjects by using a concordance correlation coefficient (CCC) and coefficient of reproducibility (CR) in a prospective multicenter observational study. Since there is no gold standard instrument of blood velocity measurement in small retinal vessel segments, we could not compare the RFI to a gold standard but rather wished to determine its reproducibility.

Methods

This prospective study was performed at three institutes: the Jacobs Retina Center at Shiley Eye Center, University of California, San Diego, The Retina Service of Wills Eye Institute in Philadelphia, PA, and the Department of Ophthalmology, Edith Wolfson Medical Center in Holon, Israel. Informed consent was obtained from all participants. All procedures conformed to the Declaration of Helsinki for research involving human subjects, and were approved for this prospective study by the Institutional Review Board of the University of California, San Diego, Thomas Jefferson University, Philadelphia, PA, and Tel Aviv University, Tel Aviv, Israel. Time period for this study was from January 2011 to March 2011. All participants were normal subjects without any ocular disease or history of ocular trauma or ocular surgery. Patients with any systemic disease such as diabetes, hyperlipidemia and hypertension were not included. Eyes with poor media clarity and high refractive error (>3 diopters) were also excluded. The Retinal Function Imager (RFI-3005, Optical Imaging, Rehovot, Israel) consists of a standard fundus camera, a stroboscopic lamp system and a computerized digital camera, which takes high-resolution images and detects functional changes in the retina without any dye [30]. For the blood flow velocity-operating mode, a green (“ red-free ”) interference filter with transmission centered at 548 nm and a bandwidth of 17 nm is used. The RFI recognizes the motion of erythrocytes in retinal vessels by comparing eight images of the retina within a single short interval of 0.14 s (17.5 msec between flashes). The apparatus has been described in detail in previous publication [23]. All subjects underwent a comprehensive ophthalmic evaluation, including medical history, assessment of best- corrected visual acuity, detailed slit-lamp biomicroscopy, intraocular pressure measurements with a Goldmann applanation tonometer, and indirect ophthalmoscopy. Blood pressure was measured before RFI imaging, and heart rate was recorded directly by the RFI instrument. All subjects underwent RFI imaging in two separate sessions 15 min apart by a single experienced photographer at each center. An average of 5 – 7 serial RFI images (field of view= 35°), centered at the fovea were taken after pupillary dilation during each session. All the images were taken in synchronization with the patient’s systolic pulse by attaching a probe to the subject ’ s finger. RFI images were transferred electronically to UCSD, and a single observer (JC) assessed the quality of images and performed analysis.

Image analysis

Only images with visible blood flow in the “ratio” mode were used for analysis. Multiple (12-20 per eye) segments of secondary and tertiary branches of arteries and veins were selected with the help of the RFI software using the manual drawing tool with automatic vessel correction by the software. We chose a blood vessel segment length of 100 microns or less in order to avoid any blood vessel crossings. The selected vessel segments from each first session were overlaid onto the second session images with the built-in software to avoid any selection bias. Unfocused segments, mostly in the periphery, were excluded. The built-in software measured average velocity and relative standard deviation or coefficient of variance (calculated as standard deviation over the mean) for each segment for both sessions. Vessel segments with relative standard deviation of more than 0.45 in either session were considered unreliable and were automatically flagged in the results display by the built-in software (Figure 1).

Statistical analysis

The inter-session reproducibility of blood flow velocity was assessed by the concordance correlation co-efficient (CCC), coefficient of reproducibility (CR) and coefficient of variance (CV). Bland Altman plots were used to assess the clinically relevant magnitude of the difference between the measurements from two sessions.

Results

This prospective study included a total of 18 eyes from 15 healthy subjects (6 men, 9 women) without any ocular or systemic disease. Mean age was 47.3 years (range 21-65 years). Intraocular pressure was within normal limits in all subjects. Mean systolic and diastolic blood pressure among the subjects was 116 ± 11.4 and 73 ± 8.9 mmHg. Mean heart rate among the subjects was 73 ± 5.4 .

Total number of segments analyzed was 229 microns with an average segment length of 78.1 microns. Total number of arterial and venous segments was 109 and 119 respectively. Average number of arterial and venous segments in each eye was 5.4 and 6.1 respectively.

Inter-session CCC for blood flow velocity was 0.97 (95% Confidence Interval (CI), 0.966 to 0.9797). Coefficient of reproducibility (CR) was 1.49 mm/sec (95% CI, 1.39 to 1.59 mm/sec). Coefficient of variance (CV) was 10.9%. Bland-Altman plot of difference against mean blood flow velocity is shown in Figure 2 (left panel).

The average systolic arterial blood flow velocity was 3.16 mm/sec, and average venous blood flow velocity was 3.15 mm/sec. The coefficient of reproducibility for arterial blood flow velocity was 1.61 mm/sec (Standard deviation= 0.82 mm/sec) (95% CI, 1.47 to 1.74 mm/sec). Coefficient of reproducibility for venous blood flow velocity was 1.27 mm/sec (Standard deviation=0.64 mm/sec) (95% CI, 1.14 to 1.41 mm/sec). There was no statistically significant difference between the arterial (paired t-test $p=0.95$) and venous (paired t-test $p=0.57$) velocities in two sessions. Mean difference between two sessions among arteries, veins and both was -0.008, 0.09, and 0.03 respectively. There was no statistically significant difference between the heart rate in two sessions ($p=0.1$). A Bland – Altman plot of difference against mean blood flow velocity for veins and arteries is shown in Fig. 2 (middle and right panel).

Discussion

Study of average retinal blood flow would be interesting in diseases such as vascular occlusions, diabetes, sickle cell disease, multiple myeloma, etc. It would be helpful to evaluate blood flow velocity of individual vessels in conditions such as tributary vein occlusion and branch retinal artery occlusion. Our co-authors previously reported the total velocity variability by RFI was up to 10% (Christian K, et al. IOVS 2010;51: ARVO E-Abstract 1054), but total flow velocity average errors may give a falsely high reproducibility. To avoid this, we determined reproducibility for individual vessel segments. In our study, the coefficient of variance for blood flow velocity for individual segment was 10.9%, which is comparable to previous reports on reproducibility for other devices. The coefficient of variance reported for blood flow velocity measurement by Canon LBF, Laser speckle method, and single point LDV were $16.4 \pm 12.8\%$, $9.5 \pm 2.5\%$, and 18% respectively [20, 21, 31]

RFI software permits comparison of retinal blood flow in any selected vessel section between multiple image sessions, and can be used to follow a disease progression or the effect of therapy. We used this software to determine reproducibility of the measurements

between a first and second imaging session. The RFI is capable of calculating average retinal artery and vein blood flow velocity per eye, which is similar to previous instruments. An advantage of RFI is that the flow velocity can be measured for arterioles and venules in the same image by drawing the vessel segment of interest, following image acquisition. Advantages of RFI include direct imaging in the blood flow plane, relatively shorter image acquisition time, simultaneous measurement of all vessels in the field of view (20 or 35°) of fundus camera, and a wider range of vessels that can be analyzed. The RFI also allows easy drawing of the vessel of interest, and the software automatically highlights unreliable measurements ($CV = >0.45$). Observation of erythrocyte movement as moving particles on “ratio” mode confirms the retinal blood flow, a feature that is not available on other instruments. In contrast to other technologies, the advantage of RFI is that it provides measurements in standard units, (mm/sec) unlike laser speckle and SLDF, which only provide measurements in relative units. Measurements in standard units help to compare velocities within each eye and between the two eyes of a patient.

The RFI uses eight flashes of a stroboscopic lamp system with a wavelength of 540 nm to get an image in 0.14 s (17.5msec between flashes). The Canon LDV has an eye tracking system [20], but the use is still limited to cooperative patients. Due to the short imaging interval, the RFI is less affected by saccades. The exposure to light wavelengths of 530 – 590 nm for approximately 0.14 s is well within permissible limits (460 mW/cm² for 10s) [32]. Laser Doppler velocimetry requires a longer duration for measurements, with strict fixation and fine alignment of the laser beam, whereas RFI does not need any alignment, and is similar to taking a color photograph of the retina.

The RFI can detect moving erythrocytes in retinal vessels as small as 8 μ m in diameter, i.e., capillaries. Therefore, the RFI may be useful in retinal vascular diseases affecting small vessels. The RFI differentiates “artery from vein” by the flow direction, and marks the segments a different color and the measurements a different sign. Red color with a negative value defines the artery; purple color with a positive value defines the vein.

In our study, the intersession concordance correlation coefficient was excellent (0.97). Concordance correlation coefficient for Canon laser blood flowmeter for intersession was 0.91, and that for SLDF was – 0.70 to 0.85 [20 , 33]. This excellent correlation may be attributed to mapping of the selected segments using built-in software that is observer-independent.

This is helpful to assess the changes in retinal blood flow with changes in the heart rate. RFI quality, like other devices, depends on optical media clarity. The RFI provides only blood flow velocity; however, flow volume can be calculated by incorporating vessel diameter measured by other software. In conclusion, the RFI provides reproducible measurements for retinal blood flow velocity. The advantages include short image acquisition time, simultaneous measurement of multiple vessels, and measurements in standard units. Better knowledge of retinal blood flow velocity with RFI will be helpful to better understand the pathophysiology of the retinal changes in various ocular and systemic disorders.

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Figure 1.

Left panel shows retinal blood flow velocity mapping in a healthy subject, average velocity values with standard deviation in vessel segment of arteries (red) and veins (purple) are shown. Right panel shows vessel segments flow velocity report including various columns displaying segment identification (SegID), Size of the segment, mean blood flow velocity, standard deviation, relative standard deviation (standard deviation/mean) and blood flow velocities of each segment in each series. Values with purple background and pink background are used for veins and arteries respectively. Measurements in white numbers are unreliable (relative SD >0.45).

Figure 2.

Intersession reproducibility for overall (left), venous (middle) and arterial (right) blood flow velocity: Bland-Altman plot of difference against mean blood flow velocity shows no significant change in intersession variability for the range of blood flow velocity measurements. Dashed lines: mean difference and 95% limits of agreement ($1.96 \times$ Standard Deviation (SD)).